The Origin and Nature of the Egg Membranes in Chirocephalus diaphanus.

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With 8 Text-figures

Introduction.

The presence in the Anostraca of glands opening into the uterus was originally noted by Buchholz (1886) in Branchipus grubei. He suggested that they serve to secrete the egg-case, and later workers on Branchipus and on Artemia, notably Spangenberg (1875) and Claus (1886), accepted this view. Smith (1909), referring to Chirocephalus diaphanus, states that 'Short diverticula of the walls of the uterus receive the ducts of unicellular glands, the bodies of which contain a peculiar opaque secretion, said to form the egg-shells'.

The present research was designed to investigate the truth of this statement, and also to compare the nature and mode of formation of the egg-case in the Anostraca with that in the Decapoda. In the latter, as exemplified by Homarus, the egg-case consists of two membranes, an inner one of chitin secreted by the walls of the oviduct, and an outer one of cuticle secreted by the cement glands in the pleopods (Yonge, 1938).

Chirocephalus diaphanus Prevost was selected as the most suitable animal for this research. The majority of the specimens were collected on Dartmoor and were fixed in Bouin's fluid, which gave excellent results. Females at different stages of maturity were selected, the criterion of this being the length of the egg-sac. Serial sections were cut through those regions of the body containing the reproductive organs. Various combinations of stains were used, notably Mallory's triple stain, Delafield's or Heidenheim's haematoxylin in combination with

acid fuchsin, biebrick scarlet or eosin, and Mann's methyl blue eosin. Comparisons were then made between the condition of the glands and of the reproductive organs at different stages in the elaboration of the ovarian egg and its later passage into the egg-sac.

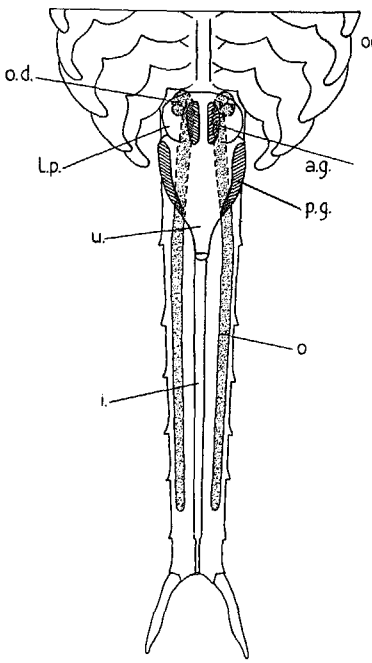
Living specimens were also obtained through the kind offices of Mr. A. G. Lowndes of Marlborough. By a fortunate chance, when two of these were fixed in Bouin's fluid eggs were observed to pass from the oviduct into the egg-sac. The condition of these eggs provided evidence of great value.

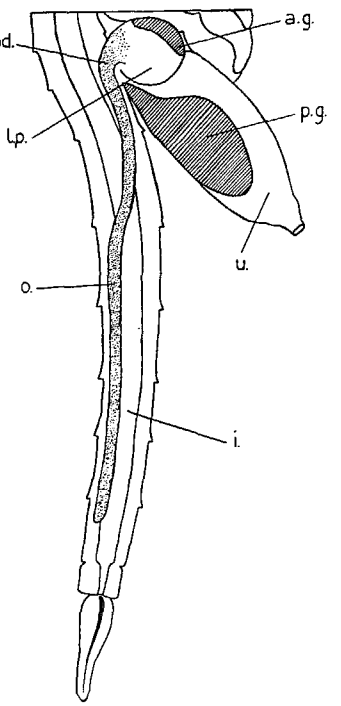
Finally, simple chemical tests were carried out on the egg-case to determine its composition.

Anatomy of the Female Reproductive System.

The female reproductive system (Text-figs. 1 and 2) consists of a pair of long tube-like ovaries (*o.*) which extend down the sides of the abdomen as far as the sixth abdominal segment. Towards their anterior end they tend to approximate on the ventral side. The oviducts (*od.*) appear as anterior prolongations of the ovaries, there being no definite line of demarcation between the two (see Text-fig. 3). In the region of the twelfth thoracic segment, the oviducts bend down sharply to open into a median unpaired uterus (*u.*) which is contained within the projecting egg-sac. In the very young individual the uterus is a simple elongated tube, but in the adult, two lateral pouches (*l.p.*) arise antero-ventrally and into these the oviducts open (Text-figs. 1, 2, and 3).

The uterine glands consist of two paired masses. The anterior pair (*a.g.*) are ventral, one on each side of the median line. The posterior and larger pair (*p.g.*) are situated one on each side of the body of the uterus extending round to the dorsal side, where they meet and extend forwards, in fully mature females, almost to the anterior limit of the anterior group. Each mass consists of a group of paired gland-cells with ducts communicating with the uterus. In the larger, more mature, individuals, the gland-masses are proportionally larger. This was found to be due not to an increase in number of the cells but to a notable enlargement of the individual gland-cells (compare Text-figs. 4 and 7).





TEXT-FIG. 1. TEXT-FIG. 2.

All the figures are of Chirocephalus diaphanus.

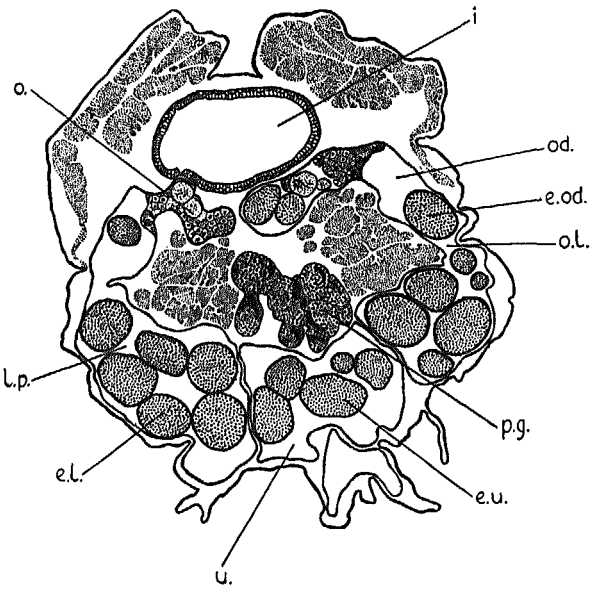
Fig. 1.—Ventral aspect showing the female reproductive organs. ×4. *a.g.*, anterior uterine glands; *i.*, intestine; *l.p.*, lateral pouch of uterus; *o.*, ovary; *od.*, oviduct; *p.g.*, posterior uterine glands; *u.*, uterus.

Fig. 2.—Lateral aspect showing the female reproductive organs. ×4½. Lettering as before.

THE UTERINE GLANDS.

The gland-cells may best be described in terms of their development, details of which are given below. Like those of Branchipus stagnalis and Branchipus torticornis, described by Buchholz (1866) and by Claus (1886) respectively, the gland-cells are arranged in pairs surrounded by a common membrane. In Branchipus grubei, on the other hand, Buchholz states that the gland-cells are separate, although a

single duct serves a pair of glands. The glands consist of cytoplasm, which is gradually replaced by granular secretion, and of a characteristically large cup- or basin-shaped nucleus. The



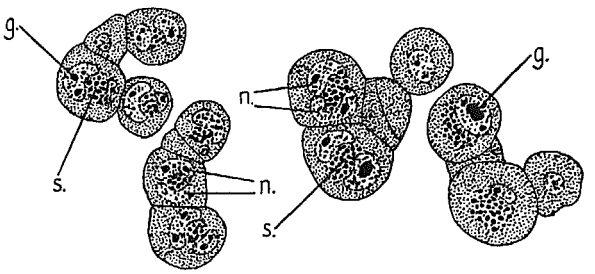
TEXT-FIG. 3.

Transverse section through a female at the junction between the oviduct and the uterus. In this animal the stimulus of fixation caused eggs to pass from the oviduct into the uterus, they do not therefore possess the rugose outer membrane. ×50. *e.l.*, eggs in lateral pouch; *e.od.*, eggs in oviduct; *e.u.*, eggs in uterus; *o.l.*, opening of oviduct into lateral pouch of uterus; *p.g.*, posterior uterine glands full of secretion. Other lettering as before.

concavities of the nuclei of associated gland-cells face one another. In the early stages of development the nuclei contain large granules which first increase and later decrease in number. These granules, and the secretion which forms in the cytoplasm, give similar staining reactions, red or orange, with Mallory's triple stain, red with Mann's methyl blue eosin, red with Delafield's haematoxylin and with acid fuchsin, and black with iron haematoxylin. It appears, therefore, that the secretion is

elaborated in the nucleus and passed into the cytoplasm. The cytoplasm and the smaller granules in the nucleus stain in the usual way with cytoplasmic and nuclear stains respectively.

The earliest stage in which the glands were studied was in animals which possessed no egg-sac and no yolk in the ovarian eggs. The uterine glands (Text-fig. 4) were small, the pairs being about 29μ in diameter. The nucleus (*n.*) had a granular



TEXT-FIG. 4. TEXT-FIG. 5.

Fig. 4.—Section through uterine glands from female with no egg-sac and no yolk in the ovarian eggs. ×300. *g.*, large granule in nucleus; *n.*, paired nuclei; *s.*, secretion in cytoplasm between paired nuclei.

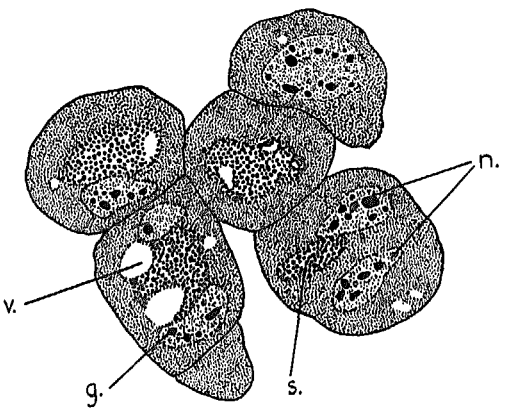
Fig. 5.—Section through uterine glands from female with egg-sac 0·64 mm. long but with no yolk in the ovarian eggs. ×300. Lettering as before.

appearance, but the darkly staining bodies (*g.*) were not numerous. The secretion (*s.*) was confined to small masses lying chiefly within the cup of the nucleus. It consisted of granules somewhat smaller than the largest of those in the nucleus.

The next stage was found in animals with an egg-sac 0·64 mm. long and 0·18 mm. wide, yolk being still absent from the ovarian eggs. The glands (Text-fig. 5) were considerably larger, pairs averaging some 45μ in diameter. The size of the nucleus and the amount of secretion in the cytoplasm had increased proportionally. Irregular, vividly staining bodies were conspicuous in the nucleus, and the granules of secretion were larger as well as more abundant. The secretion was still mainly situated within the cups of the nuclei.

The third stage examined was in more mature animals where

the egg-sac was some 2·2 mm. long and 0·9 mm. wide. Here yolk was present in the ovarian eggs, and fully developed eggs were present in the oviduct and in the uterus. The pairs of glands (Text-fig. 6) had increased in diameter up to 80μ. The

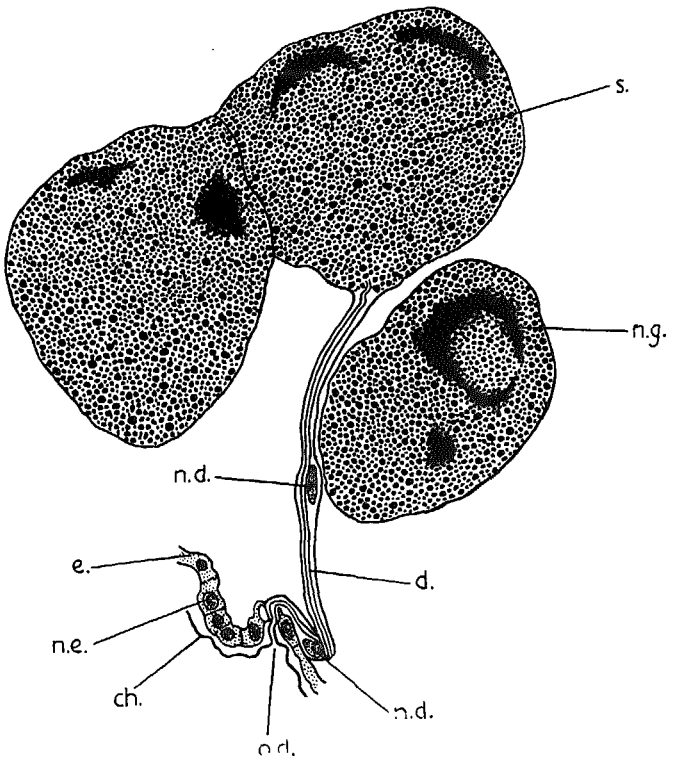


TEXT-FIG. 6.

Section through uterine glands from female with egg-sac 2·2 mm. long and with yolk in the ovarian eggs. ×300. *v.*, vacuole in cytoplasm. Other lettering as before.

granular, brightly staining, masses in the nucleus were more conspicuous and had increased in proportion to the general substance of the nucleus. The granules of secretion in the cytoplasm were slightly larger, and had increased in amount to a greater extent than had the nucleus. Occasional large vacuoles (*v.*) appeared within the secretion.

The last series of animals sectioned consisted of fully mature individuals with egg-sacs up to 3·2 mm. long and 1·35 mm. wide. As before, eggs were present in the oviduct and in the uterus (see Text-fig. 3). The paired gland-cells (Text-fig. 7) in these animals attained a diameter of 130μ, almost five times that of the glands in the earliest stage (compare Text-figs. 7 and 4). In these the nuclei (*n.g.*) broke down, becoming eventually no more than irregular masses of darkly staining material. The remainder of the cell became finally exclusively filled with secretion (*s.*),



TEXT-FIG. 7.

Section through uterine glands of fully mature female with egg-sac 3·2 mm. long and with eggs in the oviduct and uterus. ×300. *ch.*, chitinous intima of uterus; *d.*, duct of uterine gland; *e.*, epithelium of uterus; *n.d.*, nucleus of duct (two are shown not belonging to the same duct); *n.e.*, nucleus of uterine epithelium; *n.g.*, nucleus (degenerating) of gland-cell; *o.d.*, opening of duct into uterus; *s.*, secretion filling gland-cell.

the granules of which were much larger than in earlier stages. No trace of the original cytoplasm remained.

DUCTS OF THE UTERINE GLANDS.

The structure of the ducts in the fully developed glands is shown in Text-fig. 7 (*d.*). They consist of long, very narrow

tubes extending from the wall of the uterus to the paired gland-cells. Each duct is unbranched and communicates with a single pair of gland-cells. The lumen of the tube, which is very well defined, has a diameter about equal to the thickness of the walls. Each duct possesses a single oval nucleus (*n.d.*) which is situated in a small swelling in the wall of the duct. Except for their oval shape, conditioned by the nature of the duct, they are very similar in appearance to the nuclei of the uterine epithelium (*n.e.*), possessing usually a single nucleolus and many small granules. This epithelium secretes a thin integument (*ch.*), presumably of chitin, and this appears to pass into the lumen of the ducts. If such is the case it would explain the sharp definition of the lumen. In this connexion it is interesting to note that Kinzig (1914) believed that the ducts of the tegumental glands which line the statocyst of Decapoda are bounded with chitin; this has also been the impression of one of us (C. M. Y.) in the case of the tegumental glands of the oesophagus and labrum in Homarus.

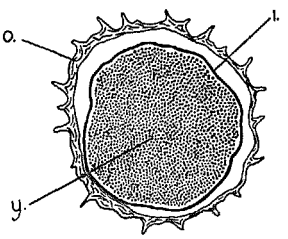
These similarities between the ducts and the uterine epithelium suggest that the former may represent ingrowths from the epithelium to the glands. Careful study of sections of the earliest stage available in the elaboration of the glands revealed that ducts are present though clearly not functional and still in process of elaboration. The epithelium of the uterus is very irregular at this stage, indicating a possible inward growth of the cells which are forming the ducts. The nuclei of these are much larger, at this stage, than those of the epithelium, but this may be due to their great activity. There is no definite evidence that the ducts are not growing inwards; on the other hand, this cannot be regarded as proved.

Nature and Formation of the Egg-case.

The structure of the egg-case is shown in Text-fig. 8. The yolky egg-mass (*y.*) is surrounded by a thin inner membrane (*i.*) which adheres closely to the egg-mass even after sectioning. Surrounding this is the thicker, rugose outer membrane (*o.*) which gives the characteristic spiny appearance to the eggs of

Chirocephalus. This membrane tends to come away from the inner one in the process of sectioning.

Neither of these membranes is present around the ovarian egg. Sections of mature females with eggs in the oviduct and in the uterus reveal that the former possess the inner, smooth membrane only, whereas the spiny outer membrane is also present around those in the uterus. This indicates that the inner



TEXT-FIG. 8.

Section through uterine egg. ×150. *i.*, inner, chitinous membrane; *o.*, outer, rugose, non-chitinous membrane; *y.*, yolk.

membrane is formed by the oviduct and the outer one by the uterine glands. Fortunate confirmation of this was obtained from sections of animals in which the act of fixation had brought about the passage of eggs into the uterus. Text-fig. 3 represents a section through the reproductive region of one of these animals. It will be seen that in this case not only are the eggs in the oviduct (*e.od.*) devoid of the outer membrane but also those in the uterus (*e.u.*) and in the lateral pouches of this (*e.l.*). Apparently, therefore, fixation brought about contraction of the oviduct, causing expulsion of eggs already coated with secretion from the oviducal epithelium into the uterus; but the uterine glands were unable to discharge their secretion, so that the eggs failed to possess the outer membrane which in all other cases surrounded the eggs in the uterus. This view was confirmed by the condition of the uterine glands (*p.g.*) which were filled with secretion.

The structure of the epithelium of the oviduct is indistinguishable from that of the uterus, consisting of flattened cells,

the boundaries of which are difficult to determine in sections, and with somewhat irregularly disposed rounded or oval nuclei. But, whereas a very thin chitinous integument can be seen in favourable preparations of the uterus (see Text-fig. 7, *ch.*), this appears to be absent in the oviduct.

Chemical examination of the egg-membranes provided further evidence of value. Eggs possessing both membranes were tested for chitin by the method of Campbell (1928). After the initial boiling in NaOH in a glycerine bath at 160° C. for 15 minutes the outer membrane was dissolved, indicating that it is not chitinous, but the inner membrane remained. When this was treated with iodine followed by sulphuric acid the deep mauve colour characteristic of chitosan was obtained. The inner membrane is therefore chitinous. Eggs were also subjected to the action of concentrated hydrochloric acid for one day, and the outer membrane remained intact. This provided further evidence that it is non-chitinous and also indicated possible similarity to the superficial cuticle of the integument of Homarus (Yonge, 1932) which also forms the outer membrane around the eggs of Decapoda (Yonge, 1938).

Discussion.

It is interesting to find that the egg-membranes in Chirocephalus diaphanus are of the same dual character as those of Homarus vulgaris, and with little doubt of all other Decapoda which attach the eggs to the pleopods (Yonge, 1938). In both the inner membrane is chitinous and is secreted by the walls of the oviduct. The outer membrane in the case of Chirocephalus possesses at least one of the properties of the cuticle which form the outer egg-membrane in Homarus (Yonge, 1932, 1938), namely resistance to attack by concentrated hydrochloric acid, and, from its mode of formation, probably also that of low surface tension. The presence of two egg-membranes has also been recorded by Ziegelmayer (1926) for Copepoda.

When discussing the significance of these two membranes in Homarus (Yonge, 1938) it was pointed out that egg-laying is associated with ecdysis, occurring at some definite period after

this, and involving the same two processes of secretion: formation of underlying chitin by an epithelium (of the ectodermal surface generally or of the oviduct), and of a superficial cuticle by glands (tegumental in the one case, cement in the other, the two being structurally indistinguishable). Certainly in the Decapoda the cement glands secrete a substance indistinguishable chemically or physically from that produced by the tegumental glands. The properties of the cuticle secreted by the latter—low surface tension, slow solidification in water, final hardness, and limited permeability—are exploited to provide an outer egg-membrane which is much harder than chitin, provides an attachment to the pleopods, and, by its strictly limited permeability (Yonge, 1936), provides not only a greater insulation of the developing egg from changes in the environment, but also the possibility of osmatic hatching (see Needham, 1931, pp. 1600–2, for resumé of literature on this subject).

In Chirocephalus the outer egg-case is purely protective in character, its substance being not prolonged into a 'funicula' providing attachment to the female. Protection is of supreme importance in a species the eggs of which must resist prolonged desiccation and, presumably in correlation with this, the outer membrane is relatively much thicker than it is in the Decapoda. The cement glands of the Decapoda being modified tegumental glands, there appears some reason for regarding the uterine glands of the Anostraca as homologous with the very numerous body and leg glands. Spangenberg (1875) came to this opinion on purely morphological grounds, but Claus (1886) strongly opposed this view, stating that not only was there difference in details of structure, but that, whereas the uterine glands are formed by inpouching of the uterus, the abdominal and leg glands originate as ectodermal structures. The former point appears of minor importance, while the latter depends on the truth of the statement that the uterus is mesodermal in origin, which, if the possession of chitinous intima be regarded as evidence of an ectodermal epithelium, can reasonably be disputed. A study of the leg and body glands of Chirocephalus lay outside the range of this research, but it was observed that their structure was of the same general character as that of the

uterine glands. Other work carried out in this Department, and briefly referred to elsewhere (Nicholson and Yonge, 1935), indicates that the leg and body glands may be concerned with cuticle formation, and, in view of the conditions prevailing in the Decapoda, the fact that the uterine glands certainly secrete a cuticle- or cement-like substance lends support to this view. It appears probable that the conditions in the Anostraca and in the Decapoda are essentially the same.

Acknowledgements are due to the Colston Research Society of the University of Bristol for financial assistance in connexion with this research.

Summary.

1. The anatomy of the female reproductive system in *Chirocephalus diaphanus* is described with especial reference to the paired anterior and posterior masses of uterine glands.

2. The gland-cells are arranged in pairs surrounded by a common membrane and served by a single duct formed by a separate duct-cell, representing possibly an ingrowth of the uterine epithelium.

3. The glands increase greatly in size during development owing to the formation of a granular secretion which is apparently formed in the nucleus and gradually displaces the original cytoplasm.

4. The egg-case consists of an inner membrane which is chitinous and is formed by the oviducal epithelium, and an outer thicker, rugose membrane which is non-chitinous and is secreted by the uterine glands.

5. Attention is drawn to the close resemblance to conditions in the Decapoda. The inner membrane in both cases is chitinous and formed in the oviduct, while the uterine secretion has much in common with the secretion of the cement glands, but is concerned with protection only and not also with attachment to the body of the female.

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