The Structure and Mode of Functioning of the Reproductive Organs of *Tritonia hombergi* (Gastropoda Opisthobranchia)

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With one plate (fig. 4)

SUMMARY

Routine observations on *Tritonia hombergi* Cuvier dredged from an area off the south coast of the Isle of Man indicate that the species has an annual life-cycle similar to that of many dorid nudibranchs.

The ovotestis communicates with the anterior genital mass through numbers of collecting tubules which unite to form a stout hermaphrodite duct. This duct functions as a vesicula seminalis in adult specimens; spermatozoa stored here are physiologically immature. The hermaphrodite duct bifurcates to form male and female ducts; the region of the bifurcation forms a valve ensuring that oocytes shall not enter the purely male regions of the genital system during oviposition. The male duct is the glandular and muscular vas deferens which connects with the base of the protrusible penis. The oviduct opens into the lumen of the mucus-gland. A region of convoluted tubules close to the point of the opening of the oviduct into the mucus-gland is the albumen-gland. The bursa copulatrix is a blind tubular invagination of the lateral body-wall which in mature specimens stores spermatozoa received at copulation. There are thus three external genital openings.

Copulation is almost invariably reciprocal, the penis of each individual being inserted into the bursa copulatrix of the other. Secretions from the glandular linings of the bursa and of the vas deferens render spermatozoa exchanged at copulation physiologically mature; they are active and capable of fertilizing oocytes.

At oviposition oocytes are expelled from the ovotestis and transported by cilia to the main hermaphrodite duct. They then travel (through the dense masses of spermatozoa in this duct) along a narrow longitudinal ciliated tract of lining epithelium; this phenomenon was clearly observed in vivisected individuals. At the hermaphrodite valve the oocytes are deflected into the oviduct and are carried by cilia into the mucus-gland. In the lumen of the latter organ they meet active 'foreign' spermatozoa which have made their way from the bursa copulatrix. The oocytes and spermatozoa are now conducted along a tortuous predetermined ciliary path through the albumen-gland (where the primary egg-cases are secreted and where fertilization occurs), and then through the peripheral tubules of the mucus-gland (where successive mucous coats are added). The completed egg-string is expelled through the external female aperture and forms a ribbon which is attached to the substratum by pressure of the parent's foot upon it. Active spermatozoa may be observed moving within the primary egg-cases for some days after oviposition.

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INTRODUCTION

ALTHOUGH the gross morphology of the reproductive organs of a number of species of nudibranchs is known from scattered systematic writings by many authors, investigations into the microscopic structure and functioning of the various regions of the system are few. The most important studies are those of Chambers (1934) on Embletonia fuscata, Lloyd (1952) on Archidoris pseudoargus and other opisthobranchs, and MacGowan and Pratt (1954) on A. montereyensis. Chambers summarizes work done before 1934. The present paper is concerned with the reproductive mechanism of one of the most primitive living nudibranchs.

METHODS

Excised pieces of the genital tract were examined alive, often after intravital staining with neutral red. Material for sectioning was fixed in the fluids of Zenker (without acetic acid), Perényi, Bouin (made up with seawater), or Lewitsky-saline (Baker, 1958). Of these, the last gave by far the best results for all types of tissue. The embedding medium employed was Hance’s rubber wax (Gurr). Sections were cut at from 4 to 10 µ. Stains used included the Azan and iron haematoxylin of Heidenhain, and Mayer’s haemalum counterstained with eosin and alcian blue 8 GS (Steedman, 1950).

THE LIFE-CYCLE AND THE STRUCTURE OF THE OVOTESTIS

Fig. 1 is a diagrammatic representation of observations made on samples of Tritonia hombergi Cuvier dredged at intervals through the year. The area from which the samples were taken was the Modiolus modiolus bed off the south coast of the Isle of Man (see Jones, 1951, p. 137). The picture which emerges is not as clear as might be desired, because the weather sometimes made it impossible to procure regular samples. Nevertheless, there can be little doubt that these observations show T. hombergi to have an annual life-cycle similar to those found in some dorid nudibranchs (Thompson, 1957, 1958a). Study of sections of the ovotestis of individuals taken from the samples removes any
possibility of an alternative explanation; gonad development proceeds through the year in one direction, that leading towards sexual maturity; there are none of the resting or regression stages characteristic of perennial sexual cycles.

Fig. 2, A–E shows the structure of the ovotestis in specimens killed at various times of the year. There is little point in describing in any detail gonad maturation in *Tritonia*, for the essential features are known in many gastropods

and *T. hombergi* does not differ from the usual pattern. Franzén (1955) describes stages in spermatogenesis of *Tritonia*. Much of his description was confirmed in the course of the present investigation, the only item eluding confirmation being the ‘spiral keel’ (Franzén, 1955, p. 426, sk) running down the sperm tail.

The ovotestis of a sexually mature specimen killed immediately after having deposited a spawn mass showed that mature oocytes remained in only a small number of the tubular follicles (fig. 2, D). Within a few days of spawning, a fresh batch of oocytes begins to enlarge by the accumulation of cytoplasmic yolk, and in due course they in turn are expelled as a spawn mass. The number of such cycles of oocyte production in the life of a single *Tritonia* is not known, but is certainly not less than three. Possibly a phenomenon similar
FIG. 2. Appearance of the ovotestis at various phases in the life-cycle. A, section through a single ovotestis tubule of an immature specimen collected on 26 Oct. 1958, showing immature oocytes and spermatocytes (immature oocytes have little or no cytoplasmic yolk and each of their nuclei contains a large nucleolus). B, portion of a section through an ovotestis tubule of an immature specimen collected on 17 Dec. 1958 (one of the oocytes shown in this plane is growing and accumulating cytoplasmic yolk). C, portion of a section through an ovotestis tubule of a mature specimen collected on 7 Jan. 1959, showing two oocytes, one full-grown, the other not (the nucleus of a mature oocyte lacks the nucleolus). D, portion of a section through an ovotestis tubule of a mature specimen collected in Jan. 1959 and killed immediately after spawning on 23 Feb. 1959 (only immature oocytes remain in the ovotestis). E, section through a whole ovotestis tubule of a mature specimen which had not spawned before death (February 1959).
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to this provides an explanation for the enigmatic short-period cycles of spawn production in the dorid *Adalaria proxima* (Thompson, 1958b).

Spermatogenesis also continues throughout the period of sexual maturity, but the cycles of sperm production are not well marked and each of the genital tubules of the gonad of a single individual contains male gametes in all stages of formation (see fig. 2, E).

In the dorid *A. proxima* it was found (Thompson, 1958a) that, as the spawning season proceeds, the resources stored in the digestive gland are exhausted until a stage is reached when the organ is virtually shrunken out of existence; the adults die when this happens, even though the ovotestis may still contain numbers of apparently healthy oocytes and spermatozoa. Although some evidence points to the existence of a similar phenomenon in *T. hombergi*, no moribund post-sexual specimens were ever seen; it was, however, established by observation that the size of the digestive gland does decrease as the spawning season proceeds.

**THE STRUCTURE OF THE MATURE REPRODUCTIVE COMPLEX**

**Ovotestis** (fig. 2, E). This displays a rather primitive organization, the separation of the constituent tubules into male and female regions being rudimentary compared with other nudibranchs (except for some ascoglossans and the eolid *Coryphella*, Pelseneer, 1894). The ovotestis usually covers the whole external surface of the digestive gland. It consists of a large number of tubules, all orientated radially with regard to the centre of the digestive gland. Each tubule opens at its innermost extremity into a factor of a complex duct system (see fig. 2, E), lined by a simple ciliated epithelium and leading anteriorly to the hermaphrodite duct. In any individual tubule, the female region is distal to the male (fig. 2, E); in some tubules the female region may be divided into two or three lobules separated from the proximal part of the tubule by slight constrictions. A fine network of muscle-fibres lies around each tubule. At maturity, the tubules each contain a small number (about 4 to 6) of mature oocytes and a great number of morphologically mature spermatozoa. The former are attached to the wall of the tubule while the latter lie free in the lumen. The mature oocytes give a cream-yellow colour to the gonad in life; this colour may be seen through the foot of a live specimen.

**Hermaphrodite duct** (figs. 3; 4, c, d, e). The hermaphrodite duct is a simple tubular structure; there is no obvious division into male and female regions. The initial region after leaving the ovotestis is narrow and lined by a simple ciliated epithelium (fig. 4, e). Outside this epithelium is a layer consisting of bundles of circular and longitudinal muscle-fibres and outside this in turn a spongy layer consisting of blood-spaces.

The duct soon widens to form the vesicula seminalis (see figs. 3; 4, c); here huge numbers of spermatozoa collect in the lumen, which is bordered by an epithelium of complex aspect. A narrow region running longitudinally through the vesicula seminalis is ciliated, the remainder of the epithelium being composed of columnar, non-ciliated cells. The ciliated zone is clearly
visible in preparations of live material. Outside the lining epithelium is a thick layer consisting of bundles of circular and longitudinal muscle-fibres and outside this in turn a layer of blood-spaces. Live spermatozoa in squash preparations from the ovotestis and vesicula seminalis appear mature in all but one respect: they are, except a few sluggishly moving individual spermatozoa, immobile and lacking any common orientation. Since both these

qualities are indicative of physiological (as distinct from gross morphological) maturity in gastropod spermatozoa, it is clear that there is no possibility of self-fertilization occurring in the hermaphrodite tubules of the ovotestis. This is borne out by other observations.

**FIG. 3.** Diagram showing the relations between the various parts of the anterior genital mass in a mature individual. The proportions are somewhat distorted in the interests of clarity. The arrows show the approximate path followed by oocytes entering the mucus-gland from the oviduct during the spawning process. Anterior is to the right of the page.

**FIG. 4 (plate).** Photomicrographs of sections (stained with Azan) through regions of the adult reproductive tract.

A, bursa copulatrix after copulation.
B, portion of the same, at greater magnification.
C, wide region of the hermaphrodite duct (vesicula seminalis).
D, portion of the same, at greater magnification.
E, narrow region of the hermaphrodite duct.
F, vas deferens.
bl, blood spaces; cil, ciliated region of lining epithelium; gl, glandular lining epithelium; m, muscle layer; non-cil, non-ciliated lining epithelium; non-or, non-orientated spermatozoa or, orientated ‘foreign’ spermatozoa.
Fig. 4

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The vesicula seminalis, which appears grey in life owing to the colour of the spermatozoa within, lies in a few loose coils, bound together by connective tissue, dorsal to the anterior genital mass (fig. 8). At its anterior extremity it narrows and appears to enter the mucus-gland; actually it runs along a furrow between the two lateral halves of the gland. Deep inside the body of the mucus-gland the hermaphrodite duct divides into two. One of these further ducts is the oviduct, the other the vas deferens: it is at this bifurcation that the separation (such as it is) of the male from the female genital products occurs. The bifurcation may be best visualized as a straight tube (one end of which is the vas deferens, the other the oviduct) into the lumen of which projects a small papilla, through which opens the hermaphrodite duct (fig. 5). The whole region is closely surrounded by muscle-fibres and aggregations of nerve-cells. The mode of functioning of this 'hermaphrodite valve' clearly involves the contraction of regions of this musculature, the degree of coordination required being reflected by the unusual aggregation of nervous elements near by. A valve of this type was described for Archidoris montereyensis by MacGowan and Pratt (1954), but it is quite unlike that which Chambers (1934) found in Embletonia fuscata.

In Tritonia this valve appears efficiently to prevent the entry of oocytes into the vas deferens during the spawning process, for female gametes were never found in the male regions of the system. However, the valve by no means prevents spermatozoa from entering the oviduct from the vesicula seminalis, although they do not do this in any quantity. Spermatozoa which have obviously passed through the valve into the female parts of the tract may be found in any part of the oviduct. Duncan (1958) calls attention to a similar phenomenon in the pulmonate Physa fontinalis. These 'escaped' sperms are not physiologically mature and almost certainly cannot result in normal circumstances in self-fertilization.

Vas deferens (figs. 3; 4, f). From the point where the vas deferens branches off from the hermaphrodite duct (fig. 3), it widens into a thick, muscular duct, leading after a few coils to the inner extremity of the penis. The lining epithelium is columnar and the cells are loaded with secretory globules of various sizes and staining qualities; this secretion may be termed, to facilitate comparison with other gastropods, the prostatic secretion. These columnar cells are ciliated. Outside the epithelium is a thick zone of circular and longitudinal muscle-fibres, lying in bundles in a connective-tissue matrix. In life the vas deferens appears white.

Penis (fig. 6). The penis is a large organ lying in an ectodermal sheath.
Distension of the connective tissue surrounding the final region of the vas deferens is responsible for the erection and eversion of the penis. When fully extended (fig. 6, B), the penis is a translucent white organ, bearing on its anterior face a small, keel-like structure; this keel is concerned with guiding the penis into the bursa copulatrix of a copulating partner. The whole organ is mobile and the tip is capable of considerable movement.

Extrusion of the penis is by no means invariably a prelude to copulation but is a frequent sign of impending death in an unhealthy specimen.

**Oviduct** (fig. 3). After leaving the hermaphrodite valve the oviduct lies in the furrow separating the two lateral halves of the mucus-gland; it is a simple, non-glandular tube, lined by a ciliated epithelium. It opens into the lumen of the mucus-gland, close to the external female genital opening.

**Albumen-gland** (figs. 3; 7, A). This small organ appears pale pink in life and consists of a coiled tube, held to the outside of the mucus-gland by connective tissue, and lined by a glandular epithelium. The secretory cells are columnar and are separated by narrow ciliated cells. The tubular gland opens at both its extremities into the main lumen of the mucus-gland, close to the entry of the oviduct (fig. 3).

**Mucus-gland** (figs. 3; 7, B, C). The mucus-gland is a large organ, pale cream to white in life; the outlines of many of the convoluted tubules of which it is composed may be seen with the naked eye in dissections (fig. 8). The gland consists of a broad central lumen (lined by a low non-glandular ciliated epithelium), into and out of which open numerous convoluted tubules. These tubules are lined by columnar secretory cells and narrow ciliated cells. The
secretory cells are of two kinds (fig. 7, B, C); regions consisting of one type lead abruptly into regions lined by the other without any apparent governing rule. The main central lumen of the gland takes the form of a V-shaped space in sections through the organ and this lumen leads down to the external female aperture. Close to this external opening are the openings of the oviduct (one) and the albumen-gland (two) (fig. 3). The final portion of the female system is clothed with cilia, all of which serve to convey particles out of the tract.

**Bursa copulatrix** (figs. 3; 4, A, B). The bursa is a blind, tubular, ectodermal invagination, opening to the exterior through a ciliated duct close to the oviducal aperture. It has no open connexion with any other part of the reproductive system. In mature specimens the bursa appears pink to pale purple owing to the presence of a pigment in the wall. The organ serves for the reception and storage of ‘foreign’ spermatozoa received during copulation. The lining epithelium of the bursa consists in sections of a peculiar ‘bubbling’ layer of cells. These cells are columnar and each contains a huge vacuole; the bursting of numbers of these vacuoles gives a hazy appearance to the adjacent lumen. This epithelium is not ciliated.

In individuals which have copulated the bursa is distended with greyish masses of spermatozoa all of which are orientated with their heads facing (but not embedded in) the wall (fig. 4, A, B). These sperms are active and it is inferred (in view of the fact that spermatozoa from the vesicula seminalis are immobile) that this motility was instigated by secretions from the prostate or from the bursa or from both.

An interesting phenomenon is the apparent synchrony of sperm-tail movement in sperms which face the same way. Sections show that the waves of action passing along the sperm-tails (which presumably all ceased at the precise moment when the fixative entered and they were killed) are in the same state in all the sperms visible in any section. This may give a striated appearance to masses of sperms in histological preparations (fig. 4, B).
Physiologically mature spermatozoa. Motile sperms of Tritonia progress by spiral waves passing backwards along the sperm-tail. Each sperm measures approximately 0.28 mm in length and approximately 20 waves may be present along the tail at any one time. Spermatozoa from the vesicula seminalis or from the ovotestis also show the tails to be spiral, but in this case the spiral is stationary and the sperms immobile.

External genital apertures (fig. 3). There are three of these. From anterior to posterior they are the external male opening (that of the penis or the penis sheath), the female opening (that of the mucus-gland), and the opening of the bursa copulatrix. These are capable of great dilation.

The Mode of Functioning of the Reproductive Organs

Copulation. Copulation is almost invariably reciprocal, two individuals coming together head to tail, with their right sides apposed. On rare occasions pairs were found to consist of one ‘passive’ individual acting as a female alone, the other acting as a male. Individuals are promiscuous and copulation occurs numerous times, not always being followed directly by oviposition. Pairs may be found in copulation throughout the breeding season. Copulation may continue for many hours without the animals appearing to move.

The erection of the penis and its extrusion (fig. 6) are brought about through the uptake of fluid by the connective tissue surrounding the base of the organ. During copulation the penis of each partner is inserted into the bursa copulatrix of the other and spermatozoa are forced through the central duct of the penis by muscular contractions of the vesicula seminalis and the vas deferens.

Oviposition (figs. 8, 9). Oviposition or spawning occurs usually, but not always, during the hours of darkness (under laboratory conditions). The production of even a relatively short spawn ribbon takes many hours.

Spawn from the field had usually been attached at oviposition to colonies of Alcyonium digitatum (on which the adults feed) or to shells of M. modiolus. In the laboratory, however, the glass sides of culture vessels were almost invariably preferred to Alcyonium colonies and Modiolus shells. This peculiar phenomenon has also been noted for the dorid nudibranch Adalaria proxima (Thompson, 1958a).

The process by which oocytes are expelled from the tubules of the ovotestis has never been directly observed, but it seems probable that contraction of the muscle-fibres surrounding the tubules is the effective agent. This was also suggested by Chambers (1934) for Embletonia. In the ductules leading towards the main hermaphrodite duct, the oocytes are carried along by the lining cilia. They travel through masses of morphologically mature spermatozoa, but self-fertilization does not occur; there is ample reason for believing that these non-motile sperms are physiologically immature. While the oocytes are moving along the ducts their nuclei begin the final phases of maturation.

Large numbers of oocytes come to collect in the ductule system near the anterior extremity of the digestive gland, for the initial region of the hermaphrodite duct is so narrow as to permit the entry of only one oocyte at a time.
The oocyte cytoplasm at this stage is highly plastic, and when numbers are packed together their shapes are modified readily to allow a high degree of concentration. They do not possess any power of independent movement.

Oocytes are carried through the initial narrow region of the hermaphrodite duct by the action of the lining cilia (fig. 4, E). In the wide region of the duct (the vesicula seminalis) only a narrow longitudinal strip of the lining is ciliated (fig. 4, C, D); this strip lies in a 'ventral' position all along the coiled duct, but, since the animal itself may be attached to a vertical surface while spawning, this is probably without significance. The longitudinal ciliated tract serves to conduct the oocytes through the masses of non-motile spermatozoa filling the vesicula seminalis. If a spawning specimen is vivisected, the stream of oocytes passing through the hermaphrodite duct may be observed (fig. 8). To see this, in a dissection from the dorsal aspect, part of the duct must be lifted and turned over. The stream of oocytes is not held to the longitudinal ciliated tract simply by the influence of gravity. The speed of their passage along the tract was between 2 and 3 mm/sec; some oocytes pass along in clumps, others singly. No peristaltic movements of the wall of the vesicula seminalis were observed to occur, and it is certain that the movement of oocytes through this region of the system is brought about solely by ciliary action.

At the bifurcation of the hermaphrodite duct (figs. 3, 5) the oocytes pass into the oviduct; none was ever found in the vas deferens. A number of
non-motile sperms enter the oviduct along with the oocytes, but play no part in the fertilization process, as far as could be ascertained. The oviduct is ciliated throughout.

The oviduct opens into the main lumen of the mucus-gland, near the external female aperture (fig. 3). The oocytes are immediately taken up by ciliary currents and impelled into the tubular albumen-gland, together with a stream of mature, motile spermatozoa from the nearby bursa copulatrix. The means by which these sperms travel the few millimetres from the bursa to the mucus-gland lumen is not certain; ciliary currents in the relevant areas are in the contrary direction, and a contraction of the musculature of the bursa would appear to be most likely to result merely in a loss of sperms to the exterior. The most probable explanation is that the spermatozoa travel from the opening of the bursa up into the lumen of the mucus-gland by their own efforts. Analogous phenomena are well known in other groups of animals.
A mixture of mature spermatozoa and oocytes enters the ciliated lumen of the albumen-gland; fertilization occurs, and the spherical shape of the fertilized ovum is assumed. Secretions from the columnar epithelium of the albumen-gland form the primary egg-cases, which are ovoid. In some instances a single primary case may enclose more than one ovum. Sperms are usually enclosed within the egg-cases, the number so trapped varying from egg to egg and from adult to adult. After having traversed the coiled albumen-gland, the ova, each enclosed in a single case, are again passed into the main lumen of the mucus-gland.

In the mucus-gland ciliary action assembles the ova into a string which is then conducted on a complex, winding journey culminating in the expulsion of a ribbon-like egg mass from the external female genital opening (fig. 9, A). The string of ova passes into and out of the numerous tubular diverticula leading from the main lumen of the mucus-gland; in these tubules the protective mucous coats of the spawn mass are added, and the string travels slowly along its path, which is predetermined by ciliary tracts. As the completed egg string emerges from the greatly dilated female genital aperture, the pressure of the foot upon it causes the free edge to become attached to the substratum (fig. 9, A). This can be demonstrated in a live spawning specimen; after a few minutes it becomes impossible to detach the ribbon from its substratum without causing damage to it. As more and more of the spawn is extruded through the genital opening, the animal moves in a slow spiral so that the completed ribbon has this form (fig. 9, A).

The Structure of the Egg Mass

A portion of a spawn mass is shown in fig. 9, B; fig. 9, C shows a single ovum with its membrane. Spermatozoa remain visible within the egg-cases (fig. 9, C) for up to a fortnight after oviposition, but then are no longer seen; they lose their motility within a few days after oviposition.

The maximum range of variation in the size of the ova of *T. hombergi* was from 0.19 to 0.21 mm in diameter; these figures were obtained by examination of ova from a large number of spawn masses. The number of ova in a single spawn mass is also greatly variable. A single example will suffice to show how many were encountered: a ribbon which measured 42 cm when uncoiled (but without any attempt to correct any of the minor kinks) contained an average number of 1,236 eggs per cm. Thus the total number of eggs in the ribbon was approximately 52,000. The parent individual in this case was 14 cm in length.

The relative abundance of twin, triplet, and quadruplet ova varied considerably from one mass to another; my observations may be loosely summarized by stating that twins were often seen, triplets rarely, and quadruplets only very rarely. There are always a number of empty egg-cases at each end of a spawn ribbon, confirming that the primary membrane is not a true fertilization membrane. (The egg-membranes are secreted by the albumen-gland.)
This work was begun during my tenure of a Leverhulme Fellowship in the University of Liverpool and completed at University College, Cardiff. I am deeply grateful to Mr. J. S. Colman and to Professor James Brough for their critical reading of the manuscript and for the provision of laboratory facilities. In addition it is a pleasure to record my indebtedness to Mr. R. G. Hartnoll, Mr. P. J. Miller, and Dr. K. Reddiah, for their help in collecting the material on which this paper is based. Dr. H. H. Williams and Mr. W. O'Grady gave advice and help in taking the photomicrographs.

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