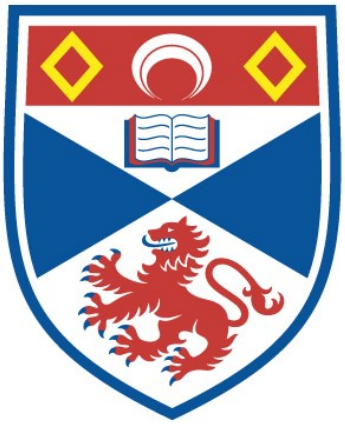
HELMINTH PARASITES OF MARINE FISHES: THE BIOLOGY OF DICLIDOPHORA DENTICULATA A MONOGENETIC TREMATODE

Helga Maud Toynbee Frankland

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1953

HELMINTH PARASITES OF MARINE FISHES:

THE BIOLOGY OF DICLIDOPHORA DENTICULATA, A MONOGENETIC TREMATODE,

being a thesis presented by

HELGA MAUD TOYNBEE FRANKLAND

to the University of St. Andrews,
in application for the degree of
Ph.D.

DECLARATION.

I hereby declare that the following Thesis is based on the results of experiments carried out by me, that the Thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The Research was carried out in the Department of Natural History, United College, St. Andrews.

(Helga Frankland)

CERTIFICATE.

I hereby certify that Helga Maud Toynbee Frankland has spent nine terms at Research Work on Helminth Parasites of Marine Fishes, that she has fulfilled the conditions of Ordinance No. 16 (St. Andrews) and that she is qualified to submit the accompanying Thesis in application for the degree of Ph.D.

1952

CAREER.

I matriculated in the University of St. Andrews in October, 1938 and followed a course leading to graduation in Natural History until 1949.

On October 18th, 1949, I commenced the research on helminth parasites of marine fishes which is now being submitted as a Ph.D. Thesis.

I was appointed to a University Post-Graduate Scholarship in the Department of Natural History, United College.

THE BIOLOGY OF DIGLIDOPHORA DENTICULATA,

A MONOGENETIC TREMATODE.

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INTRODUCTION.

The over-riding economic importance of digenetic trematodes due to their pathogenic activities has led to much research being devoted to their study. Monogenetic trematodes have no bearing on the field of applied science except in so far as some of them cause epidemics in fish-hatcheries and, as a result, most of the work which has been done on them has been confined to simple description of species with no attempt to determine their ecology or even their life-cycles. However some of the free-swimming larvae have been described and, in a handful of cases, the whole life-history has been worked out. The present study adds one species, Diclidophora denticulata, to this short list. In addition to the elucidation of the life-history, the anatomy of the fluke has been re-examined with a view to confirming and elaborating the descriptions of earlier workers, and an attempt has been made to learn something of the biology of the species.

The work was carried out during tenure of a Post-Graduate Scholarship for which I am indebted to the University of St. Andrews.

I wish to express my very grateful thanks to Mr. D.R.R. Burt, B.Sc., F.L.S., F.R.S.E., who, as my Supervisor, has given encouragement and much valuable advice throughout the work, and to Professor H.G. Callan, D.Sc., F.R.S.E., for his help and sympathetic consideration. I am also grateful to

Mr. J. Llewellyn of Birmingham University, and to Mr.D.C.W. Smith and Miss E.M. Shelswell, both of St.Andrews University, for permission to use the unpublished material referred to in the text.

MATERIAL AND METHODS.

The Host.

The flukes used for this work were obtained from the gills of *Gadus virens*. The fish were drawn almost exclusively from St. Andrews' Bay, being caught with hook and line, usually from a dinghy, over rocks. A few were obtained through the kindness of other fishermen in the neighbourhood and, of these, a small number were caught in the Firth of Forth.

In the later part of the work some scales from each fish were mounted under cover-slips in the dry state.

The Method of Detection and Removal of Flukes.

Fish for examination were killed and the gills removed immediately to petri dishes of sea-water. The filaments were scanned under low power binoculars and the flukes removed with blunt needles and a pipette or, where sections of the fluke *in situ* were required, the filament bearing the worm was cut off.

Records Kept.

The length of the host fish and the date on which it was killed were recorded. In a few cases, in which the heads of the fishes alone were available for examination, their total lengths were estimated by using a formula devised by Smith (unpubl.). He found that the relationship between length of head (H) and total length (L) was in

Gadus virens

$$H/L = 0.2405$$

with a possible error in estimating total length from head length of ± 0.4 cms. This error can be ignored. There is a correlation between this ratio and total length such that there is an increase in relative size of head with size of fish. Where, as here, the fish measured do not vary greatly in size, this again can be ignored. Total length was, accordingly, estimated from the formula

$$\text{Total Length} = \frac{\text{Length of Head}}{0.2405}$$

In every case it was noted from which pair of gills the flukes were removed, and all flukes obtained were classified according to their developmental stage.

Methods of Observation on Living Flukes.

The general behaviour of living flukes was studied in sea-water with low power dissecting binoculars, but more detailed observations of their bodily functions were made under higher power, the worms being suspended in hanging drop preparations or lightly compressed under a coverslip.

Culture of Eggs.

Eggs set aside for development were placed in sea-water in small, deep petri dishes which were put in a larger, covered vessel partly filled with water, to prevent evaporation and the whole kept in a temperature-controlled tank. The sea-water was renewed from time to time. Sometimes

Berkfeld-filtered water was used and, though this was not essential, it helped to keep down the growth of moulds on the egg-shells.

Method of Study of Free-Swimming Larvae.

Living larvae were studied in the same way as adults. To render the excretory system visible they were strongly compressed under cover-slips by withdrawing water.

Experiments were carried out to test the response of the larvae to jarring, shade and light stimuli, to dilution of the medium and to the presence of pieces of the host's gill-tissue. Details of the methods used are included in the account of larval behaviour.

Method of Host Deparasitisation and Re-infestation with Larvae.

Hosts required for experimental infestation were first deparasitised by a modification of Alvey's (1936) technique. They were placed for five minutes in a solution of chloretone (trichlor-tertiary-butyl alcohol) made up of two parts saturated chloretone-in-sea-water at room temperature to five parts of sea-water. This leadsto total anaesthesia of the fish and all, or almost all, the flukes present died and dropped off. The fish was then revived in pure sea-water, a current of water being caused to flow over the gills aiding the process.

One or two days later experimental infestation was carried out. The fish, together with a petri dish containing larvae of three to four hours of age, was placed in sea-

water in a small aquarium fitted with an aerator. After four hours the fish was removed to permanent aquarium quarters. Four hours was found to be the optimal period for infestation. No larvae established themselves on the gills of the host when periods longer than four hours were used for infestation perhaps due to their having been killed by the accumulation of waste-products in the relatively small volume of water.

Recovery of Larvae from Experimental Infestations.

At varying lengths of time after the hosts had been exposed to free-swimming larvae the fish were killed and their gills examined in the usual way to recover the larvae and determine their degree of development. The finding of a significant number of larvae of the same stage of development was taken as being satisfactory evidence that these were the larvae supplied to the fish experimentally.

Methods of Fixation and Staining of Adults and Larvae.

Owing to the great contractility of the fluke it is difficult to fix specimens in a way which gives reliable results for measurements of size and bodily proportions. The method giving the most constant results was found to be placing the worms in 20% magnesium sulphate solution where they were left until they did not move on stimulation. After being laid on a slide, if necessary under very gentle pressure from a coverslip, they were then fixed. Free-swimming larvae for all purposes were fixed by pipetting

them suddenly into a large volume of fixative.

Various fixatives were used, including Bouin's fluid, Schaudinn's fluid made up in sea-water, and Carnoy-Lebrun's fixative, but the most satisfactory, causing least shrinkage of tissue and artificial coalescence of shell-globules, was chromo-nitric acid (Gatenby and Painter, 1937).

For whole mounts the worms were usually stained with Gower's (1939) modified carmine, occasionally with light green as a counter-stain. Paraffin-embedded sections were cut at varying thicknesses from 6 - 15μ and stained either with Heidenhain's haematoxylin and eosin or Heidenhain's azan stain (Pantin, 1946), the latter proving useful particularly in the study of the muscular system.

Methods of Fixation and Staining of Embryos.

Great difficulty is always experienced in achieving satisfactory fixation of embryos which are enclosed in impermeable egg-shells and D. denticulata is no exception. Immersing the eggs in a hypertonic solution, such as a fixative, leads to the shells collapsing and crushing the contents. Attempts to puncture the shell mechanically were unsuccessful owing to its smoothness and resilience. The following method of fixation and subsequent opening of the shell by chemical means was eventually worked out and gave tolerably satisfactory results.

Acetic sublimate (Halkin, 1901) was added to the eggs, a few drops at a time, as they lay in sea-water. They were then left in the full strength fixative for some hours. After

washing in water, excess sublimate was removed by leaving them for a while in a very dilute aqueous admixture of Lugol's solution (Pantin, 1946). After a further wash in water they were transferred very gradually to a 25% aqueous solution of eau de Javelle (Halkin, 1901) and kept under observation. When the filaments had largely dissolved away and the shell seemed on the point of rupture, usually after about three quarters of an hour, the eggs were transferred suddenly to distilled water. This, by endosmosis, ruptured the shell along the opercular line. As soon as this occurred the eggs were transferred to Belling's carmine (Gatenby and Painter, 1937), which acted as the final stain for those required as whole mounts and in any case prevented further swelling and disruption of the egg-contents which rapidly took place if they were left in water.

Eggs required for whole mounts were dehydrated, cleared in clove oil and mounted in balsam. Chatton's technique (Langeron, 1925) was used for eggs required for sectioning, staining on the slide with Heidenhain's haematoxylin and eosin following.

Micro-Chemical Methods.

Micro-chemical tests carried out comprised those used on egg-shells, shell-globules, sclerites in the clamps, 'brown cells' in the epithelium of the gut, and the contents of the gut. Egg-shells and sclerites were subjected to Campbell's (1929) test for chitin and also to the xanthoproteic reaction, the biuret reaction, and to Millon's reagent.

Brown's test for blood and blood-products was used for the sclerites and shell-globules and also for the 'brown cells' and contents of the gut.

Definition of Terms Used.

To avoid confusion definitions are here given of terms to which a specialised meaning has been applied.

The term 'ovum' is used to denote the female germ cell only.

The term 'egg' is used to denote the whole, shelled egg with its enclosed ovum and yolk-cells.

The term 'free-swimming larva' (L°) denotes the ciliated larva.

The term 'first stage larva' (L^1) denotes a larva which has lost its ciliated coat but has developed no definitive clamps.

The term 'second stage larva' (L^2) denotes a larva with one pair of definitive clamps.

The term 'third stage larva' (L^3) denotes a larva with two pairs of definitive clamps.

The term 'fourth stage larva' (L^4) denotes a larva with three pairs of definitive clamps.

The term 'fifth stage larva' (L^5) denotes a larva with four pairs of definitive clamps, the anterior pair being smaller than the other pairs.

The term 'immature' fluke (I) denotes a fluke which has four pairs of definitive clamps all of equal size but

whose reproductive system is not fully mature, that is to say, yolk is not present in the yolk reservoir.

The term 'adult' fluke (A) denotes a fluke which has yolk in the yolk reservoir and is reproductively mature.

Nomenclature Used.

The names of Monogenea adopted are those considered correct by Sproston (1946). A list of these names is given together with the synonyms which have been used by authors to whose papers reference has been made.

APPENDIX ON GADUS VIRENS.

Correlation of Size and Age.

Stuart Thomson (1902) established the fact that growth rings on the scales of members of the family Gadidae were produced annually and could, therefore, be used to determine the age of specimens.

Bertelsen (1942) found that spawning of Gadus virens began off the Scottish coast in January, but later in more northerly districts, and culminated about April 1st in Faroe waters. Bertelsen's findings of the growth rate of the species in Faroe waters are expressed in the following table.

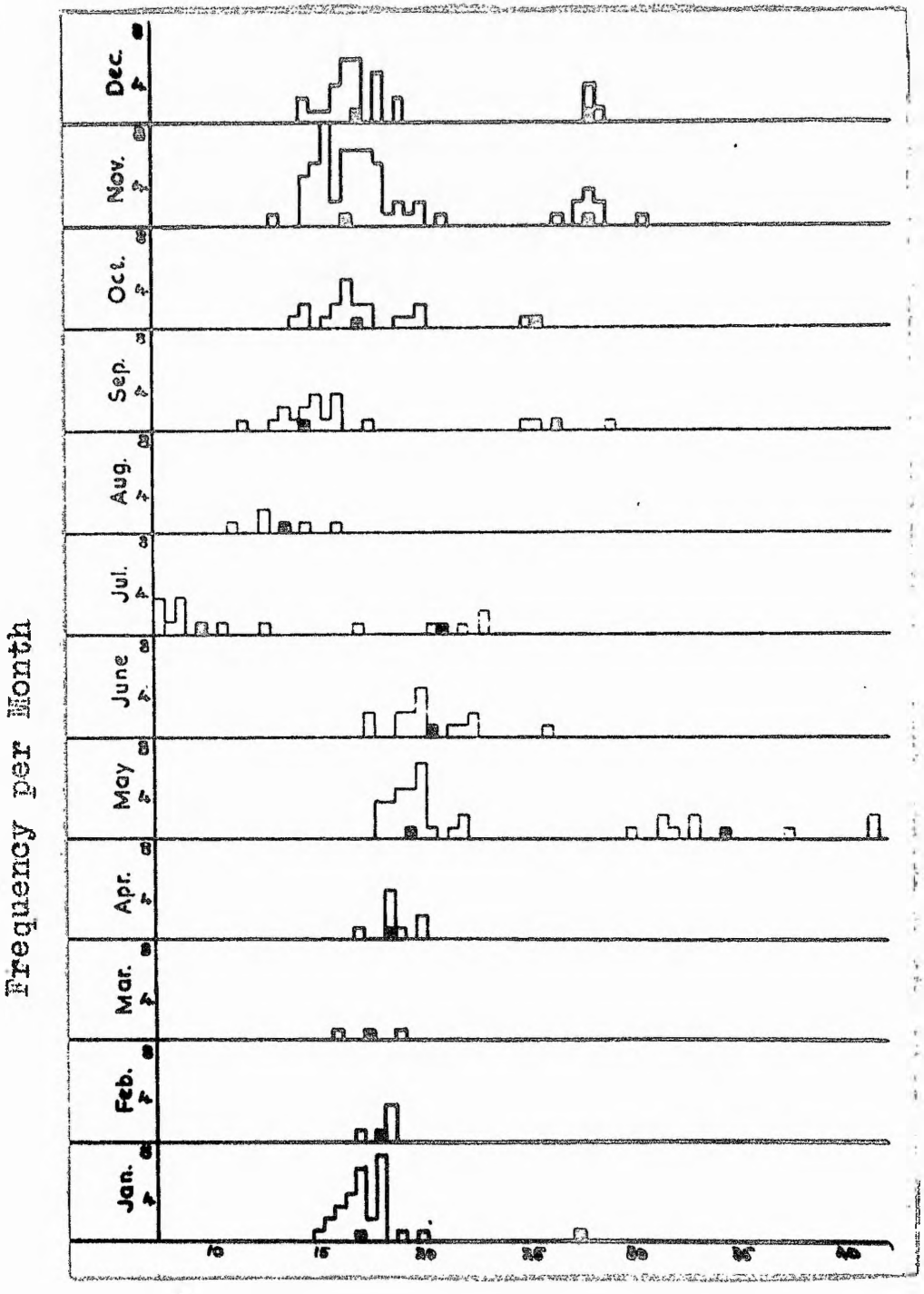
Age Period	Length at End of Period	Remarks
1st 3 months	3 cms.	Pelagic.
4th month	6 cms.	Littoral: rapid growth.
5th month		Slow growth producing a secondary ring.
6th & 7th months	10 cms.	Rapid growth again.
1st winter	13 cms.	1st annual ring.
2nd winter	26 cms.	2nd annual ring.
3rd winter	39 cms.	3rd annual ring.
4th winter	49 cms.	4th annual ring.
5th winter	60 cms.	5th annual ring.
6th winter	72 cms.	6th annual ring.
7th winter	78 cms.	7th annual ring.
8th winter	83 cms.	8th annual ring.
9th winter	84 cms.	9th annual ring.
10th winter	91 cms.	10th annual ring.

Table 1.

At St. Andrews, fry of G.virens begin to appear in rock-pools in April (Smith, unpubl.) and this confirms Bertelsen's statement that spawning begins off the Scottish coast in January, three months previously. The lengths of fishes caught in St. Andrews' Bay and neighbourhood during the present investigation are shown in Graph 1. From the composite picture thus built up, the average lengths in each natural group give the estimated growth rate of an individual fish. This is shown in Table 2.

Estimated Age	Length	Growth Period
5 months	9.0 cms.	
6 months	13.5 cms.	1st growth period
7 months	14.5 cms.	
8 months	17.0 cms.	
9 months	16.5 cms.	
10 months	17.0 cms.	
11 months	17.0 cms.	Formation of 1st annual ring
12 months	18.0 cms.	
13 months	17.5 cms.	
14 months	18.5 cms.	
15 months	19.5 cms.	
16 months	20.5 cms.	
17 months	21.0 cms.	2nd growth period
18 months	-	
19 months	26.5 cms.	
20 months	25.5 cms.	
21 months	28.0 cms.	
22 months	28.0 cms.	Formation of 2nd annual ring
23 months	27.5 cms.	
27 months	34.5 cms.	3rd growth period

Table 2.



Graph 1.

Average lengths shown solid black.

A study of the scales of these fishes revealed that in St. Andrews' Bay the average size reached by G.virens at the end of the first growth period is 16.5cms., but individuals may attain 19.5 cms. At the end of the second growth period the average length is 27.5 cms. An insufficient number of individuals of this age-group were studied to give the maximum size reached by fishes at this stage. These results accord with those derived from Graph 1.

Bertelsen (1942) found that, having spent three months as pelagic fry, the young fishes came inshore, where they remained for their first year, and then returned to deeper water, except for a few which stayed in the littoral region. This would account for the fact that the great majority of the specimens caught inshore at St. Andrews were fishes in their first year.

Distribution.

G.virens is, broadly speaking, a North Atlantic species. On the European side of the ocean Day (1880-1884) gave the range as extending from Spitzbergen to the Mediterranean, including the Norwegian coast, Skaggerack, the coasts of Britain where its incidence decreases markedly towards the south, and round the French coast. On the western side of the Atlantic Day (1880-1884) and Jordon and Everyman (1896-1900) both recorded the presence of this species from Greenland and the Davis' Straits southward to Cape Hatteras, North Carolina. It is absent from the Pacific Ocean (Jordon and Everyman, 1896-1900).

OBSERVATIONS.

Anatomy of the Adult

Diagnostic Synopsis.

HOST: Gadus virens.

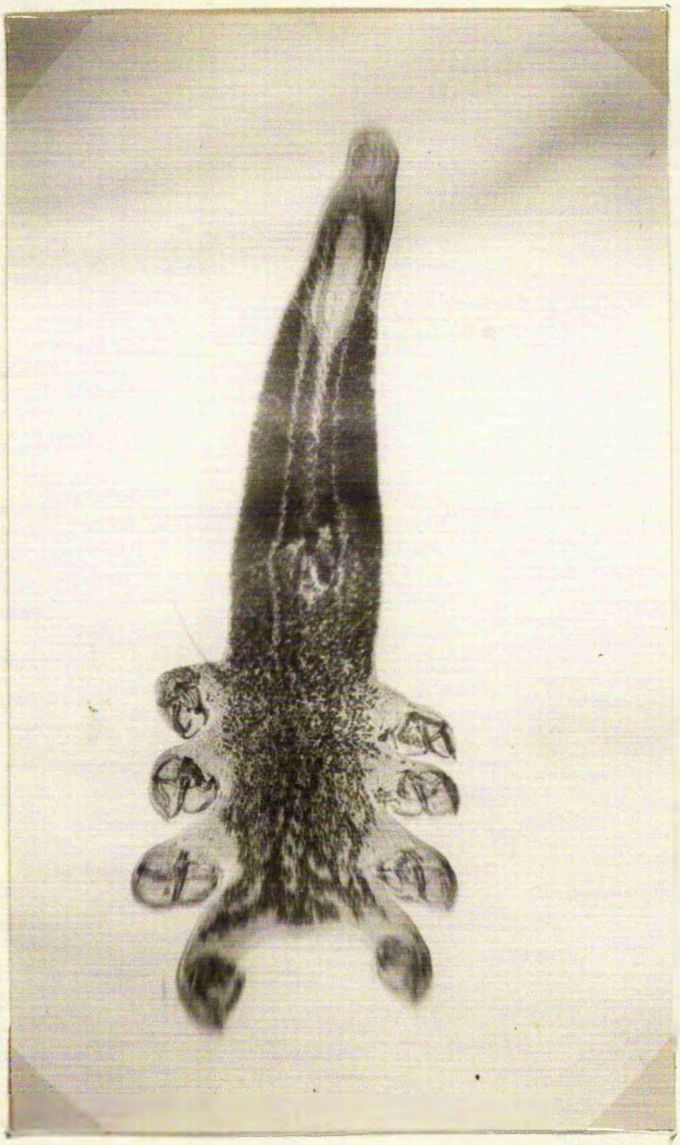
LOCATION: Gills.

SIZE: 2.100 mm. to 7.105 mm. long. FORM: lanceolate, tapering anteriorly; 4 pairs pedunculate clamps laterally in posterior third of body; dorso-ventrally flattened; no posterior hooks. CLAMPS: pedunculate cups supported by 8 sclerites; bearing teeth on outer, anterior face; all of equal size. ALIMENTARY SYSTEM: mouth almost terminal; 1 pair lateral suckers in buccal cavity; pharynx bulbous; oesophagus short; 2 intestinal caeca, blind, ramifying and anastomosing, particularly posteriorly. NERVOUS SYSTEM: supra-oesophageal commissure giving off 1 pair lateral, anteriorly-directed nerves and 2 pairs lateral, posteriorly-directed nerves. EXCRETORY SYSTEM: flame cells present; 1 pair longitudinal, lateral canals running from anterior region posteriorly and returning along same course to open anteriorly, dorso-laterally. REPRODUCTIVE SYSTEM: ovary median, broad, tubular, folded; oviduct leading to ootype; genito-intestinal canal joining oviduct to right caecum of gut; ootype surrounded by Mehlis' gland, directed anteriorly; uterus median, broad porally, opens at female genital aperture medio-ventrally, immediately behind level of gut-bifurcation; vitelline acini widespread, transverse ducts

in mid-body uniting in median vitelline reservoir opening posteriorly into distal oviduct; receptaculum seminis anterior and to right of vitelline reservoir opening, temporarily only, on ventral surface, connecting with proximal oviduct by narrow duct; testes, scattered acini posterior, and to lesser extent anterior to, ovary, ventral, confined between main gut-branches; vas deferens running anteriorly ventral to ovary to left of ootype then dorsal to uterus; cirrus ventral, median, anterior to female genital aperture, armed with 11 - 14 hooks; prostate gland immediately posterior to cirrus; eggs elliptical, measuring 191.5μ ± 6.5μ x 78.5μ ± 2.0μ with, in addition, hooked, anterior filament and longer, posterior filament terminating in crenulated disc, many eggs present in uterus together.

General Form and Size.

In shape (fig. 1) Diclidophora denticulata is, at maturity, lanceolate and dorso-ventrally flattened, especially in the mid and posterior regions. The body tapers gradually from the mid-region to the anterior tip, becoming at the same time more cylindrical in section. Behind the mid-region the sides of the body are more or less parallel and bear laterally four pairs of peduculate clamps, the most posterior pair of these being at the hind extremity of the body. The part bearing the clamps is known as the haptor. Between the last pair of clamps the posterior margin of the body extends into a slightly



Legend.
Scale

Fig. 1

demarcated median tongue. A transverse mouth, directed slightly ventrally, is situated at the anterior extremity of the animal, and a little way behind this, in the mid-ventral line are the genital apertures. The male aperture, surrounded by a crown of retracted hooks, is the more anterior, the female aperture lying immediately posterior to it. Dorso-laterally, at the level of the male aperture, there is a small pair of excretory pores, one on each side.

The form of the body is very variable, owing to its great contractility and extensibility, so that the more anterior 'neck' region and the peduncles of the clamps in particular may be greatly varied in length, and the posterior, median tongue may, at times, be obliterated. For the same reason representative measurements are difficult to make, but the range of size of adults fixed under standard conditions is given in Table 3.

Measurement.	Largest.	Smallest.
Total Length.	7.105 mm.	2.100 mm.
Length of Haptor.	2.555 mm.	0.630 mm.
Greatest Breadth.	2.345 mm.	0.315 mm.
Transverse Diameter of pharynx.	151.5μ	77.0μ
Average Transverse Diameter of 3 anterior pairs of Clamps.	688.0μ	232.5μ

Table 3.

The Body-Wall and Parenchyma.

The body-wall (fig. 18a) is composed of six layers with modifications in certain regions. Externally there is a thin cuticle beneath which lie circular muscles. Internally to the circular muscles lie longitudinal muscles separated into a very narrow outer layer and a broad inner layer by a thick parenchymatous region. Beneath the inner longitudinal muscles oblique muscles cross one another.

The cuticle is transparent and structureless. It is 1.5μ thick, but when the underlying tissues are contracted it is thrown into folds producing transverse striations on the body surface. In addition to these there are proliferations of the cuticle forming numerous rosette-shaped pores about 50μ apart. These have a central cavity open to the exterior from which exudes mucus-like material. Their bases are invaded by the underlying parenchyma.

The circular muscles are composed of closely-set, discrete bundles of fibres, very small and slender anteriorly and dorsally, but large and powerful in the ventral, haptorial region where they invade and largely obliterate the underlying parenchymatous layer.

The longitudinal muscles are similar in form to the circular muscles, but the bundles of fibres of the oblique muscles are much more widely separated from one another. No nuclei could be detected embedded in the muscles, though it is possible that nuclei associated with the development of musoular tissue may eventually become separated from it.

The parenchymatous layer is a syncytium with granular cytoplasm containing scattered nuclei. There are also fine, short fibres passing through the tissue in all directions.

The body-wall of the ventral haptorial region (fig. 18b) is not only modified as regards the thickness of the circular muscles, but is considerably thickened by the addition, external to the circular muscles, of a thick layer of closely-set muscular fibres arranged perpendicularly to the body surface with their inner ends contiguous with the circular muscles and terminating exteriorly on the body-surface as ring-shaped papillae. The cuticle in this region is modified or wanting. These perpendicular muscles and papillae are also found on the ventral aspect of the clamp-peduncles.

In addition to the musculature included in the body-wall slender strands of muscle run between the organs from the dorsal to the ventral surface and serve, when contracted, to flatten the body.

The parenchyma of the body persists between the specialised tissues of the organs as loosely arranged, unspecialised cells. However, in the posterior region the cells between the muscles, testicular acini and yolk-acini are much larger than normal parenchyma cells. They are of rather irregular outline, and, as seen after fixation with chromo-nitric acid and staining with haematoxylin, have finely granular cytoplasm; the nucleus, which is approximately spherical, contains one or two closely apposed

nucleoli embedded in a patchy, chromatic network.

The Clamps.

There are four pairs of clamps, those on one side being mirror images of those on the other. When the peduncle is extended laterally and the clamp is open, its cup-shaped cavity is directed ventrally and the rim is more or less circular (fig. 2). The cup is composed of an anterior and a posterior valve united along the mid-line transverse to the axis of the body. Accordingly, when the clamp is closed, providing it is not under any pressure and the peduncle is extended laterally, the slit marking the aperture is ventral and transverse to the body axis.

Before describing the skeleton of the clamps it is necessary to define the parts of the clamp to which reference is made. As already stated the cup is composed of an anterior and a posterior valve and the diameter marking the junction of these is the transverse diameter. At right angles to this diameter another division can be made along the longitudinal diameter. The half of the cup adjacent to the body of the animal is proximal and the other half, beyond the line of the longitudinal diameter, is distal. Thus the cup is divided into four quadrants (fig. 3); those of the anterior valve being the proximal and distal anterior quadrants and those of the posterior valve being the proximal and distal posterior quadrants. On the rim of the cup the four points marking the junction of these quadrants are defined according to their position: that between the proximal

and distal anterior quadrants is the mid-anterior point of the rim; that between the proximal and distal posterior quadrants is the mid-posterior point of the rim; that between the proximal anterior and proximal posterior quadrants is the mid-proximal point of the rim and that between the distal anterior and distal posterior quadrants is the mid-distal point of the rim. The central part of the cup is said to be basal and the parts towards the rim, peripheral. The 'inside' of the cup is ventral and the 'outside' of the cup dorsal.

The tissue of the clamp-cup shows closely packed fibres arranged perpendicularly to the surface in which are embedded eight sclerites arranged in the line of the transverse and longitudinal diameters already defined, and round the greater part of the rim.

Assuming the clamp to be open and viewed from the ventral aspect, the eight sclerites are arranged as follows (fig. 3): A stout rod, a, runs almost from the centre of the cup to the mid-anterior point of the rim between the proximal and distal anterior quadrants. A curved sclerite, b, commencing just distal to the centre of the cup and passing proximally across the base of sclerite a to which it is partially fused, runs in the line of the transverse diameter to the mid-proximal point of the rim and from there extends as a broad plate along the more peripheral part of the proximal anterior quadrant until it meets the peripheral end of sclerite a at the mid-anterior point of the rim.

Fig. 2

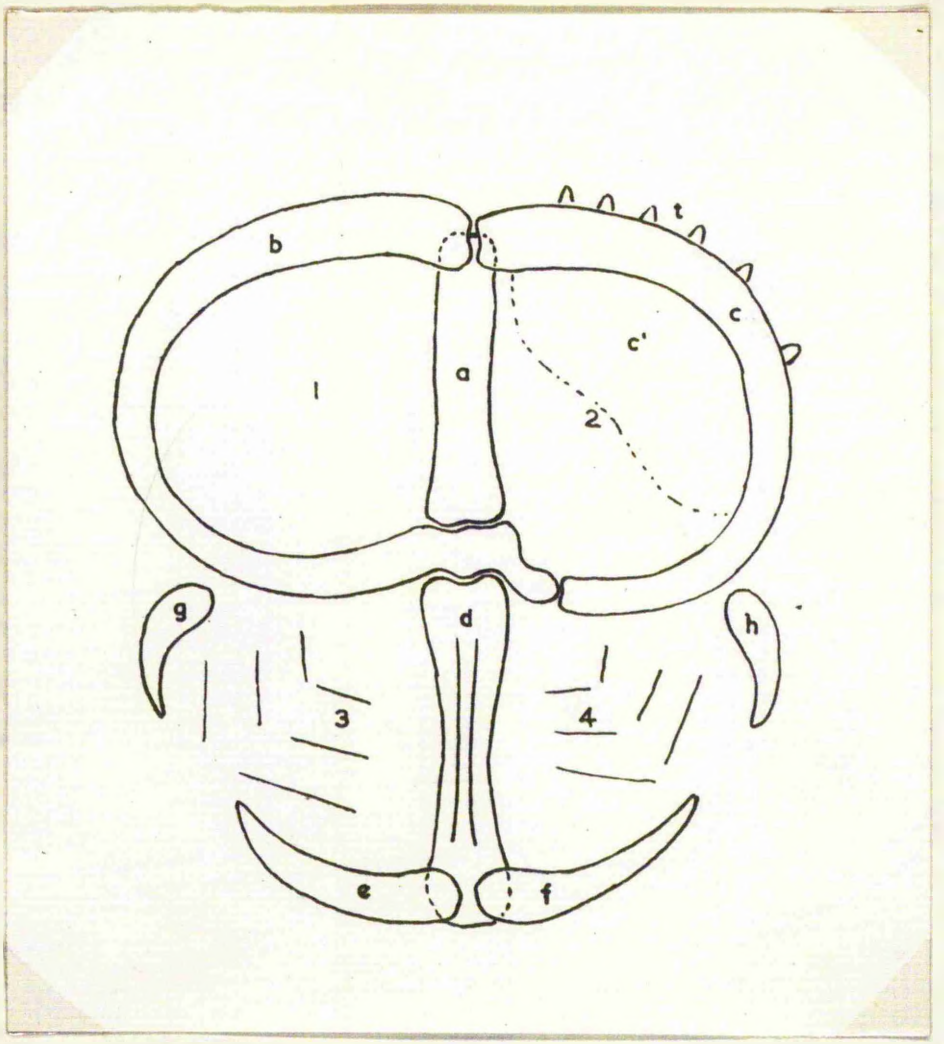


Fig. 3

1. Proximal anterior quadrant.
2. Distal anterior quadrant.
3. Proximal posterior quadrant.
4. Distal posterior quadrant.

Sclerites lettered as in text.

A second curved sclerite c runs distally from the basal end of sclerite b along the transverse diameter to the mid-distal point of the rim and from there round the rim of the distal anterior quadrant until it, also, meets the peripheral end of sclerite a at the mid-anterior point of the rim. Sclerite c is expanded into a lightly cuticularised plate, c', occupying most of the distal anterior quadrant whose dorsal aspect bears a number of conical teeth. In the posterior valve there is a median sclerite, d, running from the centre of the cup, where it is apposed to the basal part of sclerite b, along the longitudinal diameter to the mid-posterior point of the rim. From its peripheral extremity two short sclerites, e and f, pass respectively proximally and distally for a short distance along the rims of the internal and external posterior quadrants. The remaining two sclerites, g and h, are small and take origin from points on the transverse diameter shortly below the mid-proximal and mid-distal points of the rim respectively. They are directed obliquely towards the postero-lateral points on the rim, one in the proximal and the other in the distal, posterior quadrants.

The proximal and distal posterior quadrants are further strengthened by lightly cuticularised strips arranged in a series of open V's, one within another, the apices being directed toward the postero-lateral point of the rim in each quadrant.

The chemical nature of the sclerites is obscure.

Boiling in concentrated potassium hydroxide led to their solution, thus disposing of any idea that they might be chitinous. Micro-chemical colour-tests for proteins were also tried. As it was found that the results were confused by the reaction of the overlying soft tissue, the clamps were allowed to lie in distilled water for some time, while the soft tissue partially disintergrated, according to the method of Grusz (1947). The presence of protein was established by a slight positive reaction on applying the biüret test and by a more definitely positive reaction with Millon's reagent. The xanthoproteic reaction, however, gave an unexpected result, no solution or yellow coloration occurring in concentrated nitric acid and the material blackening rather than turning orange on addition of ammonia.

Musculature (fig. 4) for bringing about the opening of the clamp consists of a broad band of fibres which take origin on the dorsal surface of sclerite a, near its basal end, and the surrounding tissue. This runs across the dorsal aspect of the base of the clamp and is inserted near the basal end of the dorsal aspect of sclerite d and the surrounding tissue. Contraction of this powerful muscle draws sclerites a and d back into the same plane and, owing to the rigidity of the skeleton, opens the clamp.

There is a smaller opposing muscle (fig. 4, 1) attached to the ventral aspects of sclerites a and d near their basal ends, but not extending on to the surrounding

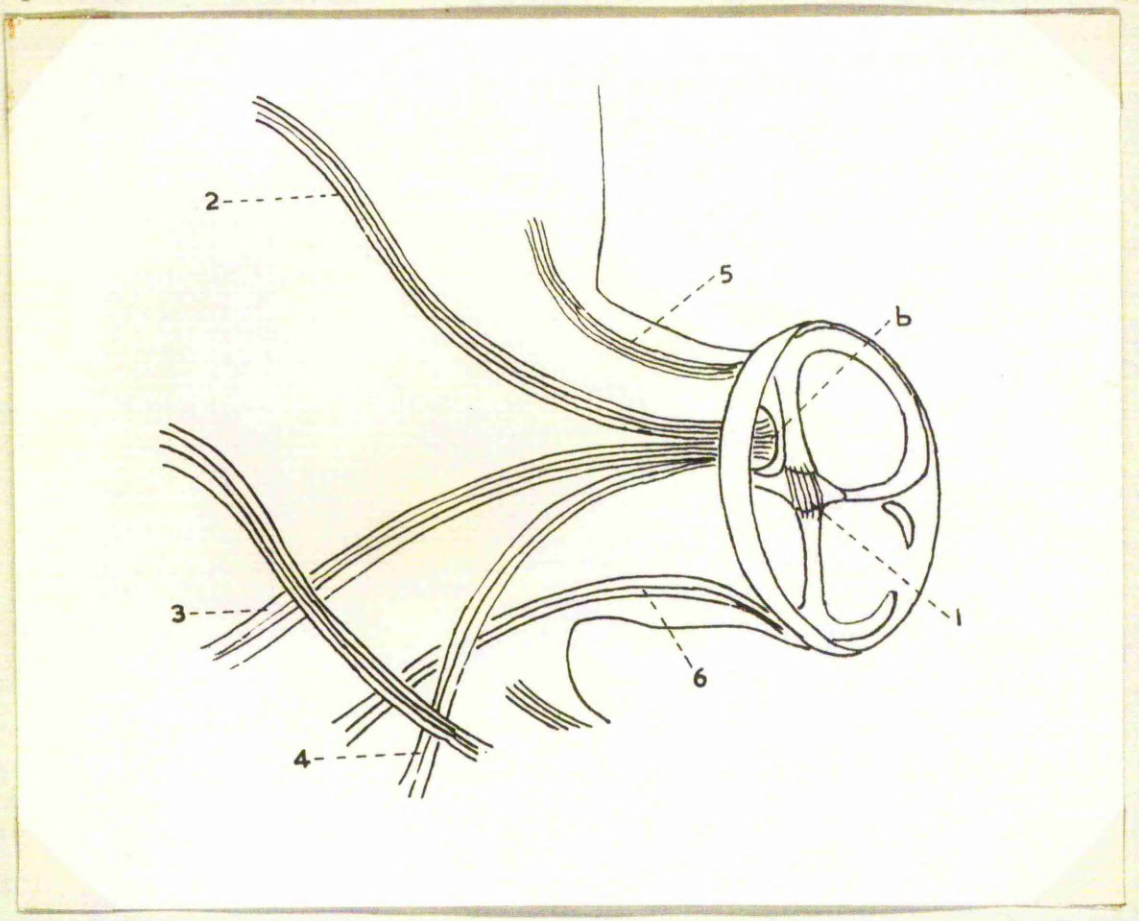


Fig. 4

b, fleshy bulb in proximal anterior quadrant.

Muscles figured as in text.

tissue, which tends to close the clamp. The action of this muscle, once the clamp is closed, is greatly re-inforced by musculature which brings about reduction of the pressure in the cavity of the cup and therefore maintains it in the closed position by suction. A prominent bulb (fig. 4, b) of fibrous tissue projects into the proximal anterior quadrant of the clamp between the bounding sclerites. On this bulb three powerful muscles are inserted. They take origin in the ventral body wall anterior (fig. 4, 2), directly opposite (fig. 4, 3) and posterior (fig. 4, 4) to the clamp which they serve. On contraction they withdraw the fibrous bulb from the cup thus reducing the pressure within it. The rim of the clamp is effectively sealed by a flange of thin, flexible material.

The three muscles mentioned as bringing about the withdrawal of the bulb from the cup also have a function in shortening the peduncle and pulling the clamp inwards ventrally to the position naturally taken up when the animal is attached to the gill-filament. In the most posterior pair of clamps the middle and posterior muscles to the bulb unite in mid-body to form a powerful band of muscle bordering the posterior margin of the haptorial region. Three other muscles are also concerned with the movement of the clamp on peduncle. Anteriorly in the peduncle there is a muscle (fig. 4, 5) which arises in the ventral body-wall and runs obliquely backward to the clamp. This muscle draws the clamp and peduncle anteriorly and ventrally. Arising

posteriorly to the clamp there is a muscle (fig. 4, 6) running forward and outward to be inserted dorsally at the posterior margin of the clamp which it serves to move in a posterior direction. Finally there is a short muscle arising from the dorsal body-wall and inserted on the proximal transverse diameter of the clamp, which, on contraction, it moves dorsally. These muscles which draw the clamp back also aid in opening it as they fan out over the dorsal face of the clamp where they are inserted.

The peduncle is extended by contraction of the circular muscle-component of the wall.

The Alimentary System.

A diagram of the alimentary system is shown in fig. 5. The anteriorly placed mouth opens into a buccal cavity leading into the pharynx, followed by a short oesophagus which bifurcates to form two blind, intestinal caeca, showing many lateral branches which anastomose posteriorly.

The mouth is terminal but directed ventrally to the extent that the dorsal lip is completely terminal and slightly overlaps the ventral lip. When fully open the mouth presents a circular aperture and, when closed, a more or less transverse slit, the ventral lip then lying in a straight line, but the median part of the dorsal lip being pinched off to form a slight pocket. The lips are composed of connective tissue and there is no sphincter muscle in their walls.

The buccal cavity has a pair of oval, buccal suckers

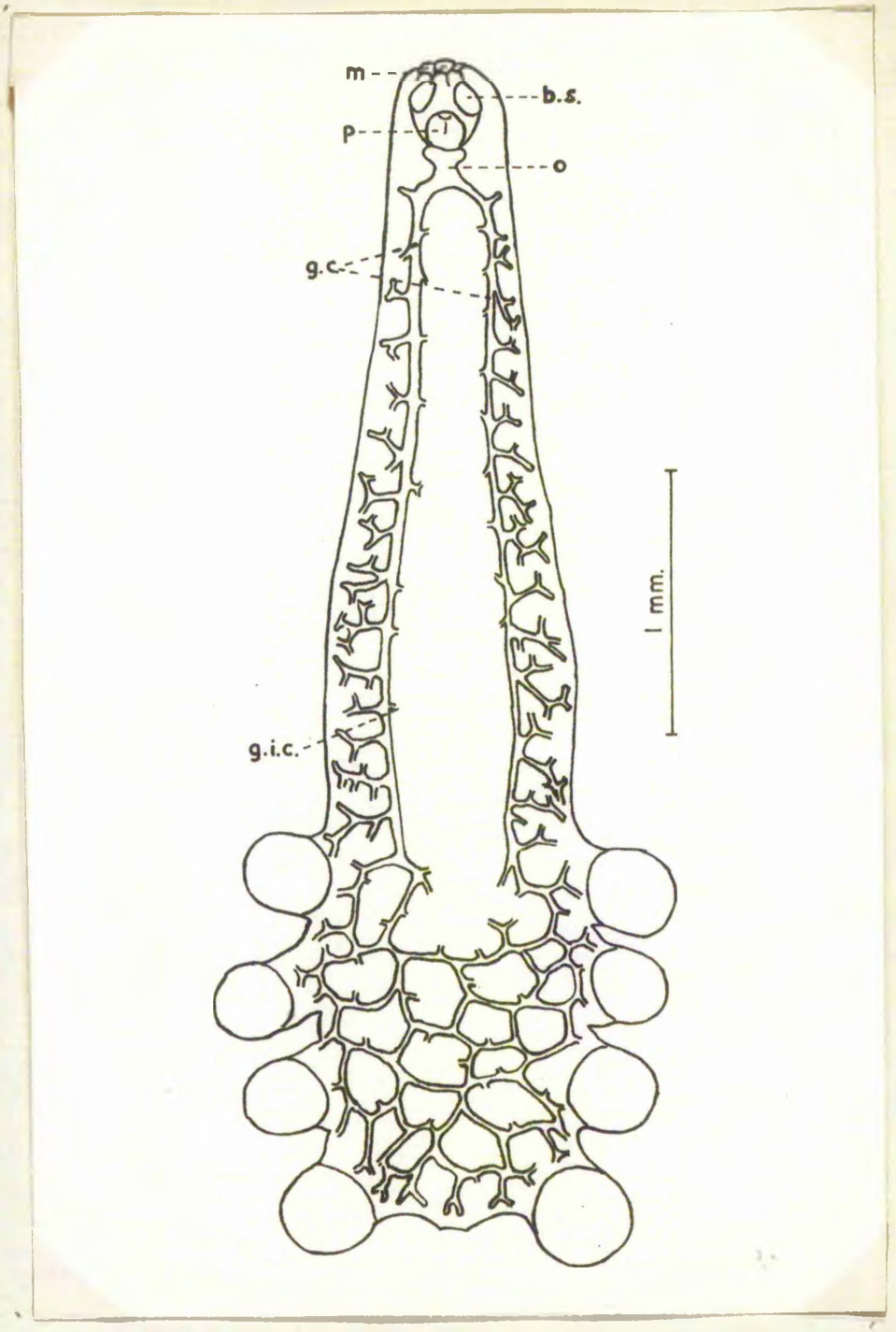


Fig. 5

m, mouth.

b.s., buccal suckers.

p., pharynx.

o., oesophagus.

g.c., gut caeca.

g.i.c., genito-intestinal canal.

(fig. 5, b.s.) incorporated in its dorso-lateral walls, their greatest diameter being longitudinal. They are composed of closely set fibres lying perpendicular to the surface. The internal surface, which is open to the buccal cavity, is papillated in the same manner as the ventral body wall in the haptorial region. Each is controlled by three bands of muscle fibres inserted on the aspect of the suckers which does not open into the mouth. A short band of fibres runs from the dorsal body-wall to the dorsal face of the sucker where it is inserted medianly and slightly anterior to the mid-point (fig. 6a, a). On contraction this muscle increases the concavity of the sucker. In addition muscles, arising from the body-wall more posteriorly in the animal, run forward in two pairs, one pair being inserted on the external lateral margins of the suckers (fig. 6a, b) and the other pair, after passing ventrally to the supra-oesophageal nerve commissure, being inserted on the internal ventro-lateral margins of the suckers (fig. 6a, c). When contracted these muscles draw the buccal suckers and the lips back past the pharynx.

The pharynx (fig. 5, p.) is a slightly pear-shaped body with very thick walls, the narrow, internal cavity being directed antero-ventrally. It is attached by its base to the surrounding tissues and projects freely into the buccal cavity except that part of its dorsal wall is fused to that of the buccal cavity. The thick walls, bounded both externally and internally by a connective tissue sheath,

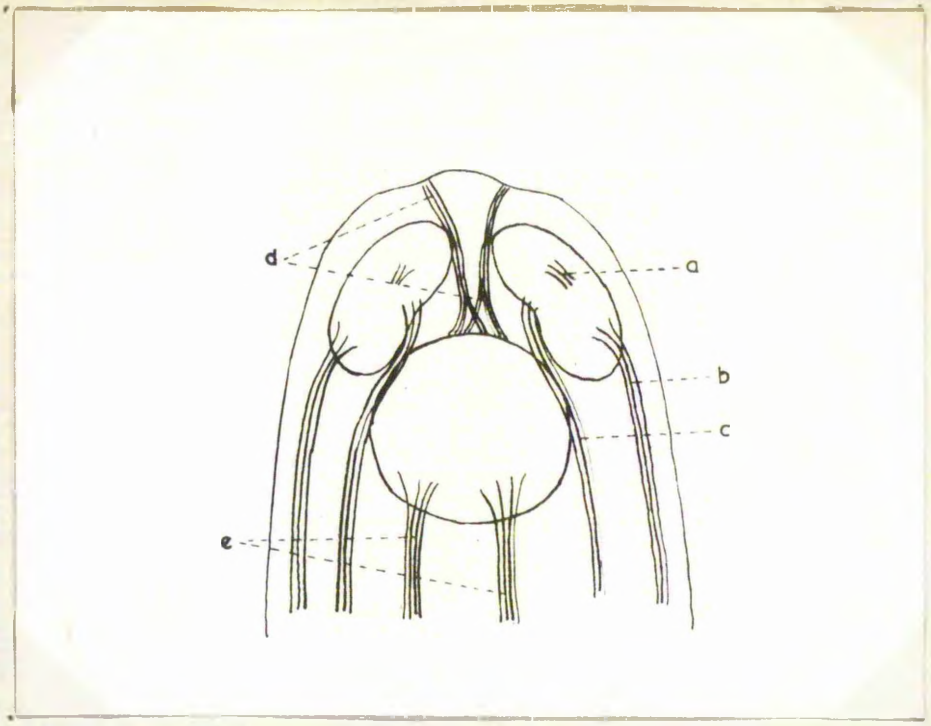


Fig. 6a

Muscles lettered as in text.

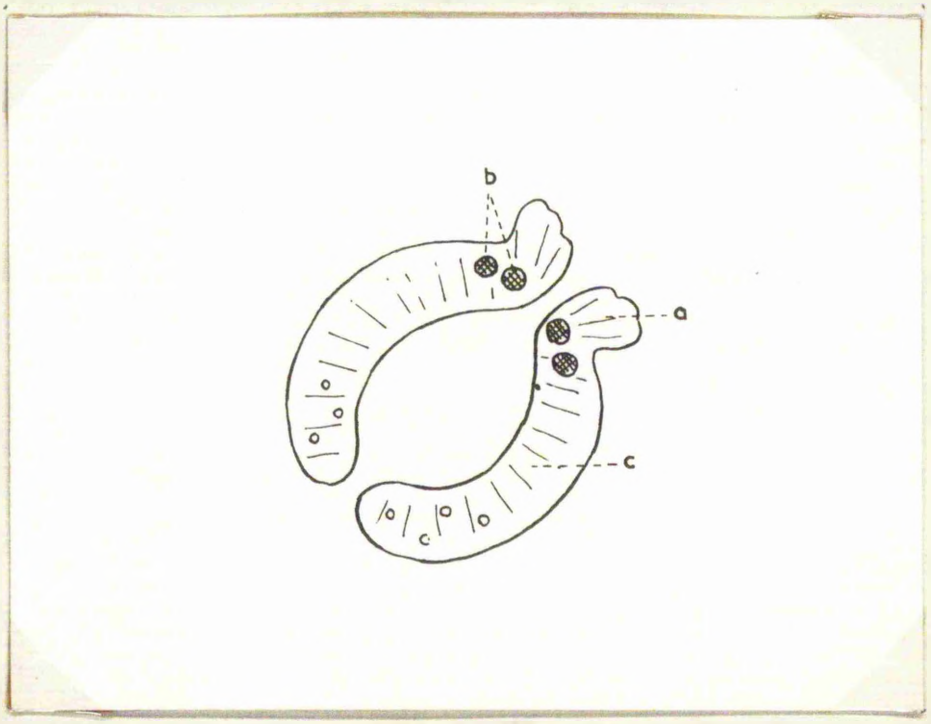


Fig. 6b

a, soft tissue.

b, sphincter muscles.

c, perpendicular muscles.

are built up of three rings of differing structure (fig. 6b). Anteriorly there is a funnel-shaped ring of soft tissue (a). At the base of the funnel the organ narrows sharply and there are two sphincters of powerful, circular muscle, one slightly posterior and external to the other, which compose the second ring (b). The third and largest ring is composed of closely set fibres perpendicular to the surface, among which, particularly posteriorly, nuclei are scattered (c). In the region of the third ring the height, but not the width, of the lumen of the pharynx increases considerably so that the canal forms a dorso-ventral slit. Posteriorly, however, the lumen decreases in height again, so that there is only a small aperture leading into the oesophagus. Apart from its intrinsic muscles the pharynx as a whole is moved by two pairs of muscles. A pair of muscles take origin close together at the most anterior point of the pharynx dorsal to the anterior aperture and, after diverging slightly, are inserted on the dorsal lip on each side of the dorsal pocket (fig. 6a, d). Their action draws the lips back and the pharynx forward. Antagonistic to these muscles is a pair running forward, from a diffuse origin on the dorsal body wall, ventral to the supra-oesophageal nerve commissure, to be inserted one on each side of the dorsal aspect of the pharynx (fig. 6a, c).

The oesophagus (fig.5, o.) is a very short, thin-walled and wide duct into which the pharynx, when at rest,

is partially withdrawn so that the oesophagus-wall rises round and embraces the posterior part of the pharyngeal region.

Immediately posterior to the male genital aperture the oesophagus bifurcates giving rise to the left and right intestinal crura (fig. 5, g.c.) which terminate blindly. These give off branches both medially and externally of increasing complexity as they pass posteriorly. There are, however, no medial branches in the region of the ovary and it is here that the right caecum receives the genito-intestinal canal (fig. 5, g.i.c.). In the haptorial region the main trunks break up into a series of ramifying and anastomosing diverticula which pass also into the clamp-peduncles where there are dilations.

The intestine is lined by a delicate epithelium with sparsely distributed, rather flattened nuclei (fig. 18c). Lying in this epithelium, and projecting freely into the lumen of the gut, are numerous irregularly shaped cells with an oval nucleus appearing reticulate in haematoxylin after fixation in chromo-nitric acid. They are characterised by fine, brown granules sometimes completely filling the cytoplasm and obscuring the nucleus, or only appearing peripherally to a clear vacuole which fills most of the cell (fig. 18c).

There are no special muscles associated with the intestinal diverticula, movement of the gut-contents being brought about by contraction of the body-wall and by dorso-ventral

muscles.

There is little evidence to show that there are glands in association with the alimentary system. The dorsal pocket contains a concentration of globules staining deeply in azocarmine and similar globules are scattered elsewhere in the lips. It is noteworthy that a sticky mucus exudes from the lips when the mouth is used suctorially. Lateral to the pharynx lie a few specialised cells whose cytoplasm stains intensely with azocarmine. Occasionally a tract of this secretion can be seen passing forward from these cells both dorsally and ventrally round the buccal suckers to the lip region.

The Nervous System.

The arrangement of the nervous system in D. denticulata (fig. 7) is characteristic of platyhelminths generally. There is a broad, supra-oesophageal band of nerve fibres from which six trunks arise: one short pair pass anteriorly and laterally to the buccal region (fig. 7, a.n.); a slender pair pass posteriorly along the lateral margin of the body for a short distance (fig. 7, e.p.n.) and finally, the main pair (fig. 7, i.p.n.) run, ventrally to the intestinal crura, posteriorly, to the haptorial region where they continue, close beneath the ventral body-wall, almost to the posterior extremity. They join in a posterior haptorial commissure (fig. 7, p.h.c.) just anterior to the last pair of clamps. There is also an anterior haptorial commissure (fig. 7, a.h.c.) at the level of the first pair of clamps. Minor branches

are given off irregularly from these main trunks and four major branches are given off from each trunk to the clamps of that side (fig. 7, n.c.). Those to the anterior clamps arise at, or sometimes just behind the level of the anterior haptorial commissure and pass forwards before turning towards the clamps. The second and third pairs run directly outwards to their respective clamps and the fourth pair continue posteriorly beyond the posterior haptorial commissure before running to the last pair of clamps. The main part of each clamp-branch passes into the retractable bulb, but minor nerves run anteriorly and posteriorly round the base of the clamp.

The nerves are composed of loose, irregularly anastomosing fibres running along the axis of the nerve trunk. Large 'nerve' cells (fig. 19d) with one large nucleolus in the nucleus and anastomosing fibrils in the cytoplasm occur singly in various parts of the body. The greatest number are seen in the pedunoles of the clamps, a few in the ventral, haptorial region, and a few in association with the more distal part of the uterus and lateral to the pharynx. There are also one or two incorporated in the posterior part of the pharyngeal wall. Very occasionally one is found lying in the nerve where a clamp-branch is given off, and sometimes those lying near a nerve trunk are seen to be attached to the trunk by extremely delicate fibres. The nerve cell bodies are thus not incorporated in the tissue of the nerve fibres.

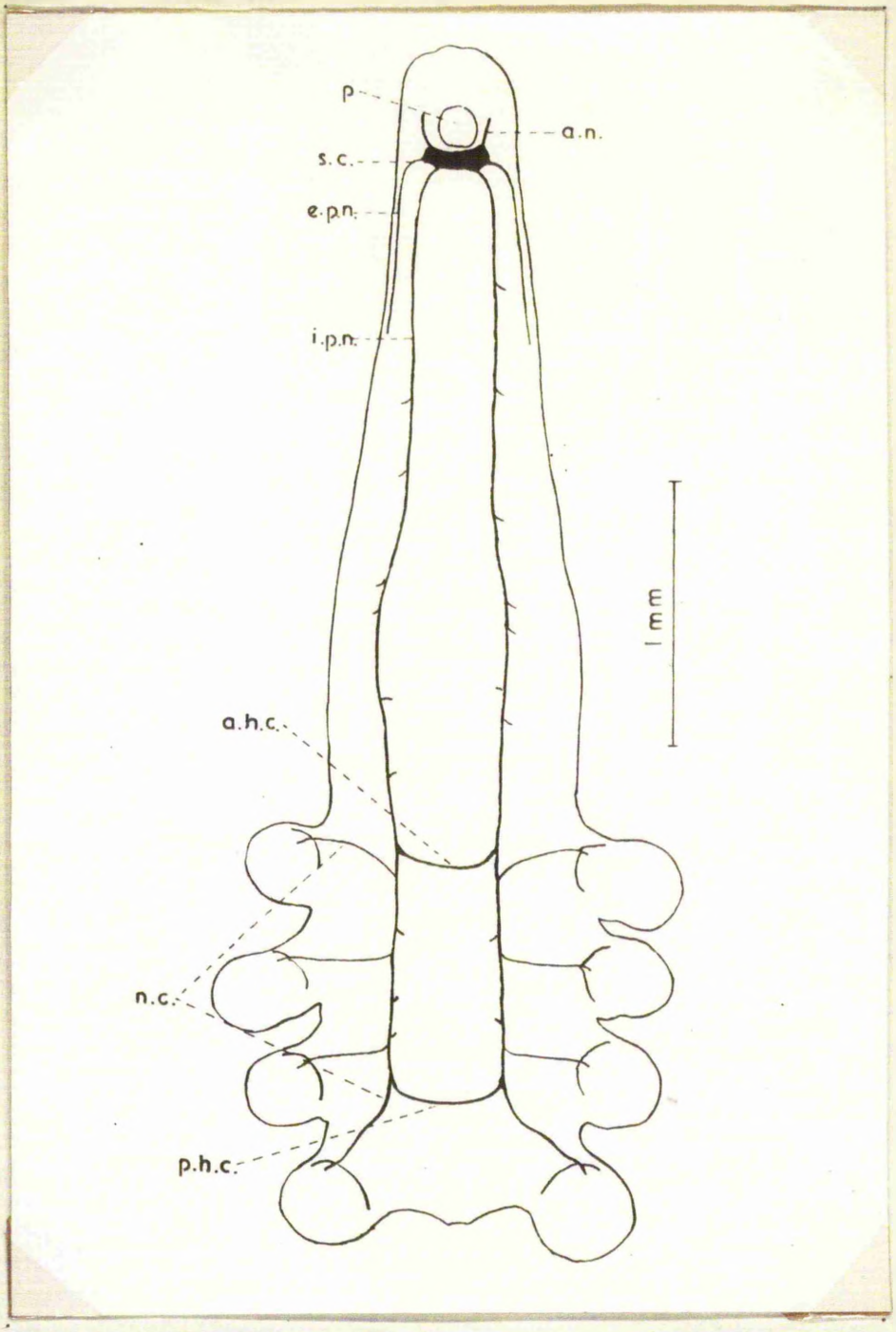


Fig. 7

p., pharynx.

a.n., anterior nerve.

s.c., supra-oesophageal commissure.

e.p.n., external posterior nerve.

i.p.n., internal posterior nerve.

a.h.c., anterior haptorial commissure.

n.c., nerves to clamps.

p.h.c., posterior haptorial commissure.

The Excretory System.

The excretory system (fig. 8) is typical of that found in monogeneans, being composed of long trunks opening anteriorly and draining finer ducts which terminate at their blind ends in flame cells. The details of the system have been found difficult to make out though both living and preserved material were studied.

Flame cells are present in various parts of the body including the extreme posterior region between the last pair of clamps, overlying the ovary, and, not only in the tissue round the sclerites in the clamps, but on one occasion a flame cell was seen apparently within the central cavity of one of the main sclerites. Fine ducts lie between the flame cells and the major ducts, but their ramifications could not be followed.

There is one main duct on each side (fig. 8). Commencing laterally to the pharynx each runs posteriorly closely applied to the dorsal aspect of the nerve trunk as far as the level of the third pair of clamps, receiving a branch from each clamp of its own side. At the level of the third pair of clamps it appears that the duct turns back on itself but also gives off a commissure to meet its fellow from the other side and, in addition, receives a duct draining the fourth clamp of its own side. The exact relationship of the transverse duct with those from the posterior clamps and the longitudinal limbs could not be established with certainty. The longitudinal trunks return anteriorly, following closely the paths of the more slender, posteriorly

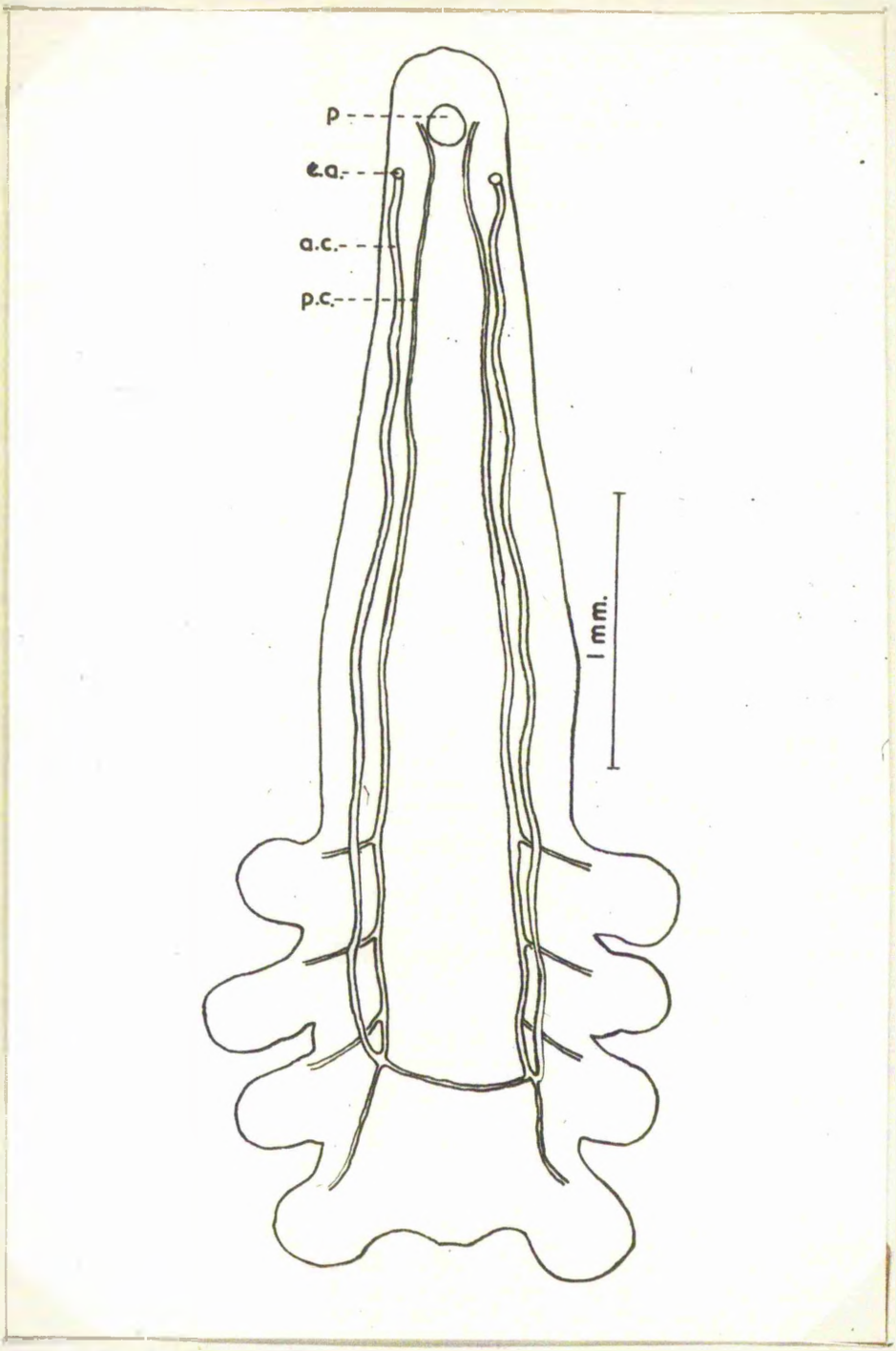


Fig. 8

p., pharynx.

e.a., excretory aperture.

a.c., anteriorly-directed canal.

p.c., posteriorly-directed canal.

directed ones, and eventually open to the exterior, after dilating into a small vesicle, dorso-laterally, on a level with the cirrus (fig.8, ea.).

The Reproductive System.

The germinal part of the female reproductive system includes the ovary, oviduct, common duct, ootype and uterus (fig.9).

The ovary (fig.9,ov.) is a broad, tubular organ sheathed in connective tissue and lying folded into an N-shape in mid-body between the intestinal caeca and rather anterior to the haptorial region. The proximal limb lies to the animal's right in the longitudinal axis, passing anteriorly into the median, oblique limb, which leads to the distal limb on the animal's left. This runs anteriorly and then turns horizontally where, near the mid-body, it passes into the strongly muscular oocapt whose walls are lined with long, cytoplasmic fibrils.

From here the oviduct (fig.9,od.), a delicate canal of decreasing muscularity, runs transversely to the right where it appears to receive a very slender duct from the receptaculum seminis (fig.9,r.s.). A little beyond this point the oviduct, now lying ventral to the right ovarian limb, turns posteriorly and gives off, on the median side, the genito-intestinal canal (fig.9,g.i.c.), a connective tissue duct which at first runs anteriorly and medial to the oviduct, but then turns outwards, ventrally to the oviduct, and joins the right intestinal caecum (fig.9,r.c.).

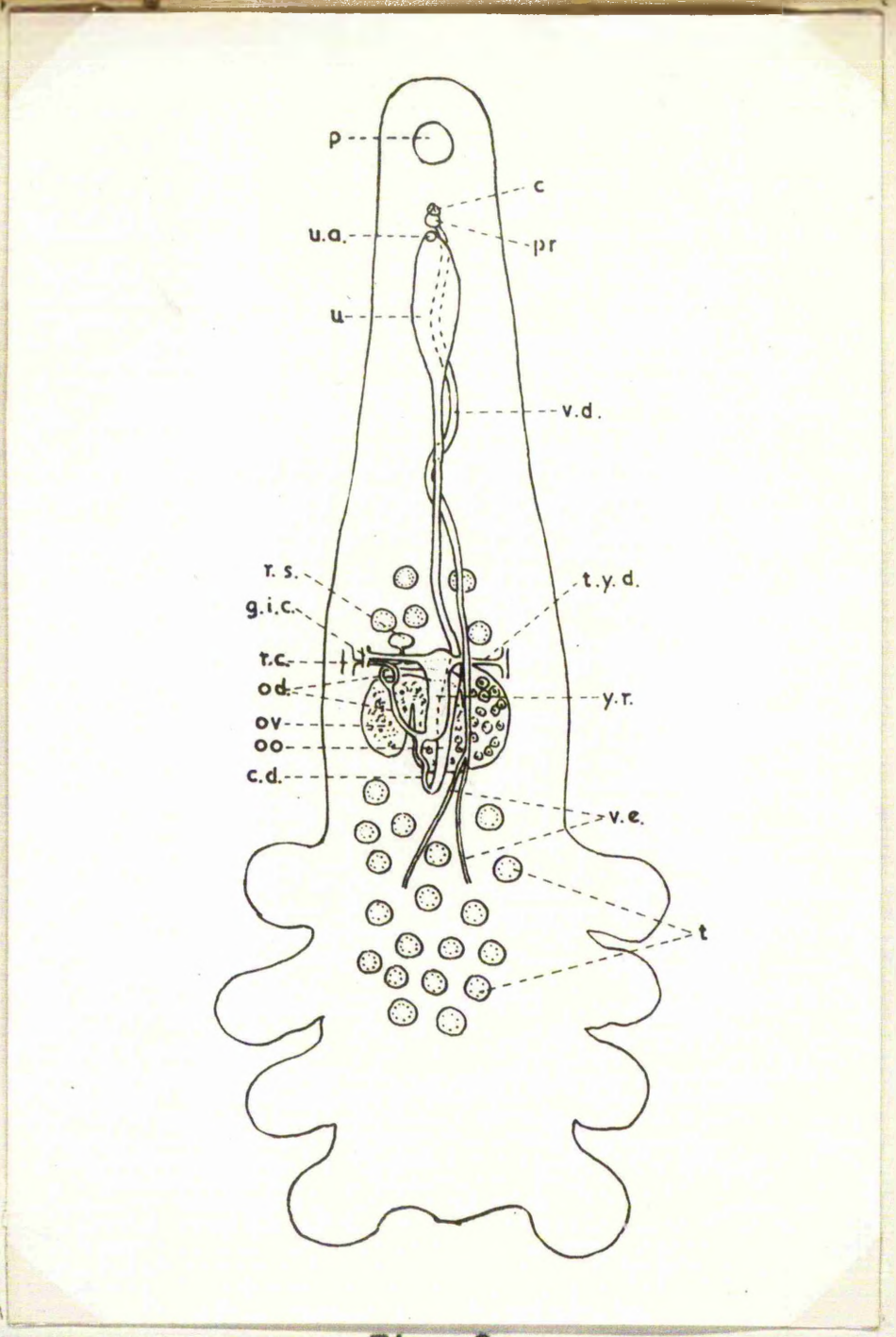


Fig. 9

p., pharynx.

c., cirrus.

pr., prostate gland.

u.a., uterine aperture.

u., uterus.

v.d., vas deferens.

r.s., receptaculum seminis.

t.y.d., transverse yolk-duct.

g.i.c., genito-intestinal canal.

r.c., right caecum.

od., oviduct.

y.r., yolk reservoir.

ov., ovary.

oo., ootype.

c.d., common duct.

v.e., vasa efferentia.

t., testes.

vitelline acini omitted.

Distal to the point of origin of the genito-intestinal canal the oviduct continues in a postero-medial direction until, by its union with the base of the vitelline reservoir, it becomes the common duct (fig.9,c.d.). This part of the oviduct and the common duct are both non-muscular, but are lined with cilia beating anteriorly.

The common duct runs posteriorly and slightly dorsally to join the ootype (fig.9,oo.), which is a broad tube directed anteriorly and slightly laterally, ventral to the oblique limb of the ovary and the oocapt. The ootype is not muscular, but numerous radiating fibres run between the cells of Mehlis' gland, in which it lies embedded, and are attached to the basement membrane of the wall. This membrane is lined with a layer of eipthelial cells which are continually sloughing off into the lumen of the organ (fig. 19d).

At the level of the oocapt the ootype passes into the uterus (fig.9,u) which is a broad, straight canal running forward in the mid-line of the body with a dilation shortly before it terminates at the uterine aperture, (fig.9, u.a.), an elastic orifice in the ventral body wall just posterior to the point of bifurcation of the gut. The walls of the uterus are composed of a loose, connective tissue-syncytium, containing scattered nuclei within which is a basement membrane. In the proximal third of the organ there is a lining of long fibrils directed anteriorly, and in the middle third, whose walls are re-inforced with

circular muscles, there is a sloughing epithelium as in the ootype. The distal third of the uterus is lined with large, longitudinal ridges of connective tissue, and, though there are no circular muscles in the wall, oblique muscles ensheath it.

There is a small, spherical receptaculum seminis (fig.9,r.s.) lying anterior to the proximal, transverse limb of the oviduct, to the right of the apex of the vitelline reservoir. It is not open to the anterior at any point, but there is a plug of scar tissue connecting it directly with the ventral body-surface (fig. 19e). The duct leading from the receptacle to the oviduct is very delicate and its path can only be deduced from the track of scattered spermatozoa lying between the two organs.

The vitelline portion of the female reproductive system includes the vitelline acini, ducts and reservoir. Vitelline acini (fig. 19b), each enclosed in a delicate connective tissue sheath, are scattered throughout the body except for that part anterior to the gut birfurcation and where they are displaced by other organs. Their greatest concentration is in the lateral fields anterior to the haptorial region. The fine, collecting ducts from these acini cannot be made out until they are presumed to have converged into the right and left, main, transverse vitelline ducts (fig.9,t.y.d.), which lie ventral to the ovary on a level with its anterior border, and unite in the mid-line of the body to form the vitelline reservoir (fig.9,y.r.).

This is a wide, elastic sac, with slight demarcation into anterior and posterior parts, lying ventral to the ovary and ootype along the axis of the body, and, where it narrows at its posterior end, joining the oviduct to form the common duct. The transverse vitelline ducts are lined with cilia beating towards the vitelline reservoir which is itself ciliated, the beat being directed posteriorly. The posterior section of the vitelline reservoir is more contractile than the anterior section. Between the two parts, when the organ is not fully extended longitudinally, there is a slight internal projection of the walls forming a shelf. Though neither part has intrinsic muscles, both are overlaid by oblique muscles.

The only gland found in association with the female reproductive system is Mehlis' gland which surrounds the ootype (fig. 19d). At the base of the ootype the lumen is narrowed by a closely encircling ring of deeply staining cells. Beyond this, where the lumen of the ootype widens, and lying between the radial strands of connective tissue, are elongate, wedge-shaped cells whose reticulate nuclei lie in the broad, external ends of the cells. The narrow ends of these cells are filled with a secretion which stains with aniline blue. No ducts leading from these cells to the lumen of the ootype could be detected.

The testes, vasa efferentia, vas deferens, prostate gland and cirrus compose the male part of the reproductive system (fig.9).

The testes consist of numerous, small acini enclosed in connective tissue (fig.9c) lying in the ventral field posterior, and to a lesser extent anterior, to the ovarian region (fig.9,t). From these fine sperm-ducts are presumed to converge, though they have not been detected, on two vasa efferentia (fig.9,v.e) which run anteriorly converging to form the vas deferens (fig.9,v.d), which continues forward across the ovarian field ventral to the female organs. It is a delicate, but quite wide, duct, with connective tissue walls running forward, with slight convolutions, in the median line dorsal to the uterus.

Immediately before passing into the cirrus the vas deferens traverses the prostate gland (fig.9,pr.), a closely packed knot of cells whose cytoplasm is filled with a dense secretion staining deeply in azocarmine.

The cirrus (fig.9,c) is a bulbous structure with a restricted lumen and surrounded by a crown of sickle-shaped hooks. The number of these hooks varies from eleven to fourteen, the commonest number being 12, and the average 12.5. The bulb is built up from three rings of tissue. The basal one (fig.10,c) is composed of fibres arranged perpendicularly to the surface. The middle one (fig.10,b) is composed of circular fibres and houses the shafts of the hooks (fig.10,f). Both these rings stain in aniline blue and differ from the uppermost ring (fig.10,a) which is composed of more homogeneous, less fibrous material. It is separated into blocks of tissue in each of which is

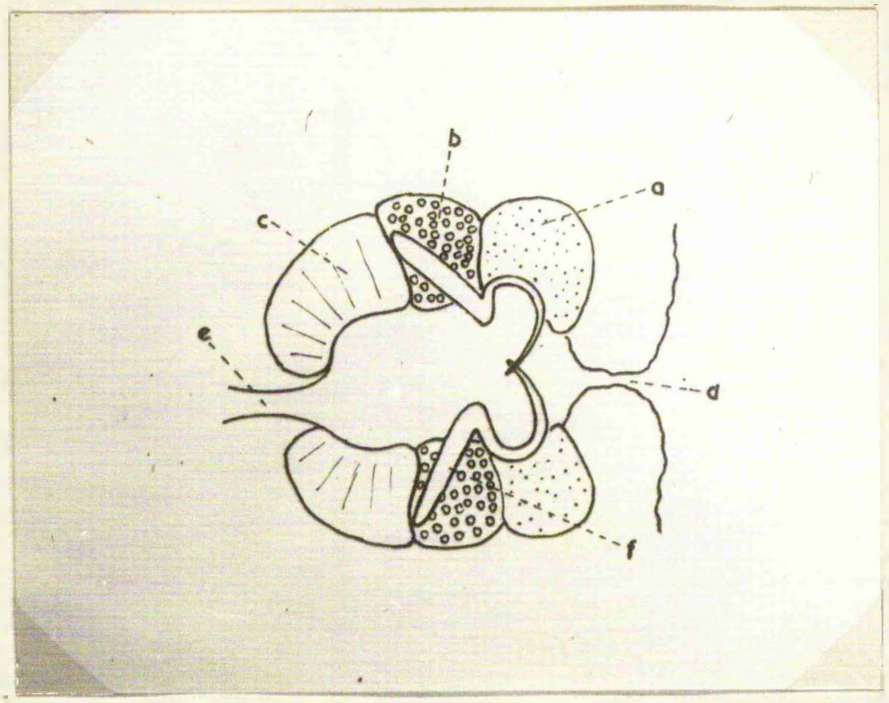


Fig. 10

a, blocks of tissue supporting blades of hooks.

b, circular muscle ring.

c, perpendicular muscle ring.

d, external aperture.

e, aperture from vas deferens.

f, hook.

embedded the convexity of the blade of one hook, the inwardly directed point of each hook projecting freely. When at rest the cirrus bulb is withdrawn into a pocket in the ventral body wall immediately anterior to the gut-bifurcation, but it can be protruded to the exterior. Contraction of the circular muscles surrounding the shafts of the hooks draws the bases of the hooks inwards, thus forcing the blades back.

Abnormalities and the Repair of Damage.

Deviations from the normal bodily form of the animal are unusual, but specimens are occasionally come across in which one of the clamps is much smaller than its fellows. Very rarely a worm is encountered with one clamp missing altogether, only the stump of the peduncle being present.

Reproduction

Spermatogenesis.

The details of cell-division during the formation of spermatozoa cannot be made out owing to the small size of the cells and only the broad outlines of the process are described here. Cytological details described are seen stained with haematoxylin after fixation with chromo-nitric acid.

How small?

The primordial spermatogonial cells are proliferated from the walls of the testicular acini (fig. 19c) where they are seen as small cells with a reticulate nucleus and but little cytoplasm, projecting separately into the acinal lumen. These cells drop off the walls singly and, while lying free in the acinus, a number of divisions occur to form a morula. During these divisions the chromosomes are often seen in a tight, intensely staining knot, but cannot be distinguished separately. Morulae of different stages occur in the acinus simultaneously. Eventually the spermatids produced by these divisions are transformed to spermatozoa by elongation of the chromatic material, at first to a 'comma' shape, and then to the final, blunt-headed, tapering form the whole of which is stained by haematoxylin. The length of the spermatozoon is approximately 8.5μ. At first the sperm heads are directed inwards and are embedded in a cytoplasmic mass while the tails radiate freely, but they soon escape from the residual cytoplasm

which is left in the acinus as an irregular mass. Ripe spermatozoa collect in the vas deferens, which may be considerably distended, but always, in the adult worm, contains at least some spermatozoa.

Oogenesis.

The same difficulty mentioned in the study of spermatogenesis occurs in examining the early stages of oogenesis: the cells are too small for it to be possible to distinguish the details. All that can be said is that oogonia are proliferated in the end of the blind limb of the ovary only, and here an undetermined number of divisions occur leading to the formation of immature, primary oocytes. The cells in this limb of the ovary show closely packed nuclei with reticulate chromatin and no clear difinition between contiguous cells. Occasionally a set of chromosomes is seen in metaphase, which gives evidence that divisions occur, but the number of these divisions undergone by each primordial cell cannot be determined.

The remainder of the ovary contains primary oocytes of gradually increasing size as they pass along the limbs of the ovary towards the oocapt. They have a large nucleus with characteristic, large, single nucleolus. In the less distal parts of the ovary the oocytes are pressed together in such a way that they form a pile of discs each traversing the diameter of the ovarian limb (fig. 19a). The cytoplasm of individual cells becomes more clearly demarcated as growth proceeds and near the oocapt the oocytes take on a

spherical shape, two or three lying across the width of the ovary. When full-grown the primary oocyte has a diameter of 24μ.

Vitellogenesis.

Vitelline cells are proliferated singly from the walls of the vitelline acini (fig. 19b), from which they are seen projecting, having, at first a reticulate nucleus. Divisions of each primordial cell may occur while it lies in the acinal lumen, but, since a division stage has only been seen on one occasion, they cannot be numerous. Only a few vitelline cells are found maturing in the acinus at one time. Very soon the nucleus develops the characteristic, small, single nucleolus and the cytoplasm stains patchily with haematoxylin. The cells grow and the cytoplasm is then seen to contain, in addition to fine granules, many discrete globules which concentrate peripherally as the cell matures.

Through the transverse vitelline canals, the vitelline cells reach the vitelline reservoir. Here they are, for the most part, in the same condition as they were before leaving the acini, with peripheral globules and an otherwise finely granular cytoplasm. However, in the lower section of the reservoir, the globules, which have hitherto taken up aniline blue now stain selectively with orange-G. A few of the cells shed their globules at this stage, but the majority remain intact. Their further history is discussed in the description of egg-formation.

Insemination.

Insemination, either between two individuals or the male and female systems of one animal, despite much study of living worms, has never been observed and therefore discussion as to how it is effected must be confined to speculation. A few relevant facts are, however, noteworthy.

In no worm examined was sperm observed in the uterus, but in mature worms sperm was found in the receptaculum seminis and also, frequently, in a scattered trail leading from there to the oviduct. The receptaculum seminis was never observed patent to the exterior, but sections of mature worms revealed scar-tissue, forming a break in the continuity of the layers of the ventral body-wall, between the receptaculum and the surface of the body (fig. 19e). Other significant points are that infertile eggs are extremely rare and that 12.8% of the mature worms producing eggs, which have been collected, were found to be solitary on the host: that is to say, no other worm, whether larval or mature, was present on the same host.

Egg-Formation and the Nature of the Shell.

The formation of eggs in D. denticulata is difficult to observe in the living animal as the natural processes are easily inhibited by the disturbance necessary for examining the worm under experimental conditions. Even worms fixed immediately on removal from the gills of the host seldom reveal an egg in the process of formation and the following account has been pieced together from many

disconnected observations.

Twitching movements of the terminal part of the ovary (fig. 9,ov.) expel the mature oocyte which passes through the muscular oocapt and so to the oviduct (fig. 9,od.) where it encounters mature spermatozoa derived from the receptaculum seminis (fig. 9,r.s.). Nothing is known of how the union of the sperm and oocyte takes place, but the oocyte now passes down the oviduct against the direction of the ciliary current. Though not actually observed in passage of this region the oocyte is thought to move rapidly by peristalsis. On reaching the common duct (fig. 9,c.d.) the oocyte is joined by a number of vitelline cells which have been expelled from the lower part of the vitelline reservoir (fig. 9, y.r.) by contraction of its walls. These contractions do not expel an exact number of cells suitable for forming one egg and those that are in excess of requirements escape up the oviduct where they move gradually toward the genito-intestinal canal (fig.9, i.c.) in the ciliary current. On reaching the genito-intestinal canal they are conveyed rapidly by spasmodic contractions to the right intestinal caecum (fig. 9, r.c.). Sometimes separate globules from yolk cells are seen floating along the same path, testifying to the fact that a few vitelline cells discharge their globules at this stage.

The egg-constituents, the oocyte and the vitelline cells, are now moved to and fro in the common duct by peristaltic action. Eventually the egg-constituents pass

into the ootype (fig. 9,oo.) where they are subjected to vigorous peristalsis during which the vitelline cells discharge their globules, these co-alescing and streaming round the oocyte yolk-cells to form the egg-shell and filaments. The crenulated disc at the tip of the posterior filament (fig.11) is moulded by the narrow, specialised part of the ootype where this joins the common duct. The formation of the anterior filament and crook (fig. 11) has not been observed. The shell hardens, to some extent at least, immediately, as the crenulated disc is not deformed during its gradual passage through the ootype.

The newly-formed egg passes gradually, by muscular action, up the uterus to the anterior dilation where it comes to rest and is joined by other eggs as they are formed. Their posterior filaments become closely entwined and the shells, at first colourless, turn a translucent, golden brown.

The chemical nature of the egg-shell is obscure. On applying Campbell's (1929) test for chitin the shells did not dissolve in concentrated potassium hydroxide, even when boiled at high temperature in a glycerine bath, thus indicating the possibility of chitin being present. However the shells did not dissolve on the addition of acetic acid and no precipitate of chitosan sulphate was formed on following this with sulphuric acid. Similarly, though a slight browning of the shell was noted on treating with iodine after boiling in sodium hydroxide, no violet-colour was developed

on replacing this with 1% sulphuric acid and solution did not occur when more concentrated sulphuric acid was added: these reactions would have indicated the presence of chitin. The shells remained unaffected on application of Millon's reagent, the biuret reaction, and the xantho-proteic reaction. The presence of protein would have been shown in the first case by a red colour, by a purple colour in the second case and a yellow-orange colour in the third case. It was observed that there was a slight positive reaction with Brown's (1911) test for iron from shell-material both before and after extrusion of the globules from the parent vitelline-cell, a greenish-blue colour developing.

That the shell is semi-permeable is apparent from the fact that eggs transferred to a hypertonic solution collapse at once. It is only with difficulty penetrated by fixatives and not at all by stains.

The Egg.

The egg of D.denticulata (fig.11) is constant in form but slightly variable in size. It is ectolecithal, there being one ovum embedded in a group of yolk-cells and enclosed in a tough, resilient shell, golden brown in colour and translucent. In shape the egg is elliptical, tapering slightly more abruptly at the posterior than the anterior end. The anterior part of the shell forms an operculum, a slightly oblique line of weakness, whose position is indicated by a dotted line in fig. 11, separating this from the main part of the shell. Even new-laid eggs may

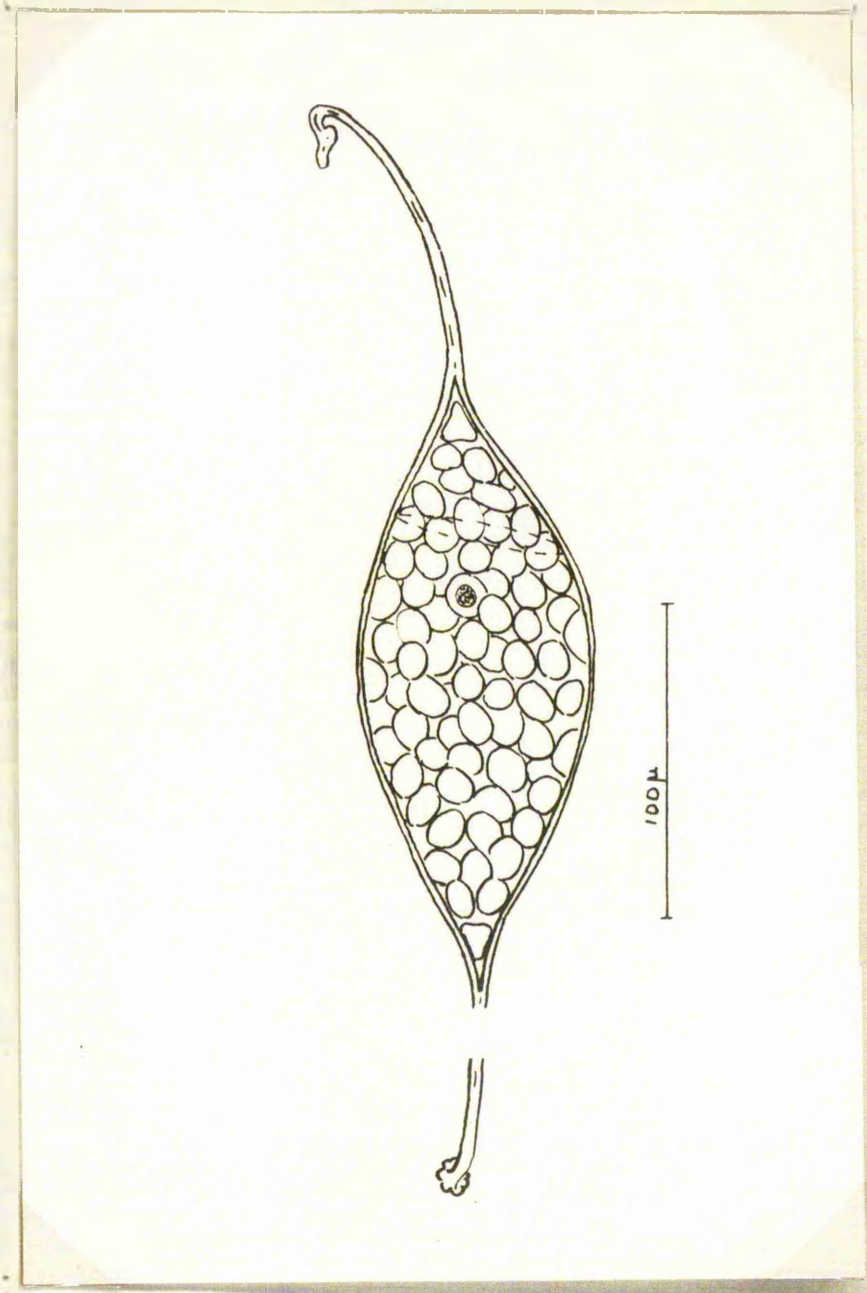


Fig. 11

Opercular suture shown as a dotted line.

rupture along this line if roughly handled. The egg proper measures 191.5μ ± 6.5μ x 78.8μ ± 2.0μ. Both ends are drawn out into intermittently hollow filaments, the anterior of which terminates in a crook (fig. 11) and is about 240μ in length. The posterior filament is longer, measuring about 800μ, and terminationg in a reflexed disc with a central depression, and crenulated border having nine projections (fig. 11).

Oviposition.

The eggs are laid in sheaves of one to two hundred together, with the posterior filaments still entwined. Oviposition is brought about by powerful contractions of the body, the head region being drawn back.

The anterior crooks do not serve the function of anchoring the eggs on the gill-filaments of the parent's host as, though new-laid eggs are sometimes found in the gill-mucus of freshly killed fishes, developing eggs have never been found attached to any part of the host.

Life History

Maturation of the Gametes and Fertilisation.

As already mentioned the small size of the chromosomes makes a study of cell-division very difficult and nothing can be said of the maturation of the spermatozoon, which must merely be presumed to contain the haploid number of chromosomes on penetrating the oocyte. It is occasionally seen as a small, lobular pronucleus lying to the side of the ovum in mature or new-laid eggs, but it was not possible to detect when or where fertilisation took place.

A little more evidence is available concerning the ovum. Polar bodies have never been seen, perhaps because they soon disintegrate, and the ovum is in any case surrounded by yolk-cells, but maturation can be inferred from the number of chromsomes seen in sections of early stages of development. Thus metaphase stages are seen in the first two hours of development before cleavage has begun in which there are nine chromosomes. Some of these may represent nine bivalents but others show the separation of the chromatids characteristic of the second meiotic metaphase. Similarly anaphase and telophase stages are found in which nine chromosomes are passing at least to one pole, though technical difficulties have prevented the establishment with certainty of the passage of nine chromosomes to each pole. In a few of these

telophase stages nine chromosomes are seen projecting in a small cytoplasmic bulge from the main part of the ovum. Other one-celled stages show divisions in which more than nine chromosomes are involved. Approximately eighteen could be counted, but their exact enumeration is difficult owing to their small size and crowded arrangement.

In prophase the chromosomes appear as delicate, thread-like bodies, which condense into short, thick rods or oval bodies in metaphase. Some of those seen in anaphase and telophase are V-shaped.

Embryology.

Cleavage begins at a varying time during the first five hours after laying as is shown in Graph 2. It is holoblastic and somewhat unequal (fig. 12), leading to the formation of an irregular, morula in which no germ layers can be distinguished. There is no evidence of spiral cleavage (fig. 12).

The interphase nuclei of cleavage stages differ from the primary oocyte in having a lobular border and a number of nucleoli.

The first few cleavage divisions follow one another relatively rapidly so that four-celled stages are common a few hours after laying, but after twenty-four hours the majority of the eggs have only reached a six-celled stage. At forty-eight hours the embryo has the form of a roughly spherical morula of about twelve cells. During the next two or three days the embryo enlarges and takes on an

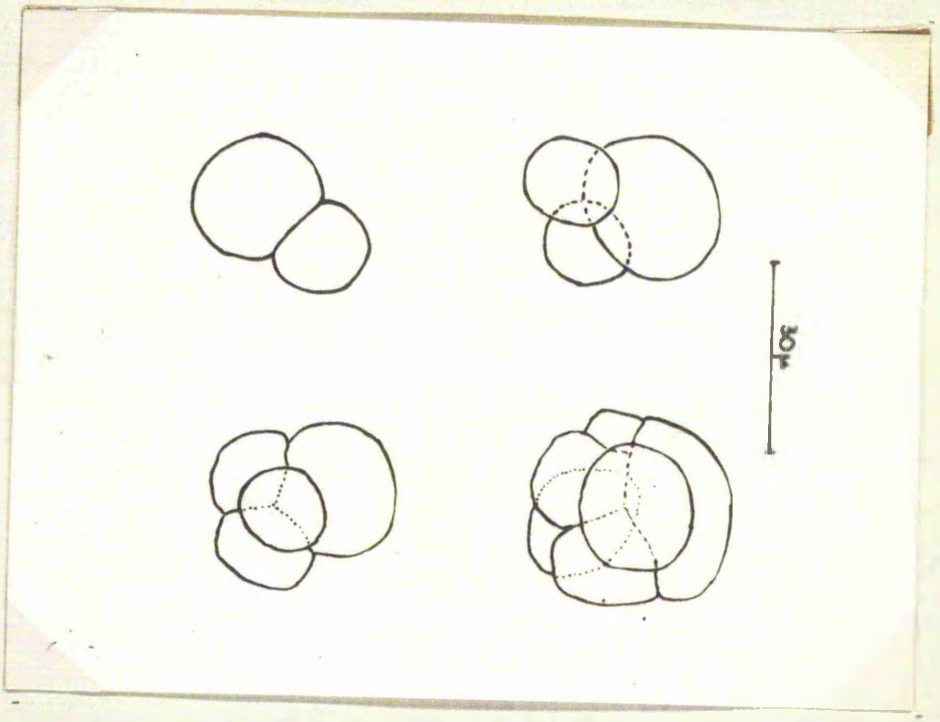


Fig. 12

oval shape. Occasionally a slight central cavity can be detected. The cells composing the embryo are all of the same type except that some nuclei are clear whereas others are coarsely granular. The two types are indiscriminately mixed.

Six days after the egg is laid the peripheral cells of the embryo have been modified to form an epithelium. The general shape of the embryo is a slightly flattened ovoid in one of whose faces there is a depression filled with adherent yolk (fig. 13a). This depression is later found to be ventral. Further growth results in the yolk adhering to the ventral depression being overgrown by the surrounding cells and incorporated in the embryo (fig. 13b). The cells of the mouth and pharynx are differentiated at about the time this epiboly is complete.

After twelve days of development the epithelium is shortly ciliated in those areas where ciliation occurs in the free-swimming larva. Four pairs of lateral larval hooks and a pair of postero-lateral hooks posterior to the foregoing are being laid down in the posterior, haptorial part of the embryo. One day later the development of these hooks is completed and the stout, posterior, median pair of hooks is forming. The mouth is clearly differentiated and from the pharynx a narrow canal can be seen running to the sac of enclosed yolk, or larval gut-sac. Refinement of the larval form follows and, with increased growth, the larva is only accommodated in the available space by being

bent into an S-shape.

Fate of the Yolk.

The yolk-cells do not break down on extrusion of their shell-globules, but persist as discrete units with a mono-nucleolate nucleus and cytoplasm through which fine granules are scattered. This structure is maintained during the first day of the egg's development but on the second day the cell-walls disintergrate somewhat and the nuclei and cytoplasm are seen lying free within the egg-shell. Further disintergration and reduction in volume of the yolk takes place as embryonic development proceeds, some of the yolk being, as mentioned above, incorporated in the embryonic body. In the later stages of development, as seen in life, refringent globules of varying size appear in the yolk, both within the embryo and round it. In the fixed condition there are corresponding 'vacuoles' which may be assumed to have arisen by the solution of the globular material.

On one occasion two eggs, out of a bunch which had been developing for three days, were found to contain no embryos. In neither case was there any sign of disintergration of the yolk-cells, which still resembled those seen in new-laid eggs.

Hatching.

For about three days prior to hatching the larva can be seen moving within the shell and the cilia start to beat.

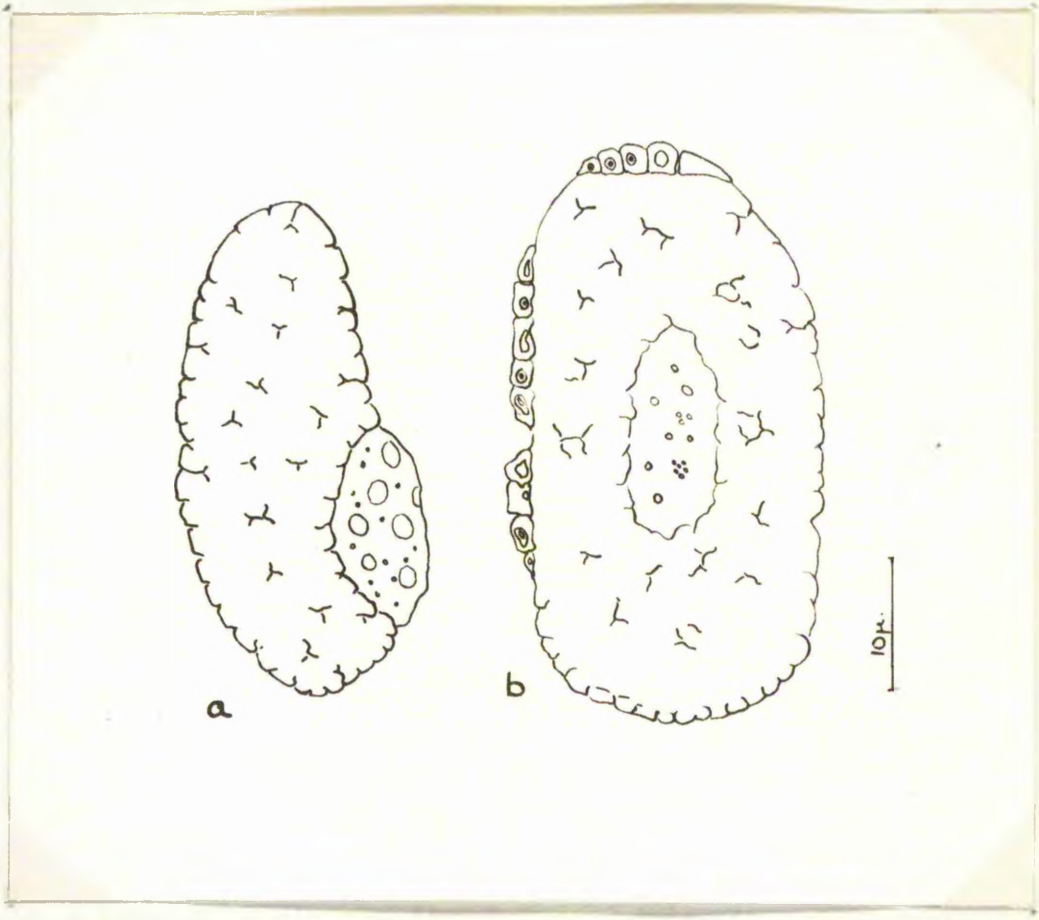


Fig. 13

a, 6-day embryo.

b, 11-day embryo.

As hatching approaches the movements become more vigorous and the larva stretches and turns convulsively. Finally the operculum is partially or wholly forced off. The larva squeezes out, remains poised on the edge of the shell for a moment, the cilia moving gently and then, with sudden vigorous beating of cilia, swims away.

The Free-Swimming Larva.

The free-swimming larva (figs. 14 and 15) which emerges from the egg is elliptical and flattened dorso-ventrally. The anterior extremity is blunt and from here the body widens somewhat, and then tapers to a rounded posterior extremity with a median cleft from which projects a small, clear vesicle. From the ventral surface of the posterior quarter of the body, but not reaching the hind extremity, there arises a haptor (fig.15, h.) expanded laterally into two wings each bearing four hooks peripherally. Posterior and median to these lateral hooks are two postero-lateral hooks and median to these is a pair of large, median hooks (fig.15, m.h.). Though very contractile and changeable in shape, at average extension the animal, when fixed, measures 138μ ± 3.5μ in length and 59.0μ ± 12μ across at the broadest part. The lateral and postero-lateral hooks are the same shape having a long, very slightly curved shaft, a short ventral backwardly directed guard and a gently curved blade. They are 20μ ±<0.5μ in length. The median hooks differ in having a straight shaft, no guard and a strongly recurved and much more robust blade. They

Fig. 14

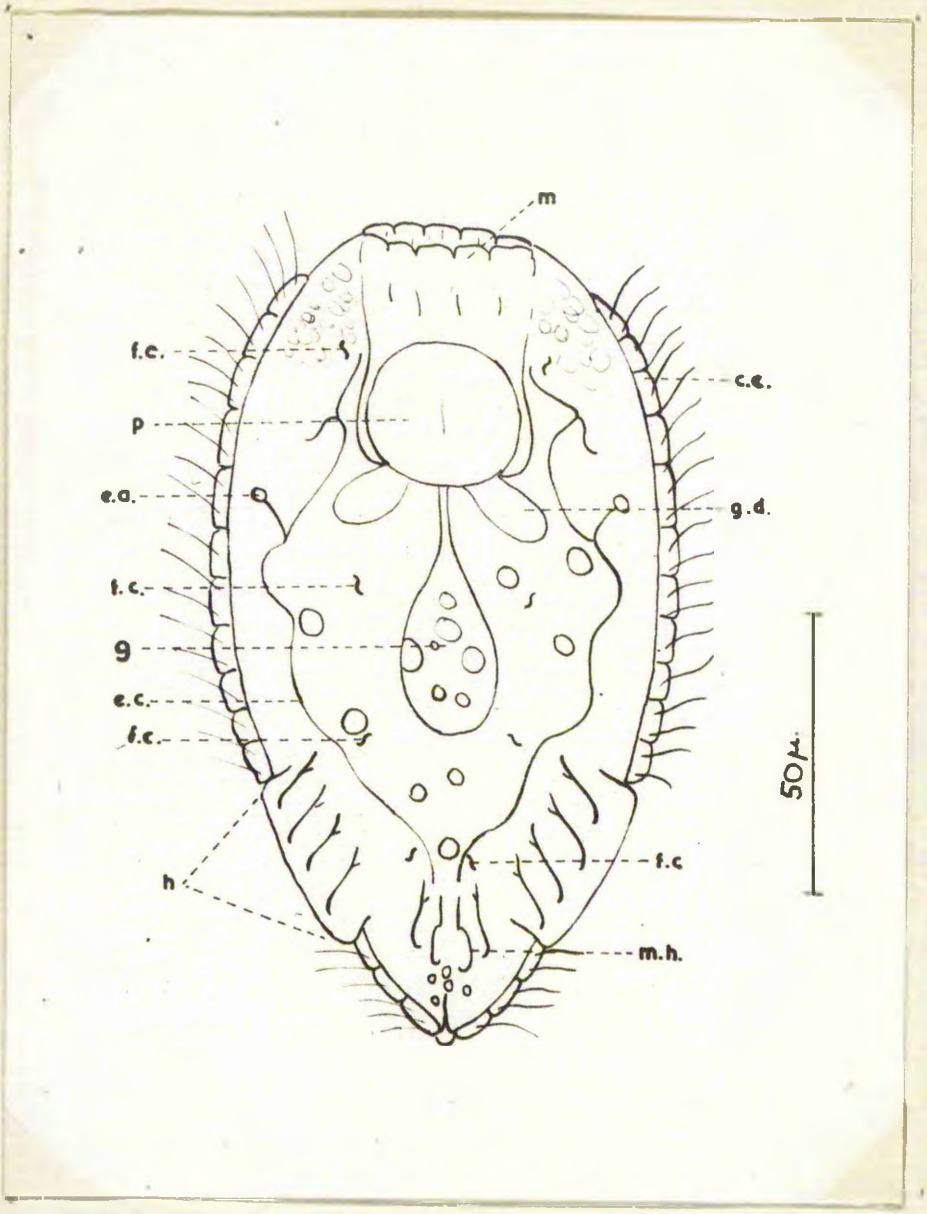


Fig. 15

m., mouth.

f.c., flame-cell.

c.e., ciliated eipthelium.

p., pharynx.

e.a., excretory aperture.

g.d., gut diverticulum.

g., gut-sac.

e.c., excretory canal.

h., haptor.

m.h., median hook.

have a length of 15μ ± < 0.5μ.

The epithelium is thickened and ciliated (fig.15, c.e.) over the posterior part of the body behind the haptor. Anterior to the haptor a strip of ciliated epithelium runs anteriorly, confined to the lateral margin of the body except for a slight expansion on to the ventral surface just anterior to the haptor. Finally it leaves the lateral margin to meet with its fellow of the other side dorsally, about 10μ behind the anterior tip of the body. The cilia are 11μ long.

The mouth (fig.15, m) is terminal and large with tumid, suctorial lips. It leads, via the prepharynx, to the pharynx (fig.15, p). The position of the pharynx in life is very variable so that at one minute there appears to be no prepharynx and at the next it is of considerable extent. It makes contact with the pharynx round the posterior margin of the latter organ which, therefore lies, as it were, in the prepharynx. The pharynx itself is a spherical body with a transverse diameter in the fixed condition of 16.5μ ± 1.5μ. It has thick muscular walls and a very restricted internal cavity whose axis is antero-ventral and postero-dorsal to the body-axis. From the pharynx there extends a restricted passage which widens out into the gut-sac (fig.15, g). This narrow cavity is, like the prepharynx, of variable length according to the position of the pharynx, and sometimes the pharynx lies adjacent to the gut-sac. This last is a wide cavity

(fig.13, g) of indeterminate and variable shape sometimes extending between the lateral hooks of the haptor almost as far as the roots of the median hooks. It contains residual yolk with globules and a few refringent, yellowish aggregates of shell-material. Also arising from the posterior aspect of the pharynx is a pair of laterally and postero-dorsally directed blind diverticula (fig.15, g.d.) which extend to the level of the anterior margin of the gut-sac. There is no differentiation of the cells surrounding the alimentary ducts apart from the muscular nature of the pharyngeal wall.

Tissue of a different nature from the remaining parenchyma and staining deeply in the vital stain, brilliant cresyl blue extends in two lateral fields between the mouth and the anterior margin of the gut-sac. Refringent spheres of varying size are seen here and there in the body.

The excretory system already shows the basic pattern of that seen in the adult. There are four pairs of flame cells (fig.15, f.c.): the first pair anterior and lateral to the pharynx, the second pair anterior and lateral to the gut-sac, the third pair posterior and lateral to the gut-sac, and the fourth pair external to the roots of the median hooks. Apparently single anterior and posterior, longitudinal collecting tubules lie on each side of the body (fig.15, e.c.). From each a short duct is given off about one third of the body-length from the anterior end and

runs forward a short distance before opening in a dorso-lateral excretory pore (fig.15, e.a.), one on each side.

No nervous system or sensory organs could be detected and the reproductive system is not yet differentiated.

The newly hatched larva is very active and the body extremely changeable in shape. The creature swims vigorously by the action of the posteriorly-beating cilia, either straight foward or spiralling on its own axis. Sharp turns are frequently made, the dorsal surface being directed to the inner side of the arc. The animal gains or loses height in the water by whirling up or down in a right-handed spiral. Frequently it comes to rest either on the bottom, supported by the surface film where it may move about by alternate contraction and elongation of the body, or, if unsupported, sinking slowly and then darting off again.

In the absence of a suitable host this phase persists for about twelve hours after which the movements become more lethargic, the larva sinks to the bottom and sloughs off the ciliated coat. Specimens may be seen creeping over the substratum, obtaining a purchase with the suctorial mouth and attempting to grip with the hooks. Death occurs within twenty-four hours of hatching if no host is found.

Various experiments were tried in an attempt to determine whether there were stimuli attracting the free-swimming larva to its host.

A dish containing a number of vigorous, free-swimming

larvae was placed on a dull, black background to out out reflections and illuminated from the side by a powerful beam of light. The larvae were observed to be swimming about in the normal manner. A black shade was then arranged so that the dish was divided vertically into two sections, one, as before, powerfully illuminated and the other only lit by low intensity, diffuse daylight. The shade was left in place for thirty minutes and the behaviour of the larvae noted. They could not be seen in the shaded region but were observed swimming normally in the lit region and making their way in and out of the shaded region without change of behaviour on encountering light of a different intensity. Some were observed making upward spirals, half of each turn being in the brightly lit zone. When the shade was removed the concentration of larvae in each zone was immediately noted and there was found to be no preponderence of larvae in either zone.

Over a period of two minutes the number of upward spirals made by a group of larvae in a dish on a dark ground and illuminated strongly from the side was counted. Twenty spirals were made. The upper half of the dish was then shaded and the spirals made in the following two minutes noted. There were eighteen.

A small piece of absolutely fresh gill of G.virens was placed in a dish containing a number of vigorously swimming larvae and the behaviour of the larvae over a period of one hour observed. They did not concentrate on

or around the gill-tissue and those which encountered it swam on without any modification of behaviour. After three hours the gill was again examined, but no larvae had attached themselves to it. A similar experiment was carried out in which some of the larvae used were old and tiring. One or two of these, having reached the gill attached themselves to it by the mouth and then embedded the hooks in the host-tissue. With many contortions the ciliated coat was torn off, the triangular, posterior part of the body being drawn out of the investing epithelium last.

Any subsequent movements, made after the larva has settled on a host, are leech-like, the larva gripping first with the mouth and then with the haptor. In this way it may move over the filaments, but is usually seen clinging by the haptor fairly near the tip of a gill-filament.

Larval Development.

When the larva has settled on the gills of the host and discarded the ciliated coat its subsequent development consists of growth and gradual acquisition of the adult characters without any sudden metamorphosis between the larval stages which are here named separately purely for convenience and clarity.

The first stage larva resembles the free-swimming larva apart from the absence of the ciliated coat. Its measurements are given in Table 4 from which it is seen that larvae of this stage are smaller than free-swimming larvae. No 'brown ' cells have been formed in the gut-

epithelium at three days of age but the gland-cells lying near the pharynx and noted in the adult are already present as are the glandular pockets in the lips. The body tissue is largely composed of parenchyma but a few larger cells with mononucleolate nuclei are seen in mid-body. The main nervous tracts, the dorsal nerve commissure and the two major longitudinal nerves, can be made out at this stage.

Between five and thirteen days after hatching the second stage of development is reached (fig.16). The most posterior pair of lateral hooks have been superseded by a pair of definitive clamps. These clamps are formed external and slightly dorsal to their corresponding larval hooks, which disintergrate when their function is taken over by clamps. The remaining hooks are fully functional. The path of the gut is difficult to follow but it is almost certainly still a median sac. 'Brown' cells have now been developed here and there in the gut epithelium. Vitelline cells are already differentiated to the extent of showing a patchy staining reaction of the cytoplasm but no other genital differentiation has taken place. Experimental injections show that this phase persists beyond thirty-eight days. The range of measurements of second stage larvae is shown in Table 4.

Experimental infections of greater duration than thirty-eight days were not successful and so it is not known when the third larval stage is reached by the formation of the second pair of adult clamps. Worms of this stage

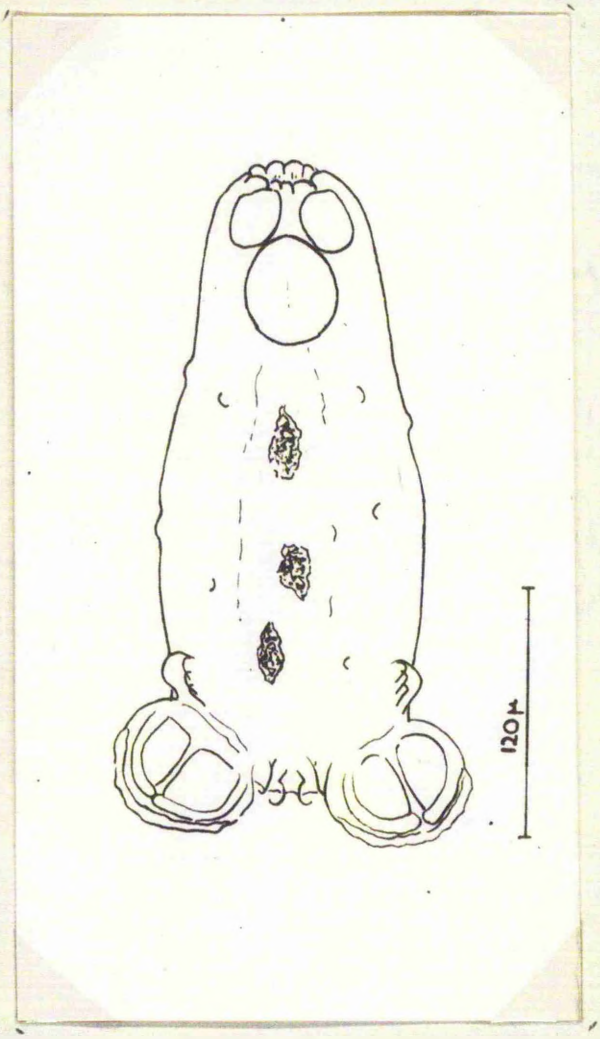


Fig. 16

collected from natural infections revealed that the second pair of clamps arises external to the corresponding, second-most posterior pair of lateral larval hooks and that the median, postero-lateral, and two anterior pairs of hooks are still intact. The gut is now plainly bifurcate, re-joining and then branching in a palmate manner in the haptorial region. There is no change in the condition of the vitelline cells but a mass of closely set cells with deeply staining, reticulate chromatin in their nuclei is differentiating in mid-body to form the genital primordium. This area is surrounded by a few large cells which have spherical nuclei containing one or two closely apposed nucleoli and a coarse, chromatic network. The cytoplasm is finely granulated and stains sharply with aniline blue. The observed range of measurements of this stage is shown in Table 4.

The attainment of the fourth larval stage is reached with the initiation of the third pair of clamps which occurs before the second pair has grown to the size of the first pair. The median, postero-lateral and most anterior pair of hooks persist. The general condition of the larva is unchanged from the previous stage except that there is increasing differentiation of the genital region. The mass of deeply-staining cells in mid-body now extends as a narrow column along the future course of the uterus and vas deferens and there may be an expansion of this column at its anterior end. The large, blue-staining cells

surround the genital area more extensively. There are slight indications of cells differentiating as testicular primordia. Each of these small cells has a nucleus containing a small, central nucleolus from which radiate a delicate network of chromatin. Measurements of the fourth larval stage are given in Table 4.

The formation of the final, and most anterior pair of clamps introduces the fifth larval stage (fig.17). This new pair of clamps is formed, as in the case of the fourth larval stage, before the preceding pair has reached the size of earlier-formed pairs. The more posterior pairs of clamps now show the external teeth characteristic of the adult. The median larval hooks are intact butthe postero-lateral ones are degenerating and all the lateral ones have disappeared. The gut shows increasing posterior anastomosis and subsidiary branching. In a few cases the genital patch in mid-body is beginning to show slight differentiation into primordia of ootype and ovary and, at the anterior end of the genital column, a ring of regular, hook-generating cells is sometimes to be seen in the position of the cirrus. A few late stages show cirrus hooks being laid down, one in each of these cells. The observed range of measurements of fifth stage larvae are shown in Table 4.

During the immature phase following the completion of the development of the fourth pair of clamps continued growth takes place as shown in Table 4, and the elaboration

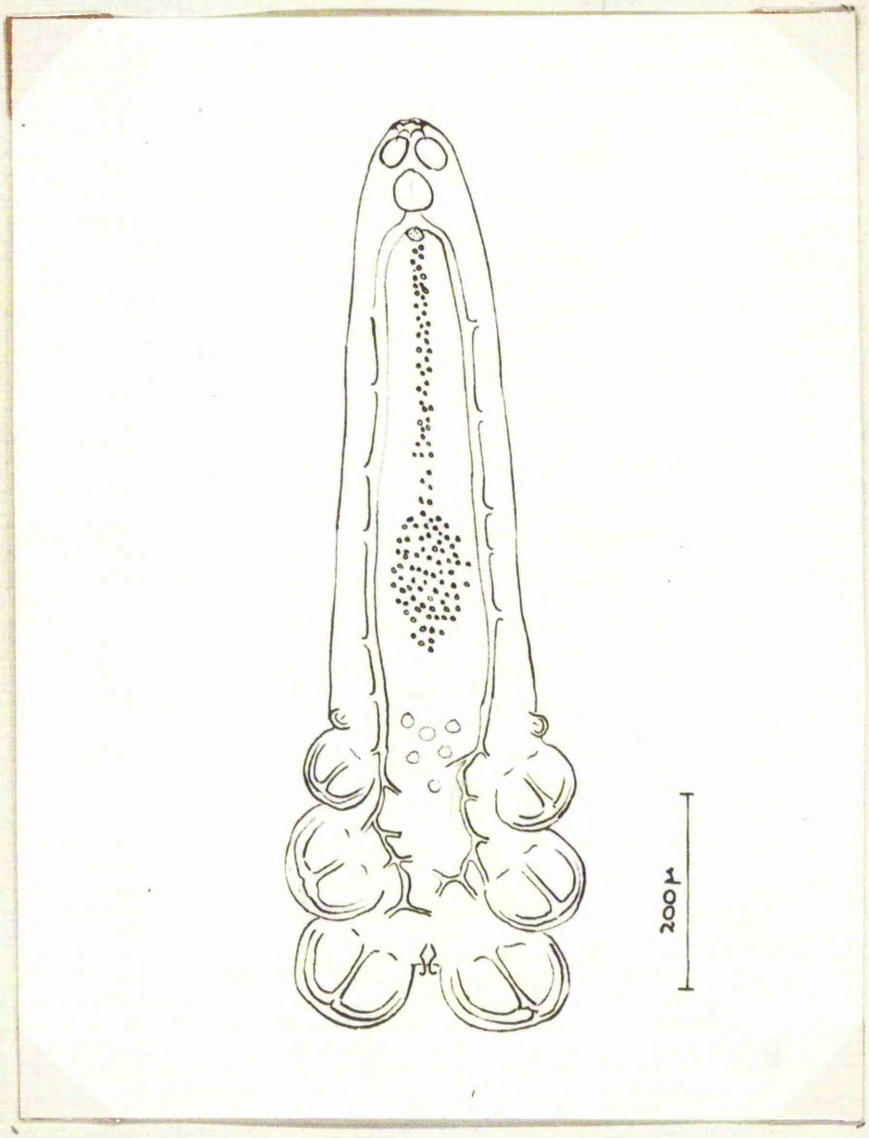


Fig. 17

of the reproductive organs is completed.

In the earliest immature adult stage the condition of the animal is unchanged from the fifth larval stage except that there are now several cells in each testicular acinus. The cirrus-hooks continue to be laid down and as each hook increases in size the cell which secretes it becomes stretched along the back of the hooks and finally disintergrates.

By the time the cirrus hooks are fully formed the branching of the gut has reached the degree of complexity seen in the adult and the 'brown' cells along its walls are very numerous. The gland-cells lateral to the pharynx are present but show the smaller proportions relative to body size characteristic of the adult. Large cells, as in earlier stages surround the genital primordium in which two parts can now be distinguished: the ovarian mass and a solid mass representing the ootype. The column of cells leading anteriorly from this mass is now separated into two solid strips of cells representing the uterus and vas deferens respectively. The receptaculum seminis has not yet been formed and the vitelline cells remain in the state reached during the second larval stage. A number of cells are seen in each testicular acinus but none have divided to form morulae. The remaining larval hooks, the median and postero-lateral pairs, are in a degenerate condition.

With increased differentiation of the ovary, in which young oocytes are formed, and of the testes where

Range of Measurements During Development.

Stage	Total Length	Haptorial Length	Breadth	Trans. Pharyng. Diam.	Av. Trans. Clamp Diam.
L^0	138μ±3.5μ	-	59μ ± 12μ	16.5μ	-
L^1	125μ - 133μ	-	53μ - 61μ	-	-
L^2	276.5μ-308.5μ	-	69.5μ - 85μ	29.5μ - 37.0μ	53.0μ - 55.0μ (only pair)
L^3	312μ - 574μ	-	89μ-158.5μ	34.5μ - 45.0μ	58.5μ - 69.0μ (post. pair)
L^4	703μ	-	148.5μ	50.5μ	61.0μ (post. pair)
L^5	772μ-831.5μ	208μ - 247.5μ	168.5μ-178μ	56.0μ -	69.0μ - (post. pair)
I	1.260 mm. - 2.975 mm.	0.368 mm. - 1.085 mm.	0.245 mm. - 0.560 mm.	53.0μ - 109.0μ	152.0μ- 286.0μ (1st 3 pairs)
A	2.100 mm. - 7.105 mm.	0.630 mm. - 2.555 mm.	0.315 mm. - 2.345 mm.	77μ - 151.5μ	232.5μ - 688.0μ

Table 4.

morulae of varying degrees of maturity appear, a change in the vitellaria, so long quiescent, occurs. Each vitelline acinus now contains several cells still showing the typical, patchy cytoplasm of earlier stages. By a breakdown of the cells of the core a central lumen is formed in the ootype, uterus and vas deferens. Finally the receptaculum seminis is differentiated, though it has never been seen to contain spermatozoa until the animal reaches reproductive maturity.

Maturity is attained by the growth and discharge of oocytes from the ovary, the filling of the vas deferens with ripe sperm, the elaboration of shell-globules in the growing vitelline cells and the passage of the latter to the vitelline reservoir.

The relative bodily proportions during growth are expressed graphically in Graphs 2, 3 and 4. Measurements of total length, haptorial length, transverse pharyngeal diameter, and average transverse diameter of the anterior three pairs of clamps have been used. The co-efficients of correlation are shown below (Table 5).

Measurements Correlated.	Co-efficient of Correlation.
Total Length/Haptorial Length.	0.90
Total Length/Transverse Diameter of Pharynx.	0.91
Total Length/Average Transverse Diameter of first 3 pairs of Clamps.	0.95

Table 5.

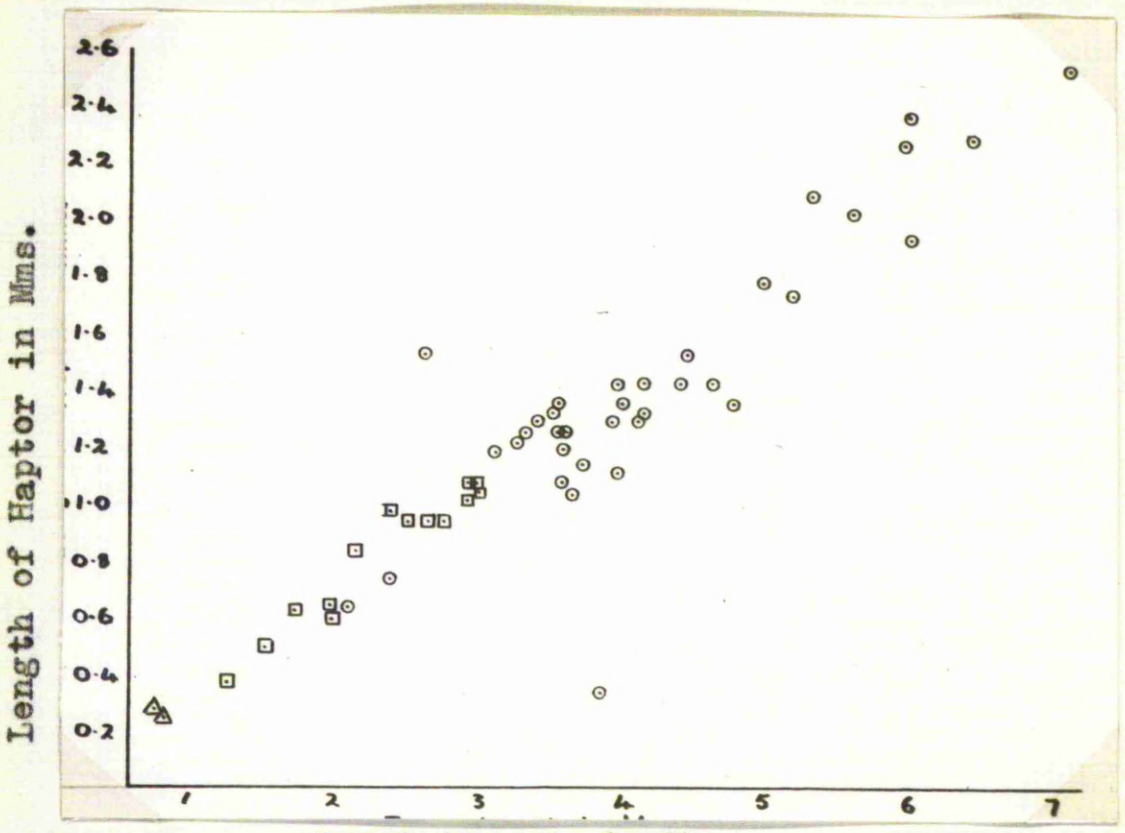
Rate of Development and Longevity.

The time-relations of development during the first seven hours after the egg has been laid are shown in Graph 5.

The temperature at which eggs were kept during development was noted. Some were kept in a constant temperature-bath. Since this bath was also in use for other purposes it could not be used for incubating eggs at abnormal temperatures. Such other temperatures, therefore, had to be chosen from those available and were liable to a certain amount of fluctuation. The effect of temperature on the rate of embryonic development is expressed in Table 6.

Temperature	Time to Hatching
7°C	Development began, then ceased.
13°C	20 days
14.25°C	18 days
14.25°C	20 days
14.25°C	18 days
14.25°C	17 days
14.25°C	19 days
14.25°C	18 days
14.25°C	19 days
14.25°C	18 days
14.25°C	18 days
14.25°C-15°C	17 days
14.25°C-15° C	17 days
14.25°C (7°C first night)	20 days
15°C	17 days
15°C	18 days
16.5°C	17 days
15°C-18°C	16 days
16°C-19°C	15 days
16°C-19°C	14 days
23°C	Development began, then ceased.

Table 6.

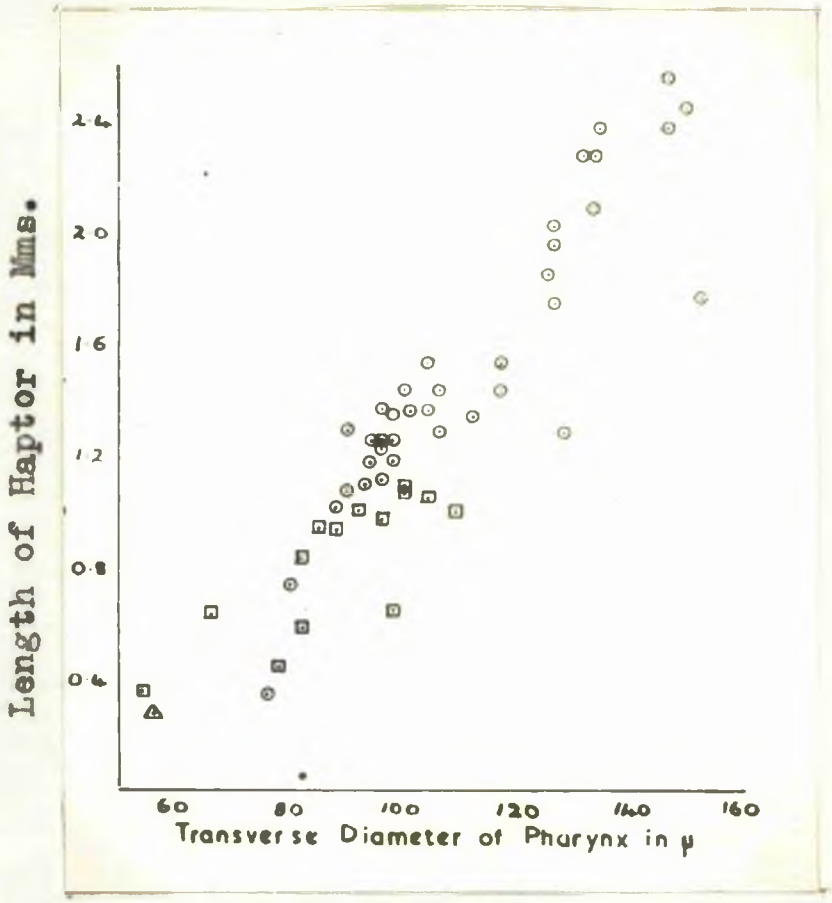


Graph 2

△ = Larvae.

□ = Immature flukes.

O = Adult flukes.

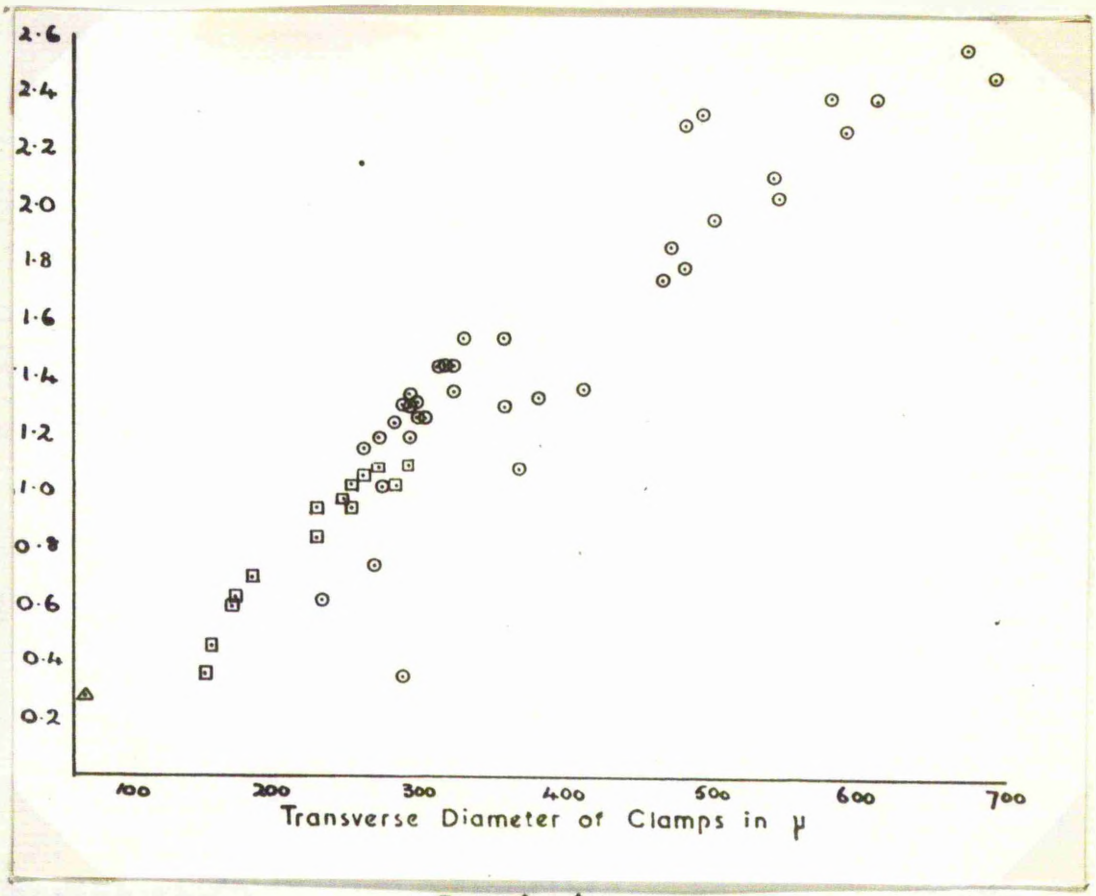


Graph 3

△ = Larvae.

□ = Immature flukes.

O = Adult flukes.

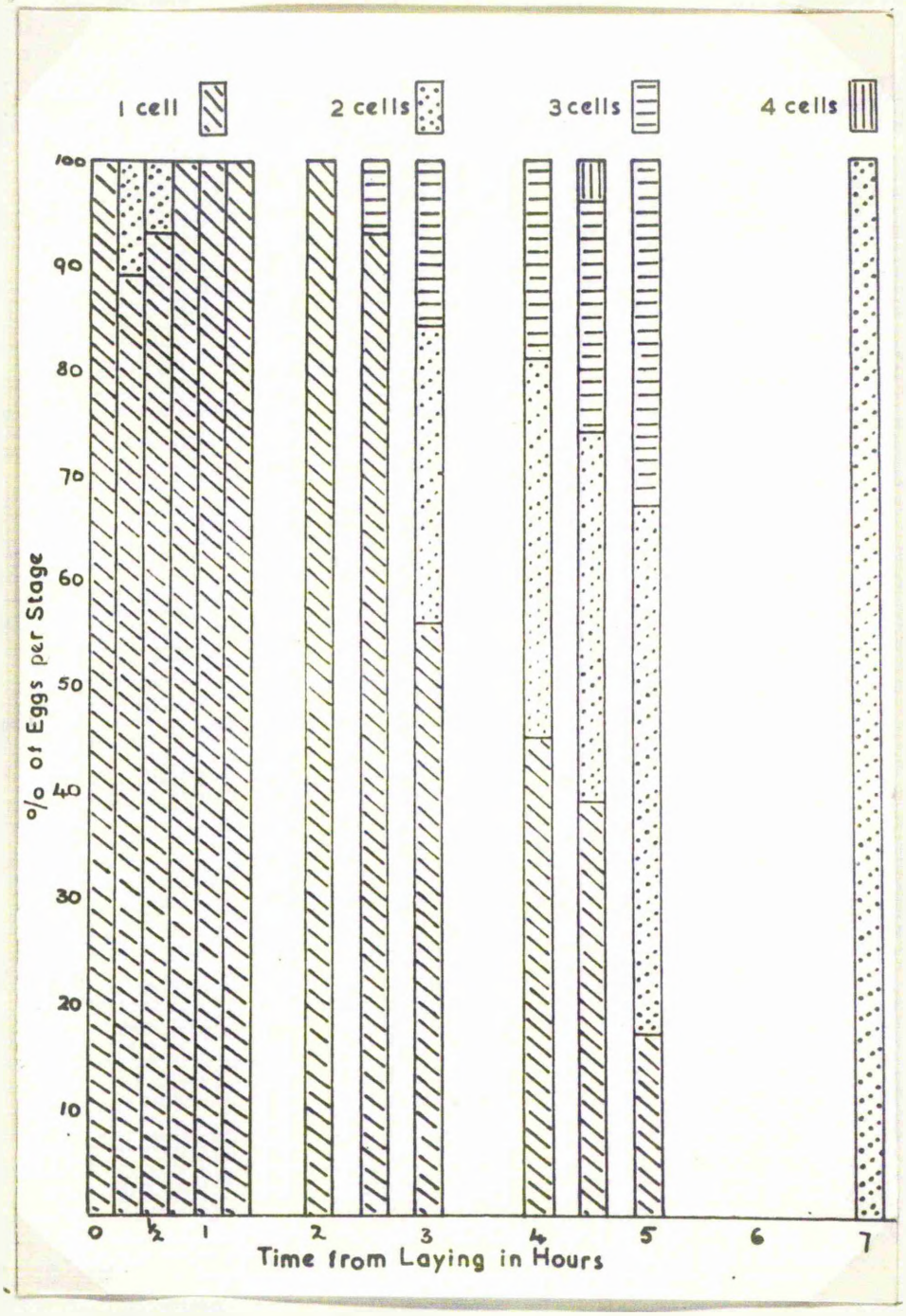


Graph 4

△ = Larvae.

□ = Immature flukes.

O = Adult flukes.


1 cell
2 cells
3 cells
4 cells
100
90
80
70
60
50
40
30
20
10
% of Eggs per Stage
0
½
1
2
3
4
5
6
7
Time from Laying in Hours

Graph 5

In view of the failure to maintain experimentally infected fishes in aquaria over a sufficiently long period observations on the rate of development must necessarily be speculative, but such facts as are either known or can be deduced from a knowledge of the age of the host are incorporated in Table 7.

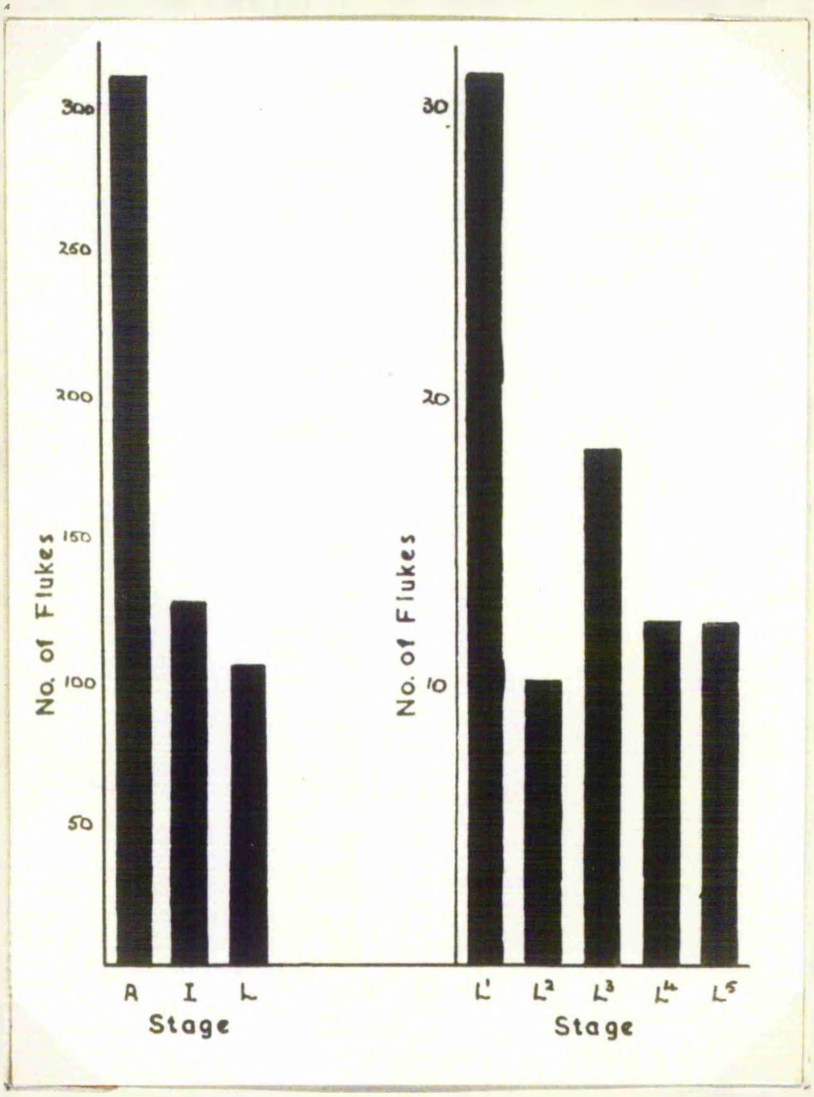
Stage	Age at Which Stage is Attained
Hatching (14.25°C)	18.3 days (average).
L^1	Within 24 hours of hatching.
L^2	Between 5 and 13 days.
L^3	More than 38 days.
L^4	-
L^5	-
I	Not more than 3 months (estimated).
A	Not more than 6 months (estimated).
A over 5.285 mm. long.	21 months or more.

Table 7.

The age-composition of the observed population of flukes is shown in Graphs 6a and 6b.

Seasonal Activity and Sexual Phases.

Reproduction took place in all seasons of the year. Every adult worm examined showed ovary, testes and vitellaria in an active state.


300
250
200
150
100
50
No. of Flukes
A I L
Stage
30
20
10
No. of Flukes
L¹ L² L³ L⁴ L⁵
Stage

a. b.

Graph 6.

Physiology and Ecology

Food and Digestion.

The newly hatched larva of D.denticulata is provided, as has already been stated, with a supply of yolk in the gut. Once it has secured a host feeding must begin in the adult manner and, within a few days, pigmented cells appear along the course of the gut. Attempts were made to determine the nature of the material upon which the worms feed.

Firstly worms were observed *in situ* on excised gills and worms which had been removed from the host were later provided with blood-clots and freshly excised gill-tissue. In no case was feeding observed, nor did the worms evince any special attraction to the food-materials supplied. Secondly a small quantity of glucose was added to the sea-water in which adult worms were being kept. The survival-time of these worms was the same as that of controls in sea-water alone.

Attention was then directed to the contents of the gut. Living worms were frequently seen to cast out at the mouth a mass of finely granular material seething with bacteria. Often, intact yolk-cells were also passed out. In a small proportion of cases the contents of the gut was coloured red in worms newly removed from the host and the red fluid, together with granules, was occasionally ejected. The red colour did not persist for long after the worms had been isolated from the host. Microscopical examination of

the contents of the gut in sections of fixed specimens revealed the presence of a number of different elements, not necessarily all present together. There was usually some finely granular material with but slight staining capacity. Lying here and there in this mass there were often brown, refringent specks, identical in appearance with those seen in the 'brown' cells of the gut-wall. No detached 'brown' cells were found in the lumen of the gut. Mature yolk-cells with their peripheral shell-globules were sometimes seen. In a few larval worms a mass of elliptical to spherical cells with clear cytoplasm and deeply-staining nuclei were seen. These were of the approximate dimensions of the host's red blood corpuscles, which could be examined on the same slide in gill-tissue, but did not have quite the same appearance. In other worms, including many adults, cells were seen which resembled those just mentioned except that they had in some cases partially, and in others completely, lost their capacity to take up stains. Where the stainability was completely lost the cells appeared rather crumpled, though the nuclear limits were still clearly defined. Cells in this last condition were seen packed closely together in worms which had been fixed at once after the observation of red contents in the gut.

Brown's (1911) test for blood and blood products was tried on both whole mounts and sections of worms, but gave negative results so far as the contents of the gut and the 'brown' cells were concerned. However, no very satisfactory

positive result was obtained from gill-tissue of the host, which was included as a control in some of the tests.

Respiration and Survival-Time.

An indirect pointer in the matter of oxygen-requirements is that worms, particularly adults, frequently survived for twelve hours or more at about 15°C after the death of the host to whose gills they were still attached.

Lowering the temperature at which detached worms were kept increased the survival-time very considerably. Adult worms kept at about 15°C lived for two to three days while those maintained at about 2°C survived for ten days.

Excretion and Osmo-Regulation.

Globules, such as are commonly seen in the excretory ducts of many trematodes were only seen in D.denticulata on one occasion. The fluid contents of the ducts were of a pinkish colour. Flame cells were active both in larvae and adults. Vital dyes, brilliant cresyl blue and neutral red were not noticeably concentrated in the excretory ducts during the time which worms remained alive after removal from the host, but were strongly absorbed by the vitelline cells.

Newly hatched larvae would only tolerate extremely dilute solutions of vital stains and here again, no concentration of the dye was seen in the excretory ducts.

Though G.virens is a marine fish it sometimes enters the mouth of streams to feed and many fish were caught in

the harbour at St. Andrews into which the Kinnessburn flows. No general estimate of the salinity of the water can be given as it varies greatly according to the state of the tide and the amount of fresh water coming down the burn. In addition, the fresh water flows out on top of the salt water and the degree of dilution to which the fish and its parasites are subjected must depend on their vertical position.

In view of the above facts and that healthy specimens of Discocotyle sagittata were recovered from rainbow trout, which had been acclimatised gradually to sea-water and, thereafter, kept for some months in sea-water, the reactions of D.denticulata to dilution of the medium were tested.

Adult worms on removal from the host were placed in sea-water diluted with an equal quantity of distilled water, controls being maintained in full strength sea-water. After twenty-four hours the dilution had provoked no ill-effects and the eventual survival-time of the two groups was the same. However, on placing worms which had been in 50% sea-water for twenty-four hours in fresh water death ensued after about one hour, the cuticle being raised from the body in blisters.

A fish, which had accidentally fallen into a tank of fresh water and remained there for a few minutes, was killed immediately afterwards. Three adult flukes were recovered from the gills in a paralysed condition, but they revived on being placed in sea-water.

A fish was placed in fresh water for ten minutes and then returned to sea-water. Six days later it died and an adult fluke producing eggs and also a fifth stage larva were recovered from the gills.

The effect of dilution of the medium was also tried on free-swimming larvae. A group of larvae was divided into three lots: the first being kept in sea-water as a control, the second placed in sea-water diluted with an equal volume of fresh water and the third placed in fresh water, the minimum of sea-water being transferred with the larvae to the experimental dish. The control larvae continued swimming vigorously throughout the experiment; those in 50% sea-water were moving slowly after fifteen minutes and were dead after one hour; those in distilled water swam actively for a short time, but soon fell to the bottom and were dead in twenty minutes. The flame-cells of all were functioning normally till death occurred and there was no enlargement of the excretory ducts. In those subjected to a diluted medium the cuticle rose in blisters before death occurred.

Position and Movement.

Adult worms have always been found lying along the inner face of a gill-filament so that the body of the worm rests between the two rows of filaments. The worm embraces the filaments with the clamps at a level which maintains the mouth approximately at the free edge of the gill when the worm is at rest. Each clamp, turned in at right angles to

the body, encloses a few gill-lamellae in its grasp. Larger worms are, therefore, attached nearer the base of the filament on which they lie than smaller worms. Larvae are much more variable in their choice of position. Their hooks and small clamps can only grasp lamellae on one side of a filament as the fluke is of insufficient breadth to span the filament as does the adult. Accordingly they are usually found attached on one side of a filament between this and an adjacent one of the same row. Though they may be found anywhere along the length of the filament, they most commonly occur near the tip.

Larval worms, particularly early stages, are much more active in moving than adults: first stage larvae, when disturbed, loop over the gills from haptor to mouth in a leech-like manner and all larvae strongly resist attempts to detach them from the gills of ther host, clinging on with the mouth if the haptor is freed and taking a fresh grip with the haptor if possible. Adults, on the other hand make little effort to resist detachment and have never been observed to succeed in re-establishing their hold if more than one or two clamps have been dislodged. When observed on the excised gill the worms are usually fairly quiescent, the larvae making very characteristic, slight, jerking movements of the anterior region more or less regularly. Adults are often seen gently shifting the grip of individual clamps without changing position. Sometimes they make exploratory movements with the neck-region.

Degree of Infestation and Distribution Over Gills.

269 host-fish were examined. Of these 172, or 63.9%, were infested with D.denticulata. In estimating the average number of flukes per fish, 247 fishes were taken into account, the remaining 22 of the total number examined being discarded as no reliable count of the number of flukes they carried could be made. 554 flukes were recovered from those of the 247 fishes which were parasitised, giving an average of 2.24 flukes per fish, including in the total those fishes which were not parasitised. The frequency distribution among those fishes which were parasitised, from 1-17 flukes per fish, is shown in Graph 7.

In considering the distribution of the flukes on individual gills it must first be taken into account that the first three pairs of gills are approximately equal in length, but the fourth pair are only 4/5 the length of the others. Accordingly, in order to render the figures for the separate gills comparable, those obtained from the fourth gill must be multiplied by 5/4. Table 8 shows the distribution per gill.

Gill	No. of Flukes	% of Total Flukes
1st	223	40.77
2nd	195	36.65
3rd	64	11.70
4th	65	11.88 (corrected 14.85)

Table 8.

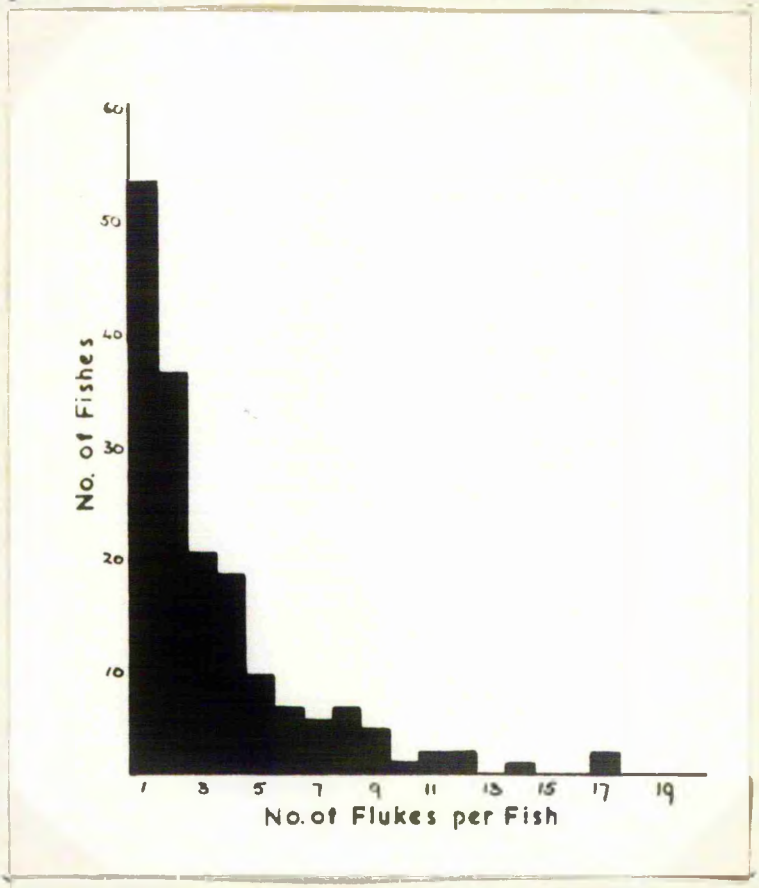
Pathogenicity.

Sometimes gills with mutilated filaments are encountered both among infested and uninfested fishes, but there is nothing to suggest that this damage is in any way associated with the activities of the flukes since the majority of infested gills are healthy and perfect in form. Sections made of worms *in situ* on the gill-filament reveal that even the lamellae which are within the grasp of the clamps show no interference with the blood supply, evidence of irritation or, indeed, any deviation from the normal condition.

Host-Specificity and Host-Immunity.

Confirmation of the reports of *Gadus merluccius* and *Gadus minutus* as subsidiary hosts has not been possible as these species were not available for study.

In order to distinguish between lack of opportunity for infestation and actual incompatibility with other hosts an attempt was made to infest a young specimen of *Gadus callarius* with free-swimming larvae of *D.denticulata* using the technique successfully employed with *G.virens*. The fish was killed on the following day and no larvae were found to have established themselves on the gills. There was unfortunately no opportunity of confirming this result by repetition of the experiment, but young specimens of *G.callarias* were often caught on the same feeding ground as *G.virens*. Though many were examined no specimen of *G.callarias* thus obtained was found to harbour *D.denticulata*.


60
50
40
30
20
10
No. of Fishes
1
3
5
7
9
11
13
15
17
19
No. of Flukes per Fish

Graph 7.

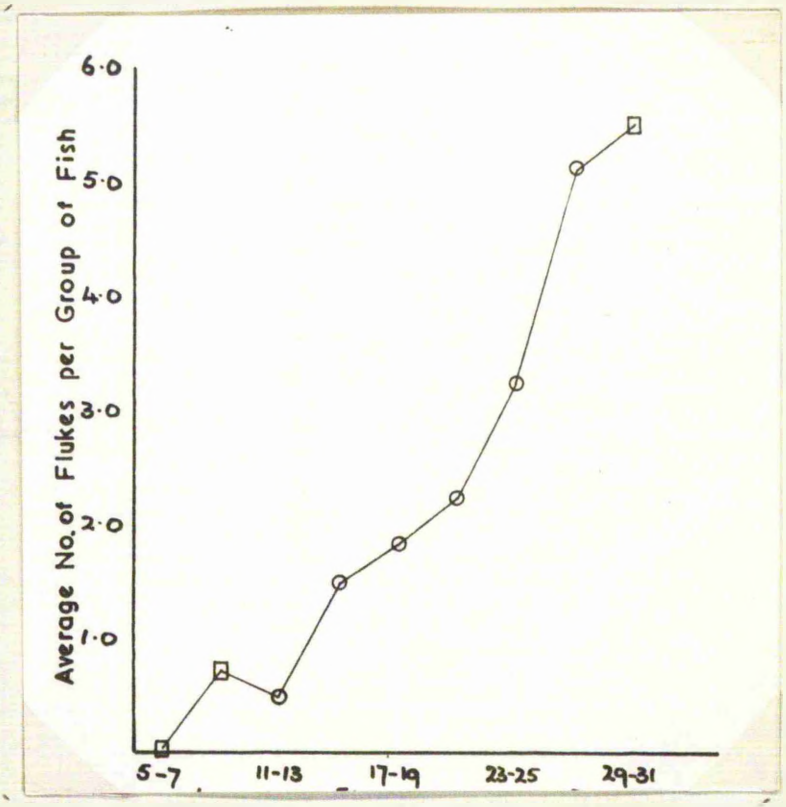
The possibiligy of the development of age-resistance and of acquired immunity through pre-munition was taken into account. The relationship between increasing age of host and degree of infestation is shown in Graph 8. The co-efficient of correlation between these values is 0.34, which is significant.

Indications of the relationship between age of host and the ability of the flukes to establish themselves are seen from the relationship between length of host and degree of infestation with adult and immature worms with a co-efficient of correlation of 0.37 (Graph 9), this being significant, and between length of host and degree of infestation with larvae only where there is a co-efficient of correlation of 0.04, which is not significant (Graph 10).

In connection with pre- the ability of flukes to establish themselves on fishes which are already parasitised is shown in Graph 11. This gives the frequency of hosts showing one, two or more separate infestations as evidenced by the presence at the same time on the same host of different age-groups of flukes. For this purpose it has been necessary to regard all adults as belonging to one age-group, which, patently, they may not, owing to the impossibility of distinguishing between them by measurement, most of them having been required for other aspects of the work.

Distribution.

D.denticulata is here reported for the first time from St. Andrews' Bay and the Firth of Forth.

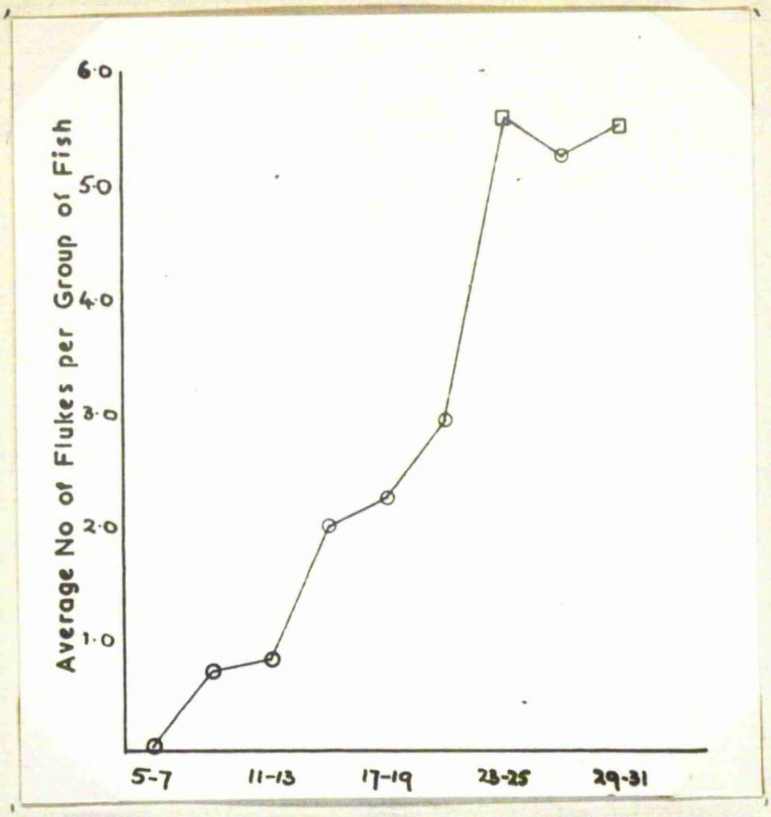


Length of Fishes in 3 cms. Groups.

Graph 8.

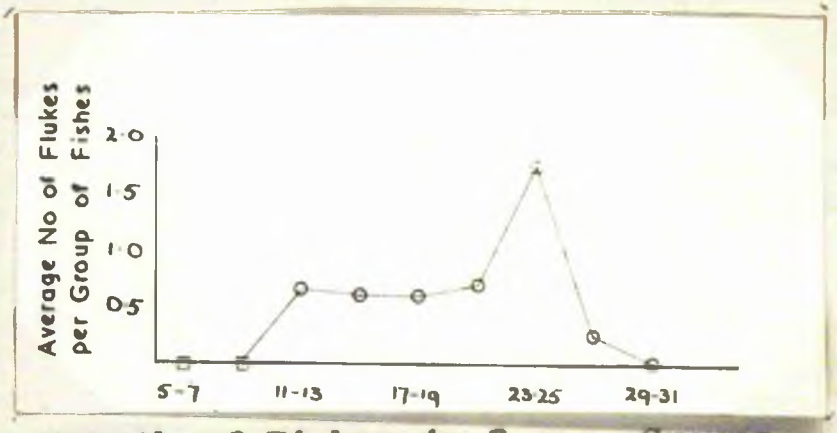
□ = averages derived from fewer than 10 fishes.

O = averages derived from 10 or more fishes.



Length of Fishes in 3 cms. Groups.

Graph 9.

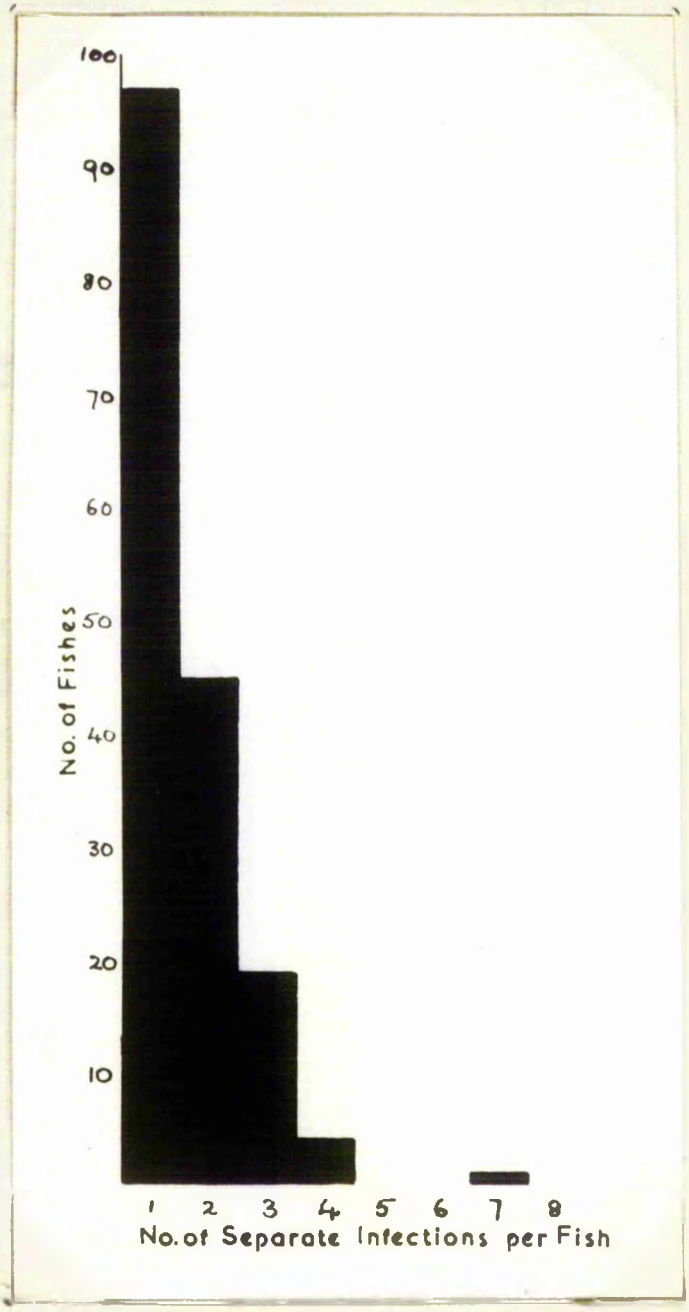


Length of Fishes in 3 cms. Groups.

Graph 10.

□ = averages derived from fewer than 10 fishes.

○ = averages derived from 10 or more fishes.


100
90
80
70
60
50
40
30
20
10
No. of Fishes
1 2 3 4 5 6 7 8
No. of Separate Infections per Fish

Graph 11.

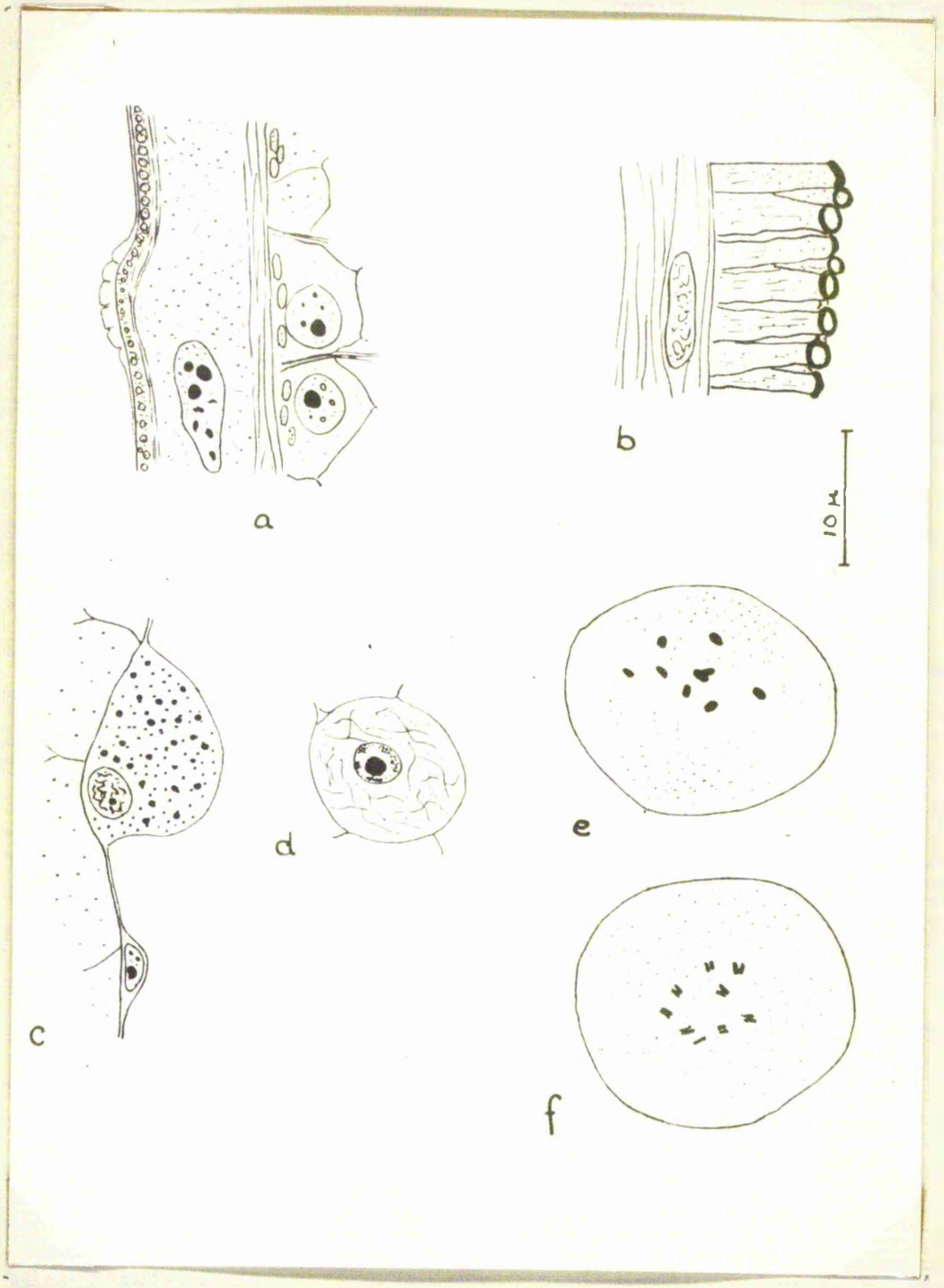


Fig. 18

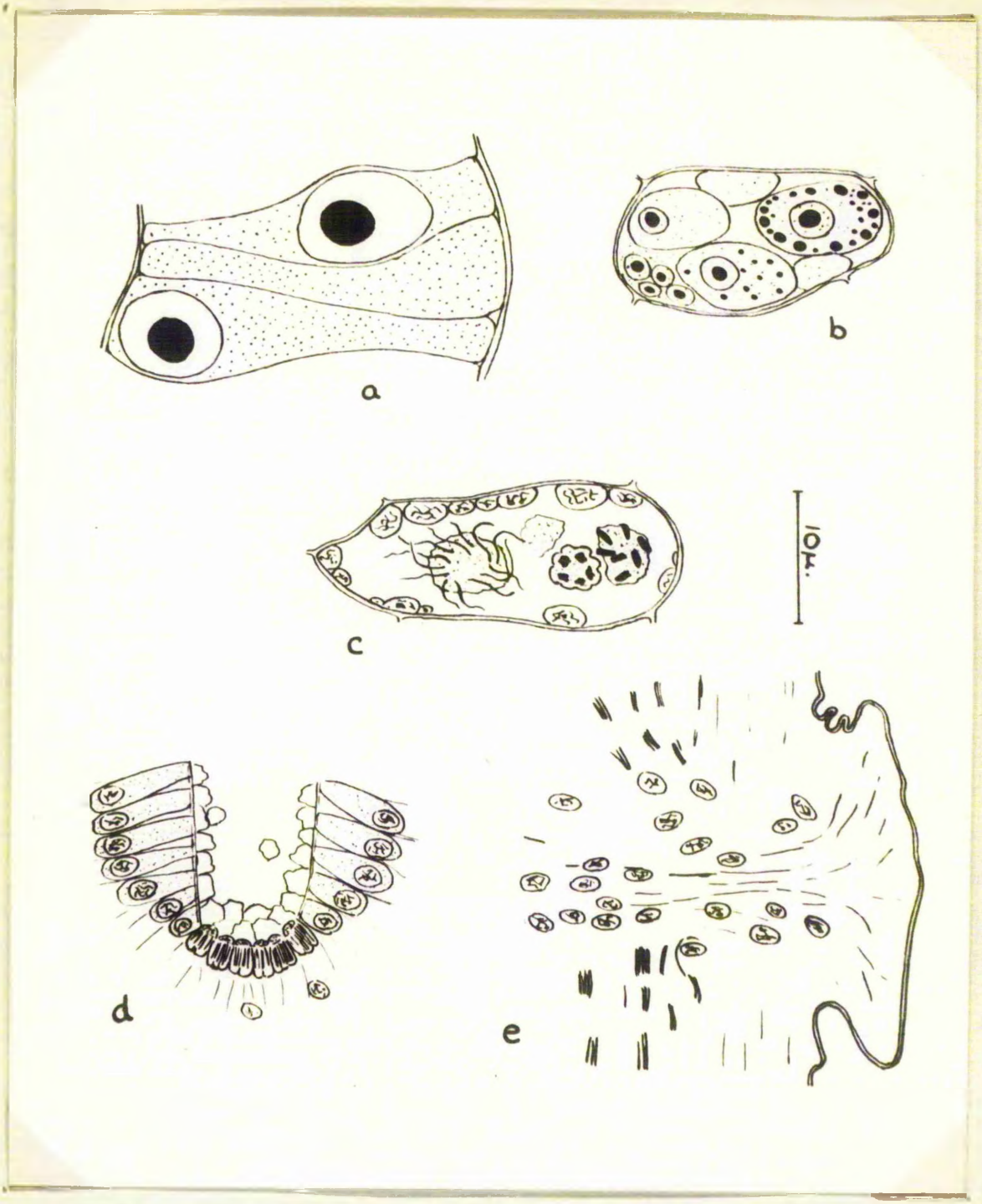


Fig. 19

DISCUSSION.

Anatomy of the Adult

Introduction.

Dicilidophora denticulata was first described in 1875 by Olsson under the name of Octobothrium denticulatum. His account, though sufficient to identify the animal and distinguish it from related species, was incomplete and contained some errors. Cerfontaine (1896) studied this fluke in detail, amplifying and correcting Olsson's account. Since then this worm has not been the subject of any further work though Price (1943) gave a diagnosis of it. However some of the literature pertaining to the more general features of the morphology of related forms has a bearing on the present case and, where relevant, is included in this review.

General Form and Size.

Olsson (1875) gave the general form of the animal as being oval and flattened, with a haptorial region amounting to half the total body-length and equipped with four pedunculate clamps on each side. He described an anterior, terminal mouth and stated that the female genital aperture was on a prominent collar not far from the mouth. His specimens were 7 mm. long and 2 mm. broad. Cerfontaine (1896) referred to the variability in the size of adults, his animals measuring from 6-13 mms. in length. In addition to the external characters noted by Olsson Cerfontaine described the posterior

margin of the animal as being a smooth curve without hooks between the hindmost clamps, its convexity facing anteriorly; he mentioned a pair of dorso-lateral excretory pores at the level of the male aperture which he found to have a bulb bearing a crown of hooks and to open medianly and ventrally; he noted the female aperture just posterior to the male one and recorded the presence of an occasionally distinguishable ventral vagina opening just to the right of the median line in mid-body. Price's (1943) account of the external characters was in accordance with that of Cerfontaine, his specimens measuring 7 mm. x 1.6 mm.

The observations of earlier workers are here confirmed as regards the general shape of adult specimens of D.denticulata. Though Cerfontaine's (1896) description of the fluke was very thorough he made no mention of the median tongue between the last pair of clamps. It may have been obliterated by distortion on fixation as sometimes happens. Evidently Olsson (1875) confused the cirrus and the female aperture. Probably he did not see the latter and must have regarded the cirrus as being the uterine aperture since he said it was on a prominent collar.

The great variability in size among adult specimens noted here bears out Cerfontaine's (1896) findings though the worms examined for this work were nearly all smaller than Cerfontaine's. Combining his findings with those of the present work it appears that the size-range of adult D.denticu-

lata is from 2.1 mm. to 13.0 mm. This indicates the caution which is necessary in giving measurements when describing a species from a few specimens only.

Body Wall and Parenchyma.

Olsson's (1875) only reference to the nature of the integument was his statement that there were 'pits' in the ventral body wall overlying the clear patch in mid-body.

No other workers having dealt with the nature of the body wall or of the parenchyma of this species, more general work must be referred to. Goto (1895) described the monogenean investing membrane as consisting of a transparent cuticle which was structureless and sometimes raisedabove the surface in watery blisters; below this came a granular or, occasionally, fibrillar, subcuticle and finally a basement membrane. Papillae might contain the endings of dorsoventral muscles or perhaps nerve-endings. He considered the whole to be a transformed epidermis. Fuhrmann (1928) gave an account in accordance with that of Goto, but thought the epidermis might either be wholly cuticularised or wanting. Among others who have referred to this matter is Dawes (1940 a), who described an investing membrane for Hexostoma extensicaudum in accordance with the above general views, but he thought it might have originated as a transformation of an absent circular muscle-layer.

Goto (1895) gave the characteristic order of the muscle layers, passing inwards, as circular, longitudinal,

diagonal and then longitudinal again with dorso-ventral strands passing from one wall to the other through the body. Fuhrmann (1928) mentioned the three components of the muscular layer, but did not state their order. Dawes' (1946) views were substantially the same as Goto's, but he did not describe an outer, longitudinal muscle-layer, and divided the longitudinal layer into superficial and deep muscles separated by a band of parenchyma. He found dorso-ventral muscles to be present and noted that there were no nuclei in any of the muscle-layers.

As regards the body parenchyma Goto (1895) divided this into two types which he called ecto- and endo-mesenchyma. In the former he said the muscles of the body-wall lay embedded and the latter filled the space between the organs, its character varying according to position in the body and species. Generally at the extremities he found it to be fibrous and reticulate, being interspersed with small nuclei, and in other parts more vacuolated and granular, approaching a syncytial condition. Fuhrmann (1928) endorsed this view as did Dawes (1946), the latter mentioning also (1940a) that parenchymatous tissue entered into the ducts of the body, these being fibrillar and without nuclei.

The cuticle in which the body of monogeneans is enclosed has been thought to arise from a transformed epidermis or muscular layer. None of the histological findings in the present work throws any light on the origin of the cuticle, but

a point which seems to have escaped the notice of those authors who have considered this matter, is that when the monogenean free-swimming larva settles on the host the ciliated coat is sloughed off. Perhaps this deciduous epithelium should be regarded as the epidermis, and, if so, an epidermis will naturally be wanting subsequently.

The arrangement of the muscle-layers composing the body-wall is evidently not constant throughout the Monogenea but shows slight variations. Dawes' (1946) general statement on the subject is in accordance with present findings. Goto (1895) mentioned papillae which seem similar to those so prominent on the ventral surface of the haptorial region in D.denticulata (fig.18b). His view that they were the terminations of dorso-ventral muscles is closer to present observations than the idea that they might contain nerve-endings. Here they are interpreted as the ends of short, perpendicular muscles which do not penetrate beyond the bodywall. They would form a re-inforcement to the integument in a region which rests against the gill-filament of the host. That similar, but much smaller, papillae and perpendicular muscles are found in the buccal suckers, where abrasion might also occur, lends support to this argument.

It appears that the large cells filling the spaces between the organs in the posterior part of the body in the adult and so prominent in larvae of D.denticulata are not typical of Monogenea as no mention of

them has been found in the literature. The nature of these cells is obscure. From their dense cytoplasm it may be inferred that they are secretory and it seems possible that they may be responsible for the mucus-like exudate seen oozing from rosette-shaped pores on the surface of the body. However it is noteworthy that though the pores, of which, again, no mention has been found in the literature, are fairly equally distributed over the surface, the 'secretory' cells are largely confined to the posterior region.

Clamps.

In his original description of the species Olsson (1875) noted that the clamps were each formed from two valves and that the anterior of these bore externally on the outer quadrant about thirty conical teeth. Cerfontaine (1896) gave a detailed description of the skeletal elements in the clamps. To avoid confusion the lettering adopted in this work has been used here to explain his findings (fig.4).

He described the clamp as being composed of an anterior and a posterior valve. Bisecting the anterior valve, running from the centre of the cup to the mid-anterior point was a sclerite, a, which then continued along the rim of the proximal, anterior quadrant (the peripheral part of sclerite b). Running from slightly distal to the centre to the mid-proximal point and a little beyond was another sclerite (basal part of sclerite b). From the basal end of sclerite b, an

element (basal part of sclerite c) ran to the mid-distal point. From here another sclerite (peripheral part of c) passed along the rim to the mid-anterior point and expanded into a plate covering the dorsal aspect of the distal, anterior quadrant and bearing about thirty conical teeth. The posterior valve was bisected by a sclerite (d) terminationg at the mid-posterior point from where two short sclerites (e and f) were directed distally and proximally respectively along the post-crior rim. The skeletal elements were completed by two small sclerites (g and h) running from the posterior margins of sclerites b and c respectively, where these elements approached the mid-proximal and mid-distal points respectively, and were directed posteriorly towards sclerites e and f respectively. Chitinous ridges re-inforced the surfaces of the posterior quadrants.

Later Cerfontaine (1898) described a small ring-sclerite, composed of two curved bars, embedded in the base of the proximal, anterior quadrant.

Goto (1895), Llewellyn (1941b), Price (1943) and Sproston (1945b) have given accounts of the skeletal elements characteristic in the clamps of the genus Diclidophora and their views co-incided substantially with those of Cerfontaine. Goto's (1895) views differed to the extent that he considered sclerite a to be fused at its base to sclerite b and to diverge into two short horns at its distal extremity. Sclerite b he considered to be broken near its point of fusion with sclerite

a, but thereafter to be continuous round the rim to the mid-anterior point. Likewise he thought sclerite c was continuous from its basal point of contact with sclerite b to its termination at the mid-anterior point.

Llewellyn (1941b) deviated from Cerfontaine in saying that neither sclerite b nor sclerite c was divided into two parts and that sclerite a did not extend along the rim of the proximal, anterior quadrant, but Price (1943) thought that it did, overlying sclerite b in this region. Sproston (1945b) did not agree with Price on this point, mentioning no peripheral extension of sclerite a. She was the only worker to suggest that sclerite d was split longitudinally, each half continuing without a break to the formation of sclerites corresponding to e and f.

Goto (1895) found that the sclerites dissolved in 35% potassium hydroxide and Remley (1942) declared that those of Microcotyle spinicirrus were not chitinous.

In the case of the musculature associated with the clamps it is Cerfontaine (1896) who provides the only account of this particular species, but Goto (1895) and Dawes (1940a) may be referred to on general points.

Cerfontaine (1896) described opposing muscles running from sclerite a to d, that for opening the clamp being on the dorsal aspect; that for closing it, on the ventral aspect. The action for opening the clamp, he said, was re-inforced by a bundle of muscles passing down the peduncle and fanning out

over the dorsal aspect of the cup. The closing action of the basal, ventral muscle was increased by short muscles running from sclerites g and h to the chitinous ridges in the posterior quadrants and also by muscles running down the peduncle, inserted on the ring sclerites and bringing about the withdrawal of the fleshy bulb in the proximal anterior quadrant and consequently increasing the volume of the clamp-cavity once a grip had been taken.

Cerfontaine did not mention the nature of the perpendicular fibres in the walls of the clamps, but Goto (1895), speaking generally, considered them to be elastic rather than contractile, noting that they stained less intensely with haematoxylin than did ordinary muscle.

Dawes (1940a), speaking of Hexostoma extensicaudum, made the generalisation that the superficial longitudinal muscles were inserted on the rim of the cup and thus acted as releasers while the deep longitudinal and dorso-ventral muscles ran to the centre of the cup thus effecting closure of the clamp.

The present findings with regard to the arrangement of the skeletal elements of the clamps are basically in agreement with the observations of other workers on the same or related species. The minor differences which obtain between the arrangement as noted here and other species or generalisations made from a number of species are probably real, but a few, small differences have been noted between Cerfontaine's (1896)

and this work on D.denticulata. Thus it has been found (fig.4) that sclerite b is continuous and not divided into two parts as Cerfontaine said, and sclerite a is not continuous with sclerite c. Similarly, sclerite c is continuous and there is a certain degree of secondary fusion between sclerite a and the adjacent portion of sclerite b. The ring sclerite mentioned by Cerfontaine (1898) as being embedded in the base of the fleshy bulb in the proximal anterior quadrant could not be detected.

The little work which has here been done concerning the chemical nature of the sclerites is inconclusive and there is no doubt that further examination of the problem would be repaid. All that can be said is that the findings of Goto (1895) and Remley (1942) that chitin does not enter into their composition is confirmed and it is evident that they are of a proteinaceous nature.

Observations made here on the musculature of the clamps are in agreement with Cerfontaine's (1896, 1898) findings. In the case of D.denticulata it does not seem possible to associate the muscles bringing about the closure of the clamp with the deep layer, and those opening the clamp with the superficial layer, of longitudinal muscles as Dawes (1940a) did in Hexostoma extensicaudum. The muscular components of the haptorial regions in D.denticulata are so greatly developed that the parenchymatous layer is obliterated and the normal characters of the body-wall are lost.

The perpendicular fibres re-inforcing the walls of the clamp are, as Goto (1895) said, elastic rather than contractile and are closely similar to those in the ventral wall of the haptor.

Alimentary System.

Olsson (1875) was not able to make out much of the alimentary system of his specimens. He observed the mouth as being transverse and terminal and noted a circular aperture in the dorsal lip which he thought might be excretory. He also saw the buccal suckers, but said nothing of the pharynx. He was unable to distinguish clearly between the longitudinal gut caeca and the vitelline canals.

Cerfontaine (1896), here again, gave a reliable account of the alimentary canal describing the transverse, terminal mouth and pair of buccal suckers, the oval pharynx with it narrow lumen, the short oesophagus bifurcating immediately at the level of the male aperture to form branching caeca anastomosing posteriorly.

Though he said the ramifying gut-branches imparted a dark colour to the animal Cerfontaine did not specifically refer to the nucleated 'brown' cells lying along the caecal walls, which were mentioned as being so conspicuous a feature of many monogeneans by Goto (1895). Among other references in the literature to these cells are those of Gallien (1935) in Polystoma integerrimum and Dawes (1940a) in Hexostoma extensicaudum.

Apart from 'brown' cells in the walls of monogenean

gut-caeca there may be (Goto, 1895; Fuhrmann, 1928; Dawes, 1946) a regular epithelium or merely scattered cells joined by a fine membrane. In this latter case Goto said there were no salivary or pharyngeal glands in association with the gut. He regarded the glandular areas which he observed in the dorsal lip of some species as being composed of 'sticky' glands. On the other hand Cerfontaine (1896) described pharyngeal glandslying in the bulb of the pharynx and opening on conical papillae at its anterior end in D.denticulata itself. Other cases which may be mentioned are those of Polystoma integerrimum where Gallien (1935) found pharyngeal glands opening posteriorly and Hexastoma extensicaudum where Dawes (1940a) only detected granular cells round the oesophagus.

Cerfontaine's (1896) description of the alimentary system is fully confirmed here and in addition the intrinsic and extrinsic muscles of the buccal suckers and pharynx are described . Though Cerfontaine described pharyngeal glands opening anteriorly in the pharynx no sign of them was found in the present work. However, lateral to the pharynx, a few glandular cells whose secretion seems to pass to the lip region have here been noted. Olsson (1875) was in error in describing a circular aperture in the dorsal lip. What he saw was the dorsal pocket, formed by the pinching-in of the lip on each side. Goto's (1895) view that this region had glands producing a sticky secretion is here confirmed.

The nature of the 'brown' cells lying along the intestinal caeca is discussed in connection with food and digestion.

Nervous System.

The nervous system, not mentioned by Olsson (1875), was described for D.denticulata by Cerfontaine (1896) as consisting of a supra-oesophageal collar giving rise to one pair of short, anteriorly directed nerves and to two pairs of posteriorly directed nerves. Of these latter he said the external pair were slender and not continued far down the body, but the internal pair, running ventral to the main gut-branches, extended through the haptorial region, giving off a branch along each peduncle, and uniting in a transverse commissure between the last pair of clamps. There was also, he said, a commissure at the level of the anterior pair of clamps, but only rudimentary ones between the second and third pairs of clamps. In the haptorial region and peduncles he observed large, specialised cells which he regarded as nerve cells.

Goto (1895) gave some generalisations concerning the structure of monogenean nervous tissue, stating that the nerves had a reticulated, fibrous structure in which no nerve cells could be seen, but that cells of this type were to be found at the roots of the nerves lateral to the cerebral collar.

Cerfontaine's (1896) description of the nervous system is here confirmed. The finding of large cells similar

to those described by Cerfontaine not only in the peduncles but also in other parts of the body where accurate nervous control would be necessary lends support to Cerfontaine's view that these are nerve cells. The argument is further strengthened by their occasional presence, as seen here, embedded in the nerve fibres themselves.

Excretory System.

Olsson (1875), as already mentioned, thought that the aperture which he saw in the dorsal lip of D.denticulata might be excretory, but made no other reference to the excretory system.

Cerfontaine (1896) only stated that the excretory apertures were paired and situated, one on each side, at the level of the main aperture, but Goto (1895) found, in the genus Diclidophora, that the excretory system consisted of fine canals converging to form a longitudinal canal running posteriorly between the main gut-branch and the nerve cord on each side and then returning on its own course to open laterally, as Cerfontaine said, at the level of the male aperture. He was unable to follow the finer canals to their origin in the tissues.

The excretory system conforms to the monogenean type. Apart from the flame-cells, which have not before been seen in this species, there is no ciliation in the ducts. Flame-cells do not seem to be arranged in pairs in the adult as they are in the larva. It must, however, be borne in mind

that they are difficult to detect and may be concealed by the opacity of the extensive vitellaria.

Flame-cells were never seen in fixed preparations and were only seen in living worms when compressed. This may account for their having been missed by other workers.

Reproductive System.

Olsson's (1875) identification of the discrete, rounded organ in mid-body as the testis and the smaller body anterior and to the right of this as the ovary was corrected by Cerfontaine (1896) who established the former organ as the ovary and the latter as the receptaculum seminis.

Cerfontaine's description included an account of the folded, N-shaped ovary opening anteriorly, as it turned horizontally, into the oviduct which, after receiving the duct of the seminal receptacle, then ran posteriorly. It gave off the genito-intestinal canal, which joined the right caecum, and then, after uniting with the yolk-reservoir, continued posterior to the ovary as the common duct. This common duct ran into the ootype which passed forward ventral to the ovary and transverse vitelline duct to join the uterus at the level of the anterior border of the ovary. The uterus continued in the mid-line to the female aperture just posterior to the gut bifurcation. The vitelline glands, he said, consisted of numerous acini lying along the gut-branches and opening into longitudinal canals which gave rise to two transverse canals meeting in the median line to form the vitelline reservoir. This lay

ventral to the ovary and opened, as stated above, into the oviduct at its base. The seminal receptacle he described as sometimes showing a vagina patent directly to the ventral surface, but frequently this opening could not be found.

The male reproductive system, Cerfontaine said was composed of many testicular acini, largely, but not entirely post-ovarial, whose products were discharged into vasa efferentia and finally into a vas deferens which ran forward ventral to the ovary, but dorsal to the uterus, and emerged in a cirrus bulb just anterior to the female aperture, there being a seminal vesicle close to the cirrus. The cirrus was armed with fourteen, or occasionally thirteen, recurved, double-bladed hooks.

Comparative evidence on the subject of the vagina is available concerning another species of the genus, D.merlangi. Odhner (1913) found that in this species the vagina was a temporary canal only, his sections indicating a plug of scar-tissue between the seminal receptacle and the ventral body-wall.

Price's (1943) diagnosis of D.denticulata gave an account of the reproductive system in agreement with Cerfontaine's except that he described the yolk-reservoir as being 'pre-ovarial.'

The only glands in association with the reproductive system referred to by Cerfontaine (1896) were those surrounding the ootype: posteriorly, at its widest part Mehlis' gland with two groups of ducts opening into the ootype and, anteriorly

to this, pyriform glands surrounding the rest of the organ. Ujiie (1936a) bore out this distinction in Echinochasmus japonicus, but Goto (1895) and Dawes (1946), speaking generally, made no distinction between the two regions, regarding the whole as a uniform Mehlis' gland. In Polystoma interexerrimum however, Gallien (1935) reported a difference between the dorsal and ventral parts of Mehlis' gland, the latter only being distinguishable during reproductive activity.

Prostate glands, though not mentioned by Cerfontaine (1896) are, according to Goto (1896), common in monogeneans. He observed scattered cells with finely granular cytoplasm surrounding the distal end of the vas deferens with fine ducts entering this organ in whose lumen discharged granules lay. Kourí and Nauss (1938) noted the similarity of the structure of prostate and Mehlis' glands in Fasciola hepatica.

Goto (1895) has also provided some general information on the histological nature of the reproductive organs. The ducts and ovarian sheath he found to have non-nucleate walls which he considered to be transformed epithelia. However Alvey (1936) noted a few nuclei in the ovarian wall of Sphyranura oligorchis. Dawes (1946) considered that parenchymatous tissue entered into the composition of the connective tissue walls of these ducts. Goto (1895) noted various modifications of the lining of the ducts such as circular thickenings in parts of the oviduct and genito-intestinal canal and occasionally 'stout cilia' in parts of the uterus. The only truly

ciliated duct he mentioned was the genito-intestinal canal. In some species he said the uterine wall included an outer layer of circular and an inner layer of longitudinal muscles, this being the only case of muscularity observed in the genital ducts. Dawes (1940a) noted that the ootype of Hexostoma extensicaudum was not muscular.

The present work largely confirms Cerfontaines' (1896) account of the reproductive system, but here the vagina described by Cerfontaine was never found patent. It is thought that the explanation lies in the condition in this fluke being the same as that described by Odhner (1913) in Diclidophora merlangi where the vagina is a temporary inbreak only. Cerfontaine gave no details of the structure of the cirrus nor did he mention that it was, when at rest, retracted below the body surface. He gave the usual number of hooks arming the cirrus as fourteen and occasionally thirteen. Examination of larger numbers of flukes shows that though from eleven to fourteen may be present, the most usual number is twelve. It is probable that Cerfontaine took the prostate gland for a seminal vesicle as he mentioned the latter but not the former, in the position here described for the prostate gland. Study of sections reveals beyond doubt that this organ is composed of cells filled with a dense secretion and is not a cavity containing spermatozoa.

Price (1943) described the yolk-reservoir as being pre-ovarial. It can only be concluded that his specimens

underwent distortion in fixation as this organ was always seen lying ventral to the ovary in worms examined during this work.

Turning to the histology of the reproductive ducts Cerfontaine (1896) only described the structure of Mehlis' gland and his view that the glands of the posterior part were histologically distinct from the more anterior ones is here endorsed. However no sign could be found of the ducts he mentioned as leading from the posterior gland to the lumen of the ootype. Gallien's (1935) division of the gland in Polystoma integerrimum into dorsal and ventral regions does not apply here.

The histological nature of the remainder of the reproductive canals is broadly in agreement with the generalisations of Goto (1895) and Dawes (1946), but it is noteworthy that true ciliation of no part of these ducts is recorded by Dawes, and Goto only found cilia in the genito-intestinal canal. During the present work, as has been described, cilia were clearly seen in the oviduct and common duct, also in the transverse vitelline ducts and vitelline reservoir, but not in the gentio-intestinal canal. While the stiff fibrils described by Goto (1895) as 'stout cilia,' of the uterus appeared clearly in sections, the true cilia were only visible in the living fluke. Thus they may well be quite a common feature of the monogenean reproductive system and yet have escaped notice where only preserved material has been studied.

Considering the vigorous peristaltic movements performed

by the ootype its lack of muscularity may seem surprising but, as Dawes (1940a) has pointed out for Hexostoma extensicaudum, there are numerous strands of connective tissue normal to the surface of the ootype running into the surrounding tissue: tension on these strands would move the ootype wall and it is thought that this obtains in D.denticulata also.

Abnormalities and the Repair of Damage.

The literature which has been studied has not revealed any mention of the capacity of monogeneans to make good injuries to the body. However the fact that worms have occasionally been seen during this research in which either a clamp was missing or where one of the clamps was much smaller than the others, is considered as strong circumstantial evidence in favour of a capacity for regeneration of lost parts. The regenerative powers of the supposedly related Turbellaria may be significant in this connection. It is unfortunate that owing to the inability to maintain worms in vitro, or examine them closely without killing their host, this suggestion could not be put to experimental test.

Reproduction

Introduction.

Information relating to the reproduction of monogenetic trematodes is very limited and incomplete. Some aspects of reproductive activity are closely similar in Monogenea and Digenea and it has therefore been thought desirable to include some mention of the latter order in this review of the literature. The references to digenetic reproduction are not, however, intended to be exhaustive, but are only included when they have a bearing on the present subject.

Since maturation of the oocyte may more properly be considered when dealing with embryology and development the details of this process are discussed in the section on development. Though maturation of spermatozoa, taking place as it does before egg-formation, is referred to here, any relevant points will be elaborated in connection with oocyte maturation.

Spermatogenesis.

Proliferation of primary spermatogonia from the wall of the testicular acinus takes place in a number of trematodes including the monogenean, Sphyranura oligorchis according to Alvey (1936) and the digeneans Zygocotyle lunata according to Willey and Godman (1941), Probilotrema californiense according to Markell (1943) and Parorchis acanthus according to Rees (1939), but in the remarkable case of Proterometra macrostoma, where the gonads are already well-developed in the cercaria, Anderson (1935) reported that spermatogonia

arose from a solid mass of testicular tissue. The spermatogonial divisions which follow to produce primary spermatocytes may be three in number. Such was found to be the case in Zygocotyle lunata by Willey and Godman (1941), in Probilotrema californiense by Markell (1943), in Parorchis acanthus by Rees (1939) and in Proterometra macrostoma by Anderson (1936), but Anderson (1935) reported Woodhead's (1935) finding of only two gonial divisions in Bucephalus elegans. Two maturation divisions follow in all the cases mentioned above producing morulae of thirty-two cells except in B. elegans when, of course, there are sixteen cells unless, as sometimes happens, the morulae coalesce in pairs. In Sphyranura oligorchis, Alvey (1936) reported a hollow morula of forty-five cells.

The spermatids produced by the second maturation division are transformed to spermatozoa. Such information as there is suggests that the filiform spermatozoa are entirely nuclear, the cytoplasm being left as a residual mass. This has been reported for Zygocotyle lunata by Willey and Godman (1941), for Probilotrema californiense by Markell (1943) and for Proterometra macrostoma by Anderson (1935), while Rees (1939) thought it probable in Parorchis acanthus.

Such observations as have here proved possible are in agreement with the general view that there is proliferation of the sperm mother cells from the testicular walls and that a number of divisions follow by which a morula is formed.

Oogenesis.

Oogonia are proliferated from the walls of the blind limb of the ovary. Examples studied include Polystoma integerrimum by Gallien (1935), Sphyranura oligorchis by Alvey (1936), Fasciola hepatica by Schubman (1905) and Henneguy (1906), Zoogonus mirus by Goldschmidt (1905) and Wassermann (1913), Zygocotyle lunata by Willey and Godman (1941) and Parorchis acanthus by Rees (1939). The cells are poor in cytoplasm and not sharply demarcated from one another. Relatively few workers have reported cell-division at this stage, but Schubman (1905) saw mitotic divisions in Fasciola hepatica and his findings have been endorsed by Gille (1914) for Gyrodactylus elegans, by Tyzzer (1918) for Collyriclum faba, by Willey and Godman (1941) for Zygocotyle lunata and by Markell (1943) for Probilotrema californiense. Anderson (1935) was the only one to give the number of divisions saying there were three in Proterometra macrostoma. While Wassermann (1913), discussing Zoogonus mirus, was followed by Gallien (1935) on Polystoma integerrimum, and Rees (1939) on Parorchis acanthus in only referring to growth of the oogonial cells, Goldschmidt (1905) stated definitely that oogonial divisions did not occur in Zoogonus mirus.

Whether by growth only, or division followed by growth, primary oocytes are eventually produced and continue to grow as they pass distally through the ovary. Schubmann (1905) and Henneguy (1906) noted that in Fasciola hepatica the oocytes maintained a peduncular connection with the ovary

wall, until growth was complete, through which nourishing particles might pass. A similar state of affairs has been described for Polystoma integerrimum by Gallien (1935) and for Parorchis acanthus by Rees (1939). In some cases nourishment of the oocyte occurs at the expense of nieghbouring oocytes as has been described by Katheriner (1904) and Gille (1914) in Gyrodactylus elegans and by Schubman (1905) in Fasciola hepatica. Anderson (1935) thought that nuclei which he found in oocytes of the cercaria of Proterometra macrostoma were derived from other oocytes which had been ingested. Cytoplasmic inclusions of various types have been reported in some other cases, notably Probilotrema californiense by Markell (1943) and Zygocotyle lunata by Killey and Godman (1941). These were thought to be food reserves but Goldschmidt (1905) described a body in the oocytic cytoplasm of Zoogonus mirus which was formed as a chromatic condensation on the nuclear membrane and then released into the cytoplasm. There is also the case of Polystoma integerrimum where Gallien (1935) described the extrusion of part of the nucleolus into the cytoplasm.

The mature, primary oocyte, when ready to leave the ovary, is more or less spherical having a rounded nucleus with a large nucleolus. Zeller (1872) is the only dissentient to the view that there is no vitelline membrane, having observed one in Diplozoon paradoxum.

There is a difference of opinion among those who have

studied the problem as to whether divisions of the cell which gives rise to an oocyte occur after proliferation from the ovarian wall. It is possible that species may differ in this respect. Evidently divisions do occur in D.denticulata, having been seen occasionally during the present study, but their rarity gives rise to speculation. If division were a constant feature at this stage, it would be expected that it should be seen with regularity. That it has been observed so seldom might indicate that inhibition of the process might result from disturbance. With this in mind fixation was often carried out immediately the host was killed, but no greater frequency of observation of oogonial division resulted. It may be that division at this stage is not a constant feature, but only occurs in a small proportion of cells.

Alternative methods of nourishment of growing oocytes referred to in the literature are ingestion of neighbouring oocytes and the passage of nutrient matter through the ovarian wall with which developing oocytes are in close contact. No positive evidence was found for either of these methods in D.denticulata though the oocytes certainly do not lose contact with the wall of the ovary until fully grown. Cytoplasmic inclusions such as degenerating nuclei or food reserves were never seen and it is probable that nourishment which may be supplied either through the ovarian wall or in ovarian fluid cannot be detected by the staining methods here adopted.

Vitellogenesis.

The first studies of the process of vitellogenesis or development of the yolk-cells in trematodes were those of Schubman (1905) and Henneguy (1906) on Fasciola hepatica, who found that the acinal walls were lined with small cells not unlike young oocytes, but characterised by two or three nucleoli (Schubman, 1905), which dropped into the lumen of the acinus and there grew to full size and developed in their cytoplasm the characteristic globules which became peripheral when the cell reached maturity. Kourí and Kauss (1938), working on the same species, reported no definite nucleolus and said that the cell-membrane had a refractile re-inforcement similar in appearance to the globules. Stephenson (1947) also made some observations on F.hepatica, and saw divisions occurring in the vitelline acini and thought it likely that the yolk-cells were haploid.

Gallien (1935) observed the migration of subtegumentary vitelline cells into the reticulate parenchyma of Polystoma integerrimum when the glands became re-activated in August. His account of their further development co-incides with the above findings for Fasciola hepatica as do the observations of Dawes (1940a) on Hexostoma extensicaudum and Markell (1948) on Probilotrema californiense, the latter stating that two nucleoli were characteristic.

The proliferation and development of yolk-cells in D.denticulata as observed here accords with those species

discussed in the literature, but it seems very doubtful whether division of the cell occurs in the acinal lumen in more than an occasional case. Accordingly there is no indication as to whether these cells are haploid, as Stephenson (1947) has suggested in Fasciola hepatica, or diploid. Gallien's (1935) observation in Polystoma integerrimum of migration of vitelline cells, prior to their development, through the parenchyma seems to be an isolated case and may be connected with the seasonal recrudescence of reproductive activity in this species.

The different staining reaction of vitelline globules in the lower part of the vitelline reservoir which has been noted here in D.denticulata has not, apparently, been seen in other species. It is thought that it may be due to the influence of the secretion of Mehlis' gland which would tend to be carried toward the vitelline reservoir by the ciliary current in the common duct.

Insemination.

Very little is known of the means by which insemination is performed in trematodes. Augustine (1929), speaking generally, considered that self-insemination was probably more common than cross-insemination, especially in digenetic forms. Dawes (1946) said that in Digenea both cross- and self-insemination have been observed.

Among Monogenea there is firstly the classic example of Diplozoon paradoxum in which Zeller (1872) described how

the pair of animals grew together and remained permanently *in copula* with mutual cross-insemination from cirrus to vagina. In *Microcotyle stenotomi* mutual insemination is impossible because the cirrus is ventral and the vagina dorsal, but cross-insemination occurs (MacCallum, 1913). Wilde (1936) observed cross-insemination in *Neodactylogyrus macracanthus* and Paul (1938) inferred that it was essential in *Polystomoides oris* from the fact that the eggs of a solitary worm were only rendered fertile when another mature worm was established on the same host. On the other hand Gallien (1935) has stated that, though cross-insemination usually takes place in *Polystoma integerrimum*, self-insemination can occur in isolated worms.

That both cross- and self-insemination is possible in trematodes is evident from the literature and Gallien (1935) has said that either may occur in *Polystoma integerrimum* . Evidence on this matter gained during the present work is purely circumstantial as worms were never observed *in copula* or performing self-insemination. From the fact that infertile eggs are extremely rare, it is apparent that, whatever the method, it is highly efficient. This high rate of fertility extended to the 12.8% of the worms producing eggs which were solitary on their host. It is, of course, possible that this figure of 12.8% is a distortion of the true facts as in some cases it is possible that the partner responsbile for insemination lost its hold during the act and was expelled from the

gill-chamber or merely died after insemination and before the host was caught and examined. However, since the 12.8% includes only those worms which were absolutely solitary on the host and takes no account of those which shared a host with larvae and immature worms incapable of providing spermatozoa, the figure is probably low rather than high. Accordingly it is thought that, though self-insemination may not be the rule, it is highly probably that it can occur. Unfortunately it was not possible to prove this by rearing a worm to maturity in isolation from its congeners.

As to the method by which insemination is carried out, again, only speculation can be put forward. The process varies in other flukes according to their morphology and in D.denticulata both physical configuration and physiological condition must be taken into account. The fact that sperm has not been found in the uterus does not exclude the possibility that it might pass rapidly through and thus escape observation, but the presence of sperm in the seminal receptacle of adults as so constant a feature is highly significant. This, together with the presence of a scar between the seminal receptacle and the ventral body-surface is taken to indicate that impregnation takes place through this temporary vagina to the seminal receptacle. It is largely self-evident that the cirrus is a functional, intromittant organ. There is no communication between the male and female reproductive systems and the cirrus is well-developed and

armed in such a way that it could readily penetrate the body-wall in making contact with the seminal receptacle. The great extensibility of the anterior region of the body would allow of the cirrus being opposed to the seminal receptacle of the same individual in the case of self-insemination.

Formation of the Egg and Nature of the Shell.

The nature of the egg-shell and the mechanism of egg-formation in trematodes have long been the subject of controversy and speculation. A study of the literature reveals that the processes involved are broadly the same in Monogenea and Digenea. Accordingly work on both orders is treated in this survey of the subject.

It was assumed by the early workers that the shell of the trematode egg was provided by a secretion of the unicellular glands surrounding the ootype; hence the name 'shell-gland.' Thus E. van Beneden (1868) stated that the shell of Diclidophora pollachii was formed from a secretion of the shell-gland. This view was upheld by Schubmann (1905) without question in his work on Fasciola hepatica. Also in 1905, Goldschmidt investigated the process of egg-formation in the aberrant, shell-less digenean, Zoogonus mirus. He noted, though apparently without appreciating its significance at the time, that the vitelline cells did not contain the globules so characteristic of the vitelline cells of trematodes with normal shelled eggs.

It was Henneguy (1906), who first suggested, as a result of his observations on Fasciola hepatica, that the egg-shell was formed, not from the secretion of the 'shell-gland,' but from globules originating in the vitelline cells. He observed that these globules were extruded during the passage of the vitelline cells through the ootype region and gave rise to the egg-shell. Since the publication of Henneguy's work an increasing body of evidence has been assembled indicating that the shell of trematode eggs is, at least in the main, provided by globules of the vitelline cells. MacCallum (1913) and more recently Yosufzai (1952) have upheld the older view. MacCallum maintained as a result of observations in life that the egg-shell of Microcotyle spinicirrus is formed from a 'chitinous secretion' of the shell-gland and Yosufzai claimed, in spite of the contrary evidence of others for the same species, that in Fasciola hepatica the secretion of the shell-gland was identical with the newly formed shell which was only temporarily re-inforced from within by yolk granules, later absorbed by the embryo.

With the establishment of the subsidiary role played by the gland surrounding the ootype in shell-formation it seems desirable, in order to avoid confusion, to refer to this gland by its alternative name, Mehlis' gland.

Goldschmidt (1909) reviewing his findings concerning Polystoma integerrimum, Dicrocoelium lanceolatum, Fasciola hepatica, Haplometra cylindracea and Opisthorchis felineus,

came independently to the same conclusion as Henneguy (1906). His reasons for this opinion were that identical staining reactions were obtained from the globules within the vitelline cells, from extruded globules lying in the ootype and from newly formed egg-shell. In this connection it is of interest to note that Tyzzer (1918), dealing with Collyriclum faba, found that there was a change in the vitelline globules on extrusion: that they only became hyaline and refringent after release from the parent cell. The characteristic staining reaction of the newly formed shell was not, Goldschmidt found, given by the secretion of Mehlis' gland. As confirmation of his theory he stated that yolk-cells incorporated in the egg lacked globules which they had previously contained and also that in an abnormal individual, which he observed, there were masses of excess shell-material in the genital ducts, this condition being associated with hypertrophy of the vitellaria, while Mehlis' gland was normal.

A number of species belonging to different groups have now been investigated with results in accordance with the hypothesis of vitelline origin of shell-material. Thus, among Monogenea, Gallien (1935) has confirmed Goldschmidt's (1909) results with Polystoma integerrimum and Wilde (1936) has found that vitelline globules provide the shell of Neodactylogyrus macracanthus. Similar results have been obtained for the digeneans Echinochasmus japonicus by Ujiie (1936a), Clonorchis sinensis by Ujiie (1936b) and Murikami

(1937), Paragonimus westermanni, Metagonimus yokogawai and Metagonimus takahashii by Murikami (1937) and Probilotrema californiense by Markell (1948). Confirmation of Henneguy's work on Fasciola hepatica has been given by Murikami (1937), Kouré and Nauss (1938) and Stephenson (1947).

There remains to be mentioned the work of Tyzzer (1918) and Dawes (1940b) both of whom considered that a basic outer layer for the shell might be provided by Mehlis' gland, and that the main part of the shell formed by the vitelline globules was laid down from within on this basal membrane. Tyzzer (1918), who also worked with Collyriclum faba, was more sure of his findings in Metorchis conjunctum where he determined that this external layer of the shell was actually formed before the extrusion of any vitelline globules. Dawes (1940b, 1946) also thought that this outer membrane might be provided by Mehlis' gland, but was not able to establish this with any certainty.

Apart from the possible exception of the layer attributable to Mehlis' gland there is no stratification of the trematode egg-shell as Dawes (1940b) has stated for Hexostoma extensicaudum. Schubmann (1905) remarked on the presence of trabeculae of shell-material, which later broke down, in Fasciola hepatica. Goldschmidt (1909), contrary to the view of Dawes (1940b), regarded the egg-shell of Polystoma integerrimum as hardening from within.

If, apart from the provision of the external, basal

membrane, Mehlis' gland does not contribute to the shell of the trematode egg, what then is the function of this gland, so typical of the whole class? It has generally been supposed that the secretion must have some influence on the shell-globules. Thus Tyzzer (1918) considered that in Collyriclum faba, their release from the parent cells was effected by this secretion because the globules were always extruded and became hyaline in the region of the gland. Ujiie was of the same opinion regarding Echrinochasmus japonicus (1936a), where he stated that the proximal, cyanophile cells of Mehlis' gland caused extrusion of the shell-globules, and also in Clonorchis sinenis (1936b). Dawes (1946), speaking generally, thought extrusion might be brought about by the secretion of Mehlis' gland. Goldschmidt (1909), on the other hand, stated that this secretion could not release the globules in Polystoma integerrimum because this occurred before the yolk-cells reached the ootype.

Henneguy (1906) considered that in Fasciola hepatica fusion of shell-globules after their release was brought about by the secretion of Mehlis' gland. Ujiie held a similar view concerning Echinochasmus japonics (1936a) and Clonorchis sinensis (1936b) adding that in the former species hardening of the shell was effected by the distal, erythrophile part of the gland. Dawes (1940b) however noted that the shells harden slowly.

The remaining investigators of this problem have mostly considered the secretion of Mehlis' gland to be suspensory and lubricatory. Augustine (1929) held this as a general view and Kourí and Nauss (1938) agreed, noting in their work on Fasciola hepatica the close similarity between the appearance of Mehlis' gland and the prostate gland. Dawes (1940b), while considering this a possibility, objected on the ground that if lubrication were the main function, Mehlis' gland would be more developed in Monogenea, where the eggs were more angular than in Digenea, a state of affairs which did not obtain.

A notable exception to the more usual views on this subject is the opinion of Stephenson (1947), who thought, as a result of work on Fasciola hepatica, that the secretion of Mehlis' gland might bring about the observed activation and aggregation of spermatozoa. He found the secretion to be alkaline and it is under conditions of alkalinity that activation and aggregation of spermatozoa take place.

Though Goldschmidt (1909) thought Mehlis' gland was not responsible for globule-extrusion he offered no other explanation of the occurrence. Tyzzer (1918) and Stephenson (1947) both thought that mechanical pressure might play a part in the process.

Concerning the chemical nature of the egg-shell MacCallum (1913) held the view that that of Microcotyle stenotomi was chitinous. Dawes (1946) noted that in some trematodes an

outer sculptured layer of the shell could be removed by treatment with sodium hydroxide, thus implying that the major part of the shell was insoluble in this reagent. These tentative findings have been overshadowed by Stephenson's (1947) analysis of the shell of Fasciola hepatica which, he found, was initially a polyphenol later becoming tanned to an ortho-dihydroxyphenol-protein. Smyth (1951) has endorsed this view.

That there is a mechanism for ensuring that each egg contains one ovum and a suitable number of vitelline cells is evident from the rarity of abnormal eggs. The few workers who have attempted an explanation are nearly all agreed that some kind of co-ordinated reflex operates so that appropriate muscular contractions are carried out in a suitable order. Schubmann (1905) and Goldschmidt (1909) held this view and Gallien (1935) described the sequence in Polystoma integerrimum. First contractions of the oviduct forced an ovum into the ootype, the contractions of the oviduct then being inhibited until the ootype was once more empty. Vitelline cells were brought to join the ovum by contraction of the vitelline duct. When the ootype was full its entrance to the vitelline duct was closed and further yolk-cells released from the vitelline duct were deflected along the genito-intestinal canal. When the newly formed egg passed from the ootype to the uterus the whole cycle was repeated. Ujiie (1936b) reported a similar mechanism in Clonorchis sinensis. Dawes (1946) referred to the presence of an oocapt in many

trematodes, which spaced the delivery of ova to the ootype, but said nothing of correlated movements of the vitello-duct and ootype.

In Monogenea the egg is usually formed and shaped in the ootype to the accompaniment of violent peristalsis, as reported by Goldschmidt (1909) and Gallien (1935) for Polystoma integerrimum and by Jahn and Kuhn (1932) for Benedenia melleni. However there are departures from this behaviour. According to Alvey (1936) the ootype of Sphyranura oligorchis is very narrow and the egg is actually formed in the proximal uterus. Intermediate is the case of Neodactylogyrus macracanthus in which Wilde (1936) found that the egg was partially formed in the ootype, but that more yolk-cells were added, and the egg sealed by the formation of the operculum, in the proximal uterus.

In some Digenia the method of egg-formation is much the same as in Monogenea. Dawes (1946) stated that this moulding of the egg in the ootype was the normal method, but did not speak of the difficulty that in many digeneans a number of eggs are formed in the ootype as one time. Henneguy (1906) thought that mutual friction might effect the shaping of eggs of Fasciola hepatica. Stephenson (1947) has put forward the suggestion as a result of work on the same animal that spermatozoa, clustered round each ovum and already aggregated by the alkaline medium, attracted and entangled yolk-cells which thus came to surround individual ova in a

suitable number and arrangement.

The formation of the operculum of the egg is not understood, but several workers have noticed that it was formed last (Ujiie, 1936b; Murikami, 1937; Wilde, 1936). Ujiie (1936b) said that this was brought about in Clonorchis sinensis by dilation of the anterior part of the ootype only occurring, and allowing shell-material to flow forward, after the rest of the shell was formed. Goldschmidt (1909) made the curious observation that no operculum is formed in the absence of an ovum. Schubman (1905) held the unsupported view that in Fasciola hepatica the line of weakness between the operculum and the remainder of the shell is not present in the new-laid egg.

The filaments of monogenetic eggs are generally held to result merely from an excess of shell-material streaming away from the egg in one or both directions during egg-formation (Dawes, 1946).

The present observations on D.denticulata show that in this species globules extruded from the yolk-cells provided the shell-material. This accords with the modern view for trematodes as a whole, though Yosufzai (1952) has recently re-affirmed the opinion that Mehlis' gland is responsible for the shell-material in Fasciola hepatica. The opinion of Tyzzer (1918) and Dawes (1940b) that an outer layer may be provided for the shell by Mehlis' gland may explain Yosufzai's findings, particularly as the latter has said that the yolk-

cells contributed to a temporary, inner shell. In D.denticulata the author has not observed any distinction into outer and inner shell-layers, the whole being homogeneous.

As to the function of the secretion of Mehlis' gland, it seems likely that in many species it does, indeed, bring about the extrusion from the yolk-cells of the shell-globules. In D.denticulata it has here been found that it is in the ootype that the majority of the shell-globules are extruded. That a few are released earlier does not invalidate this argument, for, as has been mentioned, the ciliation of the common duct must create a current which would tend to carry the secretion of Mehlis' gland toward the vitelline reservoir. The change in staining reaction of shell-globules in the lower part of the yolk-reservoir is probably attributable to the same cause. As this ciliation of the reproductive canals has not been observed before, and here only in life, it may well be that similarly ciliated ducts are present in other monogeneans and that study of living worms would reveal them. Should they be proved present in Polystoma integerrimum Goldschmidt's (1909) argument, that in this species extrusion of the globules could not be caused by the secretion of Mehlis' gland since extrusion occurred in the yolk-reservoir, would be invalidated. As an alternative cause for globule-extrusion Tyzzer (1918) and Stephenson (1947) have suggested mechanical pressure. However this seems very improbable in D.denticulata where it has frequently been noticed that pressure from a

coverslip has no effect on the yolk-cells in the ootype or anywhere else in the body.

In view of the histological distinction here noted, and also reported in many other species, between different parts of the glandular envelope of the ootype, it is probable that not only globule-releasing but also lubricatory and shell-hardening agents may be secreted in this region. In D.denticulata, however, a lubricatory function for Mehlis' gland secretion cannot be suggested on the grounds that this gland is histologically similar to the lubricatory prostate gland since there is no similarity between the structures of the two glands.

The means by which the appropriate constituents are assembled in the trematode egg and how its characteristic and remarkably constant form is achieved has given rise to much speculation. Patently the process is not the same throughout the class. Thus Stephenson's (1947) interesting view that yolk-cells might be aggregated round the ovum together with spermatozoa cannot obtain in D.denticulata or the many other species where sperm does not find its way to the ootype. Also the situation must be entirely different in those cases where many eggs are formed at once.

That some kind of nervous co-ordination is responsible for stimulating the sequence of contractions which assemble the components of the egg is generally accepted, and, though the ready interference with natural processes in D.denticulata

has prevented any definite establishment of the nature of the process, it seems highly probable that nervous co-ordination plays a part.

However it may be in those Digenea where many eggs are formed at once, it is the general rule where single eggs are concerned, that each is moulded to the definitive shape in the ootype. As reported for other species and observed here in D.denticulata this is by no means a static process, but rather takes place, albeit rapidly, to the accompaniment of vigorous peristalsis. It is not difficult to envisage this process turning out eggs of constant form with no filaments or simple, tapering filaments, but it is more difficult to understand how the ornamented and extremely characteristic filaments of some mongeneans are produced. In D.denticulata the formation of the crenulated disc at the end of the posterior filament has been watched. To be repeated with such surprising constancy a very exact and particular contraction of the base of the ootype must occur in every case. The formation of the crook of the anterior filament is a more difficult proposition which has not been observed. The only suggestion which can here be made is that by a specialised, bodily contraction at the moment when excess shell-material was flowing toward the uterus, the anterior part of the ootype might be pinched into an S-bend between the transverse yolk-duct and the oocapt and that this shape might be retained by the rapidly hardening filament.

The formation of the invisible line of weakness separating the main part of the shell from the operculum remains a mystery and no light has been thrown on it during the present study. It may be significant that where an imperfect egg is formed under disturbed conditions, it is the operculum which is missing. Perhaps this bears out reports in the literature that in many cases, it is formed last.

Finally, there has in the past been much discussion as regards the chemical nature of the shell. With the development of micro-chemical techniques, it seems only a matter of time until analyses of many shell-materials are carried out as has already been done for Fasciola hepatica by Stephenson (1947). The few tests which were here carried out on the egg-shells of D.denticulata gave ambiguous results and evidently more detailed study is necessary to settle the matter. The initial resistance to potassium hydroxide favoured the possibility of their being chitinous, but the subsequent tests failed to confirm this. Negative results on application of protein tests were puzzling. The slight positive reaction to Brown's (1911) test for iron indicated the presence of this element and that tanning takes place after formation is shown by the change in colour as the shell matures.

The rate at which egg-formation occurs is referred to in the discussion of the life history.

The Egg.

The egg of a monogenetic trematode contains one ovum and a number of yolk-cells. The form of the egg is usually fusiform though there are exceptions, for example, the tetrahedral egg of Entobdella hippoglossi. Many have no polar filaments or only a small terminal projection. In this category are Polystoma integerrimum recorded by Halkin (1901), Polystoma nearcticum and Polystomoides oris by Paul (1938), Sphyranura oligorchis by Alvey (1936), Neodactylogyrus macracanthus and Dactylogyrus crassus by Kulwiec (1927), Acolpenteron ureteroecetes by Fischthal and Allison (1941) and Diclidophora palmata by Cerfontaine (1898). Others have a long, single filament among which are the records of Siwak (1931) for Ancyrocephalus vistulensis, Sanders (1944) for Diplasiocotyle johnstoni and Zeller (1872) for Diplozoon paradoxum. Udonella caligorum has a single filament expanded into a large terminal disc (Price, 1938a). Among those eggs with filaments at both ends are Kuhnia scombri where both are straight (Gallien and Le Calvez, 1947), Microcotyle spinicirrus, the anterior one being hooked (MacCallum, 1913) and three species of Diclidophora where Cerfontaine (1896) for D. denticulata and Gallien (1934) for D. luscae and D. pollachii reported a hooked anterior filament and a longer posterior filament terminating in a crenulated disc.

In general the eggs are not found to be quite constant in shape nor in size. Those in which development has been

studied are shown, when the larvae emerge, to be operculate, though the line demarcating the two parts cannot be seen in the unruptured egg.

The form of the eggs observed during the present work agrees with Cerfontaine's (1896) description of them in his study of D.denticulata.

Oviposition.

The eggs, whether filamented or not, are usually laid singly. References to this in the literature include those of Jahn and Kuhn (1932) for Benedenia melleni, Wilde (1936) for Neodactylogyrus macracanthus, Siwak (1931) for Ancyrocephalus vistulensis, Gallien and Le Calvez (1947) for Kuhnia scombri, Alvey (1936) for Sphyranura oligorchis, MacCallum (1913) for Microcotyle stenotomi, Cerfontaine (1898) for Diclidophora merlangi and D.palmatum. In Microcotyle spinicirrus though the eggs are laid singly there is a tendency for the filaments to become hooked together so that they are laid in chains (Remley, 1942). The only cases known where the eggs are laid in a sheaf are those of Diclidophora denticulata and D.luscae reported respectively by Cerfontaine (1896) and Gallien (1934).

There are few references in the literature to the site of development of monogenean eggs, but Kulwieć (1927) said that those of Dactylogyrus anchoratus and D.crassus sank to the bottom at once as Alvey (1936) has said do those of Sphyranura oligorchis. Zeller (1872) found that the

eggs of Diplozoon paradoxum frequently remained entangled in the host's gills during development and Remley (1942) reported seeing eggs of Microcotyle spinicirrus actively entwined in the gills by the parent during laying. Cerfontaine (1896) was of the opinion that the crooks on the anterior filaments of the eggs of Diclidophora denticulata served to maintain the sheaf in position on the gills of the host during development.

It is very unusual for monogenean eggs to be laid simultaneously in a sheaf, the closely related Diclidophora luscae being the only one besides D.denticulata to be reported in the literature studied as having this habit.

Cerfontaine's (1896) view that the anterior crooks of the eggs of D.denticulata served to retain them on the gills during development is not borne out by present findings. Though eggs were sometimes found on the gills they always proved to be new-laid ones and so, it must be concluded, would have been expelled from the gill-chamber had the host not been killed just before they were laid. What then, it may be asked, is the function of the filaments? The posterior filaments keep the eggs together in a sheaf though it is doubtful whether this can be of any advantage. It is possible that the anterior filaments may entangle the eggs in seaweed and thus prevent them from being washed away from the haunts of their hosts. However this may be, it must also be borne in mind that the formation of the filaments may not have been evolved selectively,

but may be entirely fortuitous, depending on the peculiar conditions of egg-formation. If this is so they may serve no function at all. In other species fortuitously produced filaments may have been turned to the use of anchoring the eggs to the host. Thus Zeller (1872) found the eggs of Diplozoon paradoxum to develop on the host's gills and Remley (1942) saw those of Microcotyle spinicirrus being actively entwined in the gills by the parent. Apart from specific statements that eggs do actually develop while attached to the host, opinions to this effect must, in view of the present findings concerning D.denticulata, be regarded with caution as they may be based on supposition.

Life History

Maturation of the Gametes and Fertilisation.

The maturation of spermatozoa in trematodes usually follows three gonial divisions. Following on the formation of the primary spermatocyte two further divisions take place during which meiosis occurs, That the first of these divisions is the one in which reduction of the chromosome number takes place has been stated by Anderson (1935) who summarised the earlier work and also investigated Proterometra macrostoma. Rees (1939), Willey and Godman (1941) and Markell (1943) have reported the same conditions in Parorchis acanthus, Zygocotyle lunata and Probilotroma californiense respectively. The second maturation division is equational and is followed by transformation of the spermatids thus produced into mature spermatozoa.

Evidence derived from a study of oocyte maturation also indicates that reduction to the haploid number of chromosomes occurs in the first meiotic division. Goldschmidt (1902) was a notable dissentient to this view contraverting Halkin's (1901) findings in Polystoma integerrimum by saying that the second meiotic division involved a reduction from eight to four chromosomes. Halkin (1901) had given the haploid number as ten, seeing this number in the first metaphase, and, furthermore, said the chromosomes of the second meiotic division were too small to count. Goldschmidt (1905)

also claimed that reduction occurred in the second meiotic division in Zoogonus mirus, but this view was not upheld, according to Anderson (1935), by others. Concerning Dicrocoelium dendriticum Anderson (1935) reported that Goldschmidt (1908) took the conventional view. The only monogenean besides Polystoma integerrimum to have been examined in this respect is Gyrodactylus elegans where Gille (1914) found the first meiotic division to be reductional, producing a haploid number of six chromosomes.

The only mention of the shape of monogenean chromosomes is Halkin's (1901) who referred to those of Polystoma integerrimum as being 'biscuit-shaped.'

Penetration of the oocyte by the spermatozoon is generally held to stimulate maturation of the former and, accordingly, the site at which maturation begins is, to some extent, dependent on the site of sperm-penetration. However, in Proterometra macrostoma Anderson (1935) found that maturation proceded as far as the first metaphase before the entry of the sperm, which normally took place in the ootype, but occasionally in the ovary. He reported Cable (1931) as having said the same of Cryptocotyle lingua. That maturation does not necessarily follow immediately on sperm-penetration has been shown in several cases. Thus Goldschmidt (1902) said that maturation of the oocyte of Polystoma integerrimum did not take place until after laying, but Gallien (1935), though confirming this as a general rule, observed a few

cases where the first polar body was expelled in the ovary. He thought this might be the result of sperm occasionally entering the ovary. In Sphyranura oligorchis Alvey (1936) found the ovary to be the normal site of sperm-penetration. In the remaining cases discussed in the literature, all digenetic, penetration of the oocyte by the sperm takes place in the ootype or proximal uterus.

The polar bodies cast out as a result of the two maturation divisions are very ephemeral and in many cases have never been seen, but Tyzzer (1918) found that in Collyriclum faba they persisted for some time at the anterior pole of the egg and Gille (1914) found that in Gyrodactylus elegans the first one divided again before disintergrating.

When the maturation of the oocyte is complete a female pronucleus is usually formed so that the two pronuclei, one derived from each parent lie quiescent without fusing until cleavage begins. Among those cases studied in which this condition has been observed are Polystoma integerrimum by Halkin (1901) and Goldschmidt (1902), Fasciola hepatica by Schubman (1905) and Henneguy (1906), Zoogonus mirus by Goldschmidt (1905), Podocotyle atomon by Jones (1933) and Parorchis acanthus by Rees (1939), but in Gyrodactylus elegans Gille (1914) found that there was actual, irregular fusion of the karyomerites of the two pronuclei with the formation of a single zygote nucleus.

Maturation of the spermatozoon of D.denticulata has not

been seen, the small size of the cells preventing adequate observations, but in the oocyte maturation is accompanied by reduction of chromosome number from approximately eighteen to nine. Accordingly nine is thought to be the haploid number of chromosomes in this species.

Halkin (1901) gave the diploid number of chromosomes in Polystoma integerrimum as twenty, but said that ten chromosomes were present in the first meiotic metaphase. Presumably he referred to ten bivalents rather than ten single chromosomes. His findings are quite contrary to those of Goldschmidt (1902) whose evidence is unreliable in this instance.

Halkin (1901) reported that the chromosomes were too small to examine in the second meiotic metaphase of Polystoma integerrimum, but in D.denticulata the chromosomes seen in the two meiotic metaphases (figs. 18e, f) are about the same size. The second metaphase can be distinguished by the tendency of the chromatids to separate.

That polar bodies have not been seen during the present work is not surprising in view of the reports in the literature of their ephemeral nature in other trematodes.

Whether entry of the spermatozoon stimulates maturation of the oocyte in D.denticulata cannot be said, but maturation of an oocyte has never been observed prior to egg-formation, a condition reported in Proterometra macrostoma by Anderson

(1935). Similarly it is impossible to say whether in D.denticulata there is fusion of the two pronuclei after maturation of the gametes. The technical difficulties of cutting serial sections of the eggs, coupled with the extremely lobed form of the nucleus at this stage, rendered it impossible to determine whether one or two nuclei were present.

Embryology.

The embryology of very few monogeneans has been studied owing to the technical difficulties arising from the impermeable nature of the egg-shell, but in Polystoma integerrimum Halkin (1901) found that the first cleavage division was total and unequal. In Gyrodactylus elegans, on the other hand, Katheriner (1904) reported that the first division was equal and that the two cells so formed then divided unequally to produce two micromeres and two macromeres. Gille (1914) confirmed Katheriner's observations. The second division of Polystoma integerrimum Halkin (1901) found to give rise to an embryo of four cells placed spirally end to end. Of these the first did not divide again, but the others eventually gave rise to an irregular morula in which no germ-layers were discernible. Katheriner (1904) described a similar process in Gyrodactylus elegans, but a cell destined to give rise to a second embryo was incorporated in the middle of the morula at an early stage.

By a process of epiboly Halkin (1901) found that the embryo of Polystoma integerrimum became surrounded by smaller cells, the outermost of which then differentiated as an epithelium, later to become partially ciliated. He described a decreasing lobulation of the cell-nuclei and the distinction of three cell-types: epithelial cells, small cells with a chromatic network and small nucleoli, and, localised principally at the posterior of the embryo, large cells with large, clear nuclei containing a single nucleolus. Alvey (1936) noted two cell-types in the embryo of Sphyranura oligorchis: those with small, coarsely granular nuclei and others with large vesicular nuclei.

In Sphyranura oligorchis Alvey (1936) described the gut as being initiated by an anterior invagination and then progressive demarcation posteriorly. However in Polystoma integerrimum Halkin (1901) has described the excavation of a cavity in the middle of the embryo, starting from the future dorsal side and gradually enlarging, partially by cell-disintergration, to form the larval gut; the connection with the exterior is eventually lost. He described the contents of this sac as being granular and containing fatty elements.

Halkin (1901) described the further organogeny of Polystoma integerrimum as consisting in the differentiation of the organs from the cell-mass without cell-migration. He described the pharyngeal bulb as being laid down as a

solid body contiguous with the anterior end of the gut-sac and containing both large and small elements. A cerebral band was then differentiated dorsal and anterior to the pharynx. Large clear cells surrounded the gut-sac and also temporary, anterior cavities which appeared at this stage and were, he thought either extensions of the gut or of coelomic derivation. When the haptorial disc began to differentiate and some of the epithelial cells became ciliated the anterior cavities were continuous with the gut but thereafter were obliterated. Primordia of all the larval hooks appeared simultaneously, each developing in a follicle between two cells. The primordium of the prepharynx was provided by a solid column of cells which connected the pharynx to the anterior of the embryo while this latter organ sank further into the body. The gut became temporarily cyclic and then returned to the simple condition, but, though a concentration of large cells was noted at its posterior extremity, no gut-epithelium was detected. Finally pharynx and prepharynx became perforate, eye-rudiments appeared and embyronic development was completed by the larval hooks penetrating to the exterior.

Hyman (1951) has summarised the embryonic development of related platyhelminths: digenetic trematodes, cestodes, and ectolecithal turbellarians. From her account it is clear that in these forms, also, cleavage is total and leads to the formation of a morula without germ-layers, but in digenetic trematodes a propagatory cell is set aside

within the larva for provision of the next larval stage. An epithelium is differentiated and in turbellarians, digenetic trematodes and pseudophyllidean cestodes this becomes ciliated. The processes are however complicated by various devices for incorporating at least some of the yolk originally surrounding the embryo within its body. In rhabdocoels and alloeocoels the blastomeres gradually grow round the yolk which is eventually enclosed within the embryo. In triclads only a small part of the yolk is enclosed in this way, but more is ingested by the action of a temporary pharynx which is differentiated at anearly age. In pseudophyllidean cestodes certain cells are set aside to surround the yolk and these are not eventually included in the embryo. Finally in cyclophyllidean cestodes and digenetic trematodes there is no modification in this respect, yolk being absorbed from the outside.

From Graph 2 it is apparent that in D.denticulata cleavage does not always begin at the same interval after laying. Therefore the onset of cleavage must be determined by the time of egg-formation or sperm-entry rather than by any stimulus, such as contact with sea-water, which affects the whole sheaf at once on laying.

The embryological development of D.denticulata has here been shown to be broadly similar to that of Polystoma integerrimum as described by Halkin (1901). Though nuclei of rather differing appearance have been observed in embryos of D.denticulata those of one type are not

localised as they are in Polystoma integerrimum and it is thought that the differences seen in the present case depend on approaching mitosis rather than any distinction into cell-types. Perhaps the same may apply to the indiscriminately scattered two types of cell noticed by Alvey (1936) in the larva of Sphyranura oligorchis.

Concerning the formation of the larval gut-sac, Halkin (1901) described the excavation as taking place from the dorsal side in Polystoma integerrimum whereas in D.denticulata it almost certainly occurs on the ventral side. Halkin did not regard the contents of the gut in the embryonic P.integerrimum as including yolk, but it seems probable that yolk is incorporated as it is in D.denticulata. Halkin's findings with regard to a differential lining of the embryonic gut and the presence of temporary anterior cavities in P.integerrimum are not found to have a parallel in D.denticulata, nor was a nerve band seen in the embryo of the latter species.

The setting aside, at an early stage, of a propagatory cell in Gyrodactylus elegans is an adaptation to polyembryony and does not occur in P.integerrimum or D.denticulata.

The problems of development facing monogeneans, with their ectolecithal eggs, are common to other groups in which the yolk is external to the ovum, and a comparison of the methods by which Monogenea, Digenea, Cestoda and ectolecithal Turbellaria develop is therefore relevant.

In all the primitive spiral cleavage has been obliterated, a morula without germ-layers is formed, and a ciliated epithelium differentiated *in situ*. Incorporation of at least some of the yolk in the body of the embryo takes place in Monogenea, or in *D.denticulata* at least, by epiboly over a yolk mass together with excavation of the body. In rhabdocoels and alloeocoels much the same process occurs. In pseudophyllidean cestodes yolk is encircled by cells destined to play no further part in the formation of the embryo. Triclads show a remarkable development of a precocious and temporary pharynx through which yolk is drawn into the embryo. Finally in cyclophyllidean cestodes and digenetic trematodes no yolk is incorporated, but is merely made use of through the surface of the embryo. Thus it is seen that the behaviour in monogeneans so far as it is known resembles that of certain turbellarians more closely than that of digenetic trematodes.

Fate of Yolk.

It has generally been observed that the yolk-cells, which in new-laid eggs are numerous and clearly defined, undergo degeneration and absorption by the embryo as development proceeds. Evidence to this effect has been assembled by Goldschmidt (1902) for *Polystoma integerrimum*, Siwak (1931) for *Ancyrocephalus vistulensis*, Wilde (1936) for *Neodactylogyrus macracanthus* and Paul (1938) for

Polystoma nearcticum. In addition Gallien (1935) stated that some yolk was swallowed by the larva of Polystoma integerrimum before hatching. Paul (1938) observed yolk-cells in the gut of larval Polystoma nearcticum as did Gallien and Le Calvez (1947) in new-hatched Kuhnia scombri.

During embryonic development in D. denticulata, as in other Monogenea, yolk is gradually broken down and absorbed. How this is brought about cannot be said. That some digestive ferment is produced by the embryo seems evident from the fact that this disintergration was observed to be delayed in eggs which contained no embryos.

As already mentioned some of the yolk is incorporated in the body during development and Gallien (1935) reported the swallowing of yolk by the larva of Polystoma integerrimum before hatching. However Halkin (1901) said nothing of this in his work on the same animal and, in view of the present findings for D. denticulata, it may be that Gallien was in error as to how the yolk entered the larval gut.

Hatching.

Nothing has been found in the literature concerning the stimulus to hatching in monogeneans though Zeller (1872) noted that Diplozoon paradoxum larvae tended to hatch in the early morning and Remley (1942) noted redoubled efforts to escape in larvae of Microcotyle spinicirrus when a powerful beam of light was turned on them.

Vigorous movement of larvae within the eggs is common prior to hatching. Eventually the operculum becomes detached and the larva creeps out. Little reference is made in the literature to the means by which hatching is effected. Kulwieć (1927) and Remley (1942) thought that in Dactylogyrus crassus and Microcotyle spinicirrus respectively the larva actively pushed the operculum off. Paul (1938) expressed the view that cephalic glands of the larva might play a part in unsealing the operculum.

With the exceptions of Zeller (1872) and Remley (1942) no special conditions have been noted by workers on Monogenea as bringing about hatching. It seems rather that the larva simply hatches when fully developed. As to how the larva makes its escape there is little evidence of anything besides repeated pressure against the operculum. The possibility that cephalic glands may play a part in releasing the operculum has yet to be proved. From observations on D.denticulata it is clear that considerable force is used by the larva which, owing to its length being greater than that of the egg, can press strongly on the operculum merely by attempting to straighten the body. This, repeated over and over again, may be sufficient to release the operculum though secretions may also play their part.

Free-Swimming Larva.

Typically the newly-hatched monogenean larva is 'gyrodactyloid,' a term proposed by Gallien (1934) implying an elliptical and dorso-ventrally flattened larva with bands of cilia, a suctorial mouth, bulbous pharynx, simple, sac-like gut, and a posterior, haptorial disc bearing various arrangements of hooks. The larva of Sphyranura oligorchis was found by Alvey (1933, 1936) to be modified in that it was not ciliated and Zeller (1872) described that of Diplozoon paradoxum as already having, on hatching, one pair of definitive clamps with consequent modification of the haptor.

The haptorial region of the characteristic larvae, is, however, not uniform throughout the group. The three species of Dactylogyrus which Kulwieć (1927, 1929) studied, D.crassus, D.anchoratus and D.vastator were found to have a more or less winged haptor bearing laterally six or seven pairs of hooks and centrally, another pair. Wilde (1936) reported the haptor of Neodactylogyrus macracanthus as consisting of a disc bearing fourteen marginal and two central hooks. In Ancyrocephalus vistulensis Siwak (1931) described a posterior region scarcely differentiated as a disc, with marginal, larval hooks and in Acolpenteron ureteroecetes Fischthal and Allison (1941) found a cup-like haptor with fourteen hooklets. The capsalid, Benedenia melleni was examined by Jahn and Kuhn (1932) who described the larval haptor as being circular with fourteen marginal

hooks and two definitive, postero-lateral spines.

Passing to the Polyopisthocotylea, Halkin (1901) has reported for Polystoma integerrimum and Paul (1938) for Polystoma nearcticum that there are sixteen hooks round the margin of a circular disc, the former with, and the latter without, the addition of two posterior, definitive hooks. Paul (1938) found that Polystomoides oris agreed in all larval characters with Polystoma integerrimum. Alvey (1936) described a larval haptor like that of Polystoma integerrimum in Sphyranura oligorchis, but noted that in the latter case all the hooks were definitive. Rodgers (1941) found a similar condition in Diplorchis scaphiopodis.

Among diclidopheroid Polyopisthocotylea Microcotyle spinicirrus was found by Remley (1942) to have a winged haptor bearing six pairs of hooks laterally and posteriorly and also two pairs of large, anchor hooks in the mid-posterior region. Kuhnia scombri has been reported by Gallien and Le Calvez (1947) to have a winged haptor with six, bilaterally arranged pairs of hooks which were all alike. Sanders (1944) found a winged haptor with three pairs of small, lateral hooks and two, larger, median pairs of hooks in Diplasiocotyle johnstoni. Two species of Diclidophora have been examined by Gallien (1934) who found both D.luscae and D.pollachii to have a winged haptor with four pairs of lateral hooks and two pairs of median hooks.

In addition to the basic characters of the gyrodactyloid larva an excretory system has been described in a few cases. When present, it consists of paired, lateral collecting canals, each of which runs posteriorly and then returns anteriorly on its own course. Each terminates in a vesicle on a level with the pharynx, opening to the exterior just dorsal to the lateral margin of the body. These canals are fed by flame cells.

Kulwiec mentioned but did not describe the excretory systems of the larvae of Dactylogyrus crassus and D.anchoratus. However no mention of such a system was made in her work on D.vastator and Wilde (1936) was equally silent concerning the excretory system of Neodactylogyrus macracanthus. In discussing Acolpenteron ureteroecetes Fischthal and Allison (1941) did not mention the excretory system, but Siwak (1931) stated that it was not differentiated in the larva of Ancyrocephalus vistulensis. Halkin (1901) and Gallien (1935) said that the larva of Polystoma integerrimum had a typical excretory system but made no reference to flame cells. Paul (1938) for Polystoma nearcticum and Polystomoides oris and Alvey (1936) for Sphyranura oligorchis also described the excretory systems in the larvae of these species without mentioning flame-cells. Sanders (1944) made no reference to the excretory system of larval Diplasiocotyle johnstoni and Gallien (1934) and Gallien and Le Calvez (1947) found none in Diclidophora luscae

and Kuhnia scombri respectively. In Microcotyle spinicirrus and Benedenia melleni flame-cells have been described: three pairs in M.spinicirrus by Remley (1942) and ten pairs in B.melleni by Jahn and Kuhn (1932).

The presence of dorsally situated eyes, associated in some cases with the rudiments of a nervous system, have been reported in a few monogenean larvae. Thus two pairs of eye-spots have been reported in the larvae of all the species of Dactylogyrus studied (D.crassus and D.anchoratus, Kulwieć, 1927; D.vastator, Kulwieć, 1929), in Neodactylogyrus macracanthus by Wilde (1936), Ancyrocephalus vistulensis by Siwak (1931) and in Polystoma integerrimum by Halkin (1901) and Gallien (1935). One pair of eye-spots was reported by Sanders (1944) in the larva of Diplasiocotyle johnstoni and by Zeller (1872) in the larva of Diplozoon paradoxum. A single eye-spot was seen in Microcotyle spinicirrus by Remley (1942). No eye-spots were found in the remaining larvae which have been studied. A supra-oesophageal nerve band has been observed in the larva of Polystoma integerrimum by Halkin (1901) and Gallien (1935), and the larvae of Polystoma nearcticum and Polystomoides oris were found to be similarly equiped by Paul (1938).

The only other known young forms of Monogenea are those of Udonella caligorum and Gyrodactylus elegans, neither of which is characteristic of the group. Price (1938) found the former, on hatching to resemble the parent in all respects except that of size and different proportions

of the ovary and testis. Similarly Katheriner (1904) found the viviparous Gyrodactylus elegans to resemble the adult in form and size at birth. Moreover the newly born animal already contained an embryo within which a second embryo was being differentiated.

A feature other than the morphological characteristics of the larva of Diplozoon paradoxum noted by Zeller (1872) was the presence of refringent spheres distributed through the tissues. He regarded this as a common feature in trematodes.

Most monogenean larvae, which have been observed, swim erratically with the aid of their cilia, making sudden starts and stops and changing direction sharply. Many rotate spirally as they swim and they may also move by alternate elongation and contraction of the body. This type of behaviour was seen in larvae of Neodactylogyrus macracanthus by Wilde (1936), Dactylogyrus crassus and D.anchoratus by Kulwiec (1927), Acolpenteron ureteroecetes by Fischthal and Allison (1941), Benedenia melleni by Jahn and Kuhn (1932), Polystoma integerrimum by Gallien (1935), Microcotyle spinicirrus by Remley (1942), Diplasiocotyle johnstoni by Sanders (1944),&Diclidophora luscae and D.pollachii by Gallien (1934). Alvey (1933, 1936) noted that the larva of Sphyranura oligorchis swam by flapping the caudal haptor and by looping from mouth to haptor in a leech-like manner, there being no cilia. Dawes (1946), however, said there was no free-swimming stage in this species.

There does not appear to have been any work carried out on these larvae to determine their response to stimuli or the means by which they locate their host. Kulwiec (1927), however, stated that the sudden jerks performed by the larvae of Dactylogyrus crassus and D.anchoratus as they swam along could be provoked by tapping the dish. The behaviour of digenean larvae has been accorded considerable study and it is worthy of mention here that McCoy (1935), speaking generally of this group, said that while miracidia frequently respond to chemical attractions, cercariae react more usually to phototactic stimuli and may, for example, swim upwards when shadowed.

The free-swimming larva of D.denticulata (fig.15) has here been shown to be closely similar to that of D.luscae and D.pollachii as might be expected from their close relationship. The differences between the larva described here and those of the other two species concern characters such as the presence of an excretory system in D.denticulata, whereas this has not been described in the others. It is thought that further study of D.luscae and D.pollachii might well reveal the presence of this system and perhaps other common features.

As has been noted in the summary of the literature two types of haptor occur in monogenean free-swimming larvae. A study of Table 11, (p.160) in which those larvae that are known are arranged taxonomically together with the haptorial

Suborder & Superfamily	Species	Location	Haptorial Type
Monopisthocotylea Gyrodactyloidea	Neodactylogyrus macracanthus	gills	I
	Dactylogyrus anchoratus	gills	Intermediate
	Dactylogryus crassus	gills	Intermediate
	Dactylogyrus vastator	gills	Intermediate
	Acolpenteron ureteroectes	ureters	I
	Ancyrocephalus vistulensis	gills and skin	I
Capsaloidea	Benedenia melleni	skin and eyes	I
Polyopisthocotylea Polystomatoidea	Polystoma integerrimum	gill-chamber→bladder	I
	Polystoma nearcticum	gill-chamber→bladder	I
	Polystomoides oris	mouth	I
	Sphyranura oligorchis	skin	I
	Diplorchis scaphiopodis	(?→) bladder	I
Diclidophoroidea	Kuhnia scombri	gills	II
	Microcotyle spinicirrus	gills	Intermediate-II
	Diplasiocotyle johnstoni	gills	II
	Diclidophora luscae	gills	II
	Diclidophora pollachii	gills	II
	Diclidophora denticulata	gills	II

Table II.

type and parasitic location of each, indicates that this division into two groups by haptorial type accords more with the taxonomic position of the species than with the site of parasitism. It would seem therefore that the haptorial type must be determined phylogenetically rather than as a larval adaptation. It is however interesting to note that the division between the two types of haptor falls, not between the major groups Monopisthocotylea and Polyopisthocotylea, but that the whole of the Monopisthocotylea together with the Polystomatoidea of the Polyopisthocotylea constitute one group and the Diclidophoroidea of the Polyopisthocotylea comprise the other group. On this ground alone, then, there appears to be more affinity between the Monopisthocotylea and one superfamily of the Polyopisthocotylea than there is between the two superfamilies within the Polyopisthocotylea.

Absence of mention of excretory and nervous systems in descriptions of free-swimming larvae of monogeneans may, in some cases, indicate that they have not been detected rather than that they are not differentiated. In the present work on D.denticulata the excretory system was only eventually observed after much study of larvae compressed under cover-glasses. However, the case of eye-spots is rather different. These would readily be seen if present and have been described in some species but not in others. Those with eyes are scattered through the group without reference to phylogeny or any other criterion

of grouping which can be detected. Further study may reveal that the presence of eyes is related to behaviour adapted to their use. Unfortunately the behaviour of these larvae has not yet been examined, except for the few experiments here carried out on the larvae of D.denticulata.

Deviations of the larval form from the gyrodactyloid type can be interpreted as telescoping of the larval stages. Thus, on hatching, Sphyranura oligorchis has no ciliated coat, Diplozoon paradoxum has already developed one pair of definitive clamps and Udonella caligorum emerges as a small, but perfect, adult. Indeed, this may in some cases explain the apparent absence of nervous and excretory systems in the larvae of some species. Perhaps they are more precociously developed in some than in others.

Refringent spheres scattered through the body as well as lying in the cavity of the gut in the larva of D.denticulata are puzzling. Zeller (1872) noted similar spheres in the larva of Diplozoon paradoxum. Probably they represent fatty elements from the yolk which have wandered between the cells of the larval tissue prior to absorption.

The ciliated larvae which have been described all swim in much the same way, spiral rotation being a characteristic feature. As has already been mentioned, the present work is the only attempt which has been made to determine the means of attraction to the host and the results have been

singularly negative. The larvae of D.denticulata do not respond in any way either to jarring or to light or shade stimuli. Vigorous larvae ignore the freshly excised host's gill and whether the tiring larvae, which were observed to settle on a gill did so from choice is open to doubt. It is unfortunate that technical difficulties prevented controlled infestation of living fish under observation. Larvae swimming in the vicinity of a host-fish would naturally be drawn into the mouth in the respiratory current. No doubt this is how they reach the gills in the first place but they would as soon be swept out again unless they responded actively and immediately on coming into contact with the gills.

Larval Development.

Larval development in monogenetic trematodes, so far as it is known, is characterised by growth, elaboration of the adult haptor in place of the larval one and the differentiation of any other specifically adult characters including, of course, the reproductive organs.

The transformation of the larval to the adult haptor naturally involves least modification in the Gyrodactyloidea merely amounting, in Dactylogyrus anchoratus according to Kulwiec (1927) and in Neodactylogyrus macracanthus according to Wilde (1936), to growth of the whole and formation of a pair of central, great hooks and cross-bars supporting them. Kulwiec (1927) found

that in D.anchoratus these hooks were at first disproportionately large and that the rest of the animal grew at a relatively greater rate than the hooks until the adult proportions were achieved. Only the shafts of the peripheral hooks were found to increase in size (Kulwieć, 1927) and Jahn and Kuhn (1932) noted that the peripheral hooks of Benedenia mellenl did not grow at all.

In the Polyopisthocotylea a more radical metamorphosis of the haptor takes place during the acquisition of the adult characters. In Polystoma integerrimum Gallien (1935) described how each of the six adult suckers was formed round a lateral, larval hook, which might then persist in the centre of the sucker for two years. He found the suckers to be formed in pairs, commencing with the posterior pair and noted that the anterior pair were initiated before the second pair had reached the size of the posterior pair. The large, median hooks were differentiated at an early stage of sucker-development. Paul (1938) found the haptorial development of Polystoma nearcticum and Polystomoides oris parallel to that of Polystoma integerrimum.

Among Diclidophoroidea members of the Microcotylidae represent a special case as the number of pairs of clamps increases for a considerable period after maturity is reached. Remley (1942) studied the larval development of Microcotyle spinicirrus and found that the clamps were formed in pairs anterior to the larval haptor, three pairs being formed beneath the surface of the body, before

any emerged. The larval haptor persisted until the genital organs were elaborated, but disappeared when between twenty and thirty clamps had been formed. Sanders (1944) was not able to follow out the whole of the development of Diplasiocotyle johnstoni, but recoverd stages from the gills of the host in which three fairly large pairs of clamps had been differentiated and other smaller pairs were to be seen both in front and behind these.

Zeller (1872) has reported on the development of Diplozoon paradoxum, which, as already noted, hatches with one pair of clamps already differentiated. He found that larval hooks lying between the first pair of clamps persisted without growth until the adult stage. Anterior to the precociously formed pair of clamps three more pairs were formed consecutively.

Of Diclidophora luscae all that has been said is that Gallien (1934) surmised that the eight adult clamps would be organised round eight of the twelve larval hooks by analogy with the case of Polystoma integerrimum.

Sproston (1946) gave a diagnosis of the superfamily Diclidophoroidea in which she included the statement that the adult haptor in this group was developed anterior to the larval haptor.

In Dactylogyrus anchoratus and Neodactylogyrus macracanthus Kulwieć (1927) and Wilde (1936) respectively have recorded that the adult head-lappets are formed after hatching. The only other external adult character, apart

from the definitive haptor, reported as forming after hatching is the 'knob and sucker' of Diplozoon paradoxum which Zeller (1872) said were formed in the diporpa stage.

Concerning the elaboration of the alimentary canal Kulwieć (1927) described the gut of Dactylogyrus anchoratus as being bifurcate and rejoining posteriorly by the time of formation of the first pair of head-lappets. Gallien (1935) figured a larva of Polystoma integerrimum with a sac-like gut though three pairs of suckers were present, the anterior ones being very small. He stated that the adult condition of the gut was achieved late and noted that the 'brown' cells in the gut lining only appeared after feeding had begun. Other workers have merely stated that increased branching of the gut occurs as development proceeds.

There is little mention in the literature of the development of the excretory system which, if not already present in the newly hatched larva, is differentiated during development.

The reproductive organs are differentiated from concentrations of small cells. Sproston (1945a) has said that it is usual in trematodes for the testes to develop before the ovary and her statement has been borne out by Alvey (1936) for Sphyranura oligorchis, Remley (1942) for Microcotyle spinicirrus and Zeller (1872) for Diplozoon paradoxum. Price (1938) noted that in the already mature, newly hatched Udonella caligorum the testes were larger

than the ovary, this condition later being reversed. Wilde (1936) found that the vitellaria of Neodactylogyrus macracanthus developed after the germinal part of the reproductive system and Kulwieć (1927) has described the even more extreme case of Dactylogyrus minutus where sexual maturity was attained before the vitellaria were fully developed.

It is noteworthy that development of the genital organs only begins, in the few known cases, after completion of the adult haptor, as Gallien (1935) found in Polystoma integerrimum, Paul (1938) in Polystoma nearcticum and Polystomoides oris and Zeller (1872) in Diplozoon paradoxum. In Microcotyle spinicirrus with its continually increasing number of clamps, the genital organs were noted by Rawley (1942) to start developing when three pairs of clamps had been formed.

In Polystoma integerrimum and P.nearcticum, as an alternative to the normal development, neotenic forms may be produced if the larva becomes attached to a tadpole which is in the external gill stage. In this case Gallien (1935) and Paul (1938) have shown for the two species respectively that there was no migration, the host's bladder still being unformed, but that the larva rapidly developed an adult haptor, though the gut remained simple, the genital organs were differentiated and became functionally mature. No uterus or vas deferens was formed, the testis communicating directly with the ootype and the eggs passing to the exterior from there immediately after formation.

Paul (1938) also studied Polystomoides oris with a view to establishing whether such an alternative type of development might occur in this species. His findings were negative and he noted that this was the expected result in a worm whose host, a turtle, did not undergo metamorphosis.

Allometric growth has been reported by Gallien (1935) in Polystoma integerrimum, by Kulwieć (1927) in Dactylogyrus anchoratus and by Sproston (1945) in Kuhnia scombri. Dawes (1946) and Sproston (1945) both thought this might be a common feature of trematode growth and Dawes has warned of the taxonomic errors which may arise as a result of a failure to appreciate its significance.

Willey (1941) emphasised that in Zygocotyle lunata growth continued after maturity was reached and, moreover, that maturity was not reached at a specific size, the largest immature worms being larger than the smallest reproductively mature worms.

Larval development in D.denticulata has been found to comprise a gradual acquisition of the adult characters without sudden metamorphosis at any stage. This is in agreement with the findings for the larval development of such other monogeneans as are known. However, as can be seen from Graphs 3, 4 and 5, in D.denticulata the growth of the organs of which it was possible to obtain reliable measurements proved isometric, whereas Gallien (1935) and Sproston (1945a) have reported allometric growth in Polystoma integerrimum and Kuhnia scombri

respectively.

Evidence obtained here and, indeed, from the other cases where development of members of the superfamily Diclidophoroidea has been studied, show that, with the exception of Microcotyle spinicirrus, each pair of clamps of the adult haptor is developed at the level of each corresponding pair of lateral, larval hooks. In Microcotyle spinicirrus the adult haptor forms anteriorly to the larval haptor. It must have been on evidence from this species alone, though Zeller (1872) had much earlier described the development of Diplozoon paradoxum, that Sproston (1946) based her diagnosis that in the superfamily Diclidophoroidea the adult haptor is formed anteriorly to the larval haptor. Plainly, this is not the case. In view of the present findingsin D.denticulata it seems probable that the clamps of D.luscae will not, as Gallien (1934) suggested, be found to be formed round the larval hooks, but dorsally and externally to them.

D.denticulata conforms to the pattern of other monogeneans apart from Microcotyle spinicirrus in that the reproductive organs are not differentiated until the adult haptor is complete. It is curious that cells destined to become vitellaria should be distinct at so early a stage when the completion of their development comes so late in reproductive differentiation. Many other monogeneans show a tendency to protandry in that the testes are fully formed before the ovary, but this is not

so in D.denticulata where ovary and testes mature simultaneously. Table 4 shows that this fluke does not mature at a definite size as there is an overlap of size between immature and adult worms.

The peculiar feature of alternative neotenic development has so far only been discovered in the genus Polystoma and, as Paul (1938) has pointed out, it is most unlikely that such an adaptation will be found in monogeneans whose hosts undergo no metamorphosis.

Rate of Development and Longevity.

The literature reveals wide variations in the period of embryonic development in Monogenea and the rate at which development proceeds has been shown, in some cases at least, to depend on temperature. Table 9 shows the embryonic periods which are known, together with authorities.

Species	Authority	Embryonic Period
Dactylogyrus crassus.	Kulwieć, 1927.	3 days.
Dactylogyrus vastator.	Kulwieć, 1927.	3-5 days (var. with temp.).
Neodactylogyrus macracanthus.	Wilde, 1936.	5 days in warm con 10-13 days in cold cond.
Ancyrocephalus vistulensis.	Siwak, 1931.	3 days.
Acolpenteron ureteroecetes.	Fischthal and Allison, 1941.	6-9 days.
Diplorchis scaphiodis.	Rodgers, 1941.	Hatches soon after laying.
Microcotyle spinicirrus.	Remley, 1942.	5-11 days.

Species	Authority	Embryonic Period
Kuhnia scombri.	Gallien and Le Calvez, 1947.	c. 12 days.
Polystoma integerrimum.	Halkin, 1901.	17 days (or more).
" "	Gallien, 1935.	10-12 days at 20°C.
Polystoma nearcticum.	Paul, 1938.	12-13 days.
Diplozoon paradoxum.	Zeller, 1872.	15 days.
Diplasiocotyle johnstoni.	Sanders, 1944.	19 days.
Polystomoides oris.	Paul, 1938.	c. 28 days (variable).
Sphyranura oligorchis.	Alvey, 1933, 1936.	32 days.

Table 9.

The duration of the free-swimming phase has been recorded for some monogenean larvae as shown, with authorities, in Table 10.

Species	Authority	Free-Swimming Period
Dactylogyrus crassus.	Kulwiec, 1927.	Few hours.
" anchoratus.	" "	" "
" 'sp.'	Hess, 1928.	4-6 days.
Neodactylogyrus macracanthus.	Wilde, 1936.	4-5 hrs.
Benedenia melleni.	Jahn and Kuhn, 1932.	c. 6 hrs.
Polystoma integerrimum.	Gallien, 1935.	48 hrs.
" nearcticum.	Paul, 1938.	48 hrs.
Polystomoides oris.	Paul, 1938.	48 hrs.
Sphyranura oligorchis.	Alvey, 1933. 1936.	Few hours.
Microcotyle spinicirrus.	Remley, 1942.	6-10 hrs.

Species	Authority	Free-Swimming Period
Diplasiocotyle johnstoni.	Sanders, 1944.	48 hrs.
Diclidophora luscae.	Gallien, 1934.	12-24 hrs.
Diplozoon paradoxum.	Zeller, 1872.	6 hrs.

Table 10.

Less evidence is available concerning the rate of development after the larva has secured a host than in the earlier stages of monogenean development dealt with above, but in the few cases known a wide range is found. Wilde (1936) reported that in Neodactylogyrus macracanthus, when the temperature was maintained at 20°C, maturity was reached a fortnight after hatching. However, at a temperature of 13°-14°C the same species was not mature until 4⅓ weeks after hatching, and Gallien (1935) noted a similar variation in the rate of development with temperature in Polystoma integerrimum. Alvey (1933, 1936), while recording variation with temperature, said that Sphyranura oligorchis usually matured after two months. A period of at least a year was found by Paul (1938) to elapse before Polystomoides oris matured.

The only other cases which have been studied are those of Polystoma integerrimum and P.nearcticum, in both of which neoteny may occur. In the normal, slow development with passage to the bladder of the host Gallien (1935) found that more than two years elapsed before sexual maturity was attained by P.integerrimum, but neotenic

forms were mature at one month of age. Paul (1938) found the neotenic forms of P.nearcticum to mature in twenty-two days.

Gallien (1935) found that overcrowding in the host slowed down both neotenic and normal development in Polystoma integerrimum and it is worthy of note that Willey (1941) observed the same effect in heavy infestations of Zygocotyle lunata. Dawes (1946) who mentioned a number of similar cases in digeneans considered the cause to be inadequacy of food supply.

Little appears to be known concerning longevity in monogeneans. Wilde (1936) mentioned that some unspecified members of the genus Dactylogyrus died off in the winter leaving winter eggs to provide the next generation. The only other mention of the subject which has been discovered is Gallien's (1935) statement that individuals of Polystoma integerrimum could live for six or perhaps seven years.

From a study of Graph 2 the time which elapses in D.denticulata between egg-formation and laying can be deduced. It is clear that since the eggs are laid in a sheaf some will have been retained in the uterus longer than others. As has already been noted the onset of development does not depend on laying; therefore the first-formed eggs in a sheaf will undergo the first cleavage sooner than the last-formed ones. Here it is seen that the first cleavage division may occur as soon as ½ hour after laying and that unsegmented eggs persist

until 5 hours after laying. The range over which both 1-celled and 2-celled embryos are found, that is, almost five hours, represents the longest time which may elapse between egg-formation and laying of the first-formed eggs of a sheaf. No estimation of this period has been made concerning other monogeneans.

The rate at which embryonic development takes place has been found in a number of monogeneans to depend on temperature. That this is also so in D.dentioulata is seen in Table 6. It appears that some other factor may also operate here because of the variability over a range of two days which occurs at a constant temperature. The system is seen to break down altogether at more extreme temperatures. Indeed, it would not be expected that a marine animal, living as it does in conditions of relative stability, would have evolved any marked adaptations for survival of the eggs over more than a limited temperature range.

That there is a considerable range, taking Monogenea as a whole, in the period required for embryonic development is seen from Table 9. Some adaptive significance with reference to the habits of the host or conditions in which the parasite finds itself is probably involved here.

Once the larva has hatched, the free-swimming period is usually short as shown in Table 10. The one exception, Hess' (1928) record for the larva of an unspecified Dactylogyrus surviving four to six days, is curiously anomalous.

Temperature has been shown, besides its effect on embryonic stages, to affect the rate at which monogeneans develop from hatching to maturity. It was not possible to take this into account as regards D.denticulata since it could not be tested experimentally under the conditions prevailing. The variation noted by Alvey (1933, 1936) in the time taken to reach maturity by Sphyranura oligorchis may have been a temperature effect.

Table 7 includes estimates of the times taken to reach the 'immature' and 'adult' stages. These are based on information of the ages of the hosts from which certain flukes were recovered. Some slight check of these estimates is provided by Graph 6a. From this it is seen that the number present of larvae and of immature worms is of the same order and this tends to confirm the view that the larval and immature periods are of something the same length, that is, about three months. Though less reliable than Graph 6a owing to the numbers involved being smaller, Graph 6b shows the stage-composition of the larval population. From a knowledge obtained experimentally of the length of the first and second larval stages (Table 7) it is clear that the first does not occupy the relatively long period implied in Graph 6b. The drop in numbers of larvae recovered after the first stage probably indicates the comparative vulnerability of first stage larvae. Once the larva has overcome the difficulties of accommodating itself to the

host there seems little cause for differential wastage in the subsequent larval stages. Accordingly it is reasonable to assume that the lengths of duration of the second to fifth larval stages are proportional to the incidence of larvae of these stages, that is to say, approximately equal to one another with perhaps a slightly longer period spent in the third larval stage.

No use can be made of Graph 6a for estimating the longevity of adult flukes because, as has already been stressed, the full picture cannot be seen when only the younger age-groups of hosts have been studied.

Seasonal Activity and Sexual Phases.

Reproduction in Monogenea may take place throughout the year. Thus Wilde (1936) reported egg-laying in *Neodactylogyrus macracanthus* all the year round. However, Kulwiec (1927) found that eggs were only laid in the spring by *Dactylogyrus anchoratus* and *D.crassus* and Zeller (1872) said that reproduction in *Diplozoon paradoxum* was confined to the warmer months, regression of the reproductive organs occuring during winter. Gallien (1935), also, has reported that egg-laying in *Polystoma integerrimum* was normally confined to the spring and coincided with the spawning of the frog-hosts. Regression of the reproductive organs after this period was very marked, but he noted that the warm conditions prevailing in the laboratory stimulated the earlier redevelopment of

the reproductive organs. In these conditions, the frogs also spawned earlier.

Sproston (1945a) is the only worker who has observed sexual phases in Monogenea. Working with Kuhnia scombri she found there were three male and two female phases, the male organs maturing first and thereafter the phases alternating as growth continued. She thought this phenomenon might, on closer study, prove widespread in trematodes, associated with protandry. As she pointed out, Johnston and Tiegs (1922) had recorded that in Lepidotrema therapon there was a large, active testis in the young worm and that only when the seminal vesicle was full of sperm and the testis spent did the ovary mature.

Although D. denticulata was found to reproduce at all times of the year, it was thought possible that there might be greater reproductive activity at some seasons than at others. To detect this it is necessary to know whether the proportion of the adult population which is laying eggs is constant or not at all seasons and whether the proportion of larvae in the total population fluctuates seasonally.

Since all adult worms were found to have the reproductive organs in the same condition whether eggs were present in the uterus or not, it was thought that all were reproductively active and that those without eggs in utero had but recently laid a sheaf. Unfortunately it was not possible to check this from the proportion of larvae

present in the population. Since the larval period, as has been shown, is spread over about three months, only fluctuation of the proportion of first stage larvae present would give a clear indication of varying reproductive activity. The numbers of first stage larvae recovered throughout the seasons were too small to be of any significance.

There is no evidence for alternating male and female phases in D. denticulata. If, as has here been suggested, self-insemination can occur, this is not unexpected.

Physiology and Ecology

Food and Digestion.

Little is known concerning the nature of the material upon which monogeneans feed. Goto (1895) stated that some fed on mucus and others on blood, both, of course, being derived from the host. Fuhrmann (1928) mentioned epithelial cells in addition to the foregoing. Dawes (1946) has summed up the position by saying that there is probably a great deal of variation in the food-substances utilised and that much care is necessary in the interpretation of the gut-contents. In Hexostoma extensicaudum he found the gut-contents to be composed of clusters of microscopic granules and faintly staining objects which might have been denuded red blood corpuscles. Searching for corpuscles he eventually came on a perfect specimen, but nevertheless concluded that Hexostoma was a mucus-feeder and that the perfect erythrocyte had been swept into the lumen of the gut by the microtome knife. Sproston (1945a) thought there was blood in the gut of older specimens of Kuhnia scombri in spite of a negative result on applying Brown's test for blood, and Gallien (1935) reported Polystoma integerrimum as an undoubted blood-feeder. Remley (1942) thought that feeding by Microcotyle spinicirrus broke down the mucus-covering of the host's gills and therefore must have regarded this species as a mucus-feeder. A case of feeding on epithelial cells is mentioned by Wilde

(1936) who found that in Neodactylogyrus macracanthus damage to the host's gill-epithelium occurred where the hooks were inserted and that this torn up epithelium became available to the mouth when the worm moved on. Swallowing movements and extrusion of the pharynx were often seen.

In newly hatched, monogenean larvae yolk-globules have several times been reported from the gut. Thus Gallien (1935) said that yolk was swallowed by the larva of Polystoma integerrimum just before hatching. Paul (1938) observed yolk in the gut of newly hatched larvae of Polystoma nearcticum and Gallien and Le Calvez (1947) have reported this is in Kuhnia scombri. It is noteworthy that Zeller (1872) found refringent spheres, probably of a fatty nature since they did not dissolve in acetic acid, distributed through the body and not confined to the gut of the larva of Diplozoon paradoxum.

The process of digestion has received scant attention. Zeller (1872) made no reference to this subject in his work on Diplozoon paradoxum, but it is of interest to note that his figures of all but the youngest larvae of this worm show pigmented patches following the course of the gut diverticula. Goto (1895) examined these pigmented cells and advanced three suggestions as to their function: that they might contain ingested food particles, that they might secrete digestive ferments or that they might contain indigestible food residues. He observed the granules being discharged into the gut from the cells

which then filled up again. Gallien (1935) also observed these cells casting their contents into the lumen of the gut of Polystoma integerrimum and said that the cells did not make their appearance in larvae until after feeding had begun. In the epithelial cells surrounding the gut, particularly of older neotenics, he noted needle-like crystals and found that epithelial cells themselves were sometimes sloughed off. Dawes (1940a) saw 'brown' cells in the gut-epithelium of Hexostoma extensicaudum but did not relate them to the digestive processes, regarding their greater numbers in proximity to the vitellaria as a suspicious histological association. Sproston (1945a), however, regarded the brown pigment in these cells in Kuhnia scombri as being a product of digestion of haemoglobin, noting that their number increased in older worms and that they were absent from young worms which, she considered, had not started to feed on blood. Stephenson (1947) identified the pigment found in the gut of Fasciola hepatica as haematin, using chemical and spectrographic means, and considered digestion to be extra-cellular. McCoy (1935) also thought digestion in Digenea was mainly extra-cellular, as did Hyman (1951) in contrast to Turbellaria where she found food vacuoles in the cells round the gut.

Four cases, including D. denticulata as found here, of monogenean, free-swimming larvae with yolk present in the gut have been reported. Taking into account how few larvae have been examined this is a high proportion and it

is probably a common feature. Indeed such an adaptation would seem essential to tide the larva over the free-swimming period. How this yolk is digested is not known, but presumably the process may be the same as that which led to the gradual break-down and absorption of yolk lying outside the embryonic body during the embryonic period. That spheres similar in appearance to those in the gut-cavity have been seen lying in the tissues of the body both in Diplozoon paradoxum and here in D.denticulata may indicate that digestion does not necessarily take place within the gut-cavity.

There has been much discussion over the nature of food-substances utilised by adult monogeneans. Undoubtedly there must be variations from one species to another according to their ability to break down the host's defences, and their location. A gill-parasite is, for example, much more likely to be a blood-feeder than is a skin-parasite. Also it must be taken into account that one worm may not always feed on the same material. From evidence obtained here it seems highly probable that D.denticulata may not always utilise the same host-tissue.

In the absence of observation of feeding itself the evidence of the gut-contents only is available and Dawes (1946) has warned of the difficulties of interpretation. The presence of red fluid in the gut naturally suggested during the present research that blood had been ingested. The negative result with Brown's test is unexpected though

Sproston (1945a) has reported a similar failure in testing the gut-contents of Kuhnia scombri. However, microscopic examination revealed in D.denticulata the presence of cells in various stages of denudation which, if not indubitably red blood corpuscles, at least resembled them very closely. Thus in spite of negative evidence from Brown's test, the author considers, as did Sproston (1945a) under similar circumstances, that blood is taken at times. Very probably mucus and perhaps epithelial cells may also be ingested. Indeed, from the absence of pathogenic effects it is apparent that much of the feeding does not damage the host's tissues at all. That the addition of glucose to the water in which worms are kept does not prolong survival may indicate that D.denticulata cannot utilise sugars at least when supplied in solution in the medium, but the controlling factor for survival may have been bacterial activity or lack of oxygen.

In worms which have been removed from the host large numbers of yolk-cells are passed into the gut through the genito-intestinal canal and might be thought a major source of stored food were they not frequently discharged quite unchanged at the mouth. It is thought that under natural conditions relatively few yolk-cells will be passed into the gut and this represents an inhibition of egg-formation without a corresponding decrease in yolk-production.

Attention to problems connected with digestion in trematodes has largely been focussed on the question of

the 'brown' cells lying in the gut-wall. Various theories of their functions, as has already been noted, have been advanced. The present findings are in agreement with those of Gallien (1935) on Polystoma integerrimum and of Sproston (1945a) concerning Kuhnia scombri in that the pigmented cells are not present in the youngest larvae. This associates them with feeding. Evidence from the present work, and presented in the literature, that granules identical with those in the 'brown' cells are found free in the lumen of the gut favours the view that they may be excretory in function. Both Goto (1895) and Gallien (1935) claimed to have seen the granules being discharged from the cells into the gut. Dawes (1940a) regarded similar cells in Hexostoma extensicaudum as having some association with the vitellaria, but this position is not tenable in view of the findings both here and described in the literature where the 'brown' cells are developed long before the vitellaria and do not only occur in the vitellarian neighbourhood. It is natural to suspect that the pigment in the 'brown' cells, since the worms bearing them at least may feed on blood, is haematin. Stephenson (1947) has identified the pigment in the lumen of the gut of Fasciola hepatica as haematin, but on the only evidence obtained in this work, that of Brown's test, the brown cells of D.denticulata do not contain haematin. Dawes (1940a) made a very careful study of the food-material of Hexostoma extensicaudum and came to the conclusion that it did not feed on blood.

Nevertheless 'brown' cells are present. Thus 'brown' cells can be produced in the absence of blood-feeding and therefore of haematin.

Digestion in digeneans is considered to be extracellular and histological observations made here on D. denticulata do not reveal food-vacuoles or ingestatory cells in the intestinal region. If digestion is extra-cellular it would be expected that digestive glands would be present, but, as has already been mentioned, these are scanty in trematodes and none have been detected during this work on D. denticulata. The means by which digestion takes place, therefore, remains obscure. It may be that, with the relative lack of specialisation at this level of organisation, small quantities of enzymes are secreted by all cells surrounding the gut. However it is probably very significant that the gut contains such a vigorous bacterial flora and this may be the principle means of breaking down the food.

Respiration and Survival-Time.

Respiration in Monogenea has not been studied but Hyman (1951) said that oxygen must penetrate the body by simple diffusion and Dawes (1946) has presumed that in monogeneans oxygen requirements will be high in accordance with their superficial, well-oxygenated locations. No relative information can here be drawn from Digenea, inhabiting as they do sites with very low oxygen tension.

No critical work has been carried out on respiration in Monogonea. Dawes (1946) has presumed that their oxygen requirements will be high in accordance with the good supplies available. The fact that much oxygen is present does not necessarily mean that it is utilised in large quantities, but even if little is used he may be right that monogeneans are only capable of extracting it from a medium of high oxygen-tension. The survival of specimens of D.denticulata for some time after the death of the host would seem to indicate that this fluke can subsist, at least temporarily, in a medium of considerably lower oxygen-tension than normal.

Excretion and Osmo-Regulation.

Though nothing is known of excretion in Monogonea a few findings concerning related forms may have a bearing on the subject. Hyman (1951) recorded that in planarians vital dyes tended to concentrate in the mesenchyme and eventually were expelled into the gut. They sometimes passed into the lower parts of the excretory ducts, but never through the flame-cells or upper parts of the ducts. She thought therefore, that the function of the flame-cells was purely osmo-regulatory. That the whole of the excretory system in Turbellaria is closely connected with the process of osmo-regulation she deduced from the fact that in those species which are capable of living in either fresh or salt water there was much greater development of

the system in fresh-water individuals. In Digenea the same author thought the excretory system really was concerned with excretion, but also, at least in some cases, with osmo-regulation. This was shown, she said, by the flame-cells of encapsulated miracidia of Schistosoma haematobium only beating when the urine in which they were studied was diluted. She thought the fat globules seen in so many excretory canals were excretory products. Stephenson (1947) found ascorbic acid concentrated in the excretory canals of Fasciola hepatica.

From the evidence obtained here by using vital stains it appears that excretion is not one of the primary functions of the 'excretory' system in D.denticulata, but more critical work than has been possible is necessary in order to ascertain the true state of affairs. That this fluke is capable of a certain degree of osmo-regulation is evident from the tests made in dilution of the medium, but clearly this capacity does not extend to the free-swimming larvae. However, unlike the case of encapsulated miracidia of Schistosoma haematobium described by Hyman (1951), the flame-cells beat continuously in Diclidophora and not only when the medium is diluted.

Position and Movement.

There has been very little consideration of the habits of Monogenea as regards the degree of movement on the host. Among references to the subject are those of

Wilde (1936), who observed that Neodactylogyrus macracanthus moved over the host's gills during feeding, and Dawes (1946), who said that Microcotyle was continually changing its position.

Cerfontaine (1896) described D.denticulata as lying on the inner face of the gill-filament with the mouth directed toward the free end of the filament. This position he also found to be characteristic of D.pollachii, D.merlangi and D.palmatum.

The position between the two rows of gill-filaments so characteristic of D.denticulata is apparently common to the genus. From the literature it is clear that some monogeneans move about a good deal on the host, but this does not seem to be the case with adult D.denticulata. Young worms, particularly larvae, are more ready to move, and connected with this is their much greater resistance than that of adults to detachment. It is obviously necessary for the fluke to change its position as it grows and it would be advantageous to the young worms to space themselves out over the gills rather than remain wherever they happened originally to have secured a hold.

Degree of Infestation and Distribution Over Gills.

Fuhrmann (1928) noted that, generally speaking, the number of monogenean parasites on each host was small and Cerfontaine (1896) observed that there were usually two or three individuals of D.denticulata on each host.

As regards distribution on individual gills Cerfontaine (1896) considered that D.denticulata occurred most commonly on the second and third pairs of the host's gills. Later (1898) he noted similar preferences in other species of the same genus.

The average number of individuals of D.denticulata per host noted in this study is 2.24. This accords with Cerfontaine's (1896) statement that there were two or three on each host and with typical numbers per host for monogeneans as a whole. Nevertheless the importance of examining a large number of hosts to ascertain the incidence of a parasite is shown by the fact that the frequency as found here varies from one to seventeen (Graph 7).

Cerfontaine (1896) reported a preference for the second and third pair of gills not only in D.denticulata, but in other species of the same genus. Again, the examination of larger numbers has proved that this is not really the case, but that the first and second pairs of gills harbour about three times as many individuals of D.denticulata as do the third and fourth pairs (Table 8). No explanation for this situation can be suggested.

Pathogenicity.

In general monogeneans do but little, if any, harm to their hosts. Goto (1895) and Dawes (1946) testify to this. Dawes said that where hypersecretion of mucus was provoked the worm often counteracted the effect by feeding

on the excessive mucus so produced. Remley (1942) considered that breaking down of the mucus covering of the gills might admit fungi though he observed little damage in the case he studied, that of Microcotyle spinicirrus. Smith (unpubl.) has indicated the importance of mucus in forming an impermeable barrier between the fish and its environment. Dawes (1946) has said that in Discocotyle sagittata and Microcotyle sp., where there were heavy infections, suffocation of the host could occur from the excessive mucus produced as a result of irritation to the gills.

In some cases there is erosion of the gill-tissue, but harm does not necessarily result. Thus in Hexostoma extensicaudum Dawes (1940a) observed erosion of the gill-tissue at the site of attachment, perhaps by stasis, and also near the mouth of the worm. Though there was no ill effect Dawes thought the possibility of a blood vessel being perforated should not be ruled out.

Serious damage to the gills, particularly among young fish, occurs as a result of heavy infections with species of Dactylogyrus and allies. Serious epidemics in fish hatcheries are common (Kulwiec, 1927, and Hess, 1928, 1930). Link (1910) noted that little harm occured to adult carp, but parasites harboured by these fish acted as a reservoir of infestation for young fish. According to Wilde (1936), Neodactylogyrus macracanthus damages the host's gill-tissue with resultant proliferation and loss of respiratory surface.

Pathogenic effect by a parasite of the skin and eyes has been recorded by Jahn and Kuhn (1932), who found that Benedenia melleni was highly pathogenic to all susceptible fish in aquaria, often leading to blindness and in many cases proving fatal.

The present findings indicating that D.denticulata feeds, at times at least, on blood, it may be thought surprising that no pathogenic effects on the host have been discovered as a result of the fluke's activities. Apparently a balance has been struck. Harm done by monogeneans to their hosts, when it occurs, is either caused by their damaging feeding habits or the irritation produced by their holdfasts. In the case of D.denticulata the demands for blood, which would be the most serious loss, seem moderate and the animal probably frequently contents itself with mucus. That this worm is able to retain its position by gripping the delicate lamellae of the gill without damaging them is indeed remarkable and indicates a mutually satisfactory evolution of the haptor. Finally, the quiescent behaviour of mature worms will minimise irritation of the gills.

Host-Specificity and Host-Immunity.

D.denticulata has been recorded almost exclusively from the gills of Gadus virens (Olsson, 1875; Linton, 1901; Stafford, 1904; Cooper, 1915; Nicoll, 1915; Little, 1929; Rees and Llewellyn, 1941; Shelswell, unpubl.) but Baylis

and Idris Jones (1933) recorded an instance of its occurrence on Gadus merluccius and Nicoll (1915) gave Gadus minutus as a third host.

Speaking of Monogenea as a whole Fuhrmann (1928) said that species were either confined to a single host or to a group of closely related hosts. This is borne out by a study of Dawes' (1946) book in which, of the British species, forty-six are described as being confined to a single host, sixteen are restricted to a few or closely related hosts and only three as having many hosts. Baylis (1938) quoted Baer as saying that ecto-parasitic monogeneans show more host-specificity than endo-parasitic digeneans.

A few investigations have been carried out in an attempt to establish the nature of this specificity. Among these may be mentioned the work of Jahn and Kuhn (1932) who investigated the host-relationships of Benedenia melleni. Of these species which became infected they noted that none was an elasmobranch and they considered that, since pathogenic effects were observed in all cases, the true host of this parasite was not among those they studied. While some hosts eventually became partially immune others never developed any immunity to this worm. Nigrelli and Breder (1934) carried these investigations further and found that some fish never acquired an infection when exposed to Benedenia melleni while others did. Of those which it was possible to infect, some did not develop any immunity, others became immune after a time, but some became more

susceptible with increasing age. Moonfish, they found, developed a local, skin immunity and, though they could be re-infected, the new parasites occupied different sites from those of their predecessors. Nigelli (1937) followed up these findings and determined that it was the mucus of immune fishes which killed the worms and that mucus from the naturally immune elasmobranchs had the same effect. He therefore concluded that localised antibodies passed into the mucus of the affected fishes, thus producing immunity and that elasmobranch mucus was naturally toxic to the worms.

Remley (1942) attempted without result to infest fishes other than Aplodinotus grunniens with Microcotyle spinicirrus. He found that even the true host was not infested in its first year, but attributed this to lack of opportunity, the young fishes not joining a shoal until their second year. Age-resistance, he thought, was probably not acquired as a very large host was discovered to be heavily infested. Hess (1930), however, found that in Dactylogyrus-infections there was a falling off in numbers of parasites present as time went on, if the host was in good condition, and he attributed this to antibody production preventing either growth of worms already present, or the attachment of new ones. In this connection it is worthy of note that Sandground (1929) considered age-resistance not to be dependant on antibody-formation, but rather as a late development of natural immunity or host-parasite

incompatibility. His reason for this was that age-resistance was most marked in 'abnormal' hosts. Unlike acquired immunity, he found age-resistance to be general rather than specific. Ackert (1942) and Hyman (1951) uphold this view, the former supporting it by the fact that the inhibitory substances in age-resistance were thermostable while antibodies were thermo-labile. Taliaferro (1940) has noted that age-resistance may frequently be a combination of increased natural resistance and acquired immunity.

In this connection it is relevant to note that Crofton (1947) has stressed the importance of knowing the size or age of the host in assessing the incidence of a parasite in an area.

On the subject of age-resistance the only evidence concerning D.denticulata is that of Llewellyn (unpubl.), who examined twenty-five specimens of Gadus virens, from the Irish Atlantic Slope, of from two to three or more feet in length, and found adult D.denticulata only on ten of them. The lengths of these worms were from 6.0-8.0 mm. and all were sexually mature.

The fact that no harm is done to their hosts by so many of the Monogenea is an indication of the old and stable relations between them. It is under conditions of this sort that close host-specificity would be expected and is, in this group, found.

Unfortunately neither species of subsidiary host of

D.denticulata was available for examination and so no estimate of the relative infestation of these hosts could be undertaken in this study of the problem.

Since D.denticulata is parasitic on three species of Gadus it might be expected that this fluke would also parasitise other members of the same genus. Nevertheless it has not been reported from Gadus callarias and the one attempt, which was possible during the present work, to infest this species experimentally gave a negative result. More than ecological specificity, that is restricted opportunity for infestation, seems to be involved here because G.virens and G.callarias have actually been found feeding together and yet only G.virens is infested.

Species which act as host to a parasite may develop immunity either as a result of pre-munition, that is, no new infection can occur so long as parasites of the same species are already present; as a result of immunity acquired from an earlier infection which has been overcome; or as a result of increased natural resistance. The last mentioned may, as Taliaferro (1940) has pointed out, be compounded of the second and third possibilities. In the case of D.denticulata it can readily be seen from Graph 11 that pre-munition does not occur. As many as seven separate infestations on one host have here been observed.

Immunity acquired as a result of previous infection, and age-resistance, are less easily detected in a wild population, but certain pointers are noteworthy. If immunity

were acquired either as a result of increased natural resistance with age, or as a result of earlier infestation it would be expected that fewer parasites would be found on larger, and therefore older, fishes. In this case there would be a negative co-efficient of correlation between size of fish and number of parasites present. In fact, so far as D.denticulata is concerned as can be seen from Graph 8 there is an increase in number of flukes per fish with increasing host-size and a positive co-efficient of correlation. It is recognised that the fishes examined here were all under three years old and resistance might become more apparent later. However, Llewellyn (unpubl.) examined individuals of Gadus virens which were almost certainly more than four years old and yet harboured D.denticulata. Thus it is concluded that total immunity, whether as a result of previous infestation or inceased natural resistance with advancing age does not occur in Gadus virens as concerns D.denticulata.

There is, however, a modification of the same principl to be taken into account. Though parasities which are already present may be able to maintain themselves, larvae may fail to become established. Inspection of Graph 10 shows that fishes of 27 cms. and over in length have not been observed carrying larval specimens of D.denticulata. It is unfortunate that more fishes in the larger size-groups were not obtainable. The trend, in spite of the inadequate numbers, does seem downward and support is here again forth-

coming from Llewellyn's (unpubl.) record of twenty-five specimens of Gadus virens of two feet or more in length none of which carried any larval or immature individuals of D.denticulata.

There are three possible causes for the failure of larvae to establish themselves on older fish. The first is ecological: the changed habits of the host-fish, its migration to deeper water, may mean that larvae hatching from eggs laid even by worms it already carries have but slight chance of encountering a host. Secondly there may be increased natural resistance regardless of earlier infestations: in this category may perhaps be included the mechanical fact that in larger fishes water will be passed over more widely spaced gills and thus larvae will have less chance of attaching themselves as they are swept by. Thirdly there is the possibility that earlier infestations will have provoked an immunity which is of sufficient strength to resist larval attack. Of these three possibilities the first two almost certainly operate. The change of habit would be important with the chances, even inshore, of a larvae encountering a host being so slender. Increased incompatibility and mechanical difficulties associated with larger gills are at least likely to have an effect. Development of a certain degree of immunity from earlier infestations is less likely to take place. It is usually bound up with pathogenic effects and here, where none are produced, it seems unlikely that any humoral response will be

provoked.

Distribution.

Diclidophora denticulata was first recorded from the Skaggerack (Olsson, 1875) and Braun (1893) also gave this location, but was probably referring to Olsson's record. From the American side of the Atlantic this fluke has been found at Wood's Hole, Massachusetts (Linton, 1901) and from the adjacent coast of Nova Scotia (Stafford, 1904 and Cooper, 1915). From the coasts of Britain the species has been recorded from Plymouth (Baylis and Idris Jones, 1933), Galway Bay (Little, 1929), the Irish Atlantic Slope (Rees and Llewellyn, 1941), Aberdeen (Nicoll, 1915) and Shetland (Shelswell, unpubl.). Finally there is Nicoll's (1915) report of this parasite "outside British waters."

Gadus virens has been examined for trematode parasites with negative results so far as D. denticulata is concerned, at Mount Desert Island, Maine (Manter, 1926) and at Plymouth (Marine Biological Association Report, 1931). Manter only examined four fishes and, as Gadus virens is reported as "occasional" at Plymouth, it is unlikely that many individuals were examined there either.

No reference to Diclidophora denticulata or to Gadus virens has been found in the papers of Acena (1947), Bychowsky (1934), Crofton (1947), Hughes (1928), Ishii (1936), Ishii and Sawada (1938a and b), Johnston (1931), Linton (1904 and 1910), Looss (1899), Lühe (1906), Meserve (1938), Murray

(1931), Odhner (1905), Price (1938b), A. Scott (1901, 1904, 1906), T. Scott (1901, 1902, 1904, 1906, 1911a and b), Taschenberg (1880), Tubangui (1931), Wegener (1909), Woolcock (1936) or Yamaguti (1934, 1938a and b).

Speaking of the distribution of Monogenea as a whole Fuhrmann (1928) said that the distribution of the parasite usually corresponded to that of the host, but that data from which more definite conclusions could be drawn was lacking. Dawes (1946) also thought it reasonable to assume that the distribution of monogenean parasites would follow that of their hosts considering that only one host was involved in the life cycle. He noted that Monogenea, with their superficial locations, were much more subject to environmental effects than were endo-parasitic forms.

Wherever Gadus virens has been examined parasitologically, with two exceptions, D.denticulata has been reported present. Certainly in one case and probably in the other, where the host but not the parasite has been recorded, the number of fishes examined was small. Examination of larger numbers might well have revealed the presence of D.denticulata in the host-population. Therefore it is thought that D.denticulata is distributed in accordance with the distribution of the host. This is in agreement with the general views of Fuhrmann (1928) and Dawes (1946).

Nomenclature

Olsson (1875) originally described the animal discussed in this work as Octobothrium denticulatum. There has been considerable confusion as regards the nomenclatures of this and allied species and the position has recently been clarified and summarised by Sproston (1946). As the present study of the literature has led to agreement with her findings only the salient points are treated here, though the following references were also consulted: Leuckart (1827 1828), Kuhn (1829), Nordmann (1832), Chiaje (1841), Dalyell (1853), P. J. van Beneden (1856, 1858), Diesing (1858), Braun (1893), Cerfontaine (1898), Saint-Remy (1898), Pratt (1900), Monticelli (1903), Southwell and Kirshner (1937), Llewellyn (1941b) and Sproston (1945b).

The genus Diclidophora was erected by Diesing in 1850 for those species of Octobothrium which had transverse, pincer-like clamps (Diesing, 1850). P. J. van Beneden and Hesse (1864) reclassified the now much swollen numbers of the "Octocotylidés" and instituted the name Dactycotyle instead of Diclidophora without reference to previous authors. Goto (1895) used Diclidophora in a sense which, Cerfontaine (1896) pointed out, excluded Diesing's type species. Accordingly Cerfontaine used the name Dactylocotyle (= Dactycotyle) for species of type Diclidophora Diesing. E. van Beneden (1868) and Saint-Remy (1891) had already adopted P. J. van Beneden and Hesse's name Dactycotyle and

now Cerfontaine was followed by Dollfus (1922), Fuhrmann (1928), Baylis and Idris Jones (1933), Sprehn (1933), Gallier (1937), Llewellyn (1941a) and Brinkmann (1942a and b). However Price (1936) returned to Diplidophora. This paper has proved unobtainable but he made it clear in a later paper (1943) that Diplidophora is a valid genus and in this he has been followed by Dawes (1946) and Spreston (1946).

The genus Diplidophora Diesing cannot be invalidated by Goto's (1895) subsequent work because if Goto's species are congeneric with Diesing's then Diplidophora Diesing must be their generic name. If, on the other hand, Goto's species cannot be assigned to Diesing's genus they must be placed in another or new genus as the name Diplidophora is already occupied.

The present author is in agreement with Spreston (1946) that the correct name for the species under discussion in this work is Diplidophora denticulata (Olsson, 1875) Price, 1943.

SUMMARY OF THE MAIN CONCLUSIONS.

1. Cerfontaine's (1896) description of the shape of the adult animal is confirmed, but a slight median projection at the posterior extremity has also been noted.

2. The body-wall is composed of cuticle, circular, longitudinal and oblique muscle-layers, and a band of parenchyma dividing the longitudinal layer into 2 parts. Dorso-ventral muscles pass through the body. Short, perpendicular muscles re-inforce the ventral wall of the haptor. Larger cells, perhaps excretory, take the place of typical parenchyma in the mid-posterior region.

3. The clamps each contain 8 supporting sclerites of slightly different arrangement from that described by Cerfontaine (1896). These sclerites are chemically protein in nature. Cerfontine's (1896, 1898) description of the muscles associated with the clamps is confirmed.

4. Cerfontaine's (1896) description of the alimentary system is confirmed, except that no intrinsic pharyngeal glands were detected, and has been added to. Glandular tissue occurs in the lips, particularly dorsally, and lateral to the pharynx. Perpendicular fibres compose the walls of the buccal suckers, each of which is operated by a muscle to increase its concavity and 2 muscles which draw the sucker back past the pharynx.

The pharynx is built up of 3 rings of tissue: soft tissue, circular muscles and perpendicular, elastic fibres. It is extruded and retracted by muscles attached anteriorly and posteriorly. A delicate membrane with occasional nuclei lines the caeca. Prominent cells containing brown pigment protrude from the membrane.

5. Cerfontaine's (1896) description of the nervous system is confirmed, and it is agreed that the cells seen by him in association with the system are nerve-cells. They are more widely distributed in the body than he described and occasionally occur in a nerve-fibre.

6. Flame-cells are present and the course of the main, paired, longitudinal, excretory canals has been followed. They terminate at the dorso-lateral, anterior, excretory apertures mentioned by Cerfontaine (1896).

7. Cerfontaine's (1896) description of the reproductive system is confirmed with a few exceptions. The vagina is thought to be an in-break only patent at the time of insemination. What he took for a seminal vesicle is a prostate gland. The histological nature of the system is in agreement with the generalisations of Goto (1895) and Dawes (1946) but ciliation not hitherto described in D. denticulata or any other monogenean is present in the oviduct, common duct, transverse

vitelline ducts and vitelline reservoir. The cirrus is composed of 3 rings of tissue: basal perpendicular fibres, circular muscle housing the bases of the hooks and upper blocks of tissue each supporting the blade of a hook. From 11-14 cirrus hooks occur, the commonest number being 12.

8. It seems probable that lost clamps can be regenerated.

9. Spermatozoa are produced in morulae as a result of division of cells proliferated from the testicular wall.

10. Oocytes are produced from cells proliferated from the walls of the blind limb of the ovary. Occasional division may follow and growth occurs during passage through the ovary.

11. Vitelline cells are proliferated from the acinal walls. Growth follows together with differentiation of the shell-globules which become peripheral as the cell matures.

12. Insemination is thought to take place between the cirrus and the seminal receptacle through a temporary vaginamade by the cirrus hooks. Self-insemination is thought possible.

13. The egg-shell is provided by the globules contained

in the vitelline cells. Their extrusion is probably brought about by the secretion of Mehlis' gland. Shell-hardening may be effected by the distal part of Mehlis' gland. The assembly of appropriate constituents for each egg is probably brought about by nervous co-ordination. The egg is moulded actively in the ootype, the posterior filament being formed at the base of the organ

14. Cerfontaine's (1896) description of the egg is confirmed.

15. The eggs are laid in a sheaf as Cerfontaine (1896) described. They are not retained on the gills during development.

16. Maturation of the oocyte takes place after egg-formation. The haploid number of chromosomes is 9.

17. The onset of embryonic development is related to the time of egg-formation or sperm-entry. Cleavage is total and slightly unequal leading to the formation of a morula in which there are no germ-layers. The larval gut is formed by epiboly of a mass of yolk, which becomes incorporated in mid-body. Larval organs and a partially ciliated epithelium are differentiated in situ.

18. During embryonic development yolk is broken down and absorbed, some being incorporated in the body.

19. Hatching does not seem to result from any special

stimulus. Mechanical pressure by the larvae forces off the operculum, but secretions may weaken the suture.

20. The free-swimming larva is gyrodactyloid with a winged haptor bearing 4 pairs of lateral, 1 pair of postero-lateral and 1 pair of median hooks. 4 pairs of flame-cells are present and 1 pair of longitudinal, lateral excretory ducts which open antero-laterally. The larva swims by ciliary action and does not respond to jarring, light or shade stimuli or, when vigorous, to the presence of the excised gill of the host. Tired larvae are doubtfully attracted by host gill-tissue. Larval haptorial type is thought to be determined phylogenetically rather than as a result of adaptation to the type of host-tissue to which the larva attaches itself.

21. The larva attaches itself to the host's gill and sloughs off the ciliated coat. Gradual isometric growth follows with the acquisition of the adult clamps in pairs starting posteriorly, each pair replacing a pair of lateral, larval hooks. Primordia of the reproductive organs are laid down in the larval stage, but their elaboration is only achieved when the adult form has been attained. Maturity is not reached at a definite size and the ovary and testes mature simultaneously.

22. The longest time which may elapse between formation and the laying of an egg is almost 5 hours. The average

time taken for embryonic development is 18.3 days at 14.25°C, but temperature affects the developmental rate. Free-swimming larvae survive about 24 hours in the absence of a host. The 2nd larval stage is reached between 5 and 13 days after hatching, the 3rd larval stage after more than 38 days. The immature stage is thought to be reached within 3 months and the adult stage within 6 months.

23. Reproduction occurs throughout the year. The animal is functionally hermaphrodite at all times once maturity is reached.

24. The free-swimming larva subsists on yolk. The food material of attached worms is almost certainly mucus and sometimes blood. Epithelial cells from the gills may be ingested also. The 'brown' cells in the gut only appear after feeding has begun and the pigmented granules probably represent excretory products. Digestion is thought to be extra-cellular and may be aided by bacterial action.

25. Adults rest along a gill-filament on its inner face and do not move about. Larvae are more ready to move than are adults.

26. The average number of flukes per host is 2.24. The 1st and 2nd pairs of gills carry three times as many flukes as the 3rd and 4th pairs.

27. The host suffers no apparent harm from the presence of the flukes.

28. Pre-munition of Gadus virens as a result of parasitism by this fluke does not occur. Age-resistance is probably developed to the extent that larvae cannot establish themselves on older fish and is probably due to mechanical difficulties and changed habits of the host. The ability of this fluke to parasitise species of Gadus does not extend to G.callarias.

29. D.denticulata is recorded for the first time from St. Andrews' Bay and the Firth of Forth.

30. The correct name for the species is Diclidophora denticulata (Olsson, 1875) Price, 1943.

CORRECT NAMES AND SYNONYMS OF MONOGENEA DISCUSSED.

Acolpenteron ureteroecetes Fischthal and Allison, 1941.

Ancyrocephalus vistulensis Siwakowna, 1931.

Benedenia melleni (MacCallum, 1927) Johnston, 1929.

as Epibdella melleni by Jahn and Kuhn (1932).

Dactylogyrus anchoratus (Dujardin, 1845) Wagener, 1857.

Dactylogyrus crassus Kulwieć, 1927.

Dactylogyrus minutus Kulwieć, 1927.

Dactylogyrus vastator Nybelin, 1924.

Diclidophora denticulata (Olsson, 1875) Price, 1943.

as Octobothrium denticulatum by Olsson (1875), Saint-Remy (1891) and Braun (1893); as Dactylocotyle denticulatum by Cerfontaine (1896, 1898), Saint-Remy (1898), Sprehn (1933), Gallien (1937) and Brinkmann (1942b); as Dactylocotyle denticulata by Pratt (1900), Baylis and Idris Jones (1933) and Rees and Llewellyn (1941).

Diclidophora luscae (van Beneden and Hesse, 1863) Price, 194

as Dactylocotyle luscae by Gallien (1934).

Diclidophora merlangi (Kuhn, in Nordmann, 1832) Krøyer, [1851 or later]. as Dactylocotyle merlangi by Odhner (1913).

Diclidophora palmata (Leuckart, 1830) Diesing, 1850.

as Dactylocotyle palmatum by Cerfontaine (1898).

Diclidophora pollachii (van Beneden and Hesse, 1863) Price, 19

as Dactylocotyle pollachii by Gallien (1934).

Diplasiocotyle johnstoni Sanders, 1944.

Diplorchis scaphiopodis Rodgers, 1941: emend. Sproston, 1946.

as Diplorchis scaphiopi by Rodgers (1941).

Diplozoon paradoxum Nordmann, 1832.

Discocotyle Sagittata (Leuckart, 1842), Diesing, 1850.

Entobdella hippoglossi (Müller, 1776) Blainville, 1818.

Gyrodactylus elegans Nordmann, 1832.

Hexostoma extensicaudum (Dawes, 1940) Sproston, 1946.

Kuhnia scombri (Kuhn, 1829) Sproston, 1945.

as Octobothrium scombri by Gallien and Le Calvez (1947).

Lepidotrema therapon Johnston and Tiegs, 1922.

Microcotyle spinicirrus MacCallum, 1918.

Microcotyle stenotomi Goto, 1899.

Neodactylogyrus macracanthus (Wegener, 1909) Price, 1938.

as Dactylogyrus macracanthus by Wilde (1936).

Polystoma integerrimum (Frölich, 1791) Rudolphi, 1808.

as Polystomum integerrimum by Gallien (1935).

Polystoma nearcticum (Paul, 1935) Price, 1939.

as Polystoma integerrimum nearcticum by Paul (1938).

Polystomoides oris Paul, 1938.

Sphyranura oligorchis Alvey, 1933.

Udonella caligorum Johnston, 1835.

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