Thesis submitted for the Degree of Doctor of Philosophy

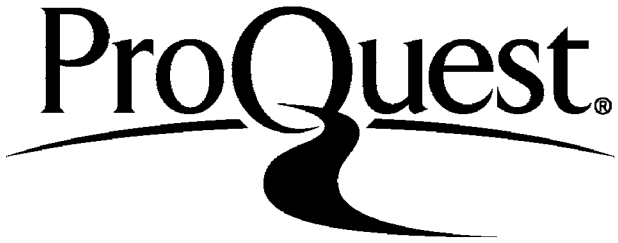
1956.

The Development of *Daphnia magna*.

by

V.M. STOUT

Bedford College, University of London.



Abstract.

A general account is given of the development of Daphnia magna from the egg to the mature adult. A review of previous work reveals that authors have concentrated on the early development, neglecting the later development. An adequate technique has been developed.

An account of the development based on living material gives a detailed series of stages. A summary of the present state of knowledge of the physiological aspects of development is included.

The development of the parthenogenetic egg is described. The early development includes a superficial cleavage and gastrulation by immigration. The mesenteron develops from a solid rod of cells in the ventral part of the egg, and acquires a central cavity which never contains yolk. The yolk cells develop from the blastoderm. The mesoderm develops a single small pair of coelomic cavities, and the heart develops from a compact group of cells. The history of the dorsal organ is described. The development of the ephippial egg resembles that of the parthenogenetic egg except in some features related to the smaller and more even sized nature of the yolk globules.

Daphnia magna hatches from the brood pouch of the mother as an immature adult. The account of the anatomy of the adult is a confirmation and extension of previous work, including histology and indicating function. The muscles of the mesenteron are striated, and the heart wall contains an

incomplete longitudinal, as well as a circular, layer of muscles. A suggestion is made for a new interpretation of the cells of the branchial sacs.

The results obtained and the importance of a large quantity of yolk are discussed.

Table of Contents.

Introduction.

"Whilst I was engaged in searching for certain small creatures which I had persuaded myself would be found in those ditches which commonly cut across our fields and divide them one from another, I came across various kinds of small creatures but not those for which at that time I was looking. I had the good fortune to see, amongst others, certain small creatures which had in their bodies such a swift power of movement that it seemed to surpass belief. These little creatures roughly equalled in bulk a coarse grain of sand, but their bodies were transparent and one could see through them. Amongst other things, I saw in one of these little creatures a clear, round little body near the head in which also it was possible to see very fast movement consisting in alternating extension and contraction. This little part I took to be the heart of the creature, and the very rapid motion, which I could see in the other parts which lay round about, I assumed was derived from this movement of the heart.

When I observed in one of these little creatures eight or nine little parts which had a greenish tinge, I began to think that these were "foetus" or "unborn little creatures". In order to attain certainty in this matter, I placed such a little creature in five or six drops of water so as to see by this means the foetus when alive and watch them when newly born swimming in the water. When however the next day I found that this little creature was dead, I opened its body, without moving it from the position it had been in when it was lying

in the water, and not only saw most clearly several unborn little creatures but also found it possible to discern plainly those organs which they would have used in swimming. This little creature was so delicately formed that I often inspected it with great wonderment; - in fact, its form so delighted me that greater creatures, seen with the naked eye, seem in comparison with these little creatures but the productions of an unskilled hand!"

A. von Leeuwenhoek, letter 121, 1699.

Since the time of Leeuwenhoek, the antics of the embryos of the species of Daphnia have fascinated many workers and there have been several descriptions of the development of living embryos but little work on the details of their embryology. The genus Daphnia has been well known since Leeuwenhoek's day and is common in freshwaters. The genus is large and the species are widespread, being frequently used for experimental and physiological work. It is therefore surprising that so little attention has been paid to its embryology.

The study of the embryological development of Daphnia magna was undertaken because our knowledge of the embryology of Cladocera is very incomplete. Earlier work is scarce and in many points the results obtained do not correspond to the general pattern of Arthropod development, in so far as it is known, but indicate that the Cladocera may have a number of interesting and unusual features in their development. For example, the indication that the midgut develops in a manner

totally different from that of other Arthropoda has been almost completely ignored. Investigation is also prompted by the knowledge that earlier workers were in considerable disagreement over a large number of important points. A review of previous work shows that attention has been concentrated almost entirely on two phases of the development, the kind of cleavage and the nature of gastrulation. Especially concerning the latter, there has been a great deal of difference of opinion. The later development of the germ layers has been almost completely neglected.

I have studied the development of Daphnia magna because the eggs of this species are larger and contain more yolk than those of other species of this genus. For the study of the development it was necessary to have a thorough knowledge of the anatomy of the adult, and this entailed a certain amount of original work together with a great deal of research into earlier papers since no single adequate account exists. I have therefore considered it essential to include a full account of the adult anatomy which deals with the structure in more detail than is found in a textbook account.

This thesis covers the whole development of Daphnia magna from the formation of the egg in the ovary, to the embryological development in the brood pouch of the mother, to the further changes in the organs during the early instars of the free-living life of the Daphnia individual and the structure finally attained in the adult. With such a broad scope it has of course been impossible to cover all the stages adequately and

detailed work has been confined to those of particular interest. Where possible an indication has been made of relationships with the development of other Arthropoda, with particular reference to those investigated recently, since recent work has often tended to disagree with the results of earlier authors.

Terminology.

Descriptions of the embryology of Crustacea have tended to use their own terms but I have endeavoured to avoid the use of terms employed only in such specialised cases.

The two kinds of egg produced by females of Daphnia magna are distinguished as ephippial ("winter") eggs and parthenogenetic or non-ephippial ("summer") eggs. Recently it has become apparent that the ephippial eggs sometimes develop parthenogenetically, although so far this has only been shown in Arctic populations (see p.100). It seems most satisfactory to retain the terms "parthenogenetic" and "ephippial" rather than to suggest new terms. The terms "summer" and "winter" are definitely unsatisfactory since in the laboratory either kind of egg may occur at any time of the year.

The term "Scheitelplatte" used by German authors means "crown of the head" plate and neither apical plate nor cephalic plate are satisfactory translations so it has been decided to retain the term as "Scheitel"-plate.

The outer egg membrane is not strictly a "vitelline" membrane since it is not present at the time of fertilisation.

The yolk cells of the egg change into the fat cells of the adult so that it is impossible to draw a sharp distinction between the two.

Acknowledgements.

I wish to express my thanks to Professor H. Munro Fox under whose guidance this work was carried out, and to acknowledge with pleasure his interest and advice. I wish to thank Miss F.E. Ince and the other members of the Zoology Department, Bedford College for their interest and stimulating criticism, in particular Miss B.M. Gilchrist and Dr. J. Green. I am grateful to Professor N. Millott for his willingness in placing the facilities of his department at my disposal during the final stages of this work.

I owe my sincere thanks to Dr. S.M. Manton for her help and encouragement.

I am also grateful to Professor P. Debaisieux, University of Louvain, for his interest.

I wish to thank Mr. H.C. Gilson and his staff for their hospitality and help during my visit to the Ferry House, Windermere.

A review of previous work.

A succession of workers have studied the embryology of the Cladocera and their results and conclusions have shown considerable variation. The investigation of the early stages of development is rendered exceedingly difficult in many species by the presence of a tough outer membrane and by a large quantity of yolk. This has resulted in controversy being centred around the cleavage and more especially the germ layer formation with a neglecting of the later development of the embryo with the formation of the organs. The history of the investigations of the embryology of Cladocera, and in particular that of Daphnia and its allies, is a varied and confusing one.

The earliest author to mention Daphnia and its embryos was Leeuwenhoek whose letter to the Royal Society in 1699 contains the extract quoted on p. 1. A copy of this letter appears in the collected works of Leeuwenhoek, published in both Latin and Dutch, as letter 121 (Leeuwenhoek, 1719).

Working at the same time as Leeuwenhoek, Swammerdam gives a more detailed description of Daphnia, which he calls Pulex arboreus or arborescens (Baird, 1850) or the Arborescent Water Flea, now known as Daphnia pulex. This description was published in his "Historia Insectorum generalis" published at Utrecht in 1669 and included in the "Book of Nature" published in Dutch in 1737 and in English in 1758.

Later Jurine (1820) in his "Histoire des Monocles qui se trovent aux environs de Genève" gives an excellent account of Monoculus Pulex (the name which was used by Linnaeus) which

includes a description and drawings of embryonic stages.

An account of early descriptions of the genus is given by Baird (1850), who himself gives a detailed description of Daphnia magna which he refers to as D. Schaefferi (according to Johnson, 1952).

Sir John Lubbock (1858) gives the first correct account of the life cycle and a detailed description of the ephippium and the formation of both ephippial and non-ephippial eggs in the ovary. He includes an account of part of the embryonic development: further details of this account are given on p. 26

In his "Naturgeschichte der Daphniden", Leydig (1860) begins with a thorough account of the anatomy and lays the groundwork for future studies. He goes on to give descriptions of the various species.

Ten years later Dohrn (1870) published a paper in which, although he was primarily interested in the carapace or shell gland, he found it necessary to include a description of the development of Daphnia longispina. Dohrn followed most of the external development, although he leaves the shedding of the egg membrane until the same time as the rupturing of the second membrane. With regard to the excretory organ, he established the incorrectness of Leydig's statement that it had no opening, but wrongly stated that the opening was into the body of the animal. Dohrn also decided that the activity of the gland bore no relation to the fat body and pointed out the strong circulation in the neighbourhood of the gland, hinting at its use as an aid to respiration.

Dohrn was soon followed by a number of authors, Claus (1876), Weismann (1876-9) and Grobben (1879). Claus gives a lengthy account of the anatomy of Daphnia, an account on which much of the later work has been based. He included a few remarks on development. Weismann was mostly concerned with reproduction, with his descriptions of the formation of the eggs in the ovary, the function of the fluid in the brood pouch of the mother, copulation and cyclic reproduction.

Grobben's 1879 account of the embryonic development of Moina rectirostris is the classic of cladoceran embryology. It describes in considerable detail the development of the external features and of most of the internal organs. Grobben's work on the development of the various organ systems in the latter half of the embryonic life is the most complete general account published. Details of his account will be referred to later but it is important to note that for the eggs of Moina, relatively poor in yolk, [redacted] he describes a superficial cleavage and determinate development. Grobben also looked at a number of Daphnia pulex embryos and decided that, contrary to the earlier observations of Metschnikoff in 1866, the cleavage was superficial and not total. He confirmed Leydig's observation of superficial cleavage in Moina and Polyphemus.

Since this time there have been continuous differences of opinion about the nature of the cleavage and of the development in Cladocera. The terms "determinate" and "indeterminate" have been used by workers on cladoceran embryology in the sense of "mosaic" and "regulative" as defined for example by

Johannsen and Butt (1941) and by Needham (1942). A determinate or mosaic egg is one in which the determination of the main features of the individuation field has taken place before fertilisation and cleavage; an indeterminate or regulative egg is one in which this determination does not take place until about the time of gastrulation. Differences in the time at which the visible morphological features of development, especially the genital cells, appear show that, for example in insects, it is possible to establish a series, the indeterminate eggs being those in which "the visible separation of regions of different prospective significance occurs after blastoderm formation, and organ segregation follows after differentiation of the germ layers and segmentation" (Johannsen and Butt, 1941). The early workers on Cladoceran development did not undertake any experiments, such as those employed by Horstadius and others on sea urchins or those employed by various workers on insects, to decide the regulative capacity of the eggs. They also did not use the terms "determinate" and "indeterminate" in the sense defined by Willier, Weiss and Hamburger (1955), that is in relation to the regularity or predictability of the cleavage patterns.

Lebedinski (1891), in a short paper on the development of Daphnia similis, found a superficial cleavage but an indeterminate development. A mesendoderm is formed and Lebedinski noted that not all the endoderm partakes in the formation of the midgut but that some surrounds the yolk and forms two large provisional hepatic pouches. This latter was not upheld by later authors.

Two years later, Samassa (1893) disagreed with Grobben about the germ layer formation. He investigated Moina rectirostris, Daphnella brachyura (now Diaphanosoma brachyurum) and Daphnia hyalina. He compared, in detail, the germ layer formation with that of Branchipus (described a year earlier by Brauer) concluding that the Cladoceran development was indeterminate, a full agreement prevailing between the three Cladoceran genera in the formation of the germ layers which took place by a proliferation of the blastozone. Samassa criticises several points of Grobben's account and also postulates hypothetical primitive Phyllopods with a true gastrulation, leading through Branchipus with invagination and polar inward proliferation to the condition in the Cladocera. He gives some information on the formation of organs in Diaphanosoma brachyurum and an account of the origin of the yolk cells, which he derives from the mesoderm.

Grobben (1893) immediately retaliated by insisting that the development was determinate - that is that the genital cells are readily distinguishable by their granular contents and present in the sixth cleavage stage, the endodermal cells distinguishable from the seventh cleavage stage and the "Scheitel"-plate from the "blastophere" stage. Grobben also replies to the majority of Samassa's other attacks with most of which he disagrees. Samassa (1894) defended his views in a short paper.

There followed two papers on the development of the ephippial eggs, Häcker (1894) and Samassa (1897). Häcker,

investigating Moina, did not observe the early cleavages up to the formation of the yolk cells but the later development he believed to be similar to the type of superficial cleavage already known for Crustacea. Samassa found a total cleavage in Moina up to the formation of eight cleavage cells, with the development at the stage of thirty-two cells of a peripheral layer of small cells surrounding a large central yolk cell.

In 1899 Sudler published a paper on the development of the Ctenopod Cladoceran Penilia schmackeri but included little detail. The egg, which is poor in yolk, undergoes total cleavage but genital cells were not distinguishable in the early stages.

Samter (1900) described the development of Leptodora hyalina as superficial and indeterminate giving a detailed account of the formation of the germ layers.

There followed a paper by Agar (1908) on the development of Holopedium gibberum in which he describes a mesendoderm formation in many respects similar to that described by Samassa. He also describes several stages of the development with some information on the formation of the organs.

A series of papers by Kühn (1908; 1911; 1912) dealt with the embryology of Daphnia pulex and Polyphemus pediculus but were devoted almost entirely to the early cleavage stages. Kühn returned to Grobben's ideas of a mosaic or determinate development and gives an elaborate account of the history of the different cells in each cleavage. He believed in the essential agreement of the development of the germ cells in

Daphnia and Polyphemus, any variations being due to the greater proportion of yolk in the Daphnia egg.

In 1912 Vollmer published a detailed account of the development of the ephippial eggs of Cladocera in which he deals mainly with Daphnia pulex, D. magna and D. longispina and finds an early total cleavage, followed by multipolar delamination resulting in the yolk cells, together with immigration of the genital cells. Ventral immigration gives the inner germ layers immediately after the resting stage. His account of the formation of the organs is extremely short and deals almost entirely with the further fate of the germ cells, referring for the rest of the development to Grobben's work on Moina.

1921 saw the appearance of two papers on Cladoceran development, that of Cannon on Simocephalus vetulus and that of Fangauf on Daphnia pulex. Cannon described a superficial cleavage and a development which he regarded as intermediate between the determinate and the indeterminate and comparable to Vollmer's results. Cannon regarded the chief difference between Simocephalus and Polyphemus to be that the endoderm is segregated very late in Simocephalus, very early in Polyphemus, and similarly to a less marked extent with the mesoderm.

Fangauf's paper was a dissertation given at Berlin University and the only trace of it that appears to be available is a list of thirteen points which suggest a list of conclusions. These indicate a superficial cleavage for the parthenogentic eggs in contrast to that of the ephippial egg

and far-reaching differences between the development of the two kinds of egg. Immigration takes place first of the genital cells and later of the endomesoderm. There are a few remarks about the development of the organ systems.

Gravier (1951) gives a lengthy account of a number of topics including copulation, egg laying, reproductive cycles, ephippia and the role of the brood pouch. He mentions very little about the actual development.

In 1937 Baldass described the embryology of Holopedium gibberum and dealt principally with the origin of the germ layers. He decided that the development was determinate and that Agar had missed many intermediate stages leading him to decide on an indeterminate development. Baldass gives no details about the development of the organs.

In the same year Wotzel (1937) described a superficial cleavage for Daphnia pulex and a determinate development. He mentions the "eight-cell" stage with cells at the surface and branches through the yolk, leading to the later establishment of an epithelium. Wotzel concludes that the yolk-poor Cladoceran eggs have a total cleavage, and the yolk-rich eggs a superficial cleavage but that otherwise the development is similar.

Finally Baldass (1942) published a work on the development of Daphnia pulex in which he asserts that the development of all Cladocera is determinate and the germ layer formation of the parthenogentic and ephippial eggs completely similar. The shape

of the egg and the amount of yolk differ in different species but in no way affect the determination of the direction of development although influencing the kind of cleavage, the division of the individual quadrants, the synchronisation of the time of division and the kind of gastrulation. Baldass suggested the plausibility of a hormonal influence exerted by the vegetal pole plasm on the development. He does not describe the development of the organs.

The dismissal by Baldass of the varying results of earlier workers which did not fit in with his conclusions on the grounds that their technique was inadequate does not seem to make sufficient allowance for the range of yolk content present in the eggs of Cladocera and its effect on the nature of development. The majority of work has been done on species, such as Polyphemus pediculus, whose eggs are relatively poor in yolk. This is to be expected in view of the difficulties imposed by greater amounts of yolk on the preparation of material. Daphnia magna possesses unusually large eggs (Green, 1956) which contain abundant yolk; and the embryological development of this species of Daphnia has not been studied except for Vollmer's work on the ephippial eggs.

uthor	Date	Species	Cleavage	Development
obben	1879	Moina rectirostris	(semi-)superficial	determinate
bedinski	1891	Daphnia similis	superficial	indeterminate
massa	1893	Moina rectirostris Diaphanosoma brachyurum Daphnia hyalina	superficial	indeterminate
dler	1899	Penilia schmackeri	total	indeterminate
mter	1900	Leptodora hyalina	superficial	indeterminate
gar	1908	Holopedium gibberum	superficial	indeterminate
hn	1908	Daphnia pulex Polyphemus pediculus	total	determinate
hn	1911	Polyphemus pediculus	total	determinate
hn	1912	" "	"	"
llmer	1912	Daphnia magna D. pulex D. longispina ephippial	total	indeterminate
nnon	1921	Simocephalus vetulus	superficial	intermediate
ngauf	1921	Daphnia pulex, parthenogenetic	superficial	indeterminate
		ephippial	total	"
ldass	1937	Holopedium gibberum	(?)total	determinate
tzel	1937	Daphnia pulex	superficial	determinate
ldass	1942	Daphnia pulex	total	determinate

Material and methods.

The eggs and embryos were obtained from adult Daphnia magna collected from various localities in London and kept in cultures in the laboratory. The cultures were maintained in run tap water and fed on the green alga, Chlorella vulgaris, which was grown on agar slopes, see Fox, Gilchrist and Phear (1951). Daphnia magna was chosen because of the large size of the eggs and the lack of knowledge of its embryological development. The number of eggs laid is variable and depends on various internal and external factors (Green, 1954; 1956, a). The eggs are laid into the brood pouch of the mother and are carried in the brood pouch until liberated as miniature adults.

For accuracy in timing the ages of the eggs and embryos, Daphnia magna females were kept individually in 2" x 1" flat-bottomed specimen tubes with cottonwool tops. The culture medium was 10 cc. of run tap water containing a suspension of Chlorella with an optical density of 0.25, measured with a Medical Research Council grey wedge photometer using a red filter (King et al., 1948). The animals were kept in a constant temperature room maintained at 18° C. in constant illumination. Constant checking led to a close approximation to the time of laying, aided by the knowledge that laying takes place about 4 to 8 hours after the release of the previous brood and about 45 minutes after moulting. The eggs or embryos could then be dissected from the mother at known times after laying, the mothers having been kept in standard conditions. In this way the variation in the stage of development reached at a given age

was minimised, although even embryos laid by the same mother at the same time were not always at the same stage of development. Some broods were fixed immediately after removal from the brood pouch; others were kept in 100 cc. of run tap water and fixed at different times.

If the eggs are dissected from the brood pouch of the mother at least three hours after having been laid, they will continue to develop normally. In this way the external development can be studied, although it is not possible to obtain much information about the development of the organs. Observations and camera lucida drawings were made of living Daphnia magna embryos both at room temperature (14° - 22°C.) and at 18°C. Drawings of the older embryos necessitated the use of urethane as a narcotic, 2% at first, but 5% with hatched young. When it was necessary to use urethane, the animal which had been so treated was not returned to the dish with the remainder of the brood but kept separate. For each observation an animal which had not previously been subjected to urethane was used, so that any effects of the urethane on development could be avoided. Of the urethaned animals kept, a considerable proportion died and the majority continued their development more slowly than other embryos of the same brood which had not been urethaned. Reflected light was used as well as direct illumination. A water bath was placed between the lamp and the microscope to prevent any marked rise in temperature.

A considerable amount of difficulty has been experienced by previous workers on the embryology of Cladocera in obtaining

satisfactory preparations, for example see Cannon (1921). The material presents extreme obstacles in a vitelline membrane and later an outer cuticle not readily permeable, and in an excessive amount of yolk. The unusually large size of the eggs of Daphnia magna in comparison with those of other Cladocera (Green, 1956, a) is coupled with a large yolk content, adding to the difficulty in obtaining good sections. The yolk greatly interferes with both sectioning and staining. It is particularly difficult to obtain preparations in which it is possible to distinguish nuclei among the yolk granules. This hinders the investigation of the early stages and of the development of the yolk cells. It was therefore necessary to devote particular attention to the selection of the most suitable reagents and procedure at each stage of the preparation of the material.

The eggs hatch out of the vitelline membrane while still in the brood pouch. Occasionally the first ecdysis also takes place while the embryos are still in the brood pouch.

The eggs or embryos were dissected from the brood pouch before fixation. Usually the whole of one brood was fixed together so that series of sections in several different planes could be obtained of embryos of the same age. Various fixatives were used. These included a number of corrosive sublimate fixatives: alcoholic corrosive sublimate, corrosive with different amounts of glacial acetic acid added, Susa, Carnoy and Petrunkewitsch. Of these fixatives, the most successful was Petrunkewitsch's corrosive-acetic with nitric

acid (Romeis, 1948). Bouin's fixative was tried with poor results, and Duboscq-Brazil was unsuitable. Cannon (1924) found that Flemming without acetic gave excellent results with Estheria larvae, but it lacked the penetration required for Daphnia embryos. At one time, following Cannon (1921), immersion for 30 seconds in boiling water was tried but this did not fix the embryos. The most satisfactory fixative for all stages of the development was found to be Smith's Formol-Bichromate, which was also used by Cannon (1921) and by Manton (1928; 1934; 1949). The specimens were left in the fixative for 24 hours, after which they were washed in running tap water for about 12 hours, in a washing tube as described by Pantin (1948). The specimens were then removed to 4% formalin in which they were left for about three days, with several changes, and then to distilled water for about four days, with several changes. This treatment with formalin and distilled water is longer than that usually recommended but was found to be necessary in order to completely remove the fixative from the specimens. It was also found necessary to leave the specimens for 24 hours in each of the lower alcohols, 30% and 50%, and for several days in 70% alcohol. The specimens were stored in 4% formalin, which was not satisfactory owing to excessive hardening of the cuticle, in 70% alcohol or in methyl benzoate.

Petrunkewitsch's fixative was also used considerably, especially for the later embryonic stages and for hatched animals, both early instar and adult. It was allowed to act for from four to four and a half hours.

For ephippial eggs both Formol-Bichromate and Petrunkewitsch were used with successful results.

For a number of other Cladocera - Daphnia longispina, Daphnia pulex, Sida crystallina and Simocephalus vetulus - the adults of which were mounted or sectioned, both Formol-Bichromate and Petrunkewitsch proved satisfactory.

A number of eggs of Triops longicaudatus were fixed, some in Formol-Bichromate, some in Susa and some in Carnoy. Of these the bichromate fixative gave the best results.

For Artemia salina, Petrunkewitsch gave better results than Bouin, although the latter was used by Weisz (1947).

Various clearing agents were used, of which the most satisfactory were methyl benzoate and methyl salicylate (wintergreen oil). Both have the great advantage that the specimens can be placed in them direct from 95% alcohol and do not harden even if left in the reagent for several months. Xylene hardened the specimens and neither cellosolve nor terpineol cleared the specimens completely. From methyl benzoate or methyl salicylate, the specimens were passed quite quickly through cedarwood oil, whence they could be either mounted in canada balsam or embedded in wax. While in 95% alcohol the specimens were tinged with eosin to make them distinguishable during embedding.

After trying 52°, 54°, 56° M.P. paraffin waxes and also ester wax the best results were obtained with 52° M.P. paraffin wax to which 1% ceresin had been added. Ester wax caused considerable shrinkage, and also, in order to ensure penetration,

it was necessary to leave the specimens in the wax baths for about two to three hours (the exact time depending on the age of the embryo) which led to brittleness and fracturing on sectioning. The specimens were passed through three paraffin wax baths, and it was not possible to reduce the time in each bath to less than 15 minutes. While the vitelline membrane was still present, a greater length of time in the waxes was required for adequate penetration. The addition of 1% ceresin to the paraffin of which the block was made improved the resulting sections. The specimens were orientated in the block by the method used by Cannon (1924). Often two or three specimens were embedded in the same block. Rapid cooling of the block by the use of iced water was necessary, and it was found advisable not to attempt to cut sections until the following day.

Sections were cut at 4μ, 6μ, 8μ, 9μ, and 10μ; the majority were cut at 8μ or 9μ. The ribbons were attached to the slide by means of Mayer's adhesive albumen, made up with albumen from a fresh egg. They were flattened on a water bath, and then left to dry for 24 hours. The method of outlining the ribbons of sections Pusey's Indian ink mixture (Pusey, 1939) was tried but did not prove useful for such small specimens.

An early tendency to the loss of whole sections, or of the yolk contents, from the slide was overcome by dipping the slide into a solution of 0.25% celloidin in equal parts of alcohol and ether between the 95% and 70% alcohols during the process of hydration. The celloidin formed a thin film over the sections

but did not interfere with the staining in any way.

A number of different staining methods were used. Heidenhain's Iron Haematoxylin proved the best stain for most purposes. The specimens were left for 12 hours in 3% Iron Alum mordant, followed by 12 hours in ½% haematoxylin. A weaker, 1%, mordant solution was tried to prevent clogging but showed no improvement. The clogging was overcome by longer differentiation and longer in xylene before mounting. The time taken for differentiation varied considerably according to the age of the embryo; the younger embryos took longer, especially before the vitelline membrane had been shed. Results were best when no counterstain was used.

Heidenhain's Azan stain was also found to be useful. By leaving the slides in the azocarmine for at least an hour and in the mordant and stain for one and a half to two hours each, the nuclei were clearly seen.

Mallory's Triple stain was especially useful for the yolk cells. Using the times given by Pantin (1948), modified by leaving for a longer time in both acid fuchsin and Mallory's stain, four to five minutes in the former and three to four minutes in the latter, the yolk cell nuclei contrasted with the yolk globules and differences in the staining reactions of the yolk were noted.

Delafield's Haematoxylin, diluted to one in five and counterstained with eosin or without counterstain, was used for sections with less success than that obtained with Heidenhain's Iron Haematoxylin.

Chlorazol Black E was used with limited success; thionin was unsatisfactory, as was v. Gieson's stain although both have been used by earlier workers on cladoceran embryology.

Both squashes and sections were stained with Feulgen, but the nuclei failed to stain adequately.

Various carmine stains were tried, the best for sections being Alum-carmine allowed to act for about three days and then differentiated to remove as much stain as possible from the yolk.

For assistance with various details of structure, especially in the study of the adult, a number of other stains including vital dyes were used. These included alizarin, methylene blue and indigo carmine.

Besides sections, whole mounts were prepared of the various stages. The most satisfactory method was to mount the embryos unstained, after clearing in methyl benzoate and cedarwood oil. Good preparations were also obtained using Ehrlich's Acid Haematoxylin diluted to a few drops of stain to 25 cc. of 70% alcohol. Delafield's Haematoxylin diluted to one in five or to one in three was used with less success. Several carmine stains were also employed for whole mounts but met with the same difficulty as most other stains: failure to penetrate through the vitelline membrane in the early stages, and excessive staining of the yolk in the older stages. The Janus green-neutral red method of staining amphibian embryos (McClung Jones, 1950) is recommended as avoiding the deep coloration of the yolk, which stains lightly or not at all. The yolk of the Daphnia embryos was lightly stained but the cytoplasm also did not stain

satisfactorily. Addition of lignin pink to Polyvinal-Lactophenol mountant provided a good medium for the study of external structures, being especially useful for hatched animals, both early instar and adult.

Various mountants were employed: Canada balsam, Euparal, Euparal Vert, and D.P.X. (B.D.H.). The latter contracts and is inclined to form bubbles, but when free of bubbles the preparations are still satisfactory after a period of two and a half years.

Reconstructions were accomplished with the aid of camera lucida drawings, of graphic reconstructions, of glass plates and coloured glass marking inks, and of plasticine models. Wax models proved unsatisfactory due to the small size and detailed structure of the embryos.

Similar methods of technique were used for hatched animals, both early instar and adult specimens. Drawings with the aid of a camera lucida were made of live animals kept in constant conditions at 18°C. for the first six instars. Observations of the living adult animals necessitated the use of a Compressorium slide or, for quick observations, a small drop of water containing the animal on a microscope slide. Hatched animals are more impermeable, requiring longer periods of time in solutions such as the wax baths and stains.

To obtain males of *Daphnia magna*, both for a study of their anatomy and for the production of ephippia, a culture was transferred to a higher temperature, crowded and given an initial generous food supply, then left without further feeding. This

treatment is similar to that used by Mortimer (1936) for D. magna, and also related to that used by Banta and Wood for D. longispina, Banta (1939).

Ephippial eggs were treated in a similar way to the non-ephippial eggs, after having first been removed from the ephippium. They required slightly longer in the wax baths (three baths of 25 minutes each in paraffin wax) and the stains. Similar lengths of time were required by the eggs of Triops longicaudatus.

Specimens of Artemia salina and of various Cladocera were treated similarly to the Daphnia magna adults, with modifications of the times in the different reagents.

The figures are camera lucida drawings unless otherwise stated, with the exception of the diagrammatic reconstructions. The diagrammatic reconstructions are based on serial sections. The photomicrographs were taken with a Zeiss plate camera and a Cooke Troughton Research microscope.

Observations made on living material.

a. The parthenogenetic, or non-ephippial, egg.

The earliest account containing any details of the development of Daphnia magna from observations of the eggs and embryos in the brood pouch of the mother was published almost exactly a hundred years ago by Sir John Lubbock (1858). Lubbock describes parts of the development of Daphnia Schaefferi = Daphnia magna, including the newly laid egg and the development of a membrane with the early swelling of the egg. He was particularly interested in the second membrane or "larval skin". He regarded the shedding of the first membrane or vitelline membrane as the hatching of the embryo and the further development in the brood pouch, inside the "larval membrane", as evidence of a metamorphosis. He writes: ".. would appear therefore that Daphnia, so far from undergoing no metamorphosis, does, in fact, enter the world in a very rudimentary condition, and that only after the first change of skin does it assume the well-known characters of the genus."

Since Lubbock, several authors have described selected stages during the embryonic development of Daphnia. These selected stages have been used to determine the stage of the instar of the mother, in both physiological and ecological work. The most thorough description of living material is that of Obreshkove and Fraser (1940) who kept parthenogenetic eggs of Daphnia magna outside the brood pouch of the mother, the authors believing themselves to be the first to do so. They had, however, been preceded by Ramult (1914), who, working with

D. pulex, besides demonstrating that embryos can develop normally outside the brood pouch in either pond water or distilled water, made a few observations and measurements during their development. Obreshkove and Fraser give a brief description of the parthenogenetic embryos, together with photographs, at three hour intervals. The descriptions are principally of changes in external features, such as appendages, but the development of the brain and eyes is also noted. The authors observed the beginning of body movements and of the beating of the heart, as well as increases in length. Later authors who established arbitary stages as indices for other work include Jancarik (1947) for D. pulex, Fox (1948) for D. magna, Kaudewitz (1950) for D. pulex, Edmondson (1955) for D. pulex, Phear (1955) for D. magna, and Green (in press) for D. magna. All these descriptions concern external changes quickly and easily determined on viewing the eggs or embryos in the brood pouch of the mother.

Several broods were observed throughout their development, both at room temperatures and when kept in a room maintained constantly at 18°C. Even when developing at the same temperature in uniform dishes and equal quantities of water, there is some individual variation in the length of time which an embryo takes to develop. At 18°C., the average time taken for development from the laying of the eggs into the brood pouch until the expulsion from the mother is about 90 hours, but times from 77 to 110 hours have been recorded. With embryos which have been removed from the brood pouch, the indications of the

time of expulsion from the mother were taken as the extension of the caudal spine and the initiation of more active swimming movements. The same indications were used by Obreshkove and Fraser (1940). It was noted that in embryos which had not been dissected from the brood pouch of the mother, extension of the caudal spine took place about five to ten minutes after expulsion from the mother. The extension occurs by the young Daphnia turning itself completely over in a somersault. The individual variation in the length of time taken for development probably depends on the amount of yolk in the egg, which will depend on the age and size of the mother, and the number and size of the eggs in the brood, see Green (1954). At room temperatures of 20-23°C., the average time taken for development is about 78½ hours; at room temperatures of 13-19°C., the average time is 109-119 hours. Other authors who have noted the length of time taken for the embryonic development of Daphnia magna at given temperatures are: Obreshkove and Fraser (1940), 46 hours at 25°C.; Green (1956, a), 116-118 hours at 13-18°C., and 69-70 hours at 22°C.

Anderson and Jenkins (1942) described the length of time spent in the brood pouch, from the entry as an egg to release as a young animal, as the brooding period and found that this varied with the duration of the instar, which in general increases with age. For Daphnia magna kept at 25°C. they found the brooding period to be about 46 to 64.5 hours. This the authors indicate as in contrast to the results of Obreshkove and Fraser (1940)

and suggest the possibility that the young animals may be in different stages of development when released. Since most of Obreshkove and Fraser's observations were made of embryos removed from the brood pouch and the times depended on the extension of the dorsal spine and initiation of more active swimming movements, this need not necessarily correspond to the time of actual release from the mother. An embryo still in the brood pouch of its mother with its dorsal spine not extended, may extend this dorsal spine and start to swim actively if dissected out from the brood pouch. Thus, although it is useful to keep a distinction between embryonic period and brooding period, the exact time of termination of the embryonic period is often doubtful. It is to be pointed out that Obreshkove and Fraser's embryos may have come from animals of the same age and size, which would explain the lack of variation in the developmental time.

The parthenogenetic eggs develop in the ovary of the female Daphnia magna during one instar and are laid into the brood pouch usually within three-quarters of an hour after the beginning of the following instar. The fully formed eggs in the ovary are surrounded by a thin membrane which enables them to be squeezed through the extremely narrow oviduct as a fine strand. This process has been described and illustrated by Jurine (1820) and v. Scharfenberg (1910). The eggs are laid at about 15-second intervals and only a few minutes are necessary for the laying of even large broods of eggs. Thus differences in stage of development are not due to an extended period of laying.

On emerging into the brood pouch the egg at first is sausage-shaped, then ovoid (Fig. 1,(a)), swelling as it does so, until after a few hours it is nearly spherical as the outer membrane becomes firm (Fig. 1,(c)). The egg is surrounded by two membranes, an outer "chorion" (Lebedinski, 1891) or cuticular membrane and an inner vitelline membrane. During an initial period of about three hours after laying the eggs do not develop if removed from the brood pouch; instead they nearly always burst. It seems likely that this is due to a failure of the egg membrane to withstand differences in osmotic pressure between the contents of the egg and the surrounding medium. The egg membrane is extremely delicate when the egg passes through the narrow oviduct and only during these few hours gradually becomes firm. When laid the egg is opaque and densely packed with yolk globules of various sizes among which are several oil droplets. Nucleus and cytoplasm are not distinguishable in the living egg. The egg when laid is smaller than a few hours later.

The following are observations made on a number of different broods of eggs developing at 18°C. As explained earlier, the times are not rigid as they vary slightly from egg to egg.

At 1½ hours after laying (Fig. 1,(a)), the egg is opaque, densely packed with yolk (y) and still slightly ovoid. A transparent peripheral layer is barely visible. There are about three fairly large oil droplets (od).

At 3½ hours, the egg is less opaque but still densely packed with yolk. The transparent peripheral zone is more obvious and the number of oil droplets has increased slightly.

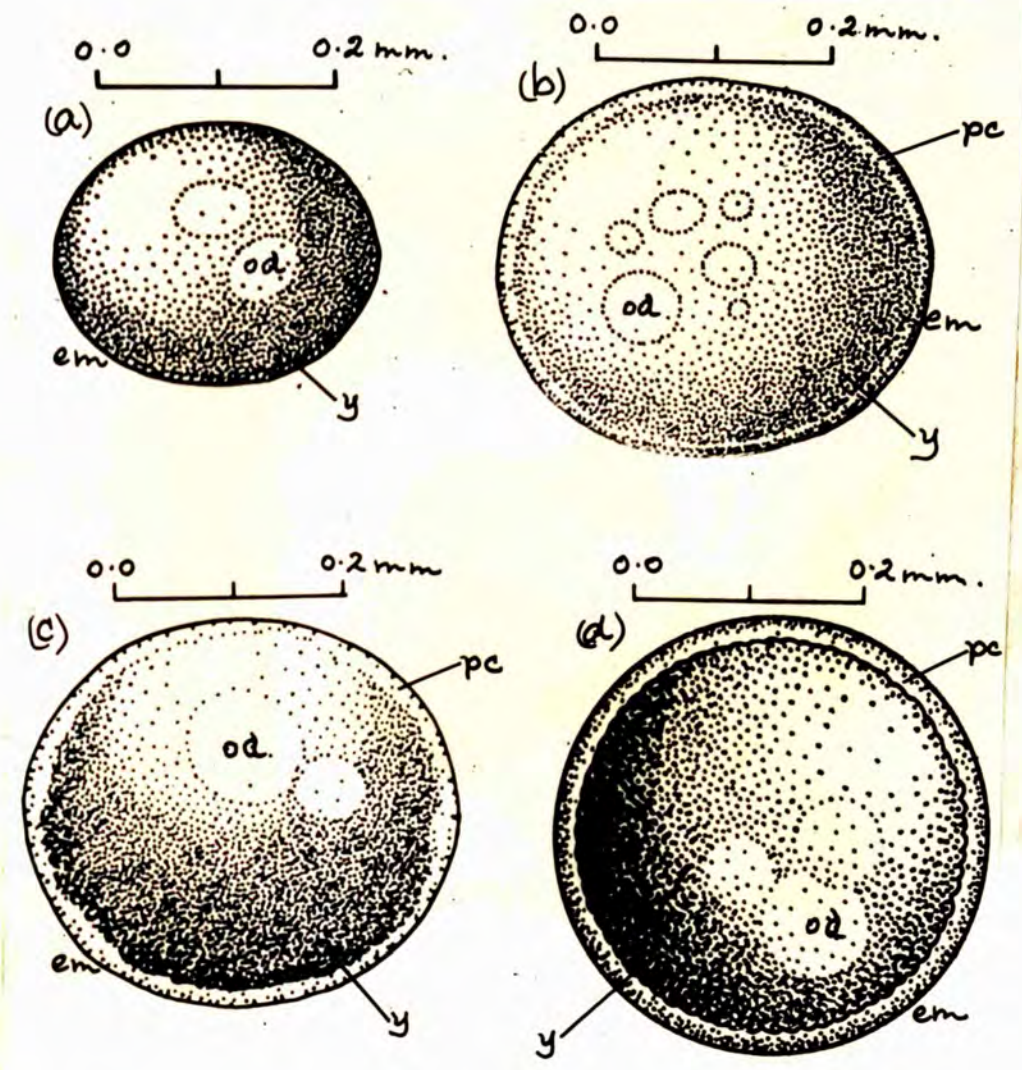


Figure 1. A series of diagrams to illustrate the external development of Daphnia magna, drawn from living material.

(a) 1½ hours after being laid into the brood pouch. em, egg membrane; od, oil droplet; y, yolk.

(b) 5½ hours. em, egg membrane; od, oil droplet; pc, peripheral cytoplasm; y, yolk.

(c) 10¼ hours. em, egg membrane; od, oil droplet; pc, peripheral cytoplasm; y, yolk.

(d) 14 hour. em, egg membrane; od, oil droplet; pc, peripheral cytoplasm; y, yolk.

At 5½ hours (Fig. 1,(b)), there is little change in the egg whose peripheral zone (pc) is moderately well defined but of uneven width. The egg contains numerous yolk globules.

At 6 hours, the egg is spherical to slightly oval, full of yolk globules, some of which appear larger and indicate the first appearance of the yolk cells. The egg is less opaque and usually yellowish-green due to the yolk. The colour may be modified by the inclusion of haemoglobin or carotenoids in the egg.

At 8 hours, the inner edge of the peripheral layer is markedly irregular. A few large globules among the yolk indicate yolk cells, the remainder of the yolk being in the form of smaller granules.

At 9 hours, there is little change in the egg. A few more yolk cells are seen.

At 10¼ hours (Fig. 1,(c)), the uneven peripheral transparent layer (pc) is still noticeable and there are two large (od) and about three smaller oil droplets.

At 12 hours, the egg is less dense, with the yolk granules becoming individually more distinct, the yolk cells in particular are more noticeable. The peripheral layer is slightly more distinct.

At 13 hours (Fig. 1,(d)), the peripheral layer (pc) is becoming granular, and the yolk granules more distinct.

At 15 hours, the peripheral layer has become granular and is thickened internally in parts. There is the first indication of indentation of the surface of the egg. The number of oil

droplets has not increased.

At 18 hours (Fig. 1,(e)), the first modification of the external shape is definitely established in the form of shallow but well marked indentations delimiting broad folds. These will form the second antennae. The central yolk contains large yolk cells which are partly granular.

At 20 hours, there is little further indentation. The peripheral layer is uneven and the oil globules appear slightly smaller.

At 22½ hours (Fig. 1,(f)), the egg has drawn away from its membrane in a few places and there is the first indication of the invaginations to form the stomodaeum and proctodaeum. The yolk globules are beginning to concentrate towards the centre of the egg which thus appears darker and denser. There are about three to four oil droplets (od) of equal size.

At 26 hours (Fig. 1,(g)), the egg is still spherical but the appendages are further developed for the second antennae (a.2) are small but distinct and the mandibles are beginning to develop. The yolk globules appear less numerous. The egg is further drawn away from its membrane (em).

At 27 hours, there is further unevenness of the peripheral layer whose inner edge is becoming less well defined. The yolk cells have increased in number.

At 30 hours (Fig. 1,(h)), the beginning of the development of the thoracic appendages (rt) is indicated by shallow indentations. Further yolk cells have been formed so that the

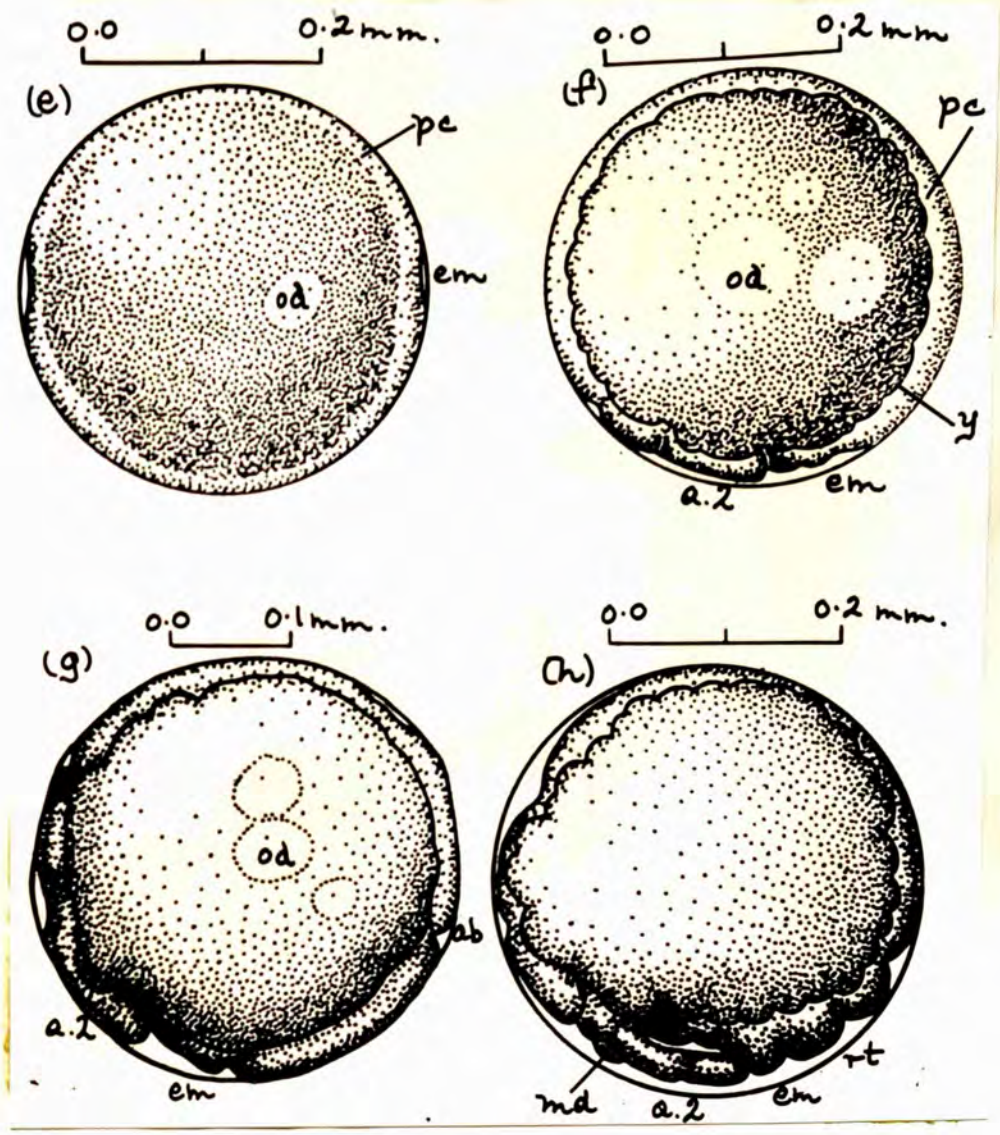


Figure 1. (e) 18 hour. em, egg membrane; od, oil droplet; pc, peripheral cytoplasm.

(f) 22½ hour. a.2, second antenna; em, egg membrane; od, oil droplet; pc, peripheral cytoplasm; y, yolk.

(g) 25 hour. a.2, second antenna; ab, abdomen; em, egg membrane; od, oil droplet.

(h) 29¾ hour. a.2, second antenna; em, egg membrane; md, mandible; rt, rudiment of thoracic appendage.

yolk appears to be formed of more even-sized globules. There is no increase in the number of oil droplets. The centre of the egg appears darker and denser. There is no change in shape, the egg membrane (em) being still present.

At 32½ hours (Fig. 1,(i)), the second antennae (a.2) are well developed each with two branches which are dividing into segments. Small first antennae (a.1) are present, and also the beginnings of the carapace folds (cf). The large dark yolk cells and still unaltered yolk globules are further concentrated towards the centre of the egg(y). The egg is still spherical, the egg membrane (em) still being present.

At 34 hours (Fig. 1,(j)), the egg membrane of some eggs has burst and the embryo begun to lengthen with the first indication of a head (h). The embryo remains surrounded by a thin second membrane. The mandibles are well developed.

At 36 hours (Fig. 1,(k)), the egg membrane (em) may be about to burst or be bursting. There is an indication of the abdomen (ab) and the carapace folds (cf) have grown.

At 41 hours (Fig. 1,(l)), the egg membrane has gone, with the formation of the head (h). The branches of the second antennae (a.2) have attained their full number of segments, the mandibles (md) are well developed, also the labrum, and there is at least one pair of small maxillae. The first antennae (a.1) are still small and the thoracic appendages (rt) still uniramous, all of them being equally developed. With the growth of the peripheral cellular layer, the yolk (y) is further towards the

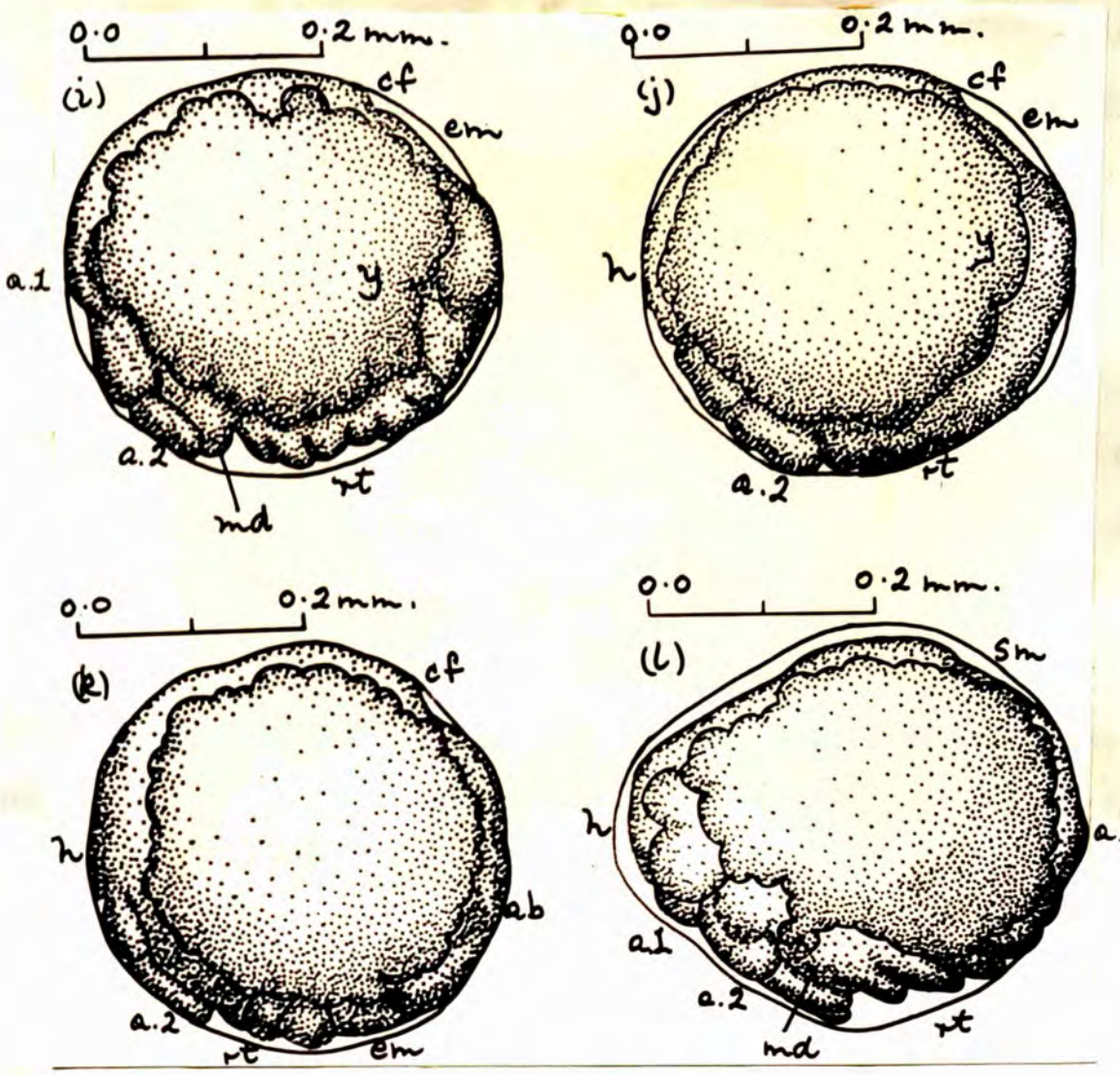


Figure (i) 32½ hour. a.1, antennule; a.2, second antenna; cf, carapace fold; em, egg membrane; md, mandible; rt, rudiment of thoracic appendage; y, yolk.

(j) 34 hour. a.2, second antenna; cf, carapace fold; em, egg membrane; h, head; rt, rudiment of thoracic appendage; y, yolk.

(k) 36 hour. a.2, second antenna; ab, abdomen, cf, carapace fold; em, egg membrane; h, head; rt, rudiment of thoracic appendage; y, yolk.

(l) 41 hour. a.1, antennule; a.2, second antenna; ab, abdomen; h, head; md, mandible; rt, rudiment of thoracic appendage; sm, second (embryonic) membrane; y, yolk.

centre and the oil droplets are not visible. There is some indication of a greater thickening of the peripheral cellular layer in the head region where the brain will later develop.

At 43 hours, the thoracic appendages are becoming biramous.

At 44½ hours (Fig. 1,(m)), the embryo has elongated further but is still enclosed in a fine membrane (sm). A distinct head (h) has developed, and also an abdomen (ab) is well formed. The carapace folds (cf) have grown down further on each side. The yolk (y) appears dark and dense.

At 47 hours (Fig. 1,(n)), there is the first sign of the development of eye pigment (re). All the segments of the second antennae (a.2) are fully developed except that they do not carry setae. The thoracic appendages (rt) are distinctly biramous. The thin embryonic membrane (sm) is still present.

At 49 hours (Fig. 1,(o)), further elongation has occurred but with the embryo still enclosed within its membrane (sm). The thoracic appendages (rt) are becoming segmented.

At 51½ hours (Fig. 1,(p)), the thin embryonic membranes of some embryos have been shed. There is further development of the second antennae (a.2) and of the carapace folds (cf). Further thickening of the peripheral cellular layer is indicated by the concentrating of the yolk (y) more towards the centre of the embryo.

At 53 hours (Fig. 1,(q)), some embryos still retain their membranes (sm). The head (h) is well developed with little eye pigment. The second antennae (a.2) extend over more than half the length of the embryo. The mandibles and labrum appear almost

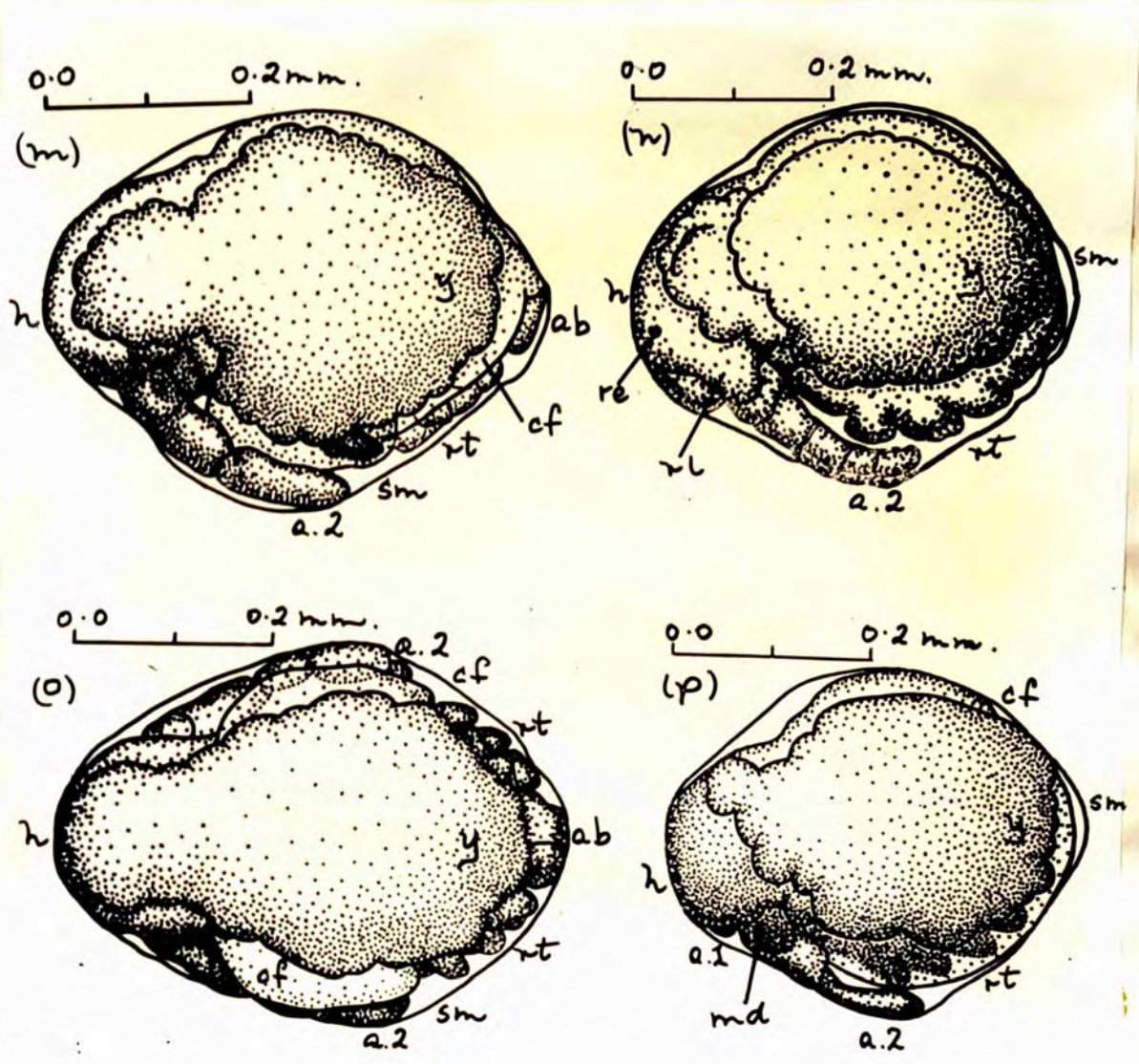


Figure 1. (m) 44½ hour. a.2, second antenna; ab, abdomen. cf, carapace fold; h, head; rt, rudiment of thoracic appendage; sm, second (embryonic) membrane; y, yolk.

(n) 47 hour. a.2, seccnd antenna; h, head; re, rudiment of eye; rl, rudiment of labrum; rt, rudiment of thoracic appendage; sm, second (embryonic) membrane; y, yolk.

(o) 49 hour. Dorsal view. a.2, second antenna; ab, abdomen; cf, carapace fold; h, head; rt, rudiment of thoracic appendage; sm, second (embryonic) membrane; y, yolk.

(p) 51½ hour. a.1, antennule; a.2, second antenna; cf, carapace fold; h, head; md, mandible; rt, rudiment of thoracic appendage; y, yolk.

fully developed. The thoracic appendages (rt) each have three segments.

At 59 hours (Fig. 1,(r)), all the embryos are free of enclosing membranes and the eye pigment is further developed. Freedom from the membrane has allowed the lateral carapace folds (cf) to spread out sideways to a certain extent.

At 62 hours (Fig. 1,(s)), there has been an increase in the amount of eye pigment (re) and a further development of the biramous thoracic appendages (rt). The shedding of the membrane has also enabled the second antennae (a.2) to project away from the body.

At 63½ hours, there has been a further lengthening of the second antennae and increase in the amount of eye pigment.

At 67 hours, the first definite movements by the embryo are observed and the thoracic appendages move slowly. The dorsal spine of the carapace is present. The area of eye pigment is beginning to divide into two.

At 68¼ hours (Fig. 1,(t)), the embryo shows more active movements. The second antennae (a.2) are well free from the body, carry setae and contain well developed muscle. The mandibles (md), maxillae and thoracic appendages (rt) are apparently fully developed. The dorsal organ (do) is visible. There is a pair of oval pinkish compound eye rudiments (re) behind which is a small pigment spot indicating the position of the ocellus (oc). The midgut caeca are seen among the yolk (y) which is becoming concentrated along the line of the alimentary canal. The carapace folds (cf) are fully grown with a well

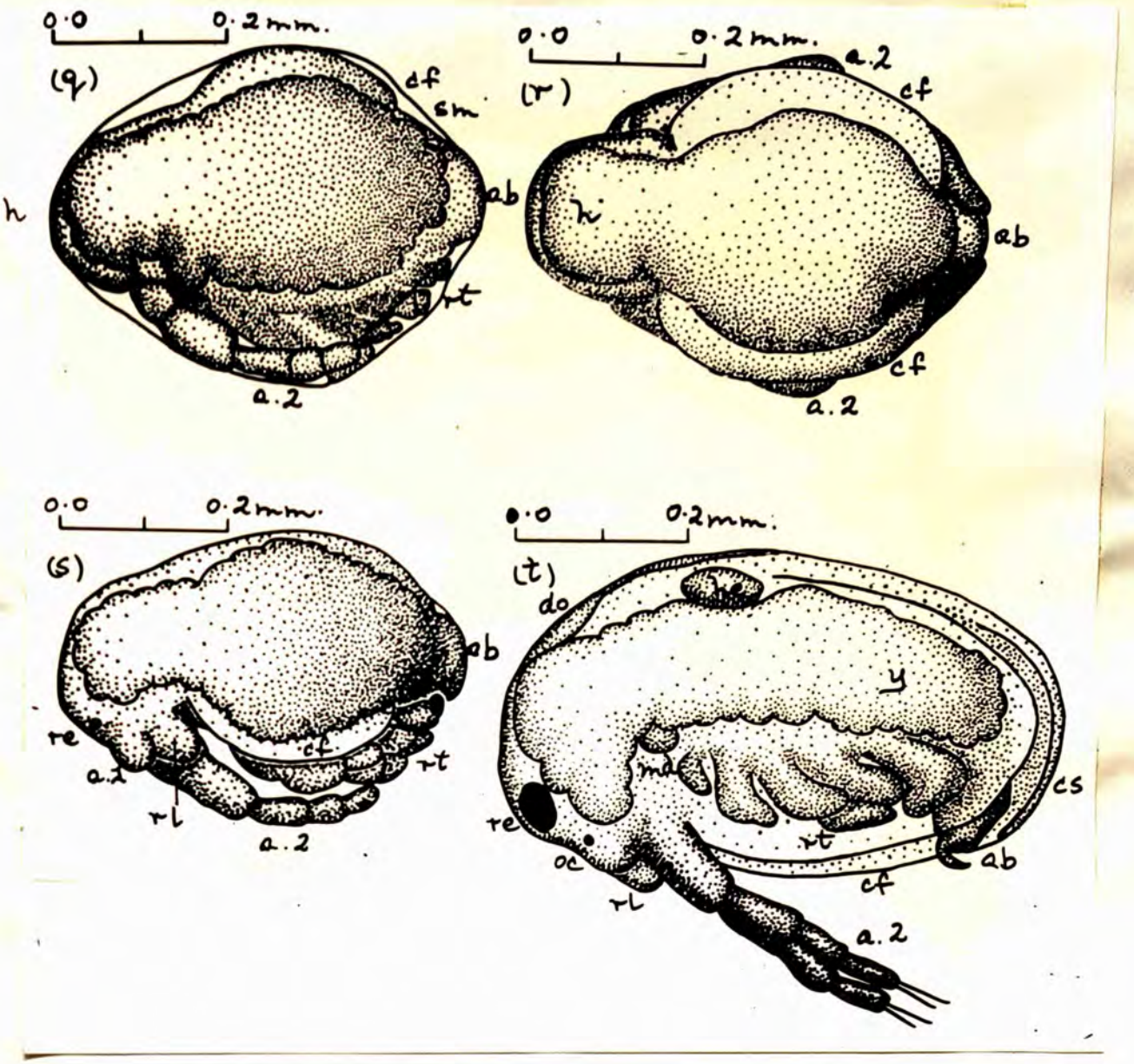


Figure 1. (q) 52¾ hour. a.2, second antenna; ab, abdomen; cf, carapace fold; h, head; rt, rudiment of thoracic appendage; sm, second (embryonic) membrane.

(r) 59 hour. Dorsal view. a.2, second antenna; ab, abdomen; cf, carapace fold; h, head.

(s) 62 hour. a.1, antennule; a.2, second antenna; ab, abdomen; cf, carapace fold; re, rudiment of eye; rl, rudiment of labrum; rt, rudiment of thoracic appendage.

(t) 68¼ hour. a.2, second antenna; ab, abdomen; cf, carapace fold; cs, caudal spine; do, dorsal organ; he, heart; md, mandible; oc, ocellus; re, rudiment of eye; rl, rudiment of labrum; rt, rudiment of thoracic appendage; y, yolk.

developed dorsal spine (cs). The heart (he) beats sporadically. There is movement of the abdomen (ab) which bears a caudal furca.

At 72 hours (Fig. 1,(u)), the embryo shows a considerable amount of movement, with the heart beating intermittently. The mandibles (md) and the abdomen (ab) also move. The first antennae (a.1) appear slightly reduced in size. The two halves of the compound eye (re) are close together, are reddish brown in colour and show a transparent outer edge which medially is common to the two of them. The midgut caeca (mc) are even more distinctly visible. Yolk globules (y) are still present both in the head and body but mostly in the dorsal region.

At 74 hours, the appendages are definitely fully formed and the heart beat has increased. There is further coalescence of the eyes which are becoming darker.

At 79¾ hours (Fig. 1,(v)), the midgut (me) is distinguishable among the yolk and approaching the dorsal part of the body. The yolk globules are surrounding the outside of the midgut. The heart beat is still sporadic. The antennal and mandibular muscles are visible in the region of the junction of the head with the body.

At 80½ hours, the two halves of the compound eye are nearly fused and almost black. The dorsal spine is still tucked in against the body.

At 84 hours (Fig. 1,(w)), there is increased movement of the second antennae, the mandibles and the heart, the latter still intermittent. Yolk is still present in considerable part of the head and body.

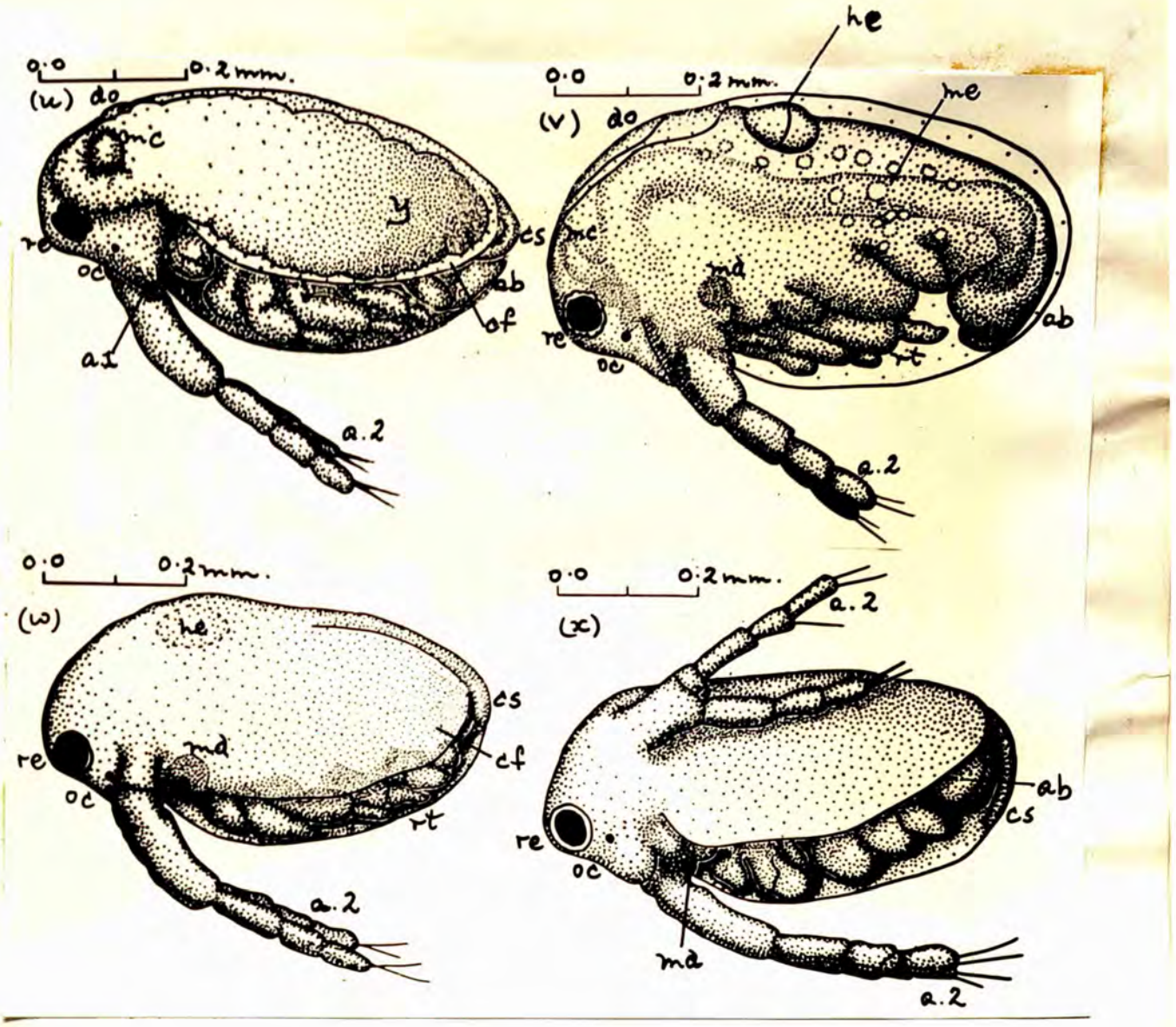


Figure 1. (u) 73 hour. a.1, antennule; a.2, second antenna; ab, abdomen; cf, carapace fold; cs, caudal spine; do, dorsal organ; mc, caecum of mesenteron; md, mandible; oc, ocellus; re, rudiment of eye; y, yolk.

(v) 79¾ hour. a.2, second antenna; ab, abdomen; do, dorsal organ; he, heart; mc, caecum of mesenteron; md, mandible; me, mesenteron; oc, ocellus; re, rudiment of eye; rt, rudiment of thoracic appendage.

(w) 84 hour. a.2, second antenna; cf, carapace fold; cs, caudal spine; he, heart; md, mandible; oc, ocellus; re, rudiment of eye; rt, rudiment of thoracic appendage.

(x) 88½ hour. a.2, second antenna; ab, abdomen, cs, caudal spine; md, mandible; oc, ocellus; re, rudiment of eye.

At 88½ hours (Fig. 1(x)), the movements are moderately vigorous but the dorsal spine of the carapace (cs) has not been extended. The two halves of the compound eye (re) have fully coalesced.

At 91 hours, the dorsal spine is still not extended and yolk globules are still present.

At 92½ hours, the dorsal spine of the carapace is extended, and the limbs are moving causing vigorous movements of the body. The first antennae are small. The heart is beating regularly. The dorsal organ is clearly seen. The shell gland, or maxillary gland, is noticeable as are the caudal setae. There are still numerous yolk globules surrounding the midgut.

The mother may take 2½ to 3 hours to release her entire brood of young. The young emerge as miniature adults except for a few minor differences noted in a later section (see p.110).

Measurements were made of the eggs and embryos at different ages. The measurements show a very gradual increase in size during the early stages of development. The egg is never quite spherical but remains slightly ovoid. With the shedding of the outer egg membrane there is a marked increase in length but throughout most of the embryonic development the dorso-ventral axis remains of about the same length. The actual measurements vary according to the initial size of the egg. Representative figures to indicate the relative increases in length are:- 0.3 mm at 4 hours; a gradual increase up to 0.37 mm immediately prior to the shedding of the outer membrane; 0.42 mm after the

shedding of the outer membrane; a gradual increase up to 0.5 mm immediately prior to the shedding of the second membrane; 0.6 mm after the shedding of the second membrane; 0.75 mm at the time of hatching.

The observations of Obreshkove and Fraser were made at 25°C. and at that temperature 46 hours were required before the extension of the dorsal spine whereas at 18°C. 90 to 92 hours were required. Therefore if Obreshkove and Fraser's times are doubled an approximation to the stage of development attained by embryos developing at 18°C. will be obtained, providing that an increase in temperature causes an increase in rate of development equal throughout the whole span of the embryonic life. This is a necessary assumption in order to be able to compare the two sets of observations, but it may not be a strictly correct assumption (compare Needham, 1931, 1942). Needham refers to the work of Brown (1926, 1927) who studied the effect of temperature on the rate of development in Cladocera. His work was done on Pseudosida bidentata in the second adult instar. Brown found that if an animal was allowed to develop for 50% of the total normal time at 15°C. and then transferred to a temperature of 25°C., it might take less or more time to finish its development than would be predicted on the basis of the fact that it had still 50% of the normal time to go at 15°C. He found that the gain or loss was statistically significant. There is no direct mention of the effect of the change of temperature on the embryos, but it seems likely that the effect on the embryos was the same as that on their mother.

The observations which I have made on the whole agree with those of Obreshkove and Fraser (a fact which suggests that the increase in the rate of development is approximately equal throughout) with the exception of one or two points. Obreshkove and Fraser's observation that at 9 hours (= 18 hours at 18°C.) there is an invagination indicating the cephalic region, may be correlated with the observation that at 18 hours there is the first modification of the external surface in the form of shallow indentations. These indentations delimit the second antennae, however, and not the cephalic region. The cephalic region is not recognisable until the egg is about 34 hours old (18°C.), that is, when the egg membranes are being shed. Obreshkove and Fraser note that at 18 hours (= 36 hours) there is the beginning of the development of the cephalic appendages, whereas according to the observations at 18°C. the second antennae are well developed with two branches which are dividing into segments. The possible explanation for this disagreement in the two sets of observations is given later. The next observation by Obreshkove and Fraser, made at 21 hours (= 42 hours), records an increase in length by 60μ. This increase in length, together with the beginning of the cephalic appendages noted in their previous observation, is almost certainly due to the shedding of the egg membrane which would enable the cephalic appendages to be more readily seen. The shedding of this membrane does not appear to have been recorded by Obreshkove and Fraser. However the time corresponds to that when the egg membrane was shed by the 18°C.

eggs, that is 34 to 41 hours after laying. The first movements were observed at much the same times in the two series, at 30 hours (= 60 hours) at 25°C. and at 63½ to 67 hours at 18°C. Obreshkove and Fraser also give measurements for the increases in length at the various stages and of the heart rate in number of beats per minute. They found that the first few irregular heart beats occurred at 30 hours (= 60 hours); that at 35 hours (= 70 hours) the rate was 20 beats per minute; that at 36 hours (= 72 hours) the rate was 32 beats per minute. Thereafter the rate of beat increased rapidly until in the second instar the rate was 180 to 200 beats per minute. The only observations on the heart rate of the embryos at 18°C. were some counts made shortly before the extension of the dorsal spine when the rate was found to be about 150 beats per minute. Thus in general the observations made by Obreshkove and Fraser on embryos at 25°C. agree very well with those made on embryos at 18°C. when the shedding of the egg membrane is taken into account. Obreshkove and Fraser note the occurrence of the two membranes surrounding the egg but do not indicate the shedding of either of them.

Edmondson (1955) comments on the apparently greater development in Daphnia magna (following Obreshkove and Fraser's description) than in D. pulex (Edmondson's own observations) of the body and its appendages at the time when the head elongates. The present observations made on the D. magna confirm that the second antennae are well developed and that the mandibles, thoracic appendages and carapace folds have appeared before the

elongation of the head. Edmondson's "early stage" apparently terminates at, or soon after, the shedding of the egg membrane.

The embryo hatched from the outer egg membrane is sometimes referred to as the "nauplius stage". The embryo is however further developed than the usual nauplius stage since the thoracic appendages are beginning to develop and the abdomen and carapace folds rudiments are also present.

b. The ephippial egg.

Apparently there is no previous description of the development of the living ephippial eggs. The eggs develop inside the ephippium, or modified brood pouch of the mother, and show very little change from the time of laying until the time of the shedding of the ephippium. The structure of the ephippium will be described in a later section (p.100). In Daphnia magna only two eggs are laid into the ephippium. The eggs remain rather more ovoid than the parthenogenetic, or non-ephippial, eggs. Earlier authors have stated, seldom with actual measurements, that the ephippial eggs are larger than the parthenogenetic eggs but this has been disproved by Green (in press) who found that the ephippial eggs may be larger or smaller than the parthenogenetic eggs. Baldass (1942) gives measurements for the parthenogenetic and ephippial eggs of Daphnia pulex indicating that the ephippial eggs are the larger but he does not state how many eggs these measurements are based on. The ephippial egg is densely packed with yolk globules which are smaller than those of the parthenogenetic eggs and appear more opaque. No oil droplets are visible in the living ephippial egg. As in the parthenogenetic egg, neither nucleus nor cytoplasm is seen.

The development within the modified brood pouch is similar to that of the parthenogenetic egg but a great deal slower. A transparent peripheral layer appears which becomes irregular and is apparently slightly wider than that in the parthenogenetic egg.

Small clear areas may often be seen in the peripheral layer during the latter part of the development in the brood pouch. A particularly well-marked unevenness of the inner edge of the peripheral layer at one end of the egg becomes noticeable, and a small circular lighter area appears about halfway along one side of the central mass of yolk approximately one day before the ephippium is shed. Occasionally a number of very small clear globules appear at either end of the egg but it is possible that this is a pathological sign. A shallow depression near one end is the only sign of change of external form before the eggs and ephippium are shed. The later development is similar to that of the parthenogenetic egg, although again slower. The young animal may hatch in about three and a half days after the shedding of the ephippium, or it may not do so for months or even years.

The small amount of change visible during the development of the ephippial egg while still carried by the mother makes a selection of arbitary stages almost impossible, and for the majority of work requiring such indices females carrying parthenogenetic eggs can be used.

Some physiological aspects of development.

a. The role of the brood pouch of the mother.

The brood pouch of Cladocera such as Daphnia is a space in the dorsal part of the animal enclosed by the carapace folds. It is in contact with the external medium, the amount of interchange varying from species to species among the Cladocera. Anterior to the brood pouch lies the heart, and ventral to it in the thorax the midgut flanked on either side by the ovaries. The ovaries communicate by minute oviducts with the brood pouch and here the eggs remain until released by the mother as miniature adults. A brood of eggs remains in the brood pouch for approximately the length of one instar. In Daphnia, the eggs are kept in the brood pouch by a dorsal process of the posterior part of the body. The brood pouch provides shelter for the eggs and embryos; it has also been claimed to provide nourishment.

In Daphnia the dorsal wall of the body, forming the base of the brood pouch, is smooth and unmodified but in Moina and in the Polyphemidae there has been a proliferation of the hypodermal cells and the cells have become taller. It has been claimed, for example by Weismann (1877), that the pressure of the blood is raised while passing through these cells and that filtration occurs from the blood into the brood pouch. Weismann believed that in Cladocera such as Daphnia filtration occurs through the lining of the carapace fold forming the sides of the brood pouch. This would be prevented in Moina by the expansion of the brood

pouch due to the large quantity of eggs and embryos causing the layers of the carapace fold to be closely pressed together and consequent stoppage of the flow of blood between them. Thus the site of the filtration apparatus has been shifted to the base of the brood pouch. In the Polyphemidae the need for a greater efficiency arises because the eggs are small and contain very little yolk yet they develop into young more than ten times their volume. In the early stages of development when the eggs have less need for outside help it is still possible for the blood to flow through the carapace folds so that less will pass the "nutrient gland" in the ventral part of the brood pouch. Later swelling of the brood pouch stops the flow through the carapace folds and all the blood is directed past the "nutrient gland", the developing embryos being now in greater need of nutriment. In the Cladocera in which such an arrangement occurs there is a corresponding increase in the efficiency of the closure of the brood pouch.

It had been recorded by Lubbock (1857) that none of the Daphnia magna eggs which he removed from the brood pouch of the mother had developed. Weismann (1877) found that eggs of Moina, Polyphemus and Bythotrephes did not develop when removed from the brood pouch of the mother and concluded that the same was true for all Cladocera. He stated that the supply of filtrate was greater when the eggs were smaller compared with the fully grown embryos, but that when the eggs were rich in yolk the filtration was still present although mixed with the surrounding medium. The latter category would include Daphnia in the case

of which he based his argument on the increase in volume during the development of the parthenogenetic eggs. Weismann believed that the ephippial eggs were initially larger than the parthenogenetic eggs and yet the resulting embryos of the same size, and that the explanation of this lay in the fact that the ephippial eggs received no nutritive fluid or "Fruchtwasser". But it is now known that in Daphnia magna the ephippial eggs may be larger or smaller than the parthenogenetic eggs (Green, 1956,a). Since Weismann's time, nourishment of the eggs by the mother has been claimed: by Gravier (1931) for Moina, Polyphemus and Bythotrephes and to a lesser extent in the yolkier eggs (but depending almost entirely on Weismann's work); by Rammner (1931) for Evadne and Podon, in contrast to D. magna where he did not find it; by Krogh (1939), again quoting Weismann, for Moina and Polyphemus; and more recently by Haget (1945; 1946) for Daphnia.

But the development of eggs outside the brood pouch, in distilled water, tap water or pond water has also been found, for example: by Agar (1913) for Simocephalus and Daphnia pulex; by Ramuźt (1914) for D. pulex; by Rammner (1931) for D. magna; by Krogh (1939), quoting Ramuźt for Daphnia spp.; by Obreshkove and Fraser (1940) for D. magna: and lately by Hoshi (1950 a; 1951 b) for Simocephalus vetulus.

These investigations, with the exception of the work of Haget, indicate that while the poorly yolked eggs probably receive and require supplementation during their stay in the

brood pouch in the form of nutrient from the mother, the more richly yolked eggs do not require such nutrient. In connection with this observation, it is interesting to note that Brammertz (1913) found that the poorly yolked eggs of Moina do not contain glycogen, whereas the richly yolked eggs of Daphnia do contain glycogen. This indicates one way in which the eggs of Moina probably require assistance from the mother.

In order to test these views a number of observations were made on eggs of Moina and compared with those on Daphnia eggs. Development of the eggs or embryos of Moina was not obtained outside the brood pouch of the live mother. If the eggs are removed from the brood pouch and placed in distilled water, run tap water or filtered water from Regent's Park Lake, or if the mother is killed without damaging the eggs, the eggs do not develop. On the other hand, the eggs of Daphnia if removed from the brood pouch of the mother at least three hours after laying will develop in any of these three media, or they will develop in the brood pouch of a dead mother. There seems thus to be this interesting difference between two closely related genera, apparently related to the amount of yolk in the egg.

It is possible, as Ramult (1914) suggested, that in Daphnia some substance essential to development enters the brood pouch at the same time as the eggs, since there is an initial period of about three hours during which it is not possible to remove the eggs from the brood pouch and obtain development. This period corresponds to the time when there is a swelling of the eggs with change of shape, and is correlated to the state of the egg

membrane, which is becoming firm. It is more likely that failure to develop is due to differences in osmotic pressure. This is supported by the fact that the majority of eggs burst on removal from the brood pouch during this period. Apparently the egg membrane is not yet able to withstand the differences in osmotic pressure on the two sides of the membrane. Also during this initial period the egg is particularly susceptible to toxic poisons. Later the egg membrane is relatively very impermeable, as is indicated for example by failure of stains to penetrate.

Haget (1945; 1946) suggested that there is a gradual increase of the osmotic pressure in the brood pouch which corresponds to the increase in the internal osmotic pressure of the Daphnia female during an instar recorded by Fritzsche (1917). The "liquide incubateur" would contain substances proceeding from the maternal blood and its osmotic pressure would thus vary in the same way as that of the mother. Haget's results were based on a consideration of the work of Ramult and Pryzlecki and on some experiments involving the substitution of older embryos into the brood pouches of animals carrying younger embryos or eggs, and vice versa. When the older embryos were substituted for younger, they freed themselves of their larval envelopes sooner than they would have done according to their "true" mother, whereas in the inverse situation there is a retardation of the bursting of the egg membrane although the animal continues to develop within the egg membrane. Since the osmotic pressure of the egg increases during development (p. 50), these results can

be explained if the osmotic pressure of the fluid in the brood pouch also increases but is initially lower. The experiments themselves however are based on small numbers.

b. The osmotic pressure of the egg and related factors.

In relation to the suggestion of a nutrient fluid in the brood pouch of the mother, it is important to consider the osmotic pressure of the eggs and that of the surrounding environment. In both parthenogenetic and ephippial eggs, the osmotic pressure rises during the course of the embryonic development until in the later stages it is about four times that in the early egg. Pryzlecki (1921, a and b) determined the osmotic pressure of the eggs of Daphnia magna, using glucose solution and measuring the concentration required to cause no change in the egg volume, that is the point of isotony. In the parthenogenetic eggs, six hours after laying into the brood pouch Pryzlecki (1921, a) found $\Delta = 0.186^{\circ}$, while at 84 hours (temperature not stated but must be less than 18°C) $\Delta = 0.759^{\circ}$. In the ephippial eggs six hours after laying $\Delta = 0.240^{\circ}$, and at 30 to 60 hours $\Delta = 0.734^{\circ}$ to 0.740° (Pryzlecki, 1921, b). Similar results were obtained for Simocephalus vetulus. Pryzlecki regarded the increase in osmotic pressure in the ephippial eggs as the cause of the stopping of development. The increase is not completely uniform but is less pronounced in the later stages of development. During the diapause of the ephippial eggs the osmotic pressure remains constant and there is no dehydration. With the recommencement of development the osmotic pressure sinks by 55%, to rise subsequently during further development. In contrast to the increase in the osmotic pressure of the eggs and embryos, the osmotic pressure of the brood pouch does not change. In general the

behaviour of the osmotic pressure is comparable to that found in Amphibia. Pryzlecki believed that most of the increase in osmotic pressure is due to osmotically active substances formed during development.

Krogh (1939) suggests the possibility of an active absorption of salts from the surrounding medium but considers this is unlikely because normal development is possible in distilled water. Pryzlecki attributed 24% of the lowering of the osmotic pressure of the ephippial egg at the time when development recommences to the absorption of water, 34% to the excretion of osmotically active substances into the perivitelline liquid, and the remainder to probably either the expulsion of osmotically active substances to the exterior of the egg or to a changing of these substances into less active bodies. The osmotic pressure of the perivitelline changes in parallel to that of the embryo but has a smaller value.

Although the osmotic pressure of the egg rises steadily, the elastic tension of the egg membrane increases less regularly. Between the sixth and the twenty-fourth hour after laying the extension of the membrane increases from 20 to 67% (Pryzlecki, 1921,a). There is then a period during which no further tension of the membrane occurs until the sixtieth hour of development when the egg membrane bursts and a period of extension of the larval membrane follows. The actual growth of the egg or embryo depends on the properties of the membranes, because although the osmotic pressure inside the egg is steadily increasing, growth is stopped for the 24 to 60 hour period due to lack of extensibility

of the membrane, whereas rupture of each membrane is followed by an increase in growth.

If the egg membrane is prevented from rupturing, differentiation continues although growth cannot and a "dwarf" embryo results. The membrane does not rupture if the osmotic pressure of the surrounding medium is increased, for example if the eggs are placed in NaCl solution. N/30 NaCl will cause "closed" development of some of the eggs of Daphnia pulex, N/15 of all the eggs. This was determined by Ramuŕt (1925). If the membrane is artificially ruptured normal development will continue even if the young are left in the salt solution. Moderately dilute calcium will also cause "closed" development (Phear, personal communication). "Closed" development occasionally occurs in eggs which are developing in the brood pouch. Ramuŕt noted that eggs of the same brood showed different powers to develop in the salt solutions. This may perhaps be correlated with the fact that in broods developing normally, eggs of different sizes are found and eggs at different stages of development.

The hatched young and adult animals will tolerate salt solutions which cause "closed" development of eggs, and their osmotic pressure is normally higher than that of the surrounding medium. The studies of Fritzsche (1917) on the adult indicated that the internal osmotic pressure is directly related to processes of growth. At a high external osmotic pressure moulting is difficult; similarly at a high external osmotic pressure rupturing of the egg membrane does not take place.

The latter indicates that the egg normally absorbs water and indeed that inflow of water is essential to normal development. This inflow is correlated with the increase in volume that takes place. Inflow of water due to differences in osmotic pressure brings about the hatching of the embryo which as it swells up breaks the membranes. A similar method of hatching is described for copepods (Needham, 1931); and Manton (1928) suggests that the bursting of the egg membrane of Hemimysis is probably mainly due to increase in the volume of the yolk by absorption of water from the surrounding medium. In this respect it is interesting to note that the eggs of Artemia salina will not hatch in strong salt solutions in which the adults normally live (an observation by Becking, quoted by Needham, 1931). Nevertheless the eggs of phyllopods such as Artemia and of cladocerans will withstand considerable desiccation without losing the power to develop.

Various experiments have been undertaken to find a salt solution physiologically balanced for the hatching of the parthenogenetic cladoceran egg. Rey (1935) reported development in a modified Ringer solution, but failure to develop in distilled water, or single solutions of NaCl, KCl or $CaCl_2$. Development may depend upon the concentration of the salt and possibly on the pH of the distilled water. The pH of the developing egg is usually neutral (Needham, 1942). Hoshi (1951,b) obtained 67% development in distilled water of pH 7.2, and 69% to 80% development in various artificial media containing NaCl, KCl and $CaCl_2$, a combination of all three being the best. None of Hoshi's Simocephalus vetulus eggs developed in the modified Ringer

solution used by Lévy for his experiments on the effect of various ions on the heart rate of Daphnia magna. 100% development occurred in filtered pond water. Obreshkove and Fraser (1940) also found that Lévy's medium prevented development. Fluid from the brood pouch of the mother gave no advantage. Ramult (1914) obtained development in distilled water and pond water but found that egg albumen killed the eggs, while the blood of the mother had no effect on the development.

Failure to develop in various artificial media may be due to an upsetting of the osmotic pressure relations or to the direct effect of one of the substances upon the egg. Little is known about the variations in susceptibility to toxic substances during the development of arthropods. In Daphnia susceptibility is greatest during the initial period before the membrane becomes firm. Van Herwerden (1921, quoted by Needham, 1931) found that Daphnia pulex eggs subjected to radium emanation proceeded to develop as far as gastrulation and all then died. This agrees with the indication found in several other animals that gastrulation is the most critical point in the development.

When irradiated with soft X-rays the early stages of the development of Daphnia proved more vulnerable than the later stages (Sneider and Kersten, 1936). The adults, as well as the eggs of the next brood, were apparently unaffected. The younger stages in the development of an animal are in general considered to be the most vulnerable (Needham, 1931), the possibility being suggested that this is concerned with the effect of the X-rays

on the absorption and utilization of some constituent of the yolk, such as lecithin.

c. The contents of the egg.

An appreciable amount of work has been done on the constitution of the insect egg but very little on that of the egg of the Crustacea. In the newly laid egg of an animal, proteins form the vitelline granules; the fats occur in the form of droplets; glycogen appears as granules scattered through the egg (Brachet, 1950 translation of 1945). Kaudewitz (1950) centrifuged the egg of Daphnia pulex at 500 g. for five minutes leading to stratification of its contents. Even in this relatively non-yolky Daphnia egg, a large quantity of yolk appeared at the centrifugal pole and a small amount of plasma and a large oil droplet at the centripetal pole.

In Daphnia magna the yolk occupies an extremely large proportion of the newly laid egg. In the parthenogenetic egg the yolk is in the form of granules of many sizes and irregular shapes, while in the ephippial egg the yolk granules are generally smaller and of more even size and shape. There appears to be a slight difference in the staining reaction between the yolk of the two types of egg and there is also a marked difference in staining reactions of the yolk of the same type of egg at different stages of development or in different parts of the egg at the same time. Thus in the parthenogenetic egg stained with Heidenhain's Iron Haematoxylin the yolk granules at first stain an even "smooth" blue, later some of them stain brown and with further development the percentage of granules staining brown increases. With Mallory's Triple stain the yolk at first stains reddish-orange, with Heidenhain's Azan stain (a modification of

Mallory's Triple) red. As the yolk cells are formed the yolk which they contain stains blue with either of these stains, the remainder of the yolk still staining reddish-orange or red. As development proceeds the proportion of yolk staining blue increases and it is noticeable that in general the larger yolk globules stain blue, the smaller red. The reactions to the three stains occur both after corrosive sublimate fixatives and after Smith's Formol-Bichromate. As the yolk cells are formed part of the yolk contained within them assumes a granular appearance. It appears that the yolk which stains pale brown with Iron Haematoxylin and that which stains blue with the other two stains is yolk in the process of assimilation. With Mallory's Triple stain and with Heidenhain's Azan stain the red or orange indicates true yolk, the blue a change to material of a more cytoplasmic nature. The yolk of the ephippial eggs stains much darker and more coarsely with Heidenhain's Iron Haematoxylin than the yolk of the parthenogenetic eggs, even when the surrounding cytoplasm has been differentiated to a very pale blue-black. With Mallory's Triple stain the yolk of the ephippial eggs stains yellow-orange as it does also with Heidenhain's Azan stain. It is interesting to note that Manton (1928) found that in the early stages of Hemimysis also the yolk stains red with Mallory's Triple stain, later staining blue.

The above observations are only differences noted in slides prepared for the study of the general embryological development. No investigations have been made of the actual chemical composition of the yolk or of its changes throughout development.

Such studies would prove extremely interesting. Possible differences between the yolk in the non-ephippial and ephippial eggs are also of importance, particularly in view of Andrewartha's (1952) suggestion that diapause in insects may be due to some metabolic process associated with food reserves in the yolk or fat.

The fat in the egg of Daphnia magna is contained within two or three large droplets which diminish slightly in volume during development towards the end of which they break up into small droplets which are apparently taken into the cytoplasm of the fat cells.

Abundant quantities of both glycogen and fat were found in the eggs of Daphnia magna by Gallistel (1936-7). She did not find any great difference in the kind of reserve material in non-ephippial and ephippial eggs.

In Simocephalus vetulus the glycogen is found in the cytoplasmic parts of the egg rather than in the yolk, according to the investigations of Hoshi (1950 c; 1951 a; 1953; 1954). This is in agreement with Brachet's general statement about the contents of eggs. In S. vetulus, the amount of glycogen gradually increases during the early embryonic development, from 0.70% of the wet weight just before the shedding of the egg membrane to 0.91% in the stage between the shedding of the two membranes, falling again to 0.71% in the young animal just released from the brood pouch, after which it again increases. The glycogen gradually appears in the muscles, the wall of the alimentary canal and the excretory organ. Both the histological

properties and enzyme tests suggest that the glycogen is combined with protein and fat. The digestibility of the glycogen increases during embryonic development and is facilitated by a combination of amylolytic, proteolytic and lypolytic enzymes. Under semi-anaerobic conditions (water containing 0.02 ml. of oxygen per litre) the glycogen content is nearly constant throughout the embryological development, that is 0.50%, 0.67% and 0.53% of the wet weight in the stages noted above. When the oxygen content of the water is varied, the amount of glycogen used by the embryos is inversely proportional to the oxygen content. When the length of time in the semi-anaerobic conditions is varied, the amount of glycogen used is directly proportional to the number of hours spent under these conditions. The maximum survival time under semi-anaerobic conditions for the stage before the shedding of the egg membrane is 11 hours.

The amount of fat contained in the oil droplet decreases during embryonic development, which suggests its utilization as a source of energy. Taken together with the information that the R.Q. increases gradually during development, these investigations led Hoshi (1950,a,c) to suggest that the energy source of development is mainly fat in the stage before the shedding of the egg membrane with some participation of protein and carbohydrate. Later, after the shedding of the egg membrane, the fat decreases in importance while the protein and carbohydrate increase in importance, until at the end of the embryonic development the carbohydrate plays the principal role in the

energy supply. These results are in contrast to the suggestion made by Needham (1931; 1942) that carbohydrate metabolism is associated with the early developmental stages; protein and fat with the later stages. Needham's conclusions were based on information from the chick, minnow, grasshopper, crab, a nematode and a gephyrean worm. However it is possible that determinations of R.Q. at a stage earlier than those so far made would indicate an initial slight decrease and thus suggest a very early carbohydrate metabolism.

A decrease in the dry weight of 16 to 25% during the embryonic development has been shown by Green (1956,a). A similar loss of dry weight during development has been shown to occur in other animals, for example the hen and the silkworm.

Other substances present in the egg of Daphnia include carotenoids and haemoglobin. Carotenoids may colour the egg green or orange, the amount present varying greatly. Their presence is apparently not essential because eggs develop normally without them. The main factor influencing the amount present appears to be the diet of the mother. Teissier (1932) first established the presence of a carotenoid in the eggs of Daphnia pulex and found that if the mother was given food devoid of carotenoids the eggs lacked carotenoid, whereas if the food contained certain carotenoids, carotenoid was also present in the eggs. The carotenoid is at first evenly distributed through the cytoplasm, but with the formation of the yolk cells it is found only in the cytoplasm of these cells (Green, personal communication).

Other carotenoids are sometimes found in the oil droplets, colouring them yellow or orange. Green has also found that at about the time of the release of the young from the brood pouch of the mother, the carotenoid protein disappears from the cytoplasm of the yolk cells (now become fat cells) whereas the colour of the fat globules becomes a much more intense orange. Green suggests that this would appear to indicate a break in the link with the protein causing a change in solubility, so that the freed carotenoid is taken into the fat globules. The carotenoid in the eggs of Daphnia has no known function.

Haemoglobin is found in the eggs and embryos of Daphnia in varying amounts. The amount present depends principally on the number of eggs in the brood (Green, in press), although the concentration of haemoglobin in the blood of the mother and indirectly the nutrition of the mother will be important. The presence of haemoglobin was detected by Teissier (1932) in the eggs of Daphnia pulex; and in the eggs of D. magna by Fox (1948) who found that haemoglobin is present in the parthenogenetic but not in the ephippial eggs. The ephippial eggs do, however, contain a considerable amount of haem. The haemoglobin passes from the blood of the mother into the eggs in the ovary a few hours before laying (Dresel, 1948). The amount of haemoglobin present decreases as the embryos develop (Fox, 1948), and as the haemoglobin decreases the amount of daphniarubin in the gut increases (Phear, 1955). The total haem content remains the same throughout the embryonic development. It has been found by Fox et al (1951) that the presence of haemoglobin

apparently accelerates the development of the parthenogenetic eggs in the later stages.

The eyes of Daphnia when first formed are pink, darkening gradually to a reddish brown and finally assuming the black colour displayed by the eyes of the adult. A similar series of colour changes occurs during the development of the eyes of Gammarus, in which Ford and Huxley (1927) noted that the red pigment is soluble in alcohol, the darkening being caused by a deposition of melanin.

d. The respiration of the egg.

The respiratory quotient increases during the embryonic development. This was determined in Simocephalus vetulus by Hoshi (1950,a) and agrees with the latter part of the curve for Carcinus moenas obtained by Needham (1933). Hoshi's determinations did not start until the stage immediately preceding the shedding of the egg membrane so that it is possible that an earlier slight decrease in the respiratory quotient may occur, as it does in Carcinus moenas. However the sea urchin (Lindahl, 1942) shows only an increase. Experiments with developing Simocephalus vetulus (Hoshi, 1952) indicate that before the shedding of the egg membrane the egg can survive longer under semi-anaerobic conditions than it can in later stages. The rH shows large changes under semi-anaerobic conditions and is less than that during development under normal conditions, when the changes are slight. Hoshi (1949) has also suggested that the eggs have a high metabolic activity, relative to the older stages.

The Development of the Parthenogenetic, or Non-ephippial, Egg.

A study of the embryological development of Daphnia magna was undertaken with a view to the elucidation of the main aspects of a type of development imperfectly known and with several interesting features. Special attention was devoted to the early stages, to the process of gastrulation and germ layer formation, to the further development of the endoderm, to the formation of the yolk cells, to the further development of the mesoderm in the formation of the heart, excretory glands and genital organs and to the early history of the dorsal organ.

No previous work has given a satisfactory account of the early development, while the accounts of gastrulation and germ layer formation have nearly all been in disagreement with each other. The method of formation of the mesenteron is of a type very rare among the Crustacea and imperfectly known. The yolk cells have been regarded as having a mesodermal origin in Daphnia, but in the majority of Arthropoda the yolk cells develop from endodermal cells and a mesodermal origin has generally been disproved. The fate of the mesoderm is of interest in view of the work of Cannon (1924; 1927) on the development of Estheria and Chirocephalus, demonstrating the existence of segmental coelomic cavities in the mesoderm and the relationship of these cavities to the formation of the heart and excretory organs of the adult. It is therefore of considerable interest to find out if there are any vestiges of metameric coelomic sacs in the embryo of a cladoceran. Such coelomic cavities are present in

all classes of Arthropoda, including a large number of Crustacea. The dorsal organ is present in the embryos and occasionally in the adults of various Cladocera and its function is not known. A study of its development and history was undertaken in the hope of adding to the understanding of this structure.

Aberrancy.

An interesting aberrant form was noted in a brood of embryos the majority of which were well developed with all the appendages present and nearly ready to shed the second, or embryonic, membrane. One of the eggs had remained spherical and was filled with granular cytoplasm, which was almost uniform and contained about six relatively enormous nuclei, their diameter being approximately one-fifth that of the whole egg. The nucleolus was proportionally large and spherical.

Disintegrating embryos were sometimes observed in the brood pouch of the mother. Either the whole or part of the brood disintegrated.

a. The early stages of development.

The newly laid egg is sausage-shaped but gradually becomes ovoid (Fig.1a; Fig.2) and remains not quite spherical for the remainder of its development until the bursting of the first egg membrane. The eggs vary considerably in size, but average about 350µ by 330µ. They are enclosed within a thin egg membrane which becomes firmer during the first few hours in the brood pouch with the formation of an outer chorion and an inner vitelline membrane. The eggs contain a large quantity of yolk in the form of globules of various sizes (Fig.2,y).

The initial or ripening division begins while the egg is still in the ovary and has been described in Daphnia magna by Mortimer (1956) and in D. pulex by Baldass (1942). The single polar body is formed about thirty minutes after the egg has been laid into the brood pouch (Fig.3; Fig.4). This division shows the nucleus in a vesicular phase which has often been observed in the nuclear divisions of D. magna. The egg nucleus then migrates to the centre of the egg. Mortimer could not identify the egg nucleus after the dividing off of the polar body and the beginning of the migration towards the centre of the egg.

In the centre of the egg the nucleus is surrounded by a small area of cytoplasm (Fig.5,nu). There is also an extremely thin layer of cytoplasm at the periphery of the egg (Fig.2,pc), varying little in width. The remainder of the egg is filled with yolk (y) and a small number of oil droplets (od). In Daphnia pulex Baldass described a single oil droplet, but in

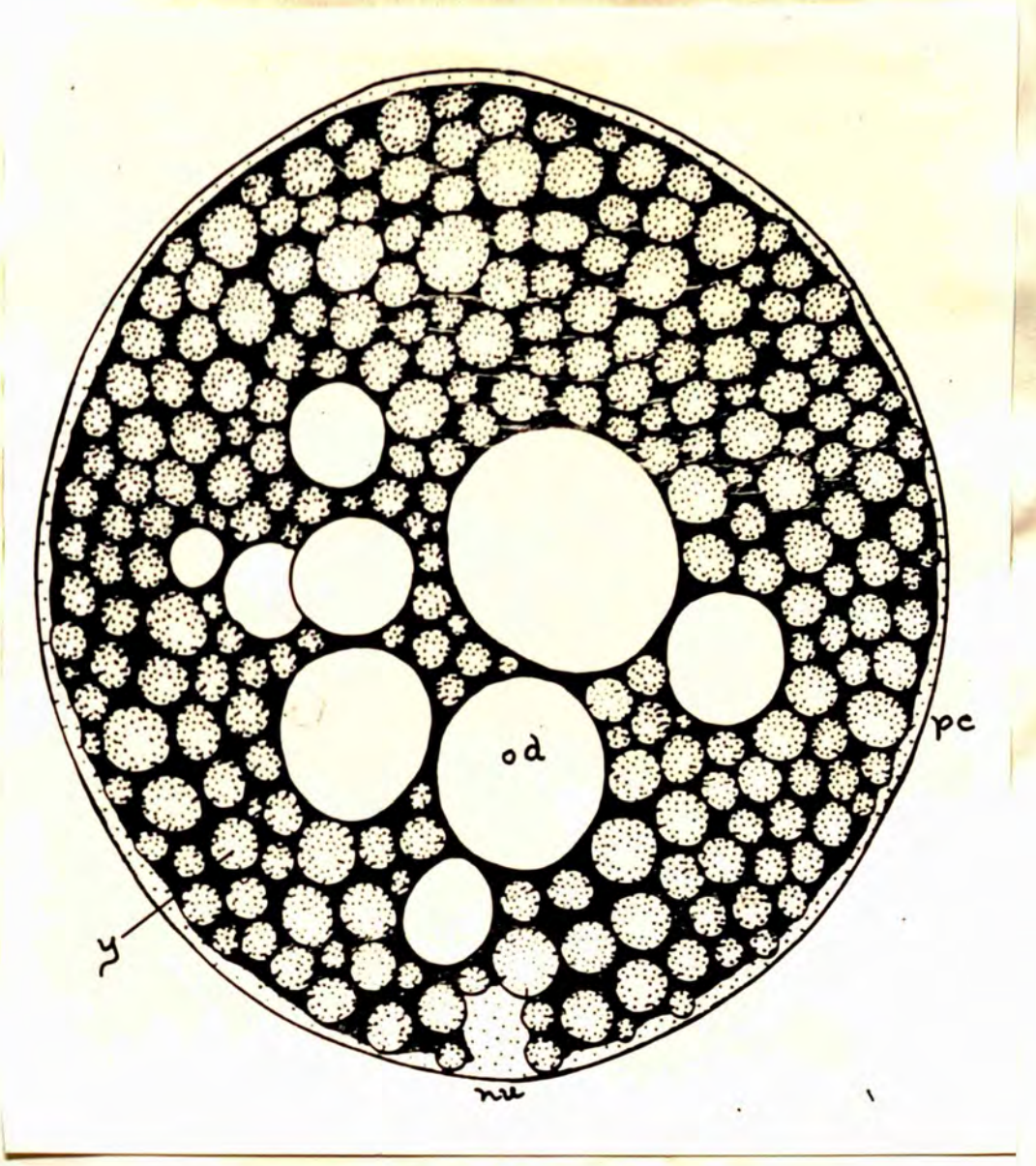


Figure 2. A diagrammatic reconstruction, based on serial sections, of the parthenogenetic egg of Daphnia magna half an hour after it has been laid into the brood pouch of the mother showing the nucleus (nu) at the periphery. od, oil droplet; pc, peripheral cytoplasm; y, yolk.

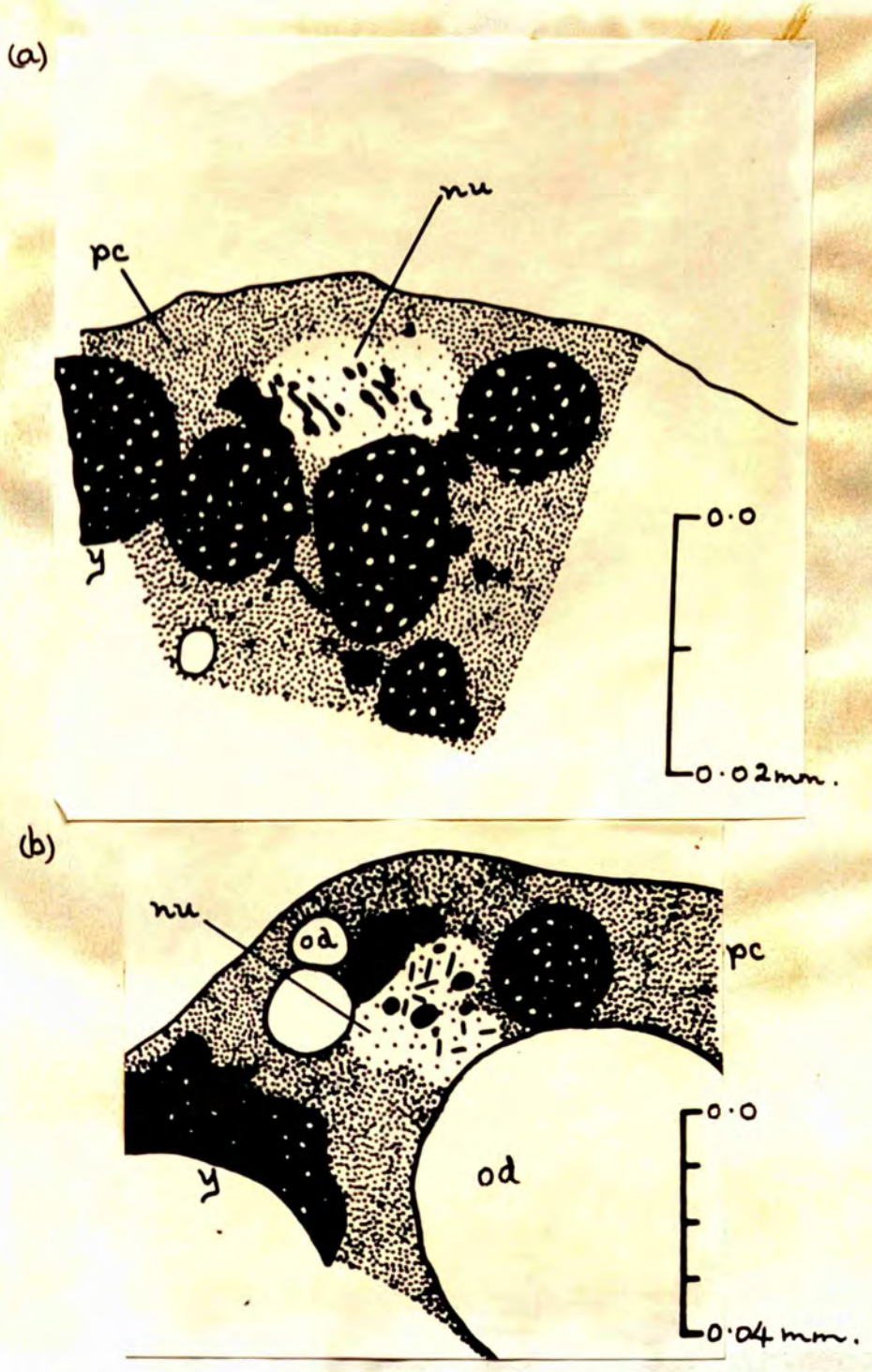


Figure 3. (a) and (b). Sections through parthenogenetic eggs of Daphnia magna at the same stage as shown in Fig. 2 showing the nucleus (nu) dividing at the periphery. od, oil droplet; pc, peripheral cytoplasm; y, yolk.

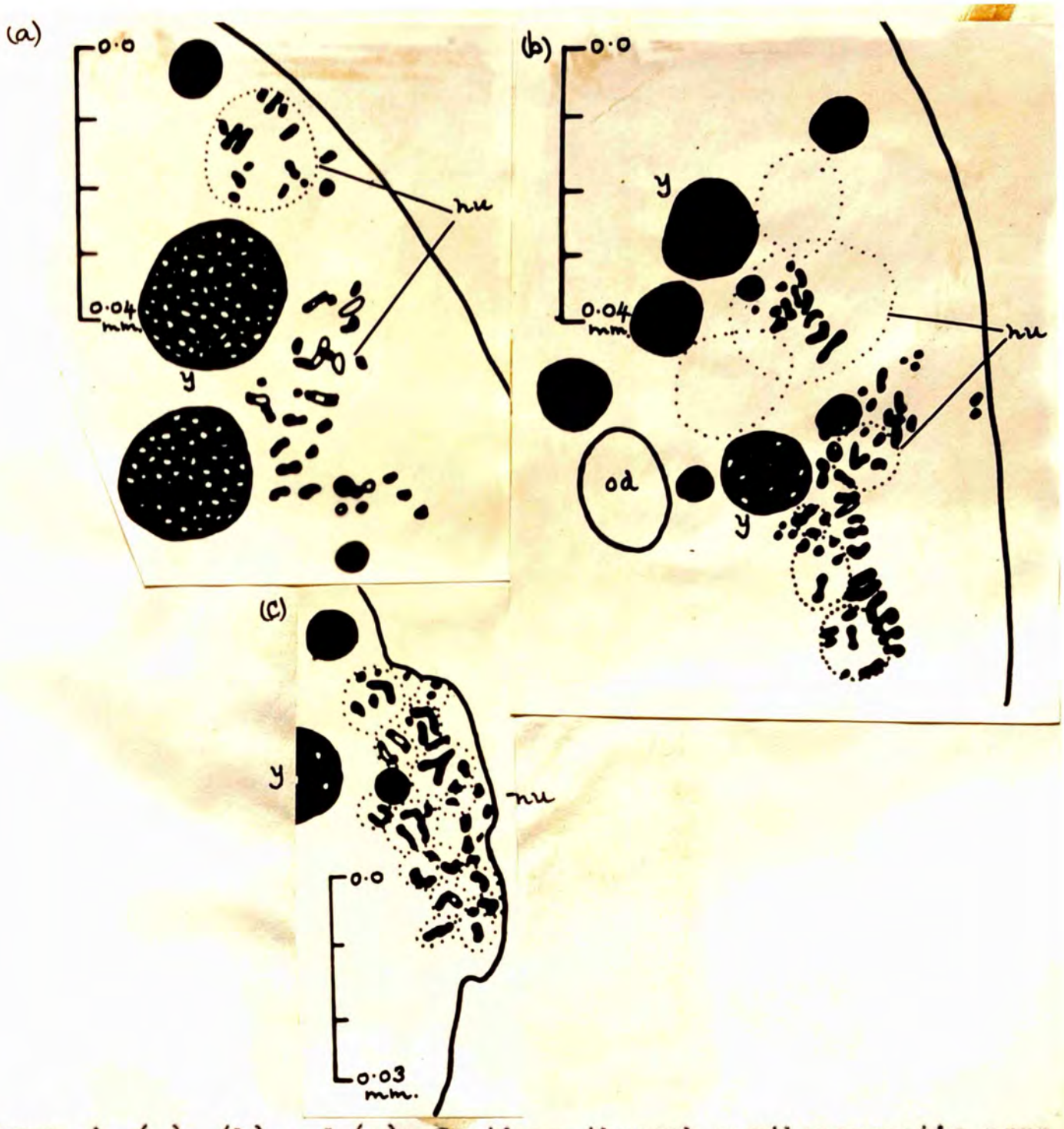


Figure 4. (a), (b) and (c). Sections through parthenogenetic eggs of Daphnia magna showing the nucleus divided into two at the periphery of the egg about one hour after the egg has been laid into the brood pouch of the mother. nu, nucleus; od, oil droplet; y, yolk.

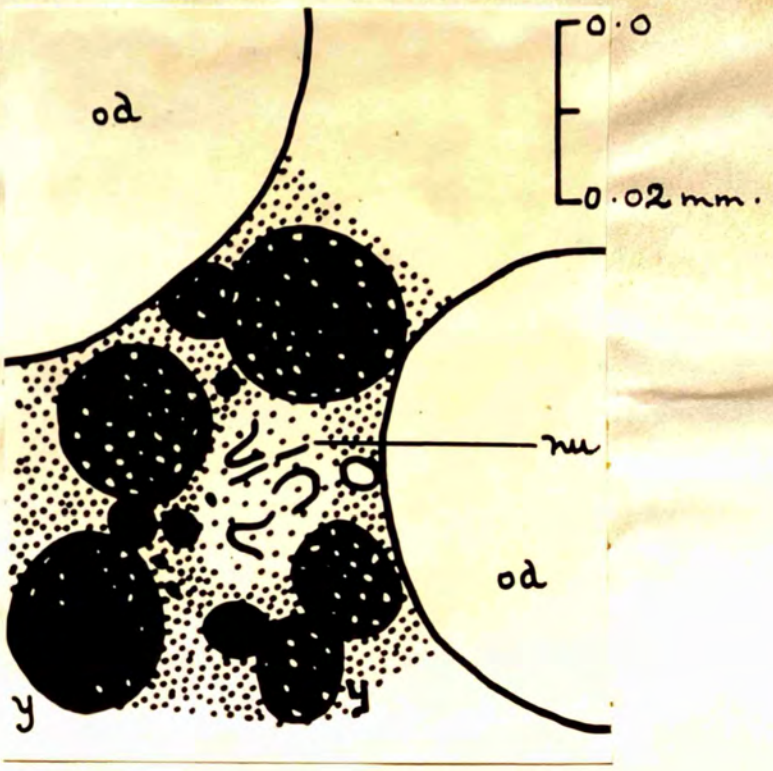


Figure 5. Section through an egg of Daphnia magna just laid into the brood pouch of the mother showing the nucleus (nu) among yolk globules (y) and about to divide. od, oil droplet.

Daphnia magna there is more than one, usually at least three, one of which is slightly larger than the others. There are also noticeable in sections numerous smaller spaces which may be due to the loss during preparation of the sections of some of the larger yolk granules but appear to show a structure similar to that of the oil droplets.

The nucleus undergoes its initial divisions while in the centre of the egg, the resulting blastomeres being represented by cytoplasmic islands (Fig. 6,bl), each with a nucleus. The cleavage affects only the central nucleus and the cytoplasm surrounding it, the remainder of the egg is unchanged. There is no external sign of segmentation. The blastomeres are irregularly scattered through the yolk at the centre of the egg, penetrating an area with a diameter about one-third that of the whole egg. The nuclei do not divide in complete synchronisation, so that 8-, 16-, 32-cell stages are not clearly defined. At about the 12- to 16-cell stage the nuclei in their areas of cytoplasm begin to move towards the periphery of the egg(Fig. 6). They do so along very fine cytoplasmic strands which extend from the thin peripheral layer through the yolk to the central cytoplasmic islands. The movements of the nuclei exhibit a slight polarity, a greater number moving towards the animal pole of the egg. The islands of cytoplasm (bl) rise through the yolk and fuse with the outer thin layer of cytoplasm (pc). They here form swellings of the peripheral layer, projecting slightly towards the interior of the egg and connected by thin cytoplasmic strands.

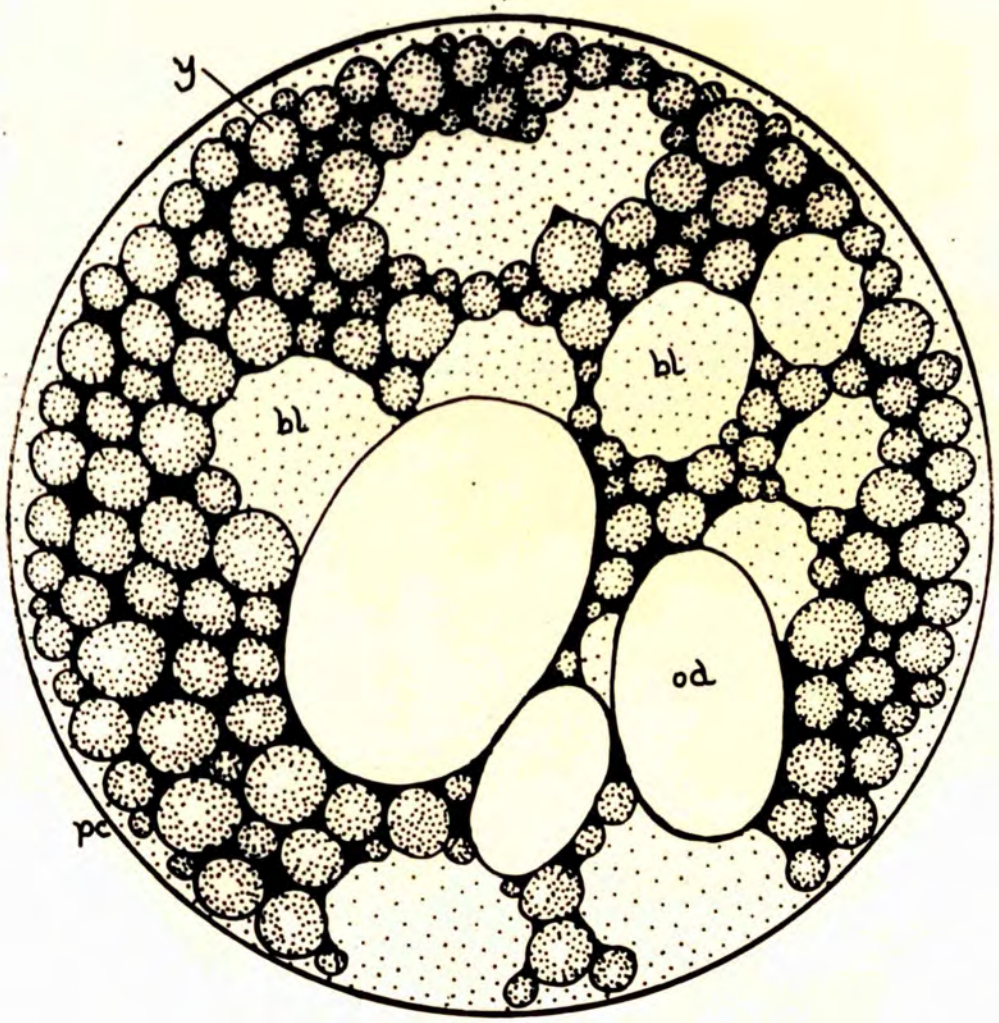


Figure 6. Diagrammatic reconstruction of a parthenogenetic egg of Daphnia magna about eleven hours after it has been laid into the brood pouch of the mother showing cytoplasmic areas (bl) scattered through the central yolk(y) and approaching the periphery of the egg. od, oil droplet; pc, peripheral cytoplasm.

The cytoplasm gradually becomes more even in thickness and the cytoplasmic strands withdrawn from the yolk (Fig. 7). Apparently all the nuclei and their surrounding areas of cytoplasm migrate to the surface of the egg, none being left behind in the yolk. The nuclei (bl) [redacted] occur at intervals in a peripheral layer of cytoplasm (pc) being slightly more numerous at one pole. The nuclei continue to divide in the peripheral cytoplasm (Fig. 8; Plate 1). With the gradual formation of cell membranes between the nuclear areas the blastula stage is reached. This consists of a thin outer blastoderm surrounding a large inner mass of yolk (Plate 2).

Figure 7. Section through an egg at the same stage as Fig. 6 showing the outer cytoplasmic layer (pc) surrounding the central yolk (y) and two of the nucleated areas of cytoplasm (bl) which have reached the periphery. od, oil droplet.

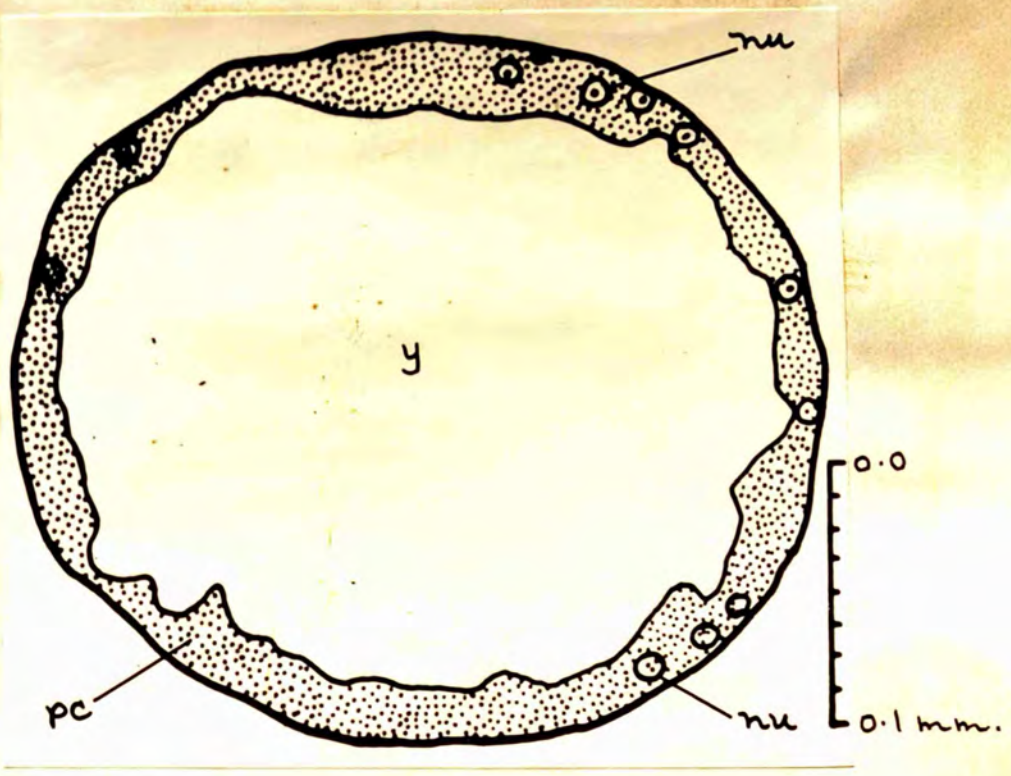


Figure 8. Section through a parthenogenetic egg of Daphnia magna about sixteen hours after it has been laid into the brood pouch of the mother showing nuclei (nu) in the peripheral cytoplasmic area (pc) surrounding the central yolk (y).

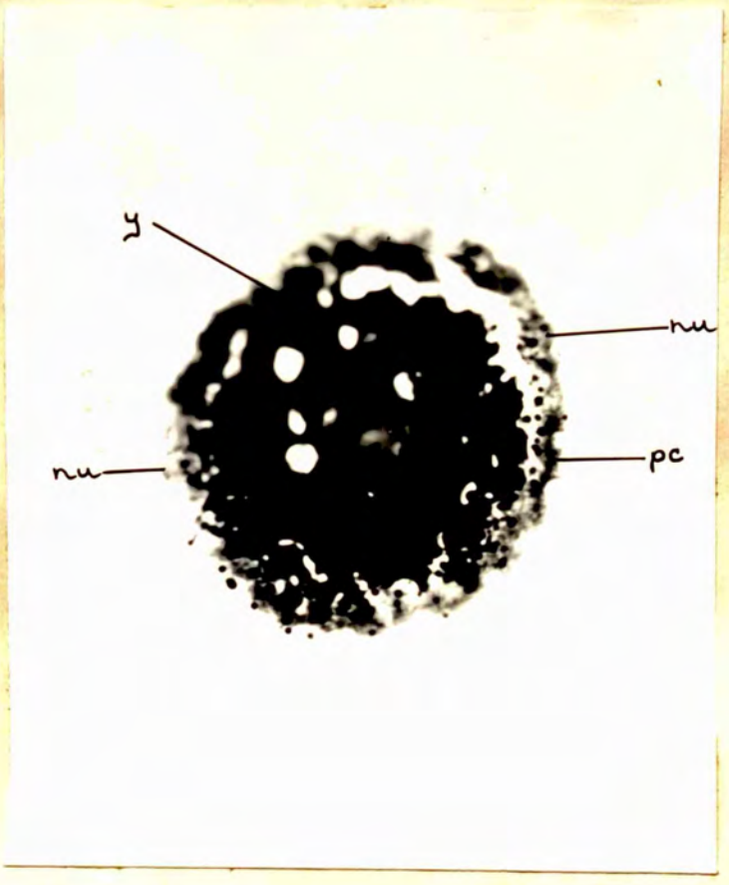


Plate 1. Photomicrograph of a transverse section through an egg at the same stage as Fig.8,16 hours after the egg has been laid into the brood pouch of the mother, showing the nuclei(nu) in the peripheral cytoplasm(pc) surrounding the central yolk(y).

Scales on the photomicrographs are approximate.

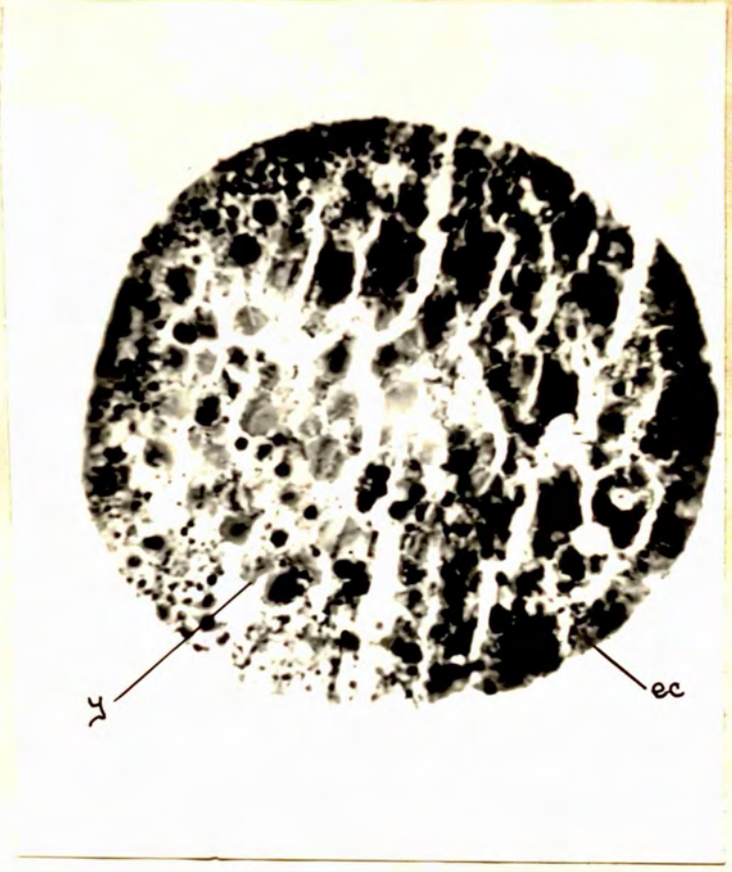


Plate 2. Photomicrograph of a transverse section through an egg just before gastrulation showing the outer blastoderm of cells(ec) and the central yolk globules(y).

b. Gastrulation.

The term gastrulation is used here in the wide sense of Pasteels: "Quelque soit l'oeuf de Métazoaire que l'on envisage, il subit après la segmentation, un changement subit de forme, un remaniement profond de la répartition de ses masses cellulaires. Cette gastrulation précède toujours de peu l'apparition des organes primordiaux" (Pasteels, 1940).

There is no visible differentiation between the cells of the blastoderm. In the future ventral region of the egg, a very small invagination appears together with a marked immigration of cells (Fig. 9). The cells immigrate separately into the interior of the egg and form an irregular mass (icm) without distinct layering, the boundaries of the cells being polygonal. The actual invagination is exceedingly small (Fig. 10). The blastoderm cells do not change in shape or alter their position, and there is no indication that part of the blastoderm itself moves into the centre of the egg. The cells pass separately from a restricted area of the surface of the egg around the small invagination into the interior of the egg. There is no visible difference between the immigrating cells except for the genital cells (Fig. 11). The genital cells (gc) are recognisable in the inner cell mass as a small group of slightly larger cells with large nuclei containing a large darkly staining nucleolus surrounded by a clear area with little chromatin. These cells do not contain yolk. The remainder of the cells of the egg contain globules of yolk within vacuoles. This applies to the

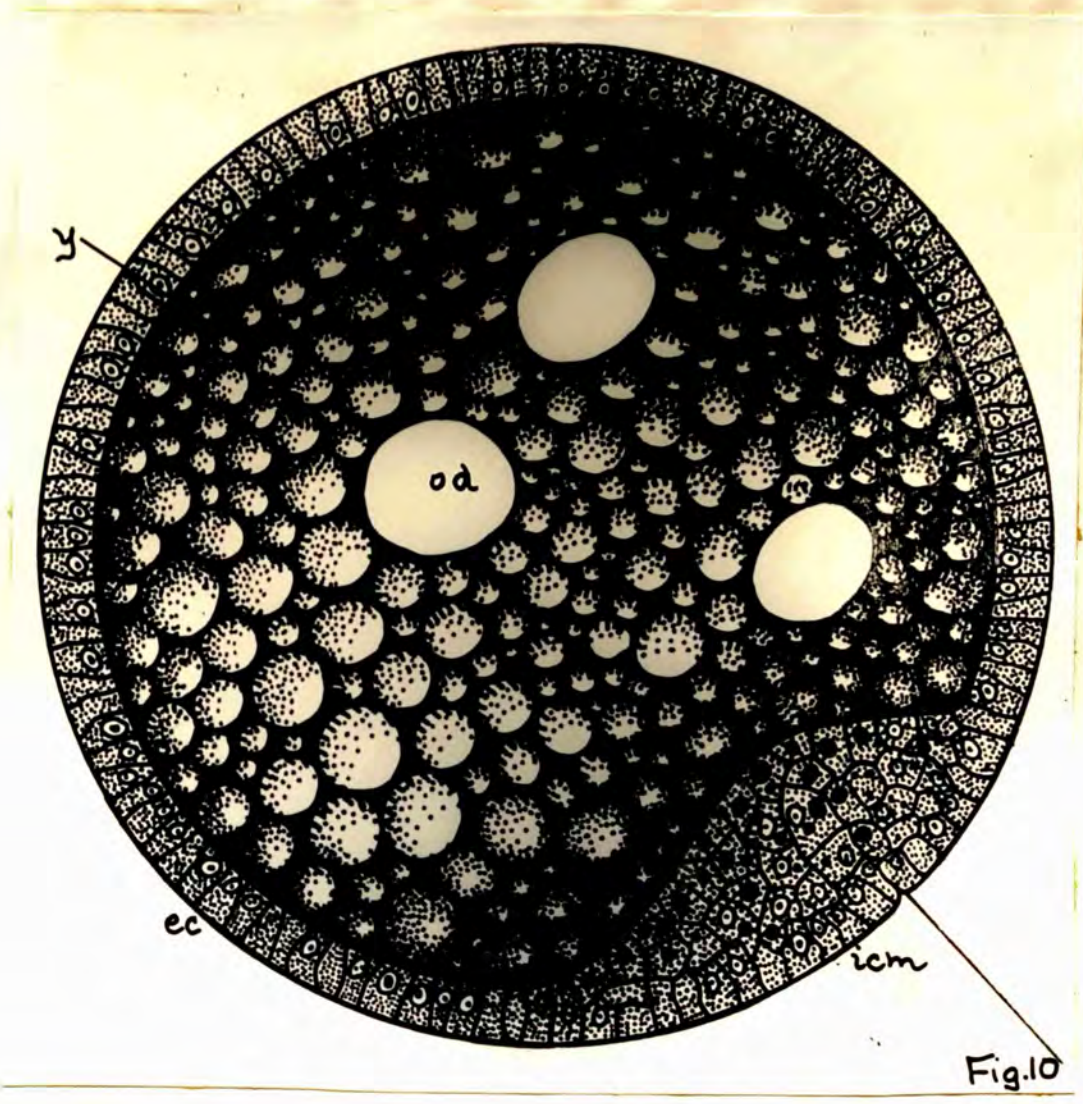


Figure 9. Diagrammatic reconstruction of the parthenogenetic egg of Daphnia magna at the stage of gastrulation. ec, outer cell layer; icm, inner cell mass; od, oil droplet; y, yolk.

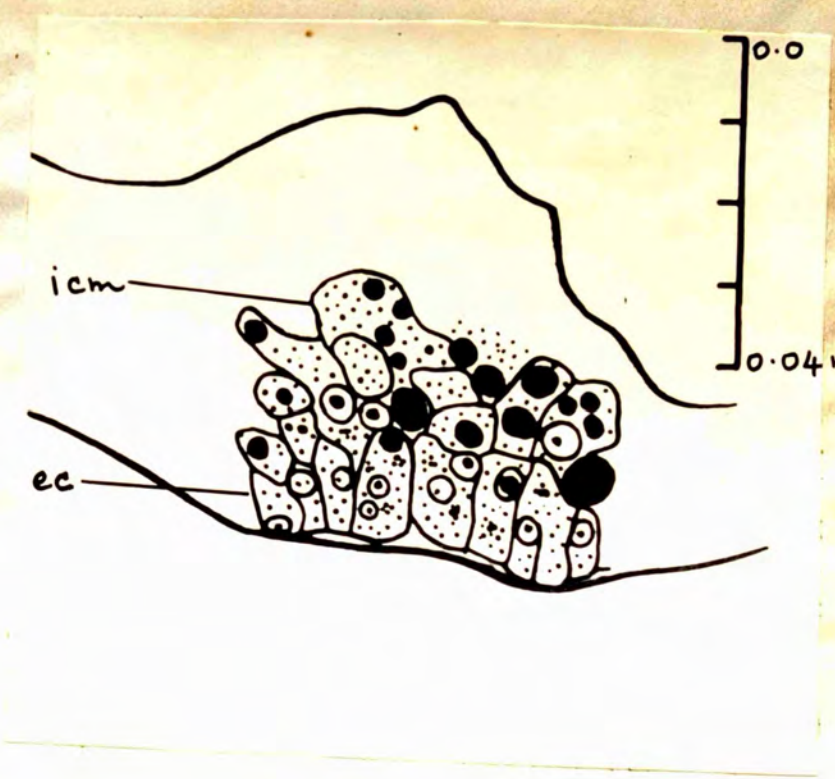


Figure 10. Section through the region of gastrulation at the same stage as Fig. 9. ec, outer cell layer; icm, inner cell mass.

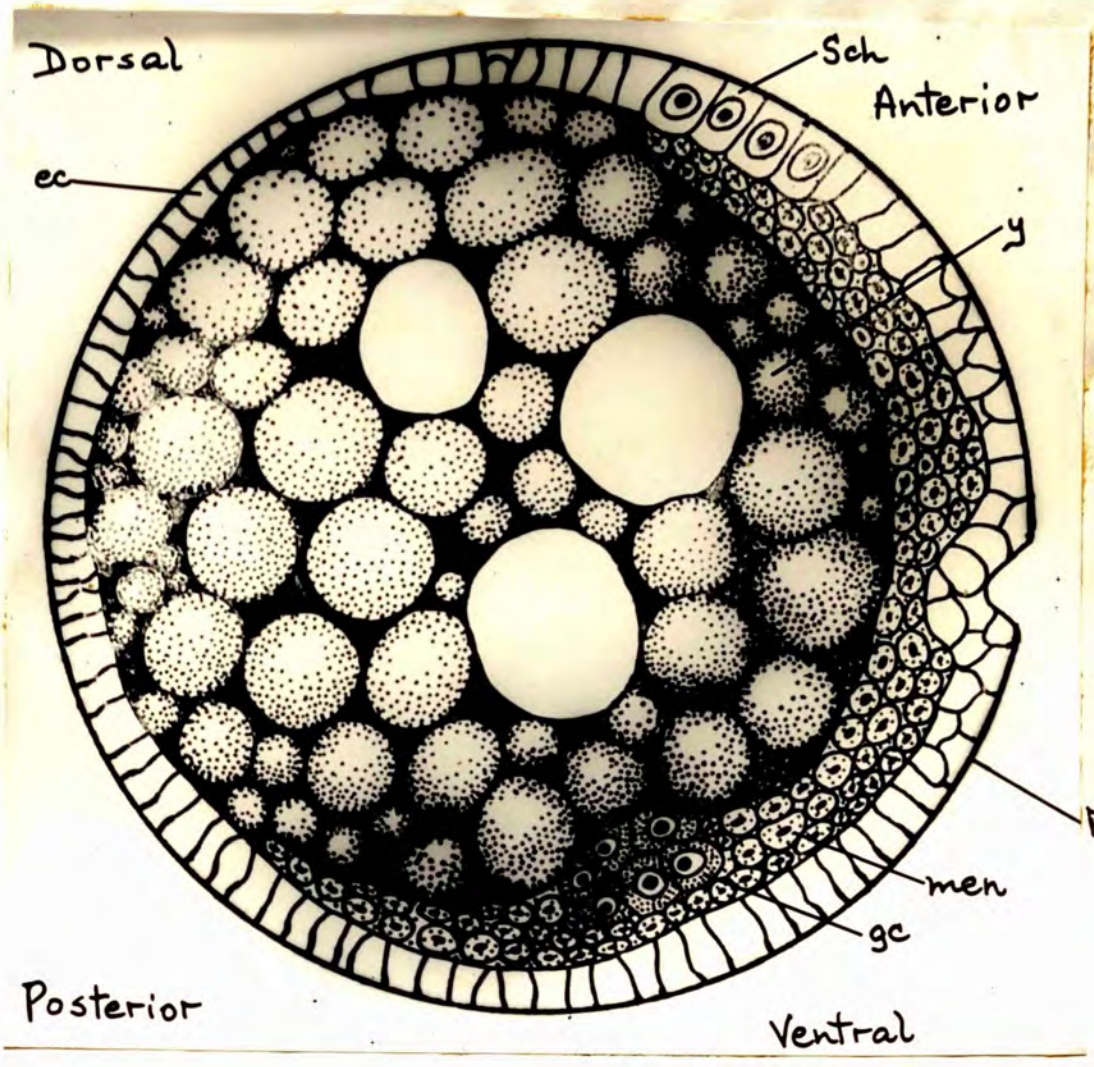


Figure 11. Diagrammatic reconstruction of the parthenogenetic egg of Daphnia magna at the end of gastrulation. The "Scheitel"-plate (Sch) does not occur in the midline. ec, outer cell layer; gc, genital cells; men, mesendoderm; y, yolk.

outer layer of cells surrounding the central mass of yolk and to the cells which have immigrated into the interior. There is no difference between the nuclei of the inner cells (Fig. 12,men) and it is not possible at this stage to distinguish mesoderm from endoderm.

Earlier workers have distinguished between an earlier ectomesoderm and a slightly later formation of a mesendoderm (Cannon, 1921), or between an early formation of ectomesoderm and a later formation of endoderm (Baldass, 1942). But in Daphnia magna no difference can be noted in yolk content, size of nuclei or in the staining of the cytoplasm between the first cells immigrating into the interior of the egg and the last cells.

The remainder of the surface of the egg is formed of a single layer of columnar cells (Fig. 11,ec). Almost simultaneously to the immigration of the cells into the interior of the egg, two groups of four of these cells situated in the future antero-dorsal region of the egg become greatly enlarged with enormous nuclei (Sch). These are the cells of the "Scheitel"-plate, or crown of the head plate, which later give rise to the nervous system and sense organs of the head. The cells are very noticeable and rival the genital cells in size.

Figure 12. Transverse section through the ventral region of an egg at the same stage as Fig. 11 showing the mesendoderm (men) of large irregular cells.

c. The alimentary canal.

At the end of gastrulation the egg (Fig. 11) has an outer layer of columnar cells (ec) surrounding an inner mass of yolk (y). There is a paired antero-dorsal "Scheitel"-plate (Sch). Ventrally an inner mass of cells (men) extends from the region of the "Scheitel"-plate to the posterior end of the egg, with a group of about a dozen genital cells (gc) lying against its postero-ventral inner edge. There are also a number of yolk cells in the peripheral region of the yolk (see p.76).

When the egg is about one day old (Fig. 13; Plate 3), it becomes possible to distinguish between the mid-ventral part of the inner mass of cells (en) and the cells forming its lateral and anterior edges (m). The mid-ventral strand is about four or five cells in width and about three cells in height (Fig. 14,en). It projects further into the interior of the egg than the cells on either side of it (m). The cells forming the strand are larger than the surrounding cells and less granular. Their nuclei are also larger and contain only a small number of scattered chromatin granules; the nucleolus is often elongate. These are the endodermal cells (en) and will form the mesenteron of the alimentary canal, they do not contribute to any other structure. The surrounding cells are mesodermal (m).

At approximately the same time as the recognition of the mid-ventral strand of endodermal cells, small invaginations appear in the anterior and posterior regions of the ventral surface of the egg (Fig. 15,st,pr). They are short simple

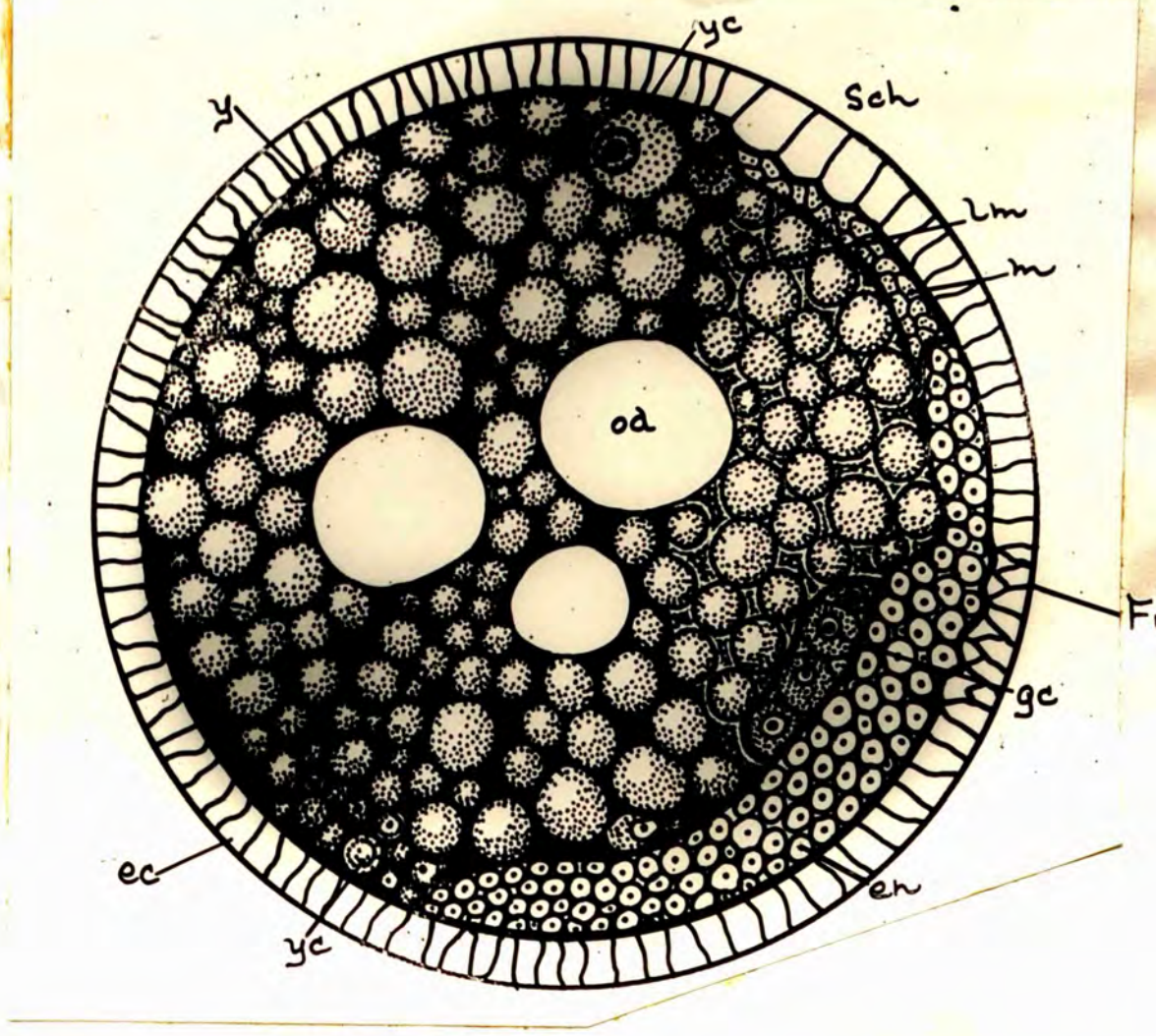


Fig. 14.

Figure 13. Diagrammatic reconstruction of the parthenogenetic egg of Daphnia magna soon after gastrulation has taken place and when endoderm (en) and mesoderm (m) have just become distinguishable. ec, outer cell layer; gc, genital cells; lm, lateral extent of mesoderm; od, oil droplet; Sch, "Scheitel"-plate; y, yolk; yc, yolk cell.

Plate 3. Photomicrograph of a transverse section through an egg at the same stage as Fig.13 showing the endodermal cells(en) with mesodermal cells(m) laterally.
ec,outer layer of cells; y,yolk.

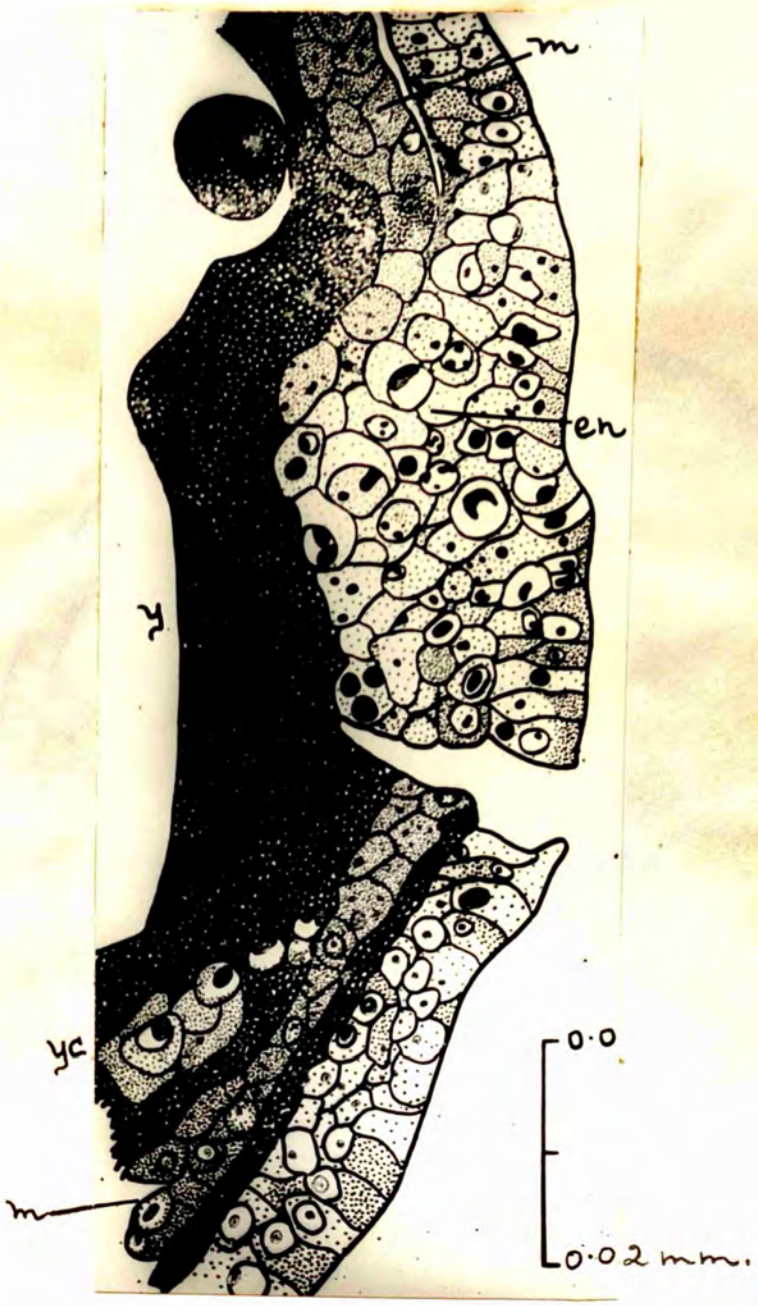


Figure 14. Transverse section through an egg at the same stage as Fig. 13 to show endoderm (en) and mesoderm (m). y, yolk; yc, yolk cell.

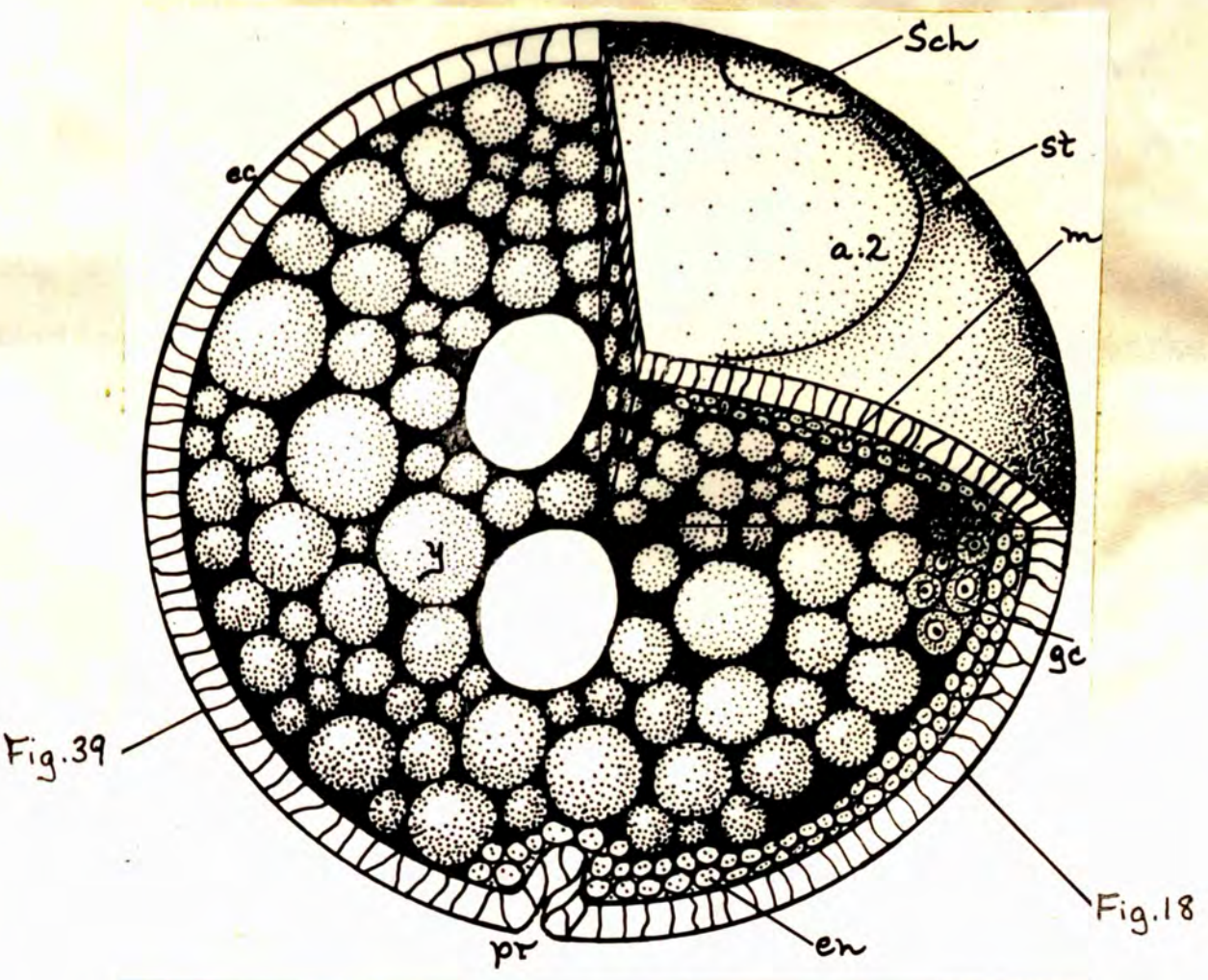


Figure 15. Diagrammatic reconstruction of the parthenogenetic egg of Daphnia magna at the stage when the antennal fold (a.2) is forming. ec, outer cell layer; en, endoderm; gc, genital cells; m, mesoderm; pr, proctodaeum; Sch, "Scheitel"-plate; st, stomodaeum; y, yolk.

invaginations of the outer cell layer. The anterior, or stomodaeal, invagination (Fig. 15,st; Fig. 16) is slightly the larger of the two. It is ventral to the "Scheitel"-plate (Sch) and close to the anterior end of the mid-ventral strand of cells (Fig. 15,en; Fig. 18). The posterior, or proctodaeal, invagination (Fig. 15,pr; Fig. 17; Plate 4) is slightly ventral to the posterior end of the inner mass of cells, and near to the posterior end of the endodermal strand. This stage is marked by the first indication of the second antennae (Fig. 15,a.2) in the form of a pair of lateral folds near to the anterior end of the egg. The fold contains cells from the lateral, or mesodermal, parts of the inner cell mass.

The formation of the mid-ventral strand of cells into a solid rod now takes place (Fig. 19). The process begins at the anterior end of the strand (Fig. 20, Plate 5; me), and to a very small extent at the posterior end (Fig. 21,me). The cells of the middle part of the strand remain at first unchanged. Anteriorly the rod consists of an outer circle of about seven to eight cells surrounding an inner core of one or two cells (Fig.20). Further posteriorly the cells of the rod are irregularly arranged (Fig. 21), indicating an intermediate stage between strand and rod. The rod lies close to the ventral outer cell layer (ec) but is distinct from it. At this stage each antennal fold (Fig. 19,a.2) has divided into two, and the mandibles (md) are beginning to develop.

At approximately the time of the bursting of the egg membrane (Fig. 22), all the endodermal cells have formed into a

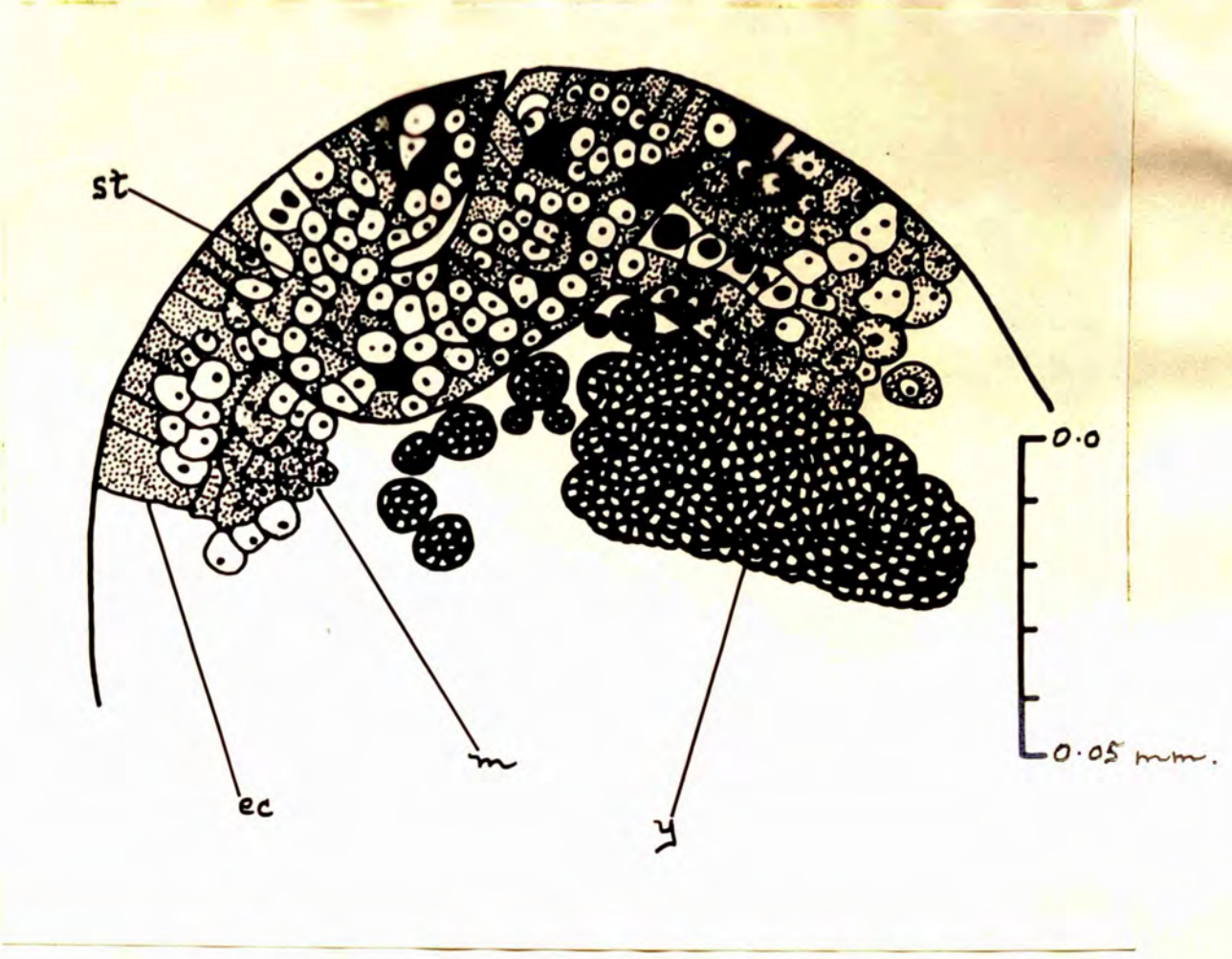


Figure 16. Sagittal section through an egg at the same stage as Fig. 15 showing the stomodaeum (st). ec, outer cell layer; m, mesoderm; y, yolk.

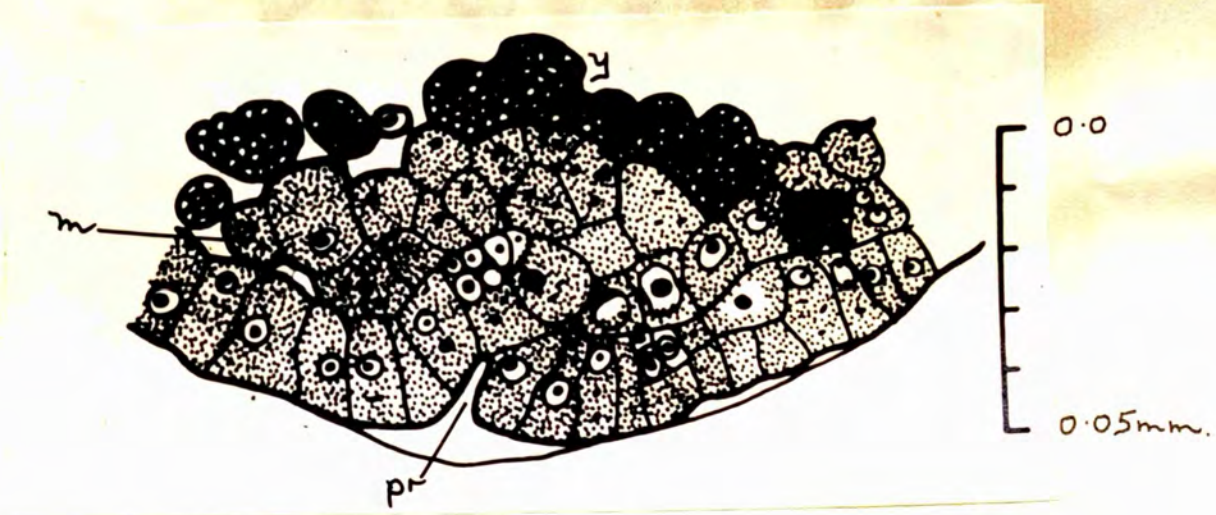


Figure 17. Vertical longitudinal section through an egg at the same stage as Fig. 15 showing the proctodaeum (pr). m, mesoderm; y, yolk.

Plate 4. Photomicrograph of the sagittal section through an egg at the same stage as Fig.15 showing the proctodaeum(pr). ec,outer layer of cells; m,mesoderm; y,yolk. X 350.

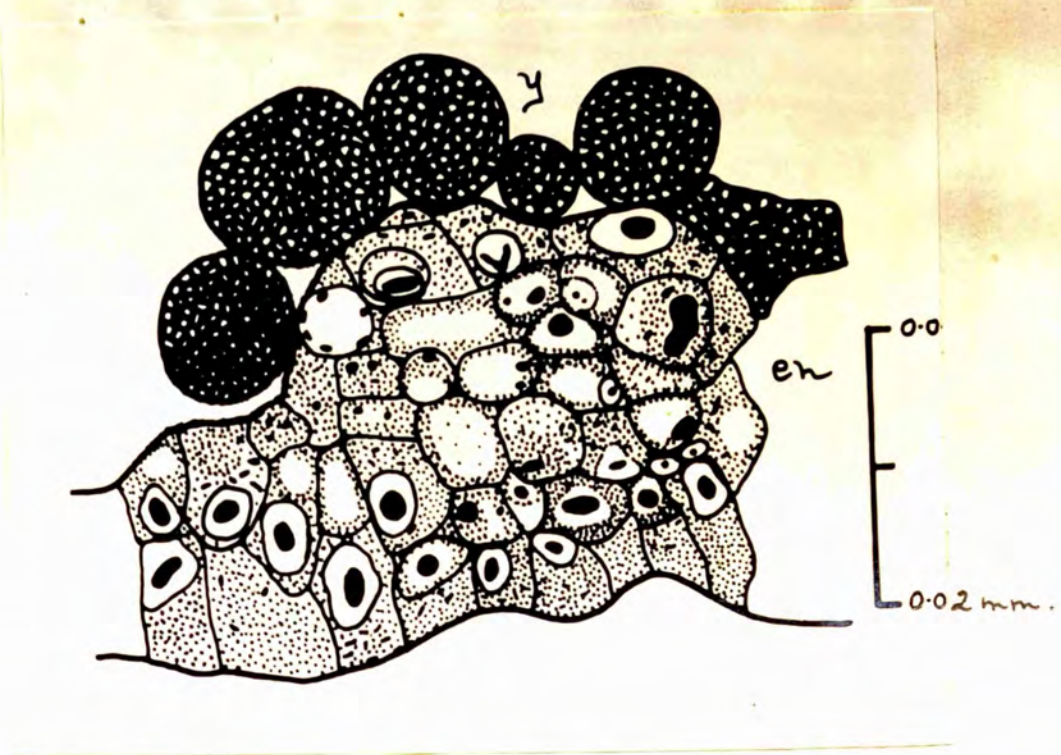


Figure 18. Transverse section through an egg at the same stage as Fig. 15 to show the endoderm cells (en). y, yolk.

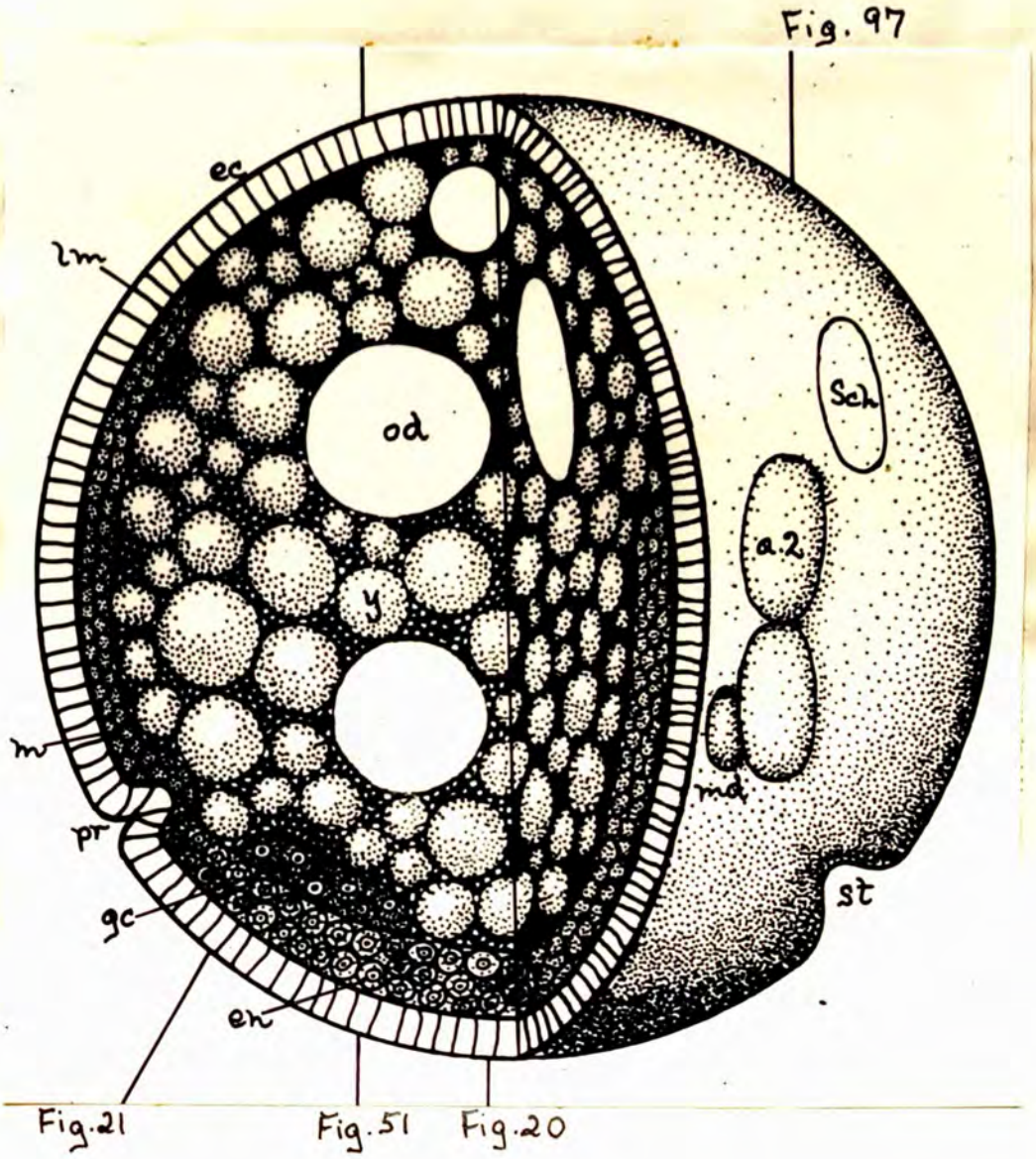


Figure 19. Diagrammatic reconstruction of the parthenogenetic egg of Daphnia magna at the stage when the rudiments of the second antennae (a.2) and mandibles (md) are present and the mesenteron (en) is beginning to form into a rod. ec, outer cell layer; gc, genital cells; lm, lateral extent of the mesoderm; m, mesoderm; od, oil droplet; pr, proctodaeum; Sch, "Scheitel"-plate; st, stomodaeum; y, yolk.

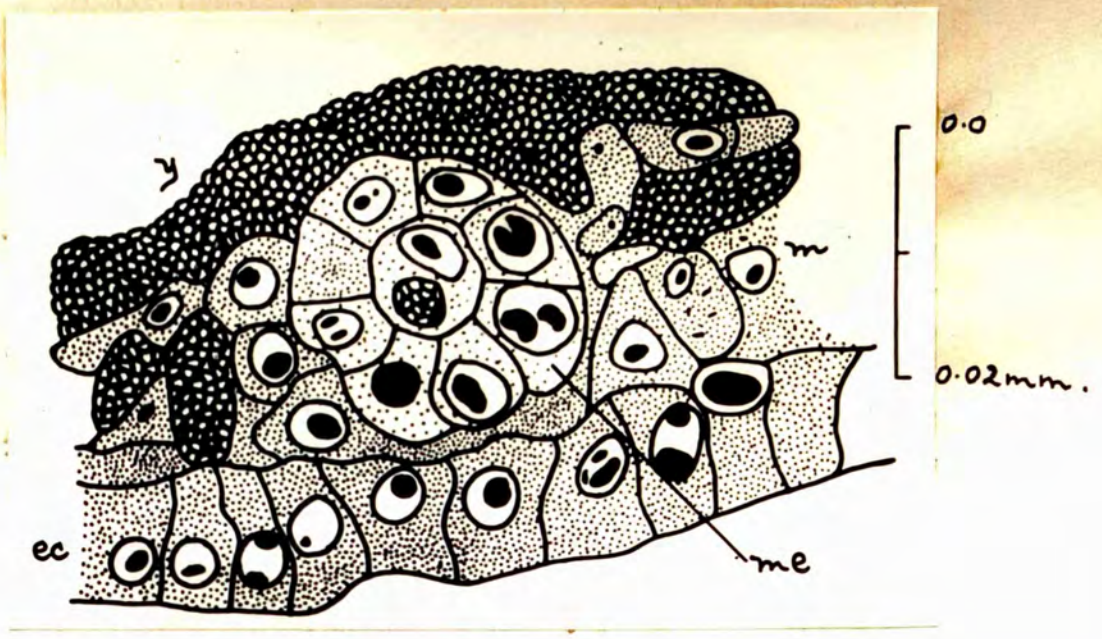


Figure 20. Transverse section through an egg at the same stage as Fig. 19 showing an early stage in the formation of the mesenteron rod (me). ec, outer cell layer; m, mesoderm; y, yolk.

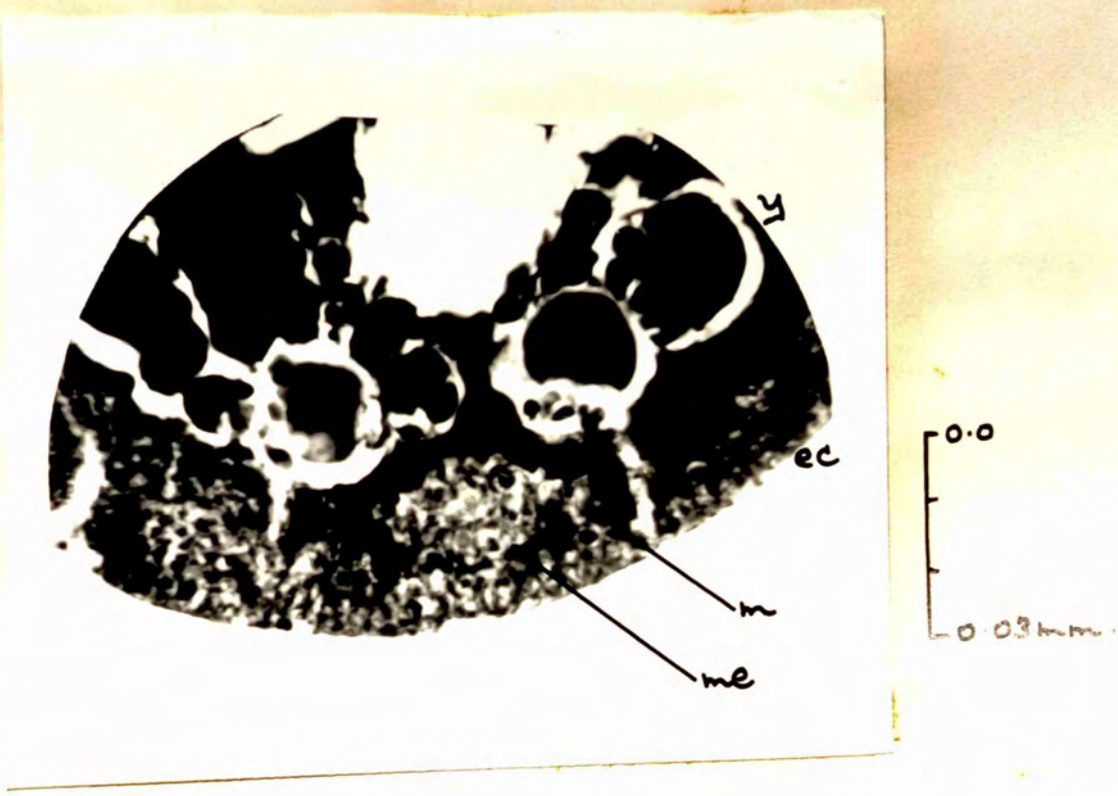


Plate 5. Photomicrograph of a transverse section through egg at the same stage as Fig.19 showing the early formation of the mesenteron rod(me). ec,outer layer of cells; m,mesoderm; y,yolk.

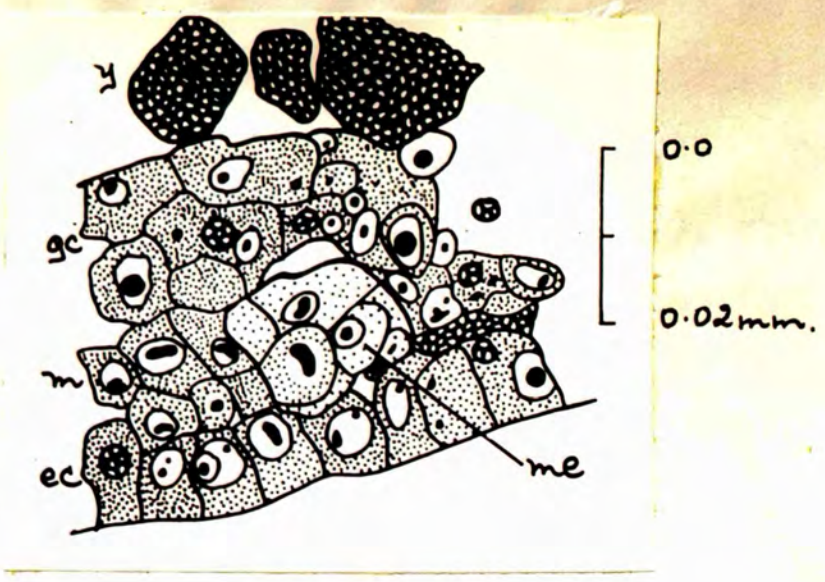


Figure 21. Transverse section through an egg at the same stage as Fig. 19 in a position posterior to that of Fig. 20 and showing the mesenteron rod (me) less well formed. ec, outer cell layer; gc, genital cells; m, mesoderm; y, yolk.

rod (me), which develops an anterior dorsal flexure with a pair of short appendages (mc) in the same region, the beginnings of the midgut caeca. There is also a small posterior flexure in the region of the junction of the mesenteron with the proctodaeum.

The alimentary canal now consists of stomodaeal and proctodaeal (Fig. 23; Plate 6; pr) invaginations connected by a solid rod of cells (Fig. 24; Plate 7; me), the future mesenteron. The mesenteron has a well developed dorsal flexure near to its junction with the stomodaeum and a poorly developed flexure near to its junction with the proctodaeum (Fig. 22). The anterior flexure bears a pair of stumpy appendages (mc), which are solid. The remainder of the mesenteron (me) is also solid except for a very short anterior region. The stomodaeum (st) and proctodaeum (pr) each have a narrow slit-like central cavity.

The mesenteron develops a central cavity when the embryo is just over two days old (Fig. 25). The first indication of the cavity is a strand of palely-staining cytoplasm in the centre of the rod (Fig. 26,me). The nuclei of the mesenteron cells accumulate towards the periphery, and the cells themselves become arranged radially into a single layer. The pale median strand collapses and a central cavity is formed. The cavity appears first at the anterior end of the mesenteron (Fig. 26), with a slight development also at the posterior end (Fig. 27,me). Most of the development spreads from the anterior end backwards. A central cavity is formed also in the short appendages, or midgut caeca (mc).

Gradually the mesenteron separates from the ventral outer

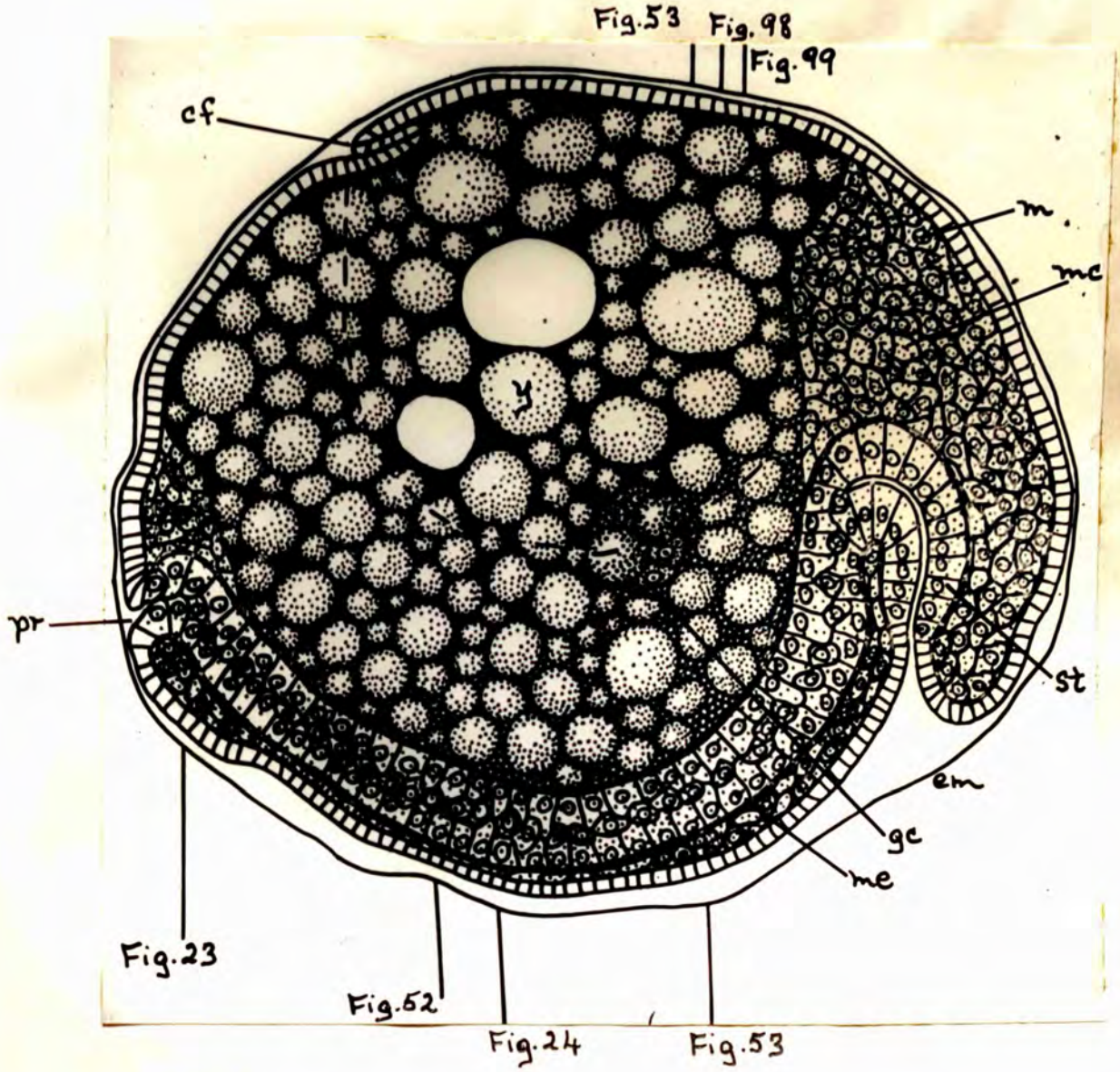


Figure 22. Diagrammatic reconstruction of the parthenogenetic egg of Daphnia magna at the stage when the egg membrane (em) is shed. cf, carapace fold; gc, genital cells; m, mesoderm; mc, caecum of mesenteron; me, mesenteron; pr, proctodaeum; st, stomodaeum, y, yolk.

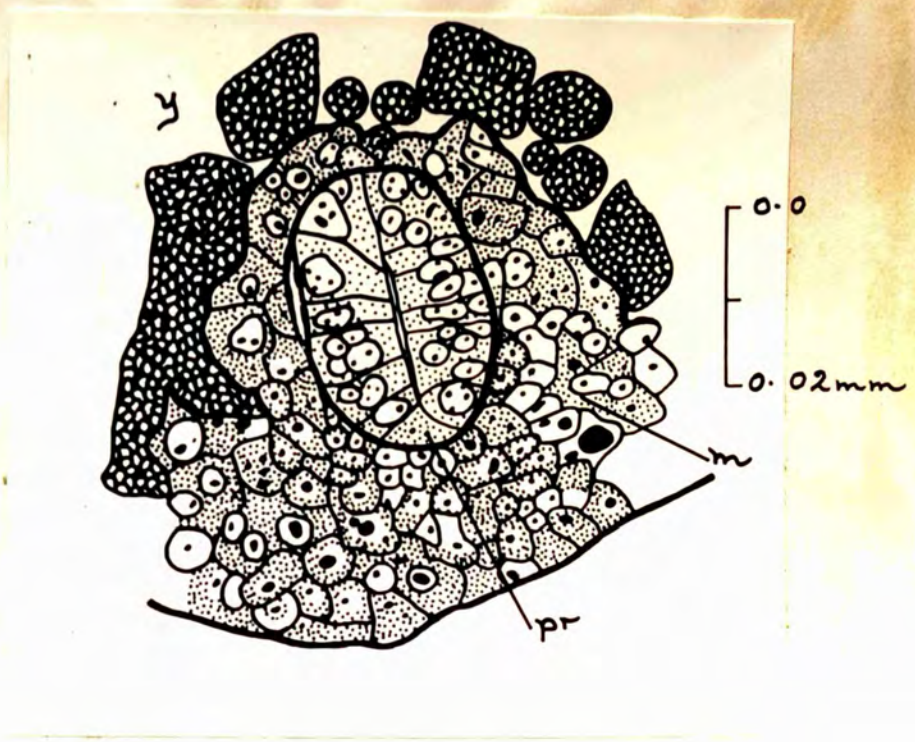


Figure 23. Transverse section through an egg at the same stage as Fig. 22 showing the proctodaeum (pr) surrounded by mesodermal cells (m). y, yolk.

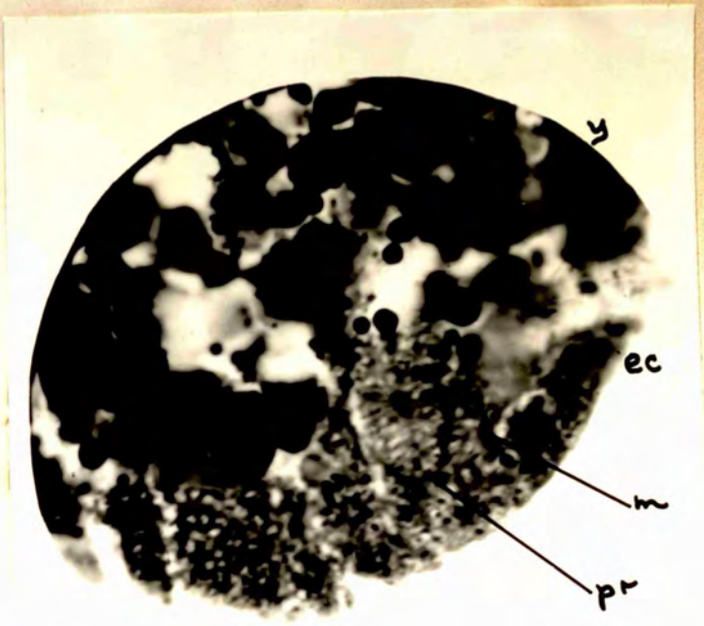


Plate 6. Photomicrograph of a transverse section through an egg at the same stage as Fig.22 showing the proctodaeum(pr) and the surrounding mesoderm cells(m). ec,outer cell layer; y,yolk.

Figure 24. Transverse section through an egg at the same stage as Fig. 22 showing the mesenteron (me). This section is anterior to that of Fig. 23. m, mesoderm; y, yolk; yc, yolk cell.

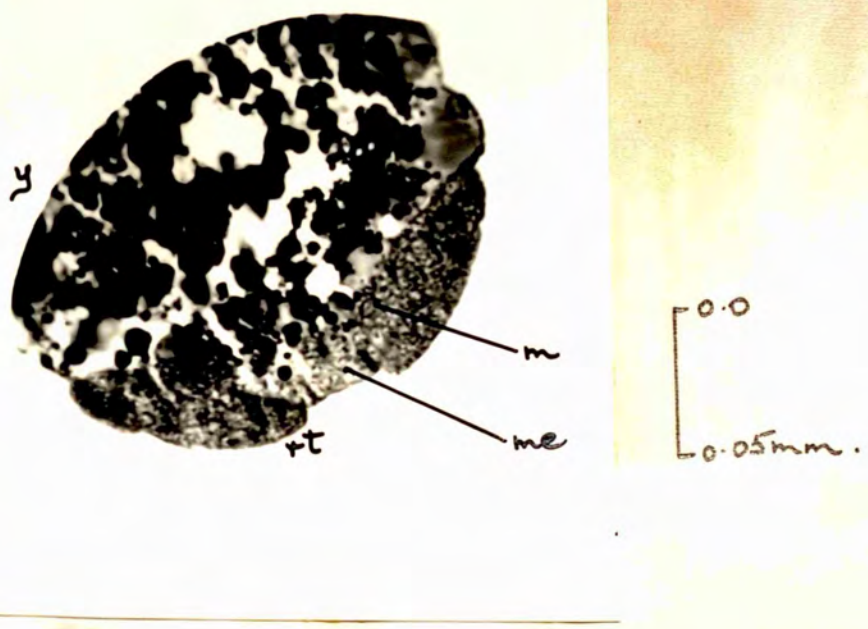


Plate 7. Photomicrograph of a transverse section through an embryo at the same stage as Fig.22 showing the mesenteron(me) formed into a rod and the surrounding mesodermal cells(m). rt,rudiment of thoracic appendage; y,yolk.

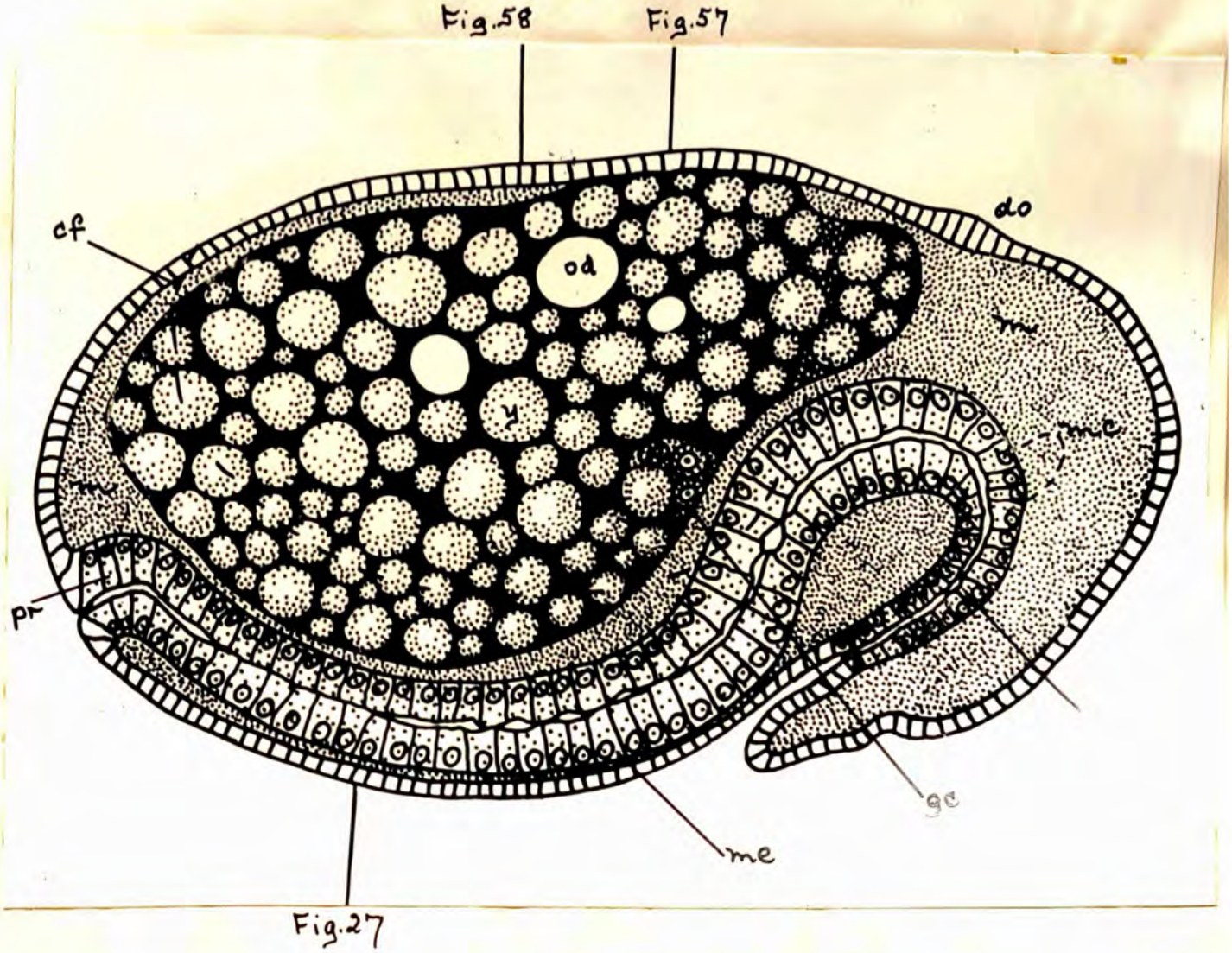


Figure 25. Diagrammatic reconstruction of the parthenogenetic embryo of Daphnia magna after the shedding of the egg membrane with the mesenteron (me) beginning to develop a cavity. The remaining membrane is not shown. cf, carapace fold; do, dorsal organ; gc, genital cells; m, mesoderm; mc, caecum of mesenteron; od, oil droplet; pr, proctodaeum; st, stomodaeum; y, yolk.

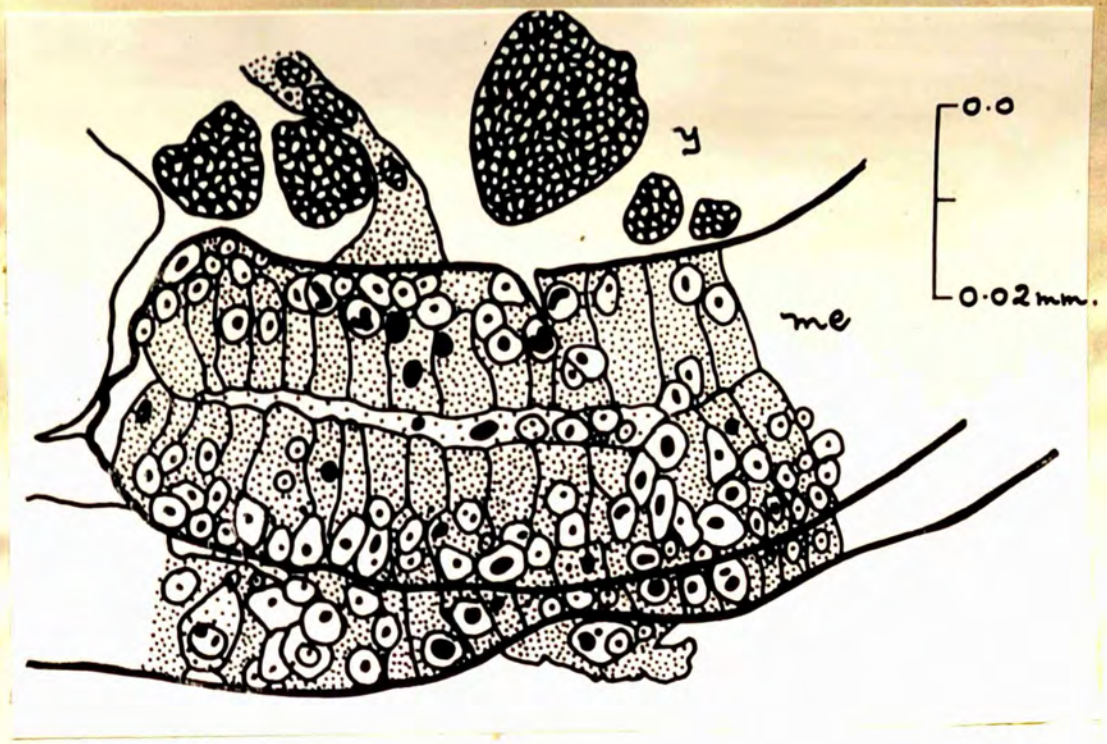


Figure 26. Sagittal section through an embryo at the same stage as Fig. 25 showing the formation of a central cavity in the mesenteron (me). y, yolk.

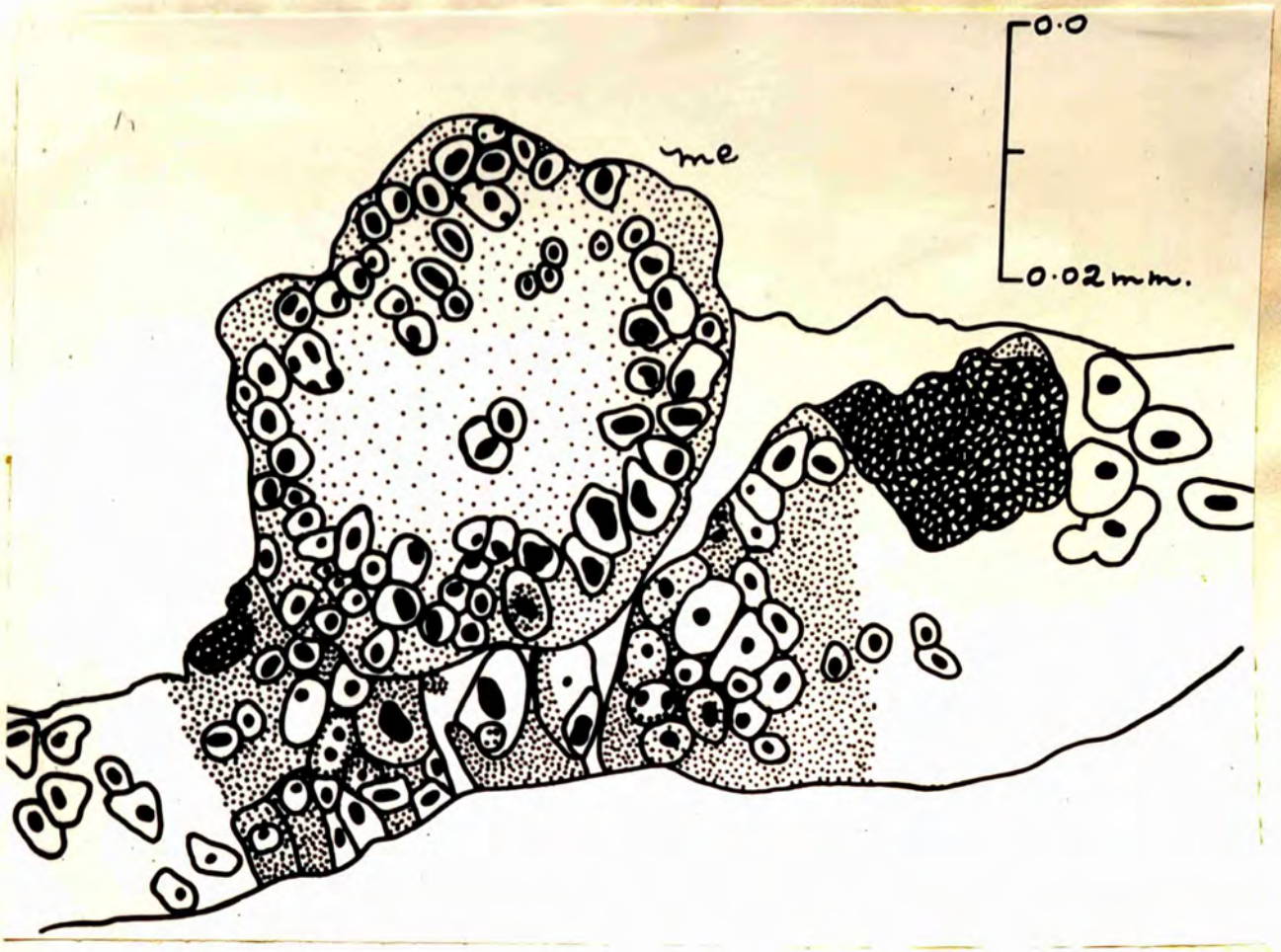


Figure 27. Transverse section through an embryo at the same stage as Fig. 25 to show the mesenteron (me).

cell layer, where the ventral nerve cord is beginning to form (Fig. 28). With the development of a central cavity the furrows at the junctions of the stomodaeum and proctodaeum with the mesenteron become conspicuous (Fig. 29). The stomodaeum (st) is surrounded by a layer of mesodermal cells which will later form the stomodaeal muscles (m, mu) and the proctodaeum is covered by an even thicker layer of mesodermal cells. The mesoderm surrounds the mesenteron laterally but not on its inner surface (Fig. 30).

With the further absorption of yolk, the mesenteron becomes further developed (Fig. 31). The mesodermal cells surround the mesenteron and its caeca (Fig. 32).

The mesenteron begins to approach the dorsal surface (Fig.33; Plate 8). The cells of the mesenteron begin to acquire the characteristics of these cells in the adult (Fig. 34,me). The outer part of each cell stains more intensely than the inner part and contains the nucleus. The cell contents are granular. The inner rods (r) and the peritrophic membrane (Fig. 36,ptm) develop as the mesenteron moves into a more dorsal position (Fig. 35; Fig. 36). This change in position results in the formerly slightly depressed middle region of the mesenteron becoming straighter (Fig. 35), and in the further development of the posterior flexure. The latter is also contributed to by the development of the abdomen.

Shortly before expulsion from the brood pouch of the mother (Fig. 35), the mesenteron is in a position similar to that of the adult except that it is still separated from the dorsal

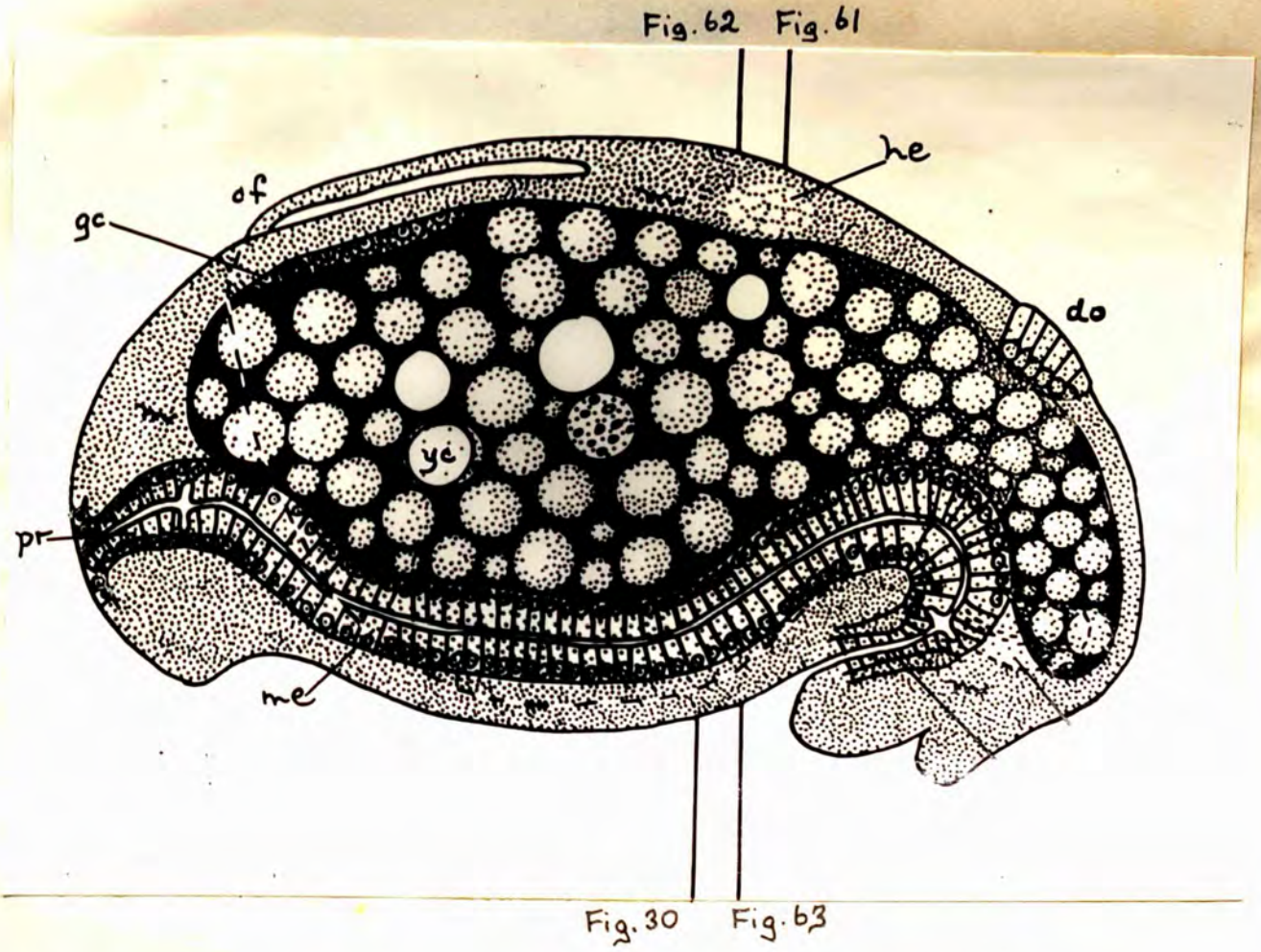


Figure 28. Diagrammatic reconstruction of the embryo of Daphnia magna at the stage when the cavity is formed throughout most of the length of the mesenteron(me). cf, carapace fold; do, dorsal organ; gc, genital cells; he, heart rudiment; m, mesoderm; mc, caecum of mesenteron; pr, proctodaeum; st, stomodaeum; yc, yolk cells.

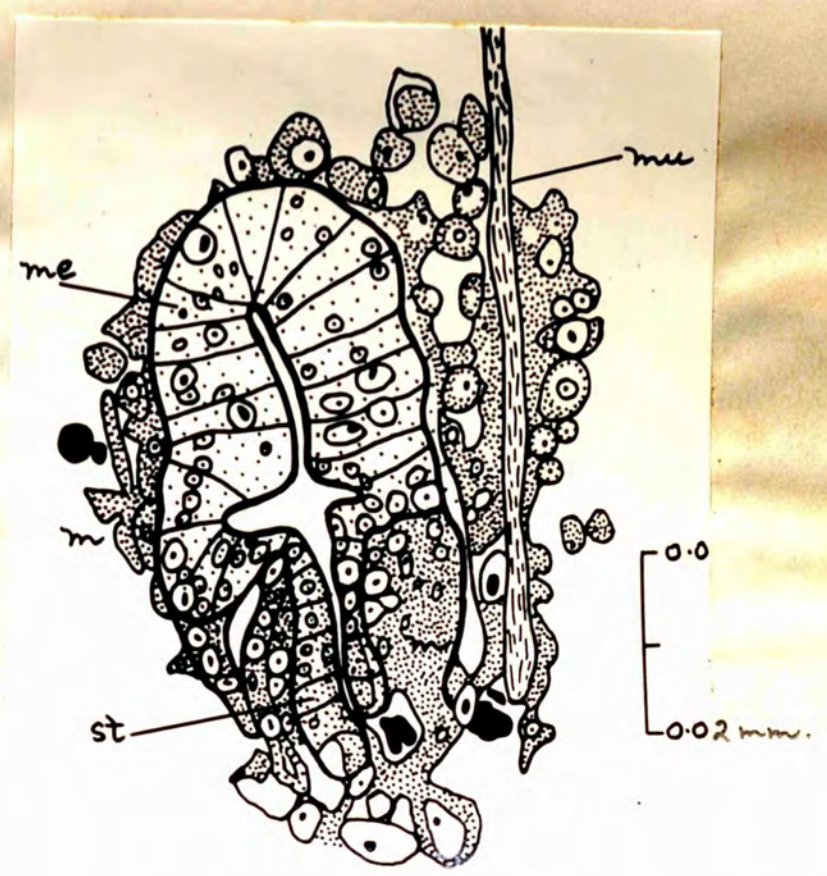


Figure 29. Vertical longitudinal section through an embryo at the same stage as Fig. 28 showing the junction of the stomodaeum (st) with the mesenteron (me) and the surrounding muscles (mu). m, mesoderm.

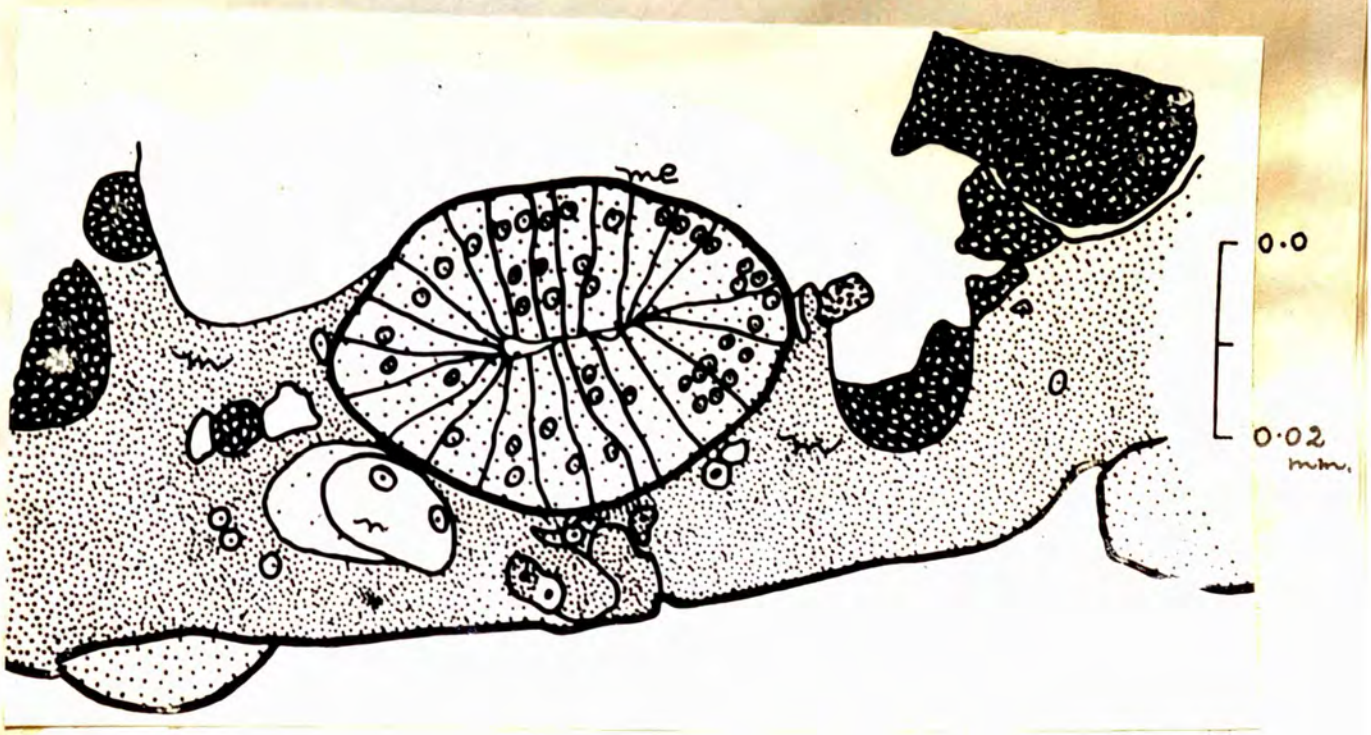


Figure 30. Transverse section through an embryo at the same stage as Fig. 28 showing the mesenteron (me) and its cavity. m, mesoderm; n, nerve cells.

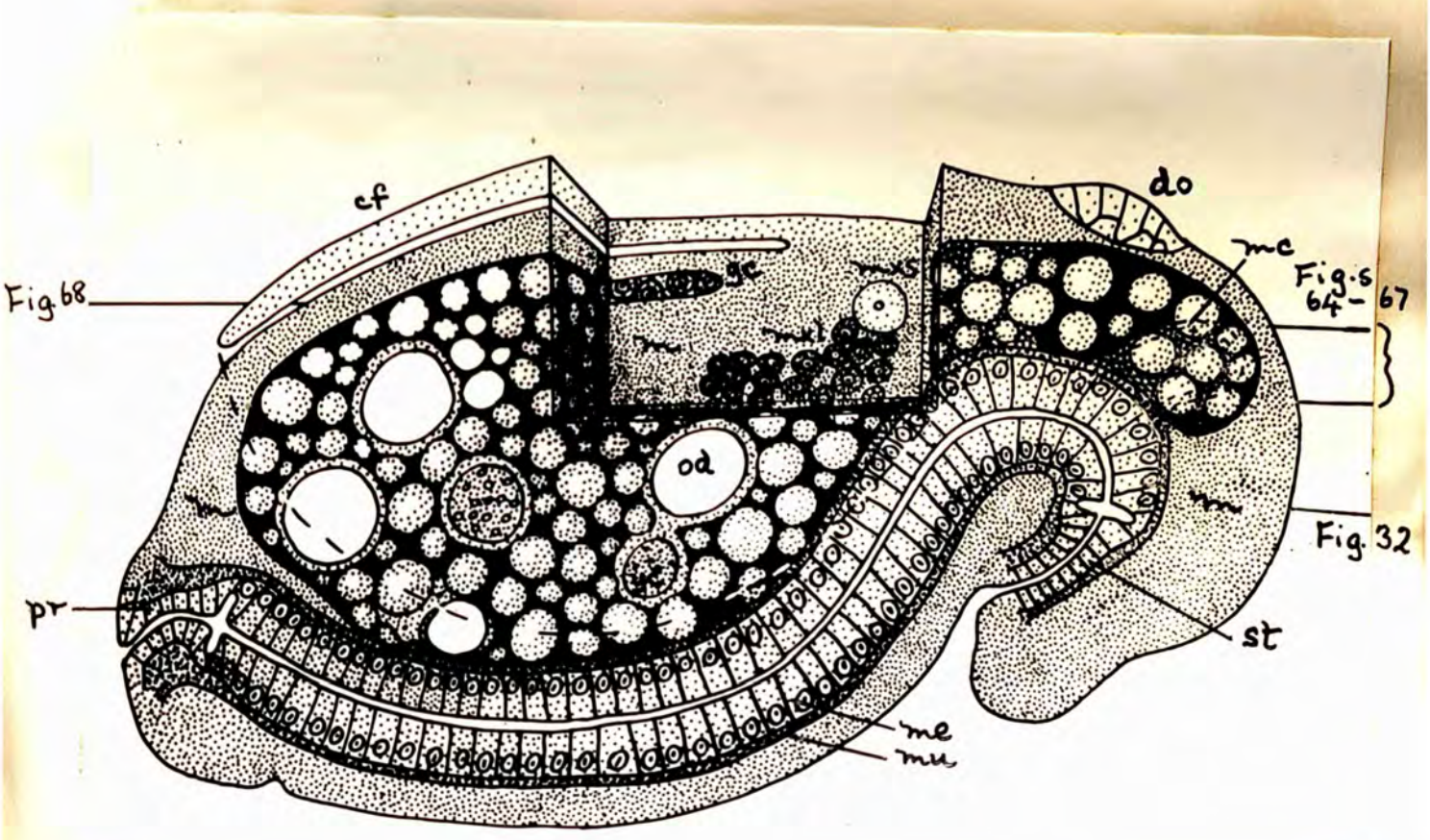


Figure 31. Diagrammatic reconstruction of the embryo of Daphnia magna at the stage when the mesenteron (me) has a cavity throughout its length and the maxillary gland is beginning to develop. cf, carapace fold; do, dorsal organ; gc, genital cells; m, mesoderm; mc, caecum of mesenteron; mu, muscle; mxl, rudiment of maxillary gland loops; mxs, rudiment of maxillary gland sac; od, oil droplet; pr, proctodaeum; st, stomodaeum; yc, yolk cell.

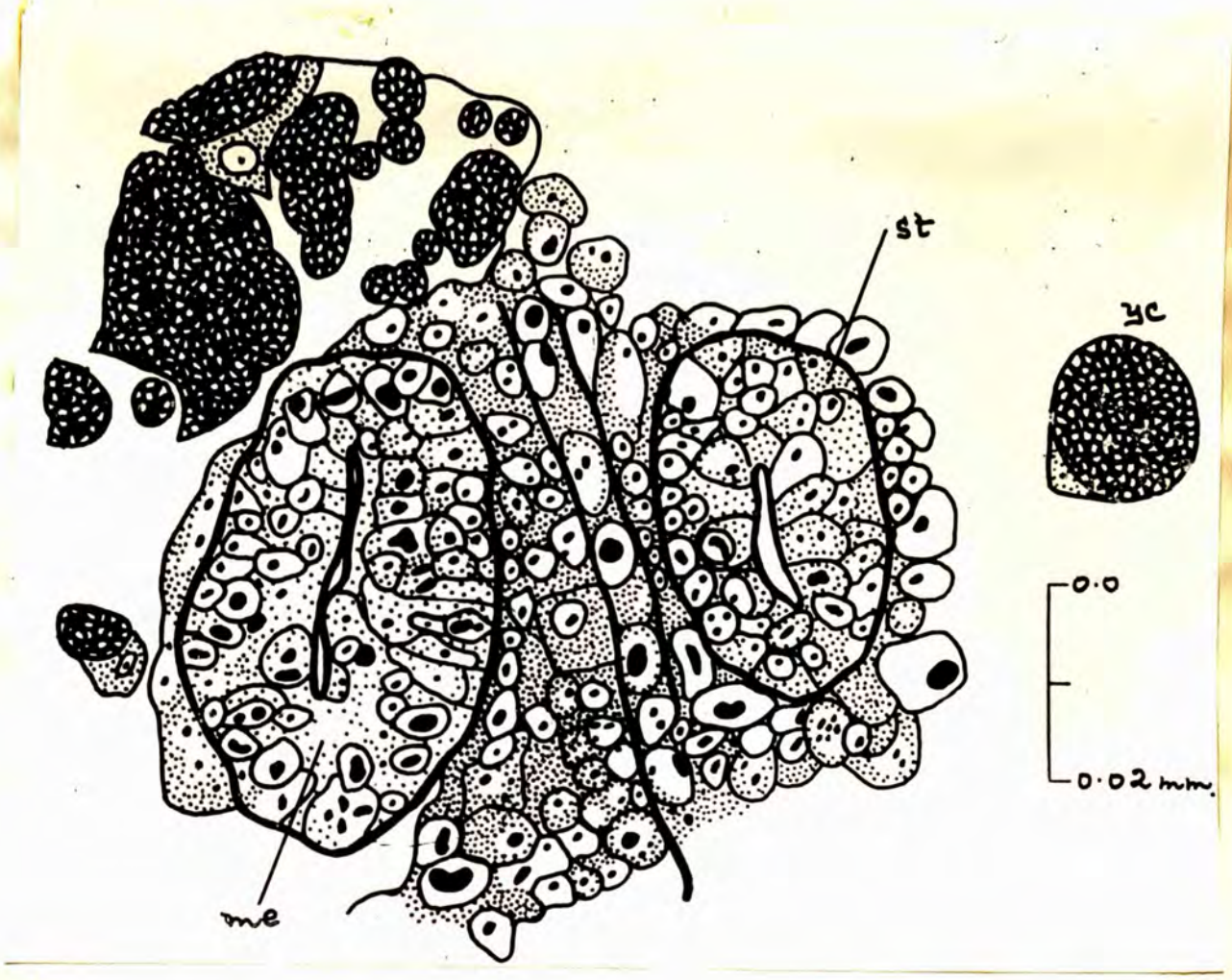


Figure 32. Horizontal longitudinal section through an embryo at the same stage as Fig. 31 showing the stomodaeum(st) and mesenteron (me). yc, yolk cell.

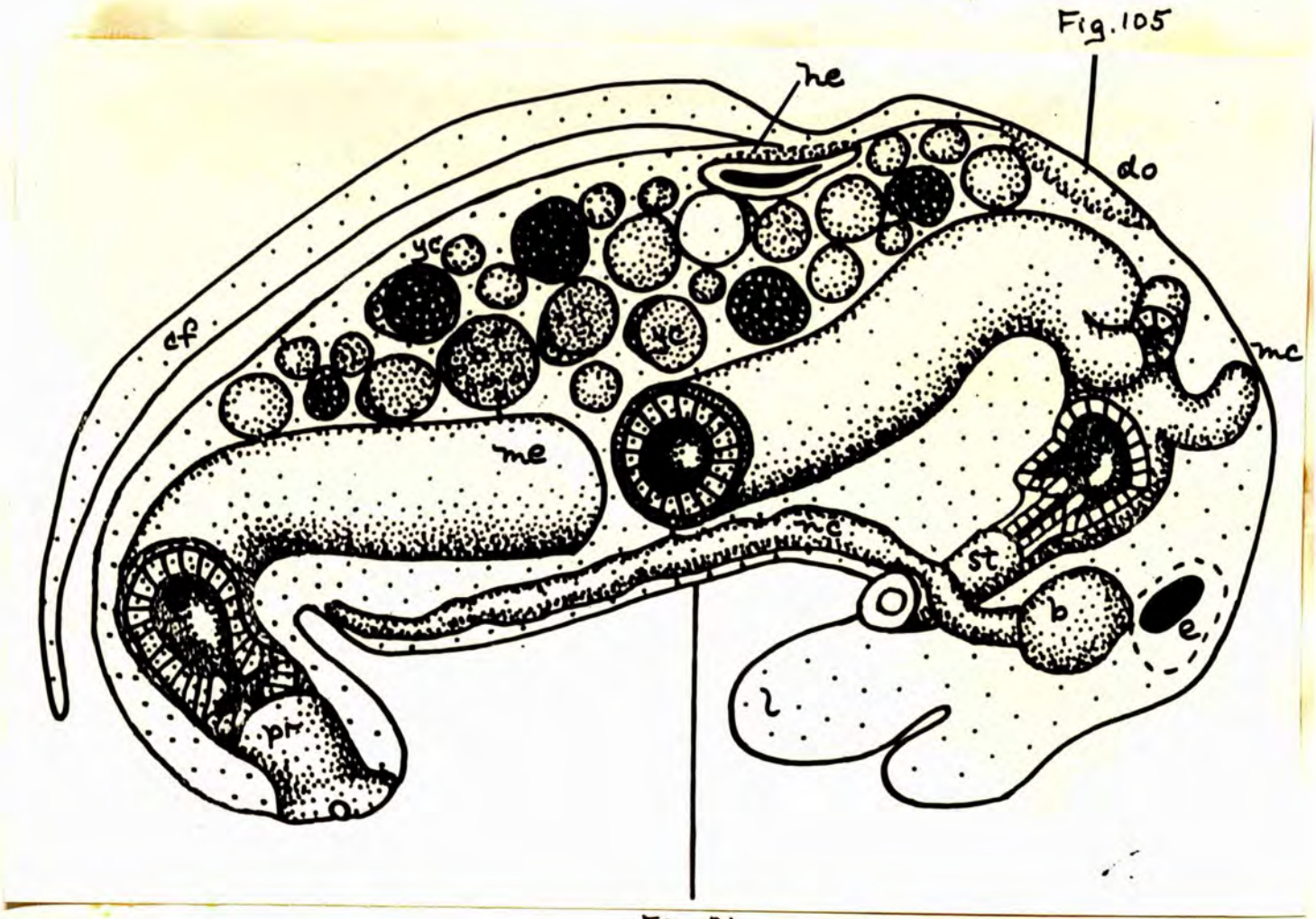


Figure 33. Diagrammatic reconstruction of the embryo of Daphnia magna at the stage when the mesenteron cavity has grown in size and the heart rudiment (he) is clearly distinguishable. The carapace folds (cf) are well developed. b, brain; do, dorsal organ; e, compound eye; l, labrum; mc, caecum of mesenteron; me, mesenteron; nc, nerve cord; pr, proctodaeum; st, stomodaeum; yc, yolk cell.

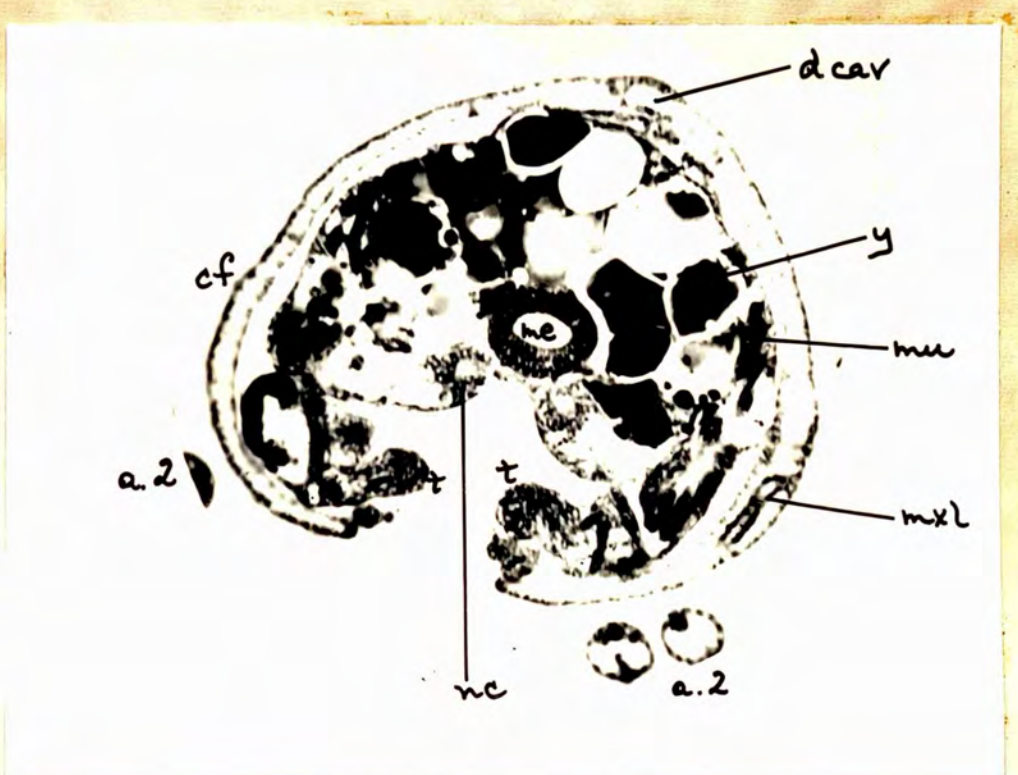


Plate 8. Photomicrograph of a transverse section through an embryo at the same stage as Fig.33,posterior to the heart,showing the mesenteron(me) and the small dorsal cavity(dcav). a.2,second antenna; cf,carapace fold; mxl,maxillary gland loop; mu,muscle; nc,nerve cord; t,thoracic appendage; y,yolk.

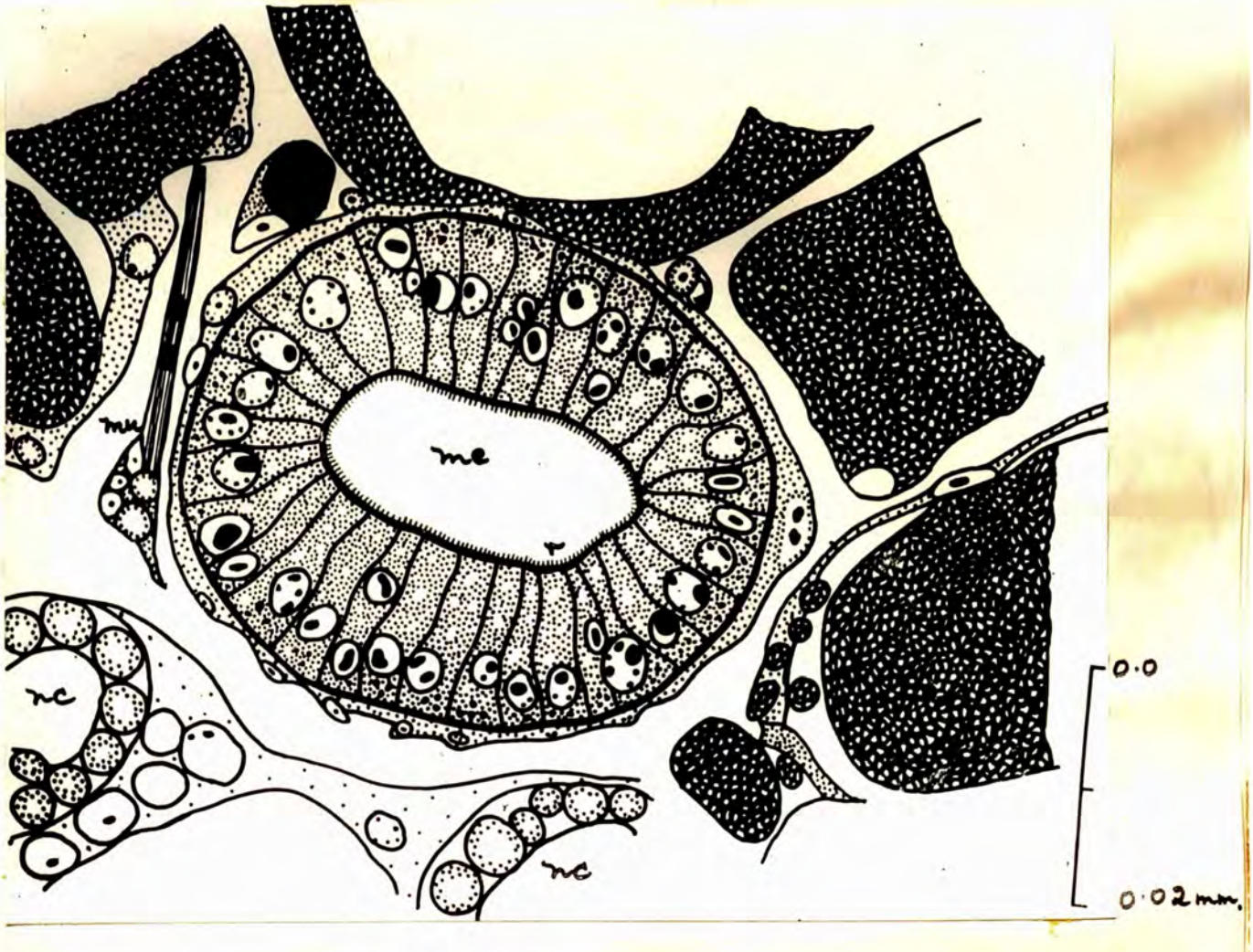


Figure 34. Transverse section through an embryo at the same stage as Fig. 33 showing the mesenteron (me). mu, muscle; nc, nerve cord; r, inner rods.

Fig.36

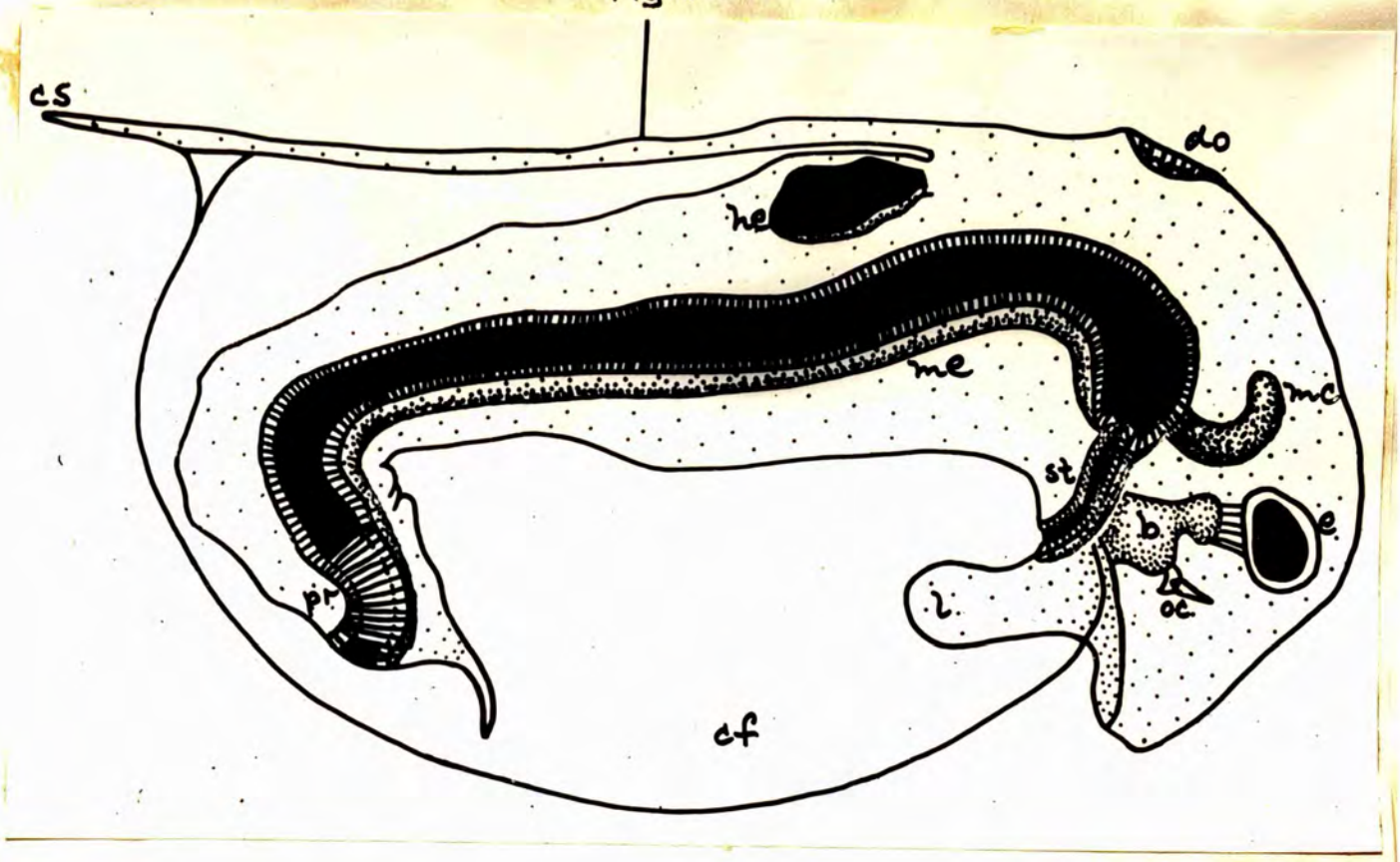


Figure 35. Diagrammatic reconstruction of the first instar Daphnia magna showing the fully developed alimentary canal. b, brain; cf, carapace fold; cs, caudal spine; do, dorsal organ; e, compound eye; he, heart; l, labrum; mc, caecum of mesenteron; me, mesenteron; oc, ocellus; pr, proctodaeum; st, stomodaeum.

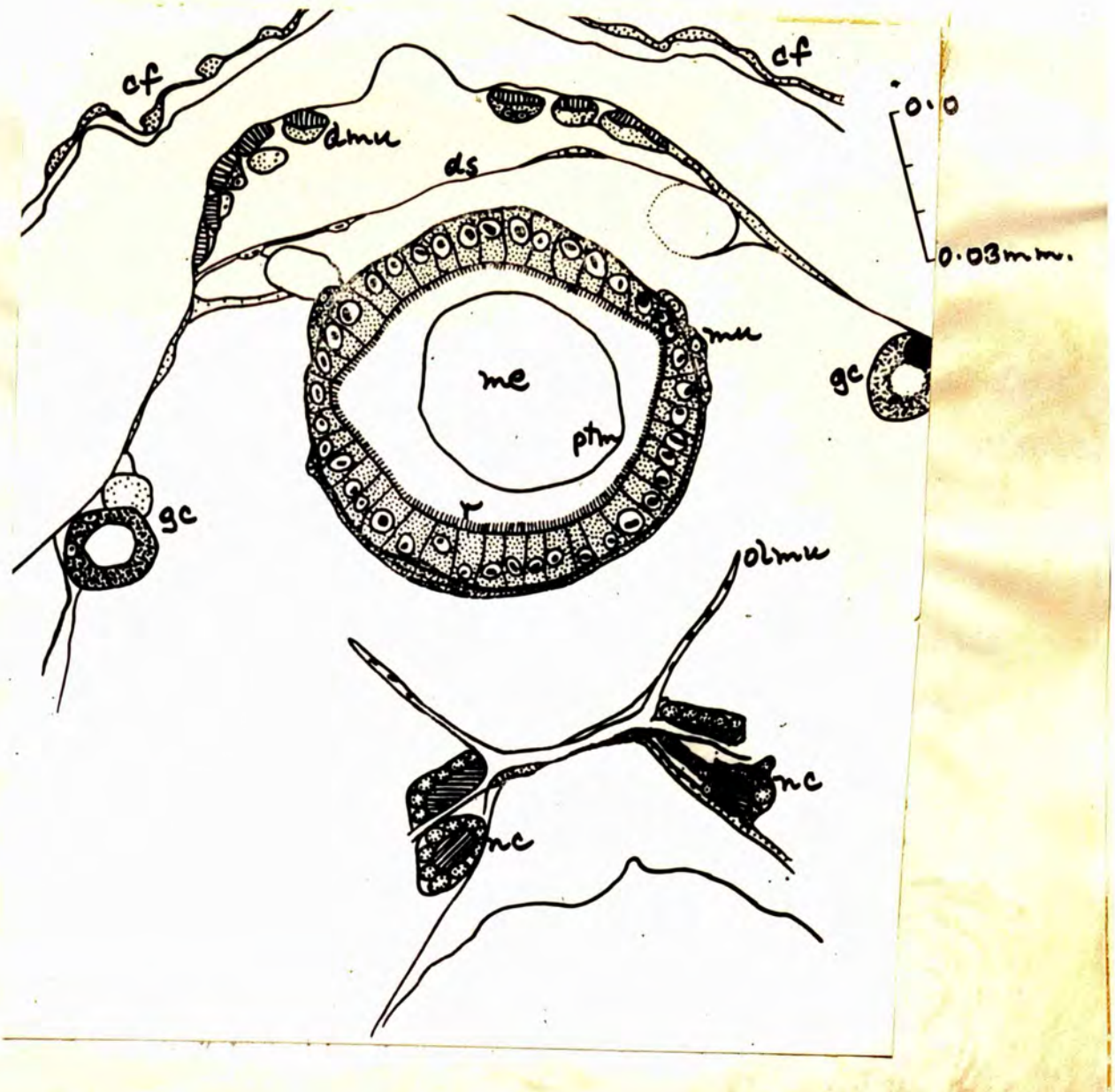


Figure 36. Transverse section through an embryo at the same stage as Fig. 35 showing the mesenteron (me). cf, carapace fold; dmu, dorsal longitudinal muscle; ds, dorsal septum; gc, genital cells; mu, muscle; nc, nerve cord; olmu, oblique lateral muscle; ptm, peritrophic membrane; r, inner rods.

surface of the embryo by a considerable quantity of yolk. The central cavity is open throughout and of the same width as that in the adult. The inner columnar cells are surrounded by muscle layers, and the muscles of the stomodaeum and proctodaeum are well developed. The alimentary canal is nearly always empty when the animal is in the brood pouch.

The amount of yolk present when the animal is expelled from the brood pouch of the mother as a first instar young is variable. Usually the yolk has disappeared by the end of the first instar and the mesenteron assumed its normal position in the body (Fig.37).

The most conspicuous difference between the early instar young and the adult animal is the smaller size of the caeca (see p.110).

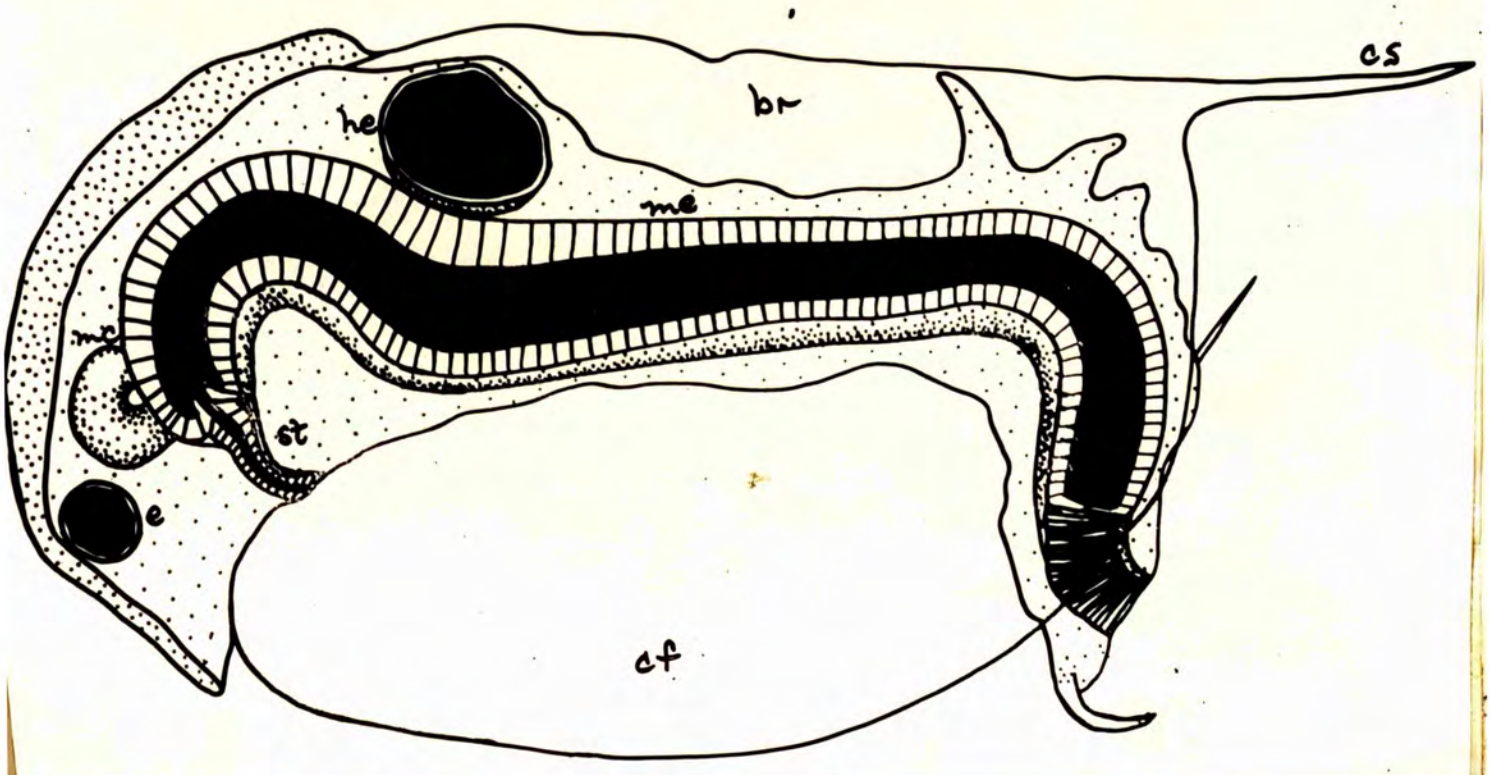


Figure 37. Diagrammatic reconstruction of the fifth instar Daphnia magna showing the final position of the mesenteron (me) close to the dorsal surface of the animal. br, brood pouch; cf, carapace fold; cs, caudal spine; e, compound eye; he, heart; mc, caecum of mesenteron; pr, proctodaeum; st, stomodaeum.

d. The yolk cells.

At the beginning of gastrulation (Fig. 9), all the cells of the blastoderm contain granules of yolk within vacuoles in the part of their cytoplasm next to the interior of the egg (Fig. 38). As gastrulation proceeds, the cells immigrating into the interior of the egg, with the exception of the genital cells, all contain yolk. The yolk within the cells has a different staining reaction from the granules remaining in the central mass and is apparently in the process of absorption.

Soon after the beginning of gastrulation, a number of cells appear in the peripheral part of the central yolk mass (Fig. 39). They arise by division of the cells surrounding the yolk, the outer cell layer (ec) and the inner cell mass. The cells engulf granules of yolk, so that each yolk cell (Fig. 40,(a) and (b),yc) consists of a large inner area of yolk surrounded by an extremely thin peripheral layer of cytoplasm, swollen in one area where the nucleus is present. The nucleus is small with scattered chromatin granules. The cytoplasm of the cell is not at first markedly granular.

The early yolk cells are arranged at moderately regular intervals in the periphery of the yolk (Fig. 41,yc), the remainder of the yolk being unchanged. In sections of the egg, especially those that have been stained with Mallory's Triple Stain, the yolk cells are conspicuous because of a different staining reaction from the remainder of the yolk. With Mallory's Triple Stain the yolk within the yolk cells stains blue, while the remainder of the yolk stains orange. With Heidenhain's Iron

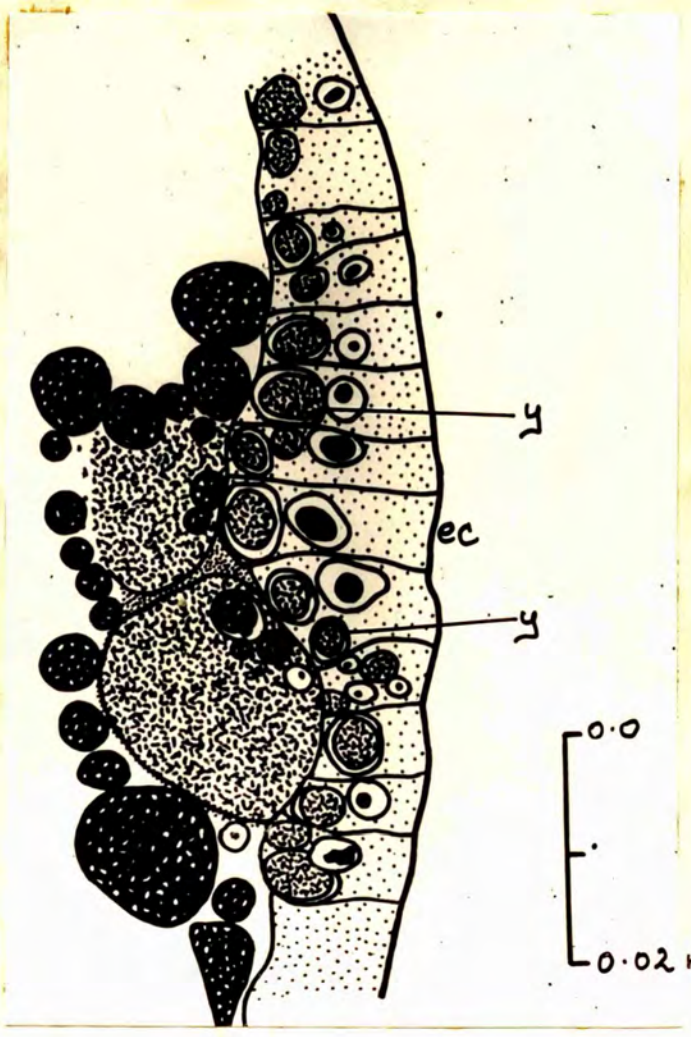


Figure 38. Transverse section through an egg at the same stage as Fig. 15 showing yolk globules (y) enclosed within the inner ends of the ectodermal cells (ec).

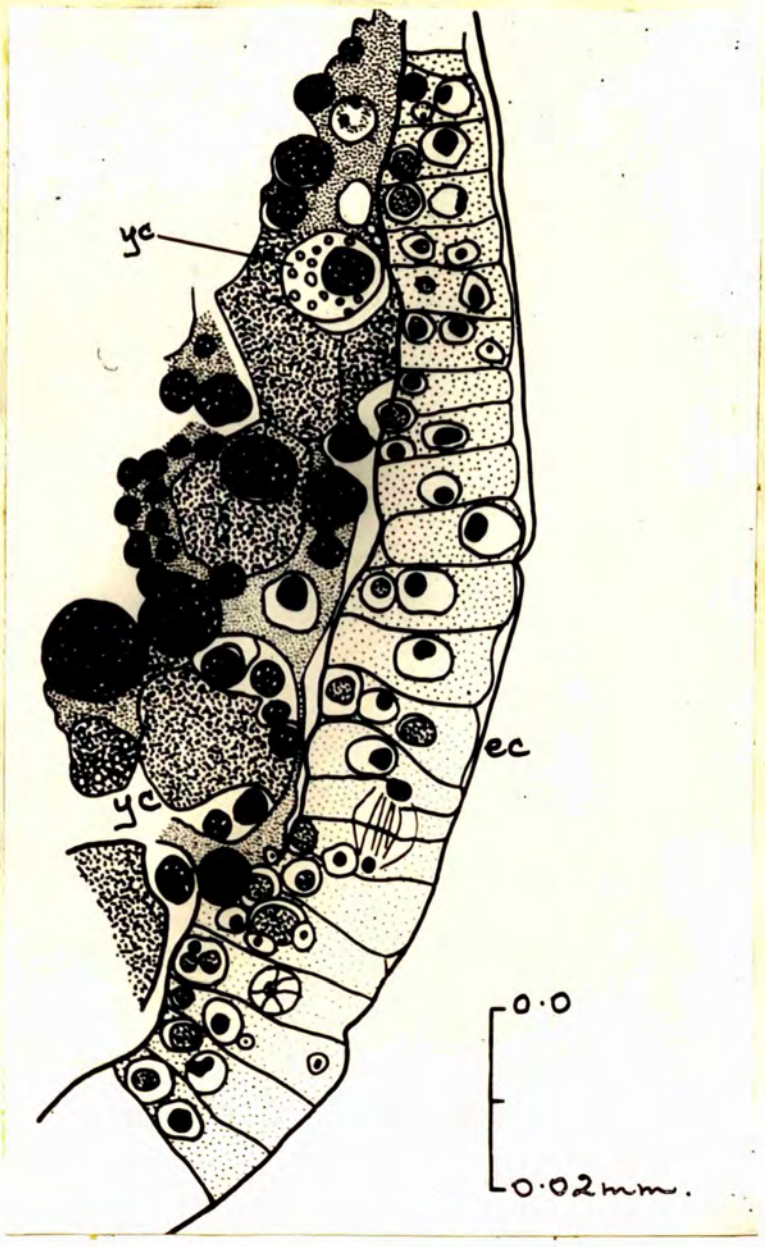
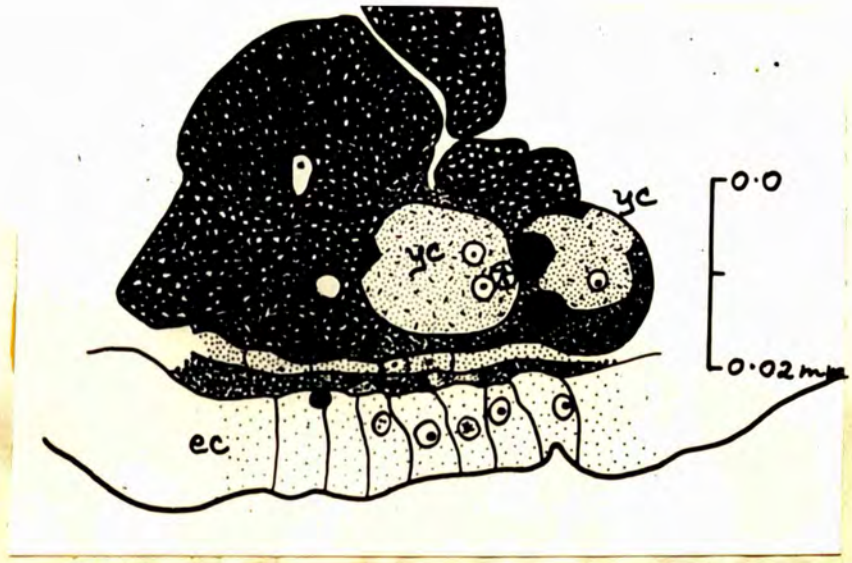


Figure 39. Transverse section through an egg at the same stage as Fig. 15 showing the formation of the yolk cells(yc) from the cells (ec) surrounding the yolk.



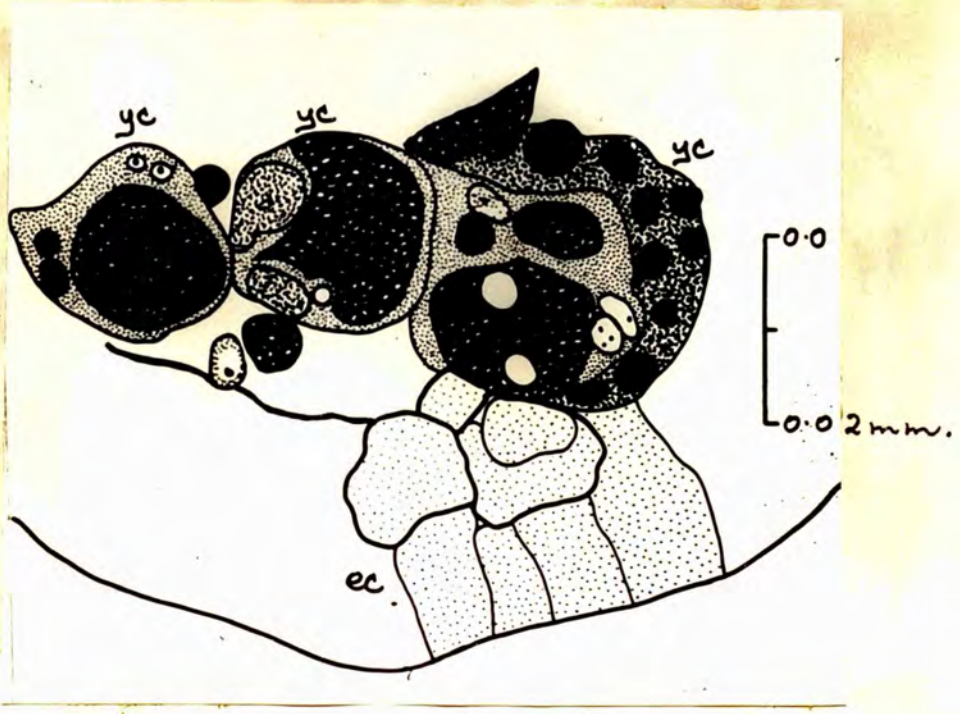


Figure 40. (a) and (b). Sections through an egg at the same stage as Fig. 41 showing early stages in the formation of yolk cells (yc). ec, ectoderm.

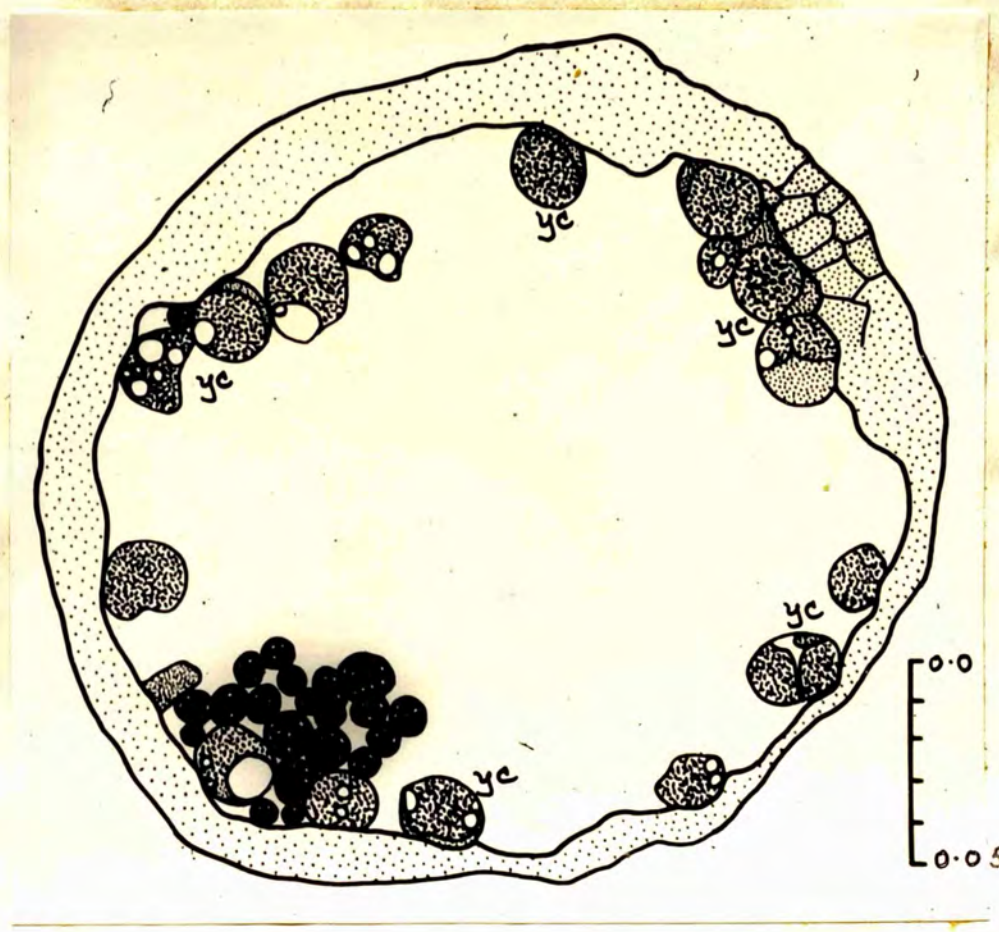


Figure 41. Transverse section through an egg at a stage between Fig. 15 and Fig. 19, when the second antenna has become two-branched, showing the formation of yolk cells (yc) from ectoderm cells surrounding the yolk.

haematoxylin, the yolk within the yolk cells shows much less affinity for the stain than the rest of the yolk, staining a pale brown instead of dark blue. The yolk contained inside the yolk cells also becomes granular in patches (Fig. 42,(a),yc) and the staining reaction becomes uneven indicating a breakdown and absorption of yolk.

The formation of yolk cells around the periphery of the yolk mass continues throughout the process of gastrulation and is still taking place when the endodermal cells have become distinguishable in the inner cell mass, when the egg is about one day old (Fig.13; Fig. 42,(a),yc). The yolk cells arise from the cells all around the central yolk mass and not from one particular area.

The yolk cells begin to extend inwards to the centre of the egg. They are most abundant in a pair of irregular bands on either side of the egg situated near to the lateral edges of the mesoderm (Fig. 13,yc). The yolk cells vary in size and in the state of the yolk contents (Fig. 42(a). In some of them the peripheral cytoplasm is beginning to increase in amount and to become granular. The nuclei show a redistribution of chromatin and the nucleolus becomes large and stains intensely. The nuclei themselves increase in size. In the regions in which the yolk cells are most numerous they are surrounded by small intensely staining yolk granules.

At a slightly later stage (Fig. 19), yolk cells are present around most of the periphery of the yolk while a number of cells occur scattered through the central yolk mass. Several cytoplasmic strands extend between the granules of the central mass

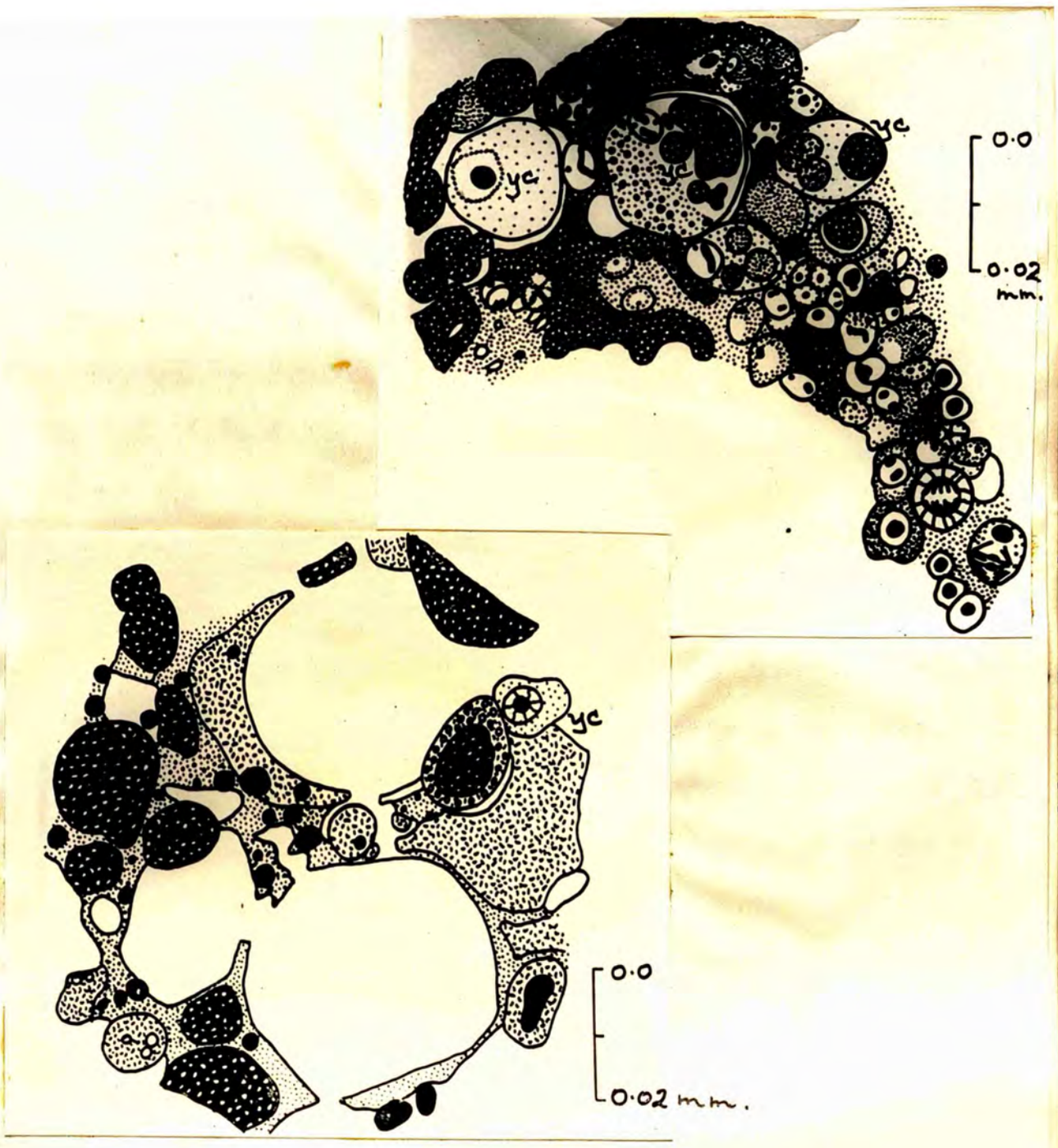


Figure 42. (a) and (b). Sections through an egg at a stage between Fig. 41 and Fig. 19 showing yolk cells (yc) in various stages of formation, and cytoplasmic syncytium extending between the yolk granules.

of yolk to form a syncytium. They are in contact with the yolk cells but are not themselves in the form of cells. The yolk within the yolk cells has become granulated and with an uneven staining reaction. It also shows, in sections, a number of small round spaces or vacuoles which are probably due to change in the composition of part of the yolk to a substance soluble in xylene. The outer part of the yolk stains less intensely than the inner area which is principally composed of small, intensely staining granules.

Shortly after the bursting of the outer egg membrane (Fig.22), the outer area of the yolk mass consists of large cells with large yolk granules with little affinity for haematoxylin (Fig.44). The inner area has a number of similar cells, together with smaller apparently unaltered yolk granules.

The cytoplasmic syncytium becomes more conspicuous (Fig.42, (b)), extending through the yolk and connecting the inner yolk cells. This syncytium was not apparent at the beginning of gastrulation. Therefore it would seem that it is not the remnant of the cytoplasmic strands along which the central blastomeres migrated to the periphery at an early stage to form the blastula.

When the embryo is just over three days old, the alimentary canal being open throughout its length (Fig.43), the remaining mass of yolk shows various stages of absorption (Fig.44; Fig.45). The innermost yolk granules remain unchanged. These occur immediately dorsal and lateral to the mesenteron and dorsal to the bases of the thoracic appendages (Fig.45). The outermost

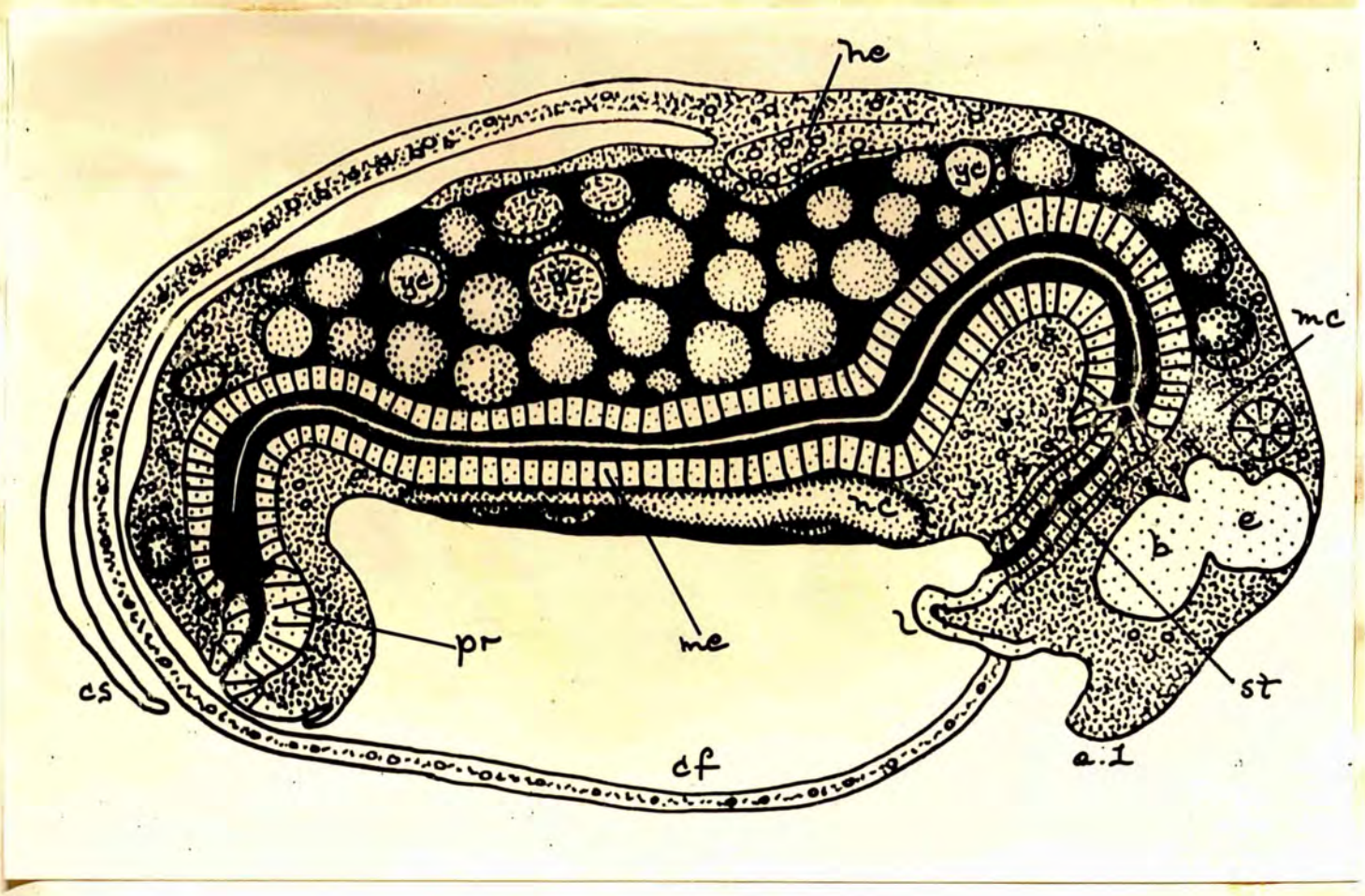


Figure 43. Diagrammatic reconstruction of an embryo of Daphnia magna at the stage when the mesenteron (me) has a well developed central cavity but is still close to the ventral surface of the animal and when most stages in the formation of yolk cells (yc) are present.
a.1, antennule; b, brain; cf, carapace fold; cs, caudal spine; e, compound eye; he, heart; l, labrum; mc, caecum of mesenteron; nc, nerve cord; pr, proctodaeum; st, stomodaeum.

Figure 44. Sagittal section through an embryo at the same stage as Fig. 43 showing yolk globules (y) being broken down. cf, carapace fold; ds, dorsal septum; yc, yolk cell.

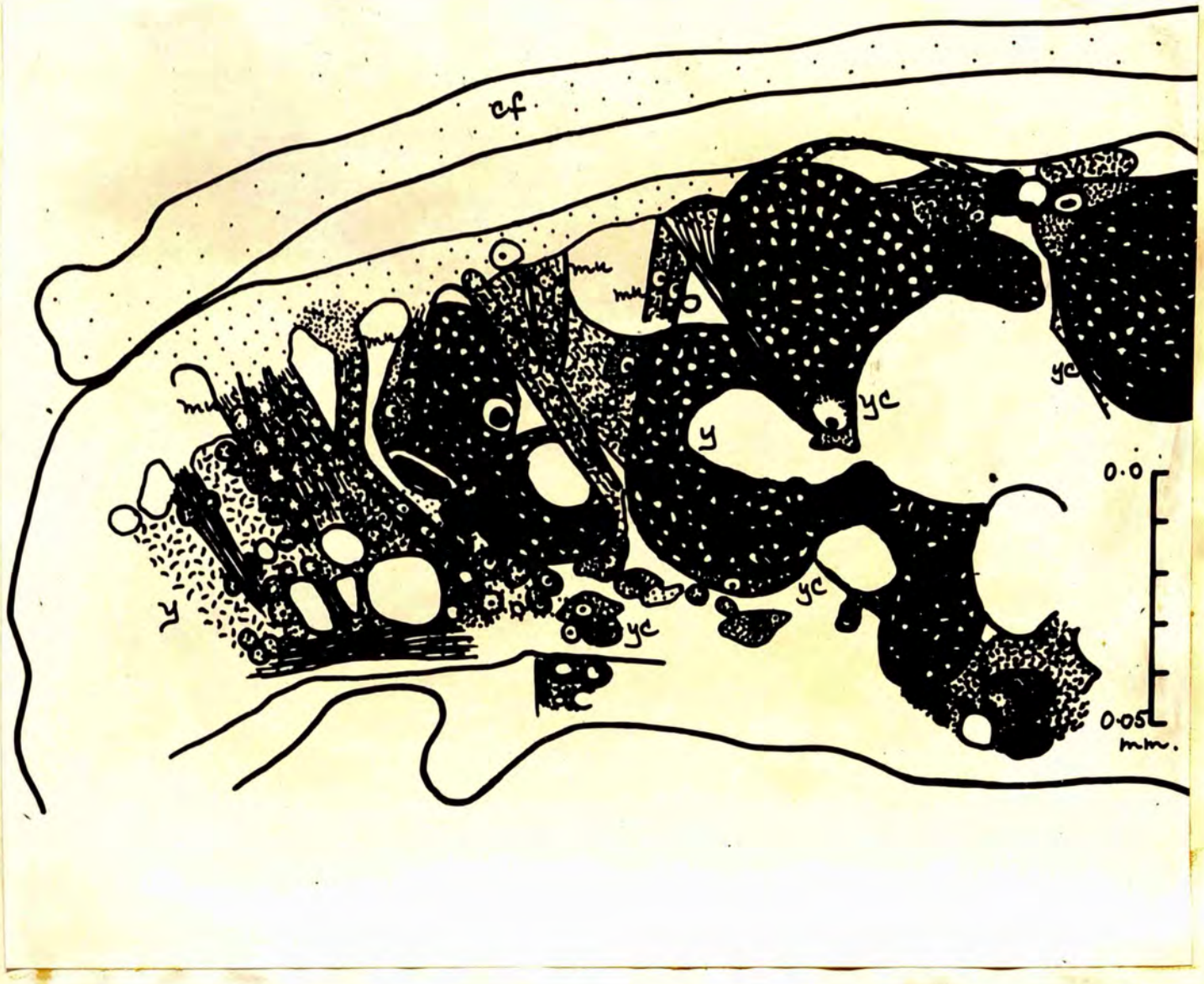


Figure 45. Sagittal section through an embryo at the same stage as Fig. 43 showing yolk cells (yc) and yolk (y) being broken down. cf, carapace fold; mu, muscle.

of these granules are partly themselves made up of small granules. Surrounding this area of yolk granules and merging into it is an area of yolk cells, with yolk granules in various stages of absorption within an outer layer of cytoplasm containing a nucleus (Fig.44; Fig.45,yc). These occur immediately dorsal to the ventral septum and in the lateral part of the body ventral to the gonad. They are also found in the bases of the thoracic limbs and near the base of the second antennae. Surrounding the area of yolk cells, forming the most peripheral part of the original yolk mass, is an area filled with a substance showing little affinity for haematoxylin, and containing numerous vacuoles. This substance occurs dorsally in and around the heart and in most of the dorsal part of the body. It fills the carapace folds and is present in the anterior region of the head. It surrounds the maxillary glands and fills the base of the second antennae. It is also present in the thoracic appendages and in the paired ventral cavities. It occurs in the abdomen and in the posterior part of the body. The staining reaction of this substance is similar to that of the contents of some of the yolk cells, and the substance appears to be an advanced stage in the breakdown of the yolk.

In the first instar animal, recently expelled from the brood pouch of its mother (Fig.46), there remain only a few unaltered yolk granules in the region ventral to the heart. The rest of the body contains cells in various stages of transition from yolk cells to fat cells, including a number of fully formed fat cells (Figs. 47-50). Surrounding the unaltered yolk

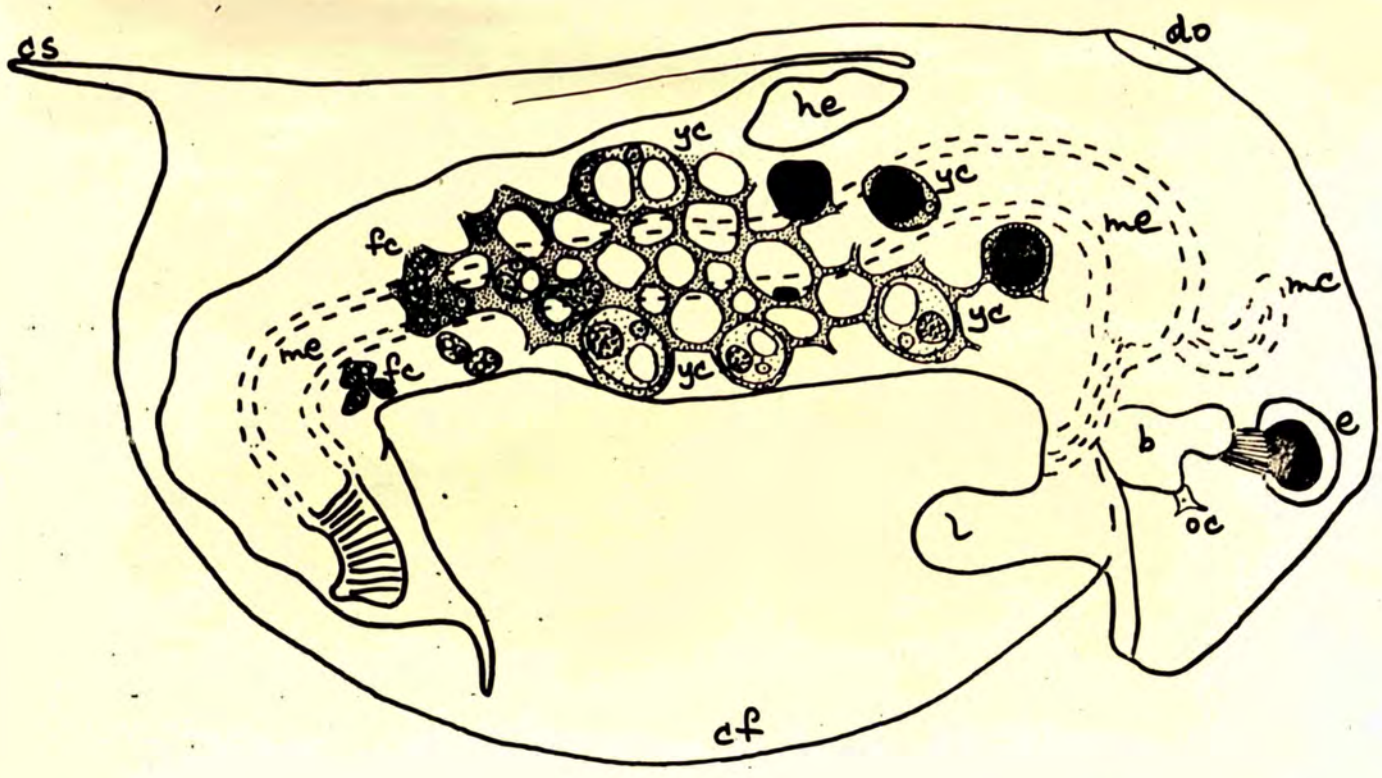


Figure 46. Diagram of a first instar Daphnia magna to show the position in the body of different stages in the formation of yolk cells (yc) and their transition to fat cells (fc). b, brain; cf, carapace fold; cs, caudal spine; do, dorsal organ; e, eye, he, heart; l, labrum; mc, caecum of mesenteron; me, mesenteron; oc, ocellus.

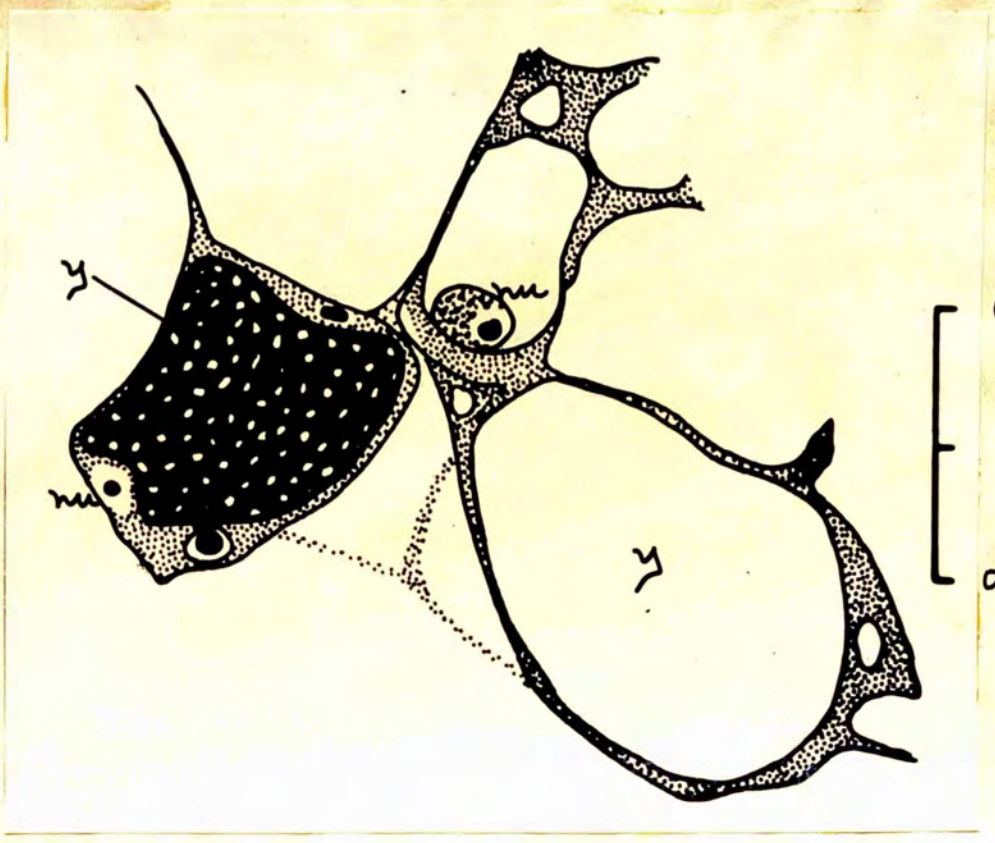


Figure 47. Vertical longitudinal section through the maxillary region of a first instar Daphnia magna at the same stage as Fig. 46 showing fat cells containing either altered or unaltered yolk (y). nu, nucleus.

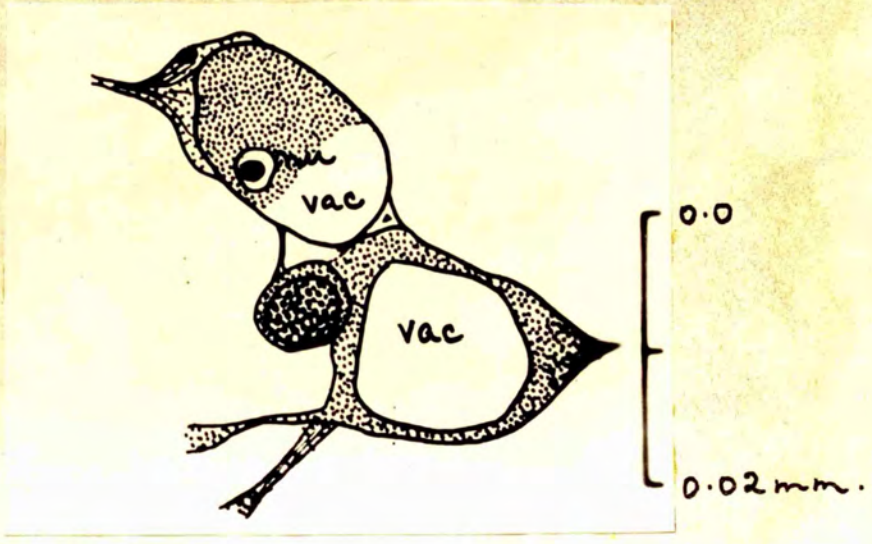


Figure 48. Vertical longitudinal section through a first instar Daphnia magna at the same stage as Fig. 46 showing fat cells each with granular cytoplasm and central vacuole (vac). nu, nucleus.

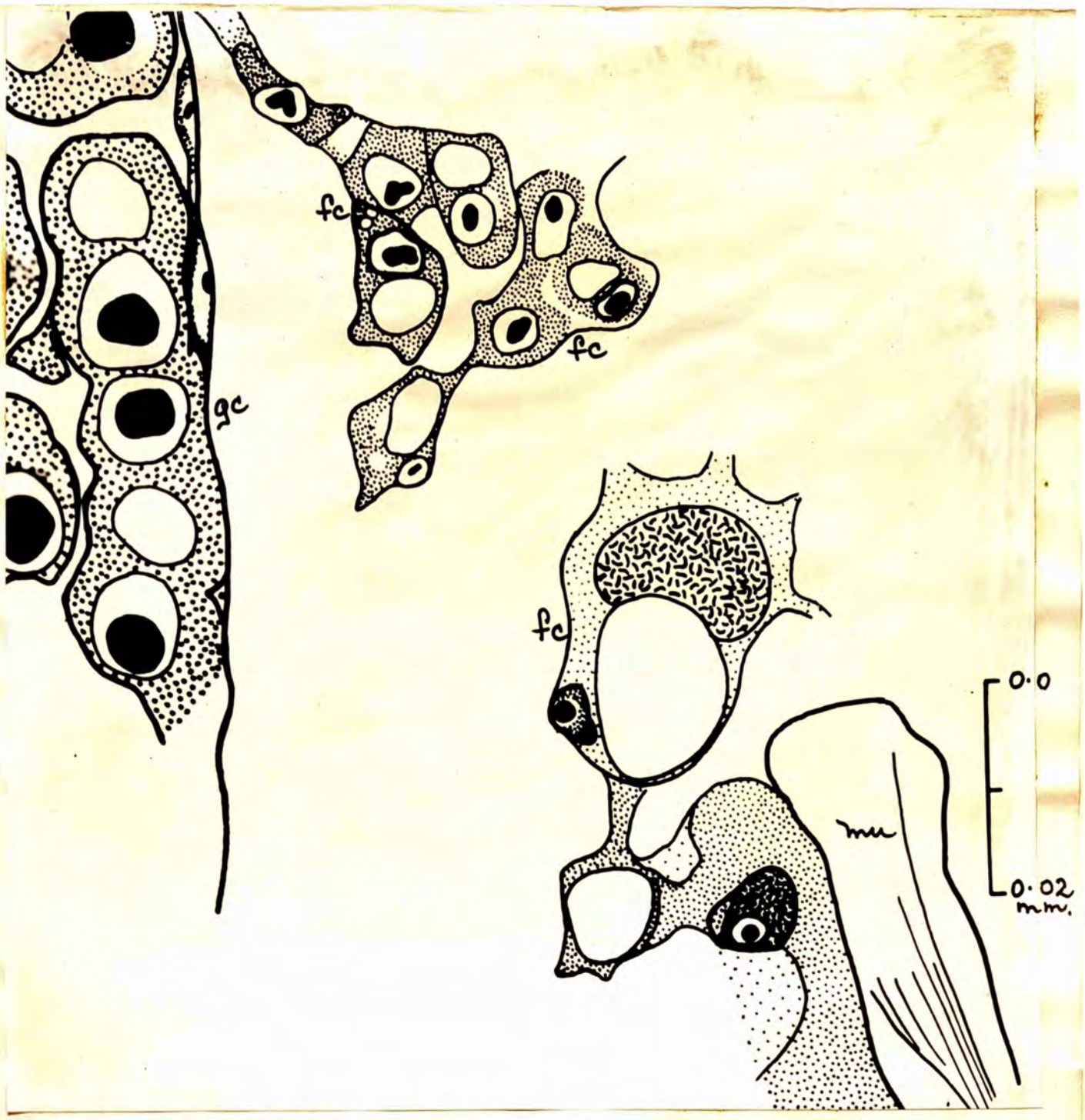


Figure 49. Vertical longitudinal section through a first instar Daphnia magna at the same stage as Fig. 46 showing fat cells (fc) fully formed or still with disintegrating yolk (y). gc, genital cells; mu, muscle.

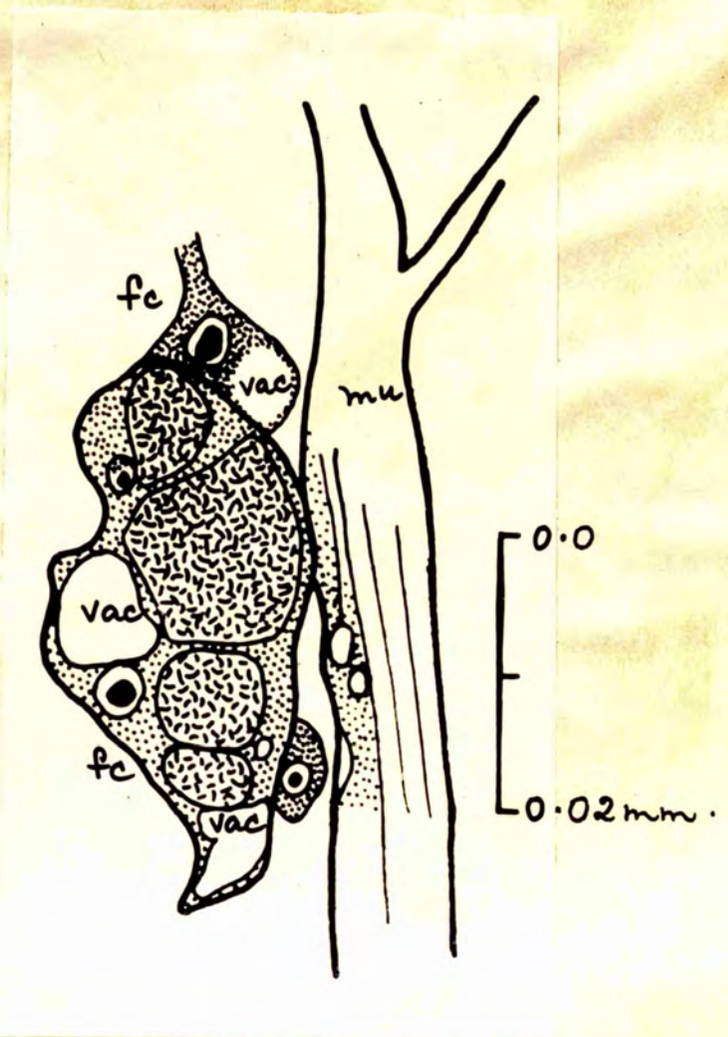


Figure 50. Vertical longitudinal section through a first instar Daphnia magna at the same stage as Fig. 46 showing fat cells (fc) containing vacuoles (vac) and lying close to one of the ventral longitudinal muscles (mu).

granules are a number of yolk cells with a thin outer layer of cytoplasm, some of them contained within a syncytium of cytoplasm (Fig.47; Fig.48). This syncytium is a meshwork of finely granular cytoplasm containing large vacuoles. It contains also a number of cells which contain a globule of disintegrating yolk and a large vacuole. There is a small amount of the vacuolated substance which shows little affinity for haematoxylin present in parts of the syncytium. The syncytium occurs principally in the lateral area of the body. Posteriorly and ventral to the gonad are a number of fully developed fat cells (Fig.49). These contain a large nucleus with a large, rod-shaped nucleolus. The cytoplasm is granular and there is a large vacuole. In the first instar animal they are present in the thoracic appendages and in the region of the posterior flexure of the mesenteron.

The yolk cells thus give rise directly to the fat cells, by an increase in the area of peripheral cytoplasm, a breakdown of the yolk contents and the formation of a vacuole in the cytoplasm. The nucleus also changes, becoming larger and developing a large central nucleolus (see p. 162).

e. The early development of the mesoderm.

At the end of gastrulation the inner mass of cells is not distinguishable into endoderm and mesoderm (Fig.11; Fig.12,men). Gradually a mid-ventral strand of cells is differentiated from the endomesodermal mass (Fig.13). These are the endodermal cells (en) and form the mesenteron. The remaining cells, lateral, anterior and also posterior to the mid-ventral strand are the mesoderm (m). They extend anteriorly to the "Scheitel"-plate, but posteriorly only just past the endoderm. The mesoderm for most of its length extends over one-quarter of the circumference of the egg in either direction from the mid-ventral line. The mesodermal cells are small, irregular, granular cells with small nuclei containing scattered chromatin granules (Fig.14,m).

The lateral part of the mesoderm begins to extend dorsalwards, especially in the region immediately posterior to the "Scheitel"-plate. Mesodermal cells are present in the antennal fold (Fig.15).

At about the time of the bursting of the outer egg membrane (Fig.19), the greater part of the egg is still filled with yolk. Anteriorly the mesoderm (m) is thickened and fills most of the head between the "Scheitel"-plate (Sch) and the second antennae (a.2). In the ventral part of the egg, the mesoderm is several layers thick on either side of the alimentary canal (en). For most of its lateral extension the mesoderm (m) is only one cell thick but posteriorly is thickened slightly. Each second antenna (a.2) has divided into two, and these pairs of branches, together with the pair of developing mandibles (md), are filled with mesodermal cells (m).

Posterior to the region of the second antenna and mandible, in the dorsal limit of the mesoderm, a single pair of tiny cavities or splits is usually present (Fig.51). Each cavity is surrounded by a single layer of mesodermal cells. This pair of cavities is the only indication of dorsal coelomic sacs. No well-defined ventral cavities are present at this stage. The only slits in the ventral region are between mesoderm and genital cells and do not resemble cavities formed in a single mass of cells. It should be noted that the dorsal edges of the mesoderm are separated from the endoderm, which is still lying against the ventral surface, and from the mid-dorsal surface by a considerable quantity of yolk. This is the most striking difference from the situation in Estheria or Chirocephalus.

At a slightly later stage, about 40 hours old, (Fig.22; Fig.52; Fig.53), the mesoderm fills the second antennae and mandibles and also the developing rudiments of the thoracic appendages. The carapace folds (cf) have begun to develop and contain a number of mesodermal cells. Anteriorly the mesoderm (m) is slightly thicker in a band immediately posterior to the midgut caeca (mc). The mesoderm in the head region is beginning to show a certain amount of differentiation.

As the embryo develops and begins to grow longer (Fig.54), the posterior part of the mesoderm extends over the dorsal surface of the yolk as a single layer of cells (m). In approximately the area of the second antennae, the lateral mesoderm is about four or five layers thick, and forms a broad band projecting inwards (Fig.55). In front of this area (Fig.56) the mesodermal

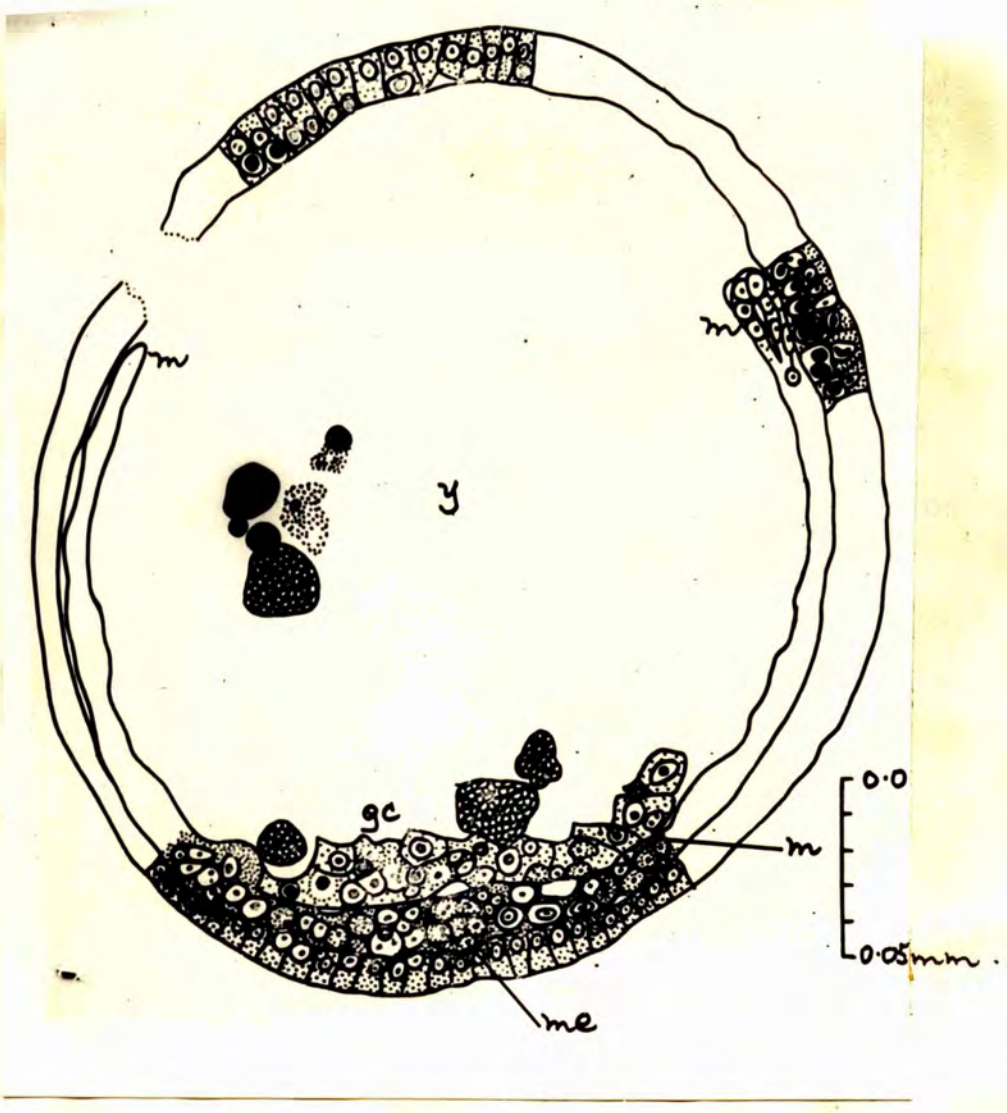


Figure 51. Transverse section through an egg at the same stage as Fig. 19 showing the dorsal extent of the mesoderm (m) laterally, with an indication of a small split in the most dorsal part of the mesoderm on one side. The genital cells (gc) lie adjacent to the ventral mesoderm cells (m). me, mesenteron; y, yolk.

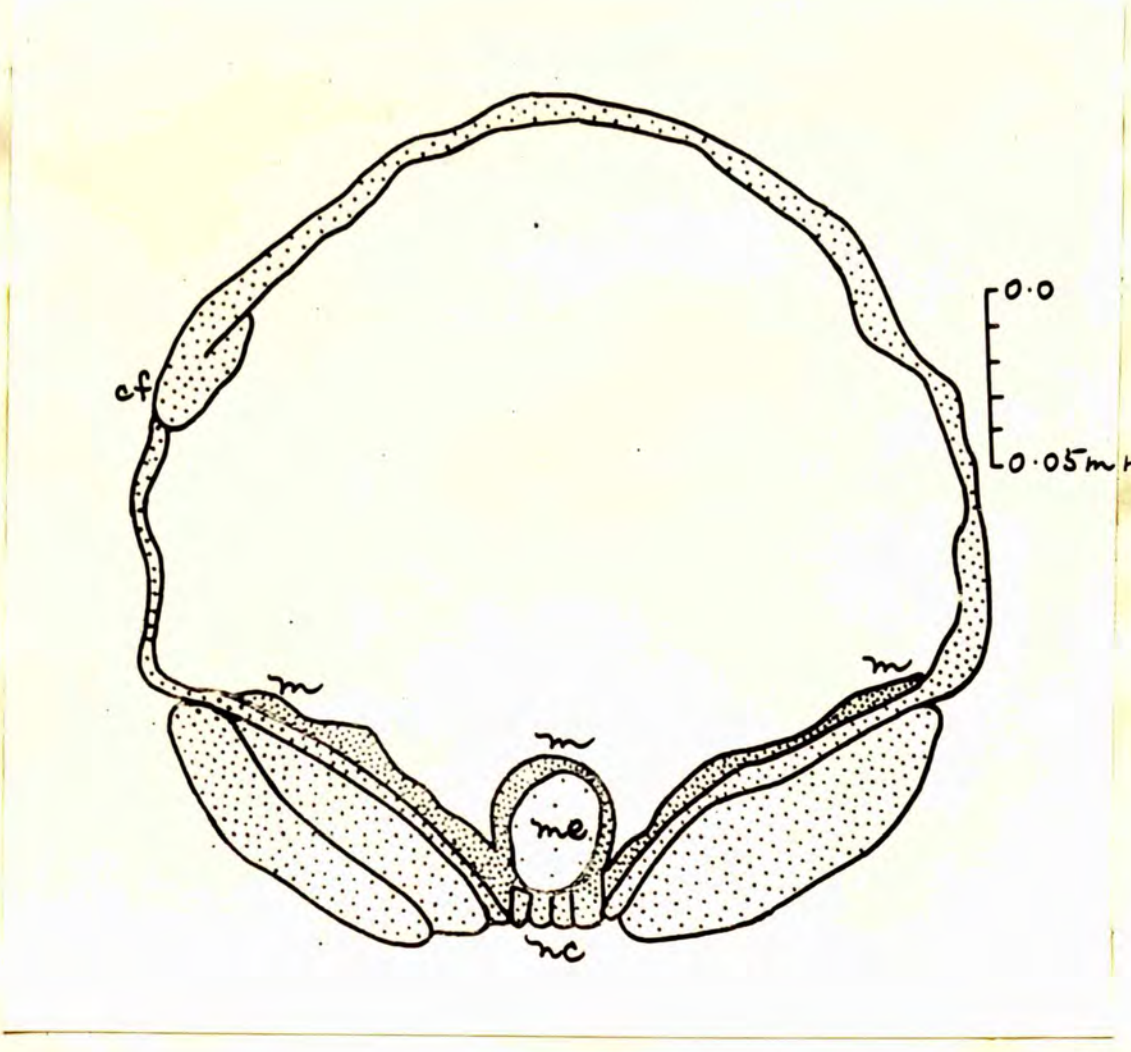


Figure 52. Transverse section through an egg at the same stage as Fig. 22 towards the posterior end, showing the dorsal extent of the mesoderm (m) laterally and also the mesoderm surrounding the mesenteron (me); the beginning of a carapace fold (cf) and an indication of the beginning of the formation of the ventral nerve cord (nc).

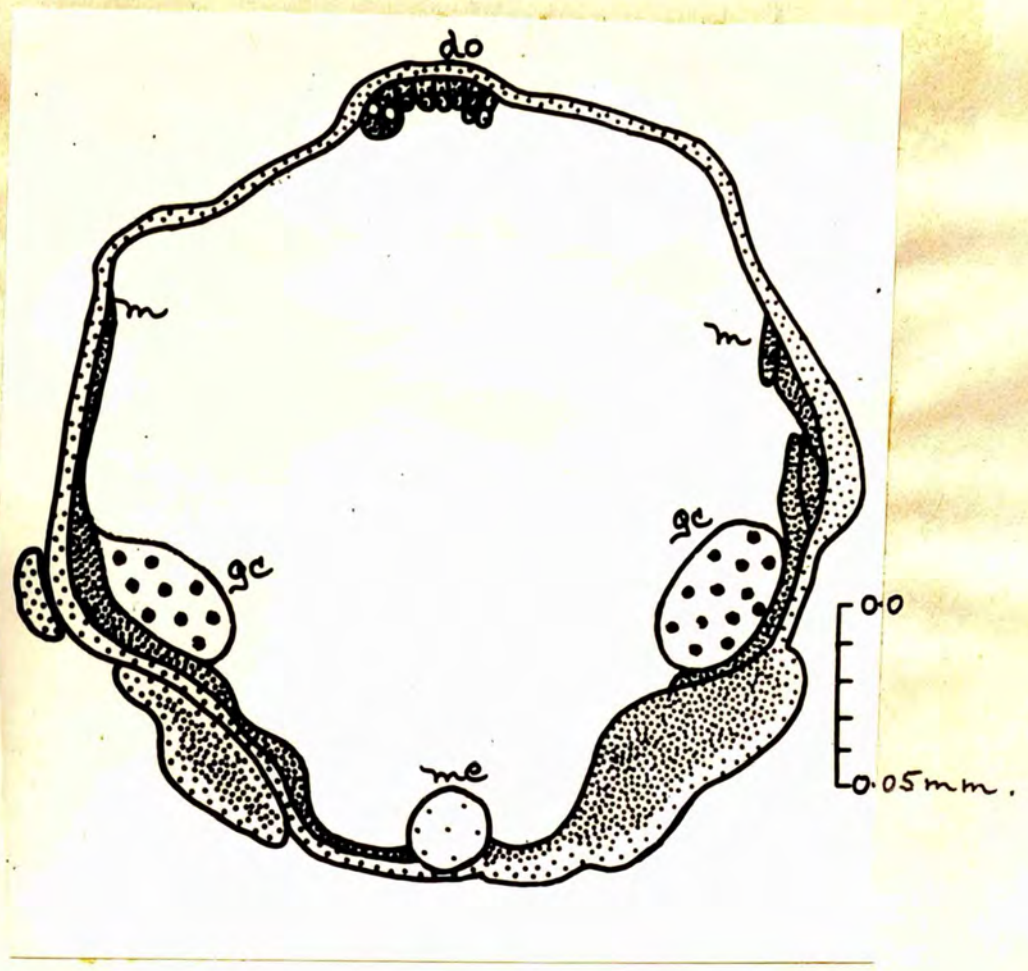


Figure 53. Transverse section through an egg at the same stage as Fig. 22, anterior to Fig. 52, showing the dorsal extent of the mesoderm (m) laterally and the paired genital rudiments (gc). do, dorsal organ; me, mesenteron.

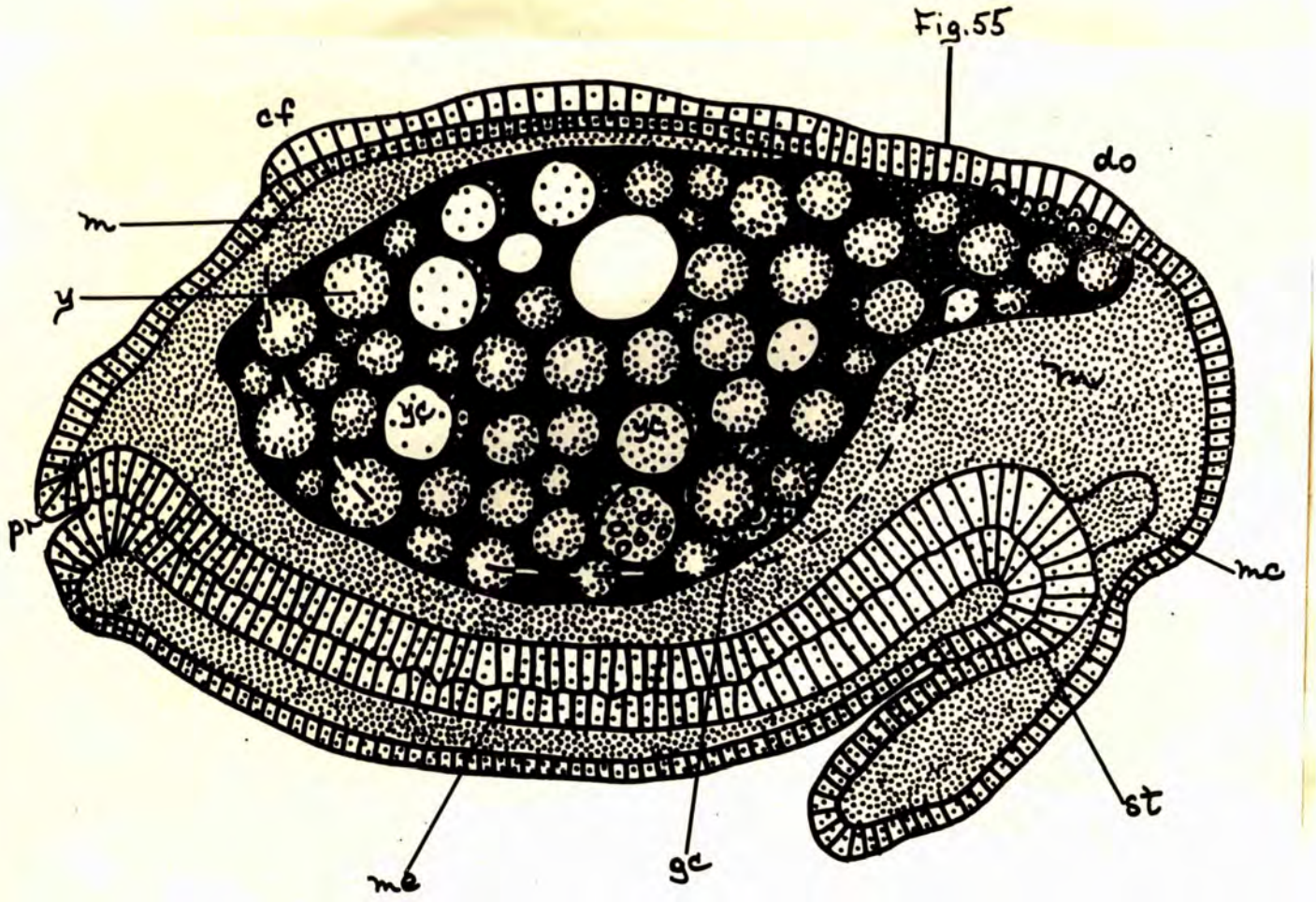


Figure 54. Diagrammatic reconstruction of an embryo of Daphnia magna at the stage when the mesenteron (me) has not yet developed a central cavity but the mesoderm (m) has extended over a large proportion of the dorsal surface of the yolk (y). cf, carapace fold; do, dorsal organ; gc, genital cells; mc, caecum of mesenteron; pr, proctodaeum; st, stomodaeum; yc, yolk cell.

Figure 55. Vertical longitudinal section close to the midline through an embryo at the same stage as Fig. 54 showing the lateral part of the dorsal organ (do) and the dorsal mesoderm (m) posterior to it in the maxillary region. cf, carapace fold.

Figure 56. Transverse section through an embryo at the same stage as Fig. 54, posterior to the dorsal organ, showing the lateral mesodermal thickening (m) in the maxillary region.

layer (m) is thinner, especially on either side of the dorsal organ (do).

When the embryo is about two days old or slightly older (Fig.25), the dorsal edge of the mesoderm has thickened slightly (Fig.58). The mesoderm (m) has extended further anteriorly along the dorsal surface but it does not yet completely cover the yolk dorsally (Fig.57).

At a slightly later stage (Fig.28), the dorsal mesoderm becomes continuous, although the cells in the future heart region are larger than the other mesodermal cells (Fig.59-62,he). In the same region but ventral to these cells, the maxillary gland is represented by a compact mass of mesodermal cells in each carapace fold (Fig.63-67). The cells are large and spherical, with a more intense staining reaction than the other mesodermal cells and are the rudiment of the loops of the maxillary gland. There is also a small, poorly-developed rectangular end-sac (Fig.63, mxs). A few smaller cells are scattered through the more posterior part of the carapace folds. A number of small mesoderm cells are present in the palely-staining brokendown yolk in the dorsal part of the body (Fig.60,m). These are possibly blood cells. The embryo has now reached the stage when the alimentary canal has just acquired a central cavity. The mesoderm is thickened in the maxillary region and posteriorly (Fig.68,m).

When the embryo is about halfway through its development, that is about 50 hours old (Fig.69), the mesoderm is still absent from the posterior dorso-lateral regions of the body, where it

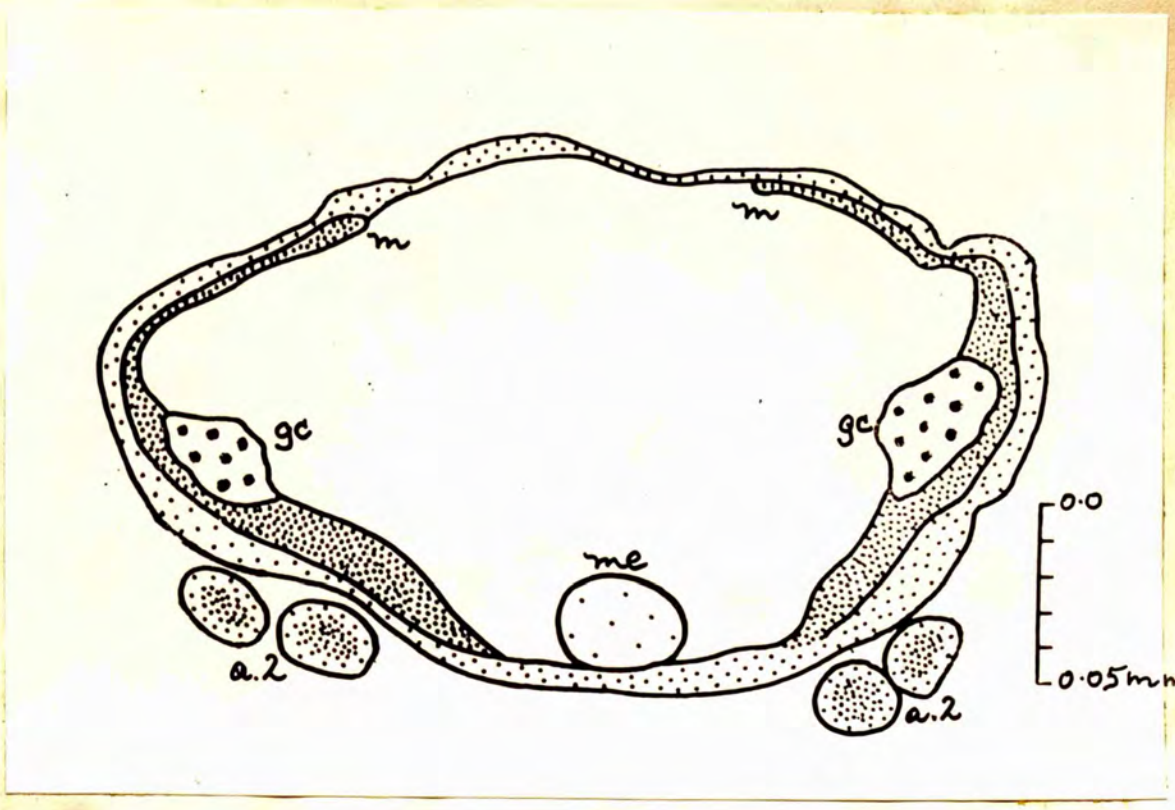


Figure 57. Transverse section through an embryo at the same stage as Fig. 25 in the region of the genital rudiments (gc) showing the dorsal extent of the mesoderm (m) laterally. a.2, second antenna, me, mesenteron.

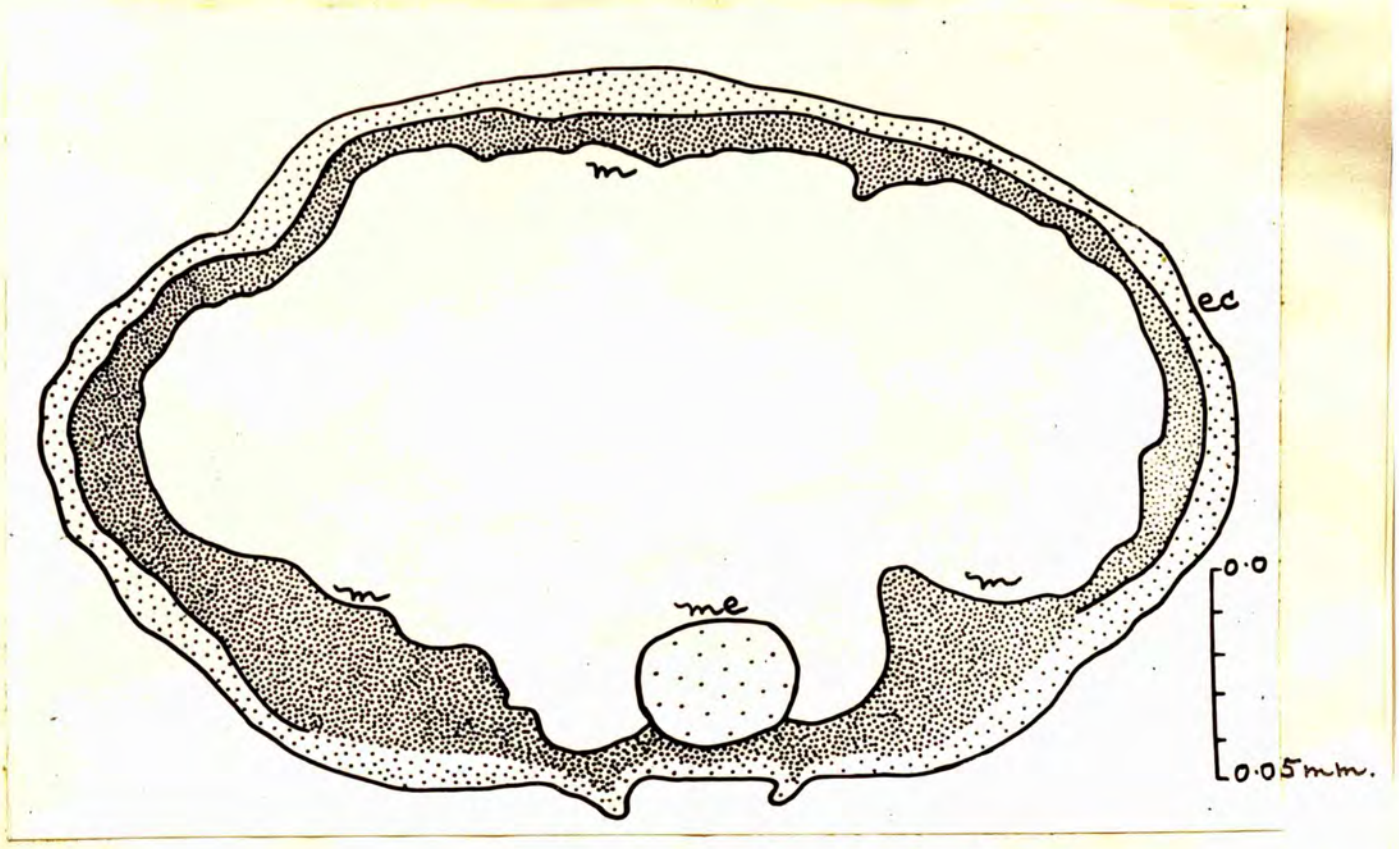


Figure 58. Transverse section through an embryo at the same stage as Fig. 25 in the region posterior to the genital rudiments showing the mesoderm (m) completely covering the yolk dorsally and thickened ventro-laterally. ec, ectoderm; me, mesenteron.

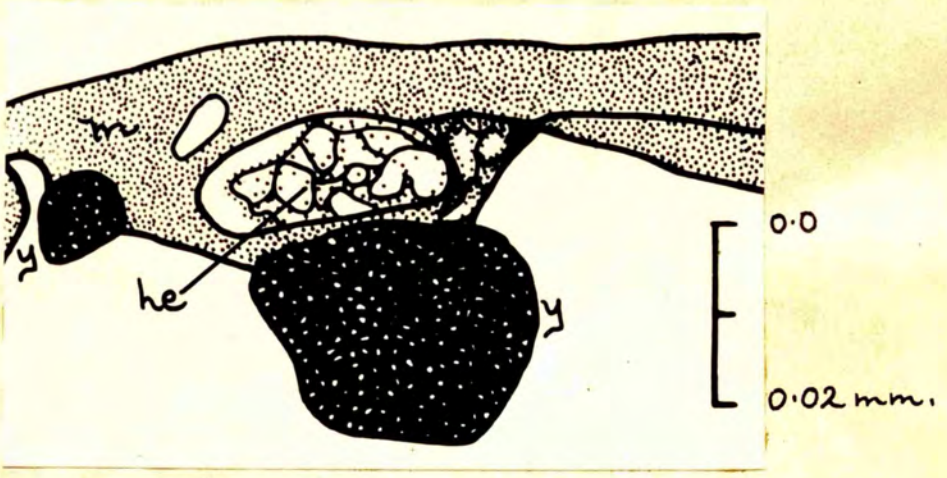


Figure 59. Vertical longitudinal section through an embryo at the same stage as Fig. 28 close to the midline, showing a thickening of the mesoderm (m) in the region subsequently occupied by the heart (he). y, yolk.

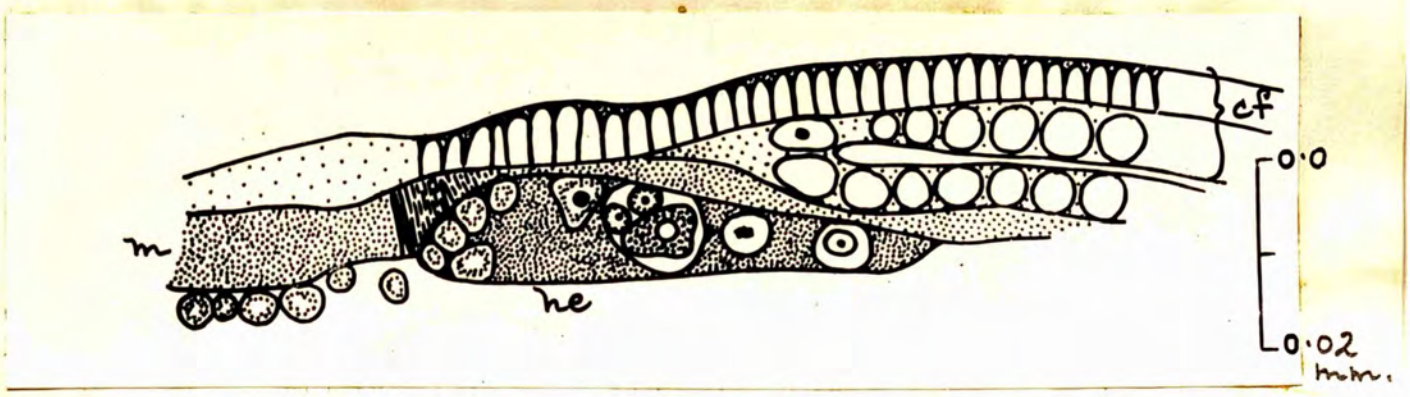


Figure 60. Sagittal section through an embryo at the same stage as Fig. 28 showing the rudiment of the heart (he) composed of a mass of mesoderm cells in which a cavity is beginning to form. cf, carapace fold; m, mesoderm.

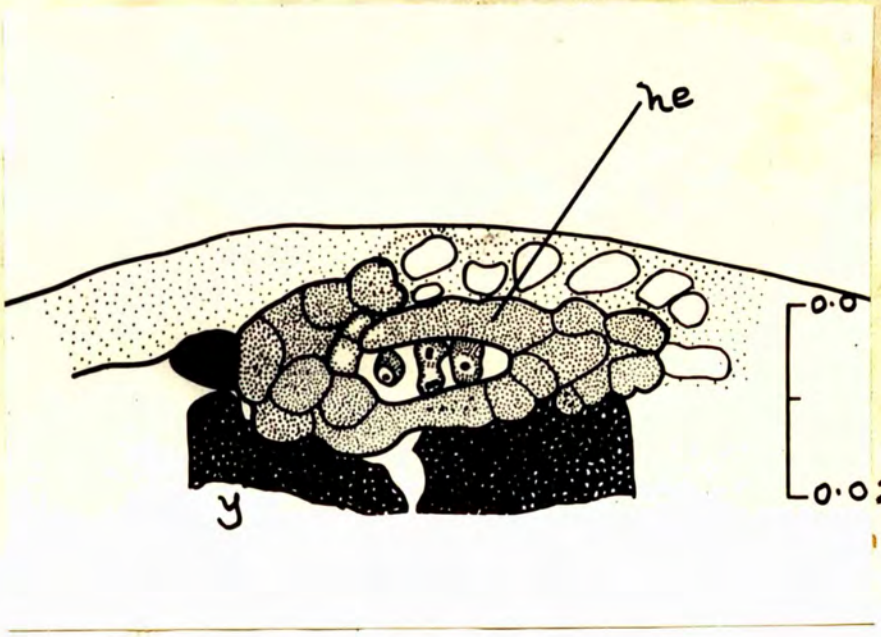


Figure 61. Transverse section through an embryo at the same stage as Fig. 28 in the region posterior to the dorsal organ showing the rudiment of the heart (he) as a thickening of mesoderm in which a cavity is beginning to develop. y, yolk.

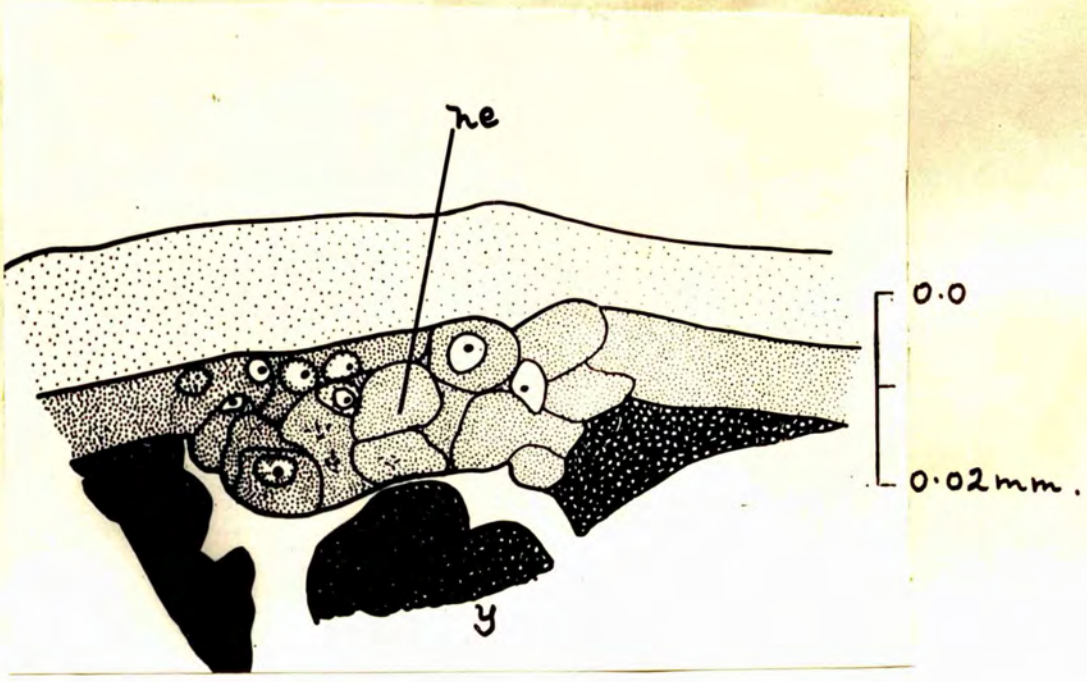


Figure 62. Transverse section through an embryo at the same stage as Fig. 28 immediately posterior to the section shown in Fig. 61 showing the rudiment of the heart (he) as a thickening of mesoderm cells without a cavity. y, yolk.

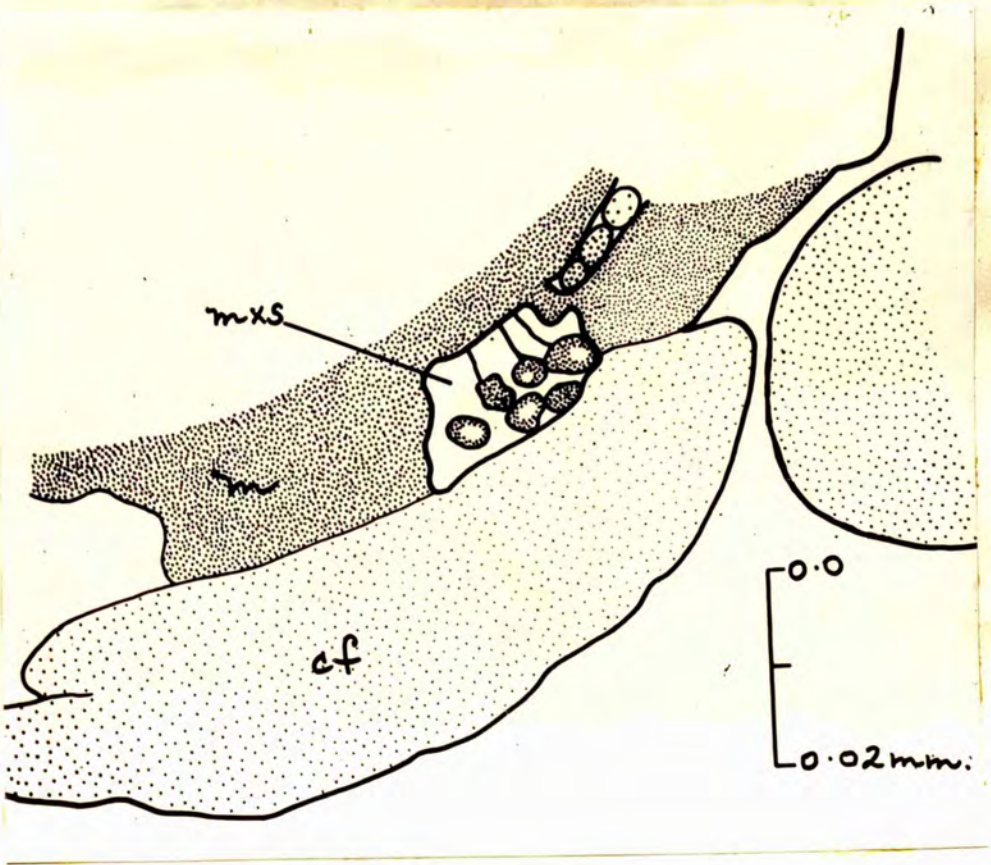


Figure 63. Transverse section through an embryo at the same stage as Fig. 28, in the region posterior to the section shown in Fig. 62, showing the carapace fold(cf) filled with a compact mass of cells and the beginning of the cavity of the maxillary gland sac (mxs) adjacent to it. m, mesoderm.

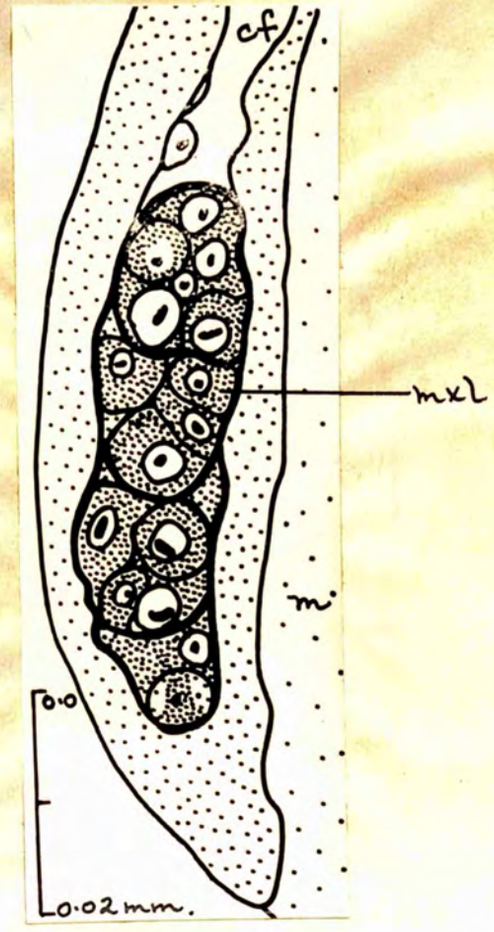


Figure 64. Horizontal longitudinal section through an embryo at the same stage as Fig. 31 showing the mass of mesoderm cells within the carapace fold (mxl) arranged as the loops of the maxillary gland without cavities.
cf, carapace fold; m, mesoderm.

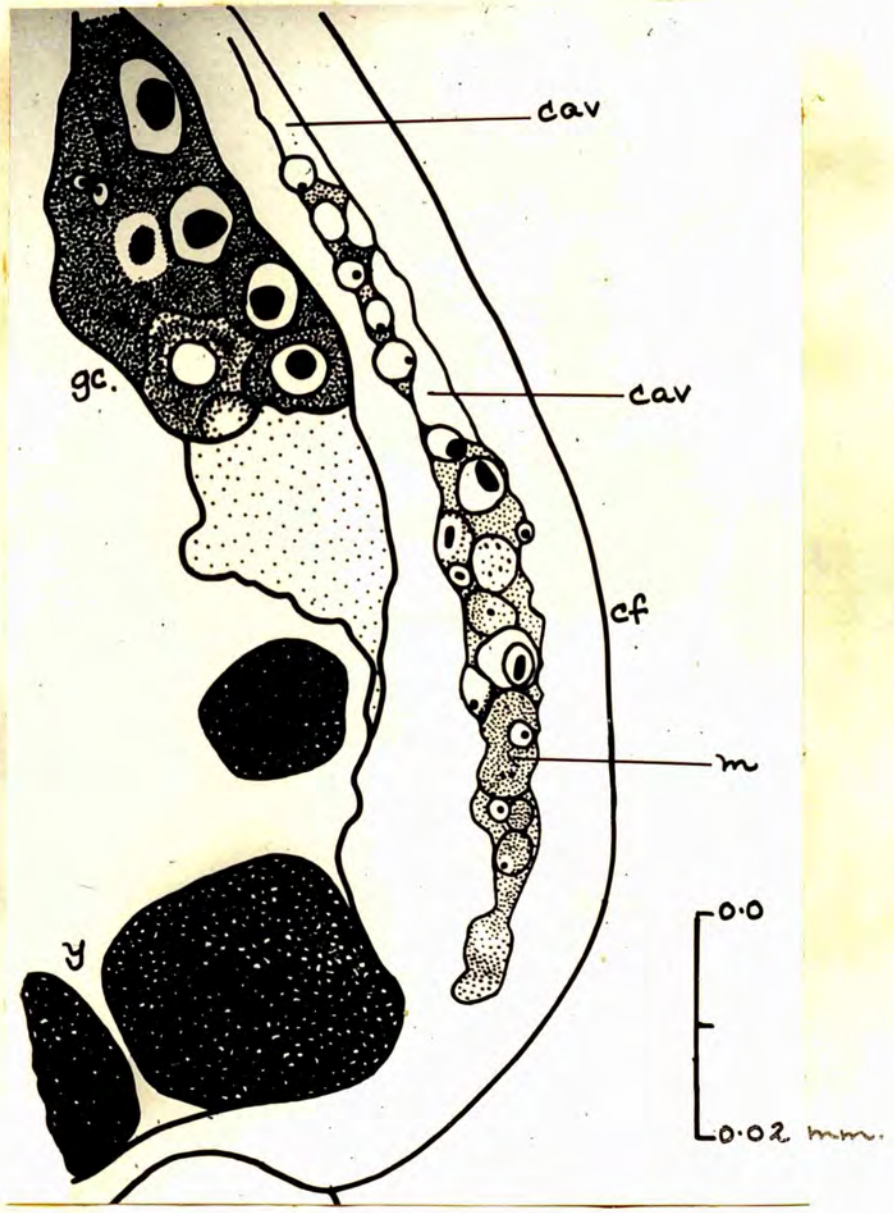


Figure 65. Horizontal longitudinal section through an embryo at the same stage as Fig. 31, in the region of the genital rudiments and dorsal to Fig. 64, showing the mesoderm cells (m) within the carapace fold not markedly arranged into loops and with the carapace cavity (cav) forming posterior to the maxillary gland rudiment. cf, carapace fold; gc, genital cells; y, yolk.

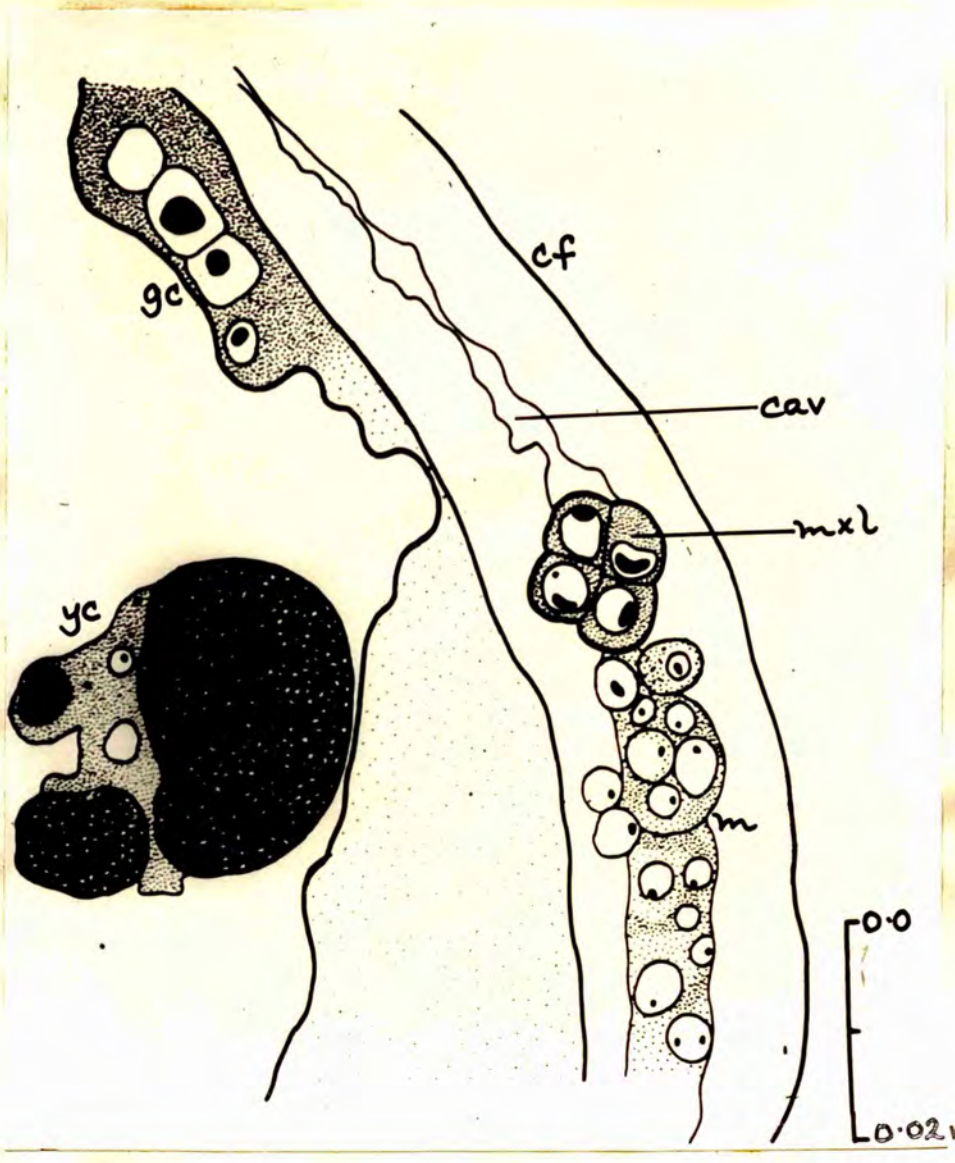


Figure 66. Horizontal longitudinal section through an embryo at the same stage as Fig. 31, ventral to the section shown in Fig. 65, showing one of the carapace folds (cf) enclosing mesoderm cells (m) four of which are distinctly arranged in a ring forming a section of one of the loops of the maxillary gland (mxl) which has not yet acquired a lumen. cav, cavity of carapace fold; gc, genital cells; yc, yolk cell.

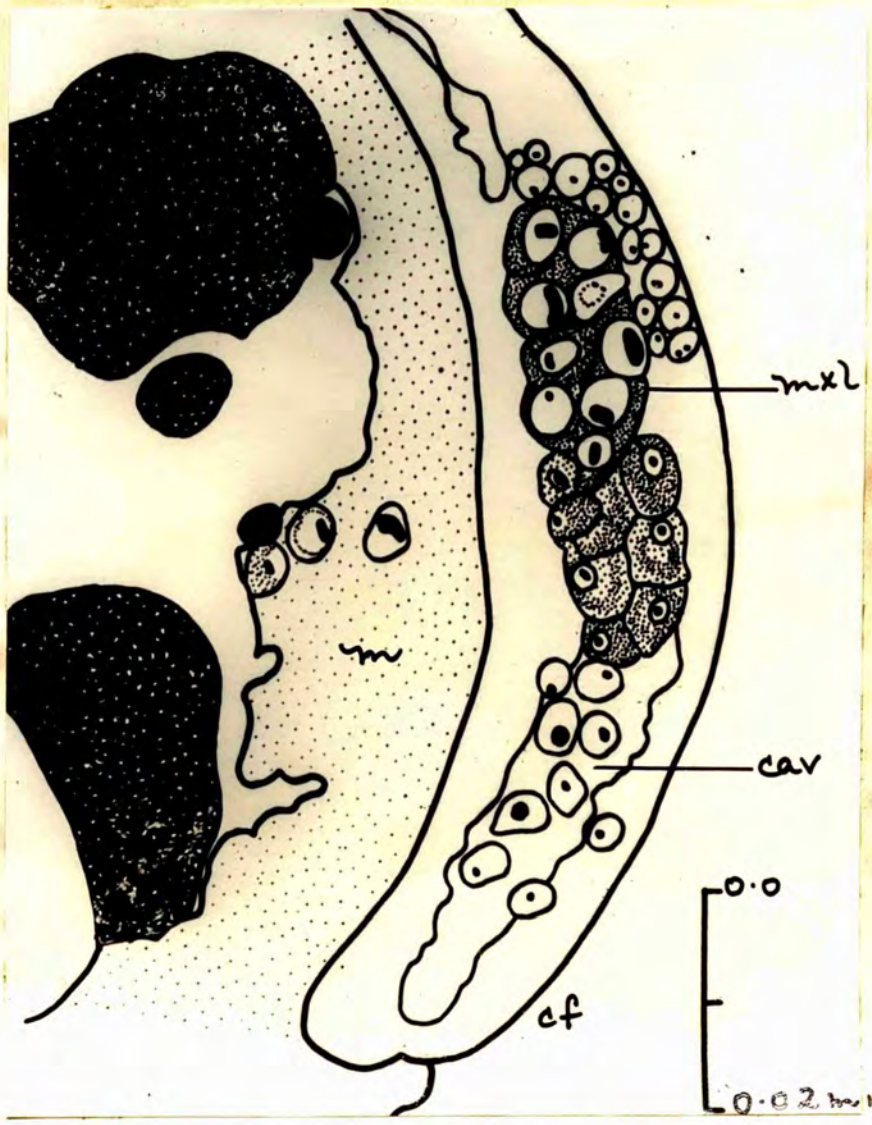


Figure 67. Horizontal longitudinal section through an embryo at the same stage as Fig. 31, ventral to the section shown in Fig. 66, showing the maxillary gland loops (mxl) forming. cav, cavity of carapace fold; cf, carapace fold; m, mesoderm.

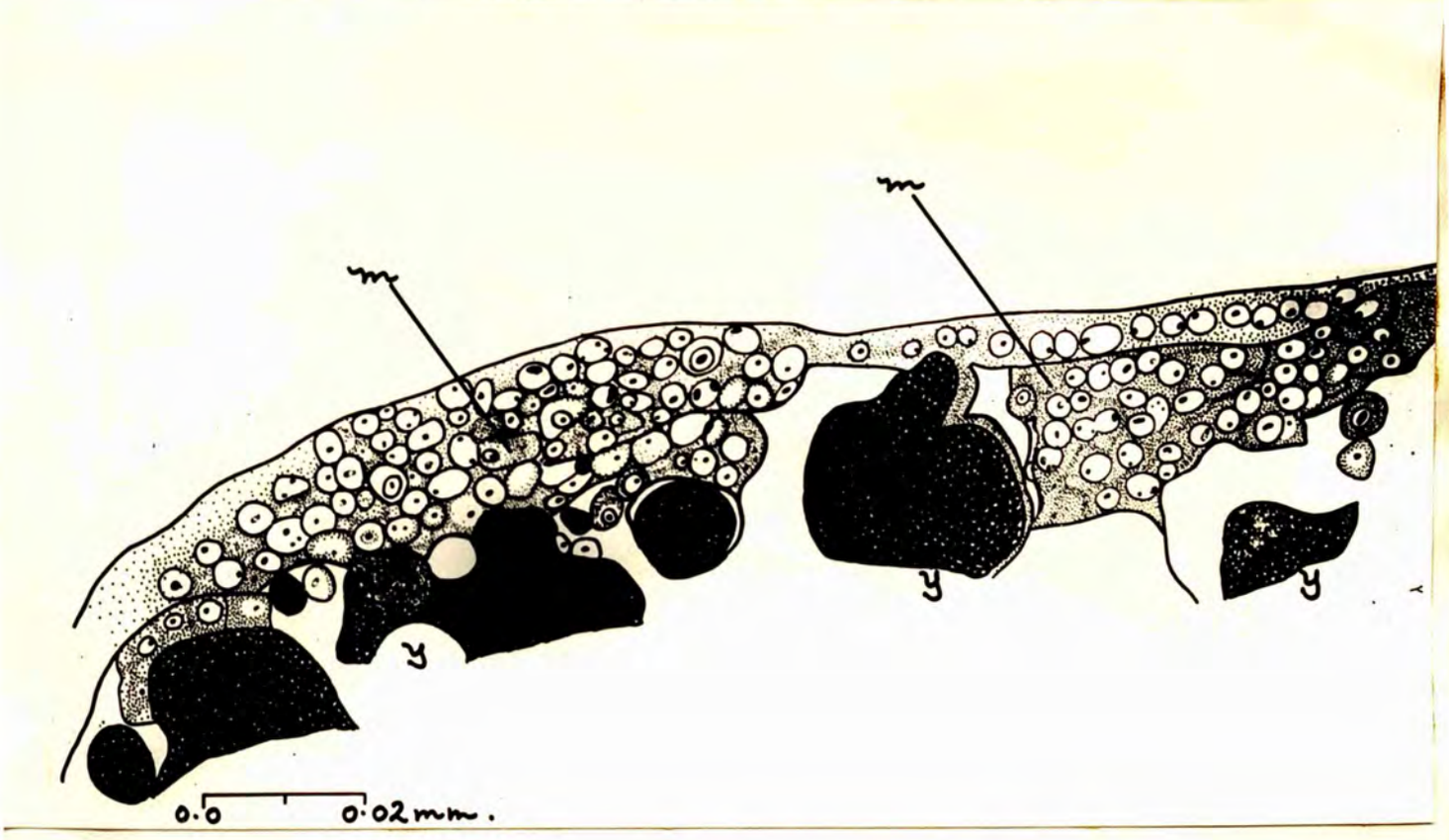


Figure 68. Horizontal longitudinal section through an embryo at the same stage as Fig. 51 showing the dorso-lateral mesodermal thickenings (m) posteriorly and in the maxillary region. y, yolk.

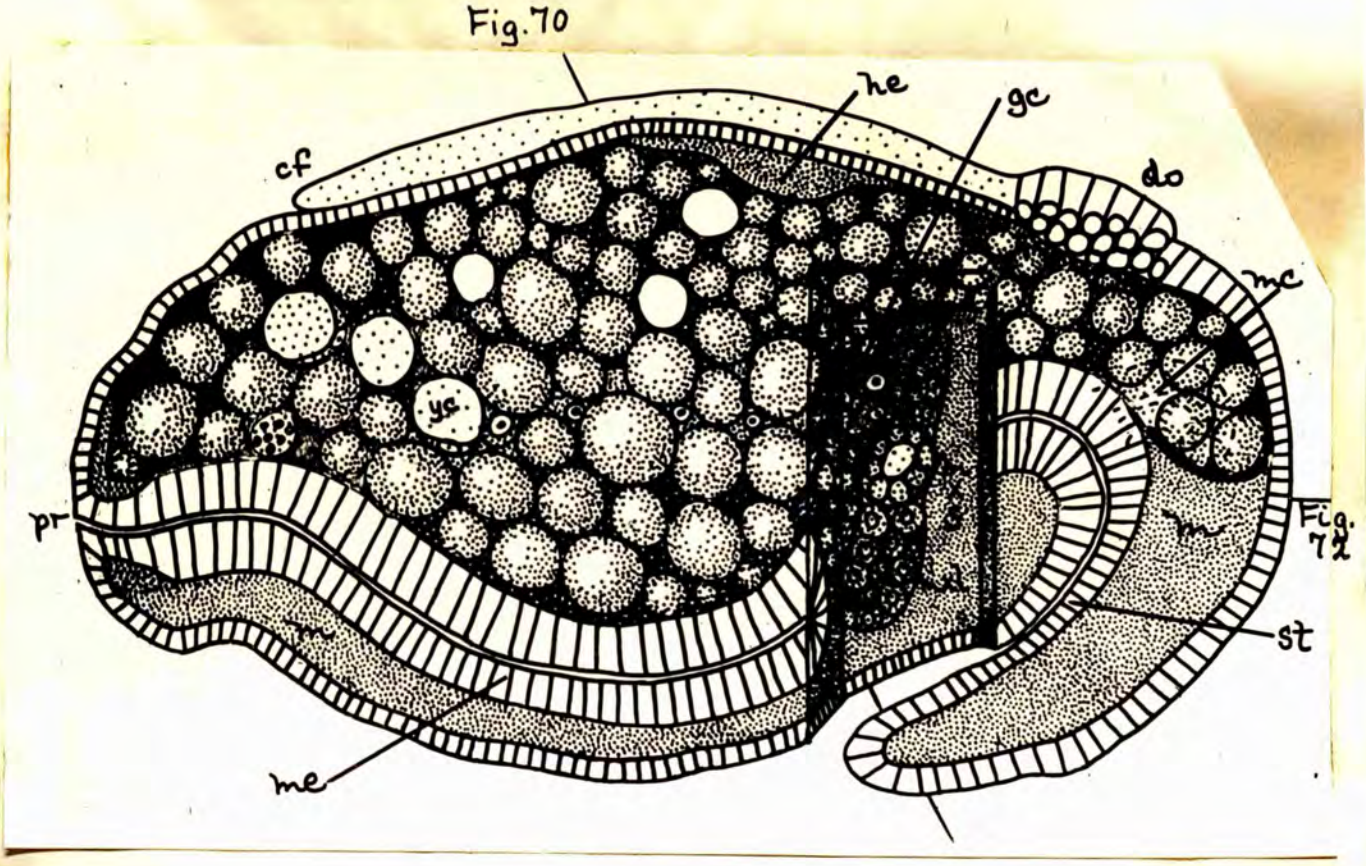


Figure 69. Diagrammatic reconstruction of an embryo of Daphnia magna at the stage when the mesenteron (me) has developed a narrow central cavity and the maxillary gland rudiment (mxl, mxs) is distinct. cf, carapace fold; do, dorsal organ; gc, genital cells, he, heart; m, mesoderm; mc, caecum of mesenteron; pr, proctodaeum; st, stomodaeum; yc, yolk cell.

does not surround the yolk (Fig.70). The thickened band of mesodermal cells in the region of the second antennae and the maxillary region has become broader and the antennal muscles are beginning to become distinguishable. These are the first muscles to develop, just as the antennae are the first of the appendages to develop. The early development of the antenna and its muscles is correlated with the importance of this appendage as a swimming organ. The mid-dorsal mesoderm is thickened internally in a region which corresponds to that of the maxillary gland (Fig.71; Fig.72). The lateral mesoderm is also beginning to extend inwards dorsal to the region of the alimentary canal. The muscles of the thoracic appendages are beginning to develop, with the consequent formation of small irregular cavities between the mesodermal cells in the bases of the appendages. The external form of the thoracic appendages is moderately well-developed. Two pairs of maxillae are present, the first pair being lateral to the second, very small and soon degenerating. The pair of first antennae are recognisable. The abdominal bristles are distinguishable and the group of cells at their bases well-developed. The distal labral glands are present in the form of large cells with cytoplasm which stains intensely and with large nuclei. The nervous system is divisible into cellular and fibrous components. There is still a considerable amount of yolk present, including a large number of granules in the dorsal part of the head.

A few hours later (54 hours old) (Fig.73) the heart (he) is represented by a compact cellular area similar in shape to the

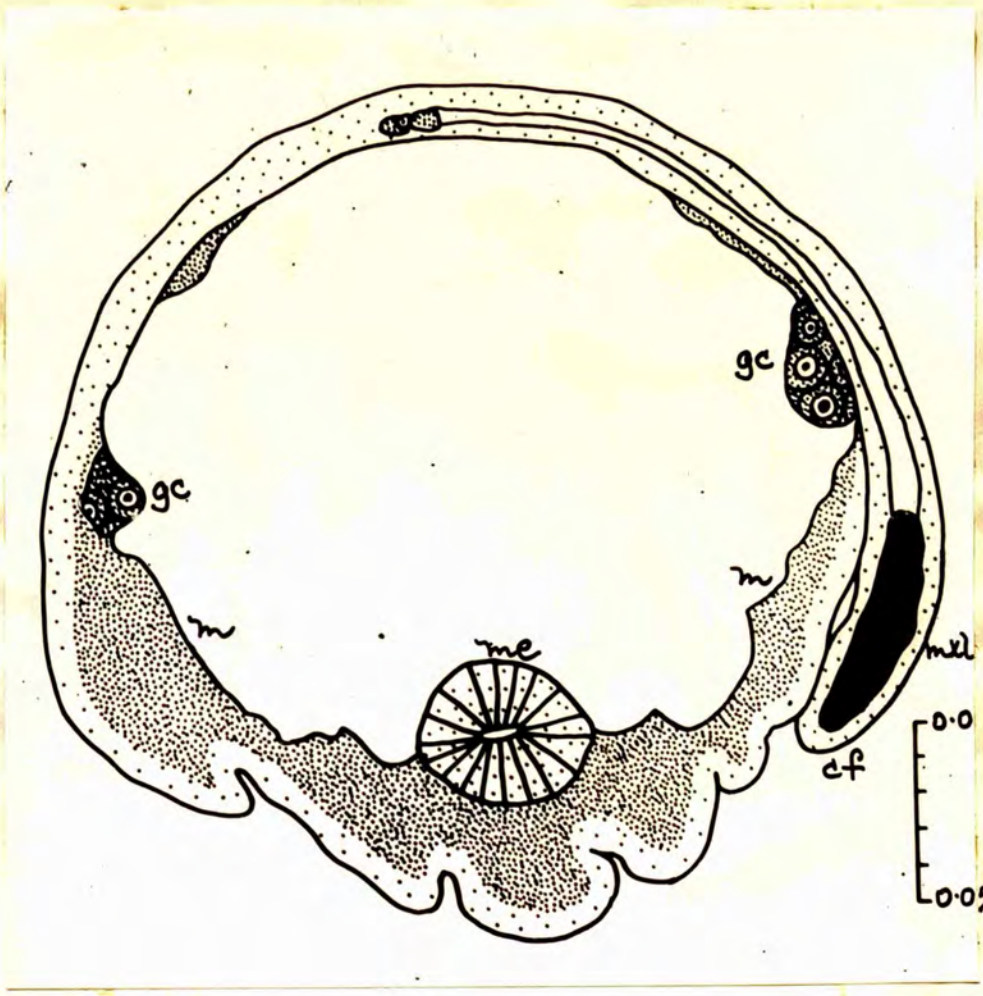


Figure 70. Transverse section through an embryo at the same stage as Fig. 69 in the region of the genital rudiments (gc) and the posterior part of the maxillary region showing the distribution of the mesoderm (m) including the maxillary gland loops (mxl). cf, carapace fold; me, mesenteron.

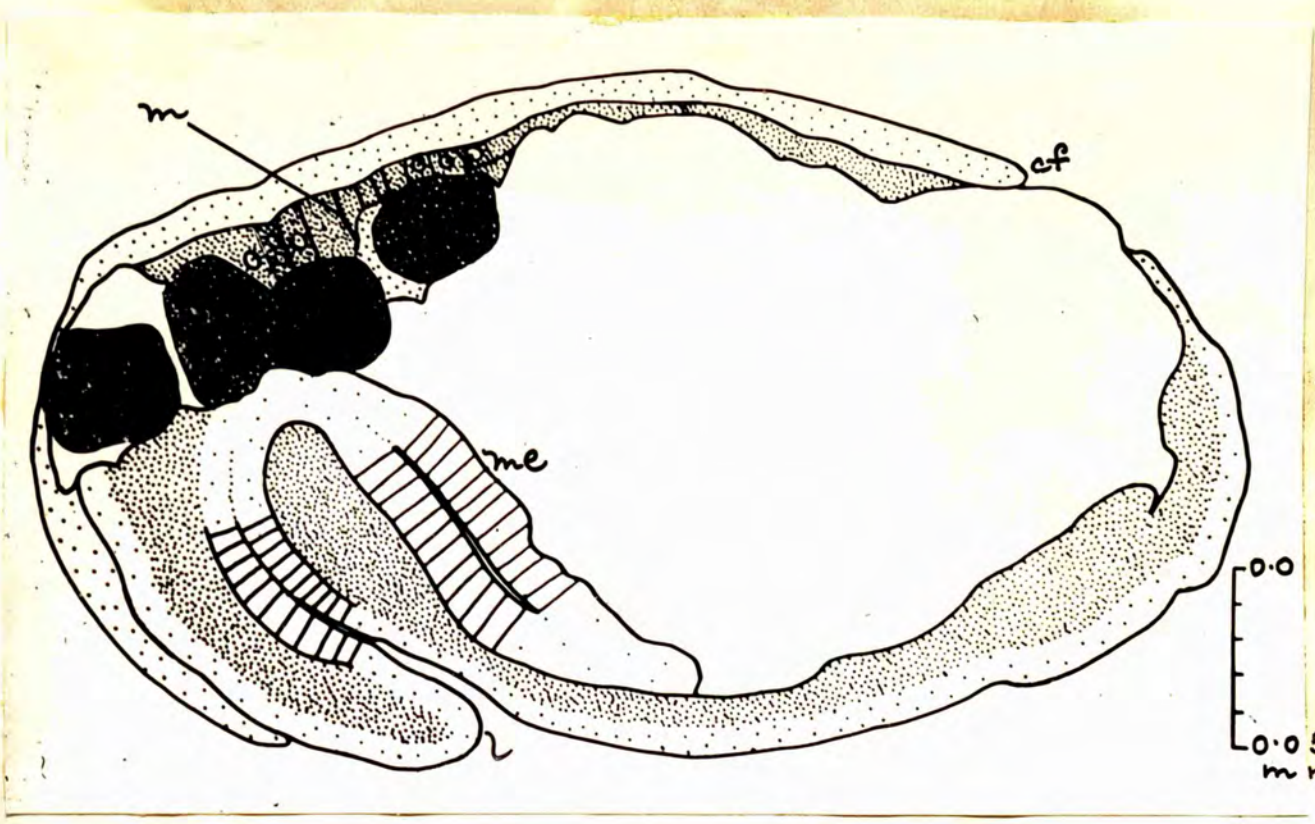


Figure 71. Vertical longitudinal section through an embryo at the same stage as Fig. 69 close to the midline showing the thickening of the mesoderm (m) dorsally in the maxillary region. cf, carapace fold; l, labrum, me, mesenteron.

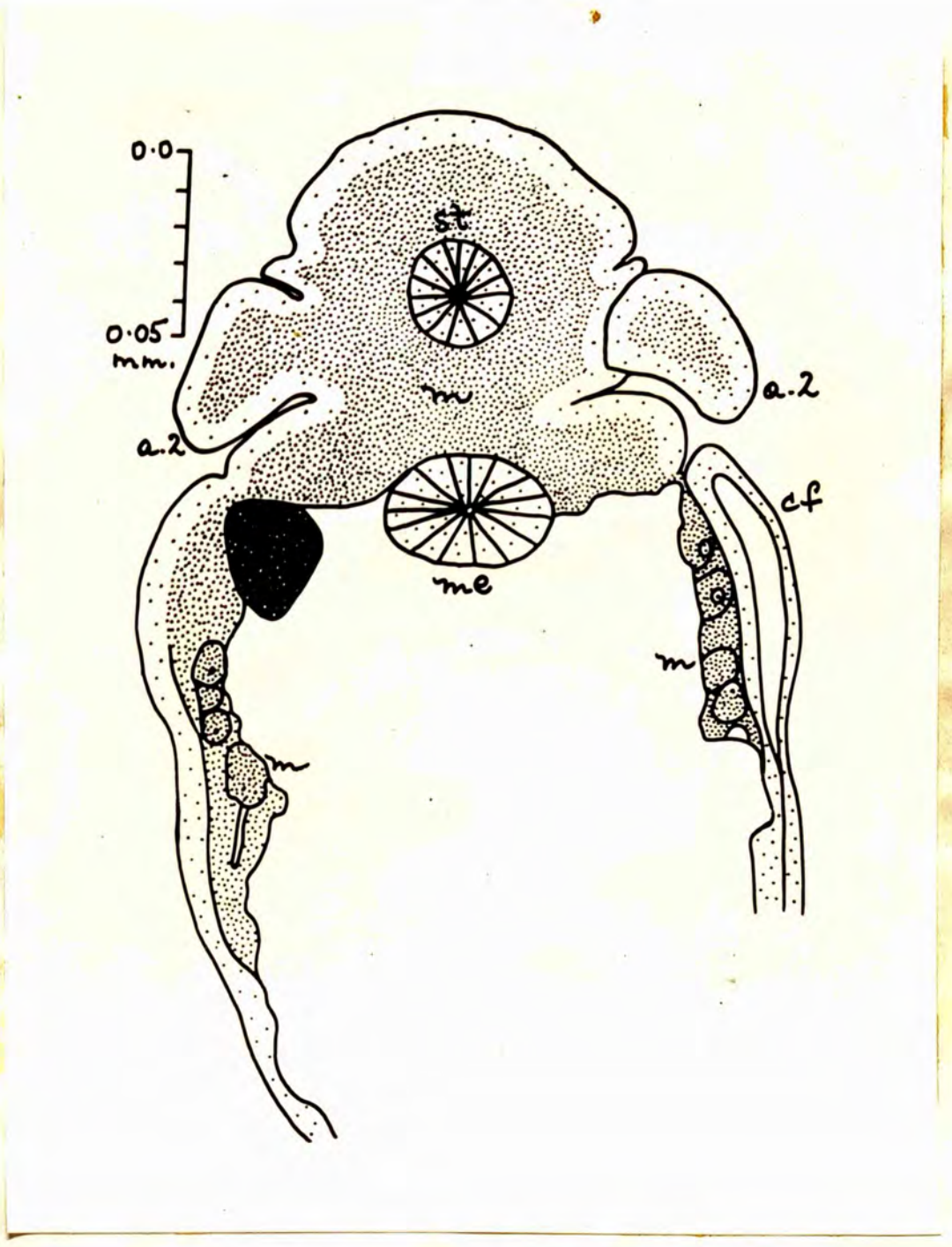


Figure 72. Horizontal longitudinal section through an embryo at the same stage as Fig. 69 showing the thickening of the mesoderm (m) on either side in the maxillary region and in the head. a.2, second antenna; cf, carapace fold; me, mesenteron; st, stomodaeum.

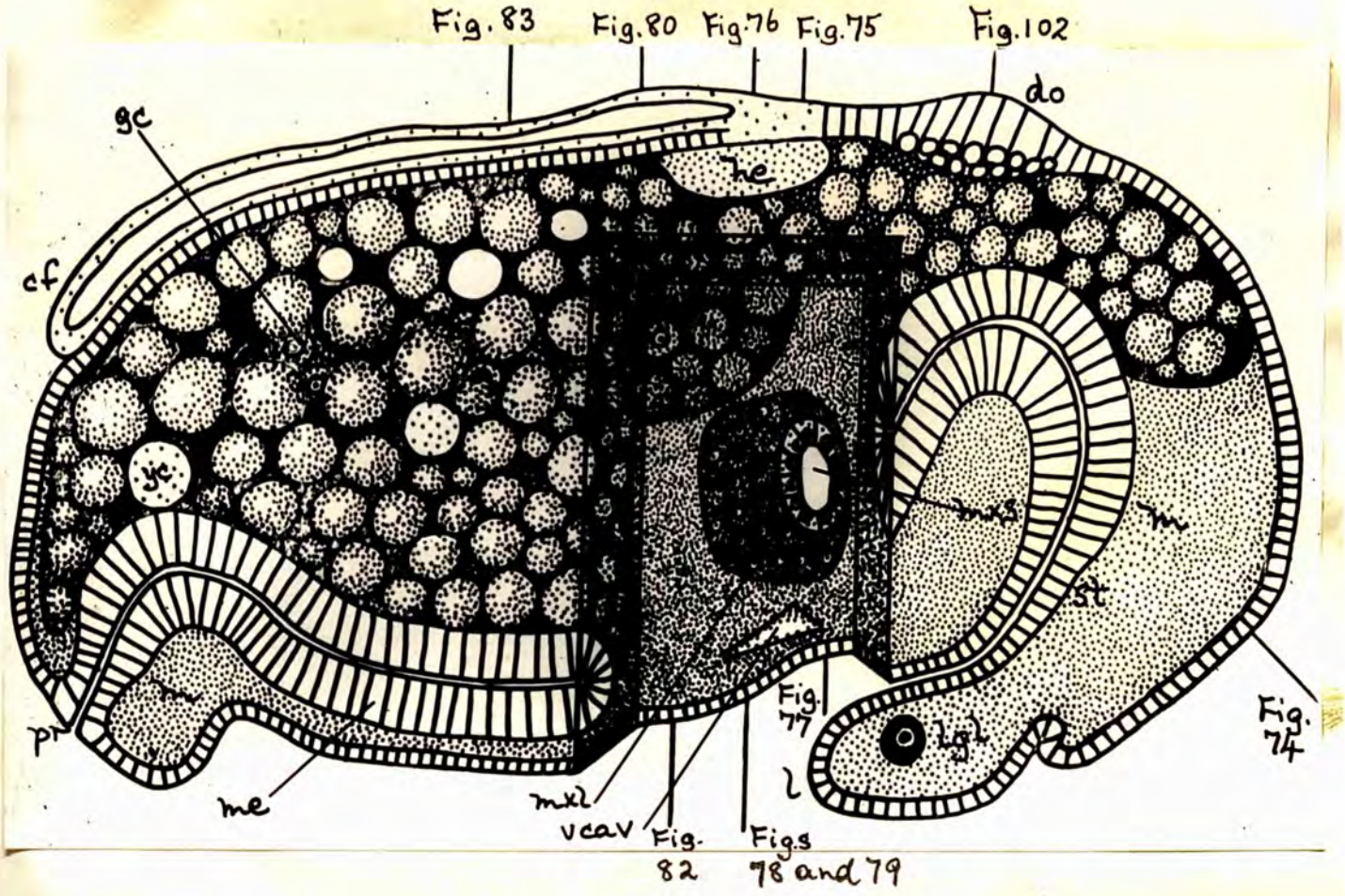


Figure 73. Diagrammatic reconstruction of an embryo of Daphnia magna at the stage when the mesenteron (me) has a narrow continuous cavity and the maxillary gland (mxl, mxs) and heart (he) rudiments are distinct as well as the beginning of the paired ventral cavities (vcav). cf, carapace fold; do, dorsal organ; gc, genital cells; he, heart; l, labrum; lgl, labral glands; m, mesoderm; pr, proctodaeum; st, stomodaeum; yc, yolk cell.

later form of the heart (Fig.75; Fig.76; Fig.84; Fig.85). The inner cells of this area are large and loosely arranged and appear to be in the process of breaking down. The cells posterior to the heart region are also beginning to collapse. Anterior to the heart, the muscles of the second antennae and of the mandibles are recognisable. The end sac of the maxillary gland has a well-developed cavity (Fig.75; Fig.80,mxs), but the loops of the gland do not possess cavities (Fig.76; Fig.79; Fig.88, mxl). The loops are distinctly recognisable and the number of coils has increased. The adductor muscles of the carapace are recognisable in the vicinity of the maxillary gland loops.

The heart continues to collapse internally. There is no dorsal mesoderm posterior to the developing heart (Fig.81; Fig.83; Fig.85). Anteriorly the heart thickening reaches to the dorsal organ (Fig.84). The mesoderm is thickened at its dorso-lateral edge and also internal to the loops of the maxillary gland (Fig.79). It is absent in the posterior dorso-lateral region. There is a wide band of mesodermal cells, several layers in thickness, in the region of the junction of the head with the body (Fig.87). The loops of the maxillary gland still have no cavities but the coils of the loops are fully developed (Fig.76,mxl). The paired ventral cavities, through which the circulatory fluid will pass, have begun to appear as splits between the mesoderm and the ectoderm (Fig.77; Fig.78, v.cav). The ventral cavities are only indicated in the anterior region of the body. The ventral septum is present as a layer of spherical cells. Each carapace fold is attached to the body by a compact region of cells (Fig.75)

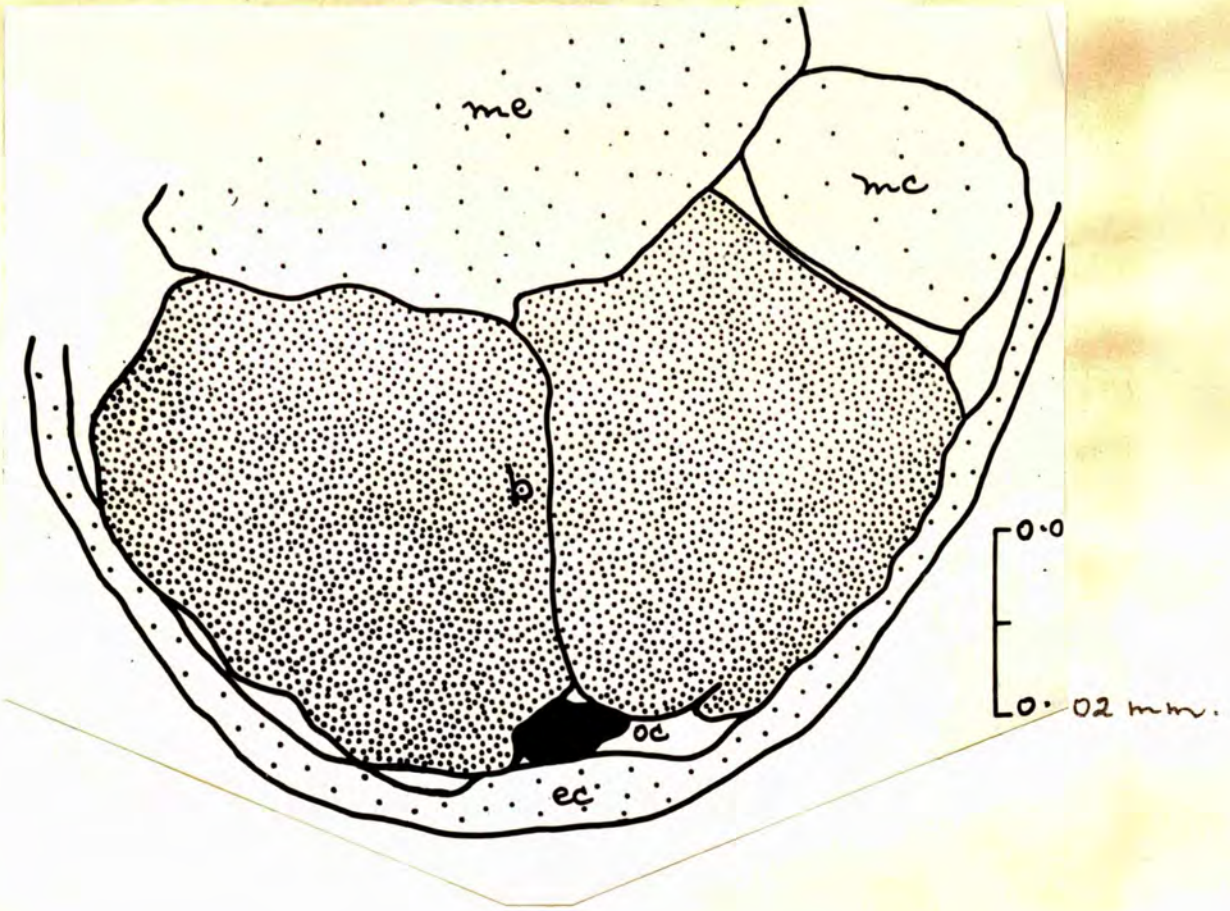


Figure 74. Transverse section through an embryo at the same stage as Fig. 73 near to the anterior end showing the brain (b) and the ocellus (oc) close to each other and to the ectoderm (ec). mc, caecum of the mesenteron; me, mesenteron.

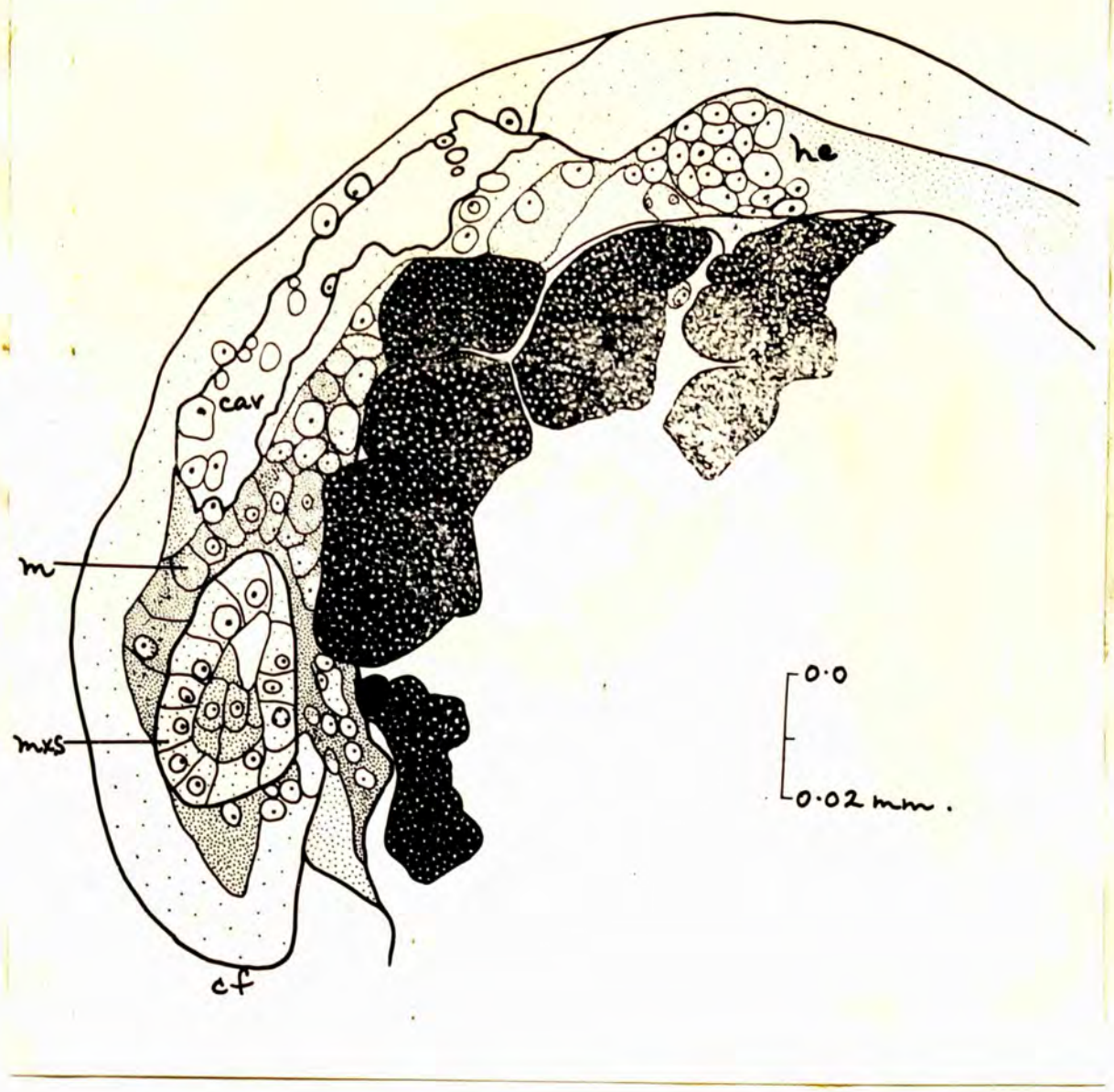


Figure 75. Transverse section through an embryo at the same stage as Fig. 73, posterior to the section shown in Fig.74, showing the maxillary gland end sac (mxs) developing a central cavity and with surrounding mesoderm cells (m); also the anterior end of the dorsal mesodermal rudiment of the heart (he). cav, cavity of the carapace fold; cf, carapace fold.

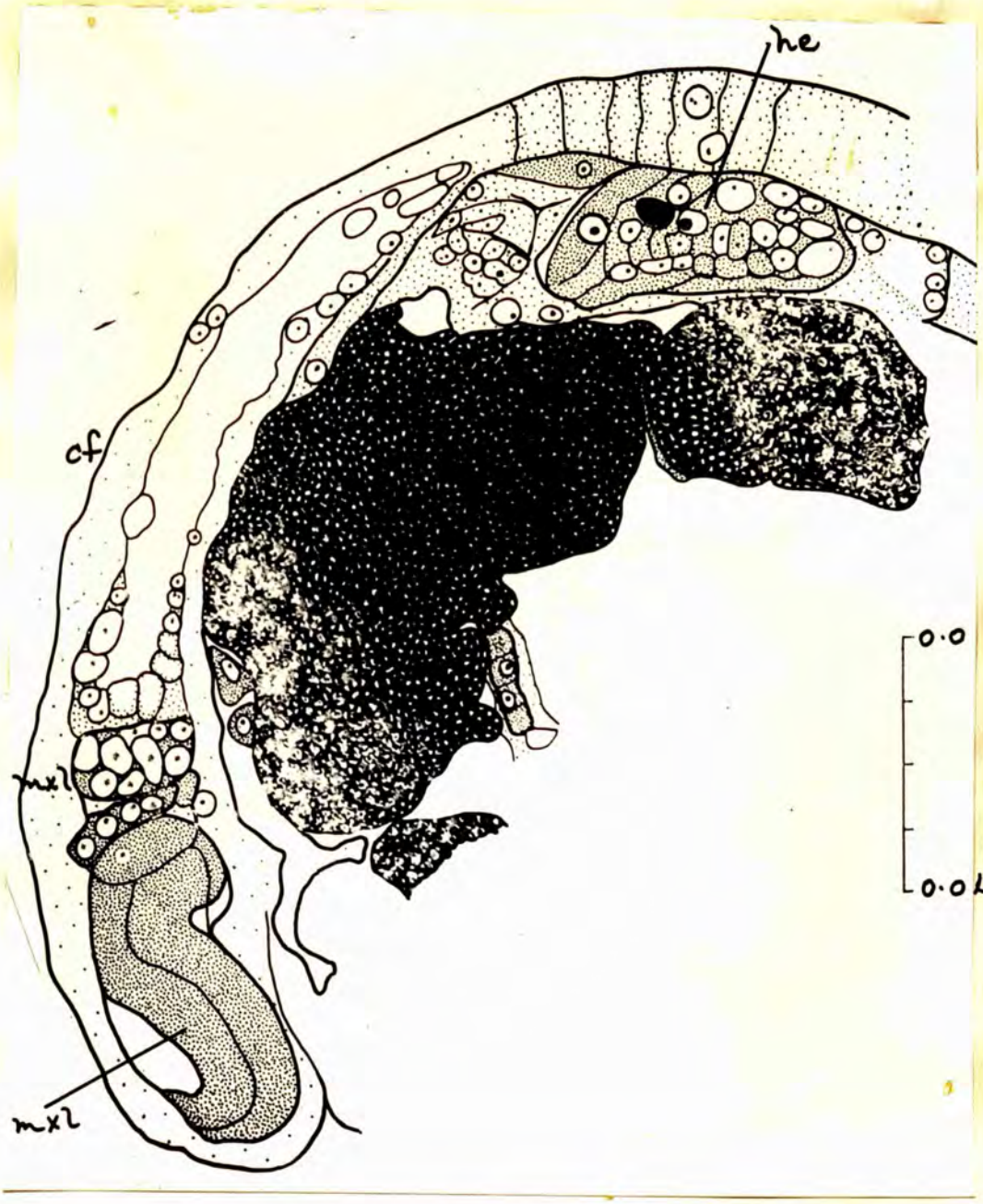


Figure 76. Transverse section through an embryo at the same stage as Fig.73, immediately posterior to Fig.75, showing the loops of the maxillary gland (mxl) and the compact cells of the heart rudiment (he).
cf, carapace fold.

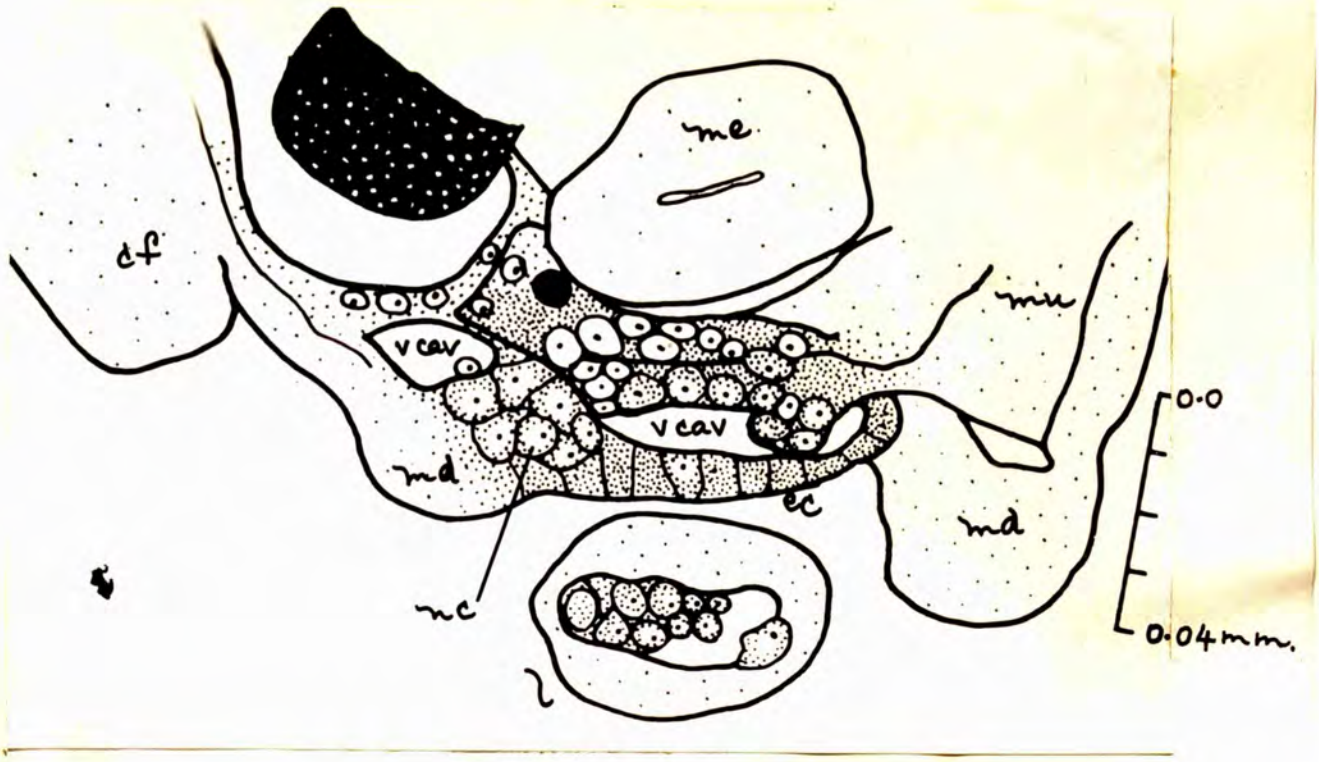


Figure 77. Transverse section through an embryo at the same stage as Fig.73 showing the beginnings of the paired ventral cavities (vcav) between the mesenteron (me) and the ventral ectoderm (ec) in the same section as Fig.76. cf, carapace fold; l, labrum; md, mandible; mu, muscle; nc, nerve cord.

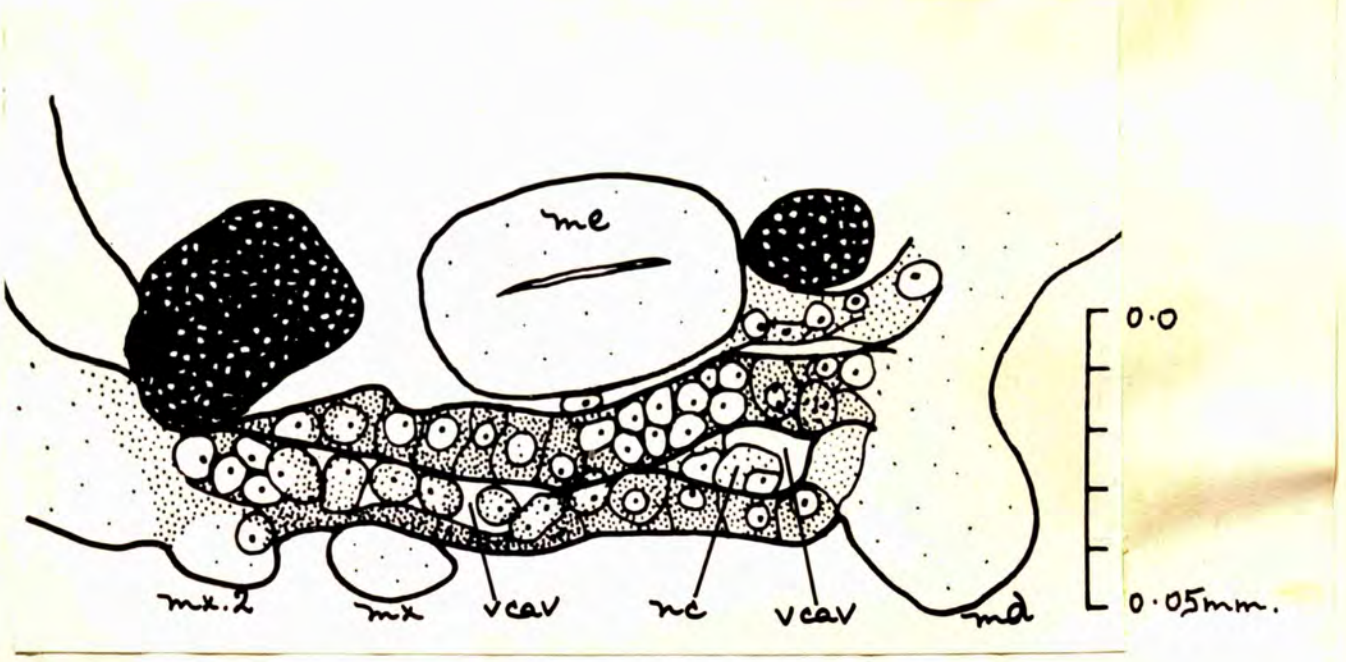


Figure 78. Transverse section through an embryo at the same stage as Fig.75, slightly posterior to the section shown in Figs. 76 and 77, showing the ventral cavities (vcav) less well developed and also one of the second maxillae (mx.2). md, mandible; me, mesenteron; mx, first maxilla; nc, nerve cord.

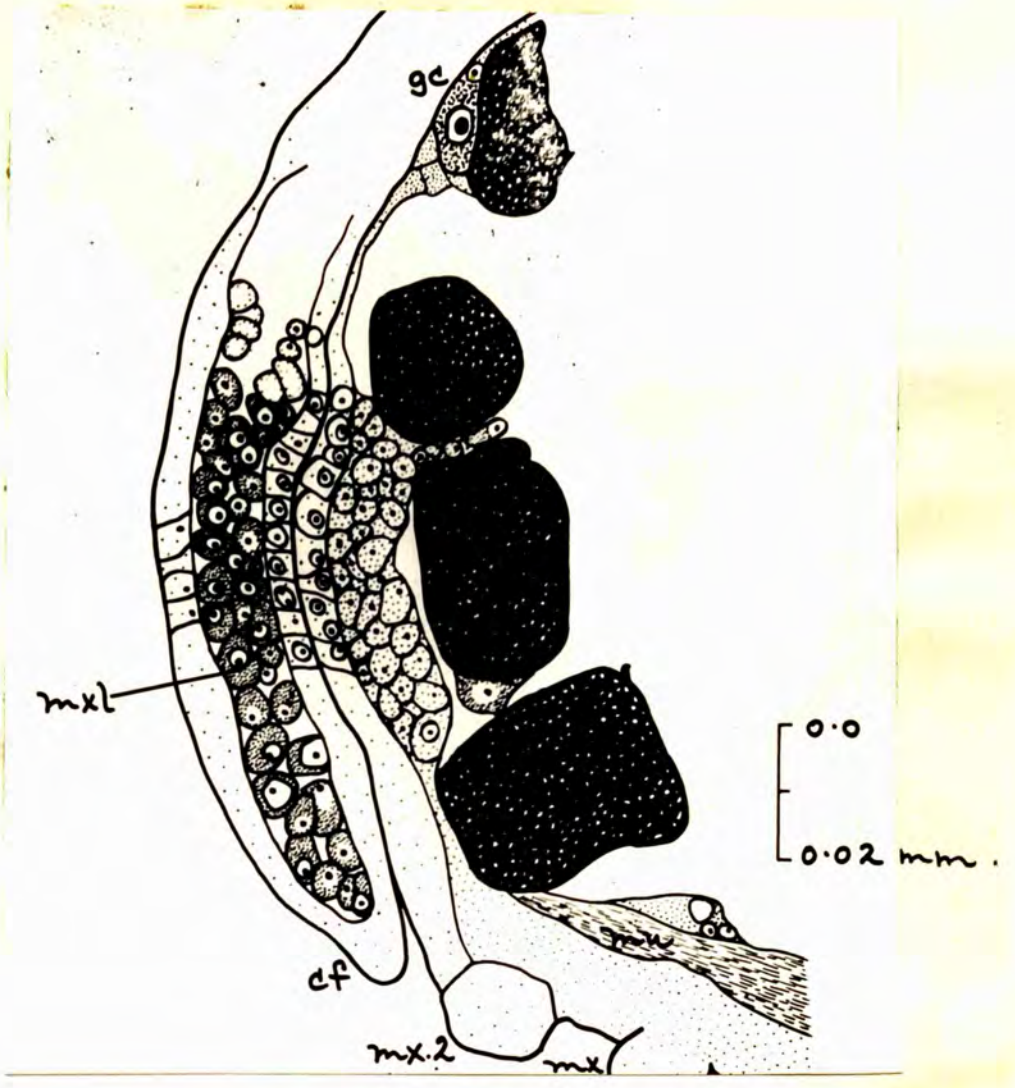


Figure 79. The lateral part of the same section as shown in Fig.78 showing the cells of the maxillary gland loops (mxl) in the carapace fold (cf). gc, genital cells; mx, first maxilla; mx.2, second maxilla; mu, muscle.

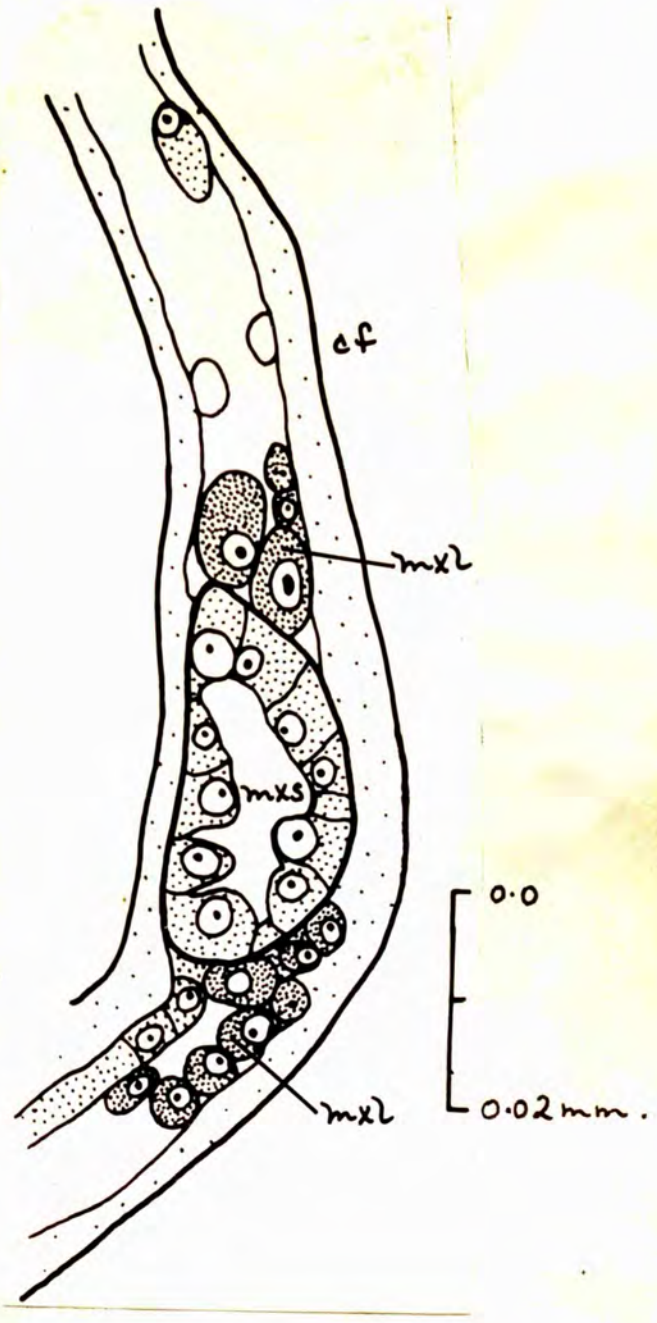


Figure 80. Transverse section through an embryo at the same stage as Fig.73, immediately posterior to the section shown in Figs. 78 and 79, showing the maxillary gland end sac (mxs) and loops (mxl). cf, carapace fold.

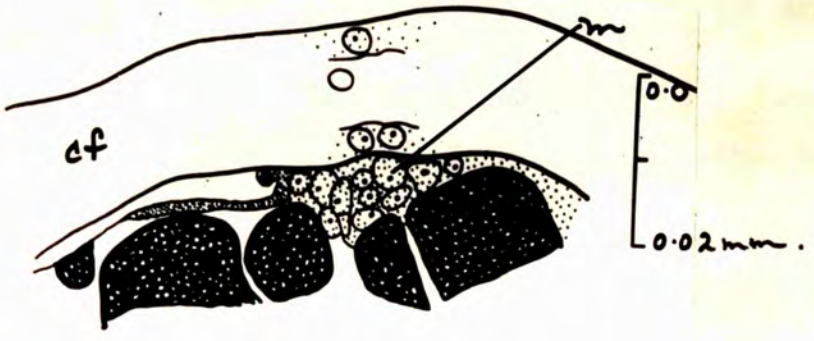


Figure 81. Transverse section through an embryo at the same stage as Fig.73 showing the small median dorsal thickening of the mesoderm (m) in the region posterior to the heart. cf, carapace fold.

and for most of its extent is filled with the palely-staining fluid which is probably a breakdown product of the yolk. There are also a number of small mesoderm cells in this fluid, and these are probably blood cells. The head still possesses a considerable quantity of yolk in its dorsal region. In the ventral part of the head (Fig.74), the nervous system is well developed and the large and well developed ocellus (oc) is directly applied to the brain (b). The post-oesophageal commissure contains a central group of cells with little affinity for stains. The paired nerve ganglia and their commissures are well developed (Fig.82). The first antennae (Fig.86, a.1) are filled with mesodermal cells and are close to the brain. The distal labral glands (Fig.86, dlgl) are seen as four enlarged, intensely-staining cells within the labrum; the proximal labral glands (plgl) as two rows of very large ectodermal cells. There are still two pairs of maxillae.

A few hours later (Figs. 89-94), when the thoracic appendages are well developed (Fig.89, t) but the mesenteron is still in the ventral part of the body (me), the heart (he) has a well developed central cavity and the pericardium (pe) is present. This has developed by the separation of mesodermal cells in the dorsal part of the body surrounding the heart from the surrounding cells and the ectoderm. The ventral septum is present as a thin strand of elongated cells which are thinner posteriorly. The heart (Fig.90; Fig.94, he) contains a number of small cells (co), which are densely granular, and are surrounded by a finely granular, vacuolated substance with little

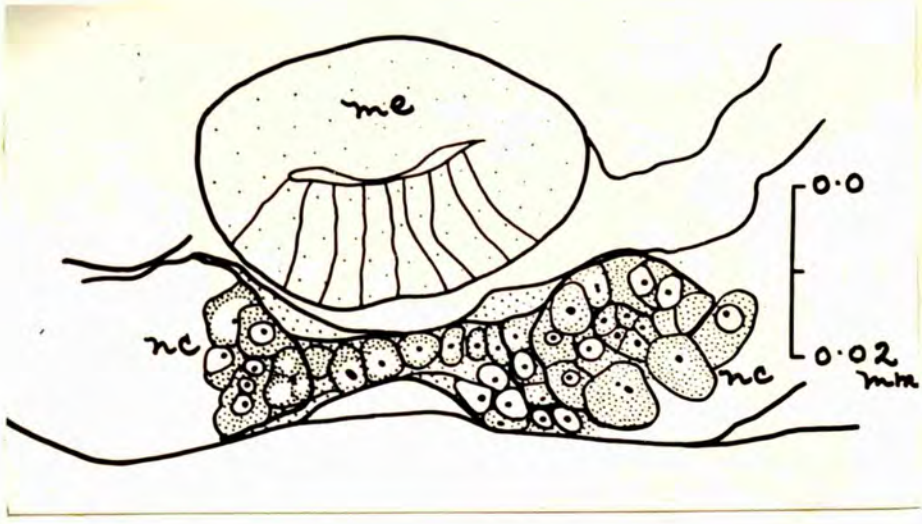


Figure 82. Transverse section through an embryo at the same stage as Fig. 73 showing the paired ventral nerve ganglia (nc) connected by a transverse commissure; the absence of ventral cavities; and the mesenteron(me) still close to the ventral surface of the embryo.

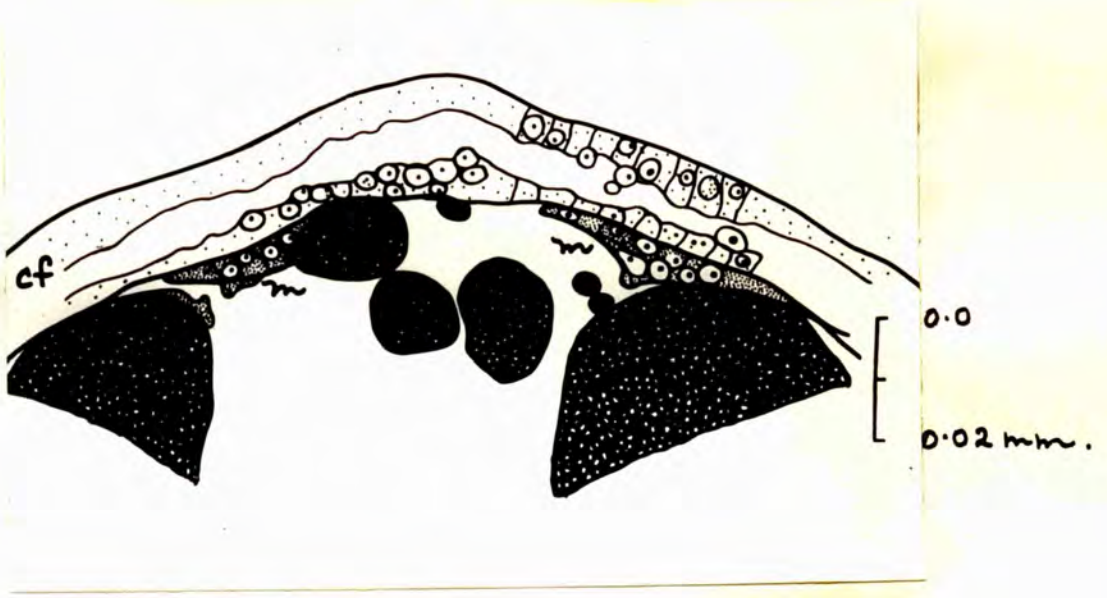


Figure 83. Transverse section through an embryo at the same stage as Fig.73, in the region posterior to that shown in Fig.81, showing the small lateral mesodermal thickenings (m) not meeting in the dorsal midline. cf, carapace fold.

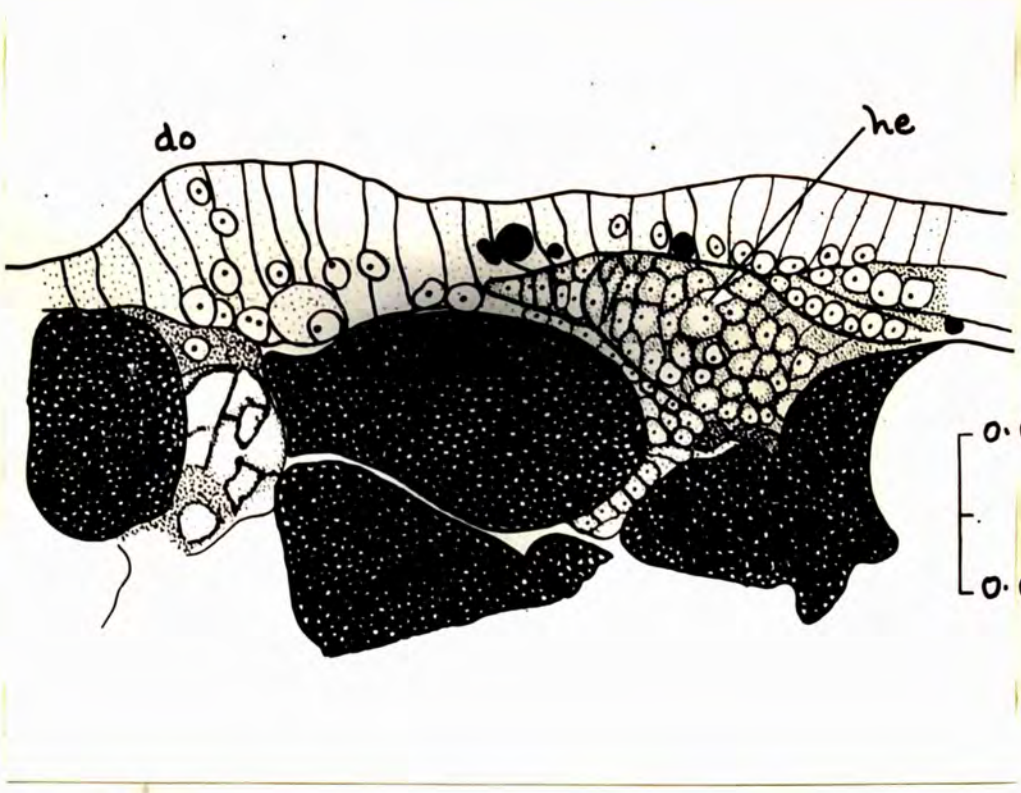


Figure 84. Sagittal section through an embryo at the same stage as Fig.73 showing the rudiment of the heart (he) formed of a compact mass of cells, those in the centre staining with less intensity. The dorsal organ (do) is situated anterior to the heart rudiment.

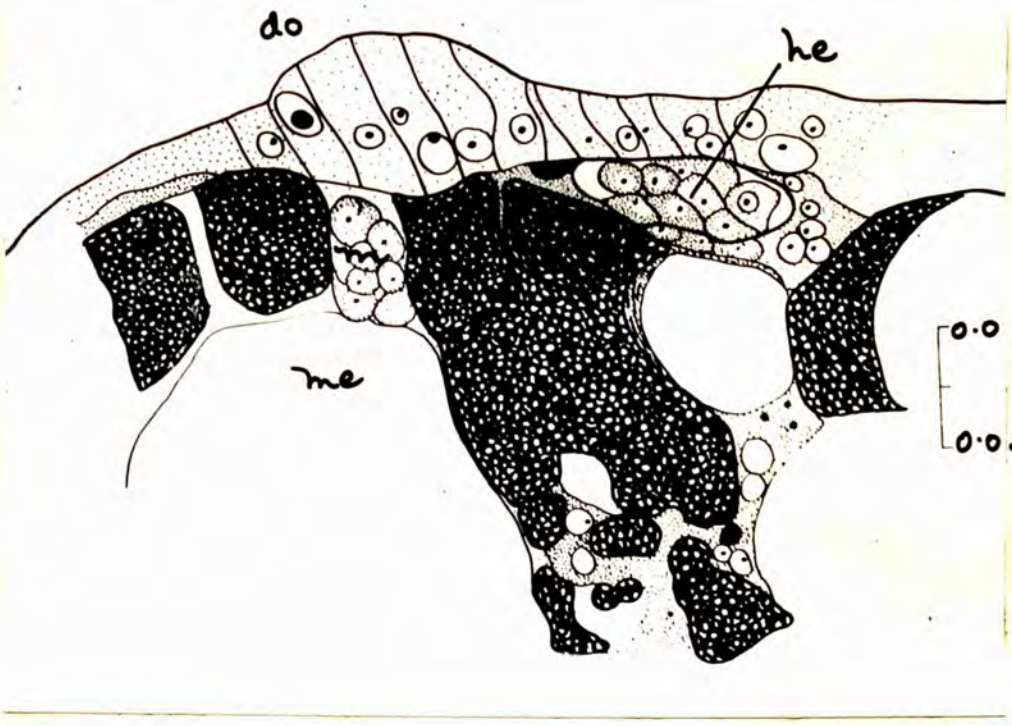


Figure 85. Vertical longitudinal section through an embryo at the same stage as Fig.73, immediately lateral to that shown in Fig.84, showing the heart rudiment (he) with the central cells beginning to break down, and the dorsal organ (do) anterior to the heart with a row of cells (m) connecting the dorsal organ with the mesenteron (me).

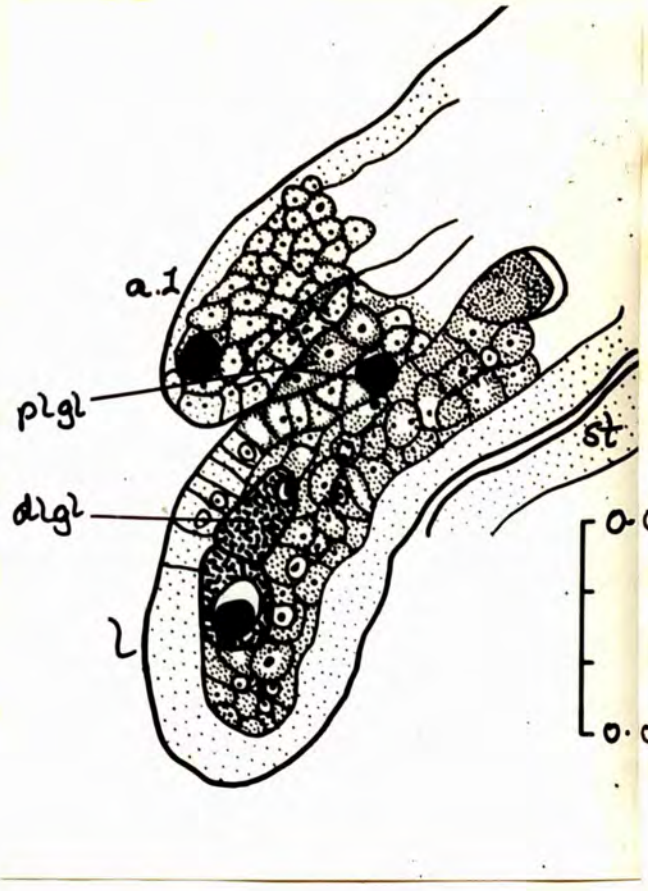


Figure 86. Vertical longitudinal section through the anterior ventral part of an embryo at the same stage as Fig. 73 showing the antennule (a.1) and labrum (l) with the proximal (plgl) and distal (dlgl) labral glands already well formed. st, stomodaeum.

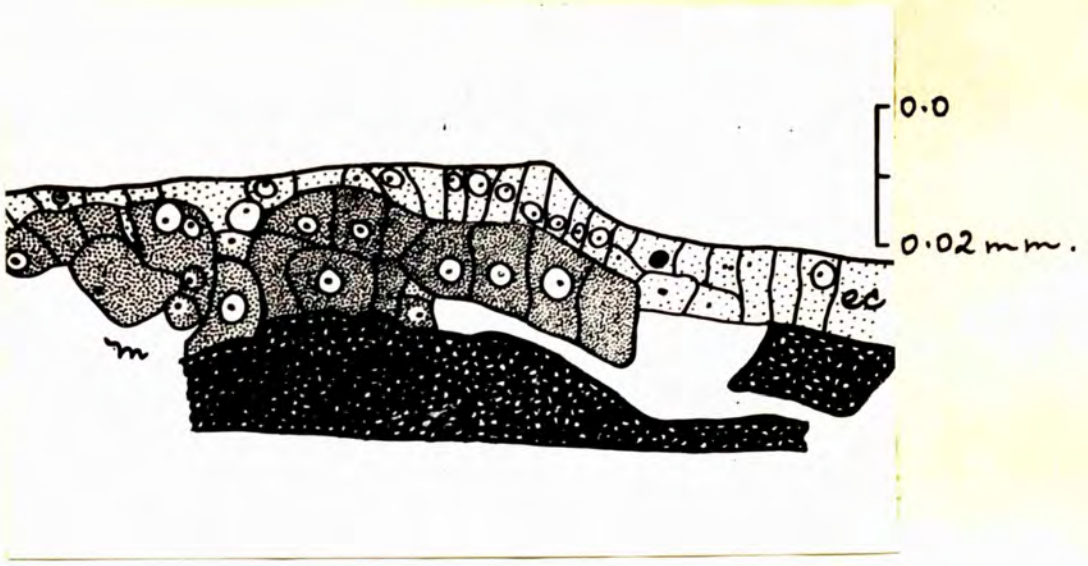


Figure 87. Vertical longitudinal section through the maxillary region of an embryo at the same stage as Fig.73. The section is lateral to that shown in Fig.86 and shows the dorso-lateral mesodermal thickening (m) in this region. ec, ectoderm.

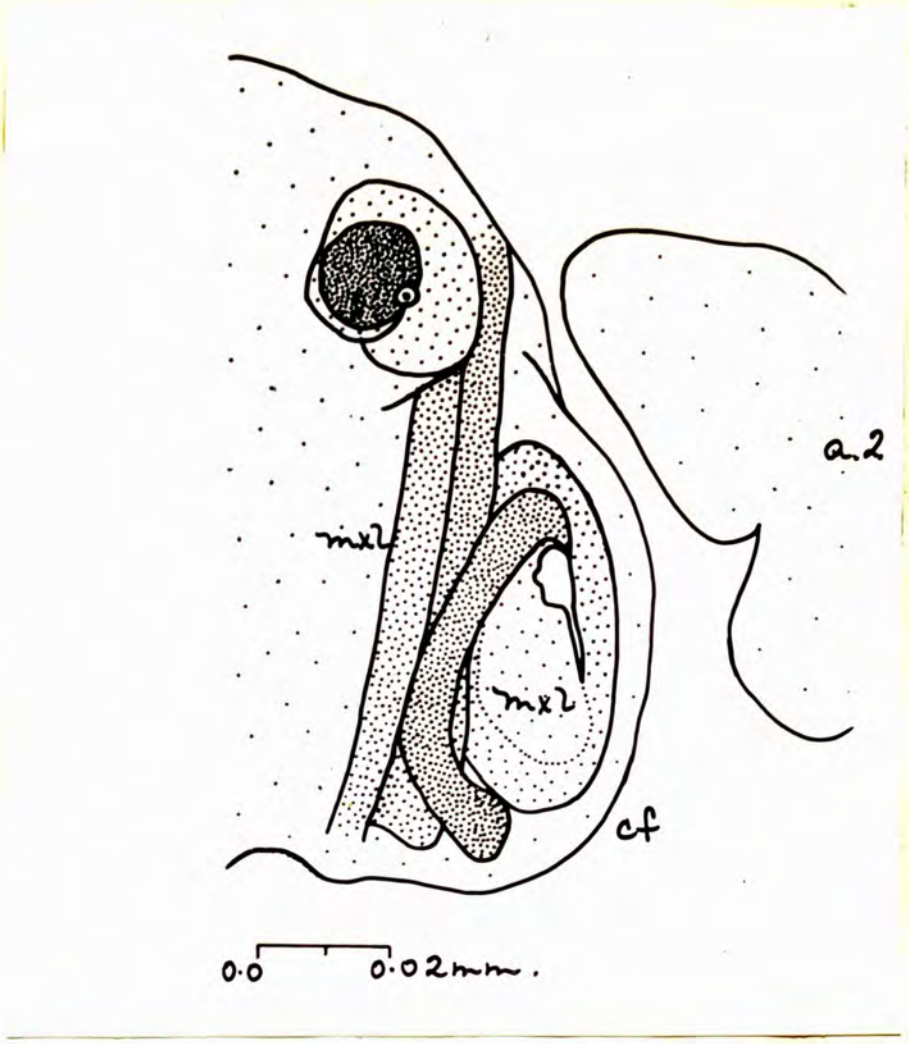


Figure 88. Lateral vertical longitudinal section through an embryo at the same stage as Fig.73 showing the coils of the maxillary gland loops (mxl). a.2, second antenna; cf, carapace fold.

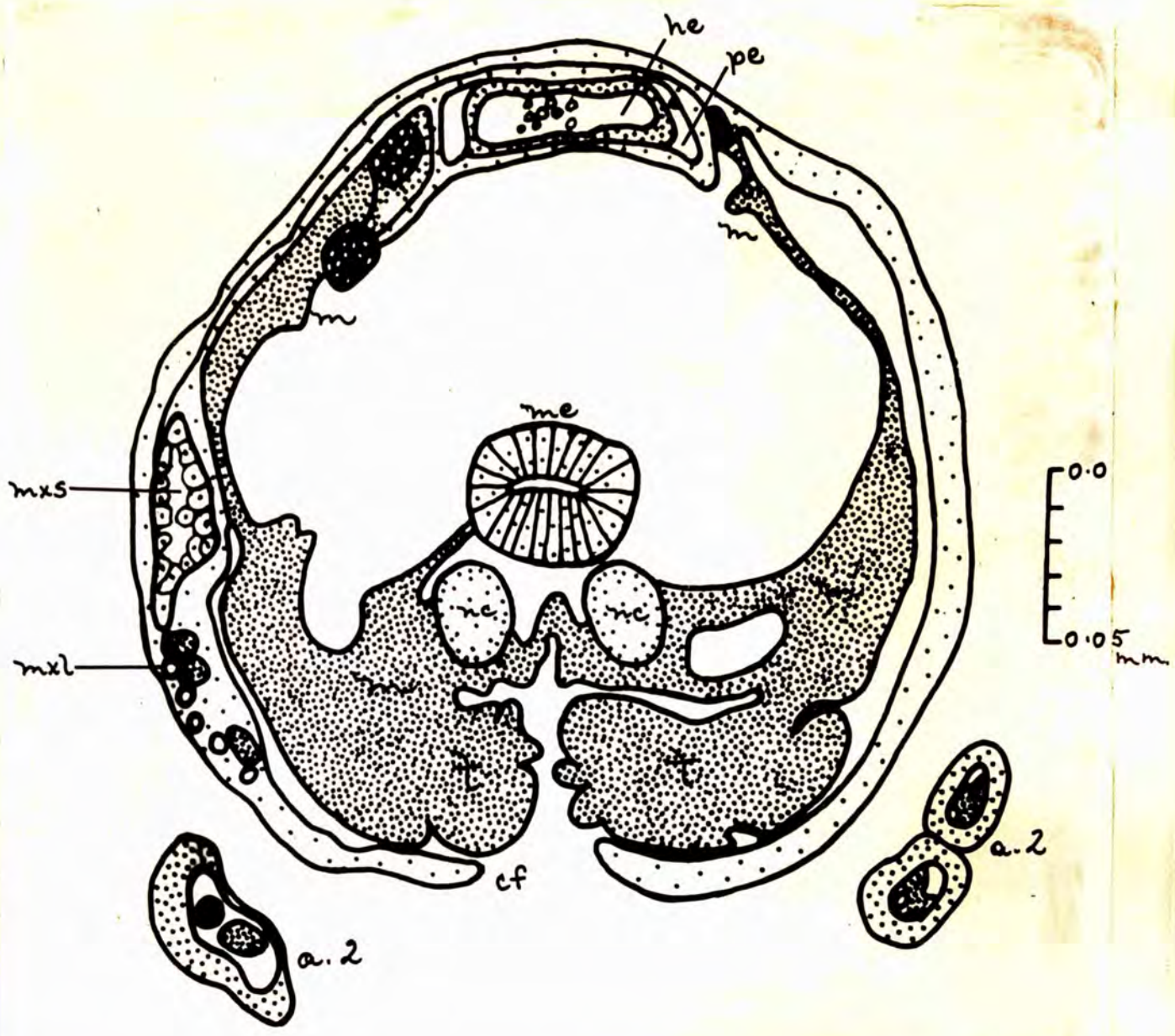


Figure 89. Transverse section through an embryo at a stage of development between that of Fig.73 and that of Fig.33 showing the heart (he) with well developed cavity and pericardium (pe); the end sac of the maxillary gland (mxs) and parts of the maxillary gland loops (mxl). The nerve ganglia (nc) are clearly distinguishable but the mesenteron (me) is still moderately close to the ventral surface of the embryo. A ventral cavity is seen on one side of the embryo. a.2, second antenna; cf, carapace fold; m, mesoderm; t, thoracic appendage.

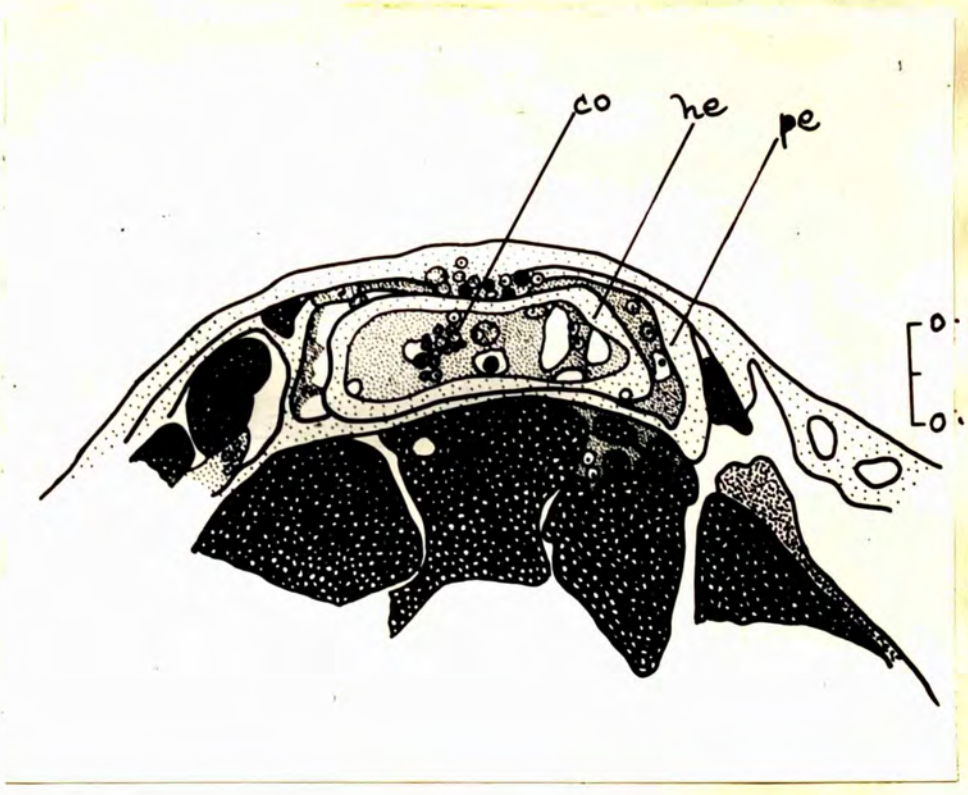


Figure 90. Detail of the transverse section Fig.89 showing the walls of the heart (he) and pericardium (pe) and the small densely granular cells (co) and finely granular substance within the heart.

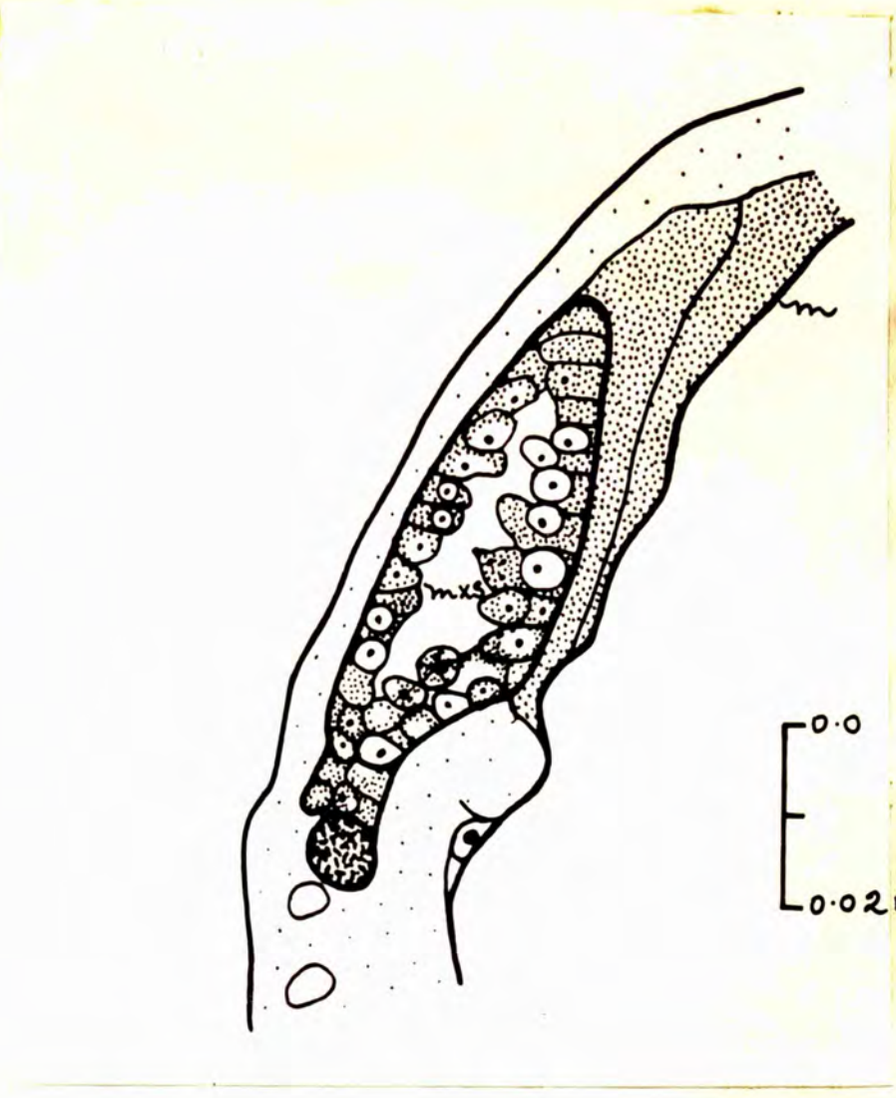


Figure 91. Detail of transverse section Fig. 89 showing the end sac of the maxillary gland (mxs) with a wall of lightly staining, loosely arranged cells.
m, mesoderm.

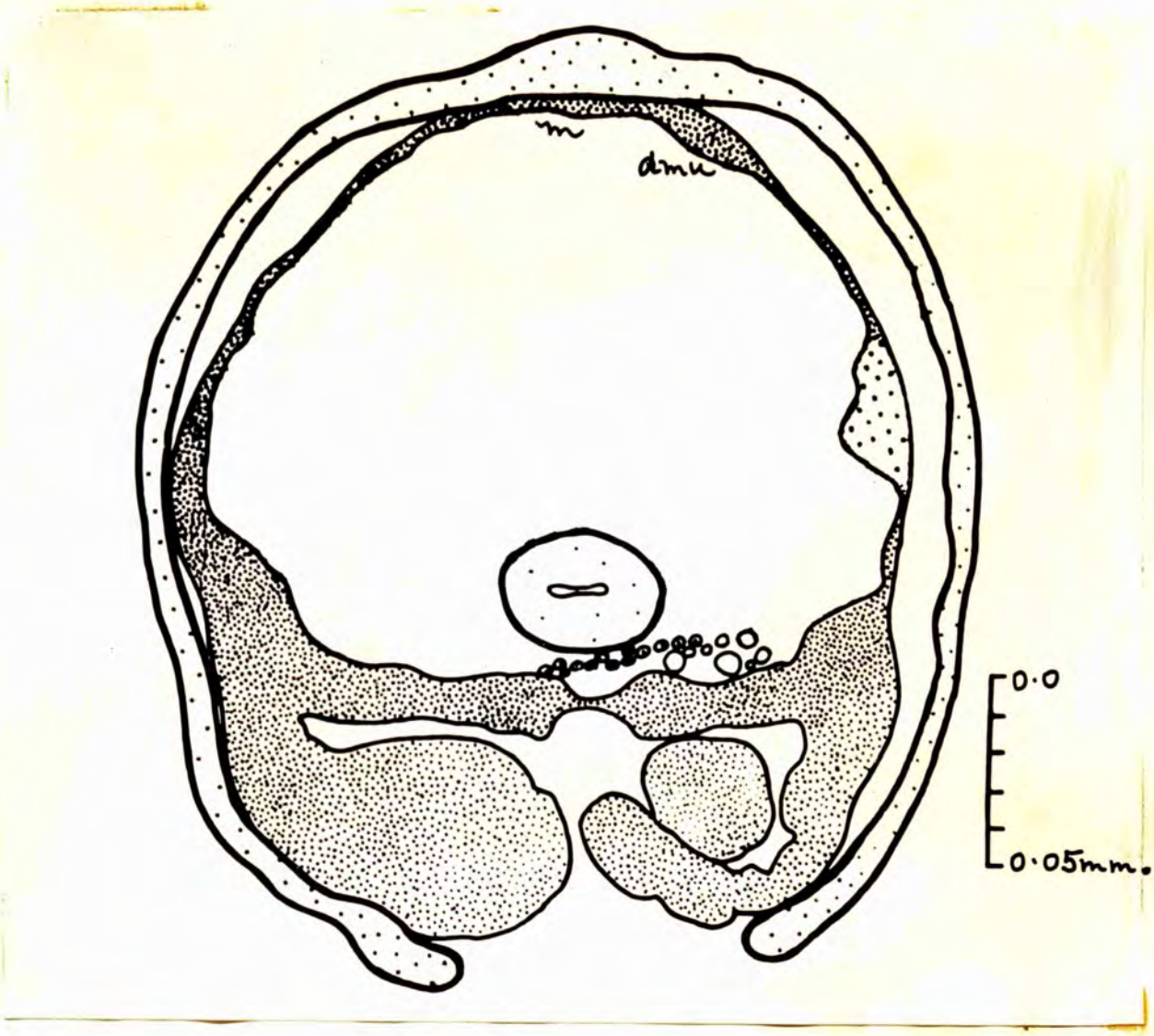


Figure 92. Transverse section through an embryo at the same stage as Fig.89. This section is posterior to the heart and shows the thin layer of mesoderm (m) surrounding the yolk with the beginning of the dorsal longitudinal muscle (dmu).

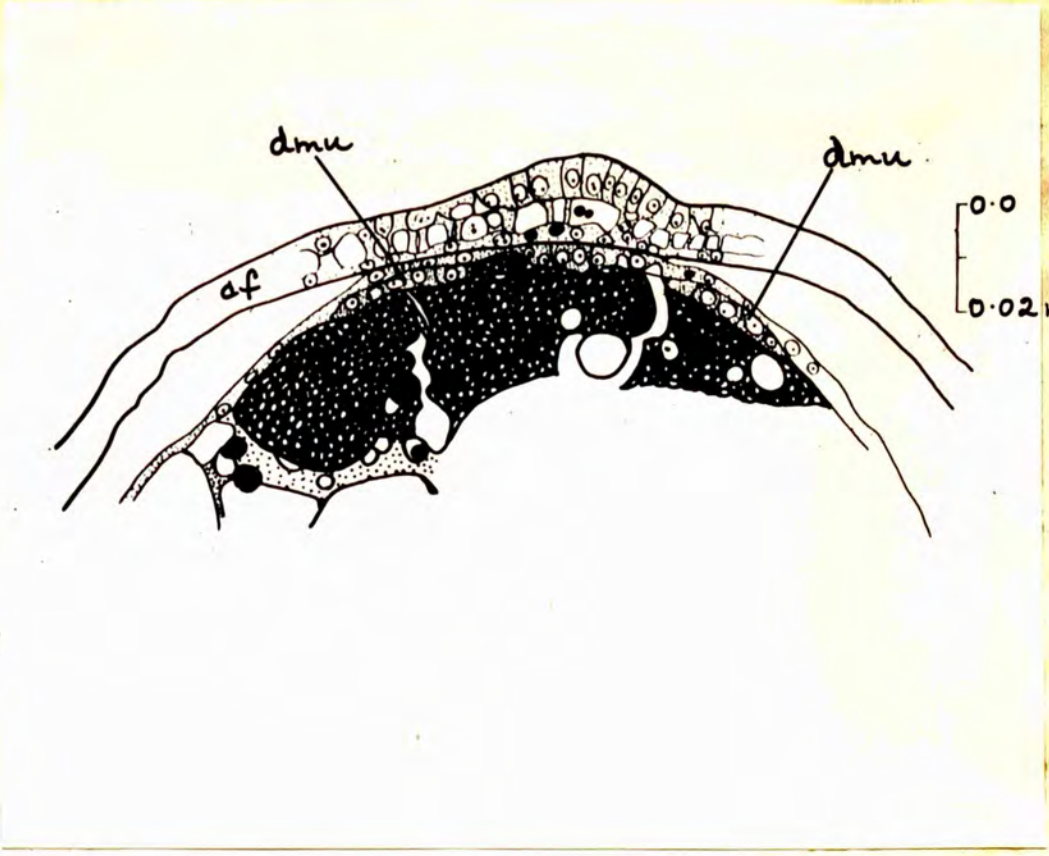


Figure 93. Detail of transverse section Fig.92 showing the beginning of the dorsal longitudinal muscle (dmu) and the well developed carapace fold (cf).

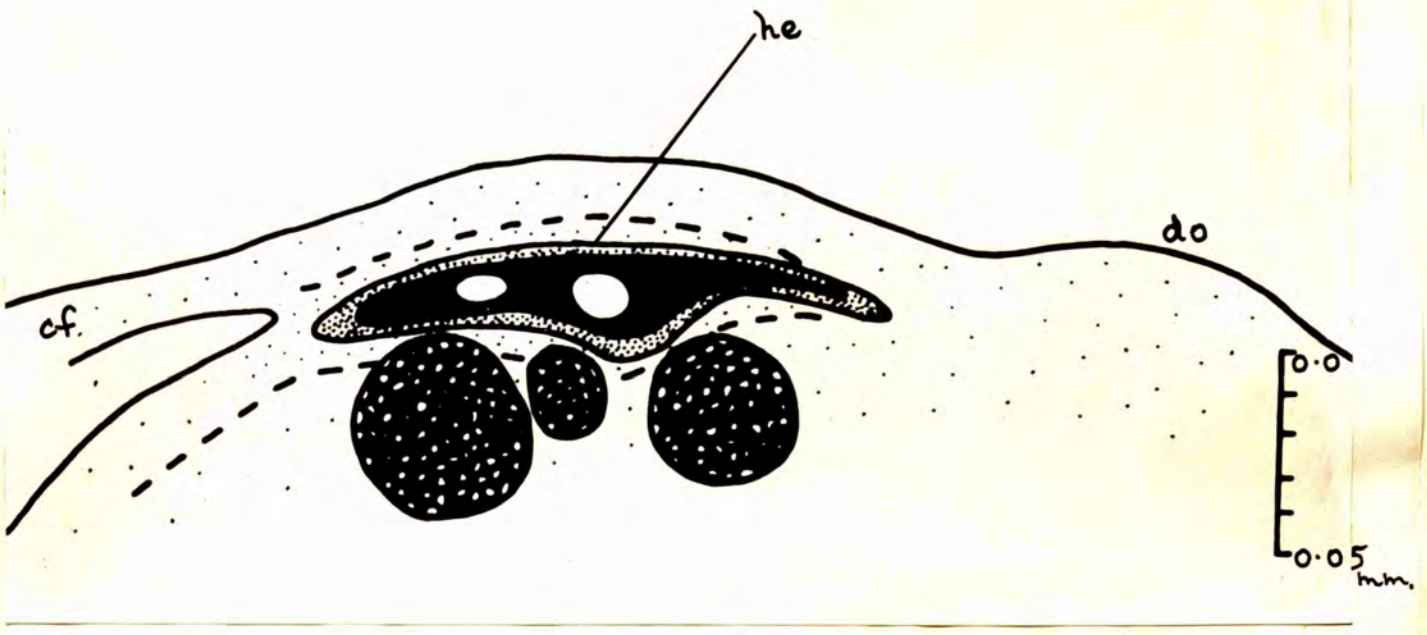


Figure 94. Sagittal section through an embryo at the same stage as Fig.89 showing the well developed heart(he) filled with a vacuolated lightly staining substance. cf, carapace fold; do, dorsal organ.

affinity for stain. The loops of the maxillary gland have developed cavities (Fig.89, mxl). The gland (Fig.91) is well developed and is situated at some distance lateral to the heart. The dorsal longitudinal muscle is distinguishable (Fig.92; Fig.93, dmu), having developed from the mesodermal cells covering the yolk dorsally. The brood pouch has not yet developed.

The heart begins to beat when the embryo is approximately 65 to 70 hours old (Fig.33; Fig.95, Plate 9; he) and the blood, containing small blood cells (co), circulates through the spaces between the septa. The maxillary gland is by now fully developed as are most of the muscles. The distal, and also the proximal, labral glands are well developed.

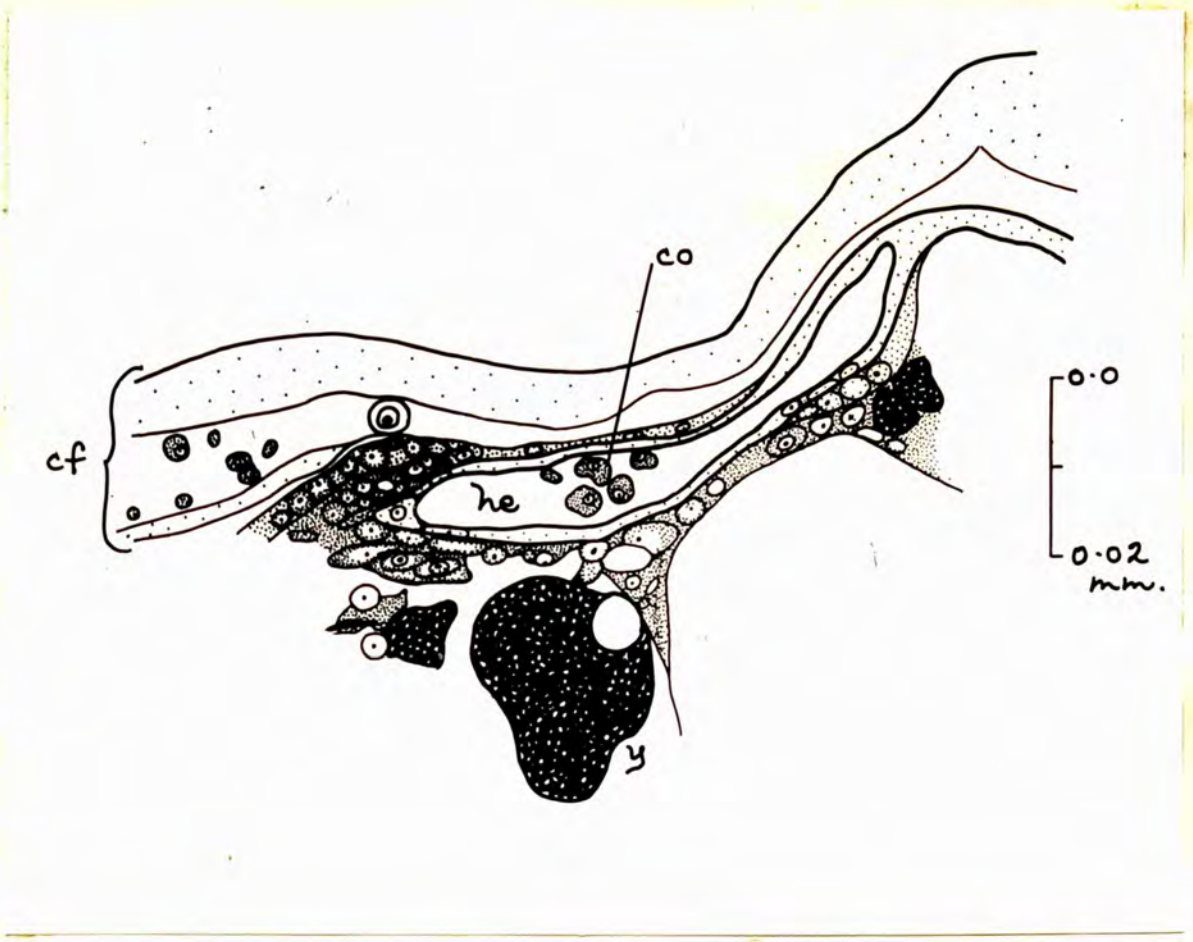


Figure 95. Sagittal section through an embryo at the same stage as Fig.33 showing the well developed heart (he) containing a few small granular cells (co) which are probably blood corpuscles. cf, carapace fold; y, yolk.

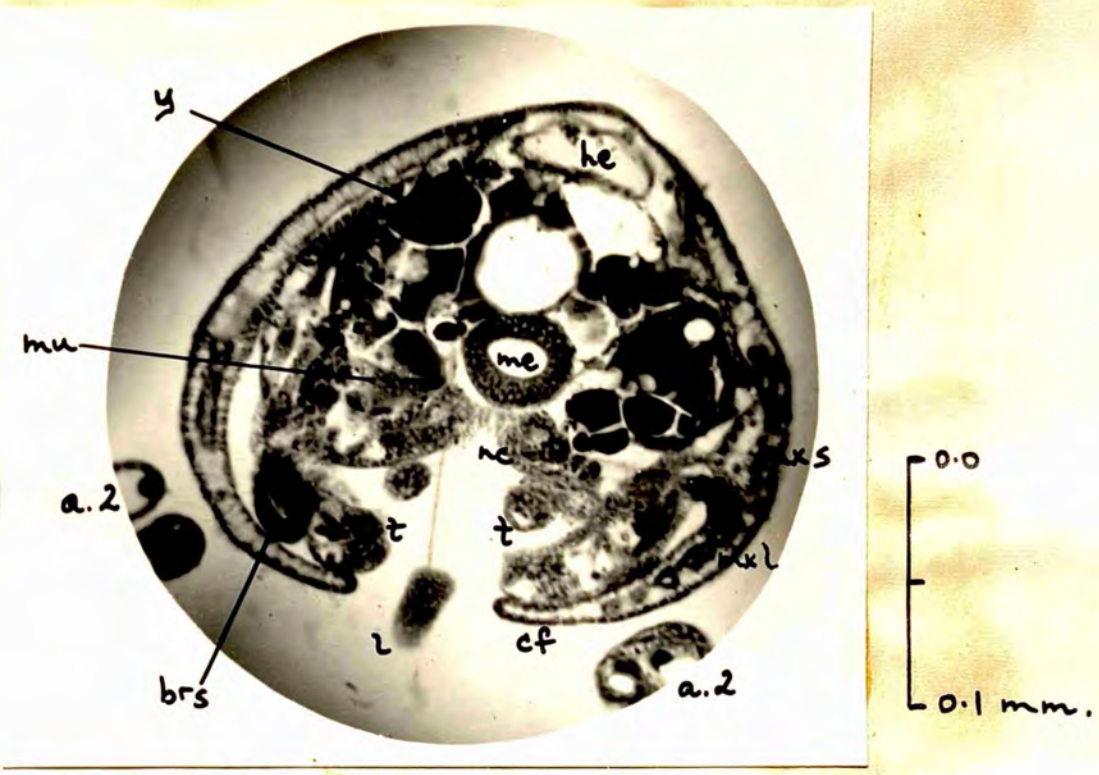


Plate 9. Photomicrograph of a transverse section through an embryo at the same stage as Fig.33 showing the heart(he) the maxillary gland end sac(mxs),the maxillary gland loops with cavities(mxl) and the mesenteron(me). a.2,second antenna; brs,branchial sac; cf,carapace fold; l,labrum; mu,muscle; nc,nerve cord; t,thoracic appendage; y,yolk.

f. The genital cells.

The genital cells become distinguishable during the process of gastrulation (Fig.11). They are large cells with coarsely granular cytoplasm and with large nuclei. The genital cells(gc) are generally found to be the largest cells in the developing embryo of any animal but in Daphnia magna they are rivalled, and sometimes exceeded in size by the cells of the "Scheitel"-plate (Sch). The nuclei soon acquire characteristic features, a large spherical nucleolus surrounded by a hyaline area (Fig.49, gc).

By the end of gastrulation (Fig.11), the genital cells (gc) lie in the mid-ventral part of the egg towards the future posterior end. They occur between the mesendodermal mass of cells (men) and the central yolk (y), forming a group of about twenty cells (Fig.96, (a)).

The genital cells remain in the same position without further division of the cells for some time, while the mesendodermal mass of cells is differentiating into endoderm and mesoderm and the mesenteron beginning to form (Fig.51, gc; Fig.96, (b)).

At about the time of the bursting of the outer egg membrane, the mid-ventral group of genital cells divides into two, and the individual cells divide resulting in an increase in number (Fig.53, gc; Fig.96, (c)). The two groups of cells are found ventrolaterally at the stage when the compact rod of endodermal cells is just beginning to develop a central cavity. There are about twenty to twenty-five cells in each group.

Gradually the two groups of genital cells progress dorsally

Figure 96. A series of diagrams to represent the positions of the genital cells during the development of Daphnia magna.

(a) A single group of cells lying against the mesendoderm in the ventral part of the egg.

(b) A slightly broader group of cells lying against the mesenteron in the ventral part of the egg.

(c) Paired genital rudiments in the ventro-lateral part of the egg.

(d) Paired genital rudiments in the dorso-lateral part of the embryo.

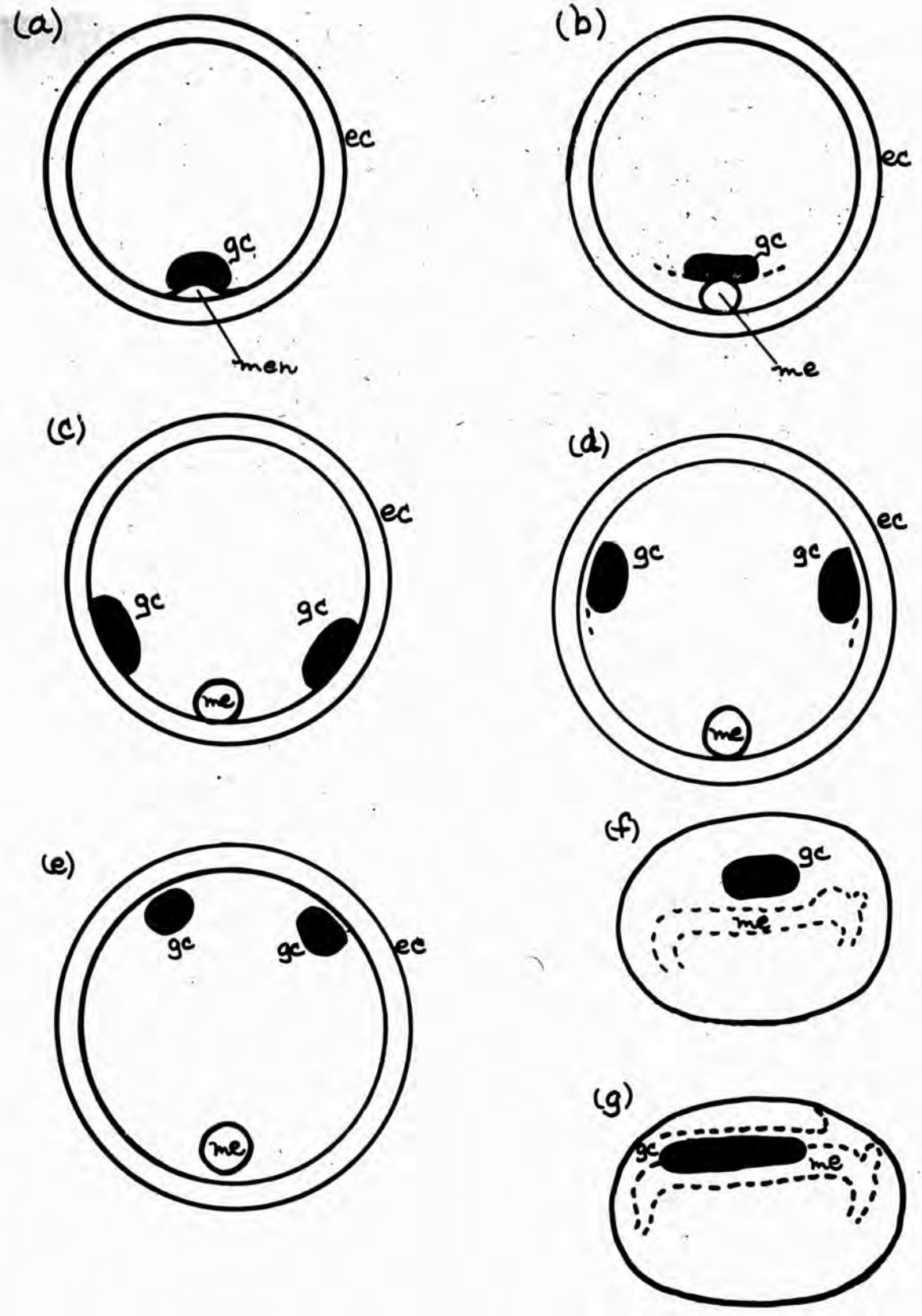
(e) Paired genital rudiments in their final dorso-lateral position.

(f) Short paired genital rudiments lying immediately dorsal to the mesenteron.

(g) Elongate paired genital rudiments lying close to the dorsal half of the mesenteron and also to the newly developed brood pouch.

(a) to (e) are transverse sections; (f) and (g) are longitudinal.

ec, ectoderm; gc, genital cells; me, mesenteron; men, mesendoderm.


(a)
ec
gc
men
(b)
ec
gc
me
(c)
ec
gc
gc
me
(d)
ec
gc
gc
me
(e)
gc
gc
ec
me
(f)
gc
me
(g)
gc
me

along the lateral walls of the body, with little increase in the number of cells (Fig.70, gc; Fig.96, (d)). The mesodermal cells are also extending dorsally.

When the embryo is about 60 hours old, the paired genital rudiments have attained a dorso-lateral position, comparable to that which they will occupy in the adult animal (Fig.96, (e)). The mesenteron is moving from a ventral into a more dorsal position During their progression dorsalwards the genital rudiments are considerably dorsal to the mesenteron and separated from it by a large quantity of yolk (Fig.96, (f)). The genital rudiments attain their final position dorso-laterally and the mesenteron becomes more dorsal until it lies between them. The genital rudiments still consist of a pair of groups of undifferentiated cells, the cells being large with coarsely granular cytoplasm and with large hyaline nuclei containing a central spherical nucleolus (Fig.65, gc).

The genital rudiments now increase in length (Fig.96, (g)), and the boundaries between the individual cells become more distinct. The increase in size of the genital rudiments occurs only in the long axis of the body of the embryo and there is still no marked differentiation of the cells.

At the time of the release of the young animal from the brood pouch of the mother, the paired rudiments of the gonads are elongate narrow cellular bodies, narrower anteriorly and widening for the greater part of their length to narrow again slightly at the posterior end. All the cells are similar, with the

boundaries between them distinctly marked. There is no internal cavity, the cells forming a compact mass (see p. 111).

g. The dorsal organ.

The dorsal organ of Daphnia forms a slight swelling on the dorsal part of the head near to the junction of the head with the body. It arises during the development of the egg and later degenerates, disappearing soon after the young animal has been released from the brood pouch of its mother, in the third or fourth instar. The function of the dorsal organ is doubtful.

The dorsal organ is first recognisable when the egg is about one day old (Fig.97, do). At this stage the endoderm is distinguishable from the mesoderm and is partly formed into a rod, while the second antennae and the mandibles are present. The dorsal organ is situated immediately posterior to the "Scheitel"-plate (Fig.19, Sch). It consists of a group of about ten elongate cells of the outer cell layer, or ectoderm (Fig.97, do). The cells have moderately large nuclei each of which contains a small nucleolus and some scattered chromatin granules. The cytoplasm of the cells is granular but its staining reaction is similar to that of the cytoplasm of the surrounding cells. The central cells have the greatest height, the more lateral cells tending to grade into the adjacent ectoderm cells so that the boundary of the dorsal organ is difficult to define. The inner ends of the cells lie against the yolk globules.

Shortly after the bursting of the outer egg membrane (Fig.22), the dorsal organ is more clearly recognisable as a group of enlarged cells in the ectoderm (Fig.98; Fig.99, (a) and (b)). It is now situated antero-dorsally (Plate 10) in a position further from the "Scheitel"-plate than in the earlier stages.

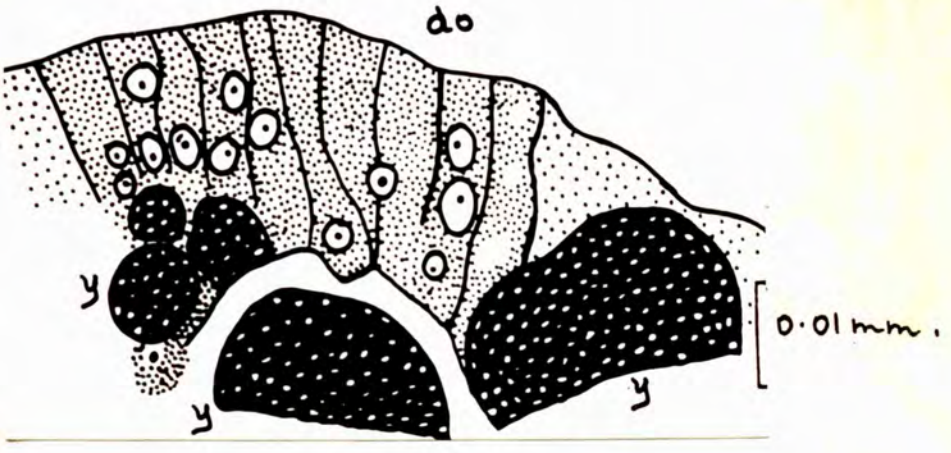


Figure 97. Transverse section through an embryo at the same stage as Fig.19 showing the dorsal organ (do) of tall granular cells. y, yolk.

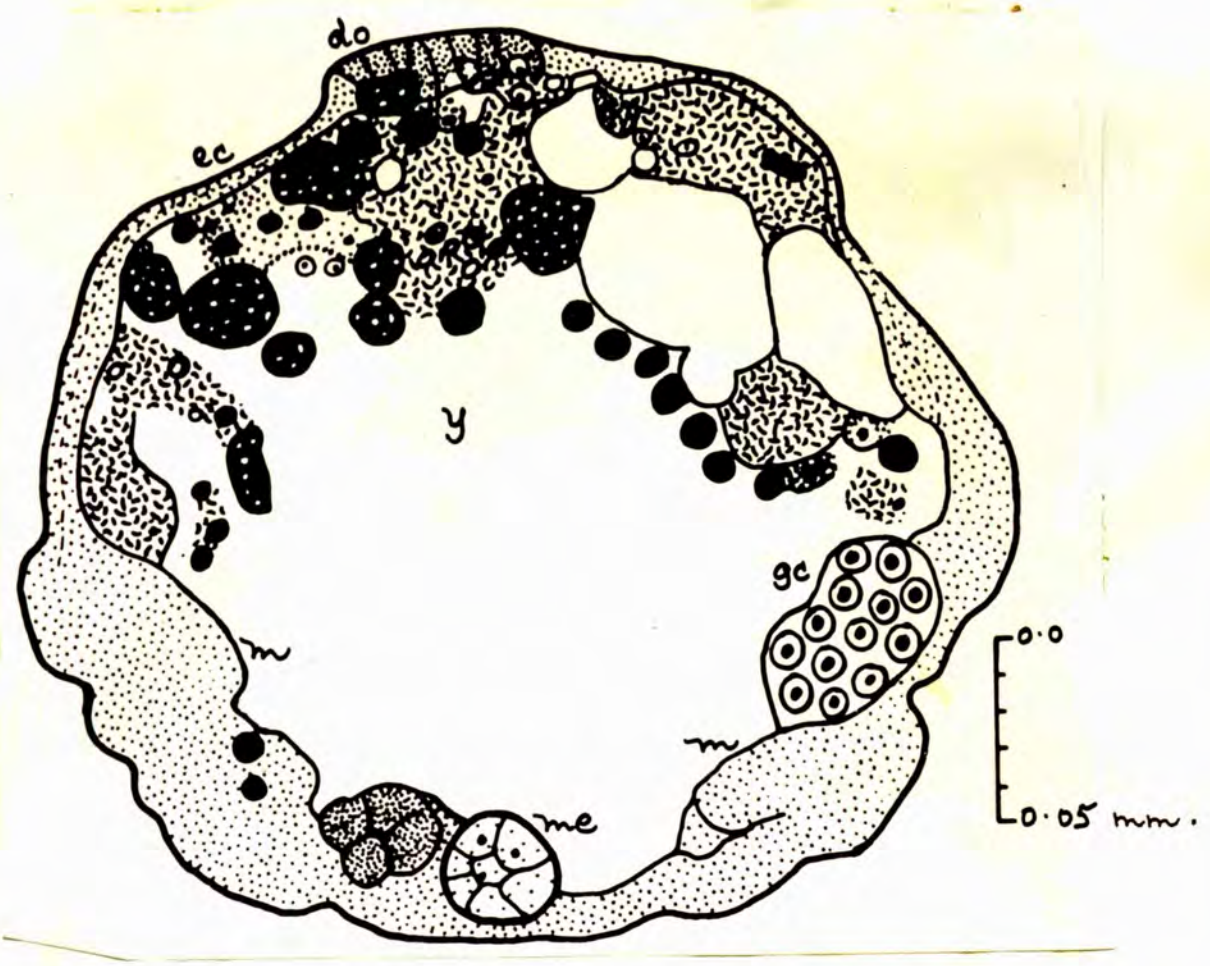


Figure 98. Transverse section through the anterior end of an embryo at the same stage as Fig.22 showing the position of the dorsal organ (do) and the thin outer cell layer (ec) enclosing the central yolk (y) lateral to the dorsal organ. The mesoderm (m) is thickened ventro-laterally. gc, genital cells; me, mesenteron.

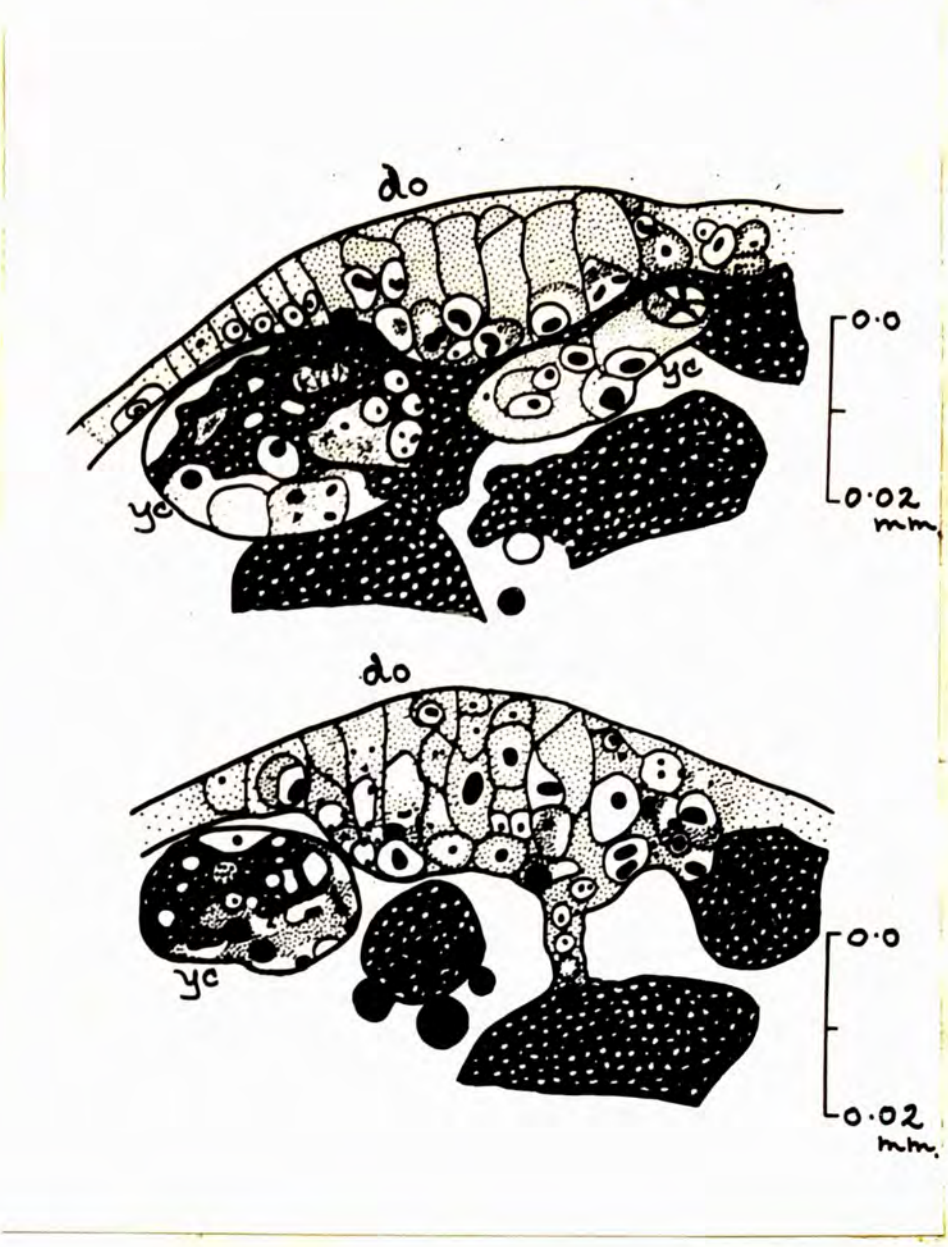


Figure 99. (a) and (b). Vertical longitudinal sections through an embryo at the same stage as Fig. 22 and Fig. 98 showing the dorsal organ (do) of large granular cells with large nuclei and with adjacent yolk cells (yc).

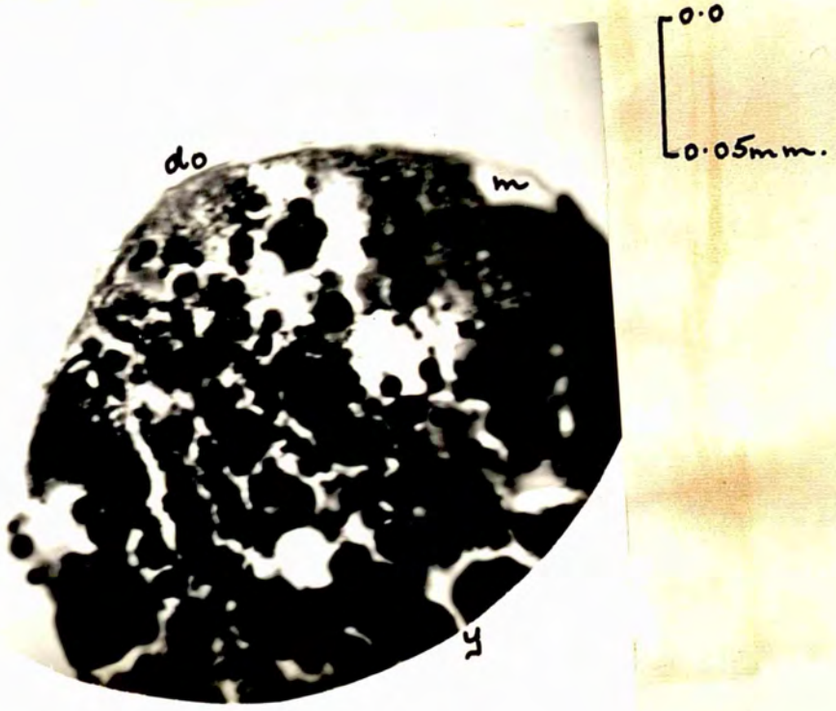


Plate 10. Photomicrograph of the sagittal section through an embryo at the same stage as Fig.22 showing the dorsal organ(do). m,mesoderm of the head; y,yolk.

The cells of the dorsal organ (do) are in height almost double that of the surrounding ectodermal cells and are also wider than the latter. The cytoplasm of the cells is granular and stains with a slightly greater intensity than that of the adjacent cells. The nuclei have become larger and contain large deeply staining nucleoli which are often rod-shaped. The inner edge of the dorsal organ is uneven, suggesting a breakdown of the basement membrane, and is next to globules of yolk among which are a number of yolk cells (yc). The yolk cells are large and usually at least part of the yolk is granular. Nuclei are visible and there sometimes appears to be more than one nucleus to a single yolk cell. The yolk in this region not contained within the yolk cells stains with the reaction characteristic of yolk in the process of being broken down, that is with Heidenhain's Iron haematoxylin it stains pale brown (see p. 57). The yolk cells are numerous in the neighbourhood of the dorsal organ. The ectoderm surrounding the dorsal organ is thin so that the boundary of the dorsal organ easier to define than in earlier stages. The dorsal organ is separated from the anterior thickening of cells in the head by a short row of ectodermal cells not thickened internally (Plate 10).

Approximately ten to twelve hours later (Fig.54), when the embryo has elongated and the carapace folds and thoracic appendages are moderately well developed, the dorsal organ is a group of cells about seven to eight cells in length (Fig.100,do), the inner edges of the cells forming an even contour. The dorsal organ is situated posterior to the anterior thickening in the

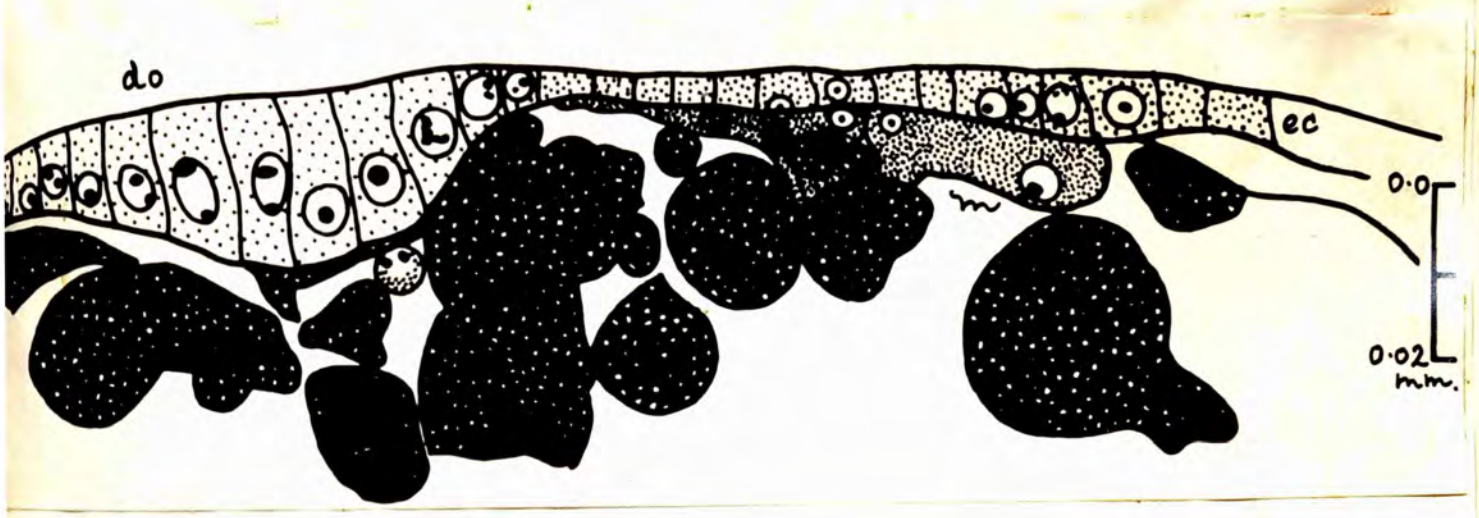


Figure 100. Sagittal section through an embryo at the same stage as Fig.54 showing the dorsal organ (do) formed of a regular row of tall cells with large nuclei. There is a thin layer of mesodermal cells (m) posterior to the dorsal organ. ec, ectoderm.

head region and it is anterior to the lateral band of thickened mesoderm found in the maxillary region. The cells are large with large nuclei containing a number of chromatin bodies. Internally the dorsal organ is still bounded by yolk.

At a slightly later stage (Fig.25), when the mesoderm surrounds most of the yolk dorsally, the dorsal organ cells (Fig.101, do) show little change except that the outer part of the cell contains a greater number of granules than the inner part of the cell. The inner edge of the cells lie against the yolk which is partly granular.

By the time that the alimentary canal has acquired a small but continuous central cavity and the maxillary gland is able to be recognised (Fig.69), the dorsal organ forms a larger swelling of the ectoderm situated dorsal to the anterior flexure of the mesenteron. The outer region of the large cells stains intensely, while the inner region contains the nuclei.

When the embryo is about two and a half days old (Fig.73), the dorsal organ (do) is still in the same position. Immediately posterior to the dorsal organ and closely connected to it (Fig.84, do), the heart (he) is distinguishable. Against the anterior end of the inner part of the dorsal organ is an area of cytoplasm with little affinity for stains but through which run a number of lines of darkly staining granules. This area is surrounded by mesodermal cells, some of which contain large nuclei. In the outer part of the dorsal organ the cells have parallel sides and the cytoplasm is granular (Fig.102, do). The cells project outwards from the ectoderm forming a swelling. The inner part

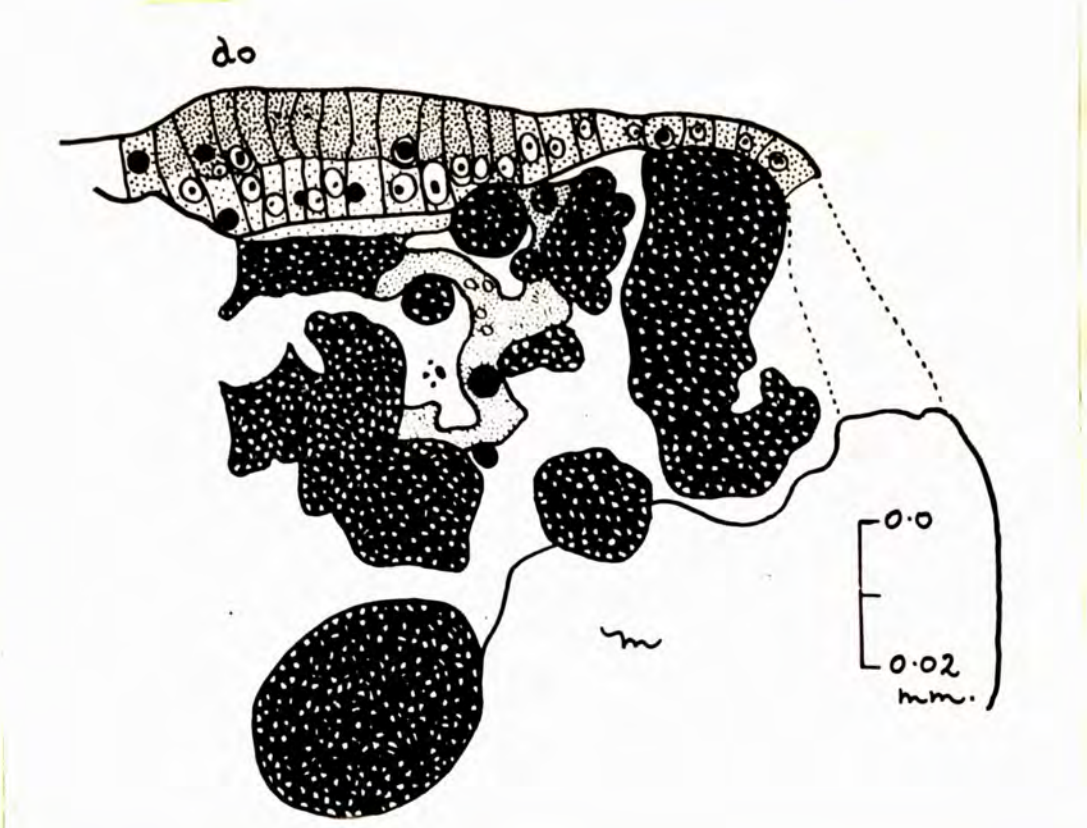


Figure 101. Sagittal section through an embryo at the same stage as Fig.25 showing the dorsal organ (do) composed of a row of tall cells, granular in their outer part and less granular with nuclei in their inner edges. m, mesoderm.

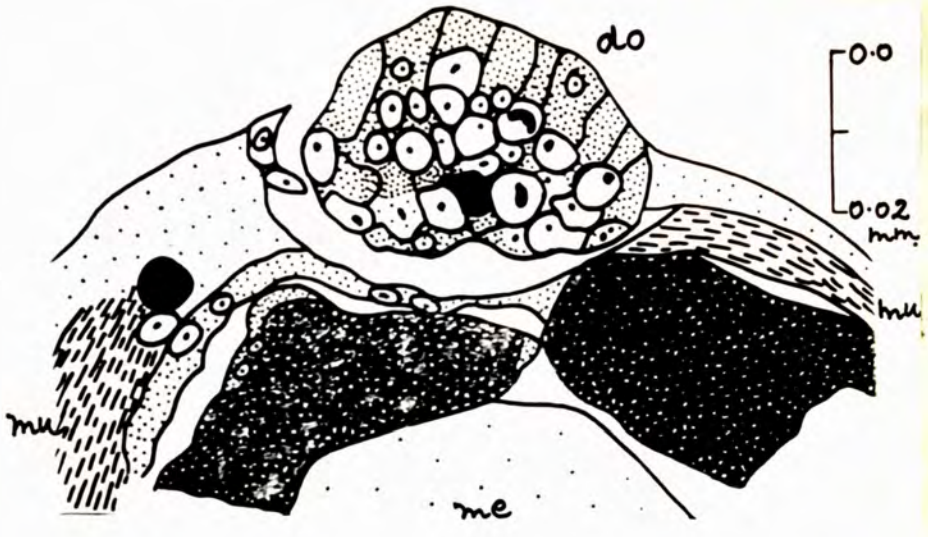


Figure 102. Transverse section through an embryo at the same stage as Fig.73 showing the dorsal organ (do) composed of tall cells with basal nuclei. This section is not vertical. me, mesenteron; mu, muscle.

of the dorsal organ is composed of numerous large spherical nuclei. The nuclei are hyaline with scattered chromatin granules and are surrounded by a small amount of cytoplasm, which shows little affinity for stains. The nuclei swell the inner ends of the cells and the inner contour of the dorsal organ is therefore uneven. The close proximity of the dorsal organ to the heart at this stage suggests that there may be some relationship between the two.

At approximately the time of the shedding of the second membrane, about 56 hours after the egg was laid into the brood pouch of the mother, the dorsal organ (Fig.103; Fig.104; do) is a group of approximately nine cells in width and about twice as long as wide. The cells are elongate and the sides almost parallel. There appear to be two rows of nuclei at the inner ends of the cells. The inner ends of the cells still lie against yolk, which is often in the form of unaltered globules. The dorsal organ is situated close to the anterior flexure of the mesenteron (Plate 11) and is bounded on either side by the developing muscles of the second antennae and mandibles. A number of small cells (m) occur near to the inner edge of the dorsal organ among the yolk. These cells are smaller than the other cells of the body irregularly arranged through the yolk. The heart at this stage of development has a central cavity and it also contains a number of small cells, loose within the cavity (Fig.89).

Shortly before the emergence from the brood pouch of the mother, when the mesenteron has a large central cavity and is

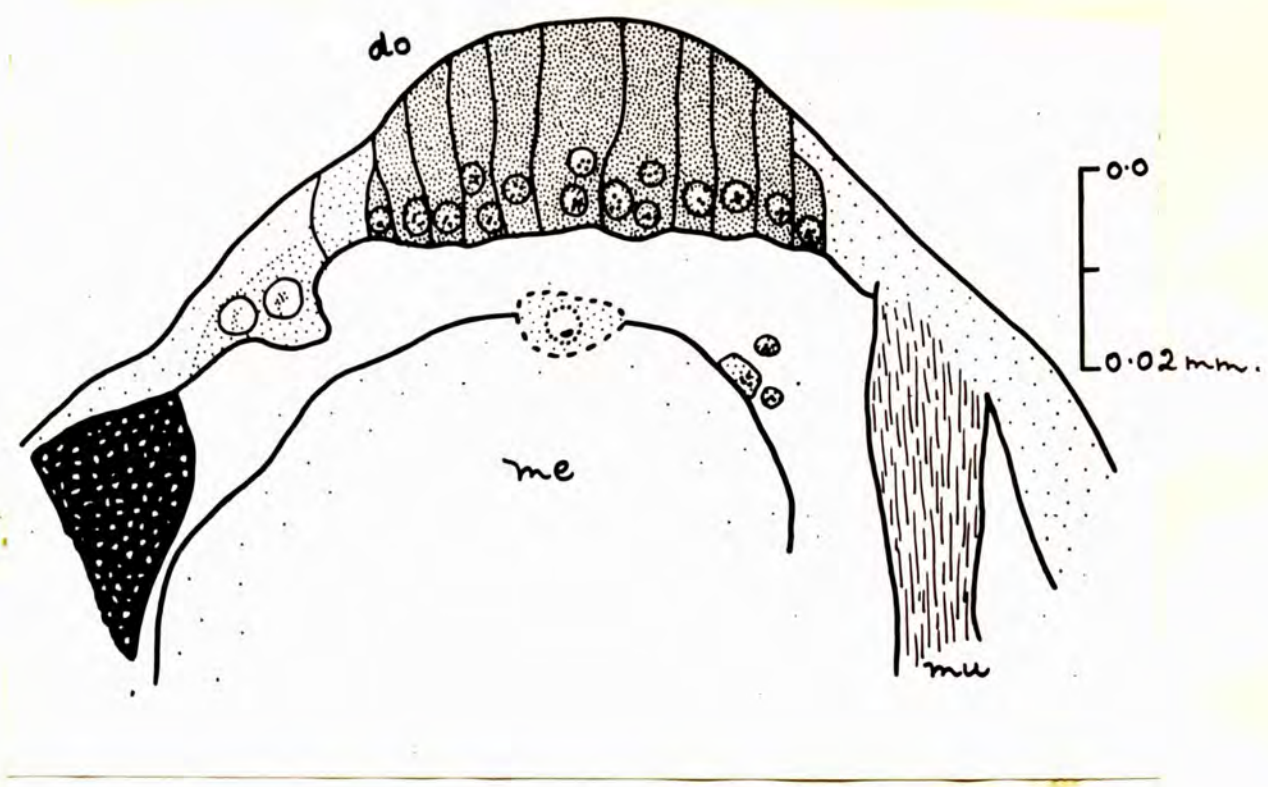


Figure 103. Transverse section through an embryo at the same stage as Figs. 89 to 94 showing the dorsal organ (do) as a row of tall granular cells with two rows of nuclei at their inner ends and lying close to the mesenteron (me). mu, muscle.

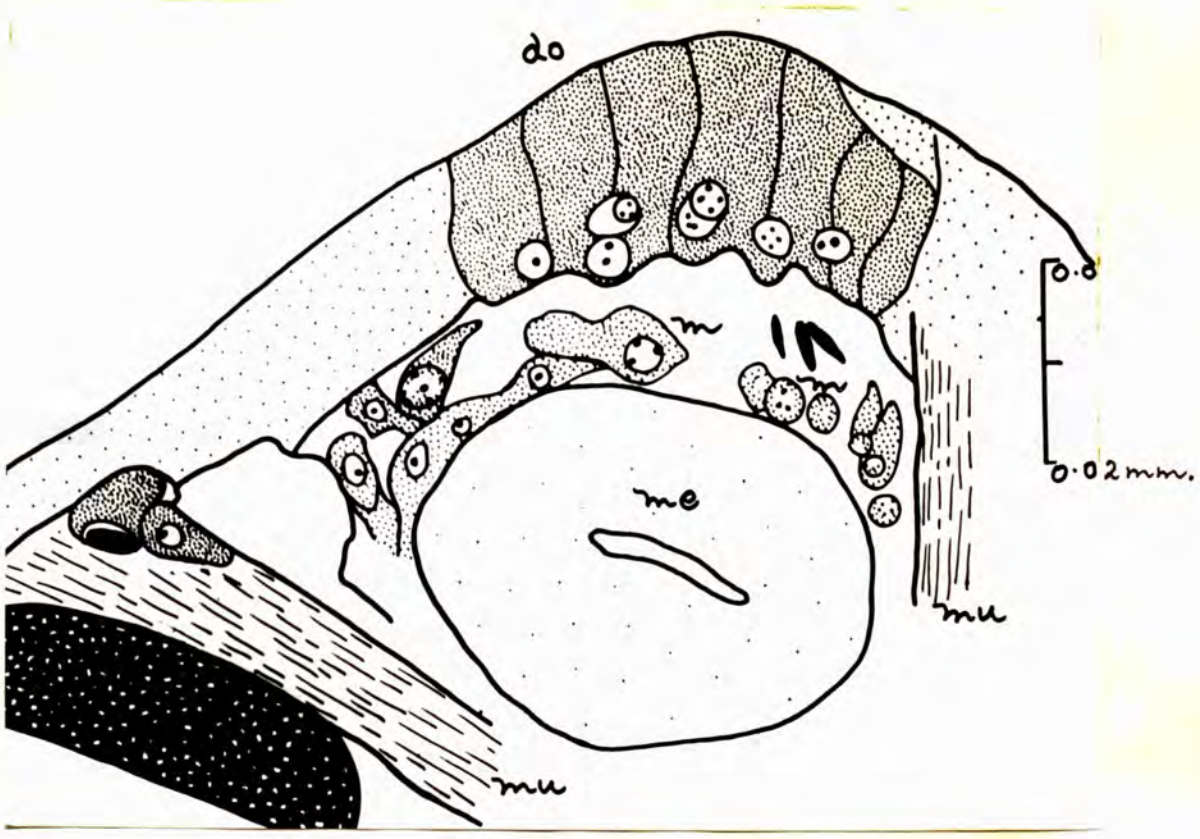


Figure 104. Transverse section through an embryo at the same stage as Fig. 103 but in a slightly more posterior region showing the dorsal organ (do) composed of large granular cells and with a number of small irregular cells (m) in the region between the dorsal organ and the alimentary canal (me). mu, muscle.

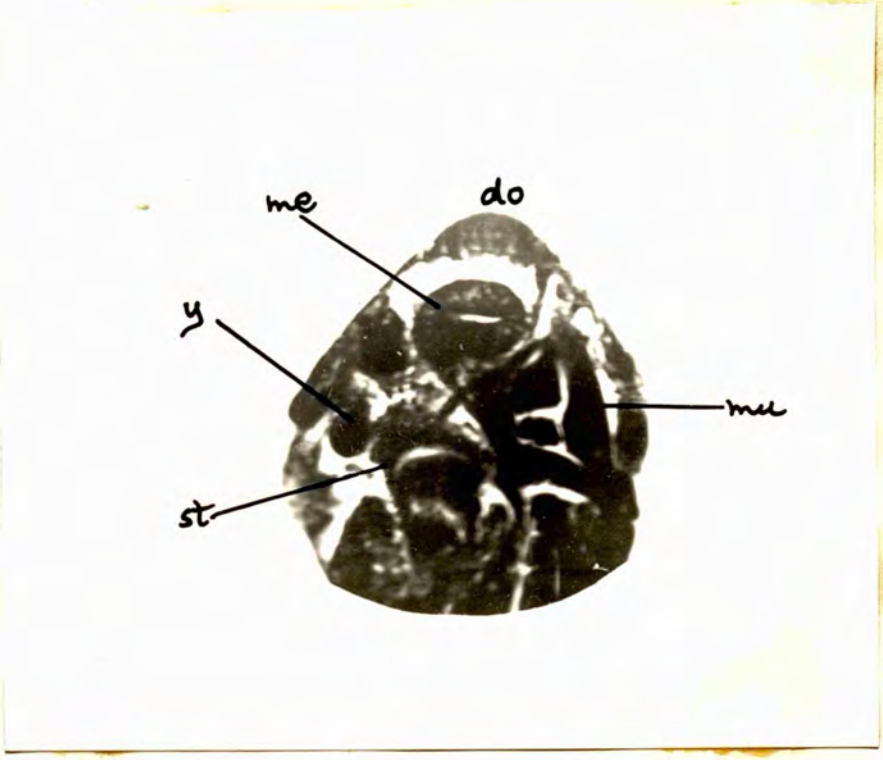


Plate 11. Photomicrograph of a transverse section through an embryo at the same stage as Fig.103 and Fig.104 showing the dorsal organ(do) close to the mesenteron(me). mu,muscle; st,stomodaeum; y,yolk.

close to the dorsal surface of the animal, the dorsal organ (Fig. 105, do) is still large and forming a swelling and it is situated immediately dorsal to the anterior flexure of the alimentary canal (Plate 12). The cells of the dorsal organ are connected to the cells of this flexure by a fine trabecular meshwork containing a number of small cells. The trabeculae enclose a faintly staining, finely granular substance similar to that identified as yolk in the process of being broken down (see p. 79). The trabeculae meet the inner, more sparsely granular region of the dorsal organ cells containing the nuclei, the outer region of each cell being more densely granular.

In the young animal newly expelled from the brood pouch of its mother, the dorsal organ (Fig.106; Fig.107; do) is slightly smaller than previously in relation to the size of the remainder of the animal, but its outer edge is still raised above the surface of the head. It forms a broad shield of thickened chitin on the postero-dorsal surface of the head opposite the anterior flexure of the mesenteron (me), which is the most dorsal part of the alimentary canal. Laterally the shield extends to just dorsal to the level of the mesenteron flexure on either side. The dorsal organ is now composed of a single outer layer of tall cells with little affinity for stains, and an inner group of cells arranged internal to the peripheral of the outer cells. There are none of the inner group of cells opposite to the central cells of the outer layer. Most of the inner group of cells show little affinity for stains but some of them stain darkly. They lie close to the wall of the mesenteron. The wall of the

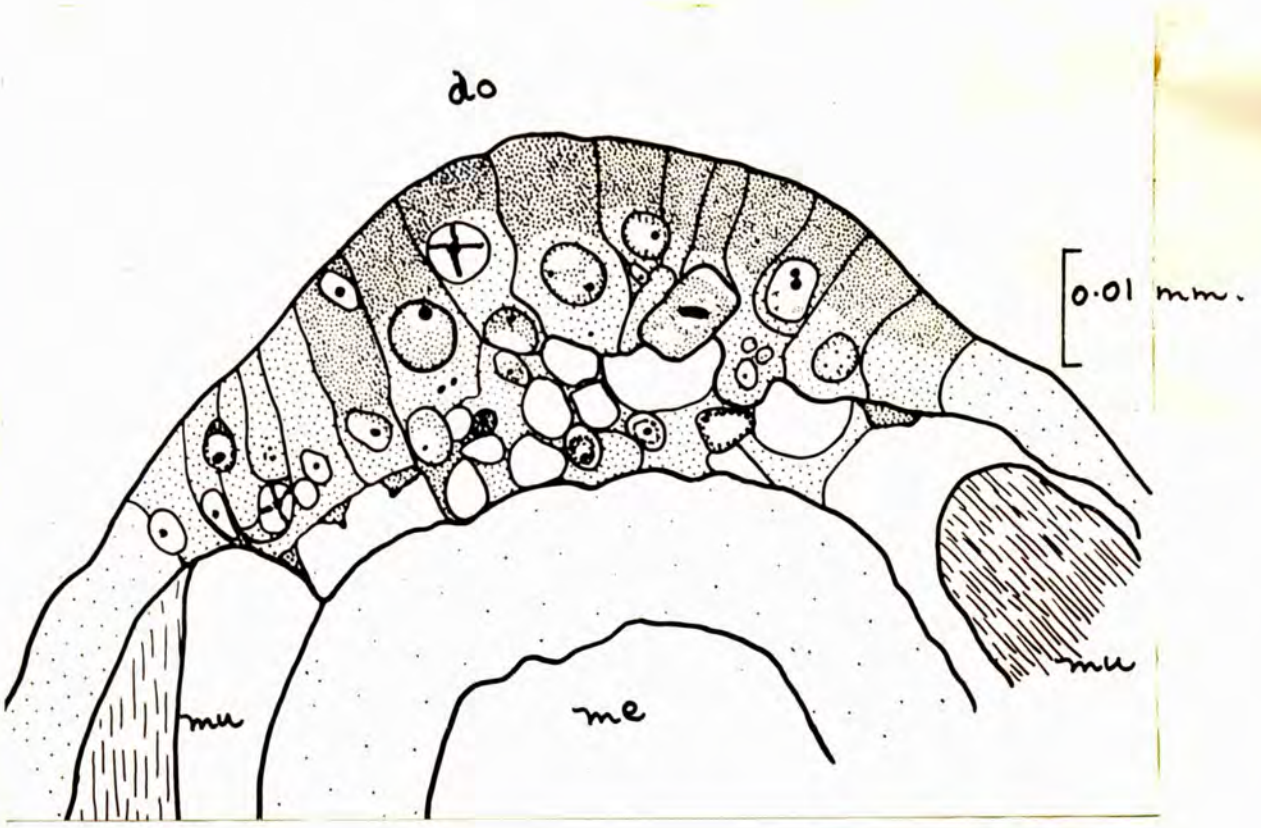


Figure 105. Transverse section through a young animal showing the dorsal organ (do) composed of a row of large cells which are densely granular in the outer part, more sparsely granular in the inner part of the cell. The cells of the dorsal organ are connected with those of the mesenteron (me) by a trabecular mesh containing a few small cells and enclosing a lightly staining, finely granular substance. mu, muscle.

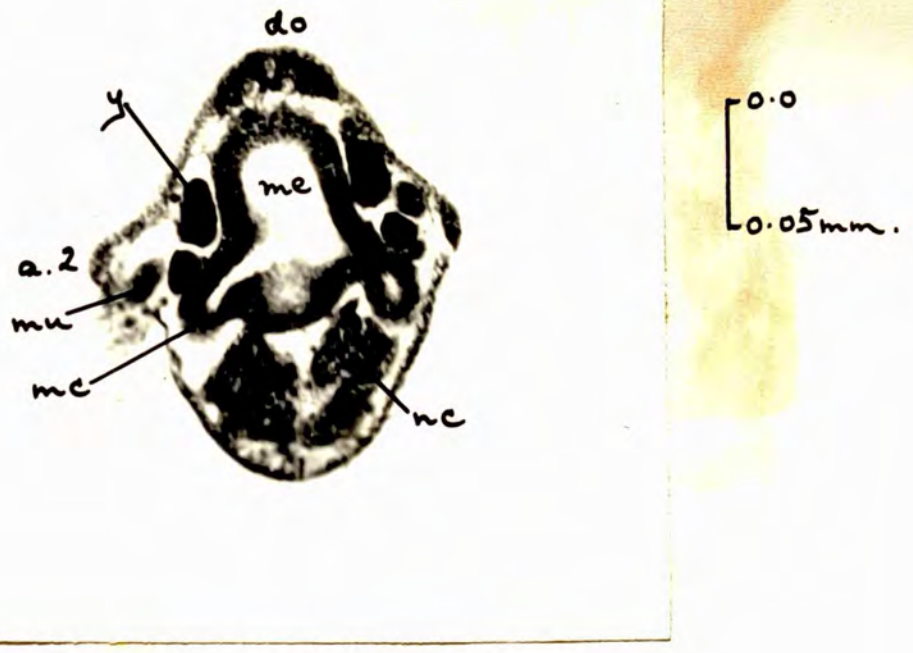


Plate 12. Photomicrograph of a transverse section through an embryo at the same stage as Fig.33 showing the dorsal organ(do) connected with the mesenteron(me) by fine strands. a.2,second antenna; mc,caecum of mesenteron; mu,muscle; nc,nerve cord; y,yolk.

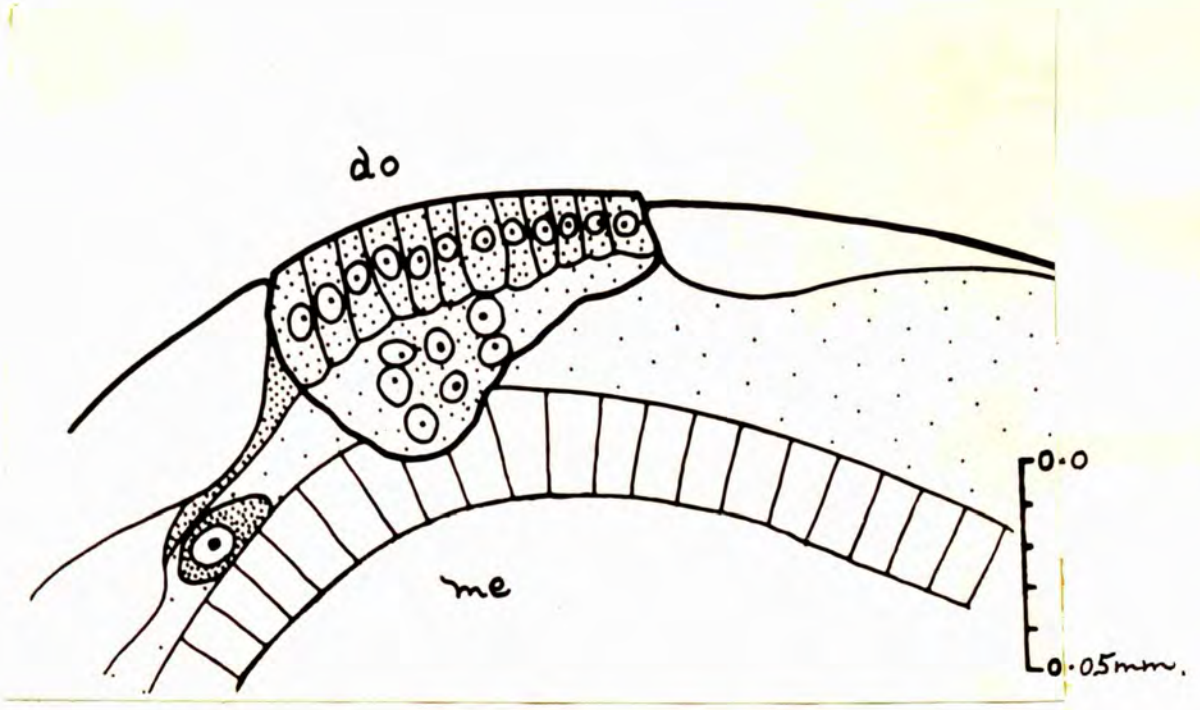


Figure 106. Diagram of the dorsal organ (do) of a first instar Daphnia magna showing the shield of cells lying close to the mesenteron (me) and an adjacent large, deeply staining cell. Freehand drawing.

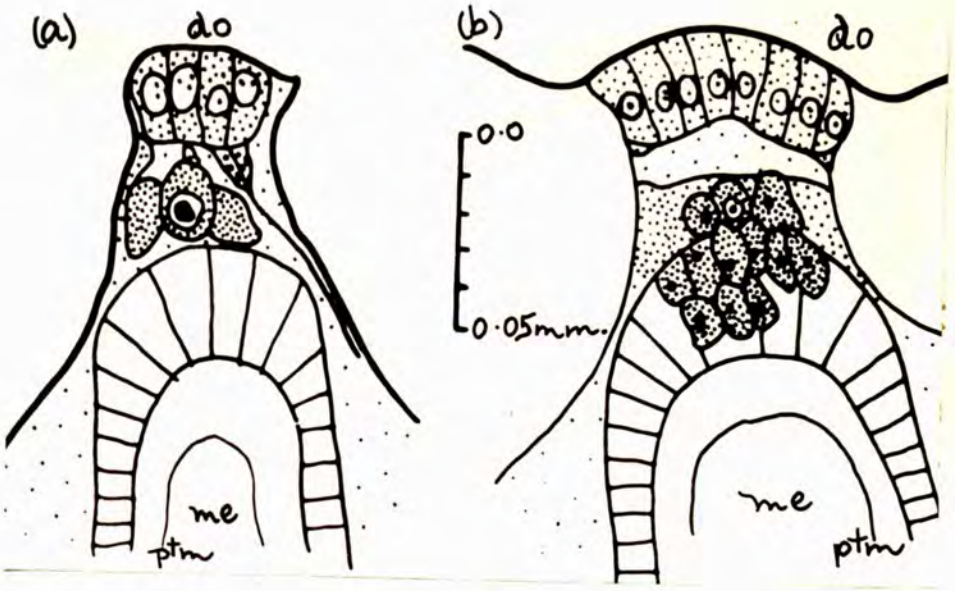


Figure 107, (a) and (b). Transverse sections through the dorsal organ (do) of a first instar Daphnia magna.

(a) the more anterior of the two sections, shows the layer of cuboidal cells and an inner group of cells lying close to the mesenteron (me).

(b) a slightly posterior section, shows the uneven nature of the dorsal surface of the mesenteron(me) adjacent to the dorsal organ.

ptm, peritrophic membrane. Freehand drawing.

mesenteron is thickened and the outer edges of the cells uneven where they lie against the cells of the dorsal organ. The outer layer of the dorsal organ is composed of large cuboidal cells the cytoplasm of which contains small granules. Each cell contains a large, hyaline nucleus with a small spherical darkly-staining nucleolus. The inner group of cells are irregularly arranged. The cytoplasm has a finely granular appearance. There are no vacuoles. The nuclei usually contain large nucleoli with a number of granules surrounding it. Some of the cells of this inner group have a more intense staining reaction and more distinct nuclei. Anterior to the dorsal organ is a pair of large spindle-shaped cells (Fig.106) with granular cytoplasm showing little affinity for stains. Their nuclei are large and each contains a large and intensely staining nucleolus together with a peripheral layer of chromatin granules. These cells are similar in appearance to the lateral frontal organ cells. At the posterior end of the dorsal organ is the beginning of a horizontal membrane, the dorsal septum (see p. 152).

In the second instar animal, the dorsal organ (Fig.108, do) is considerably smaller than in the first instar animal and shows a stronger affinity for stains. It is composed of a number of tightly packed spherical bodies each with a small darkly staining central body. It is possible that these spherical bodies are the nuclei, the cytoplasm having mostly been absorbed. The cells are covered externally by a transparent layer of chitin. The central part of the dorsal organ contains mostly colourless, but a few darkly staining, areas which tend to be arranged in two

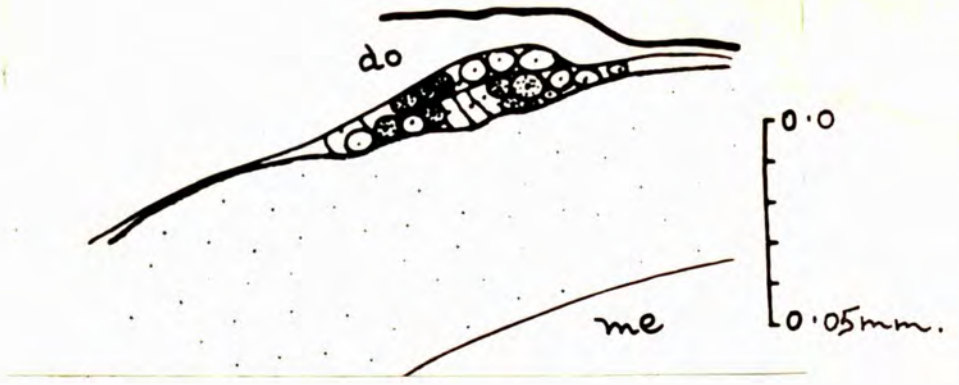


Figure 108. Diagram of the dorsal organ (do) of a second instar Daphnia magna showing the small cells staining with varying intensity. me, mesenteron. Freehand drawing.

layers. Some of the more darkly staining areas contain recognisable nuclei. The dorsal organ differs from one second instar animal to another, and appears to be in slightly different stages of degeneration. Degeneration probably takes place during the instar. Anterior to the dorsal organ is a pair of median, large spindle-shaped cells with granular cytoplasm showing little affinity for stains. Each contains a large nucleus with a large darkly staining nucleolus together with a number of chromatin granules on the periphery of the nucleus. These cells resemble the lateral frontal organ cells and correspond to those observed in a similar position in the first instar animal. They lie parallel to the surface of the integument.

The third instar animal has a greatly reduced dorsal organ (Fig.109, do) which is scarcely visible in the living animal. It is flattened so that its surface lies level with that of the surrounding integument and it does not stain intensely. The outline of the dorsal organ is irregular. The palely staining tissue contains a few small darkly staining granules and one or two recognisable nuclei - round hyaline areas with central nucleolus. The cells of the dorsal organ are compressed between the anterior flexure of the mesenteron and the outer integument. The two spindle-shaped cells are still present.

The dorsal organ of the fourth instar animal (Fig.110, do) is not visible in the living animal but there is an extremely slight indication of its position in an Ehrlich's-Haematoxylin-stained whole mount. Sections show a mass of cytoplasm with little affinity for stains lying partly against the outer

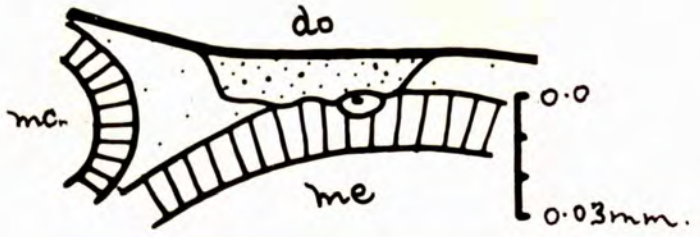


Figure 109. Diagram of the dorsal organ (do) of a third instar Daphnia magna. The dorsal organ is reduced in size and has little affinity for stains. mc, caecum of mesenteron; me, mesenteron. Freehand drawing.

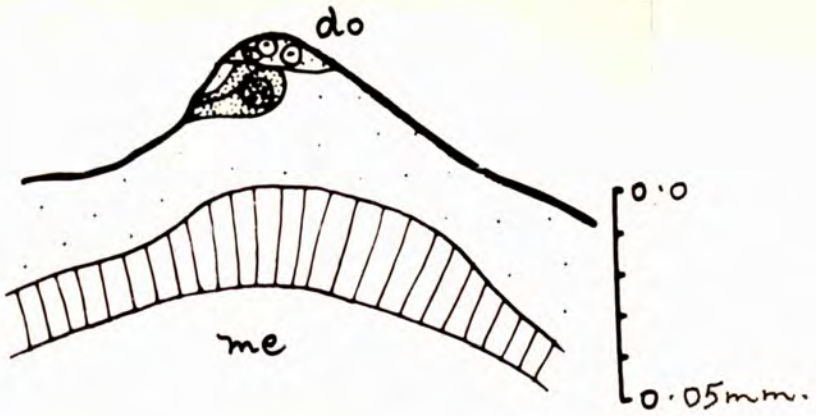


Figure 110. Transverse section through the dorsal organ (do) of a fourth instar Daphnia magna showing the small size of the organ and also the two large spindle-shaped cells adjacent to the dorsal organ.
me, mesenteron.

integument and partly loosely within the animal.

A few scattered palely staining cells lie between the anterior flexure of the mesenteron and the dorsal integument in the fifth instar animal (Fig.111, do). A few similar cells are also noted in adult animals.

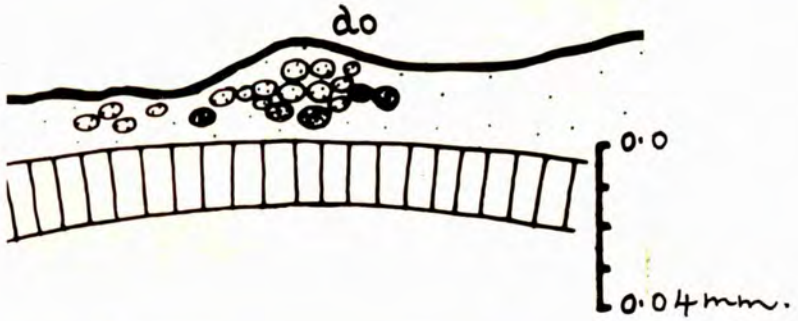


Figure 111. Vertical longitudinal section through the dorsal organ (do) of a fifth instar Daphnia magna showing the group of scattered small cells, most of which show little affinity for stains. Freehand drawing.

The Development of the Ephippial Egg.

a. Factors causing the production of ephippial eggs.

Early workers, such as Weismann, considered that ephippial eggs appeared cyclically during the life of Daphnia. It is now generally agreed that the production of ephippial eggs is due to a number of external factors which suggest a lowering of the general metabolic rate (Berg, 1951; 1954; 1956; Banta, 1939; Issakowitsch, 1905). The environmental factors responsible are scarcity of food, crowding, low temperature, and either a markedly high or a markedly low pH of the water. The labile period during which it is determined which kind of eggs will be produced occurs during the last half of the previous instar (Mortimer, 1956). The first change noted in a population subjected to adverse conditions is the production of males, which is soon followed by the appearance of females carrying ephippial eggs. Fritzsche (1917) noted that the females producing ephippial eggs have a higher internal osmotic pressure than those producing non-ephippial eggs. In a recent paper, von Dehn (1955) suggests that the formation of ephippial eggs in Moina is dependent upon the ergosterin content of the fat in the food. Earlier, Smith (1915) stated that fat storage is especially characteristic of males and ephippial females, whereas glycogen storage is especially characteristic of parthenogenetically reproducing females. Gallistel (1936-7) found that males have more abundant fat, but that there is no principle difference between the reserve material of the ephippial and non-ephippial eggs.

b. Description of the development of the ephippial egg.

The ephippial eggs of Daphnia are usually fertilised eggs and their production corresponds with the appearance within the population of males, which are however always few in number in comparison with the number of females. The early distinction between the two types of cladoceran egg as "summer" and "winter" eggs has been largely replaced by the terms "parthenogenetic" and "fertilised" or "ephippial" eggs. But recent work has shown that ephippial eggs may apparently develop parthenogenetically since populations have been found in which ephippial eggs are produced when no males are present. Instances have been cited by Banta (1926), Schrader (1926), Poulsen (1940) and Edmondson (1955), all in Arctic populations. Thus ephippial eggs are not always fertilised eggs and may be parthenogenetic.

During their development in the brood pouch of the mother, the eggs are enclosed within an ephippium. The ephippium is a darkened saddle-shaped thickening of the carapace in the region enclosing the brood pouch. It has a double wall the two layers of which correspond to those of the remainder of the carapace. The inner wall is a single delicate chitinous membrane. The outer wall is formed of tall prismatic chambers, their external edges dome-shaped and with an irregular hexagonal outline. Each prism corresponds to one of the hypodermal cells, and the prisms vary in height. In places the layer of prismatic chambers is replaced by a layer of thickened chitin resulting in a larger space enclosed between the walls of the ephippium. The median dorsal line of the ephippium is formed by a strongly elastic

ligament which causes the closure of the two valves when the ephippium is shed at the time of the next moult of the mother. The ephippium provides protection for the enclosed eggs and in most species it also acts as a flotation device, owing to the enclosure of air within the prismatic chambers. In Daphnia magna, the majority of the ephippia sink. The number of eggs in the ephippium is fixed for each species, as is the position of the eggs within the ephippium. In D. magna there are two eggs placed longitudinally end to end. Further details of the structure of the ephippium are given by Storch (1925), whose information is repeated by Gravier (1931).

The ephippial eggs are of approximately the same size as the non-ephippial eggs (Green, 1956), but of a slightly different shape, being more ovoid. The yolk is in the form of globules which are smaller and more even in size than those of the non-ephippial egg. The living ephippial egg appears more opaque than the non-ephippial egg.

The ephippial eggs develop at a slower rate than the non-ephippial so that they have only reached the stage of gastrulation at the time when they are shed by the mother at the end of the instar, whereas in the same time the non-ephippial eggs are expelled as first instar animals. In the majority of cases, further development does not take place until after the egg has passed through a period of diapause. The diapause lasts for a variable length of time and the factors causing a renewal of development, or breaking of the diapause, are not known with certainty. Sometimes the eggs will continue to develop without

entering a diapause, and Banta and his co-workers (1939) found that the best method by which to hatch young animals from the ephippia of Daphnia longispina was to keep the ephippia in dishes of fresh culture medium, without either freezing or drying. Similar conditions were employed by W.R. Green (1919) in the only one of his experiments on Simocephalus vetulus in which young hatched from the ephippia. The percentage hatch is never great under laboratory conditions, 8% to 10% being good.

A number of experiments were carried out in an endeavour to increase the percentage hatch of ephippial eggs. The experiments were based on the work of Hall (1953) with the eggs of Chirocephalus diaphanus. The ephippia were obtained from females of Daphnia which had been collected from sludge settling tanks at Hampton Water Works. The ephippia were removed from the dishes within a few hours of being thrown off by the mothers. Groups of fifty ephippia were then subjected to different conditions, but all were kept in a room maintained at 18°C. with constant illumination.

1. The first group of ephippia was neither dried nor frozen but transferred to covered 150 cc. dishes, half of which contained run tap water and the other half distilled water.
2. The second group of ephippia was left for three days in the water into which the ephippia had been thrown off. As much as possible of the water was then drained off and the ephippia left to dry completely. The ephippia were left dry for seven days and then wet again, half the dishes with run tap water and the other half with distilled water.

3. The third group of ephippia was given similar treatment to the second group except that the ephippia were left in the original water for six, instead of three, days before drying.

4. The fourth group of ephippia was left in the original water for six days and then dried. The ephippia were allowed to remain dry, some for 35, others for 49 days. The ephippia were then wet either with run tap water or with distilled water.

5. The fifth group was left in the original water for six days and then dried. After seven days, ten ephippia were removed from the dish and placed in run tap water. Another ten ephippia were removed every seven days.

6. The sixth group of ephippia was left in the original water for twelve days and then treated in a similar way to the fifth group.

7. The seventh group of ephippia was treated in a similar way to the fifth and sixth groups except that during the time in which they were dry the ephippia were also placed in a refrigerator, maintained at about 5°C.

8. The last group of ephippia was left in the original water for eighteen days, then left dry for seven days, half of the ephippia being also placed in the refrigerator maintained at about 5°C. All the ephippia were then wet.

After six months kept at 18°C., all the dishes of ephippia were removed to a room maintained constantly at 28°C., with constant illumination. The ephippia were kept at this temperature for two months.

Throughout this time, totalling eight months or more, few of the ephippial eggs hatched. Hatchings occurred at random among the various groups, about three to each group of fifty ephippia. There was no significant difference in the number which hatched between the ephippia placed in run tap water and those placed in distilled water. However the ephippia kept in distilled water had a softer more "rubbery" texture than those kept in run tap water and were easier to open. About 80% of the young that did hatch did so after the dishes of ephippia had been transferred to 28°C. This is the only significant result and the total number to hatch was only about 5%. This is a very small percentage but the fact that of this 5% the majority hatched after transference to a higher temperature seems probably due to this change in temperature rather than to elapse of time, since the ephippia had already been six months at 18°C.

The highest percentage hatch which I obtained in the laboratory was a hatch of 14 young from 30 ephippia, or 47%. These ephippia had been dried, placed in the refrigerator maintained at about 5°C. and then wet with run tap water and kept at room temperature during July, about 20°C. to 22°C.

When the diapause has been broken, the further development of the embryo until it hatches from the ephippium takes two to three days. In almost all respects the development of the ephippial egg is similar to that of the parthenogenetic or non-ephippial egg.

The development of the ephippial egg begins with the fertilisation division at the periphery of the egg. There are

two maturation divisions, the first cleavage divisions restoring the diploid number of chromosomes (Mortimer, 1936). The egg nucleus migrates to the centre of the egg, as it does in the non-ephippial egg. Three or four nuclear divisions take place in the centre of the egg, resulting in a number of blastomeres. These blastomeres migrate to the periphery of the egg where there is a thin cytoplasmic layer. They pass through the yolk along thin cytoplasmic strands. At the periphery of the egg the blastomeres form a blastodermic layer of cytoplasm (Fig.112, pc) with nuclei (nu) at intervals but without cell membranes. The remainder of the egg consists of a mass of small yolk globules (y) almost equal in size. There are no oil droplets.

Before the beginning of gastrulation, and while the peripheral blastoderm is still without cell membranes, the first yolk cells appear (Fig.115, yc). The cells have a very thin outer cytoplasmic layer, thickened where it contains the nucleus (nu). The nucleus is small and usually hyaline with a small nucleolus. The remainder of the yolk cell consists of closely packed yolk globules (y) forming a clump. The first yolk cells are visible about eighteen hours after the egg has been laid into the modified brood pouch. They arise from the peripheral blastoderm and not from blastomeres left in the central part of the egg when the other blastomeres migrated to the periphery. Unlike the first yolk cells in the parthenogenetic egg, the yolk cells of the ephippial egg can be readily distinguished and are markedly separated from each other. Each yolk cell contains in cross section about twenty yolk globules, whose similarity in

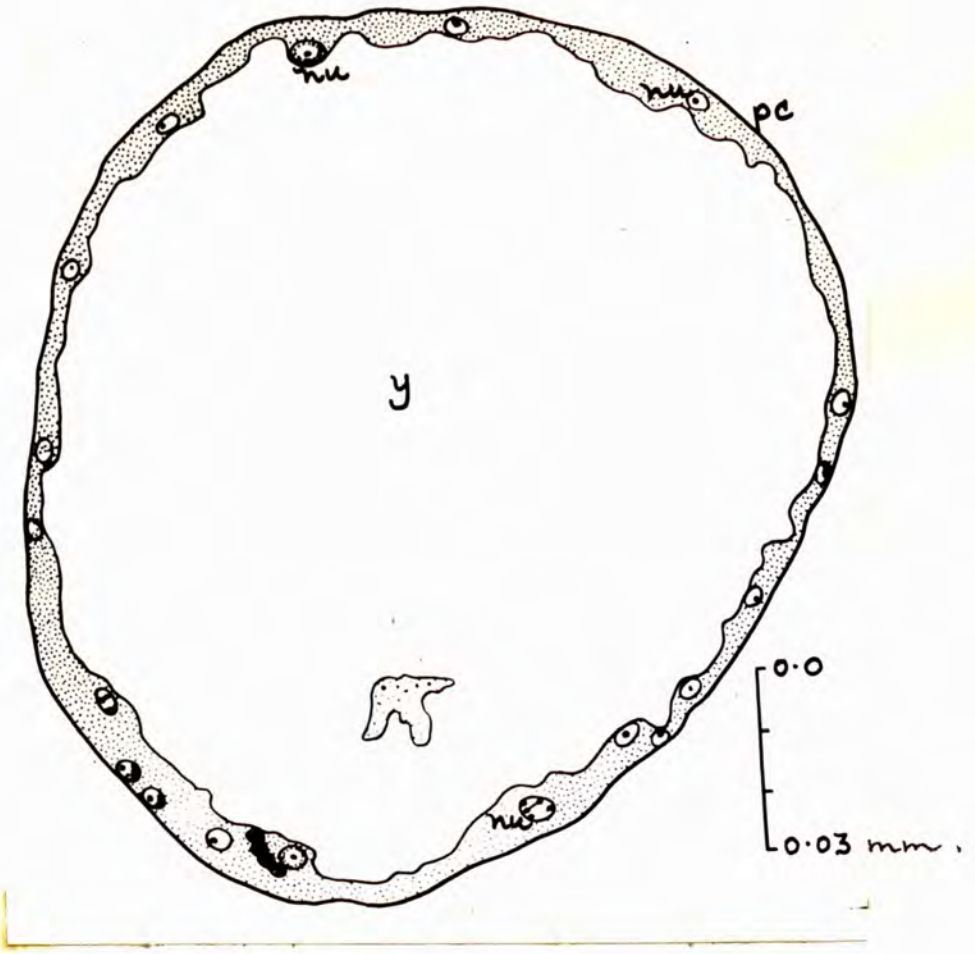


Figure 112. Transverse section through an ephippial egg of Daphnia magna a few hours after it has been laid into the brood pouch of the mother showing the thin outer blastoderm (pc) enclosing the central yolk (y) and without cell membranes. nu, nucleus.

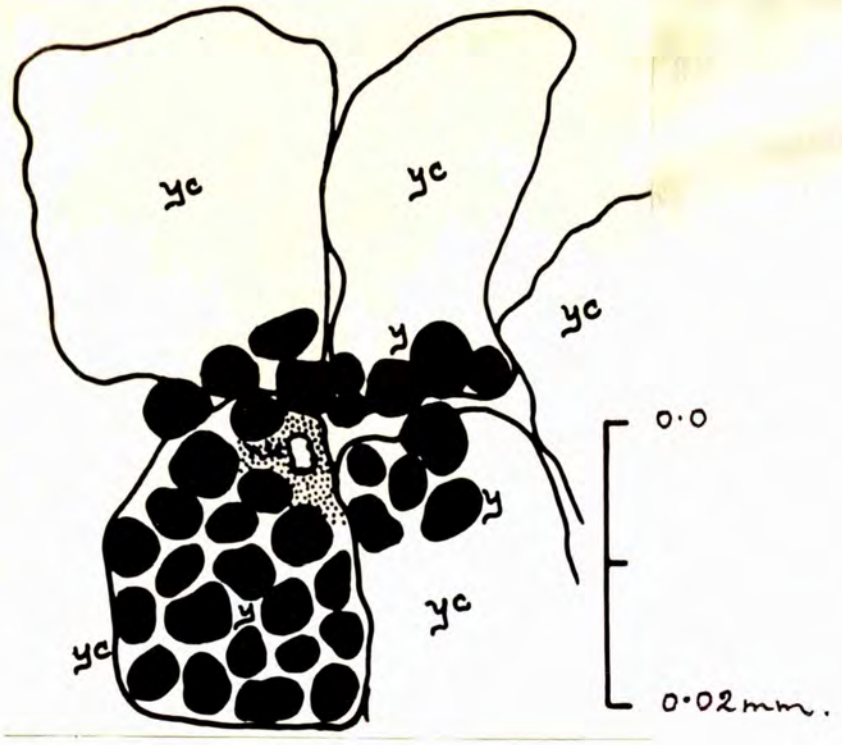


Figure 113. Section through an ephippial egg about eighteen hours after it has been laid into the brood pouch of the mother, at a stage shortly before gastrulation, showing well developed yolk cells (yc) with clearly defined nucleus (nu) and numerous small yolk globules (y).

size appears to aid the distinctiveness of the yolk cells. The nuclei are more easily recognisable than those of the yolk cells of the non-ephippial eggs. The yolk cells move to the centre of the egg at an early stage (Fig.114).

Shortly after the formation of the first yolk cells, gastrulation begins. Gastrulation takes place by immigration, as in the non-ephippial eggs, and results in an inner mass of irregular cells (Fig.117). There is a small invagination in this area. There is no distinction between the individual cells of the inner cell mass or mesendoderm (icm). The cells are irregular in outline although the outer edge of the mass as a whole is smooth. The cytoplasm of the cells resembles that of the peripheral layer and shows little affinity for stains. The nuclei (nu) are small with a central nucleolus and peripheral chromatin granules. The cells do not contain either vacuoles or yolk. Cell membranes have now appeared in the peripheral cytoplasm, but the inner membranes, parallel to the surface of the egg are difficult to distinguish. The inner part of each cell is filled with closely packed yolk globules (Fig.118, y).

The ephippial eggs, enclosed within the modified brood pouch, are thrown off by the mother at this stage of development (Fig. 119; Plate 13). Each of the two eggs in the ephippium is surrounded by an egg membrane. Within the membrane is a thin peripheral layer of small cells (ec) whose inner ends are difficult to define since they are filled with yolk globules and lie adjacent to the central mass of yolk globules. Internal to the peripheral layer of cells is an area of yolk globules and

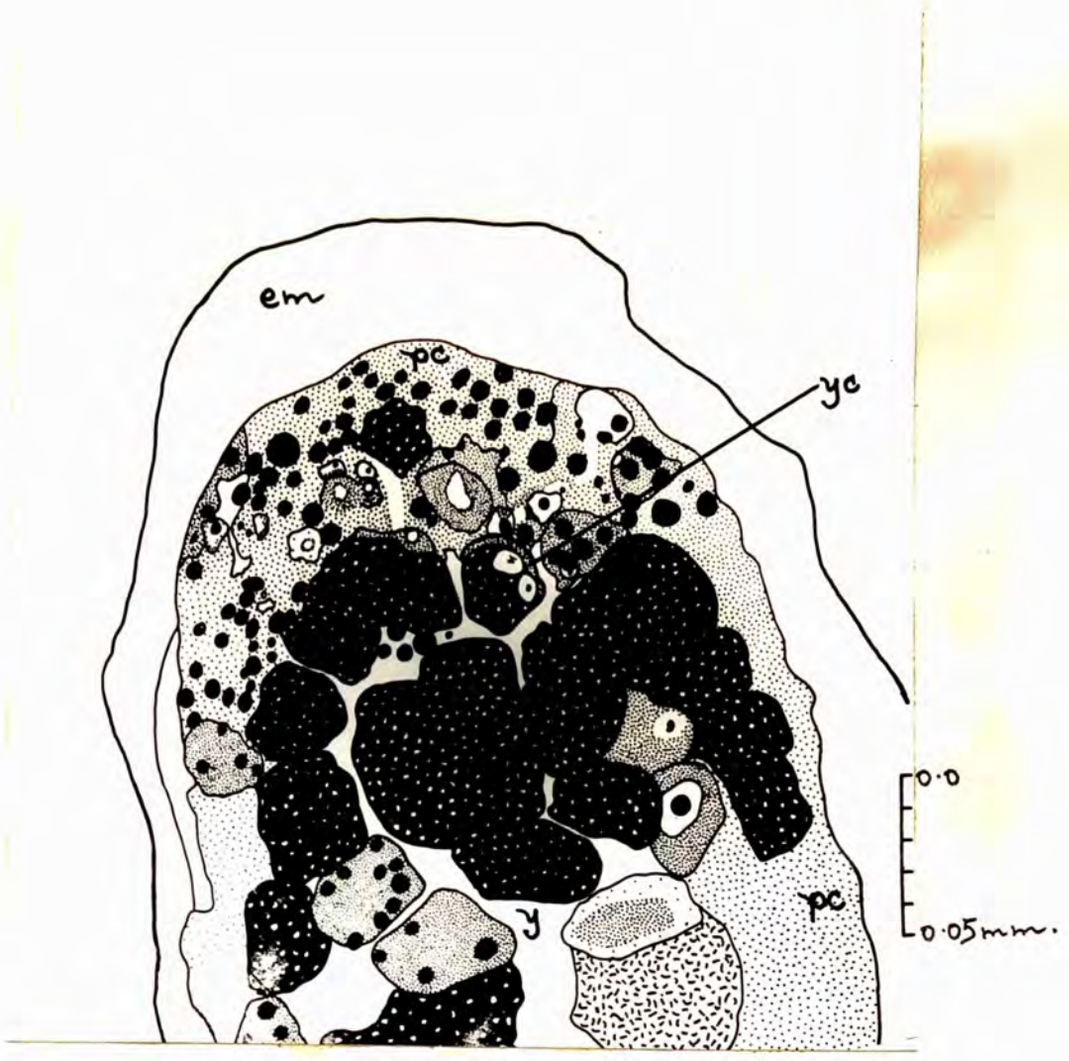


Figure 114. Section through an ephippial egg at the stage when the ephippium is shed by the mother showing the peripheral cytoplasmic layer (pc) surrounding an inner layer of yolk cells (yc) and a central mass of yolk globules (y). em, egg membrane.

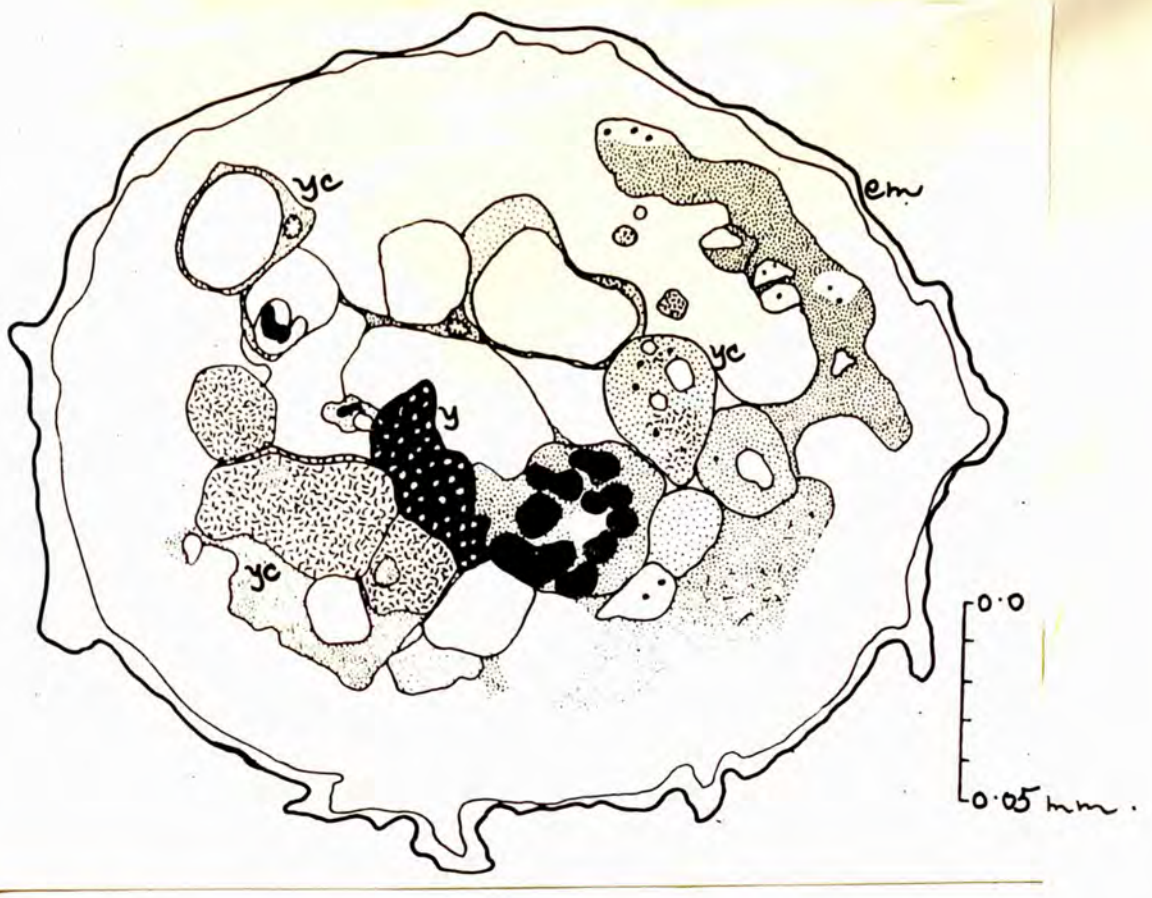


Figure 115. Transverse section through an ephippial egg at the same stage as Fig. 114 showing yolk cells (yc) and unaltered yolk (y) with yolk in various stages of breakdown. em, egg membrane.

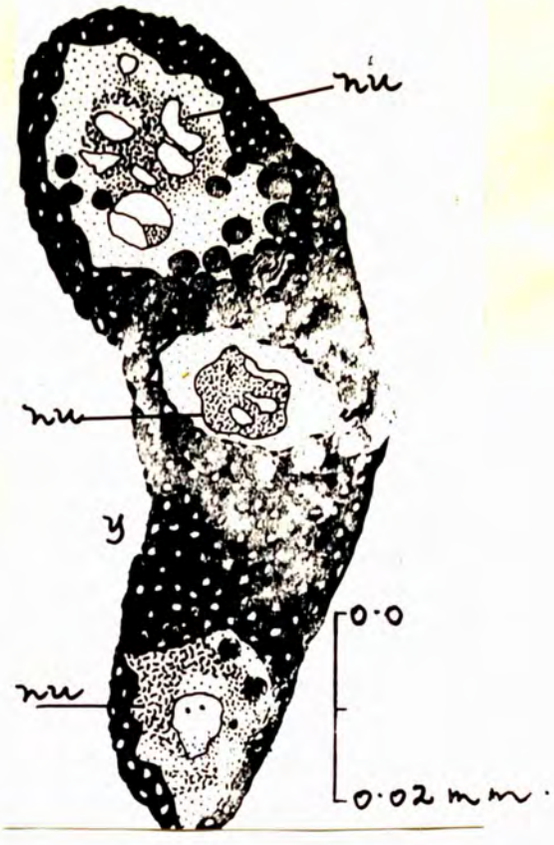


Figure 116. Section through an ephippial egg at the same stage as Fig. 114 showing nuclei (nu) in cytoplasmic areas among the yolk (y).

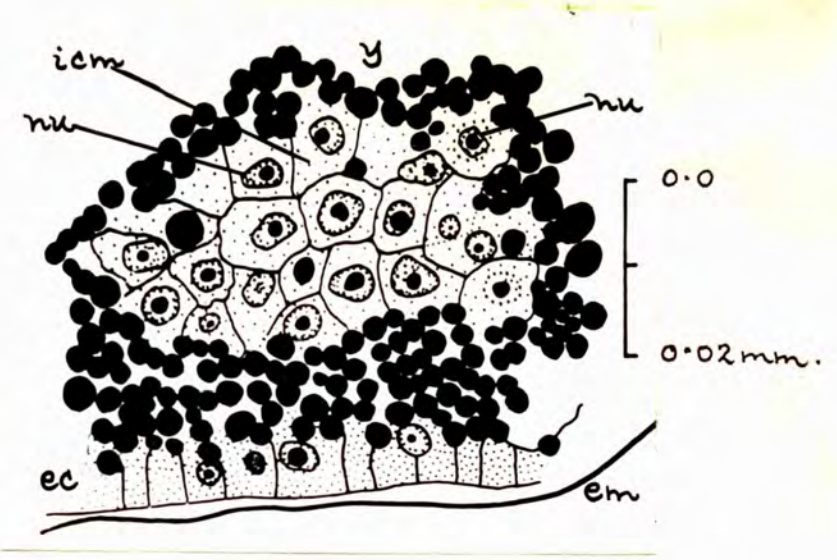


Figure 117. Section through an ephippial egg at the stage when the ephippium is shed by the mother showing the inner group of cells (icm) formed by gastrulation. The nuclei (nu) of these cells have a central spherical nucleolus and peripheral chromatin granules. ec, outer cell layer; em, egg membrane; y, yolk.

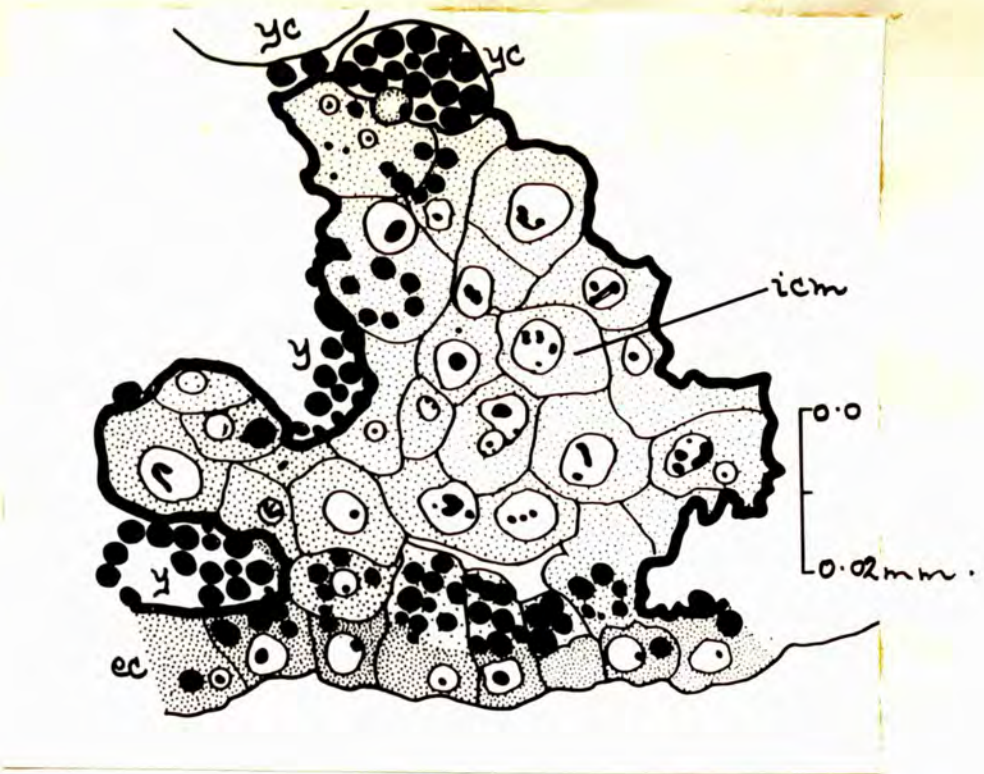


Figure 118. Section through an ephippial egg that has been shed by the mother and has entered diapause. The inner group (icm) is composed of polygonal cells with large nuclei usually containing several chromatin granules. ec, outer cell layer; yc, yolk cell; y, yolk.

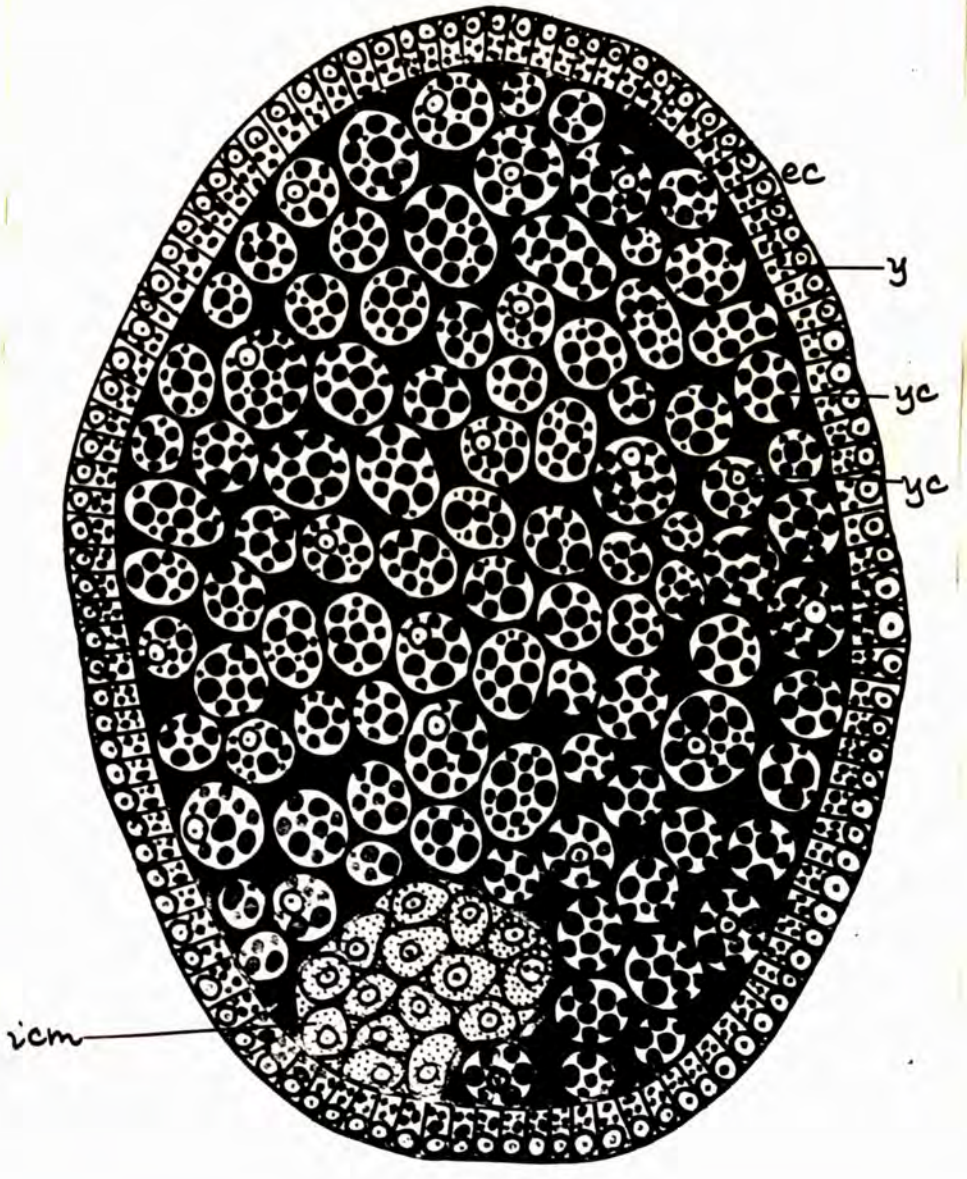


Figure 119. Diagrammatic reconstruction of the ephippial egg of Daphnia magna that has been shed by the mother showing the outer cell layer (ec), with yolk globules (y) in the inner parts of the cells, surrounding a central mass of yolk cells (yc) with an inner group of cells without yolk towards one end of the egg (icm).

Plate 13. Photomicrograph of a longitudinal section through an ephippial egg at the same stage as Fig.119 showing the inner group of cells(icm) and the yolk cells(yc). ec,outer layer of cells.

cytoplasm not divided into cells (Fig.115; Fig.116) as well as yolk cells. In one place a group of irregular cells extends from the peripheral layer through this area of unaltered yolk and cytoplasm towards the centre of the egg (Fig.119, icm). The group of cells is situated close to one end of the egg. The cells contain no yolk and form the mesendoderm. The centre of the egg contains a number of yolk cells (Fig.120, yc) together with some unaltered yolk. The yolk cells are not best developed in the neighbourhood of the mesendoderm but appear to be most numerous close to the peripheral layer. The majority of eggs found in ephippia thrown off by the mother are at this stage of development, even when several weeks old.

The paired "Scheitel"plate develops early, when the inner cell mass is still not distinguishable into endoderm and mesoderm. The nuclei of the mesendoderm (Fig.121, icm) are now large and hyaline each with a large nucleolus and with peripheral chromatin granules. The cytoplasm of the cells is granular. The "Scheitel"-plate consists of two groups of enlarged peripheral cells placed antero-dorsally. The nuclei of these cells are larger than those of the other cells of the peripheral layer, especially their nucleoli.

The mesendoderm increases and spreads over the ventral surface of the yolk. The endodermal cells become distinguishable from the surrounding mesodermal cells and form a solid rod lying mid-ventrally with the mesoderm laterally and at either end. As in the non-ephippial egg, the mesenteron develops a narrow central cavity which becomes continuous and joins with the

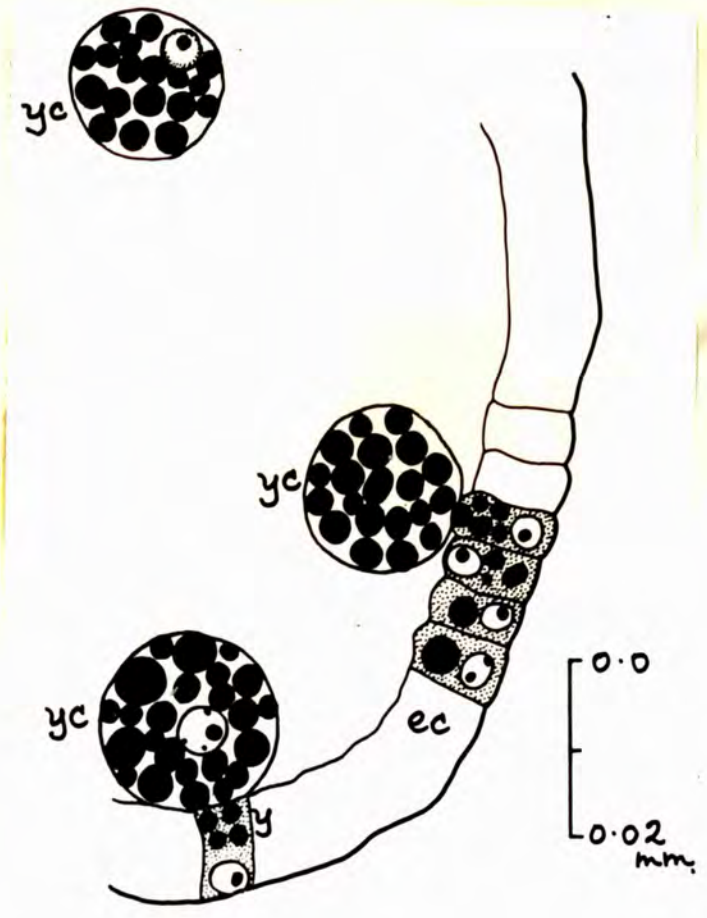


Figure 120. Section through an ephippial egg at the same stage as Fig. 119 showing the yolk cells (yc) and the outer layer of cells (ec) containing yolk globules(y).

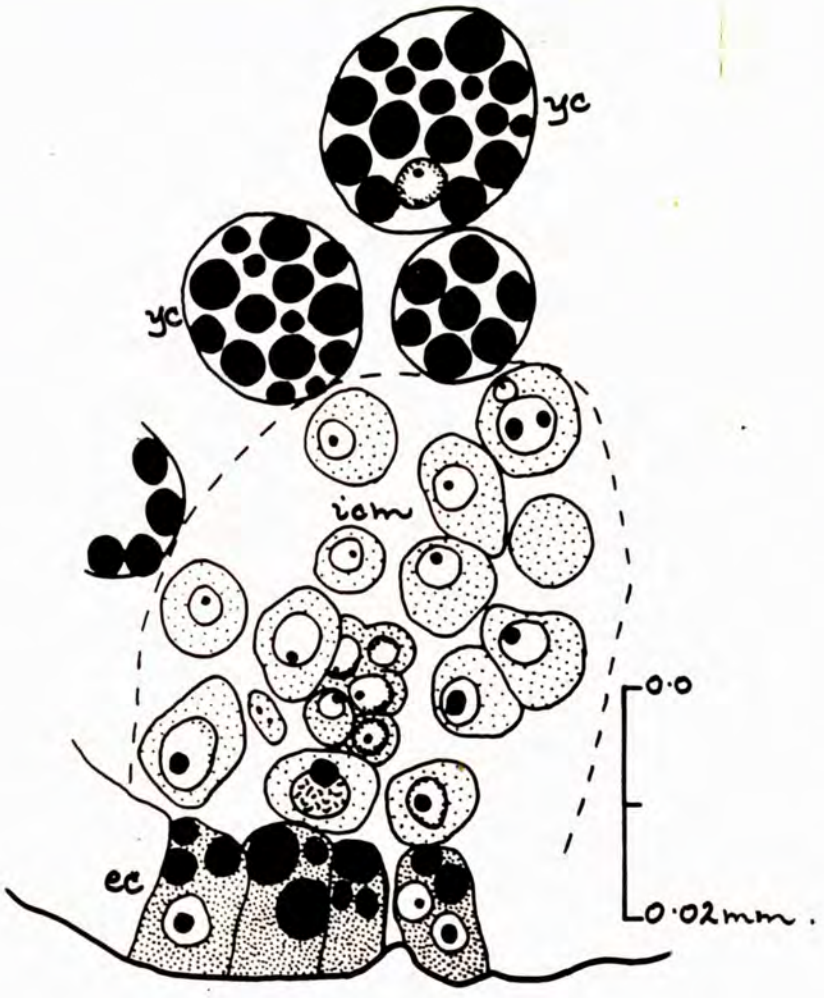


Figure 121. Section through an ephippial egg at the same stage as Fig. 119 showing the inner group of cells (icm). The cells are large and lightly staining and contain large nuclei. ec, outer layer of cells; yc, yolk cells.

cavities of the stomodaeum and proctodaeum.

At the stage when the cavity of the alimentary canal is still narrow but continuous, the heart and the maxillary gland are developing and individual muscles are distinguishable. A large dorsal organ is present and the yolk is still widespread.

The further development resembles that of the non-ephippial egg, the only difference to be noted between the embryos developing from the two kinds of eggs being the nature of the yolk. Even in the well developed embryo, the yolk in the ephippial egg can be distinguished from that of the parthenogenetic egg. In the former the globules are smaller and almost equal to each other in size. The difference in the yolk persists from the initial development of the egg until all the yolk has been incorporated into the yolk cells.

The young animal may hatch from the ephippial egg about three days after the probable breaking of the diapause, as indicated by the shedding of the ephippium by the parent or by the wetting of the dried ephippium. In other cases weeks or months may pass before the young animal hatches. In the few experiments carried out (p.102), most of the hatchings that occurred did so within four days of the transference of the ephippia from 18°C. to 28°C.

The development of the ephippial egg is essentially the same as that of the non-ephippial egg, and any differences noted appear to be due to the state of the yolk. The boundaries of the peripheral or blastodermal cells form at a slightly later

stage than in non-ephippial eggs and when the cells are formed they contain a greater amount of yolk. The yolk cells form in a similar manner in the two kinds of eggs, but the difference in the nature of the yolk globules causes the yolk cells of the ephippial eggs to be more distinct. Their nuclei are easier to recognise and each cell contains a clump of numerous yolk granules instead of a few larger granules.

The development after expulsion from the brood pouch of the mother.

When the young first instar animal is expelled from the brood pouch of its mother, it resembles the adult animal except in various features of its alimentary canal, reproductive system, fat cells, sense organs and external features, especially the dorsal organ. The heart beats at a slower rate than in the adult animal, but its beat is now regular and the heart is fully developed structurally. The animal swims and feeds actively. It is smaller than the adult and more transparent (Plate 14), the latter being principally due to the rudimentary stage of the ovaries.

Since Daphnia magna may become mature in either the fifth or the sixth instar (Anderson, 1932), not all the animals in the same instar will be in exactly the same stage of development. By the time that the ovaries, or testes, have become mature the rest of the animal is fully developed.

The alimentary canal.

In the animal newly released from the brood pouch of its mother (Fig.35), the alimentary canal is open throughout its length and differs only in a few minor points from that in the adult animal. The anterior wall of the stomodaeum is distinctly thicker than the posterior wall, the difference between the two being greater than that in the adult and due principally to the thickness of the muscle layers, especially the pair of longitudinal muscles. The midgut caeca (mc) are proportionally smaller than in the adult due to the narrowness of the lumen,

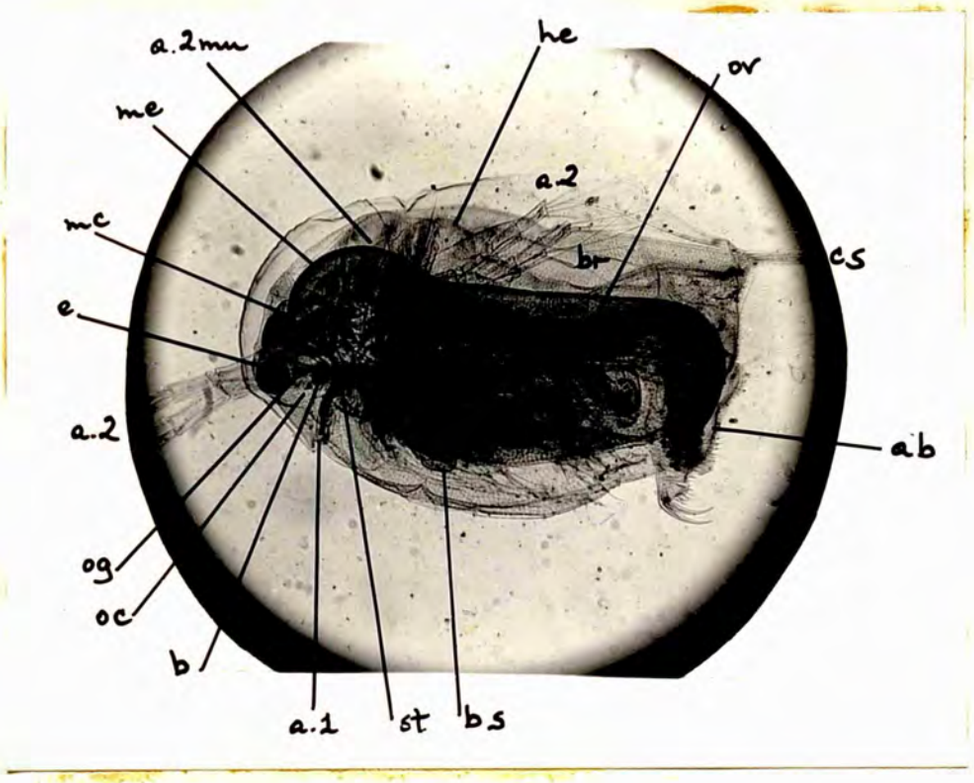


Plate 14. Photomicrograph of a whole mount of an early instar female Daphnia magna showing the general resemblance to the adult except that the ovary(ov) is less well developed. a.1,antennule; a.2,second antenna; a.2mu,muscle of second antenna; ab,abdomen; b,brain; br,brood pouch; bs,branchial sac; cs,caudal spine; e,compound eye; he,heart; mc,caecum of mesenteron; me,mesenteron; oc,ocellus; og,optic ganglion; st,stomodaeum.

the wall being equal in thickness to that of the remainder of the mesenteron. The wall of the mesenteron is thickened in the region opposite to the dorsal organ.

The second instar animal, that is after the first moult after release from the brood pouch, has the anterior wall of the stomodaeum less distinctly thicker and no sign of thickening of the mesenteron wall opposite to the dorsal organ. The midgut caeca are usually slightly larger, but may be almost as narrow as in the first instar animal.

In the third instar animal, the midgut caeca may be still small but generally they are similar in size to those in the adult animal. The remainder of the alimentary canal resembles that of the adult.

In all fourth instar animals the midgut caeca are similar in size to those of the adult (Fig.37, mc), and the alimentary canal is the same as in the adult animal.

The reproductive system.

In the first instar female, the ovaries are small and narrow. They lie on either side of the mesenteron, turning dorsally at their posterior ends. Each ovary is narrow anteriorly, broadening for the greater part of its length, and then narrowing again slightly at the posterior end. The posterior limit is difficult to distinguish.

In the second instar, the ovaries are of more even width with their anterior ends in a slightly more ventral position.

The ovaries of the third instar female are enlarged and contain distinct four-cell groups.

By the fourth instar, the ovaries are much larger but are not yet ready to lay eggs. Eggs ready to be laid are present in the ovaries of the fifth, or sometimes not until the sixth, instar.

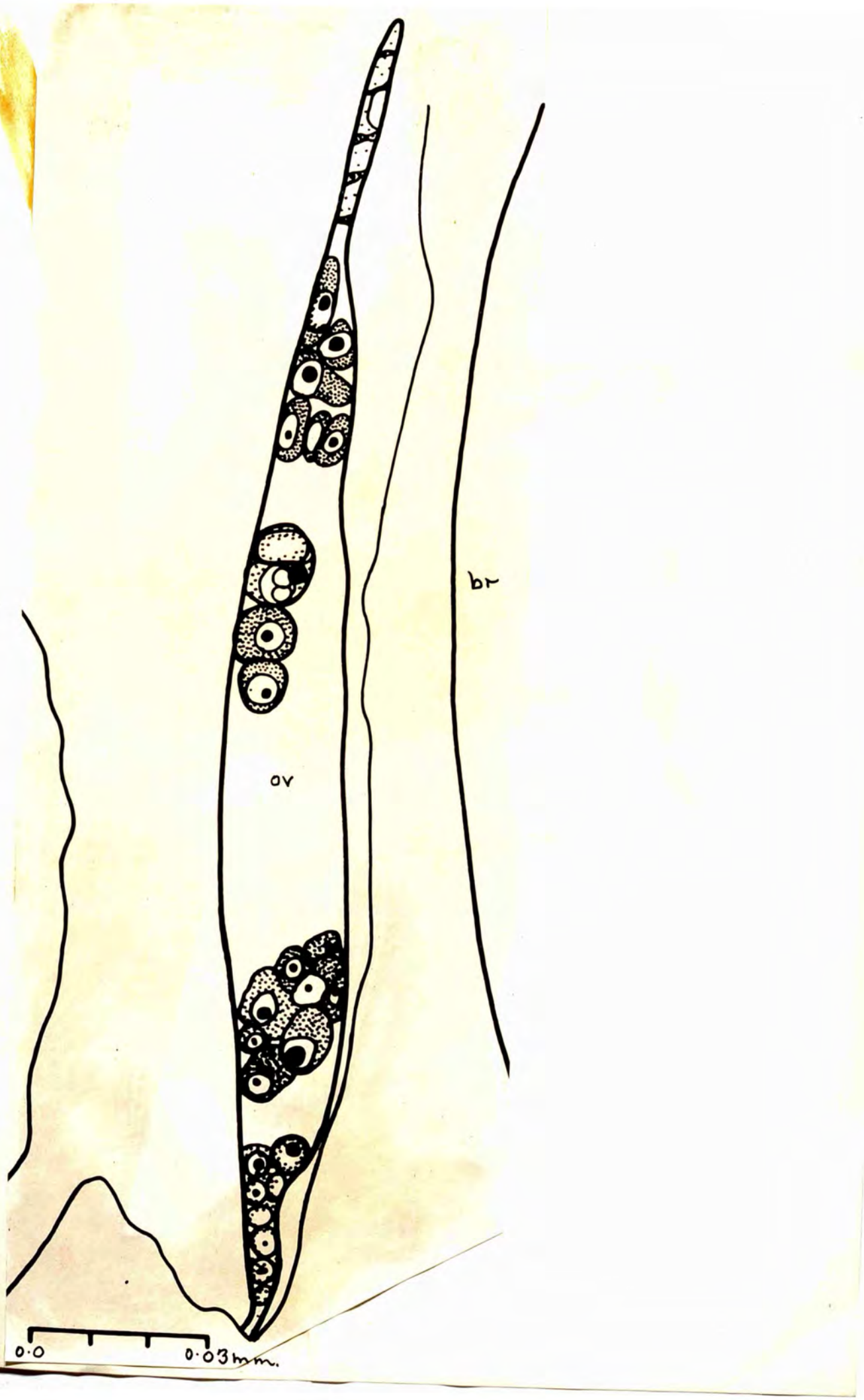
In the first instar female, the ovaries are long slender delicate strands lying slightly ventral to the mesenteron and extending from the level of the heart posteriorly to the posterior flexure of the mesenteron. Their length is thus similar to the length of the adult ovaries, but the cellular part of the ovary extends forwards only to approximately the level of the second thoracic appendage. The anterior third of the ovary consists of a very narrow strand of vesicular tissue. This anterior vesicular region consists of thin-walled vacuoles with narrow cytoplasmic areas containing a number of darkly staining granules. The posterior part of the ovary is wider than the vesicular part and consists of irregularly arranged cells with darkly staining cytoplasm and hyaline nuclei each containing a large central spherical nucleolus. A number of small granules occur round the periphery of the nucleus. There are about two cells to the width of the ovary. In the more posterior part of the ovary the cytoplasm of the cells stains less intensely and contains a greater number of vacuoles with fewer granules. Further towards the posterior end of the ovary the boundaries between the cells become irregular and less distinct and the amount of cytoplasm is greater in comparison with the nuclei. The cells are smaller further towards the posterior end of the ovary. At its posterior end the ovary becomes

narrow and grades into a narrow extension which appears to become the oviduct, or receptaculum seminis, a short tube with walls one cell thick and a narrow lumen. In the first instar animal there is a sheet of fat cells directly adjacent to each ovary, so close that it is often difficult to distinguish the boundary between them. Occasionally the cells of the ovary show an indication of an arrangement into groups of four, and there is also sometimes a beginning of a deposition of yolk.

The ovaries of the second instar female (Fig.122, ov) are slightly wider than those of the first instar animal, the widening being in a dorsal direction. The anterior vesicular area of the ovary is still present, with well-defined nuclei and cytoplasm between the vesicles. Further towards the posterior end of the ovary, the cells are irregular and stain moderately intensely, the nuclei being characteristic of the genital cells, hyaline with large central spherical nucleolus. These are bounded laterally by vesicular tissue which has smaller vesicles and a greater amount of cytoplasm than the most anterior vesicular area. The fat cells are directly adjacent to the anterior end of the more cellular part of the ovary. The cells are beginning to form into four-cell groups, but the cells are still small. The oviduct, or receptaculum seminis, is slightly longer and narrower than in the first instar animal and the lumen is open.

In the third instar (Plate 15), most of each ovary (ov) lies at the same level as that of the mesenteron (me), the posterior part being slightly more ventral. The ovaries are wider than in the previous instar and their cells larger. In the anterior

Figure 122. Vertical longitudinal section through an early instar Daphnia magna showing the ovary (ov) consisting of an anterior vesicular area, a more posterior area with cells with granular cytoplasm and beginning to form into groups of four, an area of irregularly arranged less granular cells and a narrow posterior end of small cells. br, brood pouch. Freehand drawing.


br
ov
0·0
0·03mm.

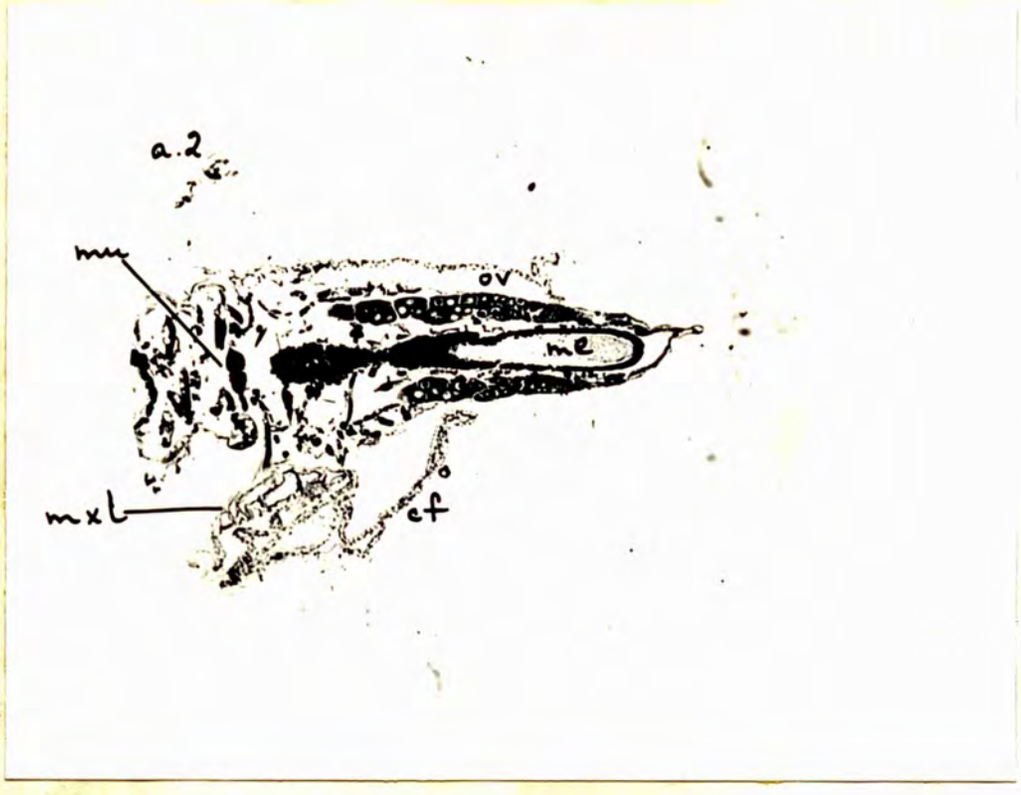


Plate 15. Photomicrograph of a horizontal longitudinal section through a third instar female Daphnia magna showing the paired ovaries(ov) next to the mesenteron(me). The anterior part of each ovary contains groups of four-cells; more posteriorly the cells are smaller and are not arranged in groups. a.2,second antenna; cf,carapace fold; mu,muscle; mxl,maxillary gland loop.

vesicular part of the ovary the vesicles are less distinctly defined and are larger. Posterior to the vesicular area the cells are arranged into groups of four, each of the four cells being of equal size. Close to these four-cell groups lie a number of fat cells. The increase in the size of the cells in this instar is due to an increase in the amount of cytoplasm in the cell. The lateral edge of each ovary is filled with vesicular tissue. Further posteriorly the cells are irregularly arranged and their cytoplasm stains less intensely and is proportionally smaller in area. A few of these cells contain fine darkly staining granules and possibly indicate the site of the formation of the ephippial eggs. Posteriorly the ovary narrows and grades into the long thin oviduct which appears at this stage to be open and to lie alongside a long narrow muscle which is attached to the integument of the animal very close to the oviduct.

In the fourth instar the ovaries lie directly adjacent to the mesenteron and resemble in shape those of the adult animal. The oviduct is recognisable as a narrow tube with walls one cell in thickness and a lumen opening immediately anterior to the second (or largest) dorsal process. There is an indication of fine muscular tissue close to each oviduct. At its anterior end each oviduct leads to the germinal part of the ovary consisting of small flattened cells with small nuclei. Anterior to the germinal part of the ovary the cells become larger until they become arranged into groups of four. The four-cell groups occur quite close to the posterior end of the ovary. The remainder, or more anterior, part of the ovary is filled with four-cell

groups and if the animal has not been supplied with abundant nourishment there is also a variable amount of vesicular tissue at the anterior end of the ovary. In well-fed animals the groups of four cells fill the ovary except for a very small anterior tip which always remains vesicular.

In the following instar (Plate 16), the shape of the ovaries (ov) resembles those of the adult and the ovaries are mature as in the adult, the eggs being laid either in this or in the following instar. The majority of the cells are in groups of four, usually with one cell larger than the remaining three and containing a number of oil droplets. All the cells contain yolk. The state of the four-cell groups depends on the stage reached in the formation of the egg and usually complete development of the eggs takes place during the instar. The four-cell groups fill the ovary with the exception of a small anterior vesicular area and a short posterior germinal region of small irregular cells (gec). The short oviduct is usually closed and is slightly broader than in earlier stages.

Since the groups of four cells may first develop into egg cells in either the fifth or the sixth instar, it is not possible to describe the exact state of the ovaries in each of the early instars. The development of the ovaries is a gradual process without reference to the occurrence of the instars.

Claus (1976) described the development of the ovaries under five stages which do not appear to correspond with the first five instars since the first description of the ovary agrees with that of a second instar animal. He apparently did not observe the

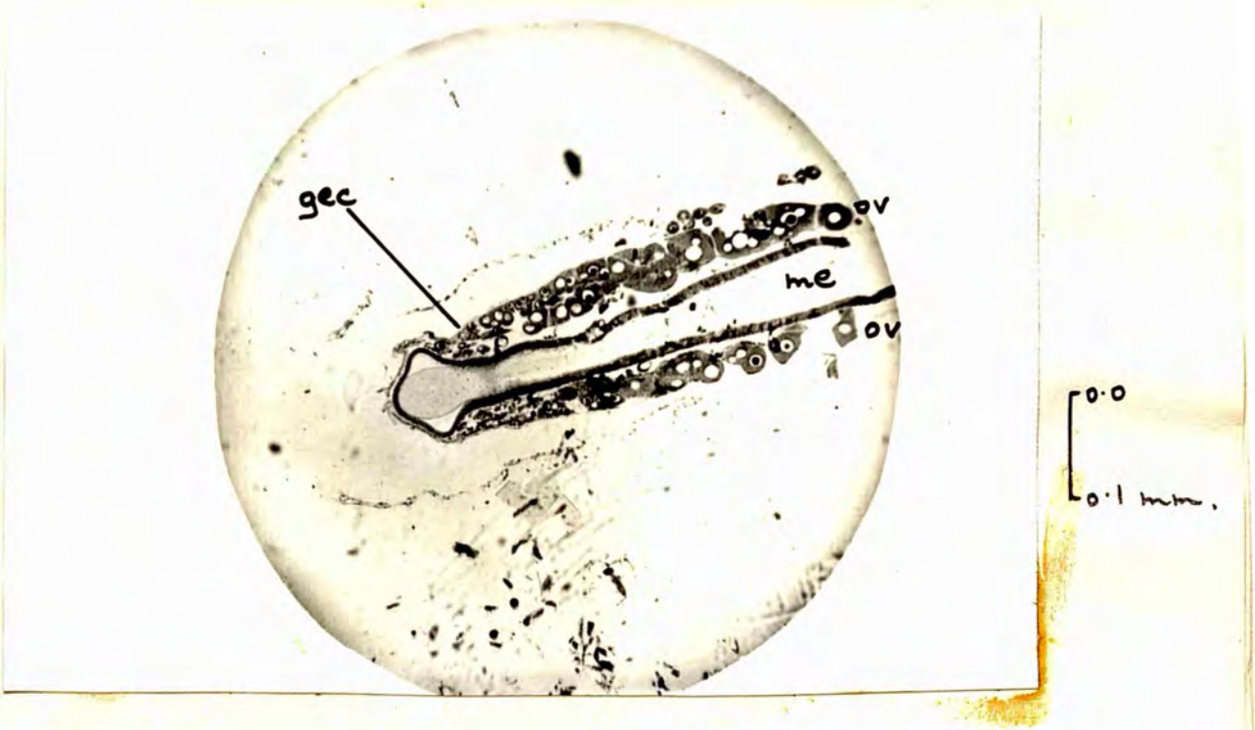


Plate 16. Photomicrograph of a horizontal longitudinal section through a fifth instar female Daphnia magna showing the paired ovaries(ov) next to the mesenteron(me). Most of the cells in the ovary are arranged into groups of four, but there is a short posterior germinal region of small irregularly arranged cells(gec).

anterior vesicular part of the ovary until the third stage. In Claus's final stage, 2 - 2½ mm. in length, the cellular part of the ovary reaches the heart region and has an increased amount of yolk and fat. The cells are in groups of four. The stage is probably that of the adolescent, or pre-mature, instar which is usually the fifth instar. It is difficult to relate the stages described by Claus to instars, especially since he says that the "Haftorgan", or dorsal organ, is still present in the third stage while it is known to disappear from view in living animals in the third instar. It therefore seems that Claus's stages do not correspond to instars but are arbitary stages of development taken probably from animals in the second to the fifth instars.

The fat cells.

The first instar animal (Fig.46) still contains a number of unaltered yolk globules, especially in the mandibular region. The fat cells are less numerous than in the fully developed animal and are generally smaller. The cells are most abundant in the inner angle of the posterior flexure of the mesenteron and at the base of the last thoracic appendage. The most anterior limit of the cells is found immediately posterior to the transverse mandibular muscles, a moderately large group of cells occurring against their posterior edge. There are few fat cells in the branchial sacs of the thoracic appendages.

All unaltered yolk globules have usually gone by the time that the animal enters the second instar. A greater number of fat cells is now present, although still less than are found in the adult. The cells are still small but are more widely

distributed and more abundant in the branchial sacs of the thoracic appendages.

By the time that the animal enters the third instar, the fat cells have become even more abundant, especially in the branchial sacs. The cells are larger, although still slightly smaller than in the adult. The cells are similar in histological structure to those of the adult animal.

The fourth instar animal has more and larger fat cells and by this or the following instar the fat cells resemble those of the adult animal both in distribution and structure.

The sense organs.

The ocellus, the lateral frontal organs, the compound eye and the bristles and setae of the young animal newly expelled from the brood pouch of its mother resemble those present in the adult animal. The only structures which show any differences are therefore the "frontal organs" scattered over the rostrum, the median frontal organs and the first antennae.

The "frontal organ" cells, ventral to the median frontal organs but probably related to them and scattered over the rostrum of the head, are smaller and arranged in a more irregular fashion in the first instar than in the adult animal. By the second instar, these cells resemble those of the adult both in size and distribution.

In the first two instars the pair of median frontal organ cells lie at right angles to the surface of the integument. During the third instar they are oblique to the surface, by the fourth instar being parallel to the surface of the integument as in the adult.

The antennules, or first antennae, of the first instar animal are situated very close to the proximal labral glands. They carry setae as in the adult and a well developed base of cells. The outer cells are large and hyaline and each contains a small dark granule. They surround the nerve ganglion. The distal tube-like cells, through which the nerve fibres run from seta to ganglion, are well developed. In the second instar animal the vacuolated cells are more abundant and less swollen, but the ganglion and the distal cells are still rather more distinct than in the adult. Two nerves can be seen going to each antennule. In the third instar animal the antennules project slightly further from the rostrum than in the second instar animal. Their structure is much as in the adult animal although the separate areas are more distinct - there is a proximal densely staining ganglionic area, a hyaline vesicular area and an area of tube-like cells leading to the "collars" at the bases of the setae. By the fourth instar the antennules are similar to those of the adult.

The external features.

Changes in the external features during the development through the early instars have been noted by several authors, of which the principal are Anderson (1930; 1932) and Scourfield (1943). Lundberg (1895) wrote a paper on Daphnia pulex. A paper by Schulze-Robbecke (1951) deals with signs of ageing in the senescent animal. The animal grows in size from instar to instar.

In the first instar animal, there is a marked tail notch,

or concave area of the carapace immediately ventral to the caudal spine (Fig.35). The brood pouch is not yet developed and the dorsal process which in the adult closes off the posterior end of the brood pouch is only feebly developed (Fig.125), being a short stump with a cluster of cells at its base giving off fine threads into the process. The abdomen has an even contour from the base of the terminal claws dorsally (or anteriorly), that is, it lacks the indentation found in this region in the adult. There are a few spines in this region. The serration of the abdominal claws is not as marked as in the adult. The dorsal organ is discussed in a separate section (p. 91).

When the animal moults to enter the second instar, the tail notch is not as well marked as in the first instar animal. The brood pouch is beginning to develop and the second dorsal process has begun to grow (Fig.124). The latter is still small, blunt and wrinkled and contains in its base a number of small round cells with small darkly staining centres. The abdomen shows the beginning of an indentation and the serrated area of the terminal claws has increased.

In the third instar animal, the tail notch is no longer present. The brood pouch has developed further and is about half the height of that in the adult (Fig.125, br). The dorsal process is larger. There are now four processes anterior to the caudal bristle, an additional process having developed anterior to the largest one. This largest, or second, dorsal process has a thickened wall and no longer has a group of cells

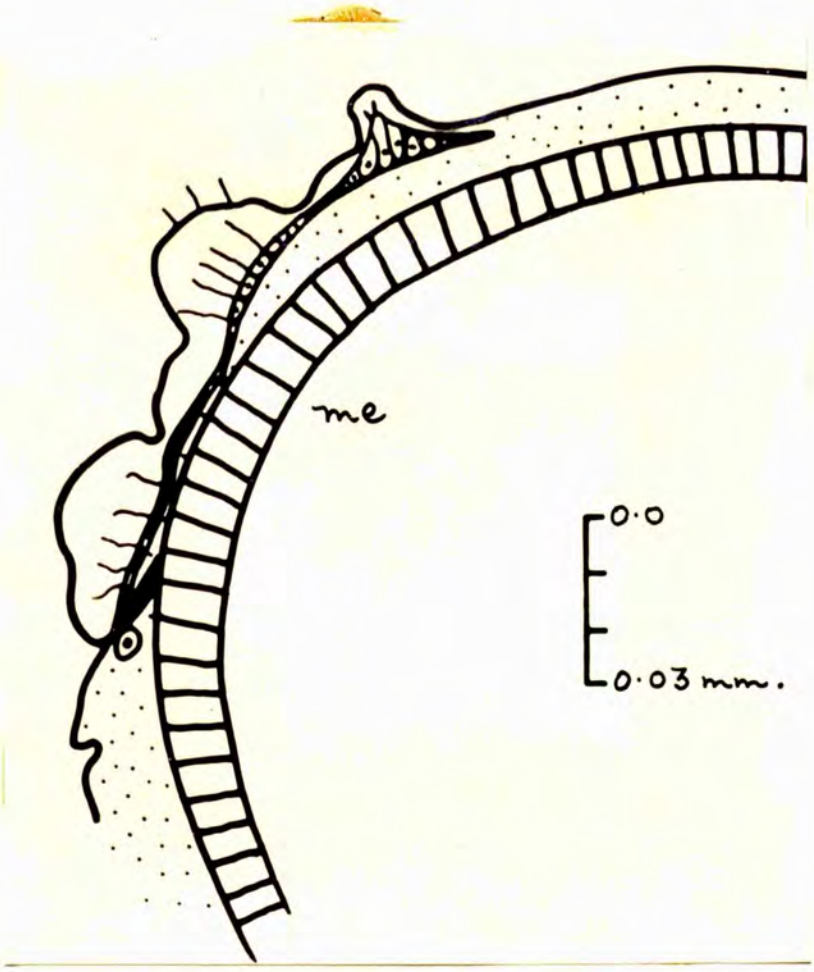


Figure 123. Vertical longitudinal section through the posterior dorsal region of a first instar Daphnia magna showing the rudimentary dorsal process at the posterior end of the brood pouch. me, mesenteron. Freehand drawing.

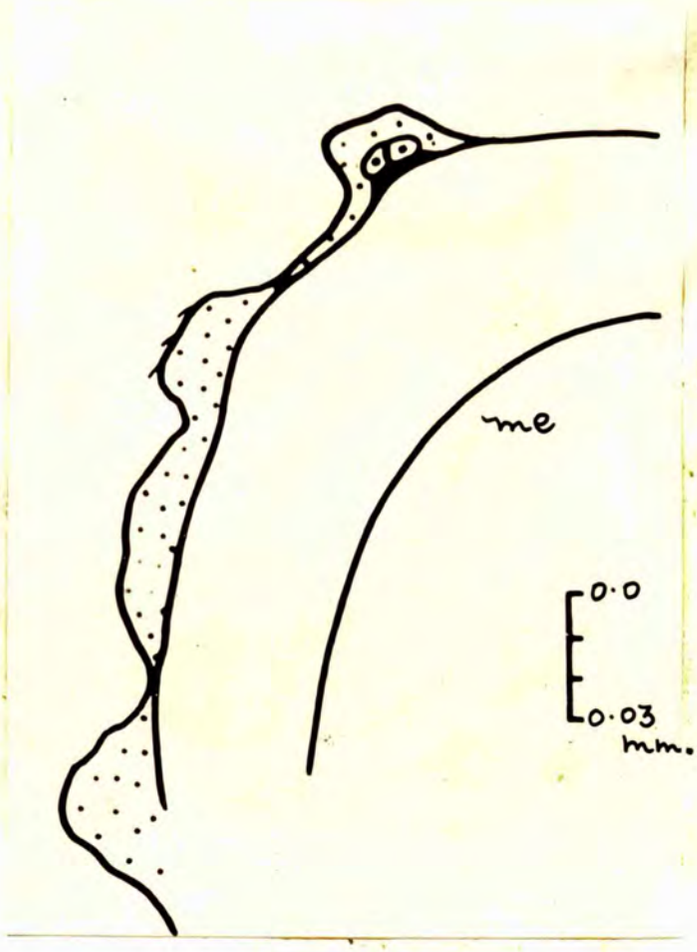


Figure 124. Vertical longitudinal section through the posterior dorsal region of a second instar Daphnia magna showing the dorsal process at the posterior end of the brood pouch still small. me, mesenteron. Freehand drawing.

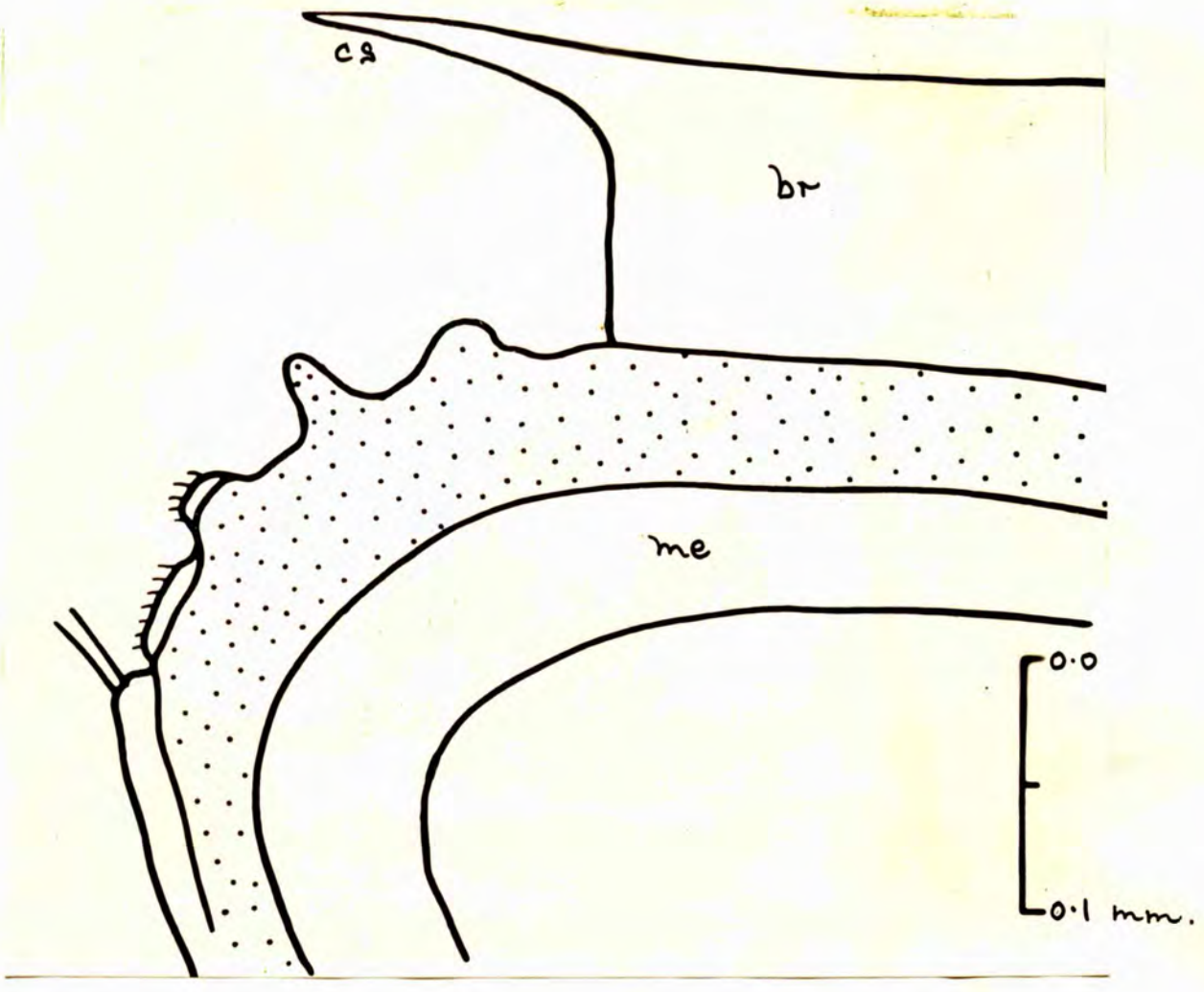


Figure 125. Vertical longitudinal section through the posterior dorsal region of a third instar Daphnia magna showing the dorsal process at the posterior end of the brood pouch (br). cs, caudal spine; me, mesenteron. Freehand drawing.

at its base. The abdomen has a more marked indentation, but the serration of the terminal claws is not yet fully developed.

By the fourth instar, the brood pouch (Fig.126, br) has grown deeper and the second dorsal process is long and wrinkled with a blunt but narrowing tip. The first dorsal process is short. There has been no change in the abdomen.

By the fifth instar the brood pouch (Fig.127, br) is usually fully formed although not as deep as in the adult animal. The first dorsal process has grown considerably into a long moderately narrow, almost pointed process formed of chitin only. There has been no change in the second dorsal process. Neither of these dorsal processes is equal in height to that of the adult. The abdomen resembles that of the adult animal.

The size of the brood pouch and of the dorsal processes in any particular instar will, of course, depend upon in which instar the animal becomes mature.

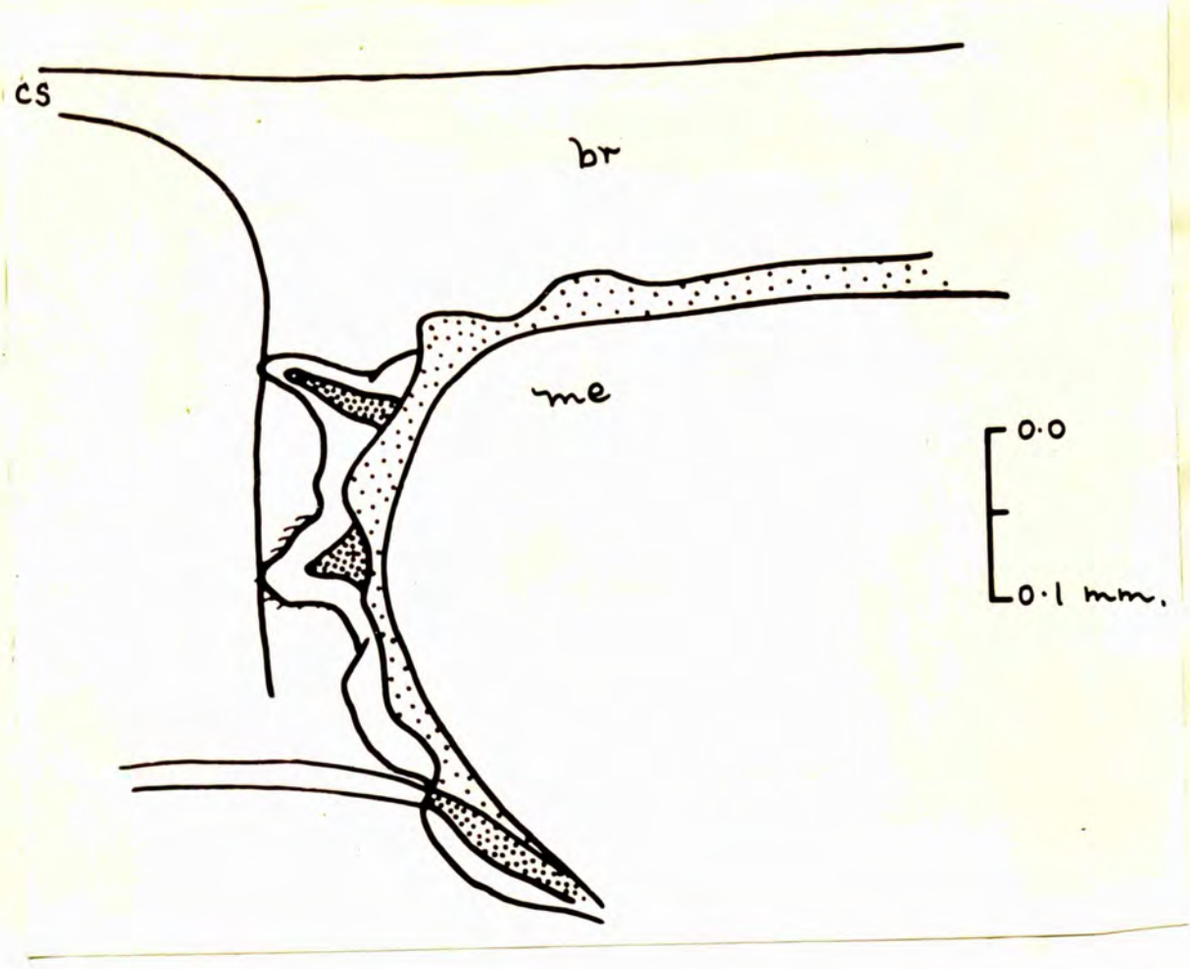


Figure 126. Vertical longitudinal section through the posterior dorsal region of a fourth instar Daphnia magna showing the dorsal processes at the posterior end of the brood pouch (br). The brood pouch has increased in depth since the third instar. cs, caudal spine; me, mesenteron. Freehand drawing.

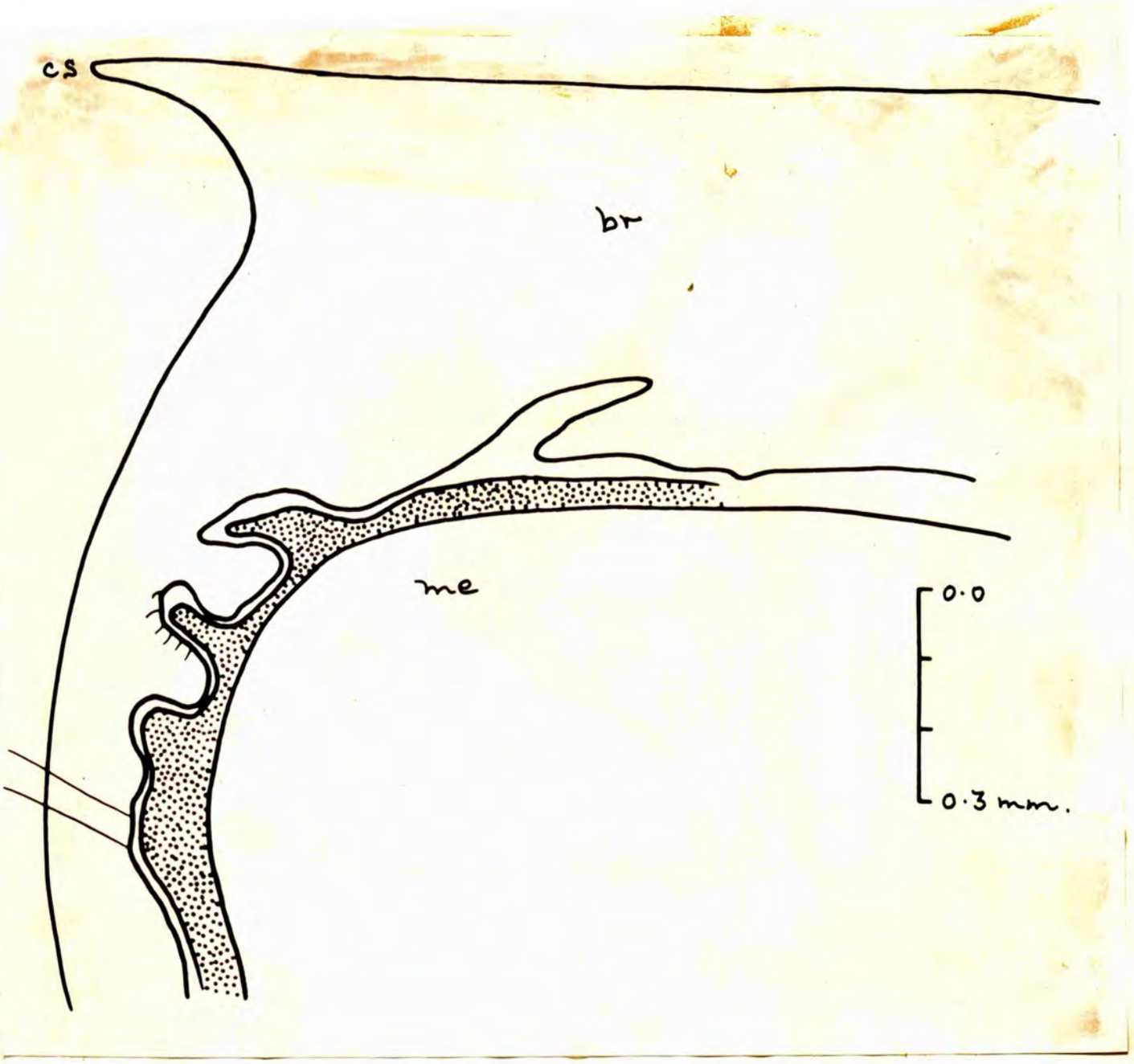


Figure 127. Vertical longitudinal section through the posterior dorsal region of a fifth instar Daphnia magna showing the fully developed dorsal processes at the posterior end of the brood pouch (br). cs, caudal spine; me, mesenteron. Freehand drawing.

The anatomy of the adult.

Introduction.

In the course of investigations of the embryological development of Daphnia magna it was found that no comprehensive up to date account of the anatomy of the adult was available. The increase in the use of Daphnia for physiological, experimental and other studies suggested that a concise account of the anatomy in greater detail than that possible in the average textbook would be useful. The standard work is that of Claus (1876), followed by a more general account by Storch (1925). Various parts of the anatomy are described by Klotzsche (1913). The works of Binder (1932) and Jäger (1935) give detailed accounts of the musculature and fat body respectively, while other accounts of individual systems are those of Cannon (1922) and Storch (1924). Useful general texts are those of Wagler (1926-7) and a short account by Lochhead (1950). An early work which has good drawings is that of Schäffer (1755).

A study of both live and fixed animals, and a survey of previous work, has led to an extension and revision of previous descriptions, especially in regard to histology, enabling the presentation of a more comprehensive revised account of the anatomy of the adult Daphnia magna.

a. The Alimentary Canal.

The alimentary canal is a simple tube with a pair of small caeca near the anterior end (Plate 17). It turns ventralwards at either end to open by means of stomodaeum (st) and proctodaeum (pr).

Claus (1876) includes an account of the alimentary canal in his general work on the anatomy of the Daphnids, while Hardy and McDougall (1893) devote a paper entirely to the alimentary canal. Further information is added by Klotzsche (1913) and a good brief account is given by Wagler (1926-7). The musculature is described by Binder (1932), and the peritrophic membrane by Chatton (1920). None of these works supply a comprehensive account of the structure of the alimentary canal and there is little reference to the histology.

The mouth.

The mouth (Plate 18, mo) is small and opens between the labrum(l) (anteriorly) and the mandibles (md) (posteriorly and laterally). It leads to the stomodaeum (st).

The stomodaeum.

The stomodaeum, foregut or oesophagus (Plate 18, st), arises as an ectodermal invagination and is short and narrow. The wall is composed of an innermost transparent cuticle, a layer of cuboidal cells with granular cytoplasm, a pair of longitudinal muscles anteriorly (lgtmu), and an outer layer of circular muscle (cimu). The longitudinal muscles may be thin and difficult to discern. A set of dilator muscles (dilmu) extends from the outer wall of the stomodaeum to the chitinous

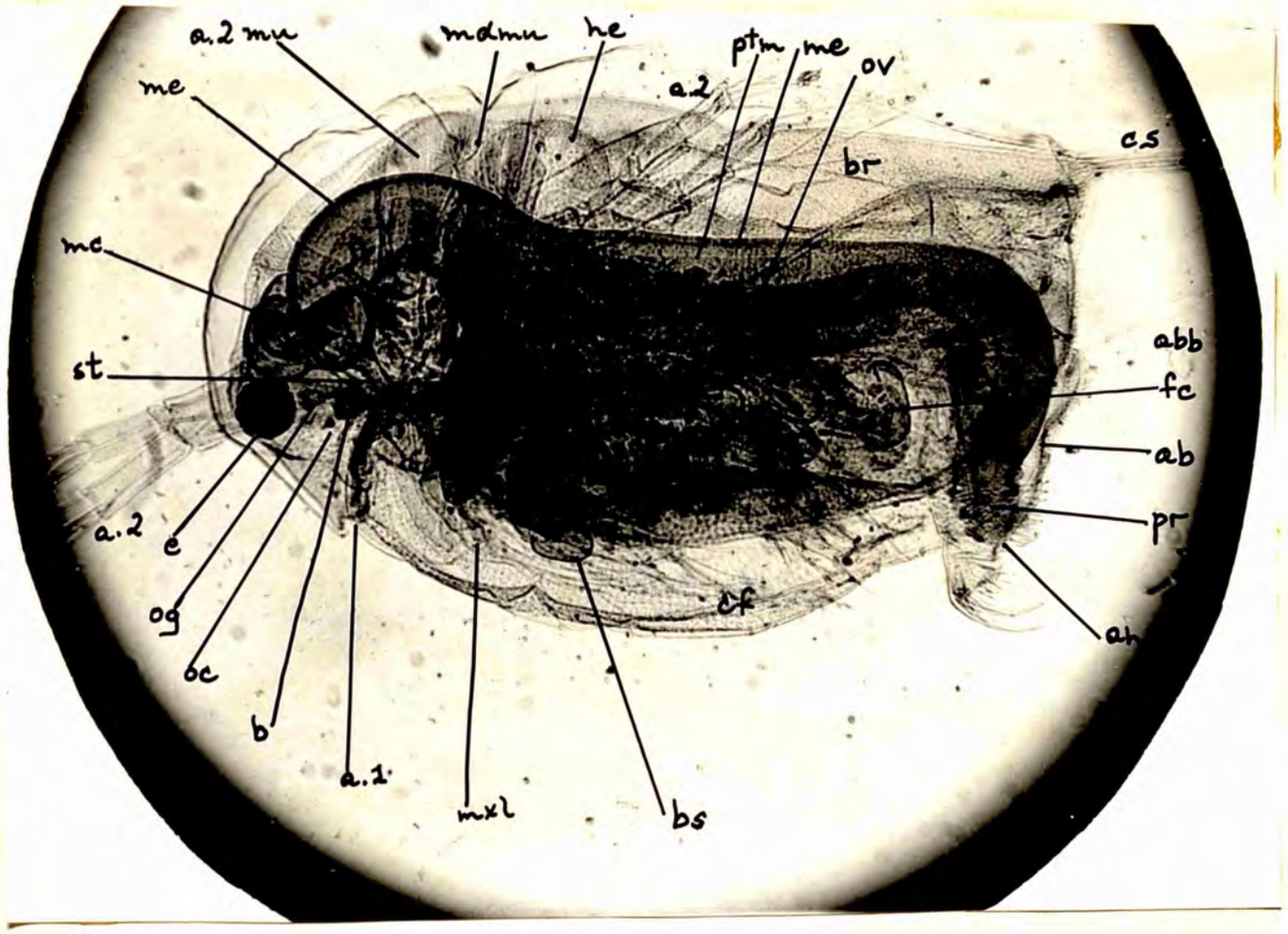


Plate 17. Photomicrograph of a whole mount of a fifth instar Daphnia magna. a.1,antennule; a.2,second antenna; a.2mu,muscle of second antenna; ab,abdomen, abb,abdominal bristle; an,anus; b,brain; br,brood pouch; bs,branchial sac; cf,carapace fold; cs,caudal spine; e,compound eye; fc,fat cell; he,heart; mc,caecum of mesenteron; mdmu,muscle of mandible; me,mesenteron; mxl,maxillary gland loop; oc,ocellus; og,optic ganglion; ov,ovary; pr,proctodaeum; ptm,peritrophic membrane; st,stomodaeum.

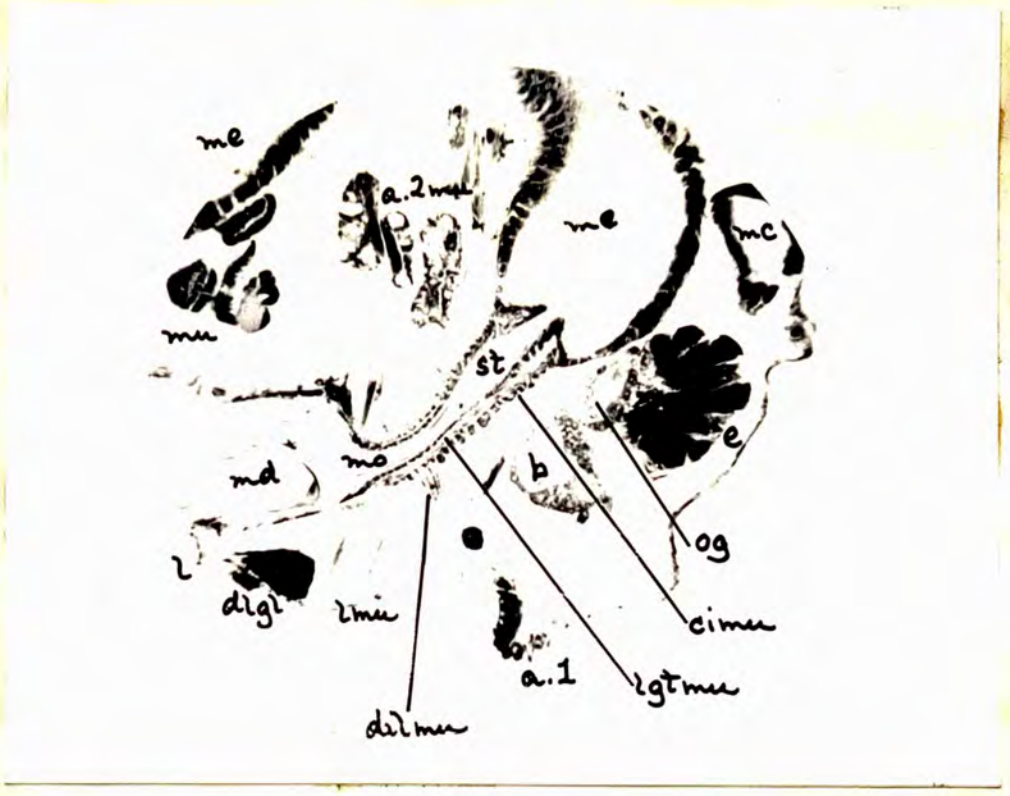


Plate 18. Photomicrograph of a vertical longitudinal section through the anterior region of an adult Daphnia magna showing the stomodaeum(st) and its junction with the mesenteron(me). The anterior wall of the stomodaeum is thicker than the posterior wall owing to the presence of a pair of longitudinal muscles(lgtmu). a.1,antennule; a.2mu,muscle of second antenna; b,brain; cimu,circular muscles of stomodaeum; dilmu,dilator muscles of stomodaeum; dlgl,distal labral gland; e,compound eye; l,labrum; lmu,muscle of labrum; mc,caecum of mesenteron; md,mandible; mo,mouth; mu,muscle; og,optic ganglion.

exoskeleton. The cavity of the stomodaeum is flattened antero-posteriorly, so that in cross section it is oval with the short axis running from the anterior to the posterior end (Plate 43,st).

The mesenteron.

The mesenteron, midgut or intestine (Plate 17, me), forms the greater part of the alimentary canal. It continues dorsally from its junction with the stomodaeum (st) to turn posteriorly above the level of the compound eye and continue parallel to the dorsal surface until turning ventrally into the abdomen (ab). There is no marked constriction, or "neck", in the mesenteron just posterior to the junction with the stomodaeum although this was mentioned by Hardy and McDougall (1895). The wall of the mesenteron partly consists of a layer of tall columnar cells of different height in the different regions, being tallest immediately behind the anterior flexure. The inner surface of the cells is very irregular. The outer part of each cell stains the more intensely and is granular; the inner part of the cell forms an almost translucent border containing a row of vertical rods (Plate 33, me). This border is slightly broader in the anterior region of the mesenteron and in the midgut caeca than more posteriorly. The rods are not always visible in the adult specimen; their presence may depend upon the state of digestion or upon the stage of the moult. Covering the layer of columnar cells is a layer of circular muscles (Plate 19; Plate 44, cimu). External to this layer of muscles is a series of longitudinal muscles (lgtmu), about 14 in number, which extend the whole length of the mesenteron. The relative positions of the sets

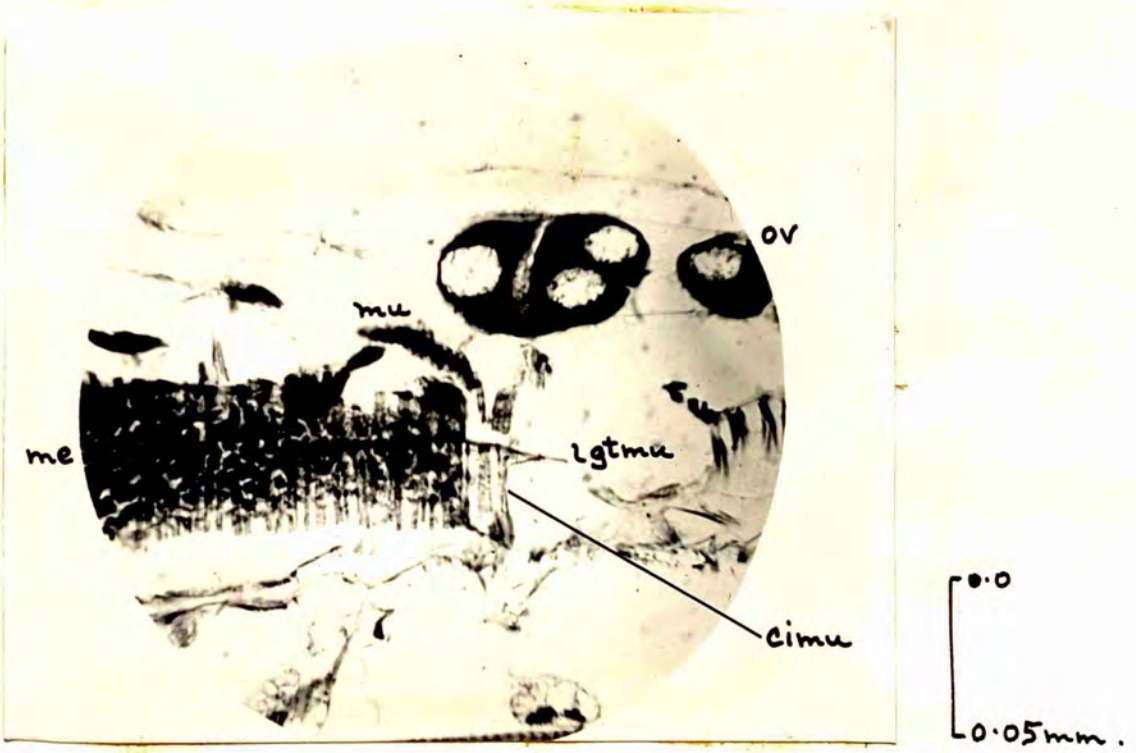


Plate 19. Photomicrograph of a horizontal longitudinal section showing the cross striation of the circular muscles(cimu) of the mesenteron(me), and also four-cell groups in the ovary(ov). lgtmu,longitudinal muscle; mu,muscle.

of muscles is thus the reverse of that present in the stomodaeum.

Within the cavity of the mesenteron lies the thin hyaline peritrophic membrane (Fig.36; Plate 37, ptm). The membrane is attached only at its anterior end where it originates in the groove found at the junction of the stomodaeum with the midgut. Otherwise the membrane hangs freely in the cavity. Chatton (1920) suggests that this peritrophic membrane is formed of a stomodaeal moult which has turned inside out to project into the mesenteron and has been augmented by agglutination in the midgut so that it now extends as far as the anus. It is interesting to note that in insects the peritrophic membrane may be produced in one of two ways: in some by a ring of cells at the anterior end of the midgut; in others by delamination of one or more layers from the surface of the midgut spithelium (Waterhouse, 1954). The membrane appears to be structureless, but it is possible that with higher magnification, such as that obtainable with the electron microscope, a meshwork of some kind, similar to that noted for example by Huber (1954) in insects, would be discernible.

The caeca of the midgut leave the mesenteron just posterior to the entry of the stomodaeum (Plate 17) and project dorsally and posteriorly. They are short with blunt ends. Their walls are similar in structure to that of the mesenteron. They do not contain the peritrophic membrane.

The proctodaeum.

The proctodaeum, hindgut or rectum (Plate 17, pr), is a short ectodermal invagination which is laterally compressed. The wall

is lined by a thin cuticle and is composed of a layer of cuboidal epithelium which resembles that of the stomodaeum and is surrounded by a strongly developed sheath of circular musculature. There is possibly a very thin layer of longitudinal muscles between the cuboidal epithelium and the circular muscles. This layer appears to be present in the first instar animal but cannot be definitely recognised in the mature adult. It is not mentioned by Binder (1932) who wrote an account of the musculature. Extrinsic muscles run to the wall of the proctodaeum. The circular muscles of the wall do not extend for the full length of the proctodaeum but internally cease before the junction with the tall columnar cells of the mesenteron. The proctodaeum contains a second or rectal peritrophic membrane which originates in the groove at the junction of the mesenteron with the proctodaeum and extends posteriorly between the proctodaeal wall and the anterior peritrophic membrane. It ends at the anus. It is probably formed of a proctodaeal moult. The proctodaeum is often coated with a large number of fat cells but it is unlikely that these are of any importance in digestion.

The anus.

The anus (Plate 17, an) opens posteriorly on the abdomen, just above the caudal furca.

Function.

Daphnia feeds principally on algae and detritus. The food is collected by the appendages (Storch, 1924; Cannon, 1933), mixed with a secretion of the labral glands and passed rapidly through the stomodaeum into the mesenteron. The stomodaeum does not

retain any of the food; the anterior one-third of the mesenteron is usually also empty of food. The food remains surrounded by the peritrophic membrane, most of the digestion and absorption taking place in the middle region of the mesenteron. The amount of movement occurring towards the anterior region and midgut caeca is greatest when the food is very plentiful. There is a rhythmic antiperistalsis forwards from the hind end of the gut, where it is strongest. It was suggested by Fox (1952) that this is due to the regular rectal swallowing of water which distends the gut. The circular muscles of the gut wall contract in response to the stretching by hydrostatic pressure. This causes defaecation from the posterior end of the mesenteron. Defaecation is sudden, and may occur several times during one minute. The proctodaeum, like the stomodaeum, acts only as a passageway. Oral swallowing probably also stretches the muscles of the gut wall, aiding antiperistalsis, and as well probably forces the food backwards along the mesenteron. Antiperistalsis causes a continual movement back and forth resulting in a mixing of the food and digestive enzymes.

Digestive enzymes found to be present include an amylase, lipase and proteinase (Hasler, 1935), together with three peptidases (Hasler, 1937). Daphnia lives long on a protein diet, while von Dehn (1930-1) found that starch digestion was slow. But Lefèvre (1942) established that the starch of algae is readily digested. The algal cell walls remain unchanged but the cell contents are removed.

The pH of the anterior three-quarters of the mesenteron is 6.0 to 6.8, of the posterior quarter 6.6 to 7.2 (Fox, 1948). This agrees with the results obtained by von Dehn and Hasler.

Acetylcholine stimulates intestinal movement, its action being inhibited by atropine (Obreshkove, 1941).

b. The excretory System.

The excretory organs of the Crustacea are the antennary glands and the maxillary glands. Seldom are both present together. The antennary gland is functional in the adult of certain Malacostraca and is the larval excretory organ of some Branchiopoda. The maxillary gland is the excretory organ of the majority of Crustacea, including adult Branchiopoda such as Daphnia. The maxillary gland is often referred to as the shell gland or "Schalendrüse". Cannon and Manton (1927) describe the arrangement of the maxillary gland in Estheria and place the remainder of the Branchiopoda into two groups, the Notostraca and the Cladocera being closely similar to the Conchostraca while the Anostraca show important differences. The derivation of the maxillary gland of Daphnia from that of Estheria is traced, and it is pointed out that one of the loops has in Daphnia developed an S-shaped twist. Earlier a suggested hypothesis for the evolution of the antennary and the maxillary glands was put forward by Cannon (1924).

Leydig (1860) gives a short account of the shell gland, while Dohrn (1870) investigated its development in Daphnia longispina and first indicates that the gland has an opening although he believes that this is internal. A description is given by Claus (1875) who decided that the shell gland was glandular and that it consisted of a vesicle or bladder, a canal and a labyrinth. In 1913, Klotzsche gave a more detailed account of both the antennary and maxillary glands and included an historical survey of the earlier work on these organs. Vital

staining experiments by Dejdar (1930) also reveal the presence of the antennary gland.

The maxillary gland.

The maxillary gland is situated principally in the anterior region of the carapace fold (Plate 20) but projecting slightly inwards in the region where the carapace is attached to the body. It consists (Fig.128) of an end sac, a short canal, a series of loops, a urinary bladder, and an excretory duct opening to the exterior.

The end sac.

The end sac lies slightly dorsal to the middle of the gland in the region where the carapace is attached to the body, but is still within the carapace fold. It is rectangular in form with slightly rounded corners. The wall is composed of cells which project into the lumen, their inner edges being uneven irregular semi-circles. Klotzsche (1913) described an inner area of each cell containing the nucleus and numerous even-sized granules, the whole part of the cell staining moderately intensely with Heidenhain's Iron haematoxylin, while at the base of each cell there was a clear, almost unstained border. The border contained similar dark-staining granules of very different sizes which Klotzsche suggested were possibly excretion or secretion products and indicated as very characteristic of the end sac. He also found vacuoles in the cells. However on staining with Heidenhain's Iron haematoxylin it has been found that the cytoplasm of the cell has little affinity for the stain throughout but that it contains granules of different sizes which stain

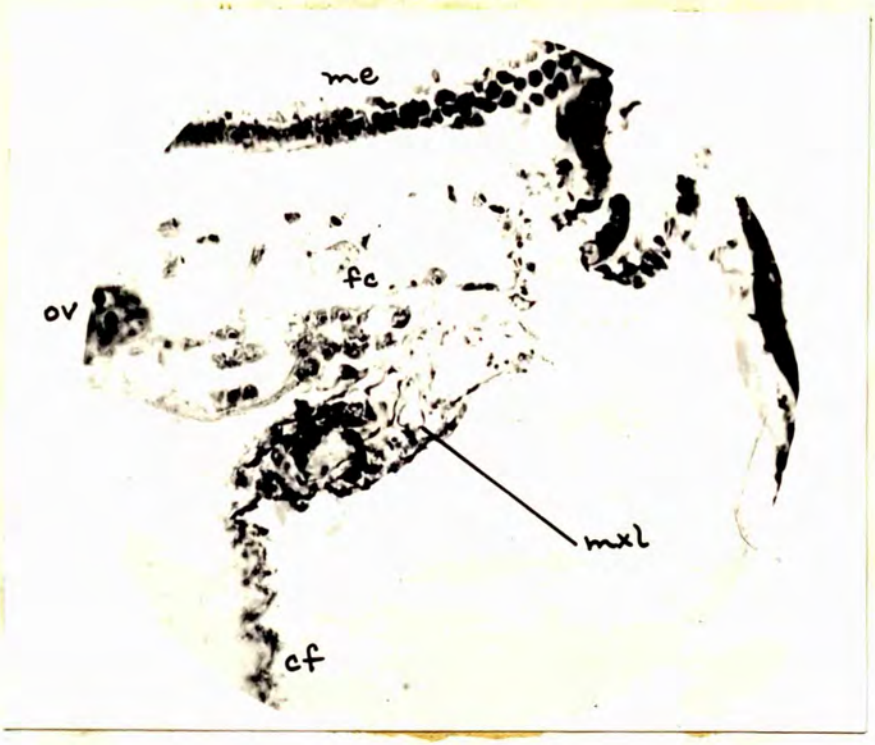


Plate 20. Photomicrograph of a horizontal longitudinal section in the region in which the carapace fold(cf) is attached to the body, showing part of the maxillary gland(mxl). fc,fat cell; me,mesenteron; ov,ovary.

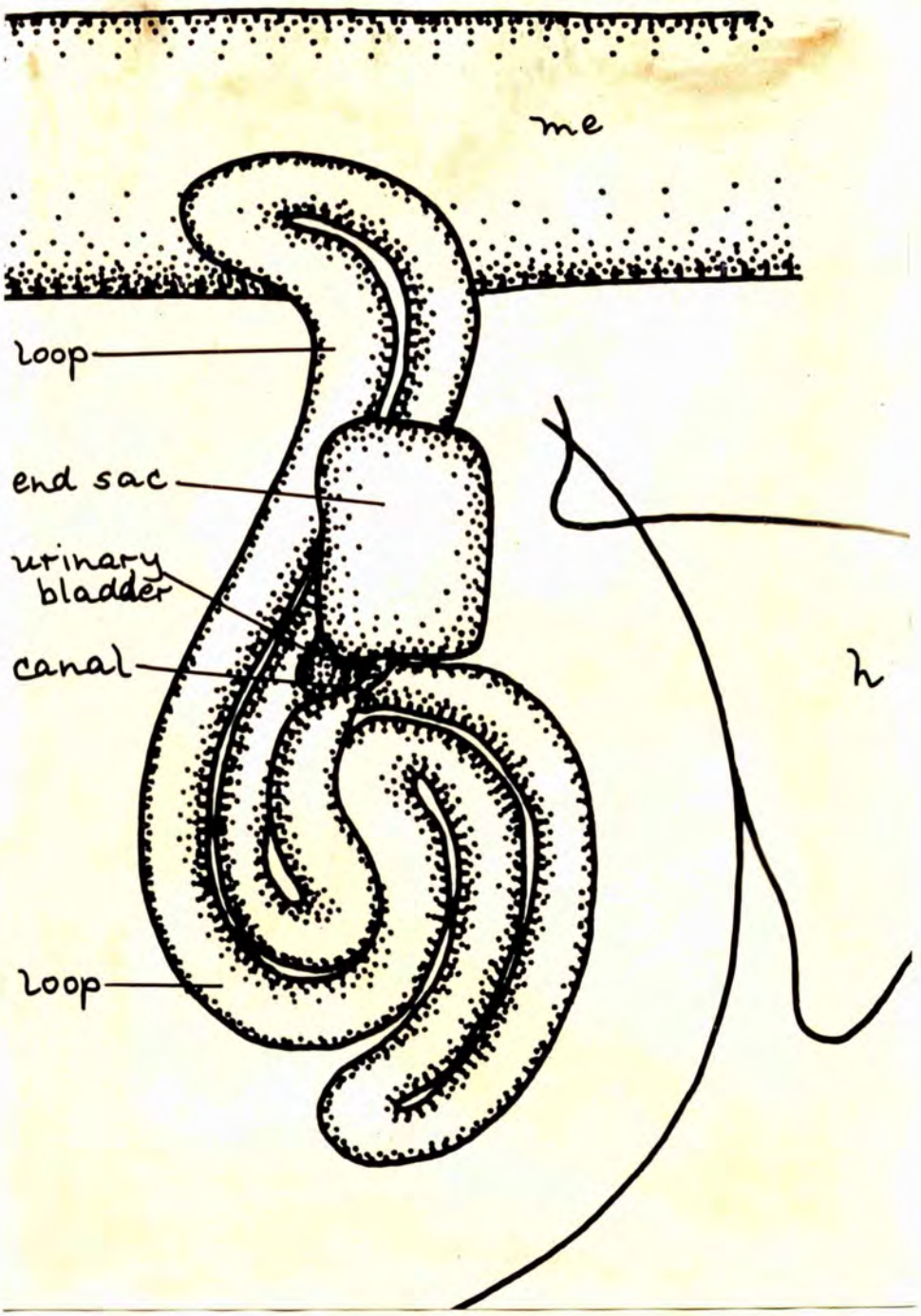


Figure 128. Diagrammatic reconstruction of the maxillary gland of an adult Daphnia magna showing the end sac, canal, coiled loops and urinary bladder. h, head; me, mesenteron.

more deeply and are more numerous in the inner part of the cell, thus giving a darker appearance to this area of the cell. The inner part of the cell also contains the nucleus which stains deeply. A few vacuoles are present in the cells. No distinctly separate border is present, the effect of one may be produced by a lesser concentration of granules. The lumen of the end sac occasionally contains spherical bodies which Klotzsche regarded as blood cells. They show little structure and take up only a small amount of stain.

The canal.

The canal is very short and narrow. It leads from the ventral end of the end sac to the series of loops and shows the same structure as that of the loops being distinguished only by its narrower diameter.

The series of loops.

This is sometimes referred to as the canal or labyrinth. The loops follow a similar pattern to that of other Branchiopoda. From the canal the loop leads ventrally, then back dorsally to a position between the dorsal end of the mandible and the heart so that the bend lies adjacent to the alimentary canal in the region of the posterior rotator muscle of the mandible, then ventrally again following the posterior edge of the previous loop until it meets the canal, when it turns anteriorly and downwards to loop up again to the level of the canal, then becoming more medial and entering the urinary bladder. Claus (1975) describes these as inner loop, outer loop, near loop and end loop; while Klotzsche (1913) refers to the first three bends as the main loop and the

remainder as the secondary loop. The most satisfactory method seems to be to number them I, II, III, IV, V, and VI, as Klotzsche does on his diagram.

In both the canal and the loops the cells of the wall are large and thin (Plate 20; Plate 21; mxl). The cytoplasm has little affinity for Heidenhain's Iron haematoxylin and does not contain granules. The cells are larger than those forming the wall of the end sac and those on the internal side of the loop appear slightly taller, or thicker, than those in the external side of the loop. Surrounding the greater part of the excretory organ but particularly noticeable between the loops is a trabecular connective tissue which forms blood sinuses.

The urinary bladder.

This is sometimes referred to as the vesicle. It is a continuation of the last loop, VI, slightly enlarged and passes median to the end sac. The cells of the wall are similar to those of the loop with the addition of several deeply staining cells containing small dark granules. These cells are against the outer surface of the wall.

The excretory duct.

This also is sometimes referred to as the vesicle. It is a straight narrow duct of even diameter with a thin wall of cells similar to those of the loops. It is situated between the end sac and the ventral septum. It leads to the opening of the gland the position of which has previously been in doubt but which occurs (Plate 21, ed) on the base of the second thoracic appendage (t2), almost opposite the mandible (md).

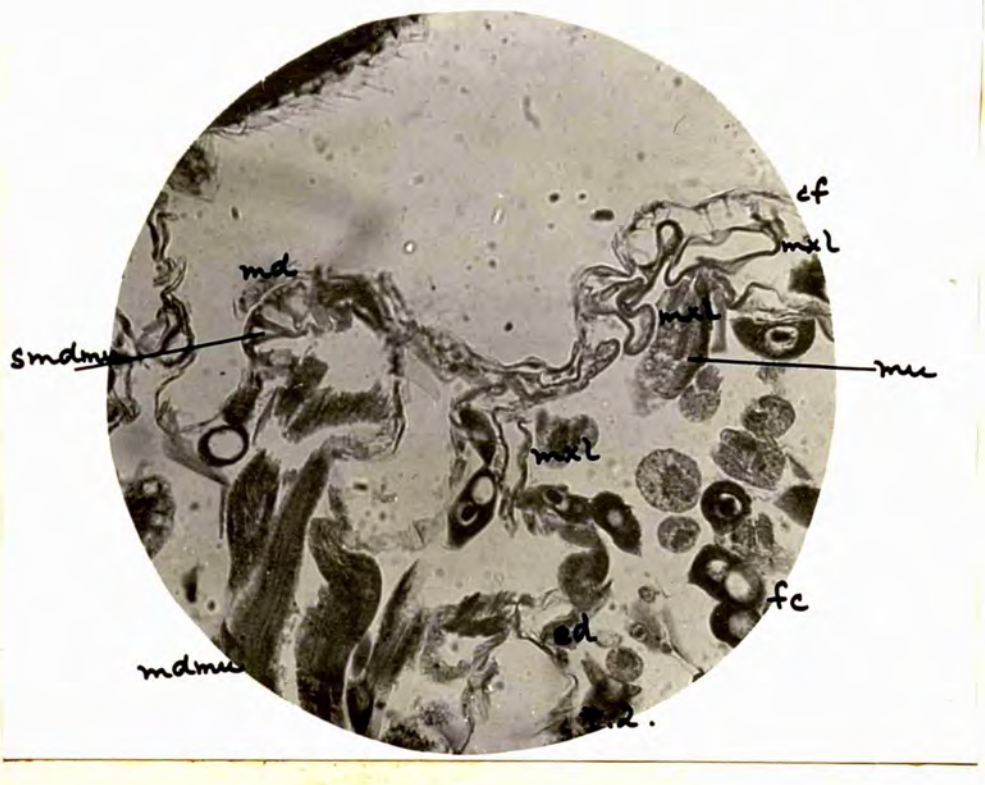


Plate 21. Photomicrograph of a horizontal longitudinal section through the region of the mandible and maxilla showing part of the maxillary gland loops(mxl) and the region of the opening of the excretory duct(ed). cf,carapace fold; fc,fat cell; md,mandible; mdmu,rotator muscle of mandible; mu,muscle; smdmu,small mandibular muscles; t.2,second thoracic appendage.

The antennary gland.

The application of vital stains shows a small area of cells which is generally assumed to be the rudimentary end sac of the antennary gland. It lies in the region of the anterior flexure of the alimentary canal at about the same level as the base of the second antenna. In sectioned animals it is extremely difficult to distinguish but is usually found as a cluster of about four cells which may be grouped into the form of a rudimentary sac (Fig. 129, a.2.gl). The cells show little affinity for stains and are highly vacuolated. They contain a number of granules. They are irregular in outline. Similar cells were described by Klotzsche as the end sac of the antennary gland. The cells are sometimes not unlike the fat cells which are found throughout the body, except in the head.

Function.

The excretory function of the maxillary gland of Daphnia was first indicated by Metschnikoff and Kowalevsky (1890) who fed different Phyllopoda with carmine suspensions and found that the end sac of the maxillary gland was stained with the carmine. A similar result was obtained with litmus, the end sac turning red. The loops, however, were not stained. Further work was carried out by Bruntz (1903) who confirmed their ideas by sections. A series of investigations using vital stains was undertaken by Gicklhorn (1951, a and c) and Gicklhorn and Keller (1925) following the earlier paper by Fischel (1908) in which the author had shown two organs in Daphna magna not otherwise seen, namely

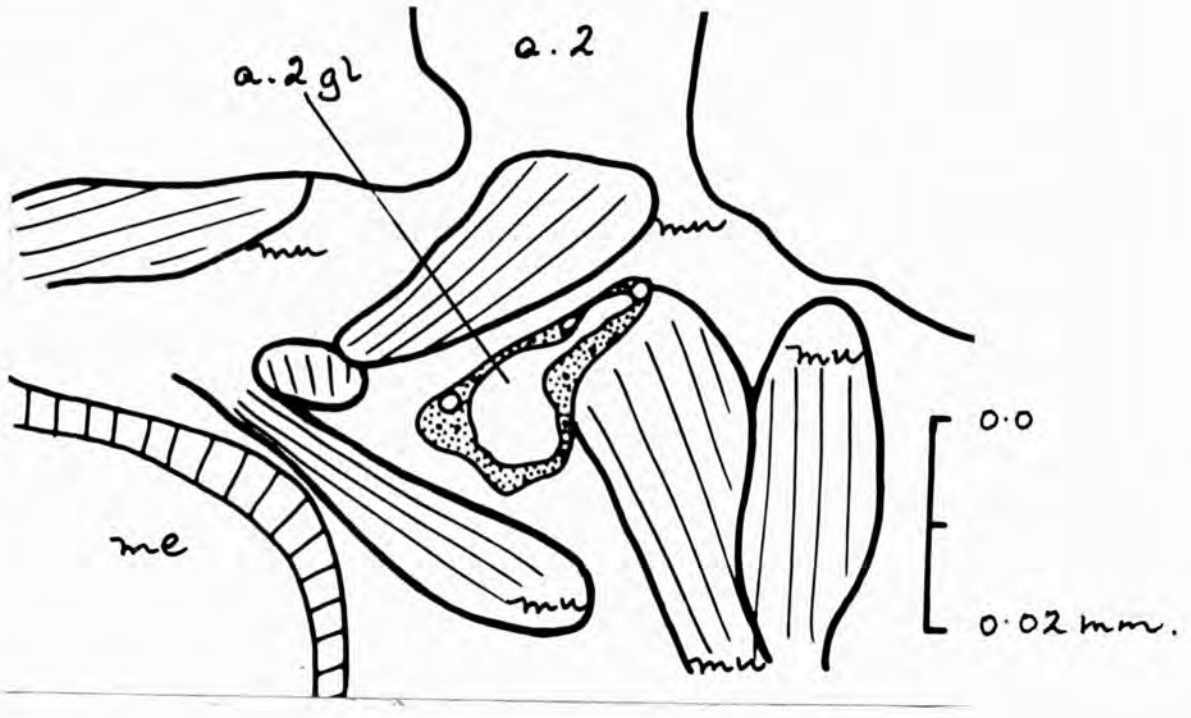


Figure 129. Transverse section through a fifth instar Daphnia magna in the region of the second antenna(a.2) showing the antennary gland (a.2gl) consisting of a small thin-walled end sac. me, mesenteron; mu, muscle. Freehand drawing.

the end sacs of the two excretory organs. Gicklhorn's vital staining showed different reactions in different regions of the loops, indicating differences in function. He concluded that in addition to an early phase of excretion there is also a later phase of resorption, both confined to anatomically distinct parts. Gicklhorn's work was continued by Sturm (1936) using fluorescent dyes, which showed that only the maxillary gland and the midgut caeca fluoresce. Similar investigations were carried out by Keller and Pisho (1947). Fischel found very variable reactions to his stains, and Klotzsche suggests that this is due to different physiological states of the gland.

Occasionally the loops of one of the maxillary glands are coloured red owing to the presence of oxyhaemoglobin in the lumen (Fox, 1948). According to Smaridge (1954), when haemoglobin is being lost from the blood of Daphnia magna, inorganic iron accumulates in the maxillary glands. She showed that iron is excreted from the walls of the loops into the lumen and from there passes through the urinary bladder lumen out of the body.

It has been suggested, for example by Bruntz (1905) and Gicklhorn (1931, a), that nephrocytes are sometimes found in the body but it seems likely that these are fat cells. Nephrocytes are mentioned by Maloeuf (1938) in a general review of excretion in Arthropoda.

c. The Labral Glands.

The labrum, or upper lip, is a large, almost club-shaped prolongation of the ventro-posterior part of the head (Plate 18,1) extending between the mandibles to the region of the maxillae. It ends posteriorly in a narrow flap or lobe. In the living animal it is seen to contain two pairs of dense, pale yellow glands, the distal labral glands (Plate 22, dlgl). With these is associated a row of enlarged cells (plgl) present on each side along the postero-lateral margin of the head. These are the proximal labral glands.

An early account of the labral glands of Daphnia is that of Claus (1876), followed in 1913 by that of Klotzsche. An account of other early workers is given in the comprehensive paper by Cannon (1922).

The proximal group.

The row of proximal cells (Plate 22, plgl) extends in a line of almost even width on either side of the head from immediately posterior and dorsal to the ganglion of the first antenna (a.1), dorsally and then dorso-posteriorly to a point just dorsal and lateral to the stomodaeum (st). The two sides of the proximal labral gland lie close together. Their anterior extension in particular is slightly different from that described by Cannon (1922) for Simocephalus vetulus, and is considerably beyond the boundary of the labrum. The single row (Plate 23; Plate 24,plgl) consists of epidermal cells of various heights, tending to be taller dorsally. The inner edges of the cells are uneven. The cells are highly vacuolated especially in the outer part of

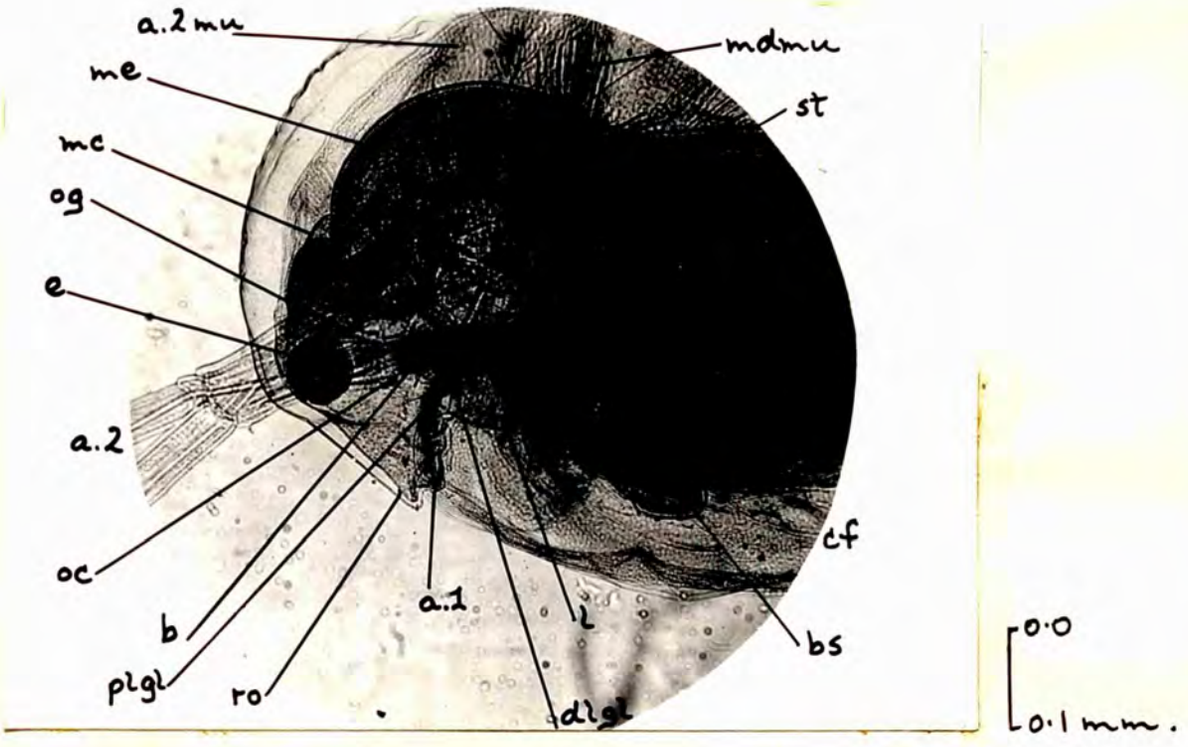


Plate 22. Photomicrograph of the anterior region of an adult Daphnia magna showing the positions of the proximal (plgl) and distal(dlgl) labral glands. a.1,antennule; a.2,second antenna; a.2mu,muscle of second antenna; b,brain; bs,branchial sac; cf,carapace fold; e,compound eye; l,labrum; mc,caecum of mesenteron; mdmu,mandibular muscle; me,mesenteron; oc,ocellus; og,optic ganglion; ro,rostrum; st,stomodaeum.

Plate 23. Photomicrograph of a horizontal longitudinal section through the anterior region showing the junction of the proximal(plgl) and distal(adlgl) labral glands and also the second pair of distal labral glands(pdlgl). l,labrum; nu,nucleus; oc,ocellus.

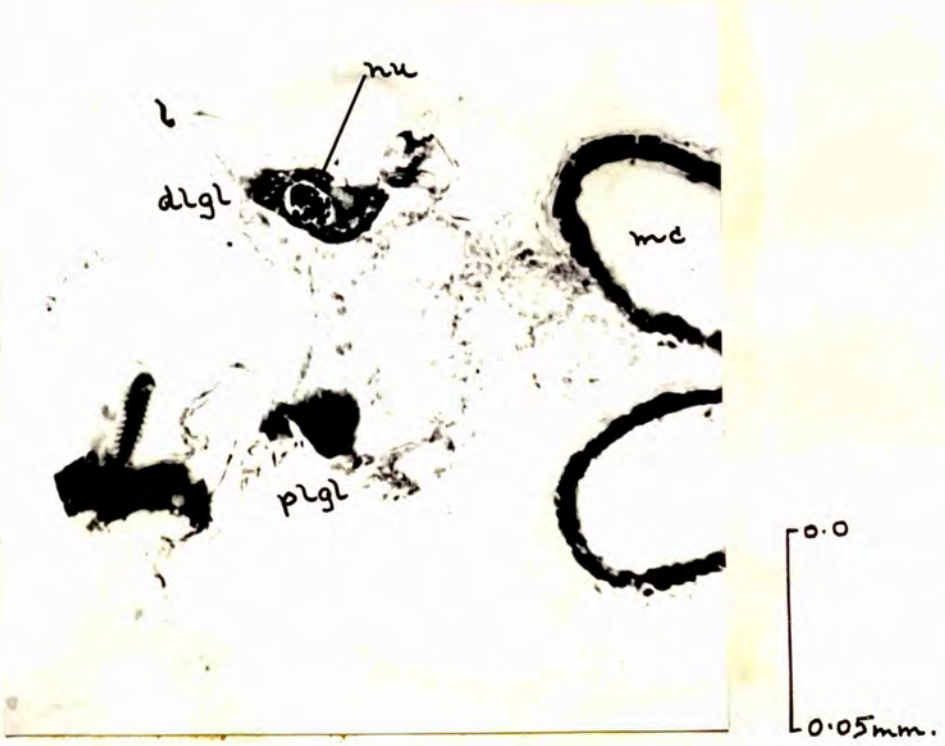


Plate 24. Photomicrograph of a horizontal longitudinal section through the anterior region showing the labrum(l) with distal labral gland cell(dlgl) containing a large nucleus(nu). mc,caecum of mesenteron; plgl,proximal labral gland.

the cell and the vacuoles tend to obscure the boundaries between the cells. The cells contain numerous darkly staining granules of about the same size as the nucleolus. Cannon found that in S. vetulus the nuclei of these cells were several times larger than those of the nerve and muscle cells. This is also true to a less marked extent in Daphnia magna. The group consists of about 25 to 30 cells, in S. vetulus there are about 20. There is no duct to the oesophagus; such a duct was described by Claus (1876) but denied by Cunnington (1903) and Cannon. There appears to be no special nerve to the proximal group of gland cells although the nerve to the first antenna passes extremely close to the cells.

The distal group.

The distal group of labral gland cells (Plate 23, dlgl) is connected with the proximal group (plgl) ventral to the brain. The group consists of five cells on each side, four large gland cells and one duct cell. The gland cells are arranged as anterior (adlgl) and posterior (pdlgl) pairs, with the duct cell attached to the posterior pair. The anterior and posterior pairs are connected by a thin protoplasmic strand. The anterior pair is situated (Plate 22, adlgl) in the base of the labrum, ventral to the stomodaeum (st), and posteriorly extends into the labrum (l) to about the level of the labral levator muscle. The two anterior pairs are partially separated by the oesophagus. The posterior pair (Plate 23, pdlgl) is situated towards the tip of the labrum, at the posterior end of the broader portion of the labrum and behind the level of the mouth. One of the two

cells is slightly posterior to the other.

The cells of both pairs contain large spheroidal nuclei (Plate 24; Plate 25; nu). None of the nuclei appear cup-shaped although in Simocephalus vetulus, Cunnington described them all as cup-shaped and Cannon the posterior pair. In Daphnia magna the nuclei are considerably larger than those of the proximal gland cells, the reverse is true in S. vetulus. The chromatin of the nucleus is scattered in large irregular clumps which are very conspicuous and stain deeply with Heidenhain's Iron haematoxylin. The cytoplasm of the cells also stains deeply and contains a number of small granules. The cytoplasm contains vacuoles which are slightly more opaque than those in the proximal group, a difference found also by Cannon. There is a peripheral area of cytoplasm which, although not distinctly demarcated, does not contain vacuoles and is slightly denser.

Between the anterior pair of distal gland cells there is a group of vacuoles forming an ill-defined reservoir, a similar reservoir was found by Cannon in Simocephalus. This reservoir is not continued into the thin protoplasmic strand connecting the anterior and posterior pairs of gland cells which however contains a few vacuoles which may serve to pass the secretion through this area. Between the posterior pair of cells is a slightly better-defined reservoir (Plate 25, res) with a number of vacuoles surrounding it. This reservoir corresponds to one found in Simocephalus but is not quite so well-defined. The duct cell forms a tube leading from this reservoir to open near the tip of the labrum, immediately in front of the terminal lobe and

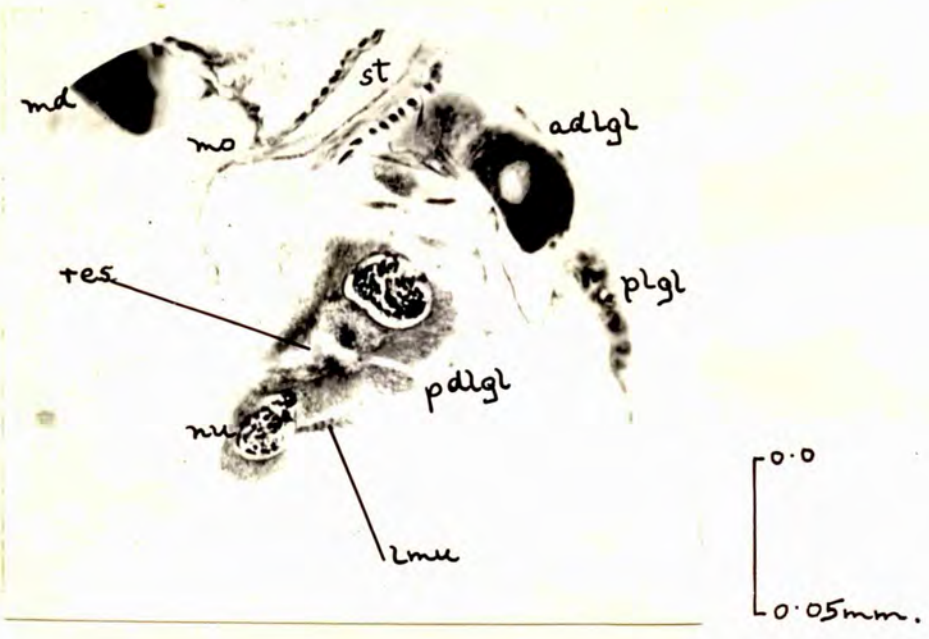


Plate 25. Photomicrograph of a vertical longitudinal section through the labral region showing a pair of posterior distal labral gland cells(pdlgl) each with a large nucleus(nu) and enclosing between them a reservoir(res) adlgl,anterior distal labral gland; lmu,labral muscle; md,mandible; mo,mouth; plgl,proximal labral gland, st,stomodaeum.

slightly dorsally. The cytoplasm of this cell does not stain as deeply as that of the gland cells and contains no vacuoles. There is a small nucleus which does not stain as deeply as that of the gland cells. Cannon found a granular coagulum in the reservoir which stained differently with Mallory's Triple stain from a similar coagulum found in the lumen of the duct cell. He suggested that the duct cell changed the nature of the secretion. With Heidenhain's Iron haematoxylin no change in staining reaction has been noted.

No nerve has been identified leading to the distal group of labral gland cells. The nerve to the first antenna passes close to the anterior pair of cells and the anterior end of this pair of cells lies immediately against the posterior part of the brain . It is possible that a nerve connection occurs in this region. Cannon found a nerve to the anterior pair of cells coming off the nerve to the first antenna. The antennular nerve in Daphnia is smaller than that in Simocephalus as illustrated by Cannon.

Function.

The function of the labral glands has been described by Cannon. They secrete a substance which is added to the food particles as they pass collect between the mandibles and maxillae. This secretion does not contain mucin.

As in Simocephalus the two groups of cells are definitely connected but it seems that the proximal group is less well developed than in Simocephalus. The posterior end of the

proximal group meets the anterior end of the distal group, and the cells show similarity in structure.

d. The Reproductive System.

Female.

There have been several earlier descriptions of the reproductive system of the female, which is not surprising since the system is both interesting and easy to observe. A description is given by Claus (1876) of the development and growth of the two kinds of eggs, followed in 1877 by a description by Weismann. Later von Scharfenberg (1910-11) gives a further description and disagrees with some of Weismann's views. More recently Baldass (1942), in his account of the development of the egg of Daphnia pulex, includes a description of the formation of the egg in the ovary.

The ovaries form long rods lying on either side of the alimentary canal (Plate 17, ov). They extend, in the adult, from the level of the first thoracic appendage and the end sac of the maxillary gland to just past the posterior edge of the fifth thoracic appendage, that is almost to the end of the horizontal part of the mesenteron before it bends downwards into the abdomen. The posterior end of the ovary is difficult to distinguish in the living animal as it lacks the greenish yolk so prominent in the remainder of the ovary. This posterior colourless area (Plate 26, ov) is composed of small slightly flattened cells which gradually increase in size towards the anterior. They comprise the germinal part of the ovary from which the cells are formed into groups of four (Plate 27, ov) and proceed to the anterior part of the ovary, sometimes referred to as the egg-container (Plate 28, ov). The egg-container forms the greater part of

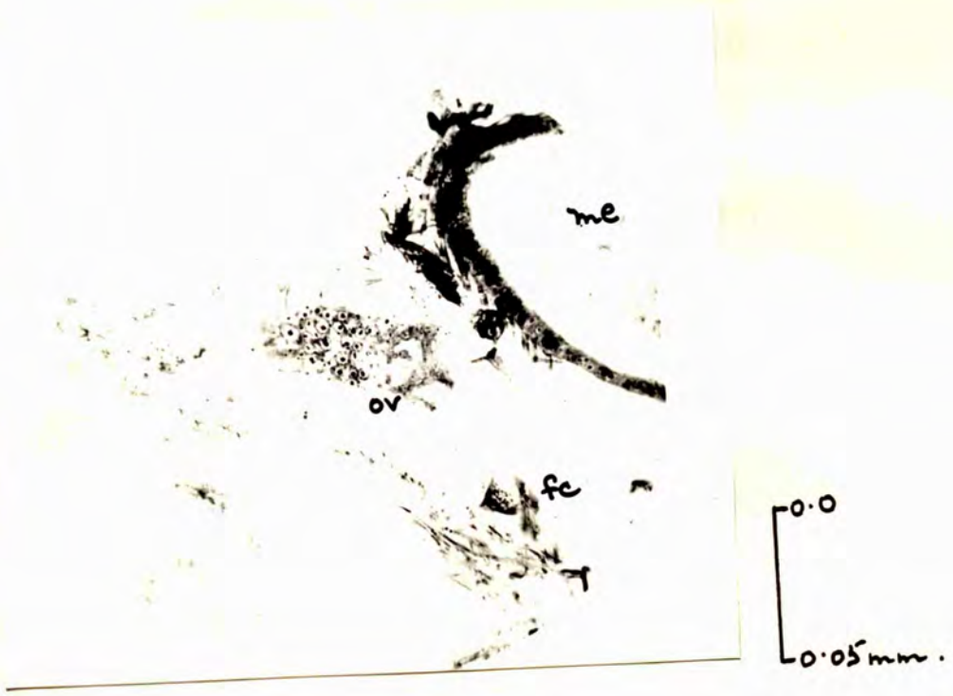


Plate 26. Photomicrograph of a vertical longitudinal section showing the posterior germinal region of the ovary(ov) composed of small cells. fc,fat cells; me,mesenteron.

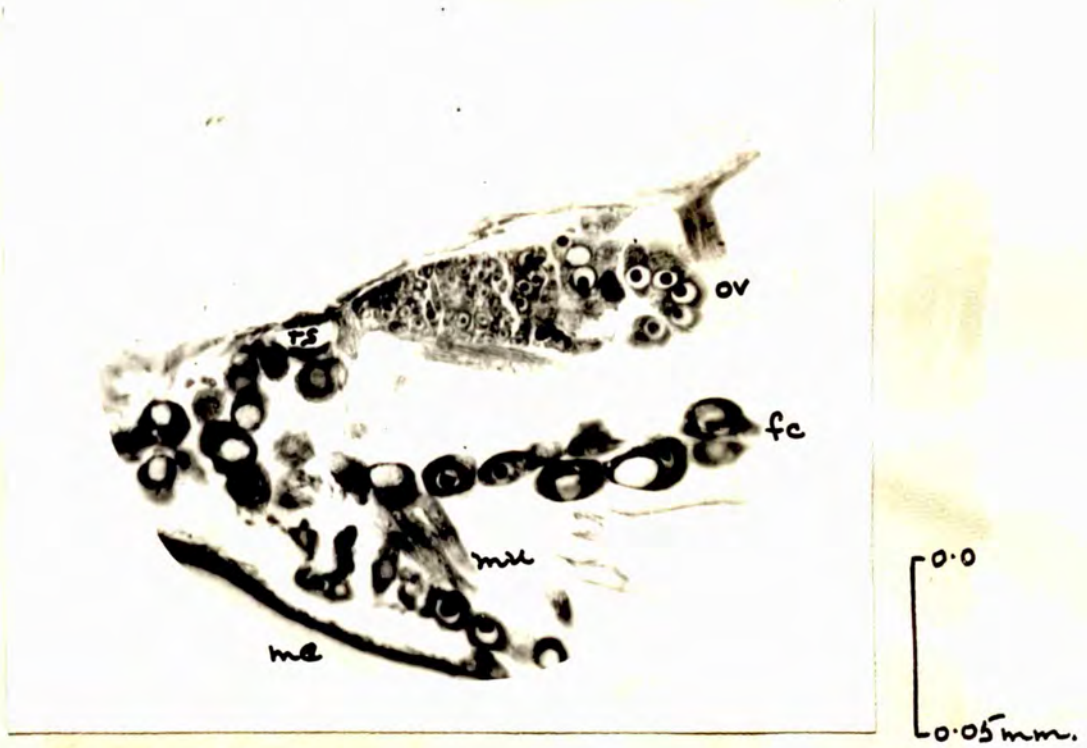


Plate 27. Photomicrograph of a horizontal longitudinal section towards the posterior end of the body showing the posterior germinal part of the ovary and the more anterior groups of four-cells(ov). fc,fat cell; me,mesenteron; mu,muscle; rs,receptaculum seminis.

Plate 28. Photomicrograph of a vertical longitudinal section showing the four-cell groups in the ovary(ov).

the ovary and is filled with developing eggs, all being at the same stage of development. It is usually possible to distinguish the individual eggs even in the living animal. The eggs are of irregular shape, filled with greenish yolk globules and cytoplasm but containing also a number of transparent spheres, the fat or oil droplets. The number of these spheres varies from egg to egg. It is not possible to see nuclei in the living ovary although they are seen distinctly in whole mounts and sections. The eggs usually fill the whole of this part of the ovary, but when there are only about two eggs present these do not necessarily meet each other, although their ends are attenuated instead of rounded as when packed together. The area not filled by these eggs, and the ovary immediately after the release of a brood of eggs into the brood pouch is composed of collapsed vesicular tissue.

The oviduct is not visible in the living animal. Its position is indicated when the eggs are being laid into the brood pouch. They are squeezed through a very narrow short duct which leads from the postero-dorsal corner of the ovary to open somewhat laterally into the brood pouch just anterior to the median dorsal process which closes off the posterior end of the brood pouch. The anterior end of the ovary is blind.

Oviduct and Receptaculum seminis.

Leading posteriorly from the ovary is a long, thin, straight tube, or duct (Plate 29; Plate 30; Plate 31; rs), which extends from the germinal part of the ovary to open near the prominent dorsal process which closes the posterior end of the brood pouch.

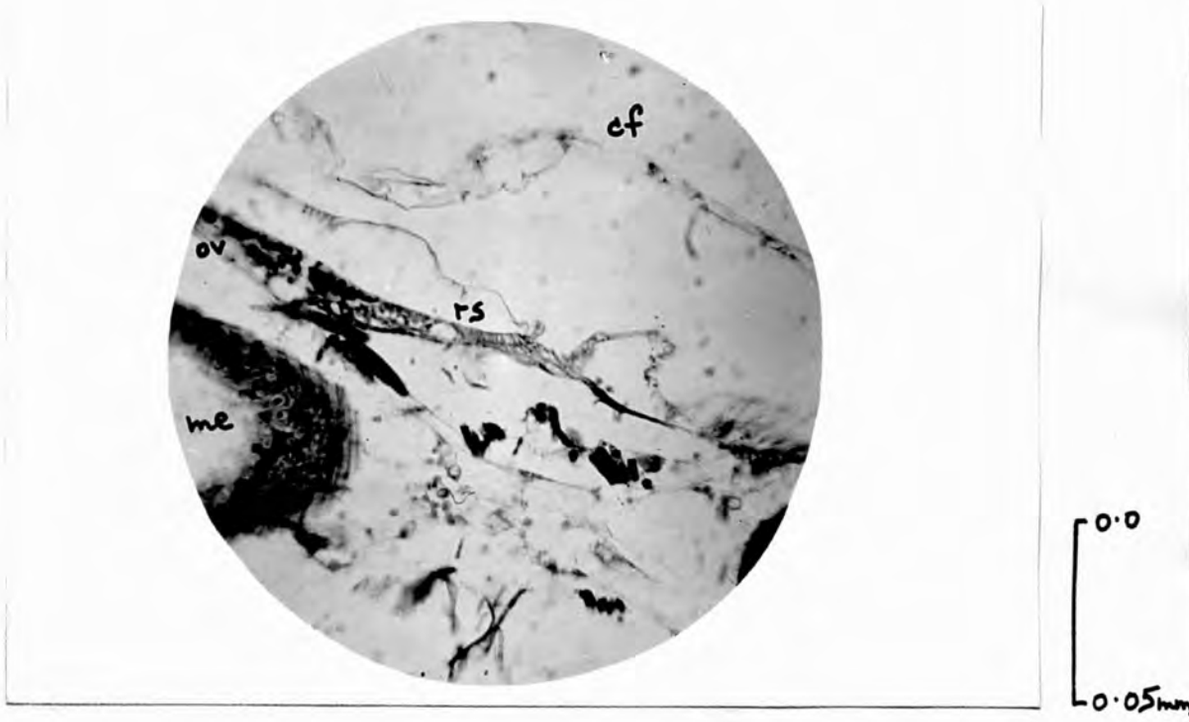


Plate 29. Photomicrograph of a horizontal longitudinal section showing the receptaculum seminis(rs) leading from the posterior end of the ovary(ov) to open close to the dorsal processes at the posterior end of the brood pouch. cf,carapace fold; me,mesenteron.

Plate 30. Photomicrograph of a horizontal longitudinal section showing the receptaculum seminis(rs) and the posterior end of the ovary(ov). fc,fat cell; me,mesenteron.

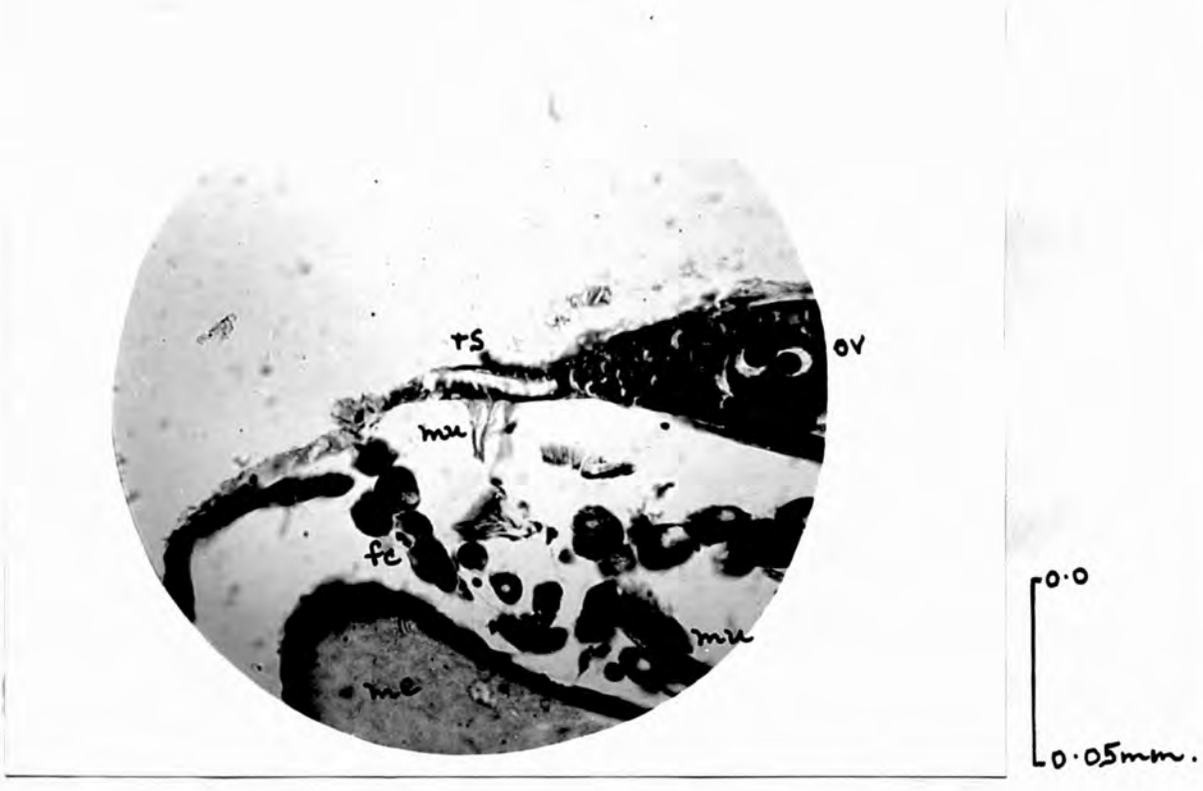


Plate 31. Photomicrograph of a horizontal longitudinal section showing the open receptaculum seminis(rs) leading from the posterior end of the ovary(ov). fc,fat cell; me,mesenteron; mu,muscle.

The wall of this tube is one cell thick, and the cells are almost square when cut in a longitudinal section of the animal. They do not stain deeply and have small nuclei. The duct turns slightly outward just before it opens and there appears to be a small thickening or rim around the opening. This duct is comparable to that described by Weismann in other Cladocera as the vesicula seminalis, although in the female the term should be receptaculum seminis. No such structure has previously been described for Daphnia. It appears to open too far posteriorly to be the oviduct and also is greater in length than observation of the passage of the eggs through the oviduct into the brood pouch indicates. The only indication of an oviduct is a very short membranous tube occasionally distinguishable with difficulty leading vertically dorsalwards from the posterior end of the ovary. This is seldom convincingly present and probably collapses to a thin strand except during the actual laying of the eggs. Observations of the process of laying suggest that the oviduct leads vertically dorsalwards from the anterior part of the germinal region of the ovary. This would allow the eggs to leave the ovary without pushing past the compact germinal cells, the receptaculum seminis leading to the posterior end of the germinal region. Attempts to fix an adult in the process of egg-laying have proved unsatisfactory.

Musculature.

No muscles have been described in relation to the reproductive organs. Muscles present close to the ovary and oviduct are the dorsal longitudinal muscles but there do not seem to be any

muscles actually connected with the reproductive organs except small muscles by the receptaculum seminis (Plate 31, mu). There is a possibility that the wall of the ovary possesses an intrinsic contractibility such as that described by Imms (1948) for insects. The only structures visible in the wall of the ovary are extremely fine plasma strands, the wall of the ovary being extremely thin (Plate 28).

The formation of the egg.

The parthenogenetic egg.

From the posterior germinal region of the ovary with small cells containing small nuclei, are formed a series of cells which pass anteriorly passing along the side of the ovary away from the alimentary canal. They eventually traverse the whole length of the ovary so that when the previous brood of eggs has been laid into the brood pouch the ovary consists of a mass of cells in front of the germinal region. These cells increase slightly in size towards the anterior end. They are arranged in groups of four, the four cells of each group usually being aligned one behind the other along the length of the ovary. Each cell contains a large nucleus with a large central nucleolus surrounded by a clear area containing chromatin threads and peripheral chromatin granules. The cytoplasm of the cell is of even granulation and does not stain deeply. All the four-cell groups in front of the germinal region develop together. They do not extend into the very anterior tip of the ovary which has a vesicular appearance, the extent of which varies according to the number of developing eggs.

Usually the ventral, or posterior, groups of cells begin to develop first. As they start to develop, all four cells of each group begin to grow in size and to accumulate yolk and fat. The first differentiation between the cells occurs when the third cell in each group counting from the germinal end begins to grow larger than the other three and its nucleolus also becomes larger. This third cell, the future egg cell of each group, then undergoes further changes. A number of oil droplets appear in the cytoplasm and the accumulation of parthenogenetic yolk increases. The structure of the nucleus of the egg cell changes. First it grows, and then growth stops and the nucleolus disintegrates, concealing the chromatin fibres. The nucleus is towards the middle of the yolk mass. The egg cell absorbs nourishment from the other three cells of the group, which are thus the food cells. The latter diminish in size and their nucleoli disintegrate to leave only remnants of chromatin within the plasma. The plasma of the food cells appears to be dissolved and absorbed by the egg cell. The vesicular tissue of the ovary probably plays an important role in the provision of nutritive material to the eggs. Claus (1876) believed that it converted the fluid nutritive material to the yolk and fat found in the eggs. Weismann (1877) stated that the fat separates from the blood first into connective tissue, identifiable with the fat cells, and then is further dissolved and separated for the egg cell. As well as deriving nourishment from the food cells, it has been suggested that the egg cell may be provided with nutritive material from

the tissue surrounding the ovary, especially where the cell is directly against the wall of the alimentary canal. The egg cell now consists of a mass of yolk surrounded by a thin plasma and with several fat droplets which fuse to give one or two spheres. The nucleus moves towards the periphery of the cell. Here it undergoes a ripening division which is an equation division since the chromosome number remains the same (Baldass, 1942; Mortimer, 1956). At this time the egg passes over into the brood pouch. The oil droplets are on the side of the egg next to the alimentary canal as are also the remains of the food cells. During the growth of the egg cells the germinal region of the ovary has been producing new groups of cells from the region opposite to the alimentary canal.

Eventually the egg cells grow to such an extent that they appear to fuse, their boundaries becoming indistinct so that the ovary has the appearance of a sausage-shaped mass filled with yolk composed of greenish spheres.

After the exit of the eggs from the ovary into the brood pouch, a number of finely granular plasma balls remain in the vesicular tissue. These are the remnants of the food cells. Occasionally they accompany the eggs into the brood pouch.

This account shows that from germinal region of the ovary to the brood pouch takes three moults. During the first the cells are produced by the germinal part of the ovary and move gradually anteriorly; during the second the differentiation and ripening of the egg cells takes place; while at the beginning of the third moult the eggs pass over into the brood pouch. The influence

of food conditions and other environmental factors will thus require at least two moults to affect the number of eggs.

The ephippial egg.

The ephippial egg may be formed at any time of the year and the same animal may produce either parthenogenetic or ephippial eggs. In Daphnia magna only two ephippial eggs are produced at a time, one from each ovary. The difference is easily discernible in the ovary when ephippial eggs are being formed instead of parthenogenetic eggs, the ovary appearing dark, being filled with opaque, dense, finely granular yolk (Plate 32; Plate 33; ov). The darkening appears first near the germinal area as patches of yolk form in the cells. The yolk formation progresses anteriorly until the whole ovary becomes dark. The ephippial eggs appear to develop from a fixed position situated posteriorly and ventrally in the ovary, at about the level of the fifth thoracic appendage. Weismann suggested that the position was fixed only by the necessity for secondary food cells in front of the ephippial egg to assist the primary food cells in the provision of nourishment. But these ideas were attacked by von Scharfenberg (1910-11) on the grounds that the ephippial egg always developed at exactly the same place, never more dorsally or slightly more anteriorly or laterally. As with the parthenogenetic eggs, the ephippial eggs are formed from four-cell groups but in this case two such four-cell groups, one behind the other, form one egg. The anterior is the larger of the two groups. Within this anterior group it is soon possible to recognise the future egg cell as it begins to grow larger than the other cells. Both groups of

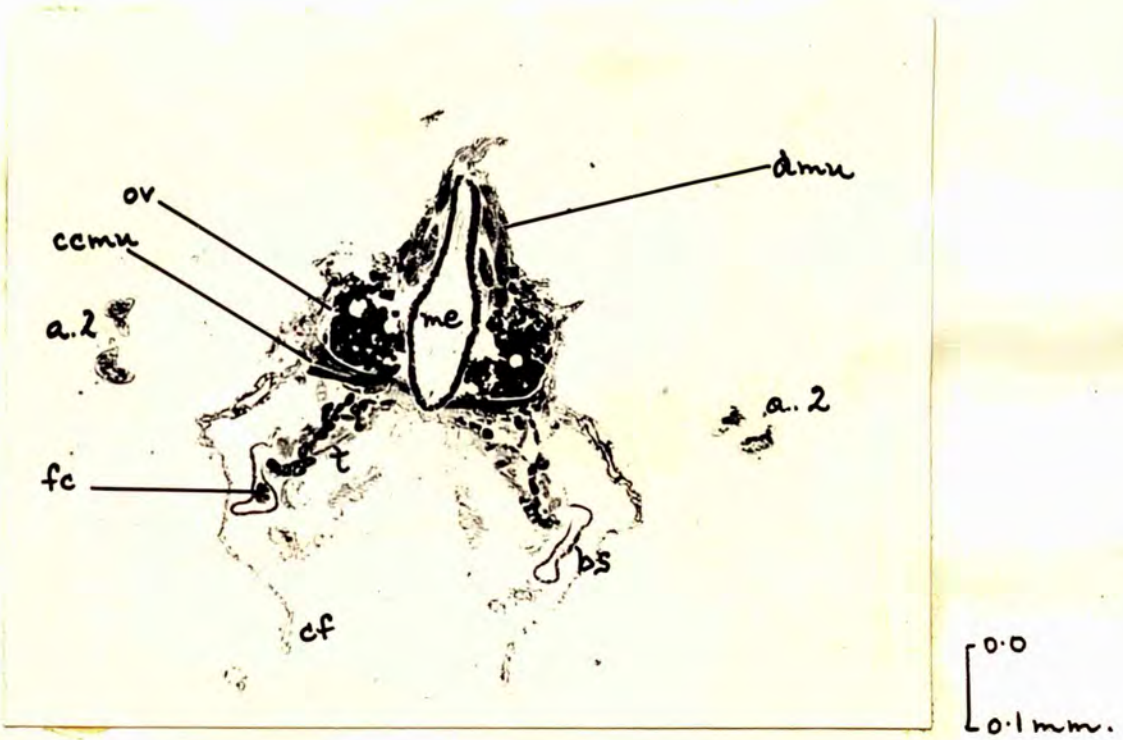


Plate 32. Photomicrograph of a transverse section through an adult Daphnia magna which is producing ephippial eggs showing the darkly-staining, dense yolk of the ephippial eggs in the ovary(ov). a.2,second antenna; bs,branchial sac; ccmu,adductor muscle of carapace; cf,carapace fold; dmu,dorsal longitudinal muscles, fc,fat cell, me,mesenteron; t,thoracic appendage.

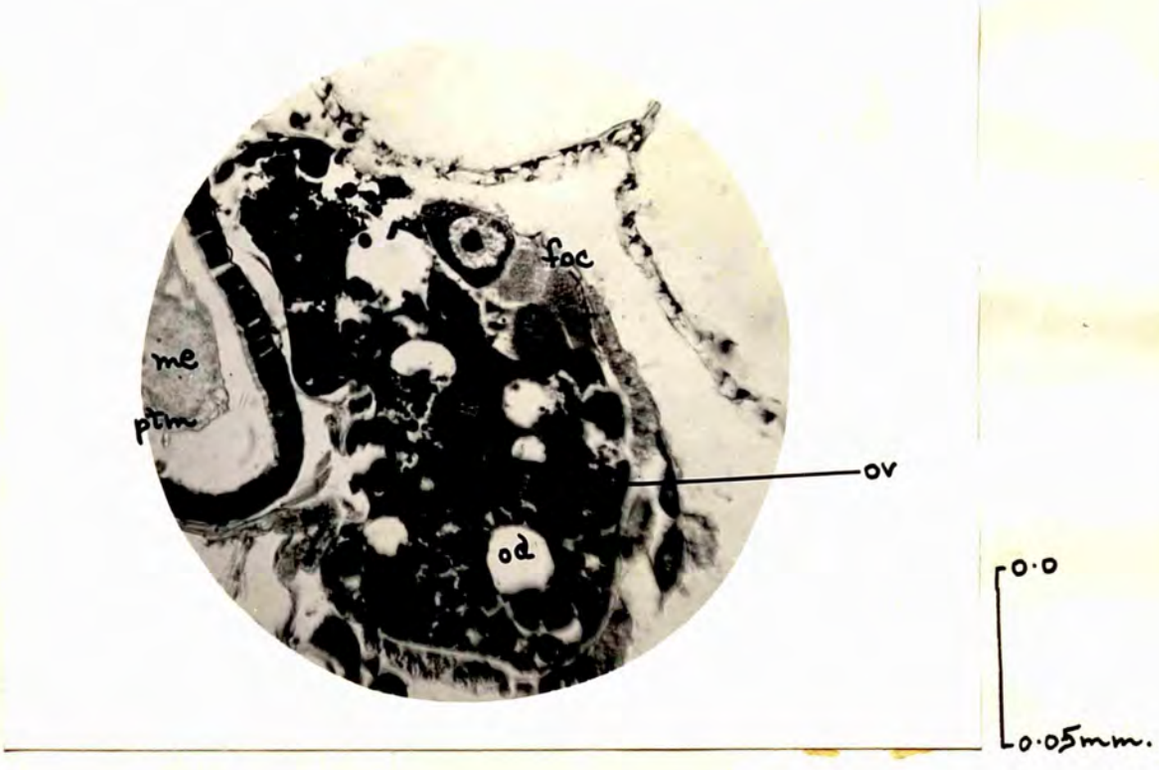


Plate 33. Photomicrograph of a transverse section through an adult Daphnia magna which is producing ephippial eggs showing one of the ovaries(ov) containing an ephippial egg which has fat droplets(od) and small adjacent food cells(foc). me,mesenteron; ptm,peritrophic membrane.

four cells increase in size and extend forwards, although the anterior remains the larger. The formation of yolk begins first in the anterior group, involving the deposition of small dark granules, smaller in size than those found in the parthenogenetic egg. Baldass (1942) found no fat droplets in the ephippial eggs of Daphnia pulex, but vacuoles of fat have been observed to occur in the ephippial eggs of D. magna while they are being formed in the ovary (Plate 33, od). The third cell of the anterior group becomes the egg cell, accumulating yolk and ripening as in the parthenogenetic egg. The three other cells of the anterior group are absorbed, functioning as food cells, as are all four cells of the posterior group (Plate 33, foc). Usually the third cell of the posterior group has also grown slightly larger than the others of its group, but like them it is resorbed and functions as a food cell. Sometimes more than one extra four-cell group starts to develop to be later used as food cells. Weismann believed that these extra four-cell groups which begin to develop are prospective parthenogenetic eggs which are used by the ephippial eggs; also that resorption of cells takes place from anterior to posterior, the dissolution of the first food cells taking place at the same time as thebeginning of the separation of the yolk in the egg cell. The primary food cells enlarge before they are resorbed. Further development is similar to that of the parthenogenetic egg. The ephippial egg extends forwards until it fills approximately half of the ovary, the anterior half of the latter being composed of vesicular tissue. Apparently this vesicular tissue has a function similar to that

in the production of parthenogenetic eggs, it provides nourishment. By the end of development of the ephippial egg, the yolky egg fills the greater part of the ovary and the nucleus moves towards the periphery of the cell. The ephippial eggs are nearly always fertilised and two polar bodies formed. Baldass believed that the ephippial egg was only laid into the brood pouch if it had been successfully fertilised. This cannot always be the case since arctic populations have been found in which ephippial eggs are produced and successfully hatch without the presence of males (Banta, 1926; Schrader, 1926; Edmondson, 1955, see later section). The eggs pass into the brood pouch immediately after copulation. Descriptions of copulation have been given by von Scharfenberg (1910-11) and Gravier (1951).

At the same time as the ephippial eggs have been developing the germinal layer has been giving off new groups of cells.

With the formation of ephippial eggs in the ovary, the brood pouch develops an ephippium (p.100).

There appears to be an initial period during which both kinds of eggs start to develop, the parthenogenetic and the ephippial. Under certain conditions the ephippial cell group gains dominance over the surrounding cells which it absorbs. In other cases the ephippial cell group stops developing and parthenogenetic eggs are formed. These are referred to as abortive ephippial cell groups, a slight darkening beginning in the neighbourhood of the germinal area, later clearing.

Male.

Early workers could not find the testes which were first

described by Lubbock (1857) and three years later by Leydig (1860). Claus (1876) refers principally to the sperm. Brief descriptions of the testes are included by Chambers (1913), Taylor (1915) and Mortimer (1936) in their accounts of spermatogenesis.

The testes of the male occupy a similar position to the ovaries of the female on either side of the mesenteron. Each testis is branched at its anterior end, while posteriorly it is continued as the vas deferens. In the early instar male the testis (Fig.130, te) is composed of irregular cells of uniform size and with granular cytoplasm. The adult testis (Fig.131; Fig.132; te) has an outer wall of large, loosely arranged cells surrounding an inner non-cellular lumen filled with a trabecular mesh. The spermatogonia develop in the cells of the wall of the testis and are discharged into the lumen. In the mature male the lumen of the testis is usually filled with spermatozoa, the walls of the testis being very thin. There are also a number of "giant cells" in the wall of the testis. They are several times the diameter of the other cells and have large nucleoli. These cells change little during the life of the animal.

A description of spermatogenesis has been given by Mortimer (1936) and also by Chambers (1913) and by Taylor (1915). The sperm are at first round, later elongate and have conspicuous chromatin.

Each vas deferens opens on the dorsal side of the abdomen close to the caudal furca. It terminates on a papilla and has a small muscle.

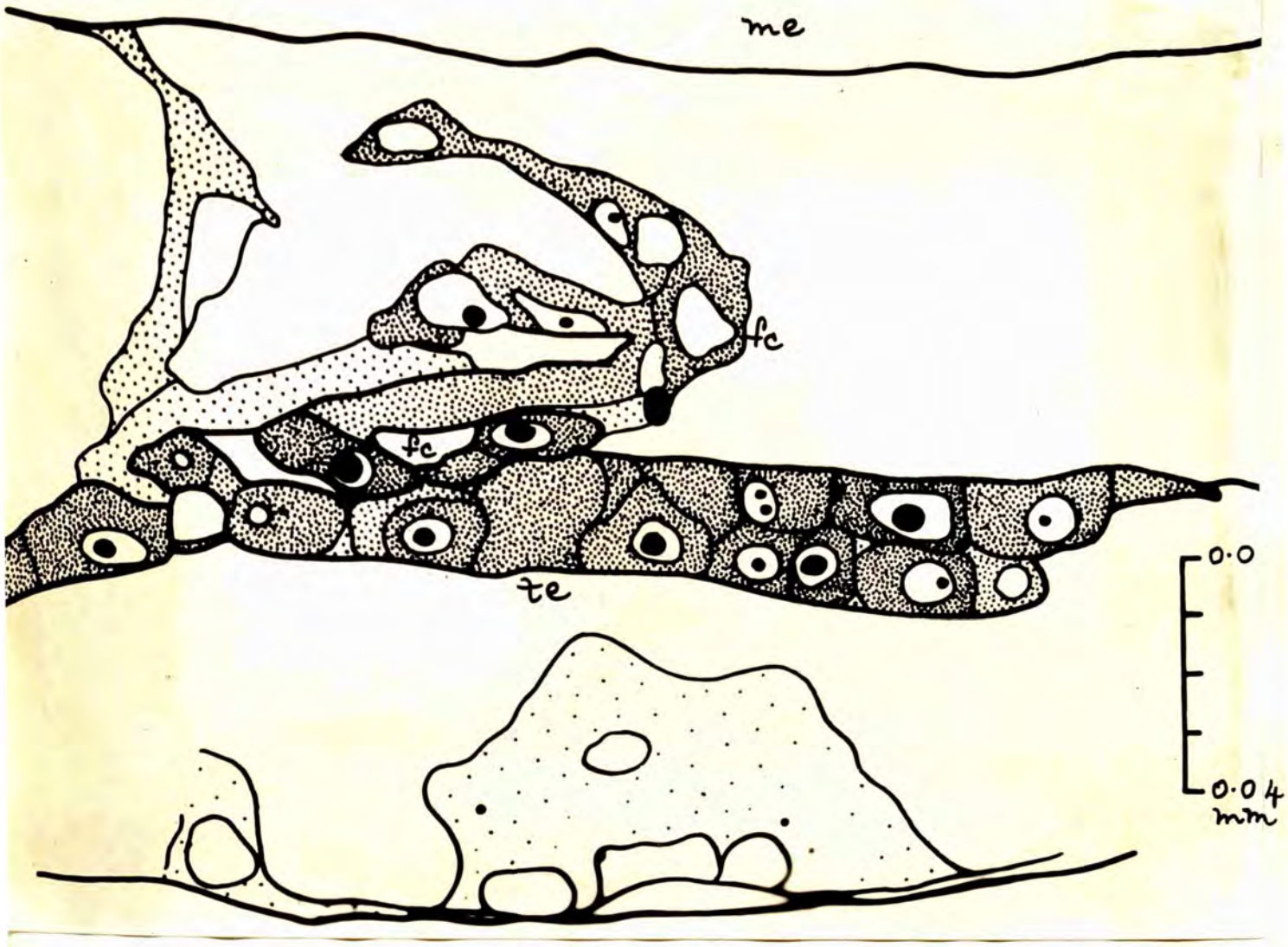


Figure 130. Vertical longitudinal section through a first instar male Daphnia magna showing the testis (te) and adjacent fat cells (fc). The cells of the testis are almost uniform in size. me, mesenteron.

e. The Circulatory System.

The circulatory system is composed of the heart together with blood spaces defined by septa or membranes.

An early description is that of Claus (1876). He describes the heart wall, composed of muscle cells together with an intima, and gives an almost completely accurate account of the circulation. Claus was followed by Hérouard (1905) who gives a more complete description of the septa enabling him to give a more exact account of the course of the flow of blood as it passes through the maxillary region. Otherwise his account of the circulation is similar to that of Claus. A description of the muscular wall of the heart is given by Binder (1952), and of the septa in relation to the distribution of fat cells by Jäger (1955).

The heart.

The heart (Fig.133, he) is situated immediately anterior to the brood pouch (br) and between the alimentary canal (me) and the dorsal surface. In front of it lie the muscles of the second antenna (a.2 mu). It has a single pair of ostia (os), one on either side of the heart. The ostia are slightly closer to the dorsal side of the heart than the ventral. The heart is a single sac-like organ, approximately ellipsoid in shape with rounded ends and slightly constricted in the middle in the region of the ostia.

The wall of the heart consists of a layer of fusiform muscle stretched dorso-ventrally together with an area of longitudinal muscle. The longitudinal muscle is internal to the dorso-ventral or transverse muscle and is present in the dorsal

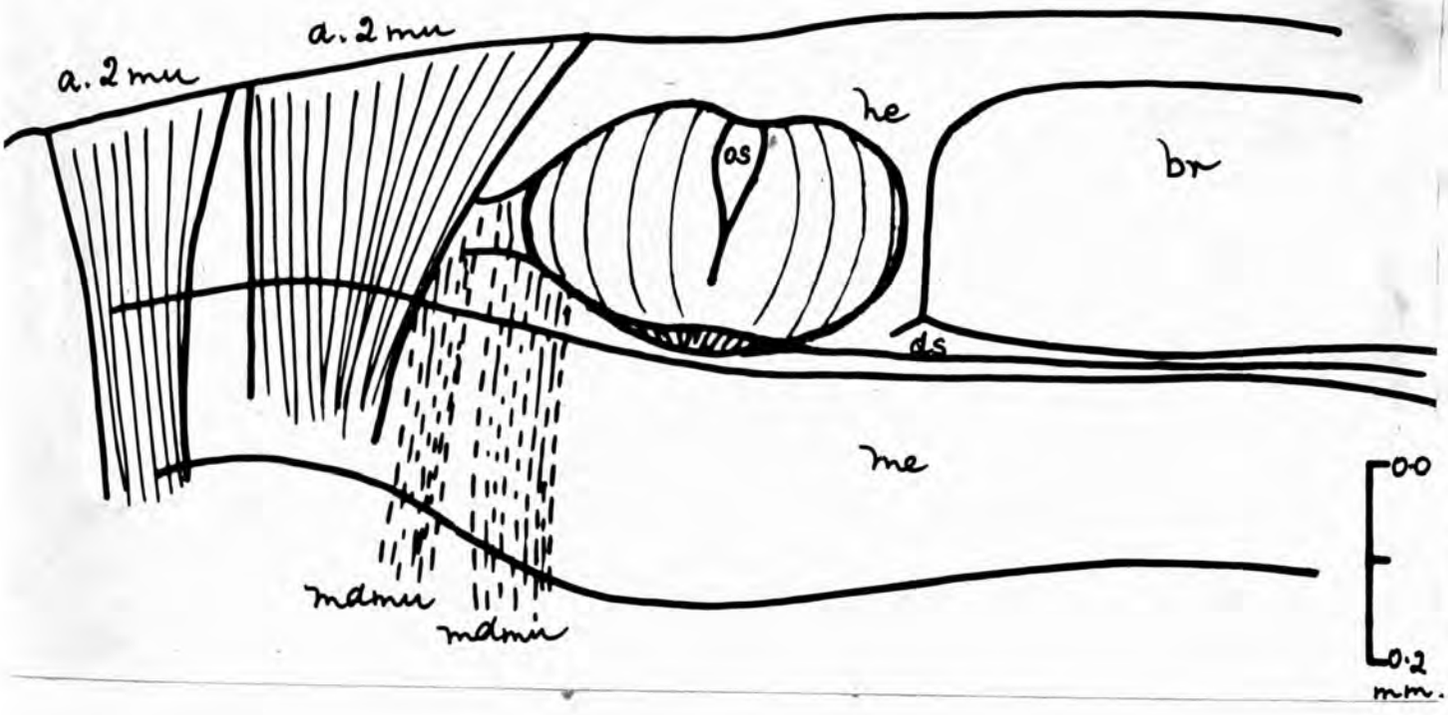


Figure 133. Diagram from a living adult Daphnia magna showing the heart (he) with one of the ostia (os), circular muscles and a very short anterior aorta.
a.2mu, muscle of second antenna; br, brood pouch; ds, dorsal septum, mdmu, muscle of mandible; me, mesenteron. Freehand drawing.

and ventral parts of the heart (Plate 34; Plate 35; lhemu). These longitudinal muscles are not mentioned either by Hérouard (1905) or by Binder (1932). They are a separate layer and not a deflection of the transverse fibres. The transverse fibres converge towards the centre dorsally and ventrally owing to the shape of the heart. At the two points of convergence the wall is slightly thickened, in part by tendinous material, and this thickening remains in position when the heart contracts. From the ventral thickening a number of trabeculae extend to the dorsal septum (Plate 34, ds). During the systole the ventral thickening approaches the dorsal and the heart is also shortened, possibly owing to contraction of the longitudinal muscles.

The muscle cells have a predominance of contractile fibres in the outer part of the cell and of protoplasm in the inner part [redacted]. There is no intima although one was mentioned by Claus. The muscle fibres show a fine cross striation and are apparently usually in pairs (Plate 36, he). They are surrounded by protoplasm, very thin on the outside, thicker on the inner side. The nuclei of the cells form a slightly curved row. Claus suggested that in the larger species of Daphnia, such as Daphnia magna, the muscular wall of the heart was composed of two bands of cells lying together and probably formed by the division of a single row. This is possibly in agreement with the observation that the fibres appear to lie in pairs.

The edges of the ostia have grown out to form a small rim. The inner lip is very narrow.

The heart is suspended in the pericardial cavity (pe).

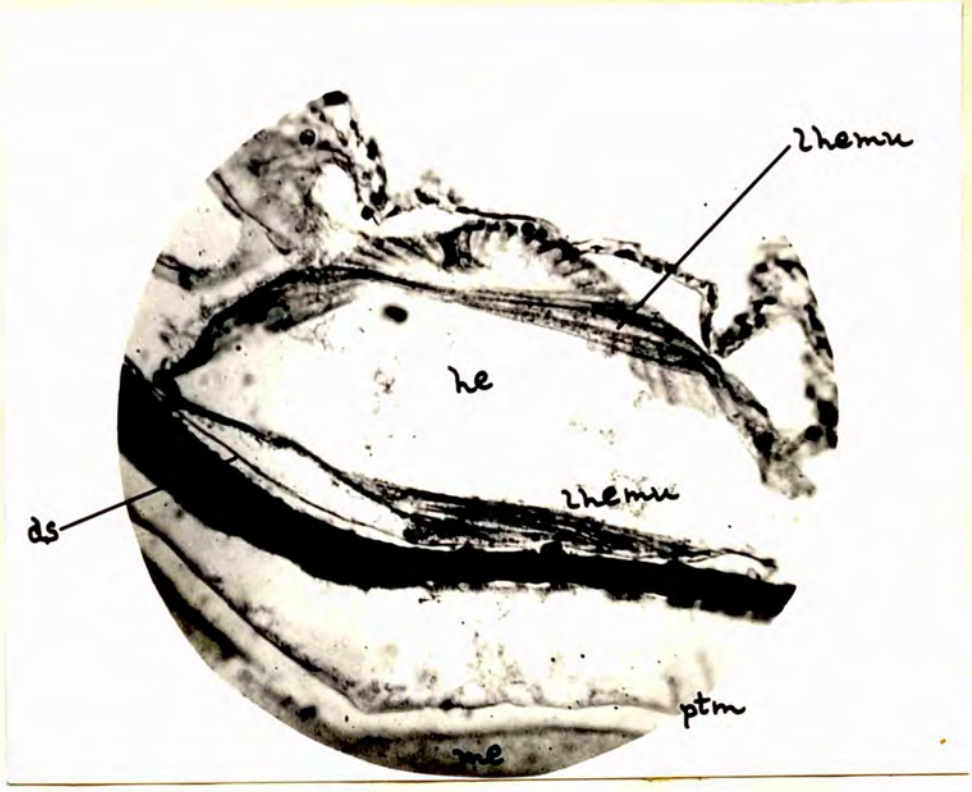


Plate 34. Photomicrograph of a vertical longitudinal section showing the heart(he) with longitudinal muscle(lhemu) dorsally and ventrally. ds,dorsal septum; me,mesenteron; ptm,peritrophic membrane.

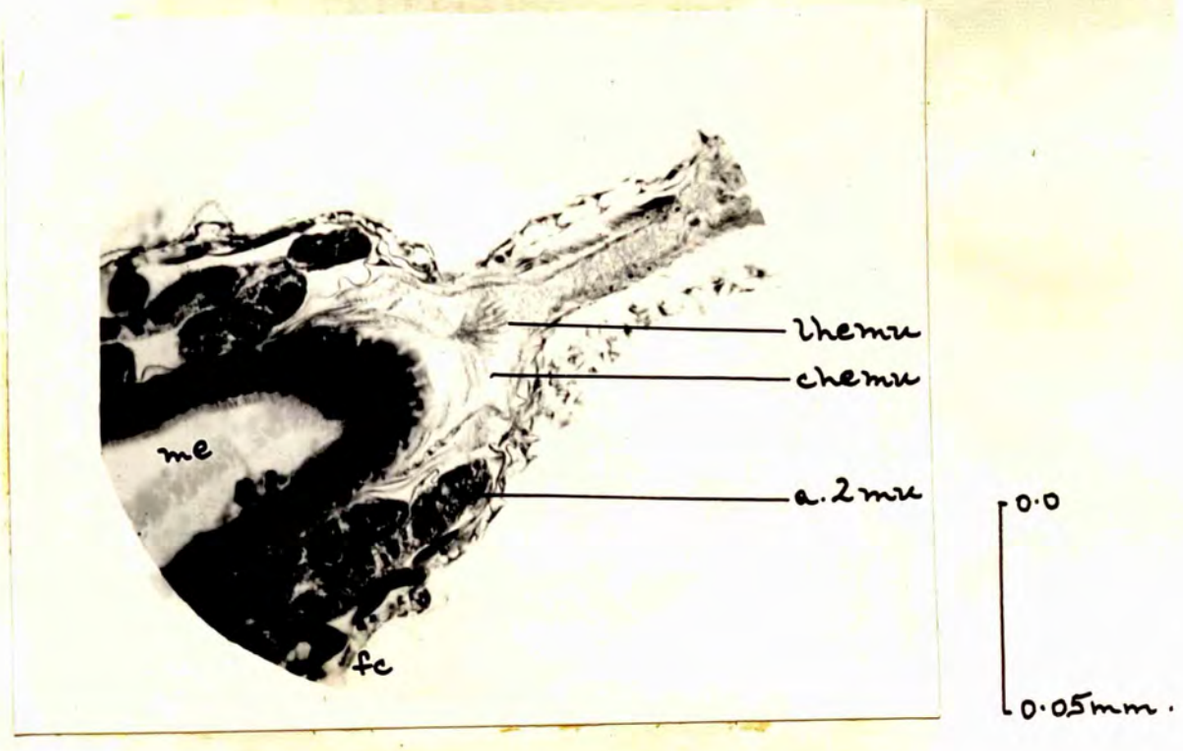


Plate 35. Photomicrograph of a horizontal longitudinal section showing circular(chemu) and longitudinal (lhemu) muscles of the heart. a.2 mu,muscle of the second antenna; fc,fat cell; me,mesenteron.

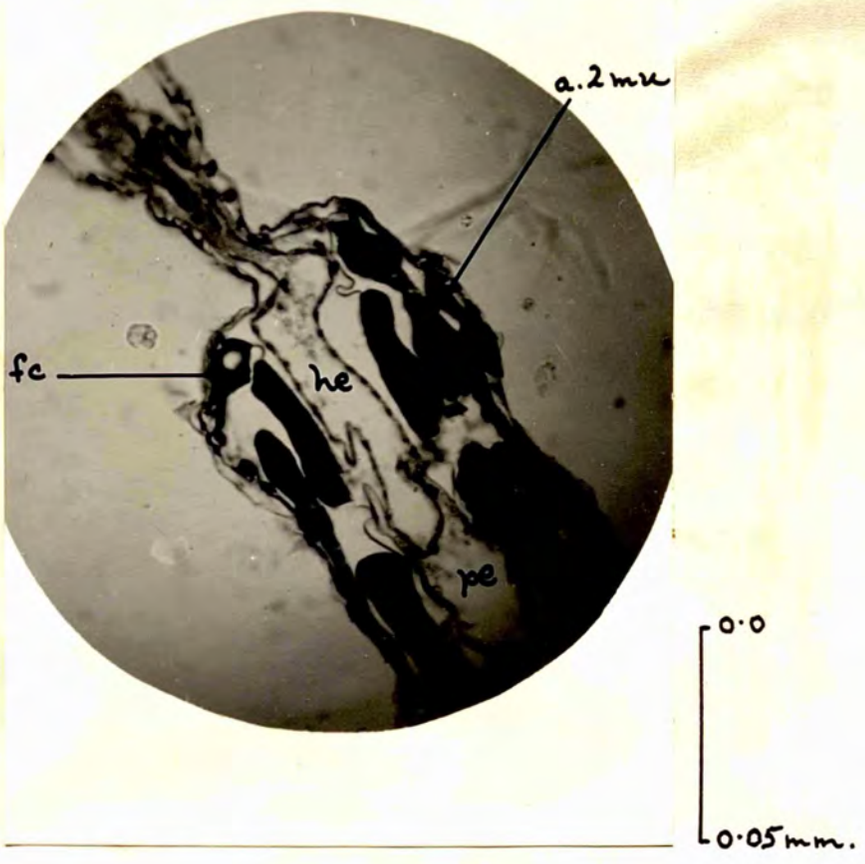


Plate 36. Photomicrograph of a horizontal longitudinal section showing the wall of the heart(he) composed of pairs of muscle fibres. a.2 mu,muscle of second antenna; fc,fat cell; pe,pericardium.

Dorsally it is attached to the hypodermis, and ventrally connected to the dorsal septum by a group of fine trabeculae. The dorsal septum is immediately dorsal to the alimentary canal and forms the ventral wall of the pericardium. The pulsations of the heart impart to it an oscillatory movement which helps to propel the blood towards the general body cavity.

From the anterior end of the heart leads a very short wide aorta (Fig.133) formed of thin [redacted] walls. It is equivalent to an anterior narrowing of the heart without the muscle fibres, and is situated in the region of the rotator muscles of the mandibles. The dorsal wall is slightly the longer and at its anterior end turns slightly dorsalwards. The aorta pulsates in synchronization with the heart beat.

The mode of action of the heart.

A review of the previous work on the mode of action of the heart of Arthropods was published in 1952 by Krijgsman. In it are summarised the results of various pharmacological experiments on the Daphnia heart which have led to the classification of the heart of Daphnia as myogenic. Acetylcholine probably has a slowing action on the heart, as also have anticholinesterases and digitalin. The action of adrenalin is doubtful, and in this respect it should be noted that the results of Lévy (1927) are open to doubt since both Obreshkove and Fraser (1940) and Hoshi (1951,b) found that the modified Ringer solution used by Lévy prevented the development of eggs. Recently Flückiger (1951, not mentioned by Krijgsman) has found that adrenalin increases the heart rate. Needham (1950) concluded that the heart of Daphnia

was myogenic, with cardiac activity outlasting all other movement except that of the alimentary canal. He pointed out that it is not necessary to conclude that a myogenic heart is devoid of nerve cells; Prosser (1942) had also concluded that the heart of Daphnia is probably myogenic but innervated; Bekker and Krijgsman (1951) reported it myogenic with extra-cardiac inhibitory nerves. It is probable that the heart of Daphnia resembles that of vertebrates more than the heart of Malacostraca. In 1952, Flückiger and Flück fed D. longispina with pure starch and found modification of the heart rate, which was restored to normal by the application of aneurine. Flückiger (1953) studied the action of various sympathomimetic agents and of dihydroergotamine on cardiac activity in D. magna and D. pulex.

The septa defining the blood spaces.

The blood spaces are defined by a series of septa: the dorsal septum, the ventral septum, the vertical septum and the abdominal septum. The septa are frequently perforated but the perforations do not affect the main course of the flow of blood.

The dorsal septum:-

The dorsal septum is a prolongation of the ventral wall of the pericardium (Fig.133; Fig.134; Plate 34; ds) and is readily visible in the living animal. Its anterior border is attached to the dorsal integument immediately in front of the heart at the posterior edge of the head and just behind the antennal muscles. The line of insertion is arched to give a posterior concavity. The septum descends to the level of the alimentary canal and then curves posteriorly just dorsal to the peduncle of the carapace.

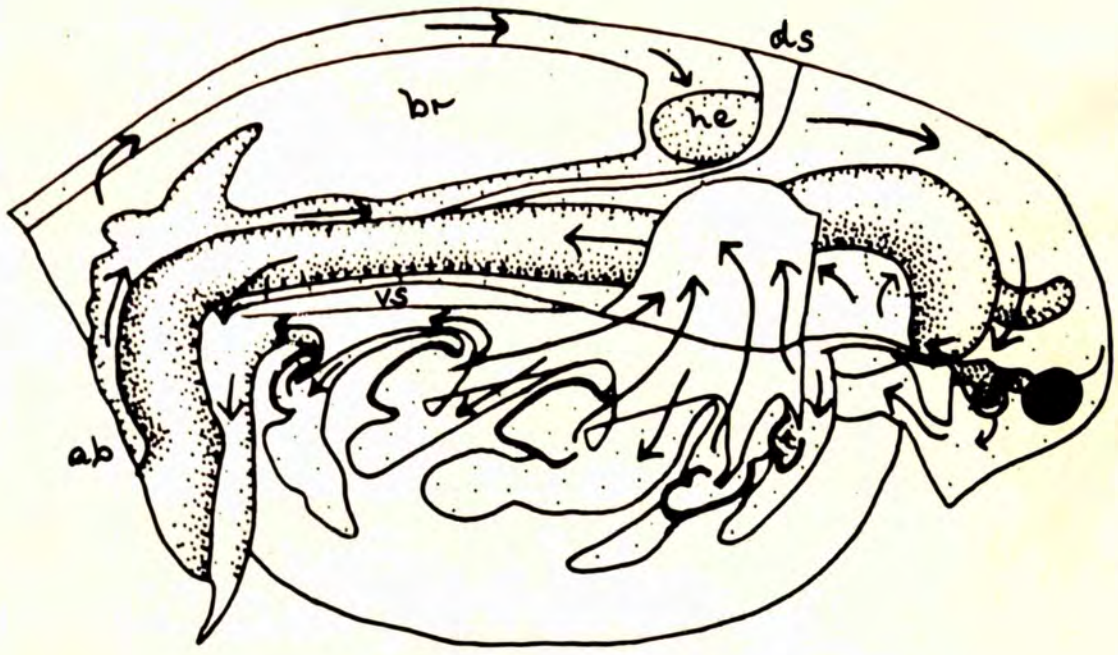


Figure 134. Diagram to show the course of the circulation of the blood through the adult Daphnia magna and the positions of the septa. (after Hérouard). ab, abdomen; br, brood pouch; ds, dorsal septum; he, heart; me, mesenteron; vs, ventral septum.

The dorsal septum bulges towards the heart in the region where the trabeculae are attached. It continues posteriorly immediately dorsal to the alimentary canal until it terminates at the beginning of the abdomen. In the anterior vertical region of the septum there is an opening for the passage of the short aorta from the heart.

The dorsal septum thus forms the anterior and ventral walls of the pericardium. The pericardial space communicates dorso-posteriorly with the carapace folds and ventro-posteriorly with the dorsal cavity of the body. Laterally it is delimited by the walls of the two deep blind anterior projections from the brood pouch (Plate 37, apr). These pouches extend forwards to approximately the level of the transverse mandibular muscles. Their diameter increases posteriorly, the pericardium becoming narrower, until they join behind the heart to form the brood pouch.

The ventral septum:-

The ventral septum (Fig.134) is not easily seen in the living animal. It extends between the alimentary canal and the ventral integument from the head region to the posterior extremity of the thorax, behind the last thoracic appendage. Laterally it is united to the hypodermis on the side of the body. At its anterior limit the ventral septum surrounds the stomodaeum medially, laterally extending further forward to the region of the optic ganglia, whence two lateral horns project to be inserted on to the hypodermis anterior to the brain and ventral to the optic ganglia. Posterior to the stomodaeum the ventral septum lies close to the ventral integument. Just anterior to the mandibular region, the

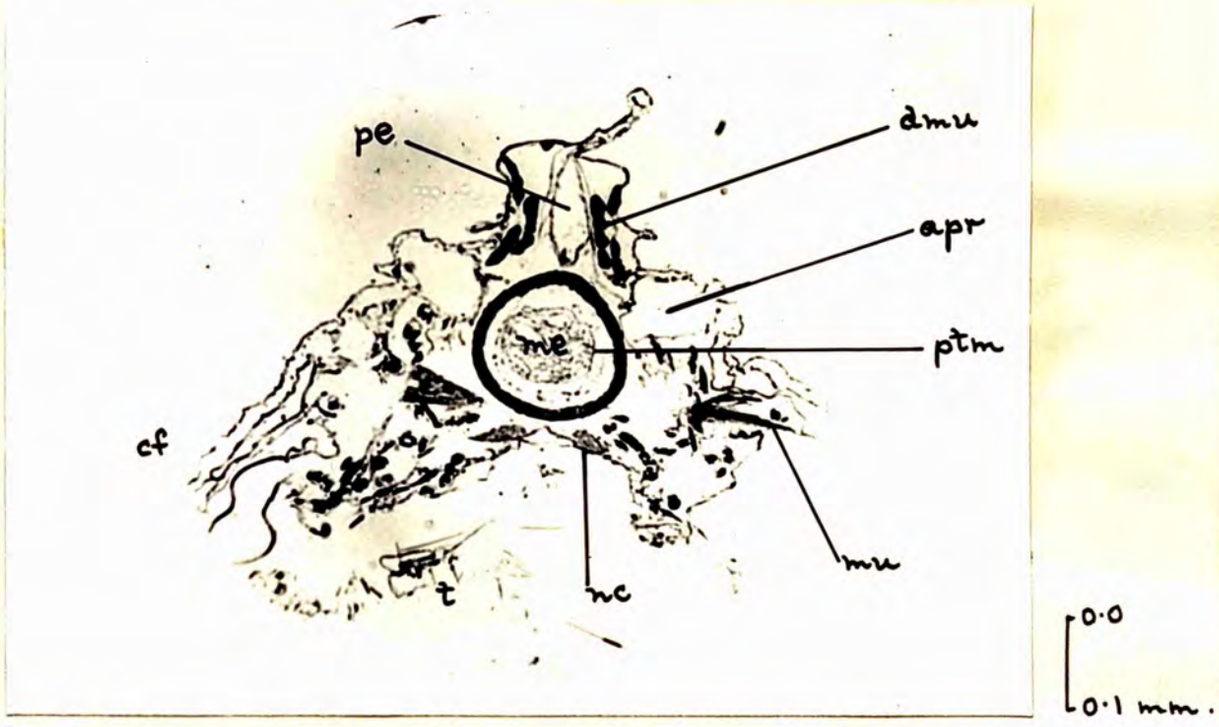


Plate 37. Photomicrograph of a transverse section showing the anterior projections(apr) from the brood pouch. cf,carapace fold; dmu,dorsal longitudinal muscle; me,mesenteron; mu,muscle; nc,nerve cord; pe,pericardium; ptm,peritrophic membrane; t,thoracic appendage.

septa form a small compartment around some of the muscles of the second antenna. Posterior to the maxillar region the septum (Plate 32, vs) is attached medially, causing a median furrow and delimiting a pair of ventral cavities. The cavities extend to just dorsal to the insertion of the thoracic appendages, lateral to the ovaries. The anterior extension of the ventral septum has therefore divided off a cavity in the anterior region of the head. This cavity extends into the first antennae and the labrum and contains the brain, the oesophageal commissures, the sub-oesophageal ganglia and part of the ventral nerve cord.

A broad lateral flap of the ventral septum begins, anteriorly, at the junction of the head and the thorax where the line of insertion of the septum turns abruptly towards the dorsal surface and reaches almost to the dorsal septum; from here the line of insertion is parallel to the dorsal surface for approximately the length of the heart; it turns abruptly towards the ventral surface again to reach the level of the fourth thoracic appendage, and then continues posteriorly parallel to the ventral groove. This flap circumscribes the borders of the junction of the carapace fold with the body and therefore delimits a cavity which communicates with the cavities of the first pairs of thoracic appendages and also with the cavity of the carapace fold, which contains the maxillary gland.

Posteriorly the pair of ventral cavities extend immediately above the thoracic appendages and communicate with the cavities of the latter. The cavities of the two sides communicate with each other only anteriorly at the level of the mouth parts and

posteriorly in the abdomen where the septum terminates.

The vertical septum:-

The vertical septum (Fig.134) divides the two symmetrical cavities formed by the ventral septum in half again longitudinally. It extends perpendicularly from the horizontal ventral septum into the cavity of each thoracic appendage where it ends in a prong. The edges of the prong are attached to the hypodermis, and the tips are attached to the base of the exopodite. Communication between the two cavities, external and internal, formed by this septum, is possible through the truncated area of each prong. The communicating narrow orifice is adjacent to the branchial sac, or epipodite, which functions in respiration.

The abdominal septum:-

A small vertical transverse membrane occurs immediately anterior to the posterior flexure of the alimentary canal, between the adductor muscles of the abdomen, and attached dorsally to the ventral wall of the mesenteron. From the lateral edges of this septum, a pair of small extensions project posteriorly, one on either side of the alimentary canal. The presence of the abdominal membrane was first mentioned by Jäger (1935), who notes that it carries a particularly abundant accumulation of fat cells. He also describes two small flaps at the posterior end of the ventral septum, the anterior one at about the level of the fifth thoracic appendage and the posterior one at about the level of the beginning of the abdomen.

Intestinal cavity.

The alimentary canal and the reproductive organs are enclosed

within a cavity formed by the dorsal and ventral septa. The lateral walls are the hypodermis of the integument, except antero-dorsally where this is replaced by the anterior projections of the brood pouch. Anteriorly the cavity encloses the internal projection of the eye with the optic ganglia, the midgut caeca and the anterior flexure of the alimentary canal. The more posterior part of the cavity encloses the alimentary canal and the reproductive organs, extending to include the rectum and ending as an open tube. The walls of the cavity are capable of being distended to allow increase in the size of the ovary, and in older females there is sometimes a fold left when the eggs have just been laid.

Distribution of muscles within the cavities.

Individual muscles do not transverse the septa, each being entirely within one cavity. Often the muscles lie close to a septum for the greater part of their length. The cavity dorsal to the dorsal septum contains the dorsal longitudinal muscles; the median, or intestinal cavity, contains the muscles of the second antennae and the dorso-ventral muscles of the body; the cavity ventral to the ventral septum contains the muscles of the other appendages together with the ventral longitudinal muscles.

Structure of the septa.

The septa are formed of extremely thin, large connective tissue cells, according to both Hérouard (1905) and Jäger (1935). My investigation has proved that this is probably correct although cell boundaries are difficult to discern. The septa have numerous perforations which enable communication between the different

cavities but do not alter the main flow of blood. The tracing out of the septa is greatly facilitated by the presence of the fat cells and their replacement cells, which tend to accumulate on the septa. The fat cells are less numerous on the dorsal septum and the more dorsal parts of the ventral septum. The distribution of the muscles is also of assistance in following the septa.

Kollman (1924) describes four different types of connective tissue in Phyllopods, with particular reference to Apus productus, now Lepidurus apus, of which two constitute a network between the organs and are probably related to the septa of Daphnia. He regards the cells as stellate and forming a meshwork by the anastomosis of their projections. This is possibly due to shrinkage caused by inadequate fixation.

The recent paper by Debaisieux (1954) indicates septa in Triops cancriformis similar to those found in Daphnia and apparently also composed of thin connective tissue cells not forming a meshwork (see Debaisieux, Fig. 42a).

The blood.

The colour of the blood varies from colourless to light yellow to red according to the amount of haemoglobin which varies with the influence of several factors (Fox, 1948; Fox et al., 1949; Fox et al., 1951; Fox and Phear, 1953; Green, in press). The blood may also occasionally be coloured green owing to the presence of carotenoids (Green, personal communication). The blood contains a number of small cells or corpuscles varying in quantity. The blood cells of Daphnia are small, about 7-8μ in diameter, amoeboid

cells with granular cytoplasm. The amount of granulation varies. The cells often adhere to other tissues, and since the capacity for adhesion varies this possibly accounts for the apparent variability in the number of the blood cells. In the case of slight injury, the blood cells collect together to block the wound. When swept into the blood stream away from their temporary attachment to other tissues, the cells remain for a while adhering by a single process which eventually breaks (Hardy, 1892). It is generally said that they reproduce by direct fission (see p.222). The blood cells are also reported to collect fat (Hardy, 1892) and Jäger (1955) believes that some of the blood cells become fat cells. The blood cells of Arthropoda are usually involved in phagocytosis and clotting, but clotting need not necessarily occur (Maluf, 1959). The question of phagocytic cells and their relation to the blood cells will be discussed in the section on fat cells (p.165).

Circulation.

On contraction of the heart the blood leaves by the short anterior aorta and flows forwards between the dorsal integument and the anterior flexure of the alimentary canal (Fig.134). The curvature of the head forces the flow of blood downwards between the midgut caeca and past the compound eye where it travels through the space between the stalks of the optic ganglia. Here the current divides into two branches. A ventral branch passes below the ventral septum into the rostrum and the anterior part of the ventral cavity, bathing the first antennae, the labrum and the brain, thence through the maxillary region to the two ventral

cavities. The remainder of the blood stream forms a pair of lateral and more dorsal branches which flow to the intestinal cavity by passing close to the optic ganglia.

The ventral branch continues through the ventral symmetrical cavities and into the cavities of the thoracic appendages, the internal and then the external section. As the blood passes through the narrow orifice between the two sections, according to Hérouard the branchial sac acts as an extensible vesicle and gives a new propulsion to the blood flow. The blood returns to the ventral cavity from the first four thoracic appendages in the area of the attachment of the carapace to the body and flows directly into the carapace folds. It passes first along the ventral border, then to the dorsal border and so to the pericardium. Some of the blood from the fourth and all the blood from the fifth thoracic appendages joins the blood flow from the intestinal cavity at the end of the ventral septum and the beginning of the abdomen.

The intestinal current flows through the intestinal cavity, past the alimentary canal and the genital organs, to the abdomen, joining here the small branch from the last thoracic appendages. In the abdomen it passes down the anterior or ventral side, then crosses the alimentary canal and flows up the posterior or dorsal side to pass dorsal to the dorsal septum and so to the pericardium.

f. The Fat Cells.

Daphnia contains a number of large cells with large characteristic nuclei and a variable amount of enclosed fat. The fat cells may be very prominent in a living animal that has been well fed.

An account of the fat cells of Daphnia was given in 1935 by Jäger on whose work much of the present description is based. Earlier Leydig (1860) had referred to the fat cells in the branchial sacs, and Claus (1876) had also recognised their presence in the thoracic appendages and recognised that these were fat-containing cells which lose their fat when the animal is poorly fed.

Distribution.

The fat cells occur attached to muscles, especially where these are in contact with the septa of the blood spaces. They are also found on the muscles of the alimentary canal. They occur on both sides of the septa themselves, with the exception of the dorsal surface of the dorsal septum. The exception is probably due to the narrowness of the space between the septum and the wall of the brood pouch and the steady flow of blood through this space. The fat cells are distinctly separated from the septal tissue and do not arise within them.

The distribution in general follows that of the septa. There are no fat cells in the head. In the carapace folds the fat cells form plates which originate from the ventral septum. In the thorax the fat cells are found in the appendages (Plate 32, fc) in both endopodite and epipodite, but not in the exopodite. There is also a plate of fat cells in the lateral part of the body

extending dorsally close to the ovary (Plate 27; Plate 30; Plate 38; fc). In the abdomen there is a plate of fat cells medially between the adductor muscles of the abdomen and two plates on both sides of the mesenteron, converging towards the abdominal membrane (Plate 17, fc). The most anterior extent of the fat cells is usually the mandibular muscle. The fat cells are especially numerous on the ventral septum, on the abdominal septum and in the lateral part of the body. They may also be present in large numbers in the thoracic appendages. The degree of development of the fat cells varies in accordance with sex, state of nutrition and stage of the reproductive cycle of the female.

The relation of the fat cells to the ovary.

In the early stages of the development of the eggs in the ovary, the fat cells contain abundant fat which increases with the growth of the four-cell groups in the ovary. With the beginning of yolk formation in the ovary the fat in the fat cells decreases. It is not clear how the fat gets from the fat cells to the ovary to form the yolk. Hérouard (1895) believed that the fat cells deliver their food reserves directly to the ovary. Jäger (1935) believed that fat transport to the ovary is probably via the blood but was uncertain if the cells or the plasma are responsible. He pointed out that while the fat cells are losing fat the blood cells often lie adjacent to them.

Histology.

The fat cells are individual cells which exist in various states. Jäger (1935) suggests that there are two kinds of cells: small, spindle shaped cells found distributed throughout the body; and

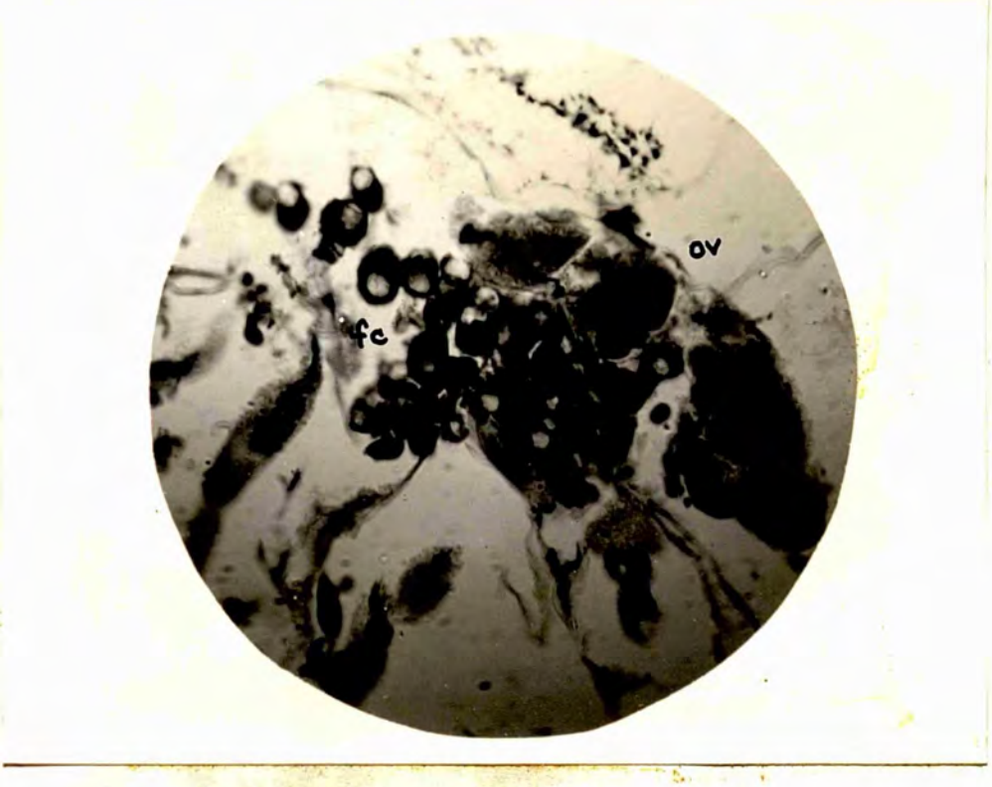


Plate 38. Photomicrograph of a vertical longitudinal section showing the group of fat cells(fc) close to the ovary(ov).

large, round cells found in the epipodites and the region of the mandibles. Large well-fed Daphnia have mostly the latter type of cell, while small poorly fed Daphnia have mostly the former.

The fat cells are nearly always conspicuous cells which stain heavily and contain a large, darkly staining nucleus and prominent droplets of fat (Plate 21; Plate 27; Plate 30; Plate 38; fc). The cells are basophilic. The most usual phase has a large nucleus containing a central nucleolus which stains intensely and is surrounded by a hyaline area. The nucleolus may contain a vacuole and apparently may break up into two or three large chromatin bodies. There may also be a number of small chromatin bodies scattered through the hyaline area of the nucleus or accumulated around the periphery or in a ring around the nucleolus. The cytoplasm is moderately dense and contains one or more fat droplets. The fat droplets are the only form of special inclusion in the fully developed cells. They are variable in both number and size. The largest are of approximately the same size as the hyaline nuclear vesicle. This phase is recognised as the 'fat-present' phase.

But the fat cells do not necessarily contain fat. In the 'fat-free' phase the appearance of the cells is similar to the 'fat-present' phase except for absence or small amount of the fat. Instead the cells contain granules which are arranged around the gradually appearing fat droplets. As the amount of fat increases, the granules decrease.

The fat cells store both fat and glycogen.

Formation.

The fat cells are formed by resorption of yolk from the yolk cells (p. 80). This process is still taking place in the hatched young. The yolk cell consists of a large quantity of yolk surrounded by a narrow peripheral area of cytoplasm containing the nucleus. The peripheral cytoplasm increases in extent and the nucleus assumes the form found typically in fat cells. A vacuole develops towards the edge of the central yolk and increases in size at the expense of the yolk material. Refractive granules appear in the yolk. The acidophilic region of the plasma is surrounded by a basophilic region and the cell gradually becomes entirely basophilic. A typical fat cell is gradually formed.

Breakdown.

When food supplies are poor, the fat cells break down to supply their reserve materials for the nourishment of the animal. Also, fat is plentiful in the fat cells when the ovary contains four-cell-groups but as yolk formation begins in the ovary the fat cells begin to break down.

The fat cells become entirely disintegrated. The nucleus breaks up, the nucleolus dividing into several pieces of various shapes and sizes until the nucleus contains a collection of small dark irregular granules. The nuclear membrane dissolves. The fat content has decreased and continues to lessen until it disappears. The cytoplasm of the cell stains less deeply and the edges of the cell curve inwards. There remains a small pale-staining cell of uneven outline with, near the centre, a large hyaline nuclear area containing a few irregularly shaped, darkly

staining bodies. Disintegration takes place from the posterior end of the body towards the anterior.

Replacement.

Simultaneous and adjacent to disintegration, replacement occurs. The renewal of the fat cells takes place from blood cells and from replacement cells (Jäger, 1935). Close to the septa, especially in the abdomen, are found round or spindle-shaped cells whose nuclei are of the kind typical for blood cells. These cells are different from the usual blood cells and the change to become fat cells is detectable. Blood cells tend to adhere to any part of the body but will only aggregate where the circulation is slowed down or checked, such as in the abdominal flexure. If some of these adhering blood cells form fat cells, while the old fat cells degenerate, the distribution of fat cells in the older animals must differ from the distribution in the younger animals, since the smooth septa do not retain the blood cells which will form fat cells only where the circulation is hindered. But the distribution in both young and old animals is similar. Further the number of blood cells found on the septa is few, while the number of fat cells found is many. It therefore appears that there is some other element which also contributes to the formation of fat cells, the replacement cells.

Replacement cells are elongate cells with nuclei similar to those of the blood cells. They contain yolk spheres which become evenly distributed lumps of yolk. The yolk is resorbed and the nucleus develops a distinct nucleolus. Later the nucleolus becomes indistinct again and the resulting cell is similar to a

blood cell and found attached to septa but it never circulates. When fat cells disintegrate, the nucleus of the replacement cell comes to resemble that of the fat cell but the cell remains smaller than the fat cell.

Neither the fat cells nor the replacement cells undergo mitosis.

Chemical content.

Jäger (1935), by microchemical tests, identified a number of substances in the fat droplets. These substances are oleic acid, sodium oleate, triolein, cholesterinoleate, stearic acid, sodium stearate, cholesterin-stearate, tristearin, as well as lecithin, linseed oil and linoleic acid. Gallistel (1936-7) found that the fat cells contain abundant fat and glycogen, and also that the males contain especially abundant quantities of fat. This was found also by Smith (1915) and is in contrast to Artemia where Lochhead and Lochhead (1941) found that the stored fat was more abundant in females than in males.

Haemoglobin is present in the fat cells of Daphnia magna (Fox, 1955). The fat cells also contain iron which is present in greatest quantity when the animal is losing haemoglobin (Smaridge, 1954). Smaridge suggests that the haemoglobin is broken down in the fat cells, and this is supported by the work of Green (1955). The fat cells also contain carotenoids.

Phagocytes.

The occurrence of phagocytosis in Daphnia magna infected with spores of the fungus Monospora bicuspidata was observed by

Metschnikoff in 1884. From his observations Metschnikoff developed his well known views on resistance to disease. In cases of infection by fungal spores, a number of blood cells were observed to cling to each spore. In addition to the blood cells, Metschnikoff noted that the only cells which attacked and ingested the spores were isolated connective tissue cells. Later Bruntz (1905), as a result of experiments with injected dyes, suggested that there were two elements responsible for phagocytosis, the blood cells and a number of phagocytic cells very much larger than the blood cells. These phagocytic cells were most numerous in the head and thorax, and later in the heart and pericardium. In the limbs they were present only in the endopodite and epipodite (as are the fat cells). The dyes collected in drops within the cells. Bruntz's work is referred to by Kollman (1924), who mentions that the phagocytic cells contain vacuoles, spheres and sometimes crystals. Jäger (1935) regards all large connective tissue cells as fat cells in various phases, and the fat cells as capable of displaying phagocytosis. However fat cells do not occur in the head, whereas according to Bruntz phagocytic cells are numerous in the head. This may mean that fat cells are able to enter the head if phagocytosis is necessary there. In Artemia, Lochhead and Lochhead (1941) suggest that the various terms, fat cells, nephrocytes, nephro-phagocytes and phagocytes have all been applied to one type of cell for which they propose the term, phagocytic storage cells. The fat cells of Daphnia appear to be comparable at least functionally.

g. Respiration.

As in many other small aquatic arthropods, respiration takes place through the general integument and also by means of the branchiae, or branchial sacs.

An early description of the structure of the branchial sacs is given by Claus (1876), followed by that of Fiedler (1908). Reactions to vital stains were examined by Fischel (1908) and Gicklhorn and Keller (1925). Lochhead (1950) also makes some original observations on the epipodites.

The branchial sacs.

The branchial sacs are the epipodites of the thoracic appendages. They occur on the external side and towards the proximal end of all the thoracic appendages (Plate 17, bs). The line of epipodites slants dorso-posteriorly, so that the first is the most ventral or furthest from the food groove. The epipodites are approximately 0.21 mm. by 0.14 mm., that is slightly larger than the compound eye. The second, third and fourth epipodites are heart-shaped (Fig.135); the first is longer and narrower but with a similar indentation at its proximal end; the fifth is shorter and broader with a slightly different form of indentation at the proximal end. This arrangement led Claus (1876) to say that the first four are heart-shaped, the fifth not, referring to proximal indentation, while Lochhead (1950) declares that the last four are heart-shaped, the first not, referring to shape. The first epipodite is placed on the thoracic appendage at a slightly different angle. The epipodites show a strong affinity for a number of stains, such as haematoxylin. The cellular wall is thicker than that of the remainder of the

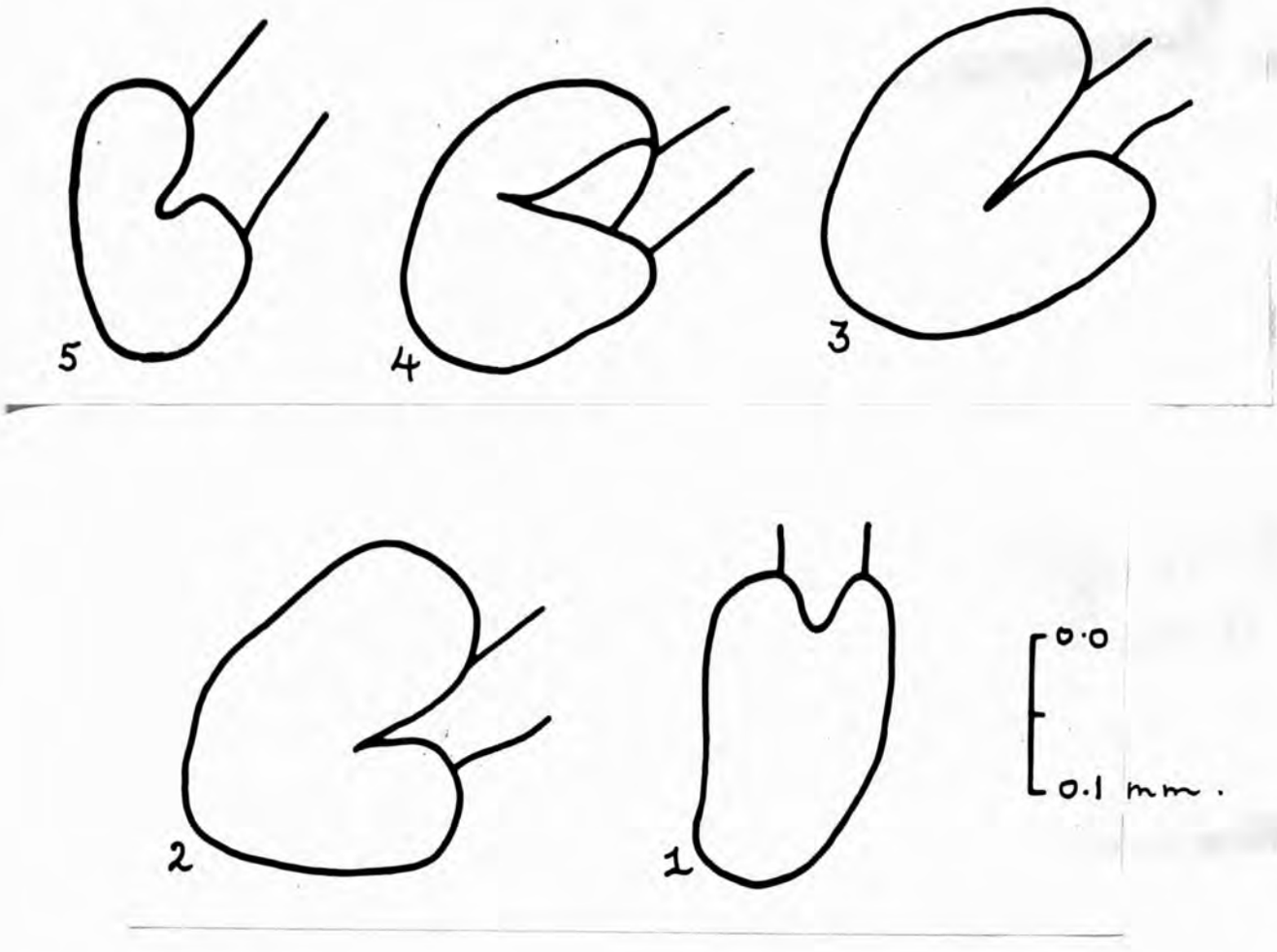


Figure 135. Diagrams showing the shapes of the branchial sacs of the thoracic appendages. 1-5, first - fifth branchial sacs.

appendages (Plate 32, bs). In very old animals the epipodites sometimes appear blocked.

Histology of the branchial sacs.

Claus (1876) described the cells of the epipodite wall as large with relatively small nuclei surrounded by a large number of small particles mostly in irregular denticular figures. The edge of the cells is irregular and uneven. In 1908, Fiedler described two types of cell. The first were large epithelial cells with wavy edges and granular contents. The nuclei were large, usually oval and stained deeply, containing a small nucleolus. The plasma contained perpendicular fibres in rod-shaped bundles towards the surface. In the second type of cell, the nuclei were generally smaller and usually round, the nucleolus being long and with an irregular outline. The plasma structure varied, but contained some fibres in thin platelets parallel to the cell boundary. Fiedler stated that the two types of cell were separated by a broad intercellular space. The cells of the second type did not touch each other, always being separated by three or four cells of the first type. Gicklhorn and Keller (1925), from their work with vital stains, distinguished the cells as "Netzmaschen" and "Netzlücken".

My study of the cells of the wall of the epipodite has led to a different interpretation of their nature. Sections show two apparently different kinds of cells. The first (Fig.156,(a),(b),(d),2; Plate 39,bs) stain with a slightly bluish tinge with Heidenhain's Iron haematoxylin, and contain coarse granules not densely crowded. The nucleus may be oval and has granular

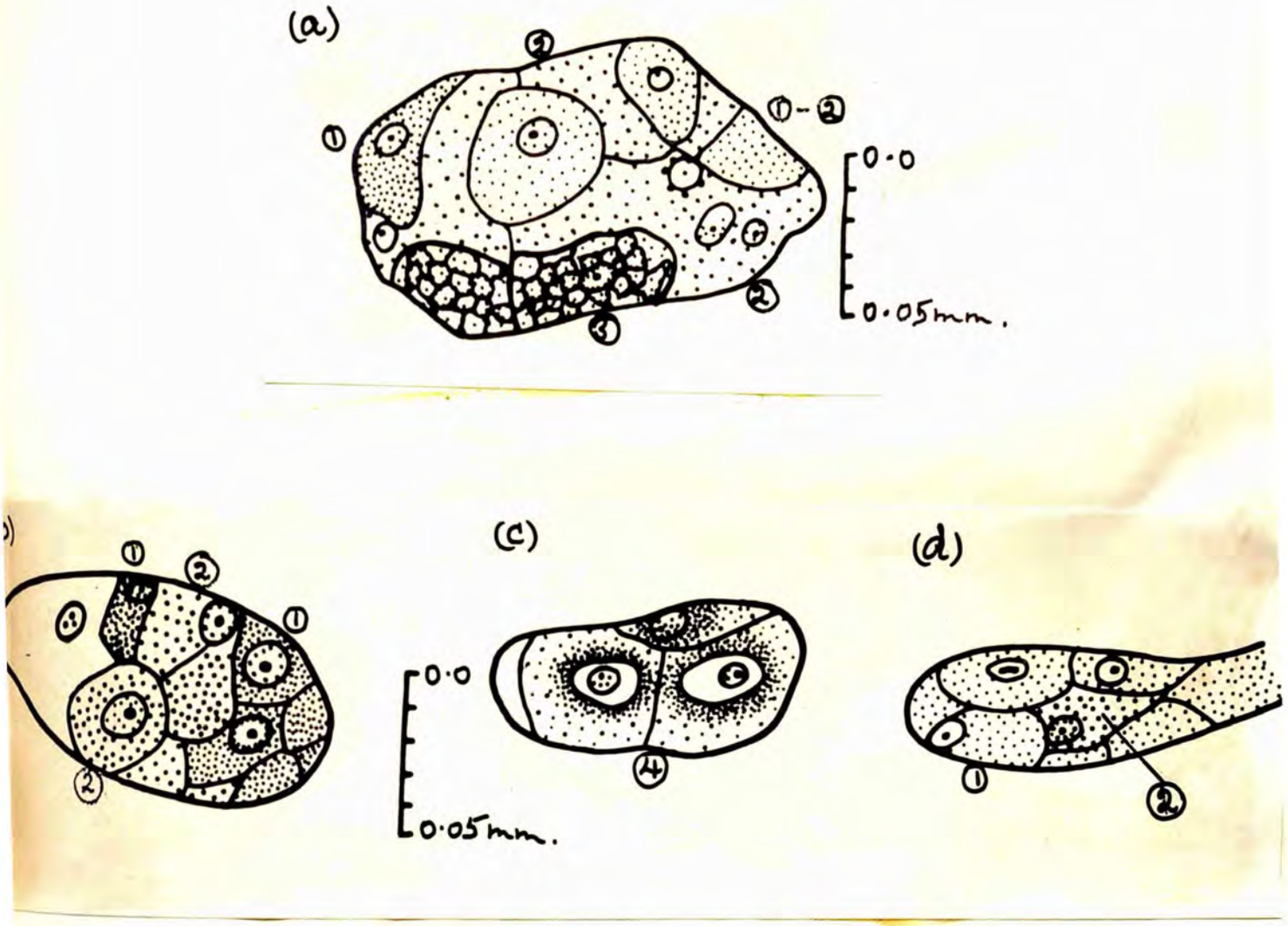


Figure 156, (a) Vertical longitudinal section through a branchial sac showing cells in the first (1), second (2) and third (3) phases and also in a phase between the first and the second (1-2).

(b) Vertical longitudinal section through a branchial sac showing cells in the first (1) and second (2) phases.

(c) Vertical longitudinal section through a branchial sac showing cells in the fourth (4) phase.

(d) Vertical longitudinal section through a branchial sac showing cells in the first (1) and second (2) phases.

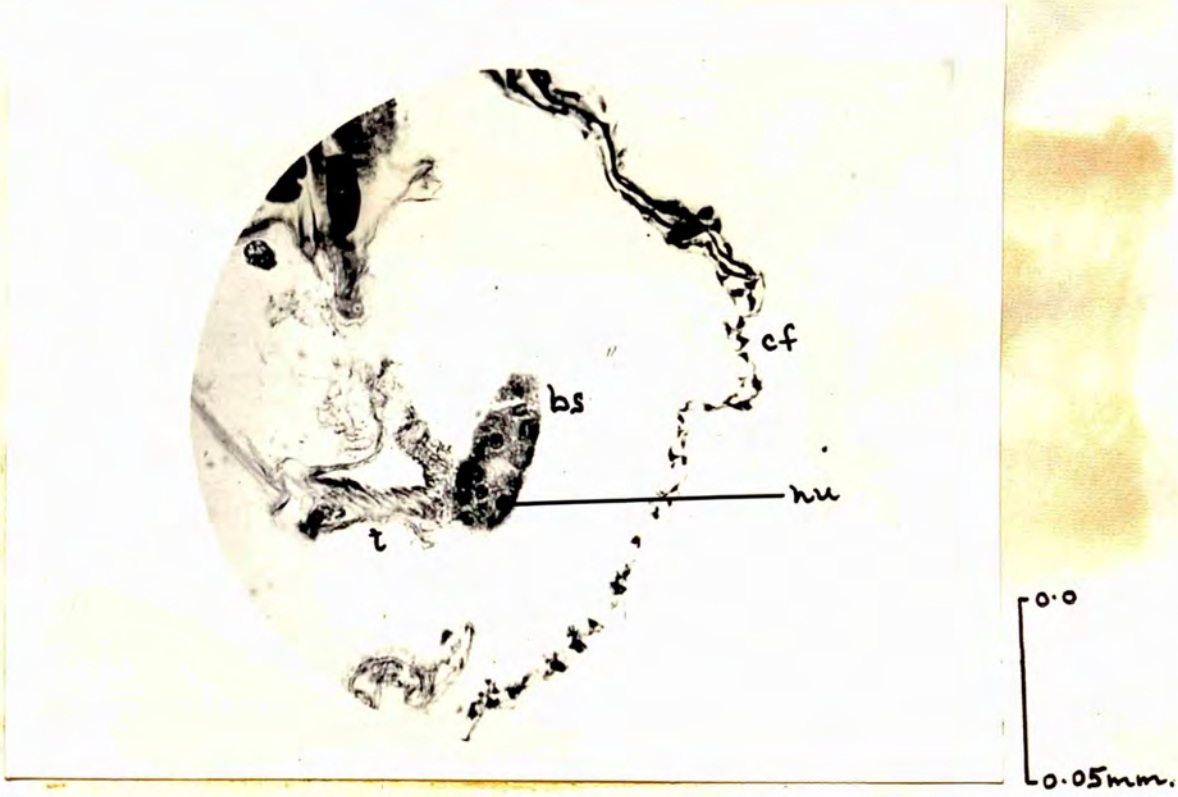


Plate 39. Photomicrograph of a vertical longitudinal section showing the branchial sac(bs) of one of the thoracic appendages(t) with the cells containing coarse granules. cf,carapace fold; nu,nucleus.

chromatin. The boundary of the cells is smooth with shallow lobes; it is not greatly convoluted. The second kind of cell (Fig.136,(a),(b),(d),1; Plate 40, bs) stains paler and with a slightly yellowish tinge with the haematoxylin, and the granules are smaller and more densely packed. The nucleus is slightly smaller than that of the first kind of cell and contains a rod-shaped nucleolus. These cells are more numerous than the first kind and occupy the spaces between them. These two kinds of cells are comparable to the two kinds described by Fiedler but differ in certain respects. There is an even distribution of granules in both kinds of cell, without rods at the periphery. It is often difficult to see distinct boundaries between the cells. Besides the cells falling into these two categories, there are a number of cells showing a slightly different structure. In some cells (Fig.136,(a),3; Plate 41,bs) the cytoplasm contains a coarsely granular mesh, and stains with definitely bluish tinge with haematoxylin. The nucleus has granules at the periphery. This kind of cell is probably comparable to Gicklhorn and Keller's "Netzmaschen"; the first kind to their "Netzlücken". In yet other cells (Fig.136,(c),4; Plate 42,bs), there appears to be a 'shadow' of bluish-staining, fine, densely crowded granules in the cytoplasm around the nucleus, while the outer cytoplasm contains granules of various sizes and less densely crowded. The nucleus may have an outer hyaline area.

This means that it is possible to see at least four slightly different kinds of cell and it seems reasonable to suggest that they are but four phases of activity of essentially one type of

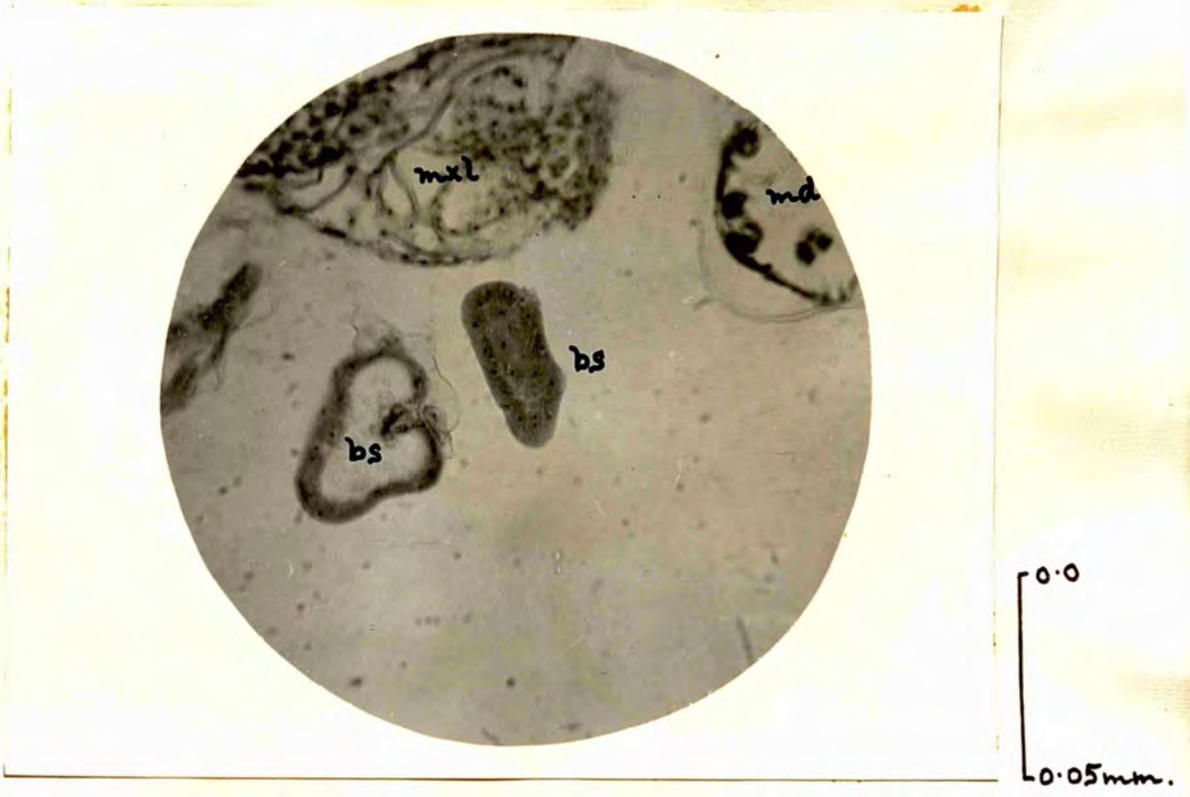


Plate 40. Photomicrograph of a vertical longitudinal section showing branchial sacs(bs) with cells containing small, densely packed granules. md,mandible; mxl,maxillary gland loops.

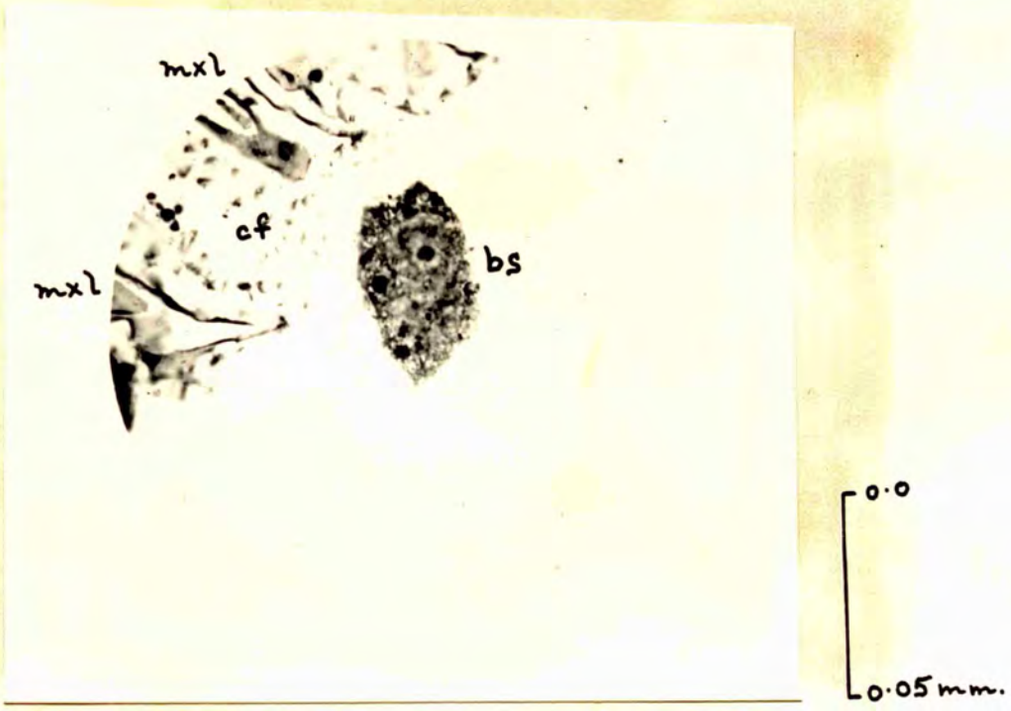


Plate 41. Photomicrograph of a vertical longitudinal section showing a branchial sac(bs) with cells containing a mesh of large granules. cf,carapace fold; mxl,maxillary gland loops.

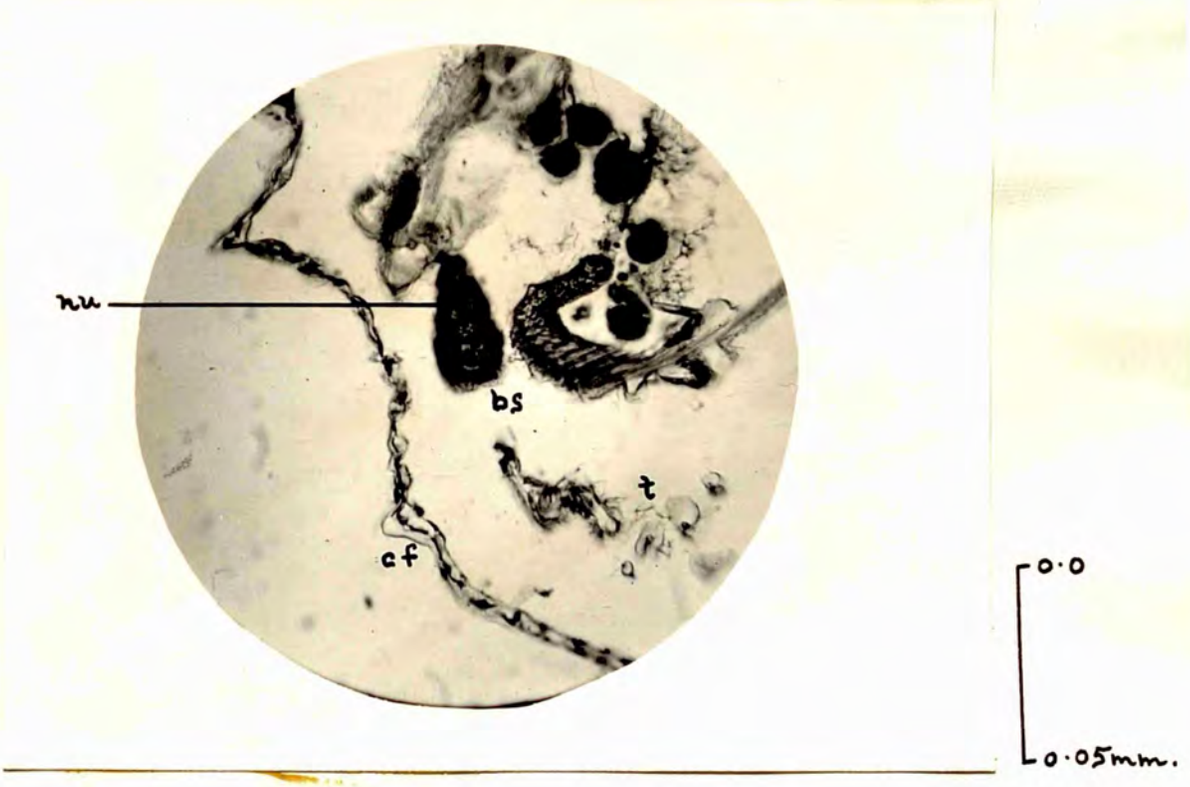


Plate 42. Photomicrograph of a transverse section showing a branchial sac(bs) with cells containing a ring of fine granules in the cytoplasm surrounding the the nucleus(nu). cf,carapace fold; t,thoracic appendage.

cell. They can be arranged in a series as follows: with fine, densely crowded granules and staining slightly yellowish (the second kind described above); with slightly coarser, less densely packed granules and staining slightly bluish (the first kind described above); with a mesh of coarse granules and staining definitely bluish (the third kind described above); and with a 'shadow' of fine, densely crowded granules around the nucleus and outer coarser, less densely crowded granules and staining bluish (the last kind described above).

Generally the first two of this series are most usually found together in the same epipodite at the same time, and the last two of this series found together, but the first and the last of the series have also been found together with the second occurring in a more posterior limb of the same animal. The various phases grade into each other.

I suggest that the cells of the epipodites are strongly active cells, acting as a region for the uptake of various substances, such as oxygen and possibly salts, from the environment. This is supported by the affinity for vital stains, by the plentiful supply of blood which the vertical septum renders greater than any other part of the appendage, and possibly by the striking difference in the nature of the cells from those of the remainder of the appendage. Strongly active cells tend to go through various phases, for example secretory cells and the fat cells, due to changes in the contents of the cell. The cells of the epipodites seem to pass through a number of phases, and the two types of cells described by Fiedler, and the two types described

by Gicklhorn and Keller, to be two phases of activity of one kind of cell.

The intercellular spaces mentioned by Fiedler, have not been observed and are a likely result of poor fixation, since contraction of the cell walls is a recognised consequence of inadequate fixation. The greater degree of convolution observed by Fiedler is probably due to the same reason. A similar explanation is possible for the rod-shaped bundles and thin platelets which may be the result of slow fixation. Fiedler notes that where the intercellular spaces are not in contact the boundaries between the cells were not observed. This is more in agreement with the condition which I have observed with better fixation, the boundaries between the cells being difficult to distinguish.

Function.

It is generally agreed that the epipodites function as respiratory organs. This was first suggested by Claus (1876). The epipodites are filled with blood which is directed past them in a steady stream. The structure of the cellular wall is distinctly different from that of the surrounding tissue. This may also suggest that besides aiding in respiration the epipodites possibly function for the intake of other requisites and salt absorption has been suggested. The epipodites stain very distinctly with a number of vital dyes, such as neutral red and methylene blue. Work on the reaction to vital stains has been carried out by Fischel (1908) and by Gicklhorn and Keller (1925). The latter found two kinds of cells: the "Netzmaschen" preponderantly

reduced and alkaline; the "Netzflicken" preponderantly oxidised and acid. But Gicklhorn and Keller also suggest that reduction or oxidation may both occur in the same cell. A paper by Gicklhorn (1925) suggested that the epipodites reduced metal salts. It is likely that the epipodites act as a region for the uptake of various substances, such as oxygen and salts, from the environment.

Suggestions for other means of respiration.

It has been suggested that respiration takes place via the alimentary canal, but this has been disproved by Fox (1952).

Gicklhorn and Keller (1925) found that in the young Daphnia the 'neck organ', or dorsal organ, reacted to vital stains in a way similar to the epipodites. They therefore suggest that the dorsal organ functions as an organ of respiration in the young animal.

h. Musculature.

A detailed account of the muscles of Daphnia has been given by Binder (1932) who considerably extended the existing knowledge. The following account is based on Binder's work, with additions based on original work and comparisons with other groups of animals.

The principal work on the muscles of Daphnia is that of Binder (1932). A brief account of the muscles had previously been given by Claus (1876) who includes little detail, and a fuller description of the antennal and mandibular muscles was given by Klotzsche (1913). In 1924, Kollman described the histology of various Branchiopod muscles and Humperdinck the structure and development of the muscles of Polyphemus. A detailed account of the histology is also given by Schneider(1908), and references on neuromuscular transmission, of which a certain amount is known in Daphnia, are given by Prosser et al (1950).

Distribution.

Muscles of the head.

1. Eye muscles.

The muscles of the compound eye consist of a pair of levators, a pair of depressors and a pair of lateral muscles. The muscles are short and very narrow and are best seen in the living animal. They extend from the equator of the eye to a point immediately dorsal to the brain. Their action produces the rolling movement of the eye.

2. Labral muscles.

On either side of the labrum is a levator muscle, while about ten

small muscles cross the labrum dorso-ventrally (Plate 18,1mu). The levator muscle (Plate 47,1mu) extends from lateral to the mesenteron but internal to the midgut caecum to its insertion the labrum near its proximal end. The levators bring about the separation of the labrum.

3. Antennular muscles.

In the female, the antennule has no muscle. In the male, the antennule has well developed muscles attached to a septum anterior to the stomodaeum (Plate 43,a.1mu).

4. Antennal muscles.

The muscles of the second antenna are derived from the metamerically arranged muscles of the thoracic appendages but are more strongly developed in relation to the greater amount of movement displayed by the antenna. Each antenna has three abductors (Fig.157, a.2ab.1, a.2ab.2), three adductors and one large levator muscle a.2L), in addition to a small extensor tendinis. The insertion of all the muscles is broad, which helps to distribute the 'pull' of the muscle. The first abductor (a.2ab.1) has its origin medially to the anterior surface of the head just dorsal to the gut caecum. It extends lateral to the mesenteron and is inserted on to the basal segment of the antenna. It is a well developed muscle. The second abductor (a.2ab.2) has its origin medially on the dorsal surface of the head at the level of the base of the second antenna and extends into the antenna to the end segment. It is less well developed than the first abductor. The third abductor originates on the integument at the level of the mesenteron and crosses to the opposite side of the animal where

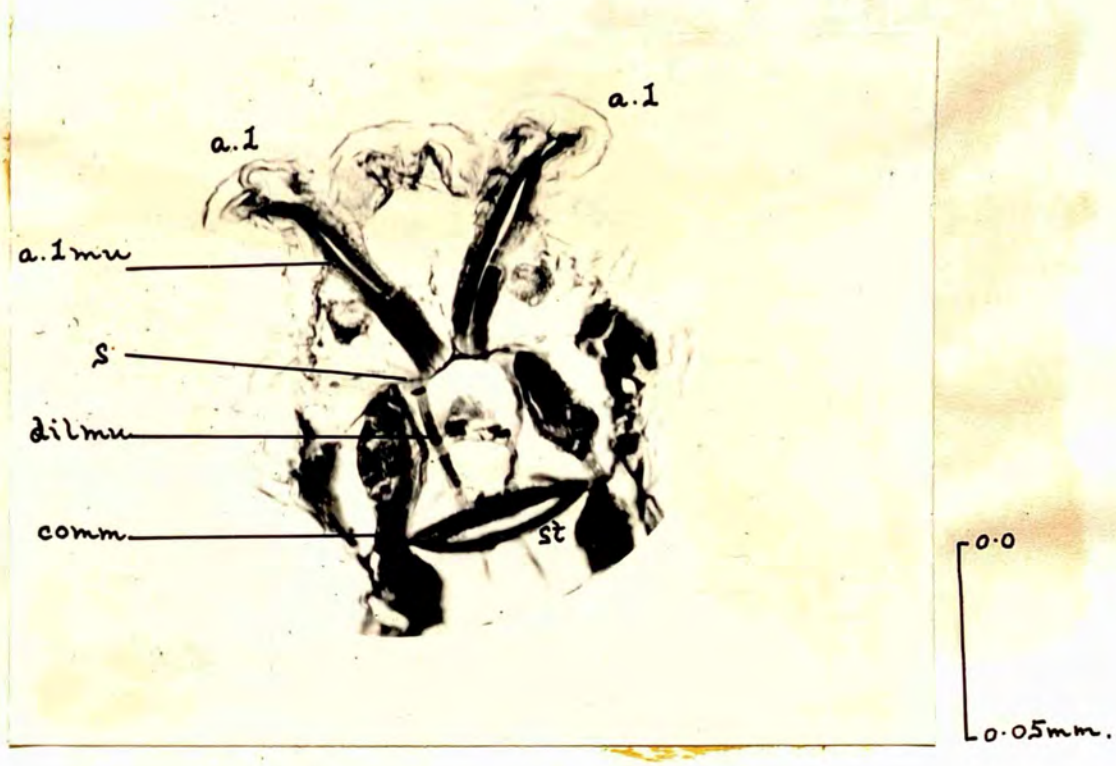


Plate 43. Photomicrograph of a transverse section through an adult male Daphnia magna showing the muscles to the antennules(a.1 mu) attached to a septum(s) anterior to the stomodaeum(st). a.1,antennule; comm,nerve commissure around the stomodaeum; dilmu,dilator muscles of the stomodaeum.

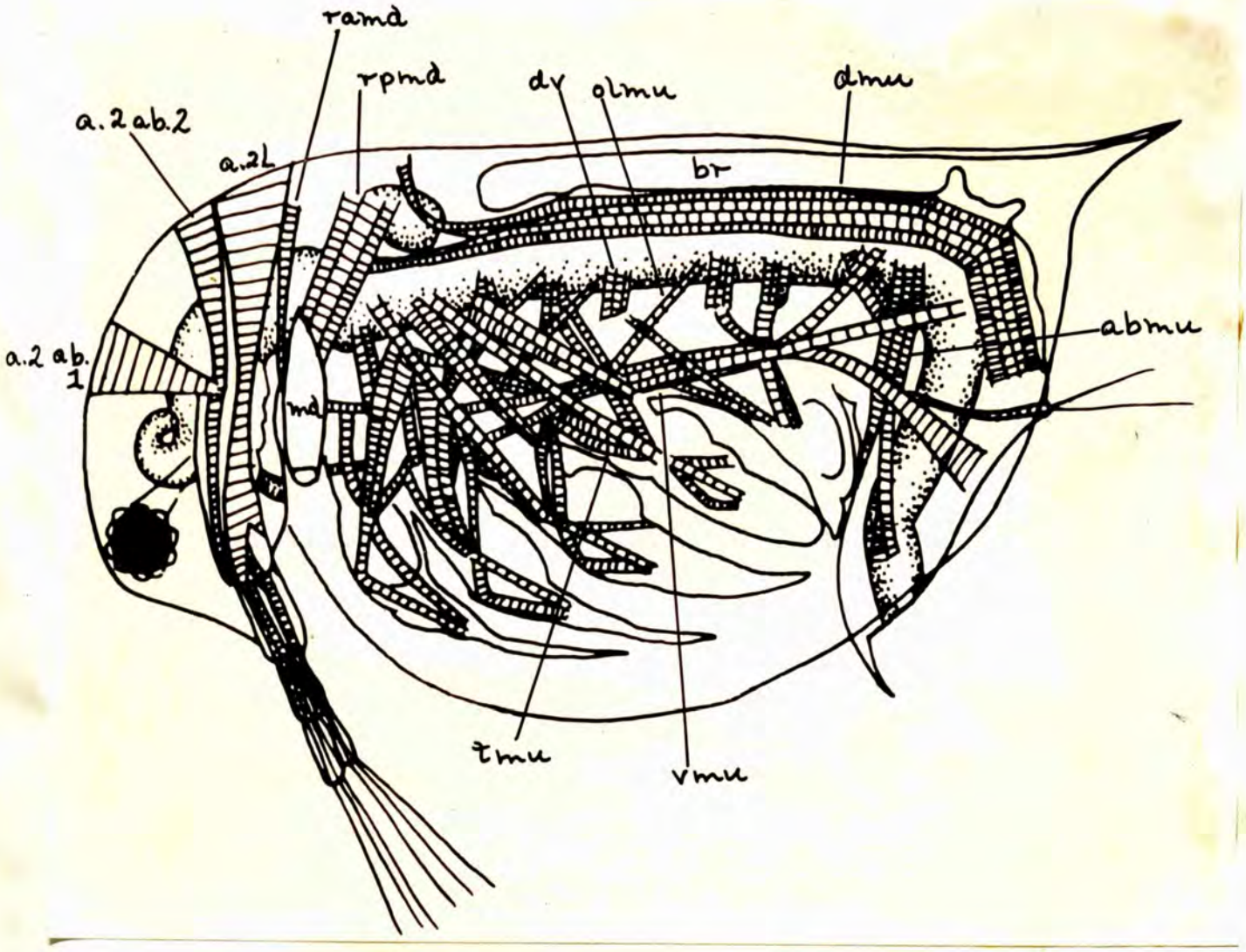


Figure 137. Diagram showing the positions of the muscles in the adult Daphnia magna. (after Binder). a.2ab.1, first abductor muscle of the second antenna; a.2ab.2, second abductor muscle of the second antenna; a.2L, levator muscle of the second antenna; abmu, muscles of the abdomen; br, brood pouch; dmu, dorsal longitudinal muscles; dv, dorso-ventral muscles; md, mandible; olmu, oblique-lateral muscle; ramd, anterior rotator muscle of the mandible; rpmd, posterior rotator muscle of the mandible; tmu, muscles of the thoracic appendages; vmu, ventral longitudinal muscles.

it enters the opposite antenna and is inserted on the end segment. A horizontal section joins the two muscles in the region where they cross. It is a well developed muscle. By their contraction, the abductors bring about the backward movement of the antenna.

The first adductor muscle has its origin just posterior to the third abductor and also crosses over to the opposite side where it enters the antenna along the ventral edge and extends to the first and second segments. There is no horizontal joining section. The second and third adductors have a common origin at a level just dorsal to the second antenna. A horizontal portion joins the two sides. There is also an oblique portion extending slightly inwards and dorsally to attach near the level of the heart. The muscles enter the antenna near its posterior edge and divide into two, one half to the dorsal branch (second adductor) and the other half to the basal segment (third adductor). The adductors, assisted by the levator, give the upwards stroke of the swimming beat.

The levator muscle (a.2L) has its origin dorsally and medially immediately posterior to the second abductor. It extends ventrally lateral to the mesenteron to join, at the level of the antenna, a well developed horizontal branch which has its origin medially and ventrally. The muscle enters the antenna and is inserted onto the distal part of the basal segment. The levator assists in the upwards movement of the antenna, especially in the directing of this movement.

The extensor tendinis is a small muscle acting as a tensor muscle on the tendon which transmits the working of the first adductor to the terminal joint. This tendon extends from the lateral wall of the antenna immediately ventral to the attachment of the second abductor to the distal part of the basal segment.

5. Mandibular muscles.

The mandibles possess anterior and posterior rotator muscles for each mandible (Fig.137,Ramd, Rpmd), two transverse adductor muscles, and a group of small muscles along the outer edge of each mandible. The anterior rotator (Ramd) is a narrow muscle which originates dorso-medially immediately anterior to the heart, and passes ventrally to the median part of the mandible to insert onto the anterior edge of the mandible. Contraction of the anterior rotator brings about separation of the mandibles.

The posterior rotator (Rpmd) has its origin slightly posterior to the anterior rotator, at the anterior end of the heart and extends to about midway down the mandible. It is considerably larger than the anterior rotator and by its action the food is pressed between the grinding surfaces of the mandibles.

The two adductor muscles are attached laterally onto the edges of the mandibles and extend between them. They are broad muscles, the dorsal one slightly the narrower. Contraction of these muscles causes the mandibles to move towards, or against, one another.

The group of small muscles (Plate 21,smdmu) form four longitudinal rows along the outer part of the mandible from the upper edge of the adductors to slightly ventral to the lower edge

of the adductors. Their inner ends are attached to an endosternite between the mandibles.

6. Maxillar muscles.

The maxilla has only two abductor and two adductor muscles. The abductors extend from the lateral wall of the maxillary segment obiquely ventralwards to the maxilla. The muscles are not well developed. Contraction of the abductors brings about a caudal and lateral movement of the maxillae. The adductors have their origin medially between the alimentary canal and the ventral surface and extend laterally to insert onto the posterior surface of the mandible. Their proportion of fibres is greater than that of the abductors. Contraction of the adductors moves the maxillae towards the mandibles.

Muscles of the body.

7. Thoracic appendage musculature.

Each thoracic appendage possesses three abductor muscles and three adductor muscles. There are modifications of the fifth pair of appendages and also in the first pair of appendages of the male. The attachment of the muscles varies slightly from appendage to appendage. The three abductor muscles have their origins slightly dorsal to the base of the appendage on the lateral integument of the body. The first has the most dorsal origin and is inserted in the region of the epipodite. The second is inserted onto the base of the exopodite. The third has the most ventral origin and is inserted just proximal to the epipodite. The contraction of the abductors brings about movement of the appendage laterally and slightly dorsalwards.

Of the adductors, the first extends from the lateral body integument, posterior to the origin of the first abductor, to the posterior edge of the base of the appendage. The second adductor usually extends from the ventral septum to the base of the epipodite, but the origin varies from one appendage to another. The third adductor extends from the ventral septum to the epipodite. Contraction of the adductors beings about ventro-caudad movement.

The fifth pair of appendages has lost the first adductor and one of the abductors, probably the second.

The first pair of appendages in the male has two extra muscles, one to the hooked bristle and one to the long flagellum.

The combined movements of the thoracic appendages cause circulatory currents and are employed in feeding, respiration and locomotion.

8. Abdominal muscles.

The abdomen has three adductor muscles (Fig.157,abmu) which extend dorso-ventrally. They originate at about the level of the mesenteron and in line with the anterior edge of the abdomen, extending between the alimentary canal and the ovary and then along the anterior edge of the abdomen. The first adductor inserts half way down the abdomen, immediately anterior to the proctodaeal muscles. The second and third adductor muscles are inserted nearer to the end of the abdomen, lateral to the proctodaeum. They are broader than the first adductor. Contraction of the abdominal muscles brings about a bending of the abdomen in the dorso-ventral plane.

9. Dorsal musculature.

There are four dorsal muscles (Fig.137,dmu) on each side of the body (Plate 52,dmu). They extend horizontally along the dorsal part of the body, just below the brood pouch and covering the dorsal part of the mesenteron. They stretch from the region of the heart to the region of the caudal seta on the abdomen. Two of the muscles arise dorsal to the middle of the heart on the lateral integument and their course is almost entirely dorsal to the mesenteron. The other two muscles arise just ventral to the anterior end of the heart on the lateral integument, and extend posteriorly at the level of the mesenteron. All four muscles lie parallel to the lower edge of the brood pouch but turn slightly ventralwards when they reach the level of the dorsal process which closes the posterior end of the brood pouch. They then follow the outline of the body into the abdomen to the level of the caudal seta where all four muscles are inserted at the same level. The muscles have connective tissue plates dividing them into four segments. Contraction of the dorsal muscles causes extension of the abdomen.

10. Dorso-ventral musculature.

There are six groups of dorso-ventral muscles (Fig.137,dv) on each side of the body. They are attached laterally to the integument immediately below the dorsal muscles and extend obliquely inwards between the alimentary canal and the gonad to the ventral septum which they join in the same position as the adductors of the thoracic appendages. The first group occurs at the level of the anterior end of the brood pouch, and the last group at the level

of the anterior edge of the abdomen. The groups of dorso-ventral muscles are apparently non-segmental. The contraction of these muscles brings the dorsal and ventral parts of the body closer together.

11. Oblique-lateral musculature.

There are five oblique-lateral muscles (Fig.137,olmu), which are branches of the ventral muscles. The muscles extend obliquely and posteriorly towards the dorsal surface to be inserted laterally in the same region as the dorso-ventral musculature. The contraction of the oblique-lateral muscles brings the dorsal and ventral parts of the body closer together. The muscles are long and narrow, in contrast to the short, broad dorso-ventral muscles.

12. Ventral musculature.

There are three long ventral muscles on each side (Fig.137,vmu), parallel to the ventral surface of the animal for the greater part of their length. They are ventral to the alimentary canal and the gonad, and lie near to the lateral integument immediately above the bases of the thoracic appendages. The three muscles have separate insertions at either end but for the greater part of their length lie together as a compact bundle. The first ventral muscle arises from the anterior edge of the base of the first pair of thoracic appendages and joins the other two muscles in a slightly more dorsal position at the level of the third pair of thoracic appendages. It leaves the other two muscles again at the level of the fifth thoracic appendage to pass towards the dorsal surface and insert lateral to the alimentary canal at the

level of the anterior edge of the abdomen. This muscle is at its anterior end the most ventral of the three, but becomes the most dorsal just before the posterior divergence. The second ventral muscle arises near the mandibular endosternite and joins the other two muscles at the level of the third thoracic appendage. It continues posteriorly after the divergence of the other two muscles and is inserted onto the integument in the region of the processes at the posterior end of the brood pouch. The third ventral muscle arises near the origin of the abductor muscles of the first and second thoracic appendages, passing ventrally to join the other two muscles. Immediately posterior to the region of the fifth thoracic appendages, the muscle diverges towards the abdomen where it is inserted below the caudal seta. A very thin branch goes to the caudal seta. Contraction of the first ventral muscle shortens the dorsal surface of the body and stretches the abdomen. The second ventral muscle helps in the bending of the abdomen when this movement is strong. The third ventral muscle also causes bending of the abdomen when it contracts.

13. Carapace-closing muscles.

The two valves of the carapace are drawn together by two transverse adductor muscles (Plate 32,ccmu). They are present in the maxillary region, and inserted adjacent to the maxillary gland and immediately dorsal to the ventral groove. The two muscles lie one behind the other, the posterior being the larger. Contraction of the muscles brings about closure of the valves of the carapace.

14. Heart muscles.

The heart has a single layer of circular muscle cells grouped in

pairs, together with an incomplete layer of longitudinal muscle cells (see p. 140). The outer part of the muscles is fibrous, the inner part is finely granular sarcoplasm. There is no intima. The muscle fibres have fine cross striations.

15. Alimentary canal muscles.

These muscles have been mentioned in the account of the structure of the alimentary canal (p.122). The stomodaeum possesses a layer of circular muscles, together with a pair of longitudinal muscles anteriorly internal to the circular muscles. The extrinsic muscles are the dilators. The anterior dilators extend from the stomodaeum to the integument at the level of the brain. The posterior dilators extend from the stomodaeum to a septum parallel to the stomodaeum. The mesenteron has a layer of circular muscles with the longitudinal muscles external to them. The proctodaeum has a layer of circular muscles, a number of dilators and a number of constrictor muscles. The dilators form a series from the anus up the lateral wall of the proctodaeum. The constrictors extend from the anterior to the posterior (morphologically ventral to dorsal) side of the abdomen, forming a row of narrow, strongly striated muscles which cover the length of the proctodaeum.

16. Reproductive system musculature.

In the female, the possibility of intrinsic musculature in the wall of the ovary has already been pointed out (p.142). In the male, there are circular muscles around the vas deferens and a fan-shaped muscle from the papilla.

Histology of the muscles.

General composition of the muscle.

Each muscle consists of sarcoplasm surrounding fibres enclosed within the sarcolemma, probably comparable to a cell membrane (Plate 55,a.2mu). The sarcoplasm contains many nuclei and is nearly always strongly vacuolated. The nuclei occur at intervals and are large, usually with a darkly staining central nucleolus surrounded by a hyaline area. The vacuoles form a meshwork with the struts of the mesh formed by finely granular, palely staining cytoplasm. The inner area of the sarcoplasm contains a large number of vacuoles with very little cytoplasm, giving a hyaline appearance. Each muscle fibre is made up of numerous fibrils which are tightly packed together to form a solid bundle and are not arranged in distinct columns. The fibres show various degrees of transverse striation from distinct to faint, and from narrow to broad.

Striation of the alimentary canal muscles.

The circular and longitudinal muscles of the alimentary canal have previously been described, for example by Binder (1952) as smooth muscles. But examination of sections of these muscles shows that they possess a fine, but well marked cross striation (Plate 44,mu; Plate 19,cimu). This is in accordance with recent work on the muscles of the alimentary canal of insects, previously recorded as smooth and now shown to be striated.

Differences between individual muscles.

Separate muscles show differences in the amount of sarcoplasm in proportion to the fibrils and in the nature of the striation.

Plate 44. Photomicrograph of a vertical longitudinal section showing the cross striation of the circular muscles(mu) of the mesenteron(me). he,heart.

In some muscles, such as the levator of the second antenna, the sarcoplasm is thicker on the external side of the fibres than on the internal. It has been observed that the sarcoplasm is particularly abundant in the antennal muscles (Plate 18,a.2mu), the mandibular adductors, the muscles of the thoracic appendages and the oblique-lateral muscles. There is less sarcoplasm in the mandibular rotators (Plate 21,mdmu), the maxillary muscles, the dorsal and ventral muscles, the dorso-ventral muscles, the carapace adductor muscles, and the stomodaeal and proctodaeal muscles. The sarcoplasm is poorly developed in the eye muscles, the labral muscles, and the group of small mandibular muscles. Where the sarcoplasm is more abundant it is usually also more vacuolated.

The striation is particularly distinct in the carapace adductor muscles, the dorsal and dorso-ventral muscles, the abdominal adductor muscles and the principal mandibular muscles. It is less distinct in the remaining muscles and especially difficult to distinguish in the antennal muscles where the striations are very fine.

Mode of insertion of the muscles.

The fibrils of the muscles are apparently each terminated by a long thin thread which penetrates between the cells of the epidermis. The chitin is often thickened in the region of the attachment of the muscle (Plate 45). The arrangement appears to be in agreement with that recently described for Triops by Debaisieux (1954). This agrees with the description by Binder (1932), and also essentially with that of Kollman (1924). The

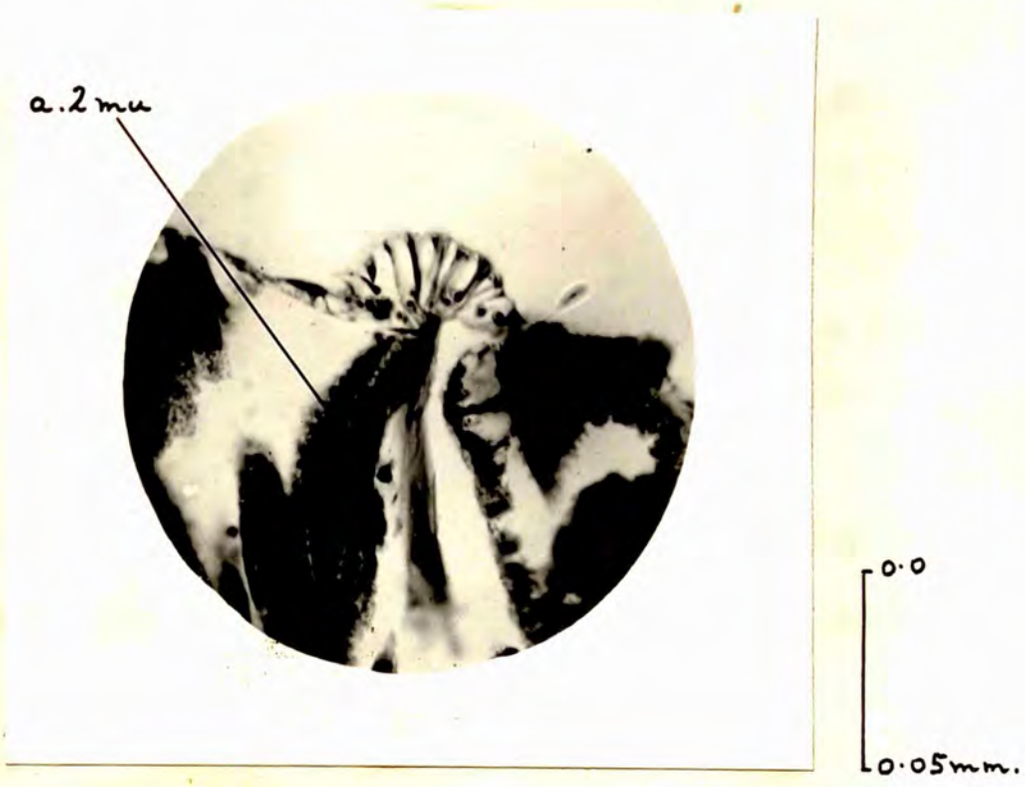


Plate 45. Photomicrograph of a vertical longitudinal section through the dorsal region of the junction of the head with the body showing the thickening of the chitin at the place of attachment of the muscles of the second antenna (a.2 mu).

mode of insertion agrees with that described for Arthropods in general by Richards (1951).

Comparison with the muscles of other Branchiopoda, and of insects.

In common with other Branchiopoda, the distribution of the muscles in Daphnia is derived from a metameric arrangement, modified in accordance with such characters as the extent of the carapace, the brood pouch and the method of locomotion.

The variation in the amount of sarcoplasm present accords with the recent work of Debaisieux (1954) on Triops and with accounts of insect muscle (Wigglesworth, 1953). The muscle of Daphnia is similar to that described by Imms (1948) for many larvae and Apterygota and by Wigglesworth for honey bee and Diptera larvae. Description of anisotrophic and isotrophic bands in Branchipus is given by Schneider (1908) who gives details of the histology of the muscles, and also of the innervation.

In insects all muscles are striated, although in some of the visceral muscles the striations are difficult to see (Morison, 1927-8; Wigglesworth, loc. cit.). The same is true in Daphnia.

There have been various opinions about the origin of the muscles, whether multicellular or unicellular. It appears most likely that some muscles have a unicellular origin and some a multicellular origin. This is indicated by Schneider (loc. cit.) and also by Humperdinck (1924). In Polyphemus, Humperdinck describes many bundles each from one cell in the labral muscles and the dilators of the alimentary canal, while the muscles of the appendages are formed from a syncytium, or a coalescence of numerous myoblasts. In a general account of Arthropod muscle,

Tiegs (1955) suggests that their origin is probably mostly multicellular.

i. The Internal Skeleton.

Various authors, such as Calman (1909) and Wagler (1926-7) have referred to an endoskeleton consisting of trabeculae lamellae, and plates of tendinous connective tissue providing attachment for the muscles. The structures referred to are often those described in this account as the septa delimiting the blood spaces; in other cases they may be tendinous attachment of the muscles to the integument.

Daphnia possesses one well developed endosternite, which is present in the mandibular region and to which the mandibular adductor muscles are attached. The endosternite is a broad transverse plate extending dorsally on either side of the alimentary canal as a long narrow phalange attached to the dorsal integument by groups of darkly staining fibres. Laterally the endosternite is connected to the mandibular integument by a series of short mandibular muscles.

The relationship of the endosternite of Daphnia to that of other Crustacea and its histology has been discussed by Debaisieux (1954).

j. The Nervous System.

The nervous system of Daphnia consists of a brain with anterior optic ganglia and posterior double chain of ganglia(Fig. 138). There is also a visceral or sympathetic nervous system supplying the alimentary canal.

An account of the nervous system of Daphnia longispina is included by Klunzinger (1864) in a description which otherwise deals only with the external features. Later descriptions are those of Claus (1876) and Samassa (1891). Cunnington's (1903) work deals principally with Simocephalus, and that of Retzius(1906) is an unsatisfactory report on earlier work. Leder (1915) gave a description of the finer structure of the nervous system, including an account of the position and functions of the various centres. A general review of neuro-muscular transmission in invertebrates is given by Katz (1949).

The Optic ganglia.

The optic ganglia (Fig.138; Plate 17; Plate 46; Plate 47; og) form the most anterior part of the nervous system. They are situated immediately adjacent to the compound eye, to which they send numerous fibres (Plate 48). There is a pair of ganglia and also a more anterior unpaired ganglion. All are composed of ganglion cells and fibres (Plate 47; Plate 48; og). Each one of the pair of ganglia has a central ball of fibres surrounded by cells. The two balls of fibres lie close to each other medially but apparently do not exchange fibres. The cellular part varies from a single to many layers in thickness, and I have found that it is thickest on the side nearest to the alimentary canal. The

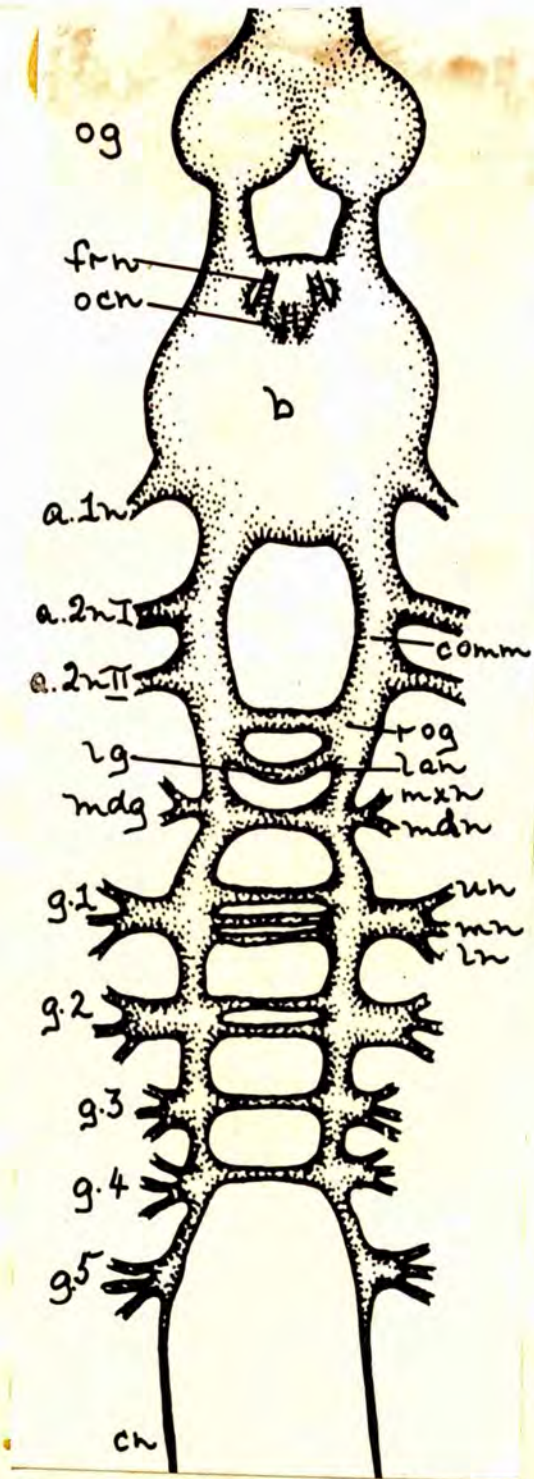


Figure 138. Diagram showing the central nervous system of the adult Daphnia magna. a.1 n, nerve to the antennule; a.2n.I, first nerve to the second antenna; a.2n.II, second nerve to the second antenna; b, brain; cn, nerve to the caudal seta; comm, stomodaeal commissure; frn, nerve to the lateral frontal organs; g.1-5, first-fifth ganglia; lg, labral ganglion; lan, nerve to the labrum; ln, lateral nerve to thoracic appendage; mdg, mandibular ganglion; mdn, nerve to the mandible; mn, middle nerve to thoracic appendage; mxn, nerve to the maxilla; ocn, nerve to the ocellus; og, optic ganglion; rog, retro-oesophageal ganglion; un, upper nerve to thoracic appendage.

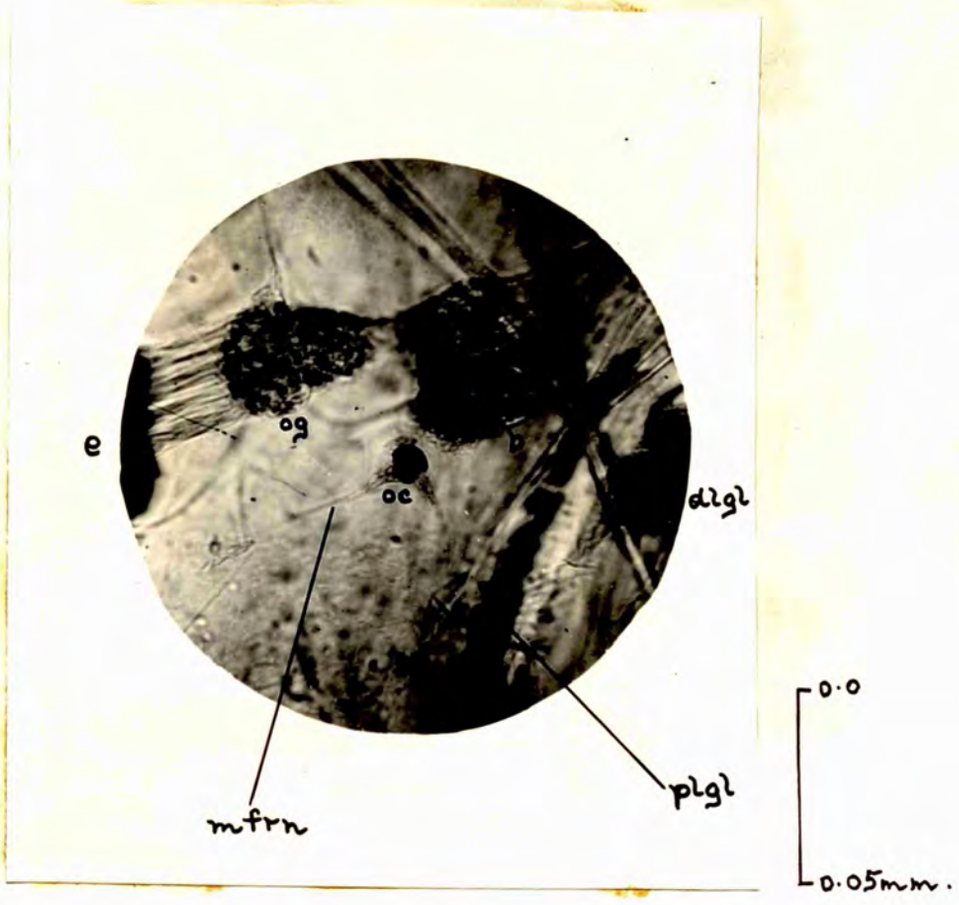


Plate 46. Photomicrograph of part of the anterior region of a whole mount of an adult Daphnia magna showing the brain(b) and the optic ganglia(og). dlgl,distal labral gland; e,compound eye; mfrn,nerve to median frontal organ; oc,ocellus; plgl,proximal labral gland.

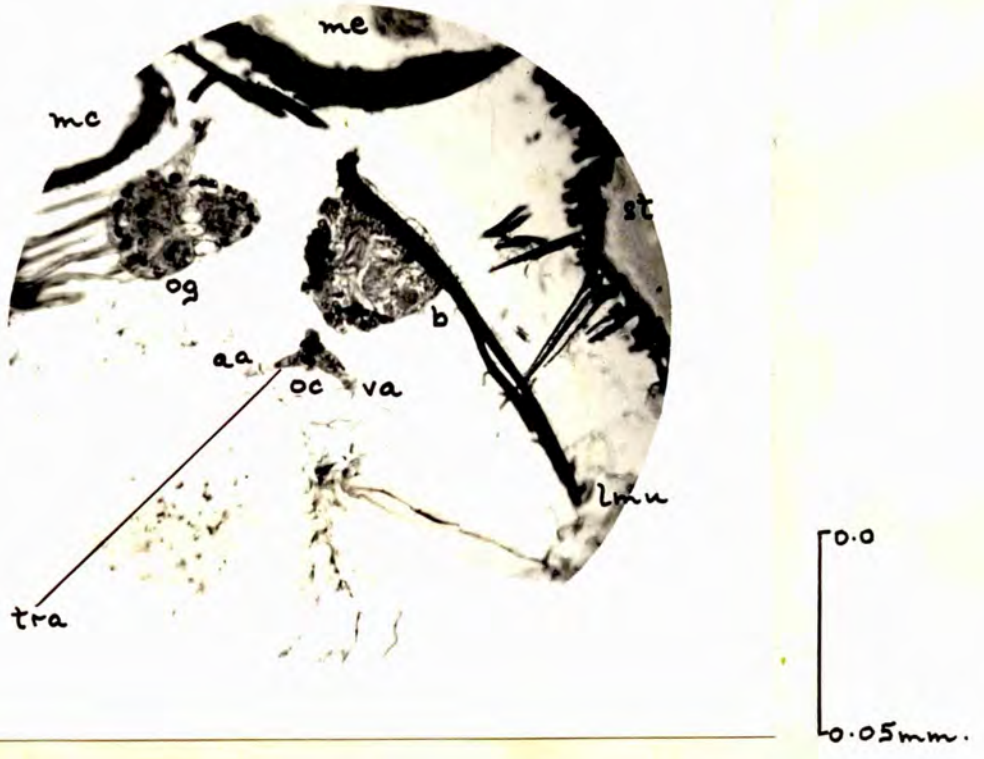


Plate 47. Photomicrograph of a vertical longitudinal section through the anterior region showing the brain(b), optic ganglia(og) and ocellus(oc). aa,anterior arm of ocellus; lmu,labral levator muscle; mc,caecum of mesenteron; me,mesenteron; st,stomodaeum; tra,transparent area; va,ventral arm of ocellus.

Plate 48. Photomicrograph of a vertical longitudinal section through the anterior region showing the optic ganglia(og) with nerve fibres leading to the compound eye(e). crs,crystalline sphere; mc,caecum of mesenteron; rh,rhabdome and retinula cells.

fibres to the eye leave principally from the dorsal side of the ganglia and those from the two sides soon join, leaving a small triangular cleft before they do so. They enter the eye posteriorly and extend to the crystalline spheres. These fibres are moderately thick; more delicate fibres innervate the eye muscles.

The paired connectives to the brain.

From each of the paired optic ganglia a short broad connective leads posteriorly to the brain (Plate 46). The connectives contain the posterior part of the fibrous balls and lie close together. They are extremely short so that they appear quadrangular in lateral view.

The brain.

The brain (Fig. 138, b), or paired supraoesophageal ganglia, is situated posterior to the eye and ventral to the anterior end of the mesenteron, or just anterior to the stomodaeum (Plate 46; Plate 47, b). The original paired nature of the brain is indicated by a shallow median indentation on the dorsal surface. This is almost absent ventrally, where there is the indication of a pair of indentations to define a median and two lateral areas. The brain is slightly larger than the optic ganglia and gives off nerves to various parts of the head, together with its posterior continuation around the stomodaeum. The central fibres are arranged in the form of centres, described later (p. 195), of which the largest pair are situated on either side in about the middle of the brain, with a smaller centre between them. A small pair occurs anteriorly and a small median centre posterior to the large pair. They are recognised as bundles of fibres surrounded by

cellular layers, which are thickest ventrally, that is on the side away from the alimentary canal. The anterior part of the brain is also composed principally of nerve cells (Plate 47, b).

The brain gives off nerves to the lateral frontal organs (Fig.138, frn), to the ocellus and the median frontal organs(ocn), and to the antennule (a.1n). All these nerves leave from the ventral surface.

The pair of nerves to the lateral frontal organs (frn) arise from near the anterior end of the ventral surface of the brain and immediately pass towards the dorsal surface, crossing the connectives to the optic ganglia and the eye muscles. Each long thin nerve branches to terminate in a number of elongate pear-shaped cells on the dorsal surface of the head. There are two main branches, one to the integument overlying the gut caecum and the other rather more ventral. The nerves arise from the anterior pair of centres.

The nerves to the ocellus, or median eye, and to the median frontal organs (ocn) leave the ventral surface of the brain immediately posterior to the nerves to the lateral frontal organs. The nerves are short and pass ventrally and slightly anteriorly. There appear to be three nerves, a delicate median nerve and a pair of lateral nerves, all of them converging towards the pigment body of the ocellus. From the anterior arm of the ocellus, a pair of long thin nerves proceed to the median frontal organs (Plate 46, mfrn), each nerve ending in an elongate cell in the integument ventral to the compound eye (Fig.139, n). The nerves arise from the small median centre.

The pair of nerves to the antennules (Fig.138, a.1n) leaves the brain towards the posterior end of the ventral surface. They are often referred to as the olfactory nerves, and the antennule as the olfactory antenna. Each nerve proceeds to the dorsal end of the posterior edge of the rostrum. It passes the proximal labral glands and along the posterior edge of the rostrum to its tip where the nerve enters the antennule. In the antennule, the nerve ends in a number of sense cells. The nerves arise from a pair of small centres at the posterior end of the brain and the beginning of the oesophageal commissures. The antennular ganglia are thus essentially part of the brain.

The oesophageal, or stomodaeal, commissures.

A pair of short broad nerves (Fig.138,comm) lead posteriorly and slightly dorsally on either side of the stomodaeum (Plate 43, comm) to the ventral nerve cord. They consist mostly of fibrous tissue, with the cells restricted almost entirely to the regions where the nerves leave the commissures.

The commissures give off two pairs of nerves to the second antennae. The first pair (a.2nI) arise from a pair of large centres situated near to the anterior end of the commissures. The nerves leave the commissures about a third of the length of the commissure from the anterior end and enter each second antenna near to its ventral edge. The first pair of antennal nerves is larger than the second. The second pair (a.2nII) arise from a smaller pair of centres posterior to the first pair. The nerves leave the commissures posterior to the first pair of nerves and enter the second antenna nearer to the posterior edge of the latter.

Both pairs of nerves are well developed and branch strongly within the second antenna.

The retro-oesophageal ganglia and ring.

The oesophageal commissures are joined together posterior to the stomodaeum by a ring of nervous tissue in the region of the labrum. The commissures broaden to form the retro-oesophageal ganglia (Fig.138, rog) which are joined medially by a pair of short commissures. From the retro-oesophageal ganglia also arise a pair of nerves (lan) which passes to the labral ganglia situated in the base of the labrum. The labral ganglia are joined medially, so that a ring of nervous tissue is formed, passing through the retro-oesophageal ganglia. The labral ganglia give off fine nerves to the sensory hairs at the tip of the labrum.

The mandibular ganglia.

The commissures become narrower and continue posterior to the retro-oesophageal ganglia for a short distance to the mandibular ganglia (Fig.138, mdg). The small mandibular ganglia are situated in the base of the maxillae, and from them arise nerves both to the mandibles (mdn) and the maxillae (mxn). The ganglia are situated close together and are joined by a short broad commissure. The commissure lies close to the ventral groove and connects the dorsal regions of the ganglia, the ventral parts of which lie below the level of the ventral groove. The nerve to the mandible arises laterally; the nerve to the maxilla arises more towards the ventral surface and slightly anteriorly.

The ventral nerve cord.

The ventral nerve cord extends posteriorly from the mandibular

ganglia between the ventral groove and the ventral septum (Plate 37, nc). Near its anterior end there is a dorsal flexure. The ventral nerve cord is short, its ganglia close together. The tissue is principally fibrous, with a thin outer layer of cells which becomes thicker in the ganglionic regions.

The ganglia correspond to the thoracic appendages and show little in the way of swelling, being principally clusters of cells. The first and second ganglia (Fig.138, g.1, g.2) are not on the cord itself but removed slightly towards the appendage; the third and fourth (g.3, g.4) are swellings of the cord itself; the fifth ganglion (g.5) is separated from the cord by a thin fibrous strand. The number of commissures which join the ganglia varies. The first pair are joined by three commissures, the second pair by two commissures, and the third and fourth pairs by one commissure each. The fifth pair of ganglia lie further apart from each other than the others and are not joined by a commissure. The commissures are short and thin. Usually there are nerve cells in the commissures, especially medially.

The nerve to each appendage has three branches, upper(un), middle (mn) and lateral (ln). The upper nerve (un) innervates the muscles of the appendage. The middle nerve (mn) enters the appendage along its inner edge and possibly innervates the end segments of the appendage. The very delicate lateral nerve (ln) is possibly sensory.

From the posterior end of the last ganglion, a long thin nerve (cn) continues to innervate the caudal bristle on the abdomen (Fig.143, n). It passes lateral to the alimentary canal

and between two spindle-shaped cells at the base of the bristle.

The ventral nerve cord also gives off nerves to the alimentary canal, especially posteriorly.

Nerve supply to the alimentary canal.

The nerve supply to the alimentary canal consists of a pair of long well developed nerves arising from the oesophageal commissure between the two nerves to the second antenna and passing dorsally. Each nerve then travels posteriorly across the base of the mandible to the mesenteron. The nerve continues posteriorly along the dorso-lateral wall of the mesenteron, giving off nerves irregularly on either side. At the posterior flexure of the mesenteron the nerve joins with a plexus which covers the posterior part of the alimentary canal and innervates the constrictors of the proctodaeum. There is a branch to the integument and carapace, and it has been suggested by Leder (1915) that there is also a branch to the heart. The presence of a nerve supply to the heart is disputed. It has not been observed in the present study.

The stomodaeum is supplied by nerves from the labral ring, together with nerves to the dilator muscles from the mesenteron nerve. The mesenteron, besides the main visceral nerve, is supplied by at least three small nerves from the ventral nerve cord. The proctodaeum is supplied by a dense plexus of nerves, connected to the main visceral nerve, which gives off a terminal plexus to the constrictor muscles.

Cells occur in the nerve to the mesenteron but not in that to the integument.

The nerve centres.

Leder (1915) has described various centres in the nervous system of Cladocera. At the most anterior end of the nervous system lie the unpaired and paired optic ganglia. The first pair of centres lie in the anterior region of the brain and supply nerves to the lateral frontal organs. The second centre is median and supplies nerves to the ocellus and the median frontal organs. The third centre is also median and is described as the central body or association centre; it co-ordinates all the other centres. The fourth pair of centres supply fibres to the compound eye muscles and to the second antenna; they are motor centres and help to regulate the general body movements. The fifth centres, also paired, supply the antennule. The sixth centres form two pairs which supply the second antenna and are principally motor, the first including a number of sensory fibres. The seventh centres are the pair found in the retro-oesophageal ganglia. The ventral nerve cord supplies the appendages with motor nerves, bipolar sensory nerves to co-ordinate the setae, and with a diffuse plexus in the integument. The nerves to the caudal bristle probably also include fibres from the fourth centres.

Leder (1915) considered that the brain consists of protocerebrum, deutocerebrum and tritocerebrum. The protocerebrum includes the first four centres and the optic ganglia; the deutocerebrum includes the fifth pair of centres, or those of the antennule; the tritocerebrum includes the two sixth pairs of centres, or those of the second antenna. The arrangement is

however similar to that present in the remaining Branchiopoda and the antennal centres or ganglia are not strictly part of the brain.

The compound eye seeks to keep a fixed position to the light, and the second antenna is instrumental in maintaining this position. It is therefore important to note that nerve fibres from the two both return to the fourth centre. Fibres from the fourth centre also supply the pinnate bristles of the second antennae and of the abdomen, and these bristles are capable of independent movement. The conclusion that the fourth centre is the regulatory centre for the body movements is supported by extirpation experiments.

Comparison with other Crustacea.

The nervous system of Daphnia is directly comparable to that of Chirocephalus illustrated by Borradaile et al (1935) and to that of Triops illustrated by Parker and Haswell (1940). The commissures here referred to as connecting the retro-oesophageal ganglia are comparable to that referred to in Chirocephalus as the antennal commissure. The nervous system of Daphnia has a shorter ventral nerve cord than these two genera, but does not show the degree of concentration exhibited for example by Leptodora and the Polyphemidae. In contrast to the Malacostraca and various other Crustacea, the nerves to the second antenna do not arise from the brain but from further posteriorly.

k. The Sense Organs.

The principal sense organs present in Daphnia are as follows: the ocellus, the median frontal organs and the lateral frontal organs; the compound eye; the antennules with their setae; and various setae and bristles, for example on the second antennae, the thoracic appendages and the abdomen.

Reference to the various sense organs is made by Claus (1876) and Klotzsche (1913). In a paper in 1894, Claus replies to a criticism by von Rath in 1892 of the term 'penetrate' to describe the relationship of the nerve fibres to the cells at the base of the abdominal bristle, and adds further remarks about this nerve. Hérouard (1895) studied the frontal organs as did Zograf (1904) who also worked on the ocellus. Moroff (1912) wrote an account of the ocellus based principally on Artemia. Moroff (1911) also studied the compound eye, but his account deals mostly with Palaemon. Other accounts of the compound eye, mostly concerned with its functioning, are those of Rádl (1901), Frisch and Kupelwieser (1913), Herwerden (1914), Koehler (1924), Schulz (1928), and Bernard (1937).

The ocellus.

The ocellus, unpaired median eye or "Nebenauge" is present in almost all adult Branchiopoda but is usually small [redacted] in Cladocera. In Daphnia there is little pigment. This appears to be correlated with the large size of the compound eye. Sida has a large compound eye and no pigment in the ocellus; Chydorids have large ocelli which may be larger than the rather small compound eye.

In Daphnia magna, the ocellus (Plate 17; Plate 46; oc) is situated immediately ventral to the brain (b) and posterior to the compound eye (e). It is close to the ventral surface of the brain, to which it is joined by very short nerves.

The ocellus consists of four pigmented "cups", or arms (Plate 47; Plate 49), one anterior (aa), one ventral (va) and two lateral (la). This is the number described by both Claus (1876) and Klotzsche (1913), the latter being contradicted by Leder(1915). My investigation has proved the earlier authors correct. The two lateral arms project slightly dorsally as well as laterally (Plate 49, la) and are joined at their distal ends to the most anterior part of the brain (b) by a group of loose cells. The ventral arm proceeds ventrally and slightly posteriorly and is continued as an elongate process which turns sharply dorsally to meet the brain just posterior to the region where this is met by the two lateral arms. A small fine nerve leaves the brain in this region and apparently goes to the integument. The anterior arm proceeds directly anteriorly and is continued at its distal end into a long delicate nerve which terminates at the median frontal organs (Plate 46). The arms consist of elongate cells (Plate 47, aa, va), the lateral arms of two cells each, the ventral of four cells and the anterior of two cells. The cells have a palely staining, sparsely granular cytoplasm with numerous vacuoles. Their nuclei are small with a small nucleolus surrounded by a hyaline area containing a few granules. The cells all meet at their proximal ends where they are packed closely together. These cells are pigment cells, and the centre of the

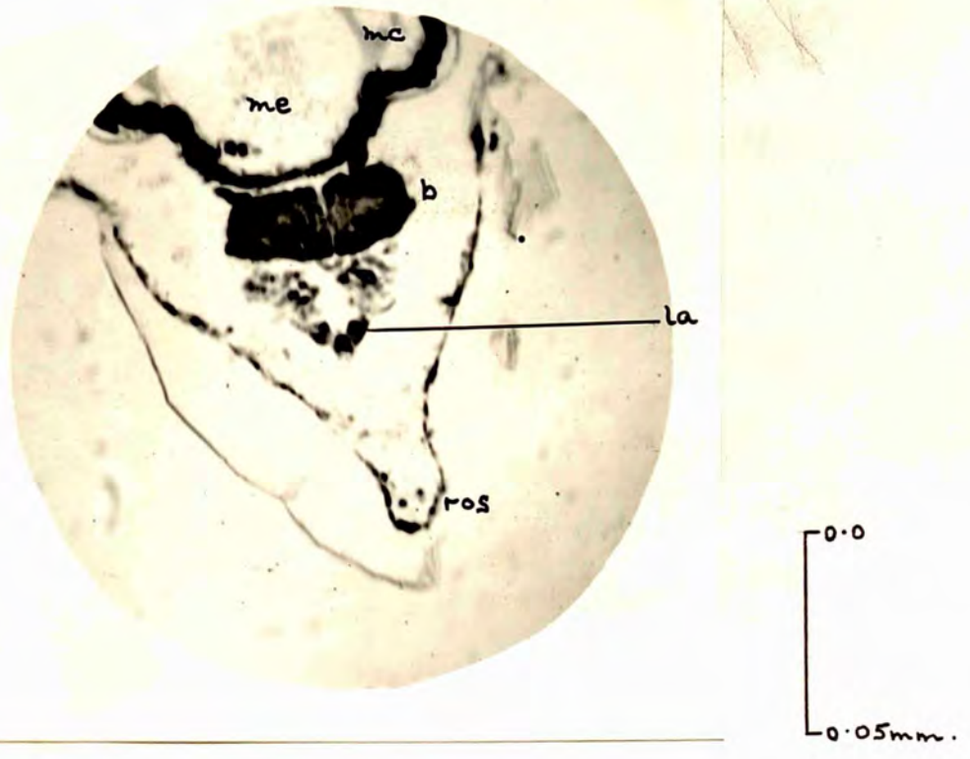


Plate 49. Photomicrograph of a transverse section through the head of a Daphnia magna showing the two lateral arms(la) of the ocellus. b,brain; mc,caecum of mesenteron; me,mesenteron; ros,rostrum of head.

ocellus where the cells converge together is filled with dense black pigment which is the most conspicuous part of the ocellus. The central pigment is indented where the arms arise. Sometimes the pigment spreads further into the projecting cells. The nerve fibres approach the cells from the outside and the eye is 'inverted'.

In the anterior and lateral arms, and possibly also in the ventral arm, I have observed two long narrow transparent areas (Plate 47, tra), each containing one small dark granule. No crystalline lens or spheres have been found. It is possible that the long narrow areas may be degenerating rhabdomes, or rods. The fact that they probably occur only in the lateral and anterior arms agrees more satisfactorily with the general Crustacean plan, in which there are usually two lateral and one median arms. The ventral arm is possibly a secondary structure.

Klotzsche (1913) suggested that the cells of the arms are sense cells in the process of involution, which he believed to be indicated by the presence of vacuoles in the cells.

The present of pigment and of nervous connections and the correlation of the development of the ocellus with that of the compound eye, indicates fairly clearly that the ocellus has an optical function which in Daphnia has been taken over largely by the compound eye. The suggestion of degenerating rhabdomes is also in agreement with this point of view.

The median frontal organs.

Situated against the integument ventral to the compound eye are two club-shaped cells (Fig.139; Plate 50; fro) connected to the ocellus by a pair of long thin nerves (Fig.139, n). The long

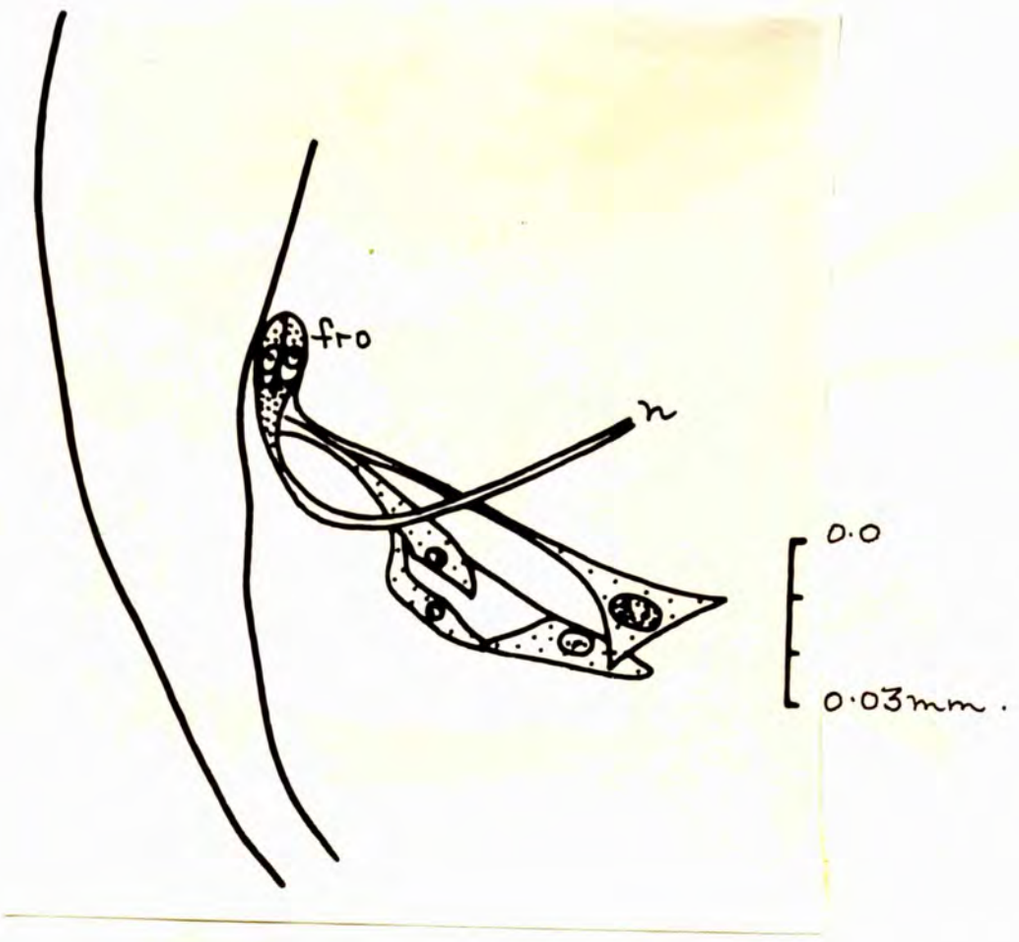


Figure 139. Drawing from a whole mount showing the median frontal organ (fro) in the adult Daphnia magna, with the nerve (n) leading to it from the ocellus and also with the adjacent group of irregular cells. Freehand drawing.

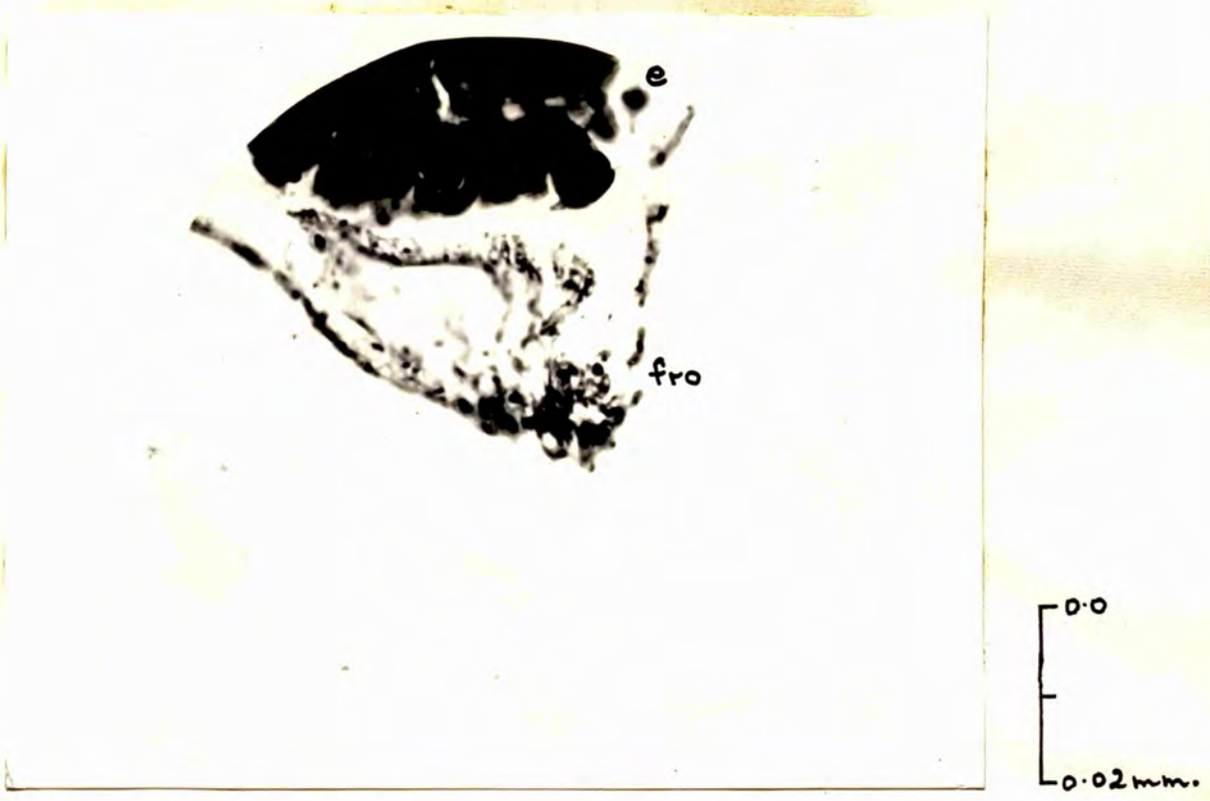


Plate 50. Photomicrograph of a transverse section through the anterior region of the head showing the median frontal organs(fro) situated ventral to the compound eye(e).

axis of these cells is almost dorso-ventral and the nerve enters at the ventral end of each cell. The cytoplasm of the cells is sparsely granular and does not stain darkly. The nucleus is spherical containing a small nucleolus surrounded by a hyaline area with a granular periphery.

On the rostrum, or antero-ventral part of the head, are a number of irregularly arranged cells of a similar nature (Fig. 139). Their shape is irregular and varied but they are mostly slightly larger than the pair of club-shaped cells. Some at least are connected to the pair of club-shaped cells by a thin strand which is probably nervous tissue. The cells are arranged into two groups, a dorsal group immediately ventral to the club-shaped cells and connected to them and a ventral group of five cells (Plate 52, fro) just dorsal to the antennule and anterior to the proximal labral glands. There appears to be a very fine strand from the ventral arm of the ocellus to the most dorso-posterior cell of this group. The two groups occur on both sides of the rostrum and lie against the integument. The cells send out fine dendritic processes in several directions. These cells sometimes contain vacuoles.

The function of these cells is not known. It is probable that the rostral groups of cells are related to the pair of club-shaped cells. Leder (1915) believed that the cells were all sense organs, probably for light perception but the only evidence to support this is the presence of a nervous supply and the connection to the ocellus. This is further discussed on p. 203.

Lateral frontal organs.

These have been referred to as the head or apical sense organs, and sometimes as the neck organ. There are drawbacks to all these names, for 'lateral frontal organs' implies relationship to the median frontal organs, 'apical sense organs' implies that they are sensory, while 'neck organ' is distinctly confusing since both the dorsal organ and the 'Haft'-organ have been referred to by this term. It is suggested that the cells bear sufficient resemblance to the median frontal organs to justify the implied relationship.

The lateral frontal organs are present in most Branchiopoda and were originally regarded as being of a glandular nature. More recently, for example Wagler (1926-7), it has been suggested that they are reduced eyes. The cells are always free of pigment.

The organs consist of groups of one or a few conical cells situated just ventral to the head ridge (Fig.140), that is lateral to the midgut caeca and dorsal to the compound eye and optic ganglia. They are spread over an area stretching from immediately dorsal to the compound eye to the level of the mouth. They generally follow the ridge of the head. There are five groups of cells, the first slightly apart from the other four. Each group receives fibres from a nerve from the brain. The nerve proceeds dorsally from the brain and divides into two when it nears the level of the dorsal edge of the compound eye. The anterior branch leads dorsally and slightly anteriorly to end in a group of four cells. The posterior branch leads dorsally and slightly posteriorly to divide into four branches each of which

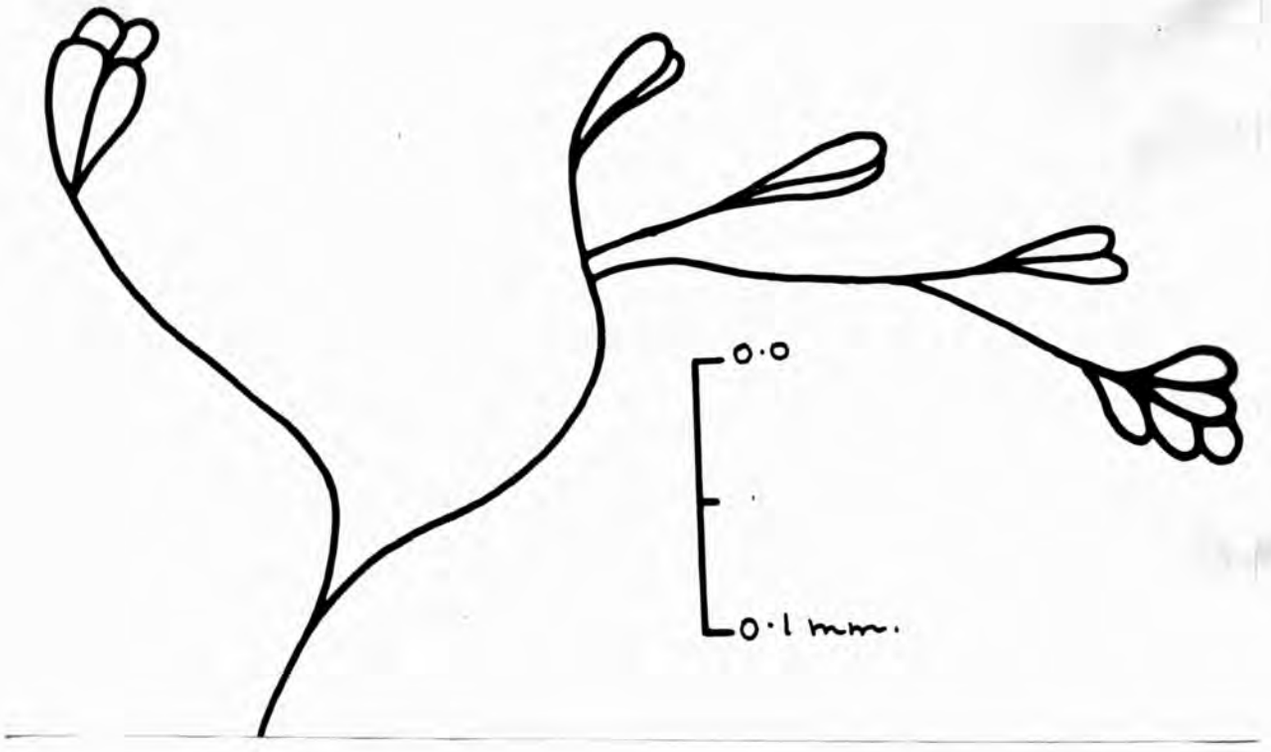


Figure 140. Diagram showing the positions of the lateral frontal organs on the left side of the head of an adult Daphnia magna. Freehand drawing.

ends in a group of cells, the first three in groups of two cells, the last in a group of six cells. However the arrangement of these groups shows a small degree of variation from one animal to another.

The cells of the groups (Plate 51) are large and pear-shaped, and the cytoplasm does not stain deeply. The nucleus is spherical with a small round nucleolus and contains a small number of granules. Vacuoles are rarely present in the cytoplasm. The cytoplasm often presents a mottled appearance, due to an irregular arrangement of granules. Between the cells of each group is a small transparent area with a small number of darkly staining granules (Fig.141; Plate 51; tra). This is possibly a "light refractive body". The size and the distinctness of these areas varies from one specimen of Daphnia magna to another. The cells are flattened against the carapace.

As well as the five major groups of cells, there are in the region of the four more posterior groups of cells a number of smaller cells arranged along the edge of the head ridge. They are produced into elongate processes which join with the nerves to the major groups.

The lateral frontal organs were described by Leydig (1860) who suggested an auditory function. Klotzsche (1915) suggested that the cells are gland cells which provide a secretion for the formation of the cuticle. Two years later Leder (1915) suggested that the cells are sense organs probably for the perception of light. He cited in evidence the strong innervation of the cells, in contrast to the innervation of the labral glands, and a

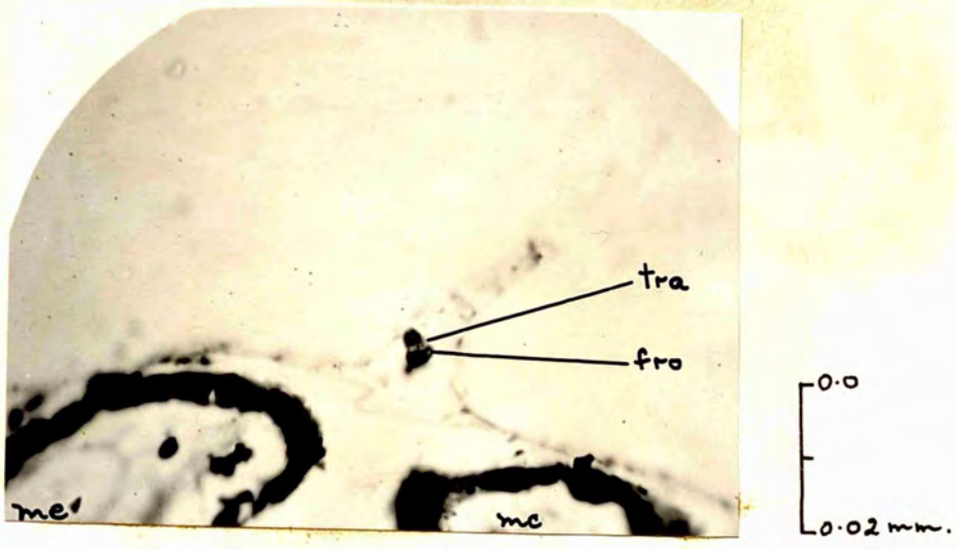


Plate 51. Photomicrograph of a horizontal longitudinal section through the dorsal part of the head region showing one of the lateral frontal organs(fro) consisting of two granular cells enclosing a small transparent area(tra). mc,caecum of mesenteron; me,mesenteron.

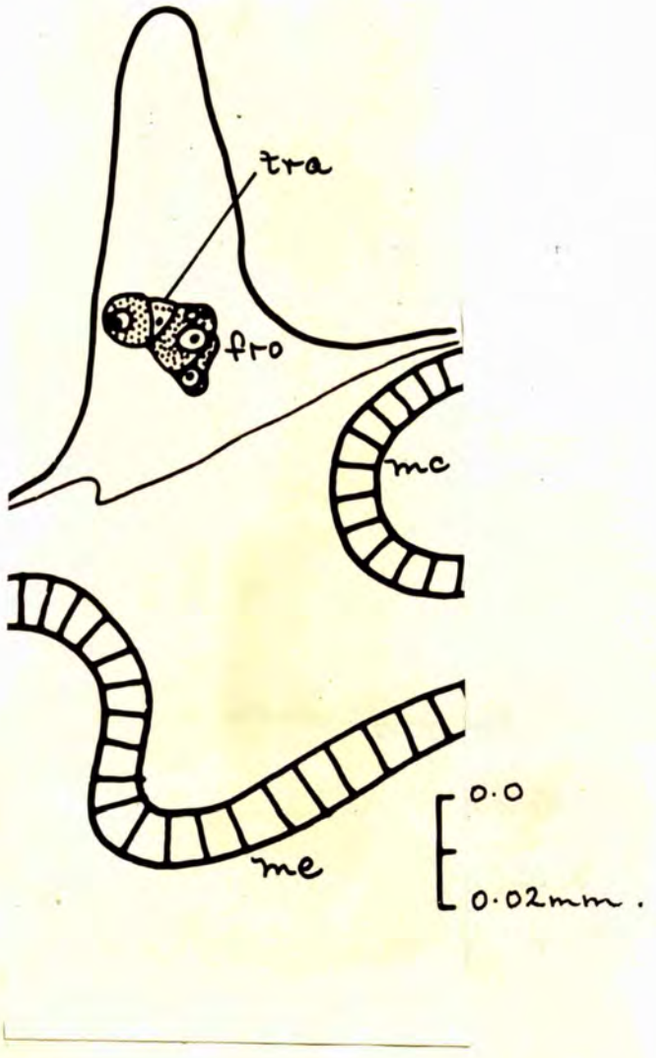


Figure 141. Horizontal longitudinal section through an adult Daphnia magna showing one of the lateral frontal organs (fro) with a central transparent area (tra). mc, caecum of mesenteron; me, mesenteron.

resemblance in cell structure to that of the cells of the ocellus. Vital staining experiments lead Gicklhorn (1931) also to suggest that the lateral frontal organs were light sense organs, but the median frontal organs not.

A possible remnant of an early organ complex?

Leder (1915) suggested that the lateral and median frontal organs together with the ocellus form the remnant of an ancient organ complex of which the most important part left is the ocellus. He believed that the form of the cells of the three groups was similar and that they represented sense organs, probably for light perception. Other workers have not stated the possibility of grouping the three together, although the median and lateral frontal organs are often regarded as comparable, and the median frontal organs and the ocellus in close association. In some Cladocera, such as Simocephalus, the nerves to the median frontal organ may be separate from the ocellus.

It seems likely that the median and lateral frontal organs are comparable structures, for their histology and general distribution are similar. Their innervation is moderately strongly developed, suggesting a sensory rather than a glandular function. The cells show little relationship to such glandular cells as the labral glands in their staining properties. There is the possibility that the small transparent area acts as a reservoir. The ocellus seems to be an organ for light perception, with pigment. There is little evidence to indicate whether the frontal organs also have, or have had, a light perceptive function or whether their function is connected with some other sense, such

as chemo-reception. Their position on the body does not indicate one sense more than another. There is a well developed compound eye, concerned with the perception of light, and the setae on the antennule and other parts of the body have been ascribed a chemo-sensory function. No definite statement is possible concerning the function of the frontal organs.

The Compound Eye.

In the majority of Crustacea there is a pair of compound eyes composed of several units. In the Branchiopoda there are various degrees of fusion. The compound eyes of Daphnia are almost completely fused, although their former paired nature is still indicated. They appear in the embryo as two separate units which later coalesce. In the adult Daphnia magna, the compound eye is a large, heavily pigmented structure at the anterior end of the animal (Plate 17, e). A large central mass of pigment is surrounded by 22 crystalline spheres. The eye has a median indentation dorso-ventrally which can be traced through the centre of the eye to give a distinct division into two parts.

Each unit of the compound eye is made up of several cells. At the proximal end is a rhabdome surrounded by five retinula cells (Plate 48, rh). Distal to these is a crystalline sphere(crs) surrounded by five sphere cells and two supporting cells. Distal again and immediately below the cornea are two small cells. All of these cells contain pigment except the two corneal cells, the pigment masking the cell boundaries so that it is difficult to distinguish individual cells. The eye is surrounded by the cornea and enclosed within an eye chamber. The eye chamber is

formed by the cuticle from each side growing over in a fold until it meets. Proximally the eye is connected to the optic ganglia by long tracts of nervous fibres. The pigment has migrated into the distal parts of these fibres.

When not filled with pigment, the distal or sphere cells have a palely staining cytoplasm and the nucleus is hyaline with a small nucleolus. There is less pigment in the postero-ventral part of the eye.

In some of the Notostraca and Conchostraca a small pore remains connecting the eye chamber with the outside environment. This pore is not present in Daphnia. The external covering of the eye chamber is composed of cuticle and hypodermis on either side, indicating its derivation from folded integument.

Function.

The eye is in almost continual movement and plays a large part in the orientation of the animal, keeping the dorsal part of the head towards the source of light. This was supported by the experiments of Schulz (1928). There is also a paper by Fox (1949).

The Antennule.

The pair of antennules is situated close together on the ventro-posterior part of the head, just above the tip of the rostrum (Plate 22; a.1). In the female the antennules are short, stumpy appendages, in the male they are longer. At its distal end each antennule (Plate 52, a.1) carries nine short setae (ss) or aesthetascs and one longer seta or bristle. The short seta have rounded tips, the longer seta is pointed.

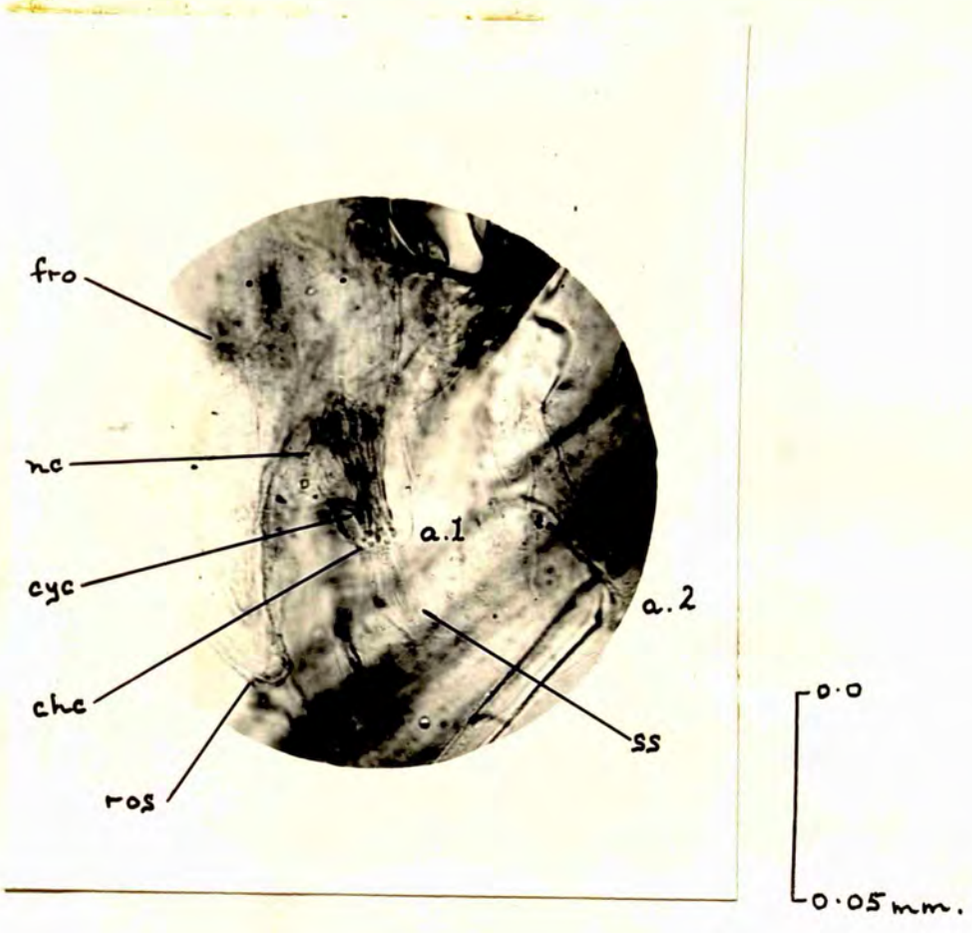


Plate 52. Photomicrograph of the ventral region of the head of a whole mount showing the antennule(a.1). a.2,second antenna; chc,chitinous collar; cyc,cylindrical cells; fro,frontal organ cells; nc,nerve cells; ros,rostrum; ss,short seta.

Each of the short setae is thin-walled and filled with protoplasm. In the protoplasm is embedded a loose mesh of nerve fibres (Fig.142). From the nerve net a pair of small nerves (nf) pass out of the seta through the short chitinous collar (Fig.142; Plate 53, chc) at the base of the seta. This collar is a thickening of the chitin with a narrow central canal. From the collar, the nerve fibres proceed to the ganglion at the base of the antennule (nc). Between the collar and the ganglion, the nerve fibres lie within narrow cylindrical cells (Plate 52-54,cyc) which appear to originate from cells among the ganglionic cells. The cells are flask-shaped with the bulb of the flask in the ganglion. From the ganglion the nerve fibres (Fig.142, n) lead proximally and soon join to form the antennular nerve (a.1n).

The longer seta has a similar structure except that it does not possess a thickened chitinous collar. The longer seta occurs at the anterior edge of the antennule, towards the tip of the rostrum, and is set distinctly apart from the other setae. This seta is often difficult to distinguish in the female, but better developed in the male.

Function.

The short seta are generally considered to be chemo-receptors, while the longer seta is thought to be a receptor for touch.

Bristles on the abdomen, second antennae and thoracic appendages.

The abdominal bristle or caudal seta.

Situated on the postero-dorsal part of the abdomen is a pair of long bristles or setae (Plate 17, abb). They are placed side by side in small sockets on a slight hump (Fig.143; Plate 55).

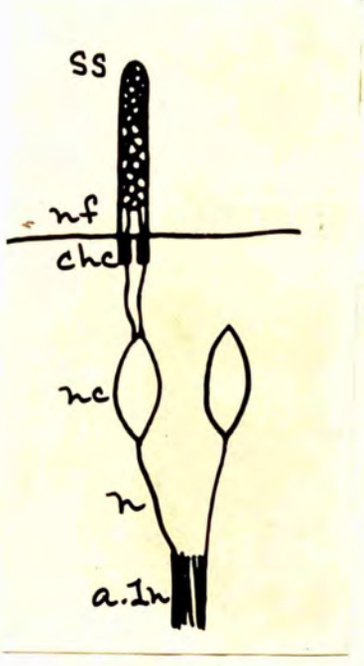


Figure 142. Diagram showing the parts of the antennule. a.1n, nerve to antennule; chc, chitinous collar; n, nerve fibre; nc, nerve cell; nf, neurofibril; ss, short seta.

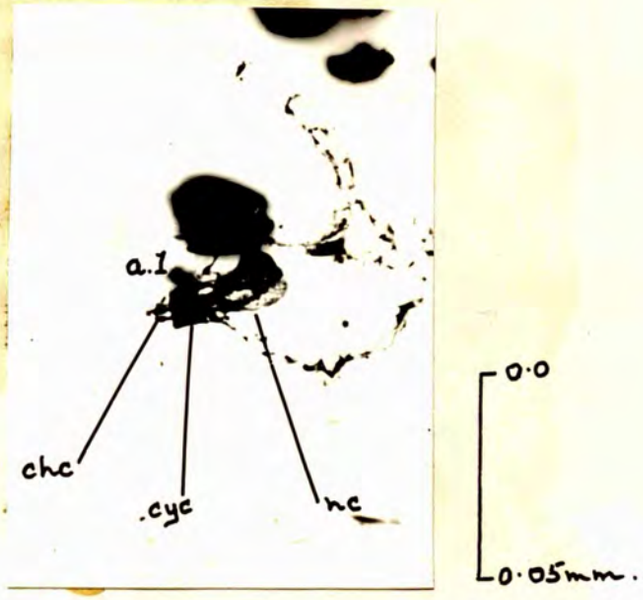


Plate 53. Photomicrograph of a vertical longitudinal section through the ventral part of the head showing one of the antennules(a.1). chc,chitinous collar; cyc,cylindrical cells; nc,nerve cells.

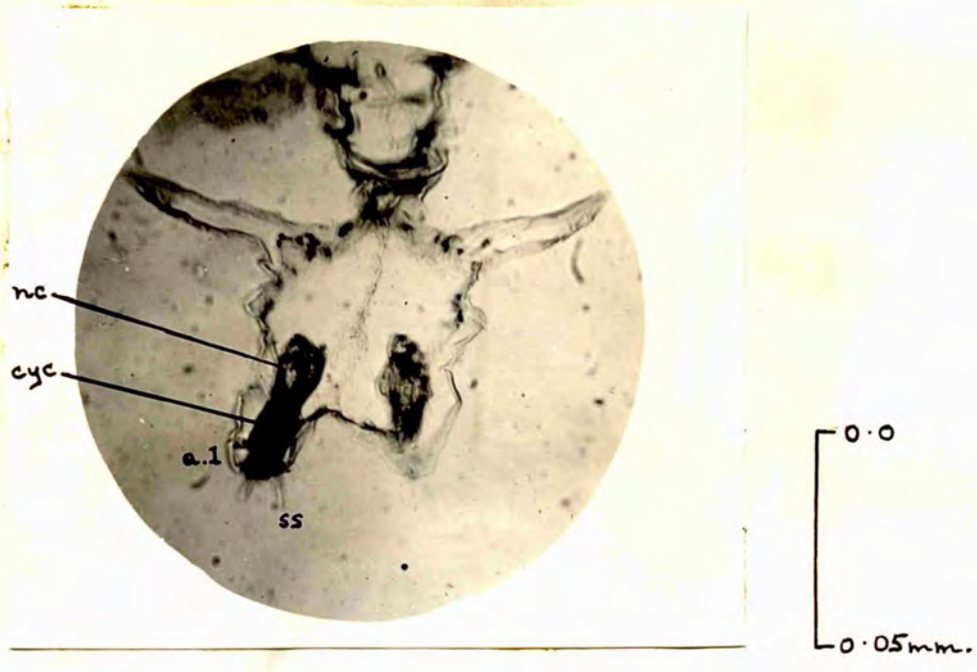


Plate 54. Photomicrograph of a transverse section through the ventral region of the head showing the pair of antennules(a.1). cyc,cylindrical cells; nc,nerve cells; ss,short seta.

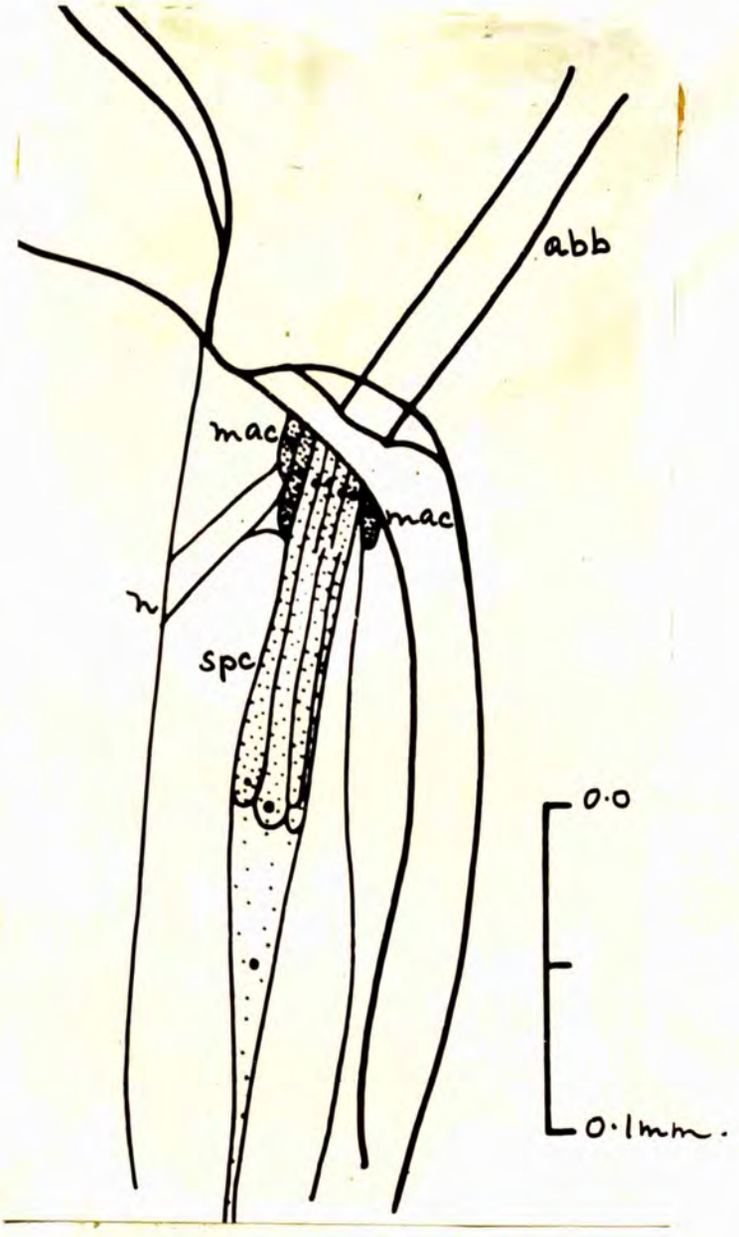


Figure 143. Vertical longitudinal section through an adult Daphnia magna showing the base of the abdominal bristle (abb) formed of spindle-shaped cells (spc) surrounded at their distal ends by a ring of small, darkly-staining matrix cells (mac). n, nerve. Freehand drawing.

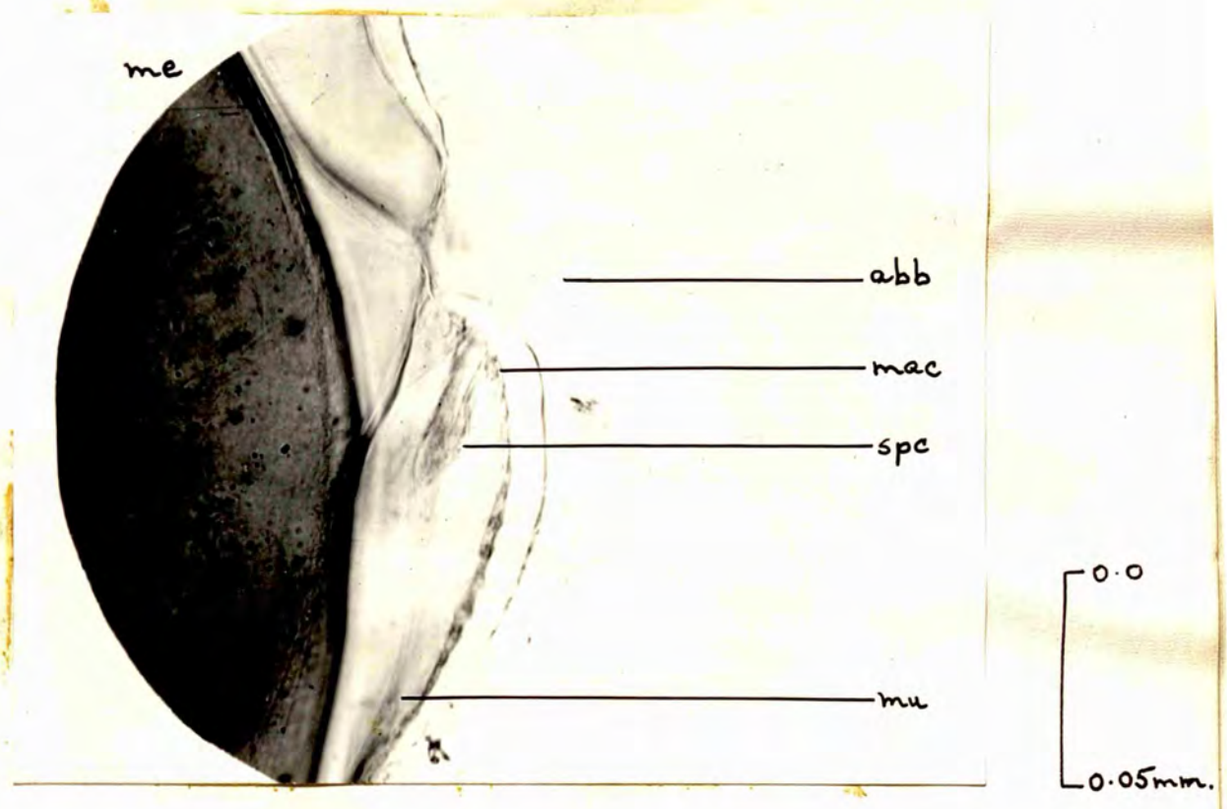


Plate 55. Photomicrograph of the posterior region of a whole mount showing the base of the abdominal bristle(abb). mac,matrix cells; me,mesenteron; mu,muscle; spc,spindle-shaped cells.

Each bristle is jointed halfway long its length and the distal part is plumose, or feathered. The group of cells at the base of the bristle is similar in appearance to that at the base of the antennule. There are four long narrow spindle-shaped cells (spc) with small nuclei. These are surrounded by a number of much smaller cells (mac) staining more deeply and with very small nuclei. These cells form a ring around the base of the bristle and enter its proximal end. There are also a number of short muscle strands in this region, the muscle being a branch of the third ventral muscle. A fine nerve (n) proceeds to each bristle and apparently divides into three strands.

The bristle is probably sensory, being strongly innervated. According to Leder (1915), the nerve fibres do not end in the last ganglion of the ventral cord but continue to the fourth centre in the brain.

Other bristles, especially those on the second antennae.

The large bristles of the second antennae are plumose for the whole of their length. They do not appear to have a cellular base comparable to that of the abdominal bristle, except that the nervous innervation is well developed.

A number of bristles occur on other parts of the body such as the thoracic appendages. They are usually innervated by nerve fibres which also form a plexus in the thoracic appendages.

The bristles are commonly ascribed a tactile function.

1. External Features.

The external features of the species of Daphnia have been described by many authors and accounts of the detailed structure are given in systematic works. The appendages have received special attention, and Bankierowa's (1933) study of the mandible and the works of Storch (1924; 1925), Eriksson (1935) and Lityński (1916) are important. Descriptions of the feeding mechanism of the appendages have been given by Cannon (1933; and others), Lowndes (1933) and Hartog (1901), and of the swimming by Scourfield (1900). A detailed description of the setae of D. carinata and of their structure, renewal and development was given by Agar (1950). Haack (1917) describes the details of the head shield. Works dealing with the structure of the carapace are those of Hardy (1892), Gicklhorn (1925), Anderson and Brown (1930) and Anderson (1933). Klotzsche (1913) also described the form of the head and various parts of the carapace and furca. Daphnia has also been the subject of a considerable amount of experimental and physiological work.

m. The Male.

An early description of the male of Daphnia magna was given by Leydig (1860), a more recent description by Scourfield (1945).

The male differs from the female principally in the larger and distinctly jointed antennule; the presence of a hook on the first pair of thoracic appendages; the presence near the end of the abdomen of a papilla carrying the vas deferens, and the frequent absence of one or more of the abdominal processes; the comparatively larger compound eye; the smaller size and the straighter dorsal margin of the carapace.

Conclusion.

An investigation of the anatomy of the adult Daphnia magna has led to the demonstration of a number of interesting points. An outline of these will serve also to clarify the extent to which the foregoing account is original work.

One of the most important advances made is the recognition of the striation of the intrinsic muscles of the mesenteron. These muscles have previously been regarded as unstriated and the identification of their fine but well defined striations parallels the recent work on insects in which it has been proved that the visceral muscles are often striated, contrary to the original belief. A more detailed study of the alimentary canal than previously available has led to a fuller description, especially of the histology of the cells. The alimentary canal is a simple tube except for the presence of a pair of caeca. These are not, in Daphnia, greatly specialised for secretion and absorption, functions which are taken over by structures corresponding to the caeca in many other Crustacea.

To the description of the excretory system is added a revised account of the histology of the cells. In particular the darker border of the cells previously described is explained as due to a greater aggregation of granules in this area of the cells. It has been established that the duct of the gland opens at the base of the second thoracic appendage. This must be a secondary shift from an original opening on the maxilla, the maxilla having become greatly reduced. The duct opens at the end of a small papilla.

The excretory organ of Daphnia can be derived directly from the general pattern of maxillary glands found in Crustacea.

The distal labral glands agree in general with the description of the glands in Simocephalus vetulus and are strongly developed. The proximal labral glands are larger and better developed than these glands in Simocephalus and show an even greater relationship to the distal glands. The labral glands form a well developed glandular system, their secretion assisting in the process of feeding. Labral glands have also been described in Anostraca (Branchipus, Chirocephalus and Artemia) and Conchostraca (Limnadia and Limnetis) and possibly occur in the majority of Branchiopods. There has been disagreement over their function in Chirocephalus, Cannon (1928, 1935, a and b) suggesting that they are associated with feeding, Nicholson and Yonge (1935) that they are concerned in the formation of the cuticular portion of the integument. In Daphnia the labral glands are distinctly better developed structures than those present elsewhere on the body and it is probable that their function is similar to that described by Cannon (1922) for the labral glands of Simocephalus, that is that they are associated with feeding.

A pair of receptacula seminis have been identified for the first time in Daphnia. Each forms a posterior continuation of the ovary and is a thin duct with a wall one cell thick. Existing descriptions of the development of the eggs in the ovary have been brought together and clarified but have been found to cover all the essential points.

A detailed examination of the wall of the heart has led to the identification of an incomplete layer of longitudinally arranged muscles as well as a complete layer of circular muscles. This longitudinal layer, originally suggested by Claus (1876), has been disregarded or denied in later descriptions. The general plan of the vascular system agrees with the general Crustacean plan of heart, short aorta, and sinuses.

In the description of the fat cells, the detailed work of Jäger (1935) is followed.

A new interpretation has been put forward for the cells of the wall of the branchial sacs, or epipodites. It is suggested that the wall is made up of essentially one type of cell which passes through a series of phases of activity. Various lines of evidence indicate that the cells are active cells and this would agree with the suggestion that they pass through a number of phases. Previous descriptions have referred to two types of cell. These two types are two phases of activity and are linked by intermediate phases. The description of Fiedler (1908) of the two phases necessitates correction in a number of points due to inadequate fixation.

Further description is given of the histology of the muscles. For their distribution the work of Binder (1932) is followed in general. It should be pointed out that Daphnia possesses a pair of well developed adductor muscles for the closure of the carapace. It is sometimes suggested that a lack of this muscle is a point distinguishing the Cladocera from the Conchostraca, for example Borradaile et al (1935).

Previous descriptions of the nature and distribution of an internal skeleton have been confused. Daphnia possesses an endoskeleton in the form of a plate in the region of the mandibular adductor muscles, as well as thickenings of the outer integument in the region of the attachment of the more highly developed muscles.

A complete description of the nervous system of Daphnia is given and the nervous system is compared with that of other Crustacea. The antennal ganglia are not included in the brain so there is no tritocerebrum. There is little concentration of ganglia, a distinct ventral nerve cord being present. Concentration has occurred only in the region of the mouth parts.

Daphnia possesses compound and median eyes, various sensory setae, and a number of frontal organs which may be sensory, being strongly innervated. The frontal organs, median and lateral, and the ocellus appear to bear some relation to each other but the frontal organs show little indication histologically of light-sensitivity. The antennule bears a well developed sensory organ, in the form of a number of setae.

Discussion.

The governing factor in the development of Daphnia magna is the presence of an extremely large quantity of yolk which fills most of the newly laid egg and remains prominent during the greater part of the development. This large proportion of yolk influences nearly every stage of the development, and to it can be attributed the superficial cleavage, the indeterminate nature of the development, the gastrulation by immigration, the unusual method of the development of the mesenteron and the yolk cells, and the feeble development of the coelomic sacs in the mesoderm. The differences between the development of the parthenogenetic and the ephippial eggs are due to differences in the state of the yolk content.

The majority of cladoceran eggs previously described have a total cleavage, exhibited for example by Polyphemus and comparable to the cleavage in the copepod Calanus. In the egg of Daphnia pulex, which has a greater proportion of yolk than that of Polyphemus but less than that of D. magna, the cleavage is related to that of D. magna but the nuclei remain nearer to the surface of the egg and each dominates the surrounding yolk (Baldass, 1942). In D. magna the initial cleavages occur near the centre of the egg and result in a number of isolated blastomeres which has no visible influence upon the surrounding yolk. There is therefore a series in the Cladocera in which yolk content and cleavage are correlated, extending from species of which the eggs are poor in yolk and have a total cleavage, such as Polyphemus, at one end of the series to species of which the eggs are rich in yolk and have

a superficial cleavage, such as D. magna, at the other end of the series. A comparable series is present in insects (Johannsen and Butt, 1942). The investigation of the early development of D. magna has broadened our knowledge of cladoceran embryology to show that in this group, as in many other groups, the kind of cleavage is related to the quantity of yolk.

The initial internal cleavage followed by the migration of the blastomeres to the surface of the egg along cytoplasmic strands and the establishment of a blastoderm at first without cell membranes, is a kind of development similar to that described not only in insects with eggs rich in yolk but also in the crustacean Hemimysis lamornae (Manton, 1928). Probably a similar development occurs in other Crustacea with yolky eggs.

The development of the eggs of Daphnia magna is indeterminate, since the genital cells are not distinguishable until the time of gastrulation (p.69). Again a continuous series appears to exist in cladoceran development, with Polyphemus at one end with determinate development and D. magna at the other end with the first sign of differentiation occurring at the time of gastrulation. An intermediate stage is seen in Simocephalus vetulus, the development of which was described by Cannon (1921). In D. magna it is still not possible at this stage to distinguish between the cells of the mesendoderm. It is difficult to relate cleavage pattern to egg pattern and the pattern of differentiation, but probably the results of the work of Baldass (1942) on D. pulex indicate the situation in D. magna also and the three axes, the cleavage axis, the egg axis and the axis of differentiation do

not coincide. The results of Baldass support the suggestion of Child (1941) that "there are considerable differences in cleavage pattern and its relation to egg pattern and to pattern of differentiation among the entomostraca".

The gastrulation resembles that of other very yolky arthropod eggs, occurring by immigration with very little sign of invagination, whereas in Polyphemus the invagination is more marked.

The most important effect of the large quantity of yolk is on the development of the mesenteron. In most Arthropoda and Annelida the endodermal cells enclose the yolk and form a cylinder, gradually absorbing the yolk. The yolk therefore lies within the cavity of the alimentary canal during the greater part of the development and the embryo develops around it. In Daphnia magna the endodermal cells do not attempt to grow around the surface of the yolk but remain in a ventral position where they form the mesenteron as a solid rod of cells. The mesenteron later develops a cavity by the breakdown of the central cytoplasm. The cavity of the alimentary canal does not at any time contain yolk. An intravitelline development is mentioned by Grobben (1879) in his work on Moina rectirostris but received no attention. The development of the mesenteron is not mentioned in the accounts of Polyphemus and has been almost completely ignored by workers on the embryology of the Cladocera. The mesenteron is figured by Agar (1908) and by Cannon (1921). When observing the development from living material, the development of the mesenteron is obscured by the yolk surrounding it. A similar development of

the mesenteron is known in some ostracods and a few isopods and schizopods (Dawydoff, 1928). A development which is so different from that of the majority of Arthropoda, including other Crustacea, is extremely interesting and important. The explanation possibly lies in the large amount of yolk in D. magna, but a similar development of the mesenteron probably occurs in all the Cladocera. It would be interesting to determine how widespread the phenomenon is, and if it occurs in other groups of Branchiopoda. The early embryology of the other groups of Branchiopoda has been little studied. The paper by Claus (1886) on Branchipus and Artemia deals with the development after the animal has hatched as a nauplius, while that of Brauer (1892) on Branchipus is mostly concerned with the early nuclear divisions. The work of Cannon on both Estheria (1924) and Chirocephalus (1926) also commences at the nauplius stage when the mesenteron is already formed and the yolk is present as yolk spheres within the cells. Weisz's (1947) paper on the development of Artemia indicates that the mesenteron forms around the central yolk. There is no indication of the situation in the Notostraca since the embryology of the group has been almost entirely neglected. Among the Branchiopoda, the Cladocera are therefore the only group known to have this unusual feature in their development.

Recent work on crustacean embryology has shown that the yolk cells usually arise from endodermal cells, and not from mesodermal cells as was often suggested by earlier workers. This has been found by Manton (1928; 1934) to be true for both Hemimysis and Nebalia. Most of the earlier workers on the embryology of the

Cladocera have also suggested that the origin of the yolk cells is mesodermal, and it was therefore interesting to determine if, in their case also the origin of the yolk cells is in fact endodermal. Investigation has shown that the yolk cells develop from the cells of the blastoderm and from any cells surrounding the yolk except for the genital cells. Their origin is neither mesodermal nor endodermal. Early in the development of the egg all the cells, except for the genital cells, contain yolk granules. The formation of the yolk cells has begun at the time of gastrulation, before the appearance of the endoderm and mesoderm. A similar development of the yolk cells occurs among some of the Orthoptera. In the case of Daphnia magna, no cells remain in the centre of the egg after the cleavage cells have migrated to the periphery although in some Orthoptera a number of yolk cells are formed from such cells. This method of formation of the yolk cells in D. magna may occur in all the Cladocera but differs from that in other groups of Crustacea that have been investigated.

As in some other Crustacea, the mesendodermal cells are not at first distinguishable into endoderm and mesoderm, nor into an ectomesoderm and a mesendoderm. When the cells become differentiated into two groups, one group forms the mesenteron only and therefore comprises the endodermal cells, while the other group is the mesoderm.

Recent work has identified the presence of coelomic sacs in a wide range of arthropods so that they have now been found in all classes of Arthropoda, including many Crustacea. Among the Branchiopoda, coelomic sacs have been identified by Cannon (1924;

1926) in Estheria and in Chirocephalus. In the nauplius of these two genera, the mesoderm of the post-mandibular region, together with the genital cells, fills the space between the endoderm and the ectoderm. A mid-dorsal split appears in the mesoderm and forms the cardiac cavity. Coelomic sacs appear, the outer walls of which form the dorsal longitudinal muscles of the trunk. In Estheria, the walls of the first four coelomic sacs form the heart, while in the ventral mesoderm a cavity appears in connection with the first coelomic sac and becomes the end sac of the maxillary gland. In Chirocephalus, the outer walls of the coelomic sacs sink in very quickly and the heart forms slightly differently in the different parts of the body, although always the process is a modification of that found in Estheria. The anterior part of the heart forms by essentially the same method as that found in Estheria. The inner angles of the coelomic sacs grow together to meet on the dorsal surface of the alimentary canal and form the ventral wall of the heart. Posteriorly, the dorsal angles meet while the inner angles remain in contact with the dorso-lateral part of the alimentary canal. In the middle region, both the inner and the dorsal angles of the coelomic sacs grow inwards to meet and close the cardiac cavity completely. The heart in this region becomes a solid plug of cells, in which a tubular cavity forms later. The maxillary gland develops from the ventral mass of mesoderm in connection with the wall of the first dorsal coelomic sac and is entirely mesodermal in origin. The end sac appears before the coils of the gland.

The development of the heart and the maxillary gland in

Cladocera has previously been briefly mentioned by Grobben (1879) in his description of the embryology of Moina. He states that each arises from a solid group of cells which acquires a central cavity.

In Daphnia magna, (Fig.144), the mesoderm in the post-mandibular region of the embryo, at the stage approximately comparable to that of the nauplius, laterally forms a thin layer of cells on the outer edge of the yolk and against the ectoderm and is thickened in the ventral region on either side of the alimentary canal. The lateral mesoderm is separated from the endoderm by a large quantity of yolk which also fills the dorsal part of the embryo. The mesoderm of the two sides does not meet in the mid-dorsal line. A transverse section of the embryo of D. magna at this stage of its development differs markedly from that of Estheria or Chirocephalus principally because of the presence of a large quantity of yolk. No mid-dorsal split appears in the mesoderm because this region is occupied by yolk. A single pair of extremely small coelomic sacs appear in the dorsal edge of the mesoderm in the maxillary region. No other coelomic sacs are present and there is no sign of segmentation in the developing ventral mesoderm. The dorsal edge of each coelomic sac, and the mesoderm on either side of it, extends dorsally until the lateral mesoderm from either side meets in the mid-dorsal line. A compact layer of mesodermal cells is now present covering the upper surface of the yolk in the maxillary region, and this mesodermal layer is thickened in the future heart region. A cavity forms in the centre of the heart, and the dorsal

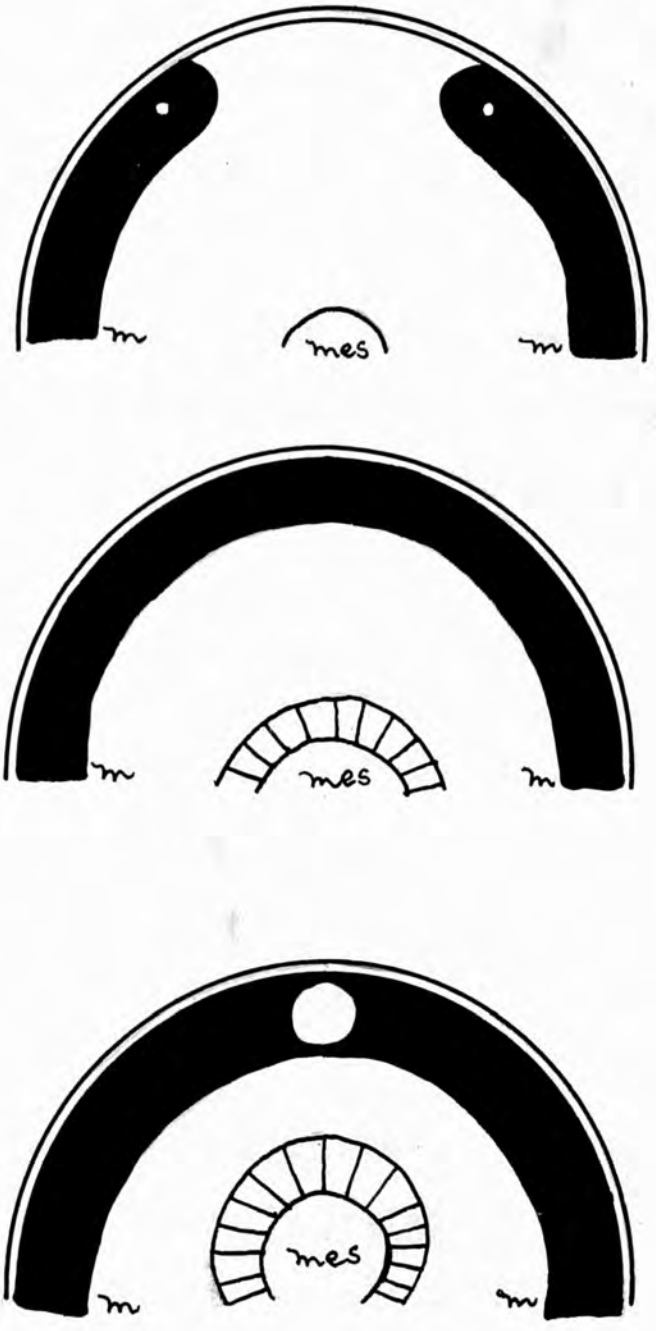


Figure 144. Diagram showing the method of heart formation in Daphnia. m, mesoderm; mes, mesenteron.

longitudinal muscles and the pericardial cavity are formed in much the same way as in Estheria and Chirocephalus by the shrinkage inwards away from the ectoderm of some of the dorsal part of the mesoderm on either side of the heart. This mesoderm is an extension of the outer wall of the small coelomic sac. The heart therefore forms in Daphnia in much the same way as the middle region of the heart of Chirocephalus. A noticeable difference is that it is not attached to the alimentary canal but separated from it by yolk. Otherwise the method of heart formation is similar.

The end sac of the maxillary gland of Daphnia magna develops as a cavity in the ventro-lateral mesoderm in the same region as that in which the coelomic sac was present. The coelomic sac is difficult to trace, but it almost certainly is connected with the end sac of the maxillary gland. The maxillary gland of D. magna is also mesodermal in origin. As in Chirocephalus, the end sac appears before the loops of the gland.

The heart develops in Daphnia magna slightly before the septa defining the blood spaces. When the heart starts beating the septa and the blood cells are present. The septa appear to develop as layers of mesodermal cells defining cavities between ectoderm and mesoderm. If there is a layer of mesodermal cells on the outer wall of the cavities, it is extremely thin. The perivisceral space, or intestinal cavity, is formed by the collapse of yolk and the formation of yolk cells and also by the movement of the mesenteron towards the dorsal surface of the embryo. The mesenteron acquires its muscle layers from the ventral mesoderm. As it moves dorsalwards, the mesenteron with its muscles separates

from the ventral mesoderm and the intestinal cavity is formed surrounding the mesenteron and the genital cells. In Estheria and Chirocephalus the perivisceral cavity is formed by the appearance of a space between the more ventral mesoderm and the alimentary canal.

In Daphnia magna, some at least of the blood cells arise from small mesodermal cells in the embryo. In Artemia, Lochhead (1941) found blood-cell-forming organs, the first description of such organs in entomostraca. The organs are situated near to the bases of the limbs and each is in the form of a nodule. No such organs have been found in Daphnia, although in a similar position there are a large number of fat cells. In Hemimysis, Manton (1928) described the origin of the blood cells which separate from the upper edges of the mesoderm and wander upwards between the yolk sac and the ectoderm, dividing mitotically. In insects, the blood cells arise in the embryo from undifferentiated mesoderm cells, and later from the mitotic division of the blood cells already present (Wigglesworth, 1953). The situation in Daphnia is comparable to Hemimysis and to insects, the blood cells arising from dorsal mesoderm cells in the embryo and multiplication by division having been suggested in the adult.

Except in themaxillary region, the lateral mesoderm remains an extremely thin layer, only about one cell in thickness, until late in the development of the embryo. The region between the endoderm and the ectoderm is principally taken up by yolk and yolk cells, making a most striking feature of the developing embryo.

The genital cells of Daphnia magna do not undergo an early secondary reduction in number, as they do in some Crustacea. In the adult, the gonads lie in the intestinal or perivisceral cavity which is formed by the collapse of yolk and are not apparently related to a coelomic sac. The ovary does not develop a true central cavity, as it does in most Arthropoda, since the whole circumference of the organ is filled with eggs. In the testis, a central cavity is formed.

Conspicuous in the ectoderm are the large cells of the "Scheitel"-plate, which in the early stages of development rival the genital cells in size, although in the embryos of most animals the genital cells are the largest cells. The "Scheitel"-plate forms the nervous system and sense organs of the head. The ventral nerve cord is formed from the ventral ectoderm. Both these processes are comparable to those in related animals.

The dorsal organ of Cladocera has aroused much interest. It is found in many Cladocera and has been compared to other structures of similar appearance in related groups. It has sometimes been called the neck organ, and at other times confused with the "Haft"-organ, or clasping organ, of Sida to which it is possibly related. The term neck organ has also been applied to a structure in a similar position in young Artemia. Weisz (1947) suggested that the function of the neck organ in Artemia is to provide support at the attachment of the antennal muscles which are large in the young animal when the antennae are used for swimming, but reduced in later life when the swimming is taken over by the thoracic

appendages. In the embryo of Daphnia the antennal muscles are not related to the dorsal organ. A dorsal organ is present in Triops, in which it survives in the adult. It may be related to the dorsal organ of Cladocera. The term neck organ has been applied to almost any organ in the Branchiopoda, usually in the form of a swelling, in the region of the junction of the head with the thorax.

In the embryo of Daphnia, the dorsal organ is present when the egg is one day old and survives until about the third instar. It consists of a group about a dozen cells in length and a few cells in width. The cells are large and contain large nuclei. The nuclei move to the inner part of the cells and the outer part contains granules. The cells stain more deeply than the surrounding ectodermal cells but not as deeply as for example the labral gland cells. During the embryological development, the basementmembrane becomes uneven and breaks down and the cells begin to degenerate so that the number of nuclei come to exceed the number of cells. The dorsal organ is bounded internally by yolk.

At one stage during the development, the dorsal organ is connected to the anterior end of the heart by a row of cells. At a later stage, it is connected to the dorsal flexure of the mesenteron by fine trabeculae. This is shortly before the animal hatches from the brood pouch of the mother and the dorsal organ is beginning to degenerate. It is possible that the mesenteron plays some part in the degeneration.

The condition of the dorsal organ in the embryo does not provide much evidence concerning the function. Earlier workers, such as Gicklhorn and Keller (1925,c) and Dejadar (1930), found that the dorsal organ shows an affinity for vital stains, while Dejadar and Gicklhorn (1933) noted the concentration of Colacium around the organ suggesting the presence of carbon dioxide. These results lead to the suggestion of a respiratory function. Further support for this theory is the relationship of the degree of development of the branchial sacs to that of the dorsal organ (Dejadar, 1930). The dorsal organ degenerates as the branchial sacs begin to function, and in forms in which the adult has no branchial sacs the dorsal organ does not degenerate. Other suggestions that have been made are that the dorsal organ functions as a secretory organ, for instance that it secretes the chitinous exoskeleton, that it is a sensory organ or that it is concerned in the regulation of osmotic pressure. The large, deeply staining cells suggest that the dorsal organ performs some function and also that this function is one which involves the contents of the cells, such as an exchange of substances, rather than a budding off of cells, such as blood cells, or any similar function. The nature of the cells and the fact that any nerve to the dorsal organ must be small, suggest that the function is not a sensory one. A secretory function is possible but a respiratory function seems the most likely one.

The development of the ephippial egg is in most respects similar to that of the parthenogenetic egg, a fact which was also noted by Baldass (1942). Fangauf (1921) stated that there are

marked differences between the development of the two kinds of eggs. After remarking that the cleavage in the parthenogenetic, or summer, eggs is superficial in contrast to that in the ephippial, or winter, eggs, Fangauf states his fourth point: "Zwischen der Entwicklung des Sommereies und des Wintereies bestehen noch andere weitgehende Unterschiede". The different conclusions arrived at by Fangauf cannot be attributed to the fact that he worked with Daphnia pulex as this was also the species studied by Baldass. The cleavage in both kinds of egg is superficial, and the differences present in the development of the eggs depend primarily on the state of the yolk content and are not far-reaching. The differences mainly concern the later formation of the membranes between the blastoderm cells and a greater inclusion of yolk within these cells in the ephippial egg, together with the more conspicuous nature of the yolk cells due to the smaller size of the yolk granules in the ephippial egg and the fact that they do not appear to be as tightly packed together. The developing ephippial egg of D. magna in the blastoderm stage is similar in appearance to the egg of Artemia as depicted by Fautrez-Firlefyn (1951) and to that of Branchipus depicted by Brauer (1892). Sections of the egg of Triops also have a similar appearance.

The majority of the ephippial eggs of Daphnia magna pass through a diapause at the stage of gastrulation. In Arthropoda, it is generally concluded that diapause occurring in the early stages of embryogenesis must depend upon the state of the mother, which influences the kind of egg produced. In Daphnia, the

cause for the production of ephippial eggs appears to be the lowering of the general metabolic rate, and the availability of food an important factor. Andrewartha (1952) considered that evidence from ecological studies indicated a central position for some metabolic process associated with food reserves stored in the fat body or egg yolk as a general cause of diapause in insects. The difference in the nature of the yolk content in the two kinds of eggs of Daphnia is interesting in this connection. But Gallistel (1936-7), in histochemical investigations, found no principal difference between the reserve material of the parthenogenetic and ephippial eggs. Also Hinton (1953) and Lees (1955) criticise the hypothesis of Andrewartha and other related theories. Hinton suggests that diapause in insects is brought about by the production of a diapause hormone and by the prothoracic glands ceasing to secrete. His ideas are in keeping with those of Wigglesworth (1953).

In insects, the factors causing the breaking of the diapause appear to vary. The recent work of Basden (1954) suggests that in Drosophila the ending of the diapause is related to a higher temperature. A similar result is indicated by the experiments with the ephippial eggs of Daphnia magna (p. 101), in which a rise in temperature from 18° to 28°C. seems to be the only factor of importance. The experiments of Hall (1953) with the eggs of Chirocephalus diaphanus suggest that desiccation is not necessary or advantageous to the hatching of the eggs. This is in agreement with the experiments with the ephippial eggs of Daphnia made by

Banta and his co-workers (1939).

The development of Daphnia magna exhibits a number of general differences from that usually seen in Crustacea, or other Arthropoda. One of these differences is the order of the development of the appendages, which do not develop in series from anterior to posterior. The second antennae appear early, which will be connected with their importance as swimming organs. The mandibles develop soon afterwards and also grow to a large size, being important in feeding. The first antennae, or antennules, develop later and are small, undergoing a slight secondary reduction during the embryonic development. The two pairs of maxillae develop almost at the same time as each other, the first pair being lateral to the second. The first pair soon degenerates, and the second pair remains small. There are no maxillary ganglia in the adult. The thoracic appendages all develop together, in contrast to the majority of other Crustacea where they are added segment by segment.

Another interesting feature is the relatively late development of the alimentary canal in comparison with other Crustacea whose development includes a nauplius stage. The large quantity of yolk available for nourishment allows for the relatively late development of a cavity in the mesenteron, which does not possess a continuous central cavity when the egg membrane is shed. The embryo which hatches from the egg membrane is sometimes referred to as the nauplius stage in Daphnia, but is further developed than the usual nauplius since it has thoracic appendages and an abdomen

as well as the rudiments of the carapace folds. The alimentary canal is less well developed than in the usual nauplius stage.

The early development of the labral glands is comparable to their early development in Estheria (Cannon, 1924) although the embryo of Daphnia does not begin to feed until it is fully developed. The large nuclei are reminiscent of the salivary glands of, for example, Drosophila.

The changes in the colour of the eyes, from pink to brownish red to black, already noted by earlier workers, are comparable to those observed in Gammarus by Ford and Huxley (1927).

An absorption of water is an important feature of the development. It accounts for the osmotic hatching, a method observed in varying degrees in other Crustacea, such as copepods and Hemimysis. A gradual increase in volume takes place throughout the development, with two major increases when the membranes are shed. If the entry of water by osmosis is prevented, the egg or embryo fails to hatch but continues to develop and a closed development results. The osmotic pressure relations may also be the explanation of the failure of the eggs of Daphnia to develop if removed from the brood pouch of the mother within about three hours after laying, since many of these eggs burst on being removed. In that case, nourishment in the brood pouch may not be necessary in any stage of development since it is not necessary after the initial three hours.

The embryo leaves the brood pouch of its mother as an immature adult, that is, direct development takes place. A

discussion of the adult anatomy is included at the end of the relevant section (p. 210). As a Crustacean, the principal modifications from an animal such as Chirocephalus which Daphnia has undergone concern an obliteration of traces of segmentation with a shortening of the body and a specialisation of the appendages. The arrangement of the musculature in part indicates a metameric origin; the nervous system shows more distinct signs of segmentation being only slightly condensed. Greater modifications are seen in the shortening of the heart to a sac-like organ of two segments, in the reduction of the two pairs of maxillae to one small pair and in the specialisation of the thoracic appendages enabling a reduction in their number. The specialisation of the thoracic appendages is correlated with the development of the second antennae as strong swimming organs. There has also been an alteration in the position of the opening of the male genital duct, and the genital organs are simple sac-like organs without trace of segmentation. The fat cells are a specialisation for the storage and breakdown of substances, highly developed in Daphnia.

Summary of advances made in this thesis.

When writing a comprehensive account of a subject in which there has been a considerable amount of previous work, the consecutiveness of the account may be impaired if the distinction between old work and new is too greatly stressed. Care has been taken throughout the text to distinguish between facts that have been acquired from earlier authors and work that is new. But in order to clearly distinguish the advances that have been made in this thesis, the most important are summarised here.

1. A technique which is adequate for the material has been developed by the fine adjustment of both conditions and times.
2. The embryos of any one brood show a variation in the time taken for development, even when all are kept at the same temperature and in uniform conditions. The embryos of a brood often show variation in the amount of yolk which they contain. The amount of yolk may influence the time taken for development.
3. Observations on the development of Daphnia magna eggs living at 18°C. give greater detail than has previously been available, and will enable the more exact definition of the stage during the instar of the mother for experimental and ecological work. The first such series of observations on the development of the ephippial eggs has been made.
4. The parthenogenetic egg of Daphnia magna always contains more than one oil droplet, in contrast to the egg of D. pulex which contains only one oil droplet. The number of oil droplets increases during development and the droplets become smaller.

5. The egg of Daphnia magna contains a large amount of yolk which has an important influence upon the development of the egg. Some of the yolk remains unaltered until after the young animal has hatched from the brood pouch of the mother.
6. The first modification of the external shape of the egg occurs when the egg is 18 hours old and marks the beginning of the broad antennal folds. The second antennae develop early, as do the antennal muscles. This early development is correlated with the importance of the antennae as swimming organs.
7. The thoracic appendages all develop together.
8. The first antennae, or antennules, develop late, when the rudiments of the thoracic appendages are already present. The antennules undergone a slight secondary reduction in size.
9. Both the pairs of maxillae are present when the embryo is approximately 55 hours old. The second pair is lateral to the first pair and disappears during the next few hours.
10. The outer egg membrane bursts when the egg is 34 to 41 hours old. The second, or embryonic, membrane bursts when the embryo is 51 to 59 hours old.
11. The pigment of the eye develops when the embryo is approximately 47 hours old. The pigment at first forms a single area, it then divides into two, and later coalesces again.
12. The first movements occur when the embryo is approximately 67 hours old, and are made by the thoracic appendages.
13. The heart starts to beat when the embryo is approximately 68 hours old. The beat is irregular until after the animal has hatched.

14. The dorsal spine is extended when the embryo is approximately 92 hours old. The extension of the spine takes place by the animal performing a somersault.

15. The development of the ephippial eggs is similar to that of the parthenogenetic eggs but slower. The only external modification present when the eggs are shed at the end of the instar is a small shallow depression near to one end of the egg.

16. Further experiments have been carried out to show more conclusively that the eggs of Daphnia magna will, while the eggs of Moina rectirostris will not, develop outside the brood pouch of the mother or in the brood pouch of the dead mother. In confirmation of earlier work, the eggs of D. magna will not, however develop if removed during the first three hours after they have been laid into the brood pouch.

17. The initial cleavage divisions take place in the centre of the egg with no external sign of segmentation. The divisions are not entirely synchronous and the resulting blastomeres are scattered irregularly through the yolk.

18. The blastomeres migrate to the periphery of the egg at approximately the 12- to the 16-cell stage. Here they form a layer of cytoplasm with nuclei but without cell membranes. No nuclei are left behind in the centre of the egg. Cell boundaries form later in the peripheral layer.

19. Gastrulation occurs by immigration of cells from a restricted area of the blastoderm, together with a very small invagination. An irregular inner group of polygonal cells is formed,

consisting of the genital cells and the mesendoderm. All the mesendodermal cells are similar and contain yolk, as do the blastoderm cells. The "Scheitel"-plate is present at this stage and its cells rival the genital cells in size.

20. The endoderm becomes distinguishable from the mesoderm when the egg is approximately one day old. It forms a group four to five cells in width and about three cells in height. The endodermal cells are larger and less granular than the surrounding cells. They contain large nuclei with few chromatin granules. The endoderm forms the mesenteron only. The stomodaeal and proctodaeal invaginations are distinguishable at this stage of development.

21. The endodermal cells form into a solid rod which is recognisable at first anteriorly. The caeca of the mesenteron and the anterior dorsal flexure of the mesenteron are formed at the same time.

22. The mesenteron rod develops a central cavity by the breakdown of the cytoplasm in the centre of the rod, when the embryo is just over two days old. The central cavity develops from the anterior end towards the posterior end of the mesenteron.

23. The mesenteron separates from its position adjacent to the ventral ectoderm and moves towards the dorsal surface of the embryo. With the movement of the mesenteron dorsally and with the development of the abdomen, the posterior flexure of the mesenteron becomes more marked. The furrows present at the junction of the stomodaeum and proctodaeum with the mesenteron become conspicuous. Mesodermal cells surround

the stomodaeum and the proctodaeum and form the muscles. The mesenteric muscles develop slightly later.

24. The inner rods and the peritrophic membranes develop shortly before the animal hatches from the brood pouch of the mother.

25. When the animal hatches the alimentary canal is fully developed but empty. It never contains yolk.

26. Within the same egg, the yolk stains differently at different stages of the development, and in different parts of the egg at the same stage of development.

27. The yolk cells first appear shortly before the time of gastrulation. Their formation continues during the process of gastrulation and the differentiation of the mesendoderm.

28. The yolk cells do not form from any one germ layer but from the cells surrounding the yolk. They occur at moderately regular intervals in the peripheral area of the yolk and gradually move towards the centre of the egg.

29. At a later stage, a cytoplasmic syncytium is formed through the yolk connecting with the yolk cells. A portion of the yolk becomes vacuolated and changes its staining reaction.

30. The yolk cells develop directly into the fat cells which are present in the adult. Fat cells, yolk cells and unaltered yolk are present when the animal hatches from the brood pouch of the mother.

31. The mesoderm develops from the lateral and the anterior parts of the mesendoderm and is composed of small irregularly shaped, granular cells with small nuclei containing scattered chromatin granules. The mesoderm thickens anteriorly, and to a smaller

extent posteriorly, but laterally remains only one cell thick.

32. A single pair of extremely small coelomic cavities occurs in the maxillary region in the dorsal edge of the mesoderm. The cavities are enclosed by a single layer of mesodermal cells. At this stage, the dorsal edge of the mesoderm is widely separated from both the mesenteron and the mid-dorsal surface of the embryo by the central yolk mass. In this respect the embryo of Daphnia magna is strikingly different from that of Estheria or Chirocephalus.

33. The mesoderm is especially thickened in the lateral part of the maxillary region. It gradually extends over the dorsal surface of the yolk from the posterior end of the animal and in the maxillary region, where the mesoderm from the two sides meets to form a compact mass of cells in the future region of the heart.

34. The heart is formed from a compact mass of cells, the central cells of which collapse. The end sac of the maxillary gland almost certainly originates in connection with the small dorsal coelomic cavity. The heart and the end sac of the maxillary gland develop at the same time, as does the rudiment of the maxillary gland loops. The rudiment of the maxillary gland loops is composed of large, spherical, deeply staining cells and the loops do not develop cavities until much later in the development. Small cells which are probably blood cells are first observed at about this stage of the development.

35. The proximal and distal labral glands develop at an early stage, when the embryo is approximately 50 hours old.

36. The dorsal mesoderm posterior to the heart collapses. The septa and the blood spaces develop secondarily, the ventral cavities between the ectoderm and the mesoderm. The septa are at first formed of a row of mesodermal cells, which become thinner. Small blood cells are present inside the heart and the carapace folds. When the heart starts to beat, when the embryo is approximately 68 hours old, the blood spaces and the blood cells are well developed.

37. The genital cells are distinguishable at the time of gastrulation. The cells are large and coarsely granular with large nuclei.

38. At the time of the bursting of the outer egg membrane, the group of genital cells divides into two and the cells increase in number. The two groups of genital cells move to a dorso-lateral position with little further increase in the number of cells.

39. By the time that the animal hatches, the genital rudiments have increased in length but the cells are all alike and there is no central cavity.

40. The dorsal organ is present when the egg is one day old, and remains visible until approximately the third instar. It consists of a group of swollen cells in the ectoderm, which soon stain more intensely than the surrounding cells. The cells have large nuclei and nucleoli. The nuclei move to the inner part of the cell, while the outer part becomes filled with granules. The dorsal organ lies close to the anterior flexure of the mesenteron, to which it is connected at a later

stage by a number of fine trabeculae. After the animal hatches, the cells become smaller and the organ gradually disappears.

41. The ephippial egg differs from the parthenogenetic egg in the yolk content. The yolk globules in the ephippial egg are smaller and of a more even size, and their staining reaction is different. Differences between the development of the two kinds of egg are mostly due to the difference in the state of the yolk. Differences in the development include the later formation of the cell membranes in the peripheral layer of cytoplasm and the greater yolk content of these cells when formed, and the more conspicuous nature of the yolk cells in the ephippial egg.

42. When the ephippial eggs are shed by the mother at the end of the instar, they have reached the stage of gastrulation and enter diapause at this stage.

43. A number of experiments have been undertaken in an attempt to determine the factors which will break the diapause. The only significant result is that a rise in temperature of 10°C. increased the percentage hatch.

44. Development after the diapause has been broken takes two to three days.

45. The newly hatched animal differs from the adult animal in various features of the alimentary canal, the reproductive system, the fat cells, the sense organs and the external features, including the dorsal organ.

46. A revised and extended account of the anatomy of the adult animal is given.

47. The circular and longitudinal muscles of the mesenteron are striated. A detailed account of the histology of the cells of the mesenteron is included.

48. A revised account of the histology of the maxillary gland is included. The excretory duct opens on the base of the second thoracic appendage on a small papilla. The position of the opening has previously been in doubt.

49. A detailed account of the labral glands is included and the glands compared to those in Simocephalus vetulus.

50. A receptaculum seminis has been identified for the first time in a species of Daphnia.

51. An incomplete layer of longitudinal muscle has been identified in the wall of the heart. This is in addition to a complete layer of circular muscles. The circular muscles are arranged in pairs.

52. It is suggested that the cells of the branchial sacs are all one type of cell which pass through a number of phases, of which four are described. The phases grade into each other. The description of the histology of the cells is revised.

53. The ocellus has four pigment cups, in confirmation of early authors but in disagreement of Leder. The cells in the anterior and lateral arms contain two long thin transparent areas which appear to be degenerating rhabdomes.

54. A number of irregularly arranged cells, of a similar nature to those of the median frontal organ, occur in two groups on the rostrum. The dorsal group is connected to the pair of median frontal organ cells, and the ventral group possibly to the ocellus.

55. The antennules are well developed as sensory organs.

References.

Agar, W.E., 1908, Note on the early development of a Cladoceran (Holopedium gibberum). Zool.Anz.,33,420-427.

Agar, W.E., 1913, Transmission of environmental effects from parent to offspring in Simocephalus vetulus. Phil.Trans.,B,203,319-350.

Agar, W.E., 1950, The swimming setae of Daphnia carinata. Quart.J.micr.Sci.,91,353-368.

Anderson, B.G., 1930, Growth and sexual maturity in Daphnia magna. Proc.Soc.exp.Biol.,N.Y.,38,334-335.

Anderson, B.G., 1932, The number of pre-adult instars, growth, relative growth and variation in Daphnia magna. Biol.Bull.,Wood's Hole,63,81-98.

Anderson, B.G., 1933, Regeneration in the carapace of Daphnia magna. I. The relation between the amount of regeneration and the area of wound during single adult instars. Biol.Bull.,Wood's Hole,64,70-85.

Anderson, B.G. and Brown, L.A., 1930, A study of chitin secretion in Daphnia magna. Physiol.Zool.,3,485-493.

Anderson, B.G. and Jenkins, J.C., 1942, A time study of events in the life span of Daphnia magna. Biol.Bull.,Wood's Hole, 83,260-272.

Andrewartha, H.G., 1952, Diapause in relation to the ecology of insects. Biol.Rev.,27,50-107.

Baird, W., 1850, The natural history of the British Entomostraca. Ray Society, London.

Baldass, F. von, 1937, Entwicklung von Holopedium gibberum. Zool.Jb.,Anat.,63,399-454.

Baldass, F. von, 1942, Entwicklung von Daphnia pulex. Zool.Jb.,Anat.,67,1-60.

Bankierowa, J., 1933, Uber den Bau der Mandiblen bei Daphnia magna Straus. Ann.Mus.zool.polon.,10,33-40.

Banta, A.M., 1925, A thelytokous race of Cladocera in which pseudo-sexual reproduction occurs. Z.indukt.Abstamm.-u. VererbLehre.,40,28-41.

Banta, A.M., 1939, Studies on the physiology, genetics, and evolution of some Cladocera. Pap.Dep.Genet.Carneg. Instn., No. 39

Basden, E.B., 1954, Diapause in Drosophila (Diptera: Drosophilidae). Proc.R.ent.Soc.Lond.,Ser.A,29,114-118.

Bekker, O.M. and Krijgsman, B.S., 1951, Physiological investigation into the heart function of Daphnia. J.Physiol.,115, 249-257.

Berg, K., 1931, Studies of the genus Daphnia with especial reference to the mode of reproduction. Vidensk.Medd.dansk naturh.Foren.Kbh.,92,1-222.

Berg, K., 1934, Cyclic reproduction, sex determination and depression in the Cladocera. Biol.Rev.,9,139-174.

Berg, K., 1936, Reproduction and depression in the Cladocera illustrated by the weight of the animals. Arch.Hydrobiol.(Plankt.).,30,438-462.

Bernard, F., 1937, Recherches sur la morphogénèse des yeux composés d'arthropodes. Bull.biol.,suppl.23,1-162.

Binder, G., 1932, Das Muskelsystem von Daphnia. Int.Rev.Hydrobiol., 26,54-111.

Borradaile, L.A., Potts, F.A., Eastham, L.E.S., and Saunders, J.T., 1935, The Invertebrata. Cambridge, at the University Press.

Brachet, J., 1945, Chemical Embryology. (Translated from the French by L.G. Barth, 1950. Interscience Publishers, Inc., New York).

Brauer, A., 1892, Ueber das Ei von Branchipus grubei von der Bildung bis zur Ablage. Abh.preuss.Akad.Wiss., 1892, 1-66.

Brown, L.A., 1926, Temperature characteristics for duration of an instar in Cladocerans. J.gen.Physiol.,10,111-119.

Brown, L.A., 1927, On the nature of the equation for growth processes. J.gen.Physiol.,11,37-42.

Bruntz, M.L., 1903, L'excrétion chez les Phyllopodes et les Copépodes. C.R.Soc.Biol.,Paris,55,652-653.

Bruntz, M.L., 1905, Etude physiologique sur les phyllopodes Branchiopodes. Arch.Zool.exp.gén.,4,183-198.

Calman, W.T., 1909, Crustacea, in A treatise of Zoology, edited by Sir Ray Lankester. Adam and Charles Black, London.

Cannon, H.G., 1921, The early development of the summer egg of a Cladoceran (Simocephalus vetulus). Quart.J.micr.Sci., 65,627-642.

Cannon, H.G., 1922, On the labral glands of a Cladoceran (Simocephalus vetulus) with a description of the mode of feeding. Quart.J.micr.Sci.,66,213-234.

Cannon, H.G., 1924, On the development of an Estherid Crustacean. Phil.Trans.,B,212,395-430.

Cannon, H.G., 1926, On the post-embryonic development of the Fairy Shrimp (Chirocephalus diaphanus). J.Linn.Soc. (Zool.),36,401-416.

Cannon, H.G., 1928, On the feeding mechanism of the Fairy Shrimp, Chirocephalus diaphanus. Trans.roy.Soc.Edinb.,55, 807-822.

Cannon, H.G., 1933, On the feeding mechanism of the Branchiopoda. Phil.Trans.,B,222,267-352.

Cannon, H.G., 1935,a, A further account of the feeding mechanism of Chirocephalus diaphanus. Proc.roy.Soc.,B,117,455-470.

Cannon, H.G., 1935,b, Function of labral glands in Chirocephalus. Nature,Lond.,136,758.

Cannon, H.G. and Manton, S.M., 1927, Notes on the segmentary excretory organs of Crustacea I-IV. J.Linn.Soc.(Zool). 36,439-456.

Chambers, R., 1913, The spermatogenesis of a Daphnid, Simocephalus vetulus. Biol.Bull.,Wood's Hole,25,134-140.

Chatton, E., 1920, Membranes peritrophiques des Drosophiles et des Daphnies. Bull.Soc.zool.Fr.,45,265-280.

Child, C.M., 1941, Patterns and Problems of Development. University of Chicago Press, Chicago.

Claus, C., 1875, Die Schalendrüse der Daphniden. Z.wiss.Zool.,25, 165-175.

Claus, C., 1876, Zur Kenntniss der Organisation und des fienern Baues der Daphniden und verwandten Cladoceren. Z.wiss.Zool.,27,362-402.

Claus, C., 1886, Untersuchungen über die Organisation und Entwicklung von Branchipus und Artemia. Arb.zool.Inst. Univ.Wien,6,267-370.

Claus, C., 1894, Bemerkungen über die Nervenendigungen in den Hautsinnesorganen der Arthropoden, insbesondere Crustaceen. Zool.Anz.,17,404-408.

Cunnington, W.A., 1903, Studien an einer Daphnide, Simocephalus sima. Beitrage zur Kenntniss des Zentralnervensystems und den feineren Anatomie der Daphniden. Jena.Z. Naturw.,37,447-520.

Dawydoff, C., 1928, Traité d'embryologie comparée des Invertébrés. Masson and Cie., Paris.

Debaisieux, P., 1954, Histogenèse des muscles et charpentes chez les Crustacés. La Cellule,56,265-305.

von Dehn, M., 1930-1, Untersuchungen über die Verdauung bei Daphnien. Z.vergl.Physiol.,13,334-358.

von Dehn, M., 1955, Die Geschlectsbestimmung der Daphniden. Die Bedeutung der Fettstoffe, untersucht an Moina rectirostris L. Zool.Jb.,Abt.3,65,334-356.

Dejadar, E., 1930, Vitale Electivfärbung der rudimentären Antennendrüse von Cladoceren. Z.Morph.Okol.Tiere,17, 768-777.

Dejadar, E. and Gicklhorn, J., 1933, Neue Untersuchungen zum Nachweis der Funktion des Nackenschildes der Cladoceren als Atmungsorgan. Z.Morph.Okol.Tiere,26,94-109.

Dresel, E.I.B., 1948, Passage of haemoglobin from blood into eggs of Daphnia. Nature,Lond.,162,736.

Dohrn, A., 1870, Untersuchungen über Bau und Entwicklung der Arthropoden. 5. Die Schalendrüse und die embryonale Entwicklung der Daphniden. Jena.Z.Naturw.,5,277-292.

Edmondson, W.T., 1955, The seasonal life history of Daphnia in an arctic lake. Ecology,36,No.3,439-455.

Eriksson, S., 1935, Studien über die Fangapparate der Branchiopoden nebst einigen phylogenetischen Bemerkungen. Zool.Bidr.Uppsala,15,23-287.

Fangauf, R., 1921, Die Entwicklung von Daphnia pulex aus dem Sommerei. Jahrb.Dissert.phil.Fakul.Berlin.

Fautrez-Firlefyn, N., 1951, Etude cytochimique des acides nucléiques au cours de la gamétogénèse et des premiers stades du développement embryonnaire chez Artemia salina L. Arch.Biol.,Paris,62,391-438.

Fiedler, P., 1908-9, Mitteilung über das Epithel der Kiemensachschen von Daphnia magna Straus. Zool.Anz.,33,493-496.

Fischel, A., 1908, Untersuchungen über vitale Färbung an Süsswassertieren, insbesondere bei Cladoceren. Int.Rev.Hydrobiol.,1,73-141.

Flückiger, E., 1952, Die Wirkung von l-Adrenalin an Daphnia magna Str., K.fysiogr.Sällsk.Lund Forh.,21,No.6,63-68.

Flückiger, E., 1953, Ueber die Wirkung der Sympathomimetica und Dihydroergotamin bei Daphnien. I. Wirkung auf Muskelfunktionen. Acta physiol.scand.,27,No.2-3,206-216.

Flückiger, E. and Flück, H., 1952, Die Wirkung von Vitamin B_1 am Herz von Daphnien. Experientia,8,223-224.

Ford, E.D. and Huxley, J.S., 1927, Mendelian genes and rates of development in Gammarus chevreuxi. J.exp.Biol.,5, 112-134.

Fox, H.M., 1948, The haemoglobin of Daphnia. Proc.roy.Soc.,B, 135,195-212.

Fox, H.M., 1952, Anal and oral intake of water by Crustacea. J.exp.Biol.,29,583-599.

Fox, H.M., 1955, The effect of oxygen on the concentration of haem in invertebrates. Proc.roy.Soc.,B,143,203-214.

Fox, H.M., Gilchrist, B.M. and Phear, E.A., 1951, Functions of haemoglobin in Daphnia. Proc.roy.Soc.,B,138,514-528.

Fox, H.M., Hardcastle, S.M. and Drezel, E.I.B., 1949, Fluctuations in the haemoglobin content of Daphnia. Proc.roy.Soc.,B,136,388-399.

Fox, H.M. and Phear, E.A., 1953, Factors influencing haemoglobin synthesis by Daphnia. Proc.roy.Soc.,B,141,179-189.

von Frisch, K. and Kupelwieser, H., 1913, Uber den Einfluss der Lichtfarbe auf die Phototaktischen Reaktionen niederer Krebse. Biol.Zbl.,33,517-552.

Fritzsche, H., 1917, Studien über die Schwankungen des osmotischen Druckes der Körperflussigkeiten bei Daphnia magna. Int.Rev.Hydrobiol.,8,17-20;22-80; 125-203.

Gallistel, H., 1956-7, Histochemische Untersuchungen über das Speichern von Glykogen und Fett bei Daphnia magna. Z.Zellforsch.,25,66-82.

Gicklhorn, J., 1925a, Uber Reduktion von Silbersalzen in den Kiemensackchen von Daphnia. Lotos,73,83-96.

Gicklhorn, J., 1925,b, Kalkinkrustationen der Schale von Cladoceren. Lotos,73,157-166.

Gicklhorn J., 1931,a, Elective Vitalfarbungen im Dienste der Anatomie und Physiologie der Exkretionsorgane von Wirbellosen (Cladoceren als beispiel). Protoplasma,13, 701-724.

Gicklhorn, J., 1931,b, Beobachtungen an der lateralen Frontal-organen von Daphnia magna M. nach elektiver Vital-farbung. Protoplasma,13,725-739.

Gicklhorn, J., 1931,c, Elective Vitalfärbung, Probleme, Ziele, Ergebnisse, aktuelle Fragen und Bemerkungen zu den Methoden. Ergebn.Biol.,7,551-685.

Gicklhorn, J. and Keller, R., 1925,a, Uber elektive Vitalfärbung zweier Drüsen von Daphnia magna. Biol.Zbl.,45,154-169.

Gicklhorn, J. and Keller, R., 1925,b, Uber elektive Vitalfärbungen der Keimensäckchen von Daphnia magna Müller als Beispiel organ - und zellspezifischer Differenzierung. Z.Zellforsch.,2,515-537.

Gicklhorn, J. and Keller, R., 1925,c, Bau und Funktion des "Haftorgans" von Daphnia bzw. des "Kopfschildes" von Leptodora und Polyphemus auf Grund vitaler Elektivfarbung. Zool.Anz.,64,217-234.

Gravier, C., 1931, La Ponte et l'incubation chez les Crustacés. Ann.Sci.nat.,Zool.,14,303-419.

Green, J., 1954, Size and reproduction in Daphnia magna (Crustacea: Cladocera). Proc.Zool.Soc.Lond.,124, Pt.3,535-545.

Green, J., 1955, Haemoglobin in the fat-cells of Daphnia. Quart.J.micr.Sci.,96,Pt.2,173-176.

Green, J., 1956, Growth, size and reproduction in Daphnia (Crustacea: Cladocera). Proc.Zool.Soc.Lond.,126,173-204.

Green, J., in press, Variation in the haemoglobin content of Daphnia. Proc.roy.Soc.,B,

Green, W.R., 1919, Studies in the life cycle of Simocephalus vetulus. Biol.Bull.,Wood's Hole,37,49-95.

Grobben, C., 1879, Die Entwicklungsgeschichte der Moina rectirostris. Arb.zool.Inst.Univ.Wien,2,1-66.

Grobben, C., 1893, Einige Bemerkungen zu Dr. P. Samassa's Publikationen über die Entwicklung von Moina rectirostris. Arch.mikr.Anat.,42,213-216.

Haack, M., 1917, Zur äusseren Morphologie einiger Daphniden. Int.Rev.Hydrobiol.,8,338-393; 417-467.

Häcker, V., 1894, Die Entwicklung der Wintereier der Daphniden. Ber.naturf.Ges.Freiburg.i.B.,8,35-53.

Haget, A., 1945, Incubation d'oeufs ou d'embryons de Daphnides échangés à des âges différents entre femelles parthénogénétiques. C.R.Soc.Biol.,Paris,139,216-218.

Haget, A., 1946, Variations physicochimiques du liquide incubateur au cours de l'intermue chez les Daphnides. C.R.Soc.Biol.,Paris,140,504-506.

Hall, R.E., 1955, Observations on the hatching of eggs of Chirocephalus diaphanus Prévost. Proc.Zool.Soc.Lond., 125,95-109.

Hardy, W.B., 1892, The blood-corpuscles of the Crustacea, together with a suggestion as to the origin of the Crustacean fibrin-ferment. J.Physiol.,13,165-190.

Hardy, W.B. and McDougall, W., 1895, On the structure and functions of the alimentary canal of Daphnia. Proc.Camb.phil.Soc., 8,41-50.

Hartog, M., 1901, Method of feeding of Phyllopods and Cladocera. J.Quekett micr.Cl.,2nd Series,8,157-158.

Hasler, A.D., 1935, The physiology of digestion of plankton Crustacea. I. Some digestive enzymes of Daphnia. Biol.Bull.,Wood's Hole,68,207-214.

Hasler, A.D., 1937, The physiology of digestion in plankton Crustacea. II. Further studies on the digestive enzymes of (A) Daphnia and Polyphemus; (B) Diaptomus and Calanus. Biol.Bull.,Wood's Hole,72,290-298.

Hérouard, E., 1895, Organes frontaux, glande unicellulaire géante et origine du vitellus nutritif chez les Cladocères. Bull.Soc.zool.Fr.,20,68-76.

Hérouard, E., 1905, La Circulation chez les Daphnies. Mém.Soc. zool.Fr.,18,214-232.

van Herwerden, M.A., 1914, Uber die perzeptionsfähigkeit des Daphnienauges für ultraviolette Strahlen. Biol.Zbl.,34,213-216.

Hinton, H.E., 1953, The initiation, maintenance, and rupture of diapause: A new theory. The Entomologist,86,No.12, 279-291.

Hoshi, T., 1949, Studies on physiology and ecology of plankton. II. Oxygen consumption and heart-beat rate in developmental stages of Simocephalus vetulus (O.F. Müller). Sci.Rep.Tohoku Univ.,Ser.4 (Biol.),18,159-161.

Hoshi, T., 1950,a, Studies on physiology and ecology of plankton. III. Changes in respiratory quotient during embryonic development of a Daphnid, Simocephalus vetulus (O.F. Müller). Sci.Rep.Tohoku Univ.,Ser.4(Biol.),18, 316-323.

Hoshi, T., 1950,b, Studies on physiology and ecology of plankton. V. Fatty substance in development of Simocephalus vetulus, with reference to behaviour of yolk granule. Sci.Rep.Tohoku Univ.,Ser.4 (Biol.),18,464-466.

Hoshi, T., 1951,a, Studies on physiology and ecology of plankton. VI. Glycogen in embryonic life of Simocephalus vetulus, with some notes on the energy source of development. Sci.Rep.Tohoku Univ.,Ser.4 (Biol.),19,123-132.

Hoshi, T., 1951,b, Studies on physiology and ecology of plankton. VII. Hatchability of the summer egg of a Daphnid, Simocephalus vetulus, cultured in artificial media. Sci.Rep.Tohoku Univ.,Ser.4 (Biol.),19,145-151.

Hoshi, T., 1952, Studies on physiology and ecology of plankton. VIII. Behaviour of embryo of Simocephalus vetulus stained vitally with rH-indicators under aerobic and anaerobic conditions, with a note on the anaerobic

metabolism. Sci.Rep.Tohoku Univ.,Ser.4 (Biol.),19, 221-230.

Hoshi, T., 1953, Studies on physiology and ecology of plankton. IX. Changes in glycogen content during development of the Daphnid, Simocephalus vetulus under aerobic and anaerobic conditions. Sci.Rep.Tohoku Univ.,Ser.4 (Biol), 20,6-10.

Hoshi, T., 1954, Studies on the physiology and ecology of plankton. X. A relation between oxygen content in medium, incubation period of animal and glycogen content of the Daphnid, Simocephalus vetulus, kept under anaerobic conditions. Sci.Rep.Tohoku Univ.,Ser.4 (Biol.),20, 260-264.

Huber, W., 1954, Die submikroskopische Struktur der peritrophischen Membran aus dem Mitteldarm von Geotrupes silvaticus (Col.). Mitt.schweiz.ent.Ges.,27, 277-279.

Humperdinck, I., 1924, Uber Muskulatur und Endoskelett von Polyphemus pediculus de Geer. Topographisches und Embryologisches. Z.wiss.Zool.,121,621-655.

Imms, A.D., 1948, A general textbook of entomology including the anatomy, physiology, development and classification of insects. Methuen and Co., London.

Issakowitsch, A., 1907, Geschlectsbestimmende Ursachen bei den Daphniden. Arch.mikr.Anat.,69,223-244.

Jäger, G., 1935, Uber den Fettkörper von Daphnia magna. Z.Zellforsch.,22,89-131.

Jančařík, A., 1947, Přehled ontogenetického vyvoje perloočky Daphnia pulex. (Review of ontogenetic development of Daphnia pulex). Biologické listy, roč. 29, cís. 3, 109-116.

Johannsen, O.A. and Butt, F.H., 1941, Embryology of insects and myriapods. McGraw-Hill Book Company, Inc., New York and London.

Johnson, D.S., 1952, The British species of the genus Daphnia (Crustacea, Cladocera). Proc. zool. Soc. Lond., 122, 435-462.

Jurine, L., 1820, Histoire des Monocles, qui se trouvent aux environs de Genève. Genève and Paris.

Katz, B., 1949, Neuro-muscular transmission in invertebrates. Biol. Rev., 4, 1-20.

Kaudewitz, F., 1950, Zur Entwicklungsphysiologie von Daphnia pulex. Arch. Entw. Mech. Org., 144, 410-447.

Keller, R. and Pisho, B., 1947, Vital staining of the Daphnia nephron in ultra-violet light. Anat. Rec., 98, 39-44.

King, E.J., Gilchrist, M., Wootton, I.D.P., Donaldson, R., Sisson, R.B., Macfarlane, R.G., Jope, H.M., O'Brien, J.R.P., Peterson, J.M., and Strangeways, D.H., 1948, Determination of haemoglobin. V. Precision of colorimetric methods. Lancet, 255, 2, 563-566.

Klotzsche, K., 1913, Beiträge zur Kenntnis des feineren Baues der Cladoceren (Daphnia magna). Jena. Z. Naturw., 50, 601-646.

Klunzinger, 1864, Einiges zur Anatomie der Daphniden, nebst kurzen Bemerkungen über die Süsswasserfauna der Umgegend Cairo's. Z. wiss. Zool., 14, 165-173.

Koehler, O., 1924, Uber das Farbensehen von Daphnia magna Straus. Z.vergl.Physiol.,1,84-174.

Kollmann, M., 1924, Notes histologiques sur les Phyllopodes Branchiopodes. Bull.Soc.zool.Fr.,49,495-506.

Kowalevsky, A., 1890, Ein Beitrag zur Kenntnis der Exkretionsorgan. Biol.Zbl.,9,33-47; 65-76; 127-128.

Krijgsman, B.J., 1952, Contractile and pacemaker mechanisms of the heart of Arthropods. Biol.Rev.,27,320-346.

Krogh, A., 1939, Osmotic regulation in aquatic animals. Cambridge Univ. Press.

Kühn, A., 1908, Die Entwicklung der Keimzellen in den parthenogenetischen Generationen der Cladoceren Daphnia pulex De Geer und Polyphemus pediculus De Geer. Arch.Zellforsch.,1,538-586.

Kühn, A., 1911, Uber determinierte Entwicklung bei Cladoceren. Zool.Anz.,38,345-357.

Kühn, A., 1912, Die Sonderung der Keimbezirke in der Entwicklung der Sommereier von Polyphemus pediculus De Geer. Zool.Jb.,Anat.,35,243-340.

Lebedinski, J., 1891, Die Entwicklung von Daphnia aus dem Sommerei. Zool.Anz.,14,149-152.

Leder, H., 1915, Untersuchungen über den feineren Bau des Nervensystems der Cladoceren. Arb.zool.Inst.Univ.Wien., 20,297-392.

Lees, A.D., 1955, The physiology of diapause in Arthropods. Cambridge Univ. Press.

van Leeuwenhoek, A., 1719, Epistolae ad Societatem Regiam Anglicam et alios Illustres Viros Seu Continuatio mirandorum Arcanorum Naturae detectorum, Quadraginta Epistolis contentorum, quae ex Belgica in Latinam Linguam translatae sunt, cum figuris aeneis, et indicibus locupletissemis. Joh. Arnold, Langerak, Lugduni Batavorum.

Lefèvre, M., 1942, L'utilisation des algues d'eau douce par les Cladocères. Bull. biol., 76, 250-276.

Lévy, R., 1927, Mode de préparation de la Daphnie permettant de faire battre son coeur en milieu artificiel. C.R. Soc. Biol., Paris, 97, 1600-1602; 1602-1605.

Leydig, F., 1860, Naturgeschichte der Daphniden (Crustacea Cladocera). Laupp and Siebeck, Tübingen.

Lindahl, P.E., 1942, Contribution to the physiology of form generation in the sea urchin development. Quart. Rev. Biol., 17, 213-227.

Lityński, A., 1916, Über den Bau der Extremitäten bei den Cladoceren und deren Bedeutung für das System. Bull. int. Acad. Cracovie (Acad. pol. Sci.), 3-30.

Lochhead, J.H., 1950, Daphnia magna, in Selected invertebrate types, edited by F.A. Brown. John Wiley and Sons, New York.

Lochhead, J.H. and Lochhead, M.S., 1941, Studies on the blood and related tissues in Artemia (Crustacea. Anostraca). J. Morph., 68, 593-632.

Lowndes, A.G., 1933, The feeding mechanism of Chirocephalus diaphanus Prevost, the Fairy Shrimp. Proc. zool. Soc. Lond., 1093-1118.

Lubbock, Sir J., 1858, An account of the two methods of reproduction in Daphnia, and of the structure of the ephippium. Phil.Trans.,147,79-100.

Lundberg, R., 1895, The post-embryonal development of the Daphnids. Bih.svensk.Vetensk. Akad.Handl.,20,IV,1-19.

MacBride, E.W., 1914, Text-book of Embryology, Vol. I. Invertebrata. Macmillan and Co., London.

McClung's Handbook of microscopical technique, edited by R. McClung Jones, 1950. Paul B. Hoeber, New York.

Maloeuf, N.S.R., 1938, Physiology of excretion among the Arthropoda. Physiol.Rev.,18,28-58.

Maluf, N.S.R., 1939, The blood of Arthropods. Quart.Rev.Biol., 14,149-191.

Manton, S.M., 1928, On the embryology of a Mysid Crustacean, Hemimysis lamornae. Phil.Trans.,B,216,363-463.

Manton, S.M., 1934, On the embryology of the Crustacean Nebalia bipes. Phil.Trans.,B,223,163-238.

Manton, S.M., 1949, Studies on the Onychophora. VII. Early embryonic stages of Peripatopsis, and some general considerations concerning the morphology and phylogeny of the Arthropoda. Phil.Trans.,B,233,483-580.

Mawson, M.L. and Yonge, C.M., 1938, The origin and nature of the egg membranes in Chirocephalus diaphanus. Quart.J.micr. Sci.,80,553-565.

Metschnikoff, E., 1884, Über eine Sprosspilzkrankheit der Daphnien. Beitrag zur Lehre über den Kampf der Phagocyten gegen Krankheitserreger. Virchows Arch.,96,

(Neunte Folge,Bd.VI),177-195.

Mori, Y., 1933,a, Kastrationversuche bei Cladoceren. I. Die Entwicklung der sekundären Sexualcharaktere bei radiumbestrahlten Männchen von Daphnia magna. Z.wiss.Zool.,144,289-316.

Mori, Y., 1933,b, Kastrationversuche bei Cladoceren. II. Die Entwicklung der sekundären Sexualcharaktere bei radiumbestrahlten Weibchen von Daphnia magna. Z.wiss.Zool.,144,573-612.

Morison, G.D., 1927-8, The muscles of the adult honey-bee (Apis mellifera L.). Quart.J.micr.Sci.,71,395-463; 563-651: 72,511-526.

Moroff, T., 1911, Uber Entwicklung des Facettenauges der Crustaceen. Biol.Zbl.,31,144-150.

Moroff, T., 1912, Entwicklung und phylogenetische Bedeutung des Medianauges bei Crustaceen. Zool.Anz.,40,11-25.

Mortimer, C.H., 1956, Experimentelle und cytologische Untersuchungen uber den Generationswechsel der Cladoceren. Zool.Jb.,allg.Zool.u.Physiol.,56,323-388.

Nair, K.B., 1939, The reproduction, oogenesis and development of Mesopodopsis orientalis Tatt. Proc.Indian Acad. Sci.,9,175-223.

Nair, K.B., 1941, On the embryology of Squilla. Proc.Indian Acad.Sci.,14,543-576.

Nair, K.B., 1949, The embryology of Caridina laevis Heller. Proc.Indian Acad.Sci.,29,B,6,211-288.

Needham, A.E., 1937, Some points on the development of Neomysis vulgaris. Quart.J.micr.Sci.,79,559-589.

Needham, A.E., 1950, The neurogenic heart and ether anaesthesia. Nature,Lond.,166,9-13.

Needham, J., 1931, Chemical Embryology. Volumes I, II and III. Cambridge Univ. Press.

Needham, J., 1933, The energy sources in ontogenesis. VII. The respiratory quotient of developing Crustacean embryos. J.exp.Biol.,10,79-87.

Needham, J., 1942, Biochemistry and Morphogenesis. Cambridge Univ. Press.

Nicholson, K.W. and Yonge, C.M., 1935, Function of the labral glands in Chirocephalus. Nature,Lond.,136,608.

Obreshkove, V., 1941, The action of acetylcholine, atropine and physostigmine on the intestine of Daphnia magna. Biol.Bull.,Wood's Hole,81,105-113.

Obreshkove, V. and Fraser, A.W., 1940, Growth and differentiation of Daphnia magna eggs in vitro. Biol.Bull.,Wood's Hole, 78,429-436.

Pantin, C.F.A., 1948, Notes on microscopical technique for zoologists. Cambridge Univ. Press.

Parker, T.F., and Haswell, W.A., 1940, A text-book of Zoology. Sixth edition, revised by O. Lowenstein. Macmillan and Co., London.

Phear, E.A., 1955, Gut haems in the invertebrates. Proc.zool. Soc.Lond.,125,383-406.

Prosser, C.L., 1942, An analysis of the action of acetylcholine on hearts, particularly in Arthropods. Biol.Bull., Wood's Hole,83,145-164.

Prosser, C.L., Bishop, D.W., Brown, F.A., Jahn, T.L., and Wulff,V.J., 1950, Comparative animal physiology. W.B. Saunders Co., Philadelphia and London.

Pryzlecki, S., 1921,a, Recherches sur la pression osmotique chez les embryons de Cladocères, provenants des oeufs parthénogénétiques. Trav.Inst.Nencki,1.

Pryzlecki, S., 1921,b, Recherches sur la pression osmotique chez les embryons de Cladocères, provenants des oeufs fécondés. Trav.Inst.Nencki,1.

Pusey, H.K., 1939, Methods of reconstruction from microscopic sections. J.R.micr.Soc.,Ser.3,59,232-244.

Rádl, E., 1901, Uber den Phototropisms einiger Arthropoden. Biol.Zbl.,21,75-86.

Rammner, W., 1931, Mitteilungen über marine Cladoceren. Biol.Zbl.,51,618-653.

Rammner, W., 1933, Wird das Cladoceren-embryo von Muttertier ernährt. Arch.Hydrobiol.(Plankt.),25,692-698.

Ramułt, M., 1914, Untersuchungen über die Entwicklungsbedingungen der Sommereier von Daphnia pulex und anderen Cladoceren. Bull.int.Acad.Cracovie(Acad.pol.Sci.),481-514.

Ramułt, M., 1925, Development and resisting power of Cladocera embryos in the solutions of certain inorganic salts. Bull.int.Acad.Cracovie(Acad.pol.Sci.),135-194.

Retzius, G., 1906, Nervensystem der Daphniden. Biol.Untersuch., N.F.,13,106-112.

Rey, P., 1935, Sur le dévelopment en milieu artificiel des oeufs parthenogenetiques des Cladocéres. Bull.Soc.zool.Fr., 60,239-241.

Richards, A.G., 1951, The integument of Arthropods. University of Minnesota Press, Minneapolis.

Romeis, B., 1948, Mikroskopische Technik. R. Oldenbourg, Munchen.

Samassa, P., 1891, Untersuchungen über das centrale Nervensystem der Cladoceren. Arch.mikr.Anat.,38,100-141.

Samassa, P., 1893, Die Keimblätterbildung bei der Cladoceren. I. Moina rectirostris Baird. II. Daphnella brachyura Liév. und Daphnia hyalina Leydig. Arch.mikr.Anat.,41, 339-366; 650-688.

Samassa, P., 1894, Keimblätterbildung bei Moina. Zool.Anz.,16, 434-436; 437-439.

Samassa, P., 1897, Die Furchung der Wintereier der Cladoceren. Zool.Anz.,20,51-55.

Samter, M., 1900, Studien zur Entwicklungsgeschichte der Leptodora hyalina. Z.wiss.Zool.,68,169-260.

Schäffer, J.C., 1755, Die geschwänzten und ungeschwänzten zackigen Wasserflöhe. Reygensburg.

von Scharfenberg, U., 1910-11, Studien über die Eibildung und den Generationszyklus von Daphnia magna. Int.Rev. Hydrobiol.,Biol.Suppl.1,1-42.

Schneider, K.C., 1908, Histologisches Praktikum der Tiere für Studenten und Forscher. G. Fischer, Jena.

Schrader, F., 1926, The cytology of pseudo-sexual eggs in a species of Daphnia. Z.indukt.Abstamm.-u.VererbLehre, 40,1-27.

Schulz, H., 1928, Uber die Bedeutung des Lichtes im Leben niederer Krebse (nach Versuchen an Daphniden). Z.vergl.Physiol.,7,488-552.

Schulze-Röbbecke, G., 1951, Untersuchungen über Lebensdauer, Altern und Tod bei Arthropoden. Zool.Jb.,Abt.Allg. Zool.u.Physiol.,63,366-394.

Scourfield, D.J., 1900, The swimming peculiarities of Daphnia and its allies. J.Quekett micr.Cl.,Ser.II,7,395-404.

Scourfield, D.J., 1943, The post-embryonal development of the male of Daphnia magna. J.Quekett micr.Cl.,4th Series, 1,276-285.

Shiino, S.M., 1942, Studies on the embryology of Squilla oratoria de Haan. MemColl.Sci.Kyoto,17B,1,77-174.

Smaridge, M.W., 1954, Iron excretion by Daphnia during haemoglobin loss. Nature,Lond.,175,782.

Smith, G., 1915, The life cycle of Cladocera, with remarks on the physiology of growth and reproduction in the Crustacea. Proc.roy.Soc.,B,88,418-435.

Sneider, G. and Kersten, H., 1936, Susceptibility to soft X-rays of Daphnia magna during its development from egg to young in the brood pouch. J.exp.Zool.,74,1-6.

Storch, O., 1924, Morphologie und Physiologie des Fangapparates der Daphniden. Ergebn.Zool.,6,125-234.

Storch, O., 1925, Cladocera, Wasserflöhe, in Biologie der Tiere Deutschlands, edited by Schulze. Gebrüder Borntraeger, Berlin.

Sturm, F., 1936, Zur Fragen der Nierenphysiologie besonders der Cladoceren nach elektiver Vitalfarbung. Z.vergl. Physiol.,23,420-428.

Sudler, M.T., 1899, The development of Penilia schmackeri Richard. Proc.Boston Soc.nat.Hist.,29,109-129.

Swammerdam, J., 1758, The book of nature. Seyffert, London.

Taylor, M., 1914-5, Note on the number of chromosomes in the male Daphnia pulex. Zool.Anz.,45,21-24.

Teissier, G., 1932, Origine et nature du pigment carotinoid des oeufs de Daphnia pulex. C.R.Soc.Biol.,Paris,109,813-815.

Tiegs, O.W., 1955, The flight muscles of insects. Their anatomy and histology; with some observations on the structure of striated muscle in general. Phil.Trans.,B,238, 221-348.

Vollmer, C., 1912, Zur Entwicklung der Cladoceren aus dem Dauerei. Z.wiss.Zool.,102,646-700.

Wagler, E., 1926-7, Branchiopoda, Phyllopoda, in Handbuch der Zoologie, III,1, by W. Kukenthal and T. Krumbach. Walter de Gruyter and Co., Berlin und Leipzig.

Waterhouse, D.F., 1954, The rate of production of the peritrophic membrane in some insects. Aust.J.Biol.Sci.,7,59-72.

Weismann, A., 1876-9, Beiträge zur Naturgeschichte der Daphnoiden, I-VII. Z.wiss.Zool.,27-35.

Weisz, P., 1947, The histological pattern of metameric development in Artemia salina. J. Morph.,81,45-89.

Wigglesworth, V.B., 1953, The principles of insect physiology. Methuen and Co. Ltd., London.

Willier, B.H., Weiss, P.A., and Hamburger, V., editors, 1955, Analysis of development. W.B. Saunders Co., Philadelphia and London.

Wotzel, F., 1937, Zur Entwicklung des Sommereies von Daphnia pulex. Zool.Jb.,Abt.Anat.,63,455-470.

von Zograf, N., 1904, Das unpaare Auge, die Frontalorgane und das Nackenorgan einiger Branchiopoden. Friedländer u. Sohn, Berlin.