# STUDIES ON THE REPRODUCTION OF *ARCHIDORIS PSEUDOARGUS* (RAPP) (GASTROPODA OPISTHOBRANCHIA)

By T. E. THOMPSON, D.Sc.

*Zoology Department, University of Bristol*

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[Plates 72 to 76]

CONTENTS



During reciprocal copulation in *Archidoris pseudoargus*, seminal fluid is pumped into the bursa copulatrix of the partner and spermatozoa then pass to the receptaculum seminis where they are nourished and stored. The breakdown of excess gametes in the bursa and the relationship between the receptacular endothelium and the stored allosperms (i.e. foreign sperms received during copulation) were investigated with the electron microscope. Intrinsic allosperm motility probably plays no part in the translocation of semen either in the male or the female tracts.

When oviposition occurs ripe oocytes are conducted along a ciliated tract through dense masses of autosperms (the individual's own spermatozoa) in the vesicula seminalis. They meet active allosperms in the capsule-gland, and fertilization occurs. Many valve-mechanisms were discovered which guide the gametes during various reproductive activities. Female gametes are transported solely by ciliary means, whereas male gametes are translocated almost entirely by peristaltic muscular activities of the ducts.

The axis of the spermatozoan tail is formed of a fibre-bundle, around which a prominent keel spirals to the tip. During flagellation in normal allosperms, uniplanar, bilaterally symmetrical waves originate in the neck and progress rearwards along the tail. In forward progression allosperms spin in a clockwise direction when viewed from the front. The suggestion is advanced that spinning is connected with differential alterations of the moving spermatozoon's resistance to torque, brought about by the spiral keel, and the helical shape of the head itself; experimental support is furnished.

A normal table of embryonic development was compiled for cultures maintained at 10 °C. Hatching occurs on the 28th day. The veligers are liberated at an earlier stage of development than those of *Adalaria proxima* and *Tritonia hombergi* (Thompson 1958*a*, 1962).

Field observations on *Archidoris* in a variety of localities yielded a confusing picture when pooled.

When the population on a single stretch of shore was studied more closely there was no doubt that the life cycle was an annual one, and the maximal life span was approximately one year. Young individuals came into the samples in early autumn. They grew rapidly and gametogenesis began. Within a month some oocytes had ripened to apparent maturity and the production of tailed spermatozoa was under way. During the winter months more and more oocytes were brought to maturity. Ripe autosperms passed to the vesicula seminalis. Adults reached their maximal size in the spring, when spawning began; copulation and oviposition took place time and again. Feeding declined, but, so long as food reserves remained, new waves of ripening oocytes replenished the gonad. Finally, death occurred when the digestive gland was so shrunken that the digestive cells rounded off and drifted away from the basement membrane of the lobules. The life cycle varies slightly in different areas around the coast of Britain. A second, autumnal breeding season may occur in some localities. There is no reliable evidence that the maximal life span anywhere greatly exceeds one year.

## 1. Introduction

Although the anatomy of *Archidoris pseudoargus* is better known than that of any other nudibranch, thanks to the work of Alder & Hancock (1845–55), Hancock & Embleton (1852), Hancock (1865), Bergh (1880–92), Vayssière (1888), Hecht (1895), and Eliot (1910), its development and life history have not received serious study. This is surprising, because it is the largest and most conspicuous dorid nudibranch of the north Atlantic (plate 76), and is one of the largest European molluscs, measuring up to 12 cm in length (Alder & Hancock 1845–55; Dalyell 1853; Garstang 1889; Odhner 1939). It has been recorded by various authors from Iceland, Norway, Skagerrak, Kattegat, Øresund, Kieler Bucht, Netherlands, British Isles, Irish Sea, North Sea, Mediterranean and Atlantic France, and Portugal. Early records from the Antarctic are probably mistaken (Smith 1903), as are records from the Pacific coast of North America (la Rocque 1953).

A great deal is known about the natural history of *Archidoris*. Records of diet are summarized by Thompson (1964); the main adult diet is the sponge *Halichondria panicea*, although other sponges may occasionally be taken in some localities. M'Intosh (1863), Garstang (1890), Herdman & Clubb (1892), Cuénot (1903) and Fisher (1936, 1937) have contributed observations on colouration and behaviour. Aspects of the histology of this (and other) nudibranchs are covered by the papers of Cuénot (1891), Henneguy (1925), Carter (1926), Millott (1937), Forrest (1950, 1953), and Runham (1963).

The development of *Archidoris* has not been completely ignored and observations have been published by Alder & Hancock (1845–55), Reid (1846), Saunders (1880), Eliot (1910), Allen & Nelson (1911), and Thompson (1958*a*). The veliger, and the larval feeding mechanism were described by Thompson (1959). The histology of the reproductive organs was described in an unpublished thesis by Lloyd (1952); the mature spermatozoa were investigated with the phase-contrast microscope and illustrated by Franzén (1955).

Records of reproductive activity are widespread in the literature; spawning has been found in many months of the year: February to July in the Cromarty Firth (Sutherland 1890); April to June in the Roscoff area (Hecht 1895); April to June in the Arcachon area (Cuénot 1903); April to May in Northumberland (Storrow 1911); April to May, October, in the Isle of Bute (Renouf 1915); January to June in the Clyde area (Elmhirst 1922); June to July, probably, in the Faroes (Lemche 1929); June in Norway (Odhner 1939); March to May in Co. Antrim (McMillan 1944); December to June in the Plymouth area (Marine

Biological Association 1957); January to May, October, in the Clyde area (Allen 1962); March to June in the Isle of Man (Miller 1962); February to June in the Isle of Man (Bruce, Colman & Jones 1963).

Some of these authors have speculated upon the probable life span of *A. pseudoargus*. Cuénot (1903) found at Arcachon in April five adults, 5 to 6 cm in length, and identified spawn of *Archidoris* in the field in April, May and June. In September, on another collecting trip, he found numerous young specimens, less than 25 mm in length. From these observations alone he concluded that this species is probably an annual, young ones hatching in spring, growing in summer, resting in winter, spawning in spring and then dying. Renouf (1915) made a number of observations on the growth and breeding activity of *Archidoris* in the Isle of Bute, filling out a little the picture indicated by Cuénot, but adding the new feature that an extra spawning period was once observed in October. This led Renouf to suggest that two generations may be produced in a year, but left uncertain the question of the natural longevity of this species. Miller (1958, 1962) has published his observations on the size and breeding activity of 46 intertidal and 5 offshore specimens collected around the Isle of Man over a period of approximately $2\frac{1}{2}$ years. From these pooled records he concludes (Miller 1962, table 1, p. 564) that Manx *Archidoris* have a single breeding season each year and that the normal life-span is 2 to $2\frac{1}{4}$ years (computed allowing for 'a period of life through the. . . breeding season'). None of these authors investigated the histology of the reproductive organs at various seasons. None has made regular observations on a single locality for a protracted period. None has verified that post-reproductive senescence and death do in fact occur. (Fisher (1936) records finding many dead and dying specimens in Co. Antrim in the month of August. Unaccountably, no spawn was present in the field and this, together with the absence of data regarding the state of the gonads or of the food reserves, renders her interesting observations completely inexplicable.) In an attempt to clarify these issues the present work was undertaken. It has been carried out while working at the Zoology Department of Bangor University College, the Marine Science Laboratories at Menai Bridge, the Dove Marine Laboratory, Cullercoats, the Marine Biological Station, Port Erin, and on field expeditions to Cornwall, during the years 1954–65.

The nomenclature of the British animals referred to herein is that of the Plymouth Marine Fauna list (Marine Biological Association 1957).

## 2. Methods

Vivisection was facilitated by placing the live specimen in Bouin's fluid for 5 s to fix the mucus-glands of the skin, washing for 10 s in running tap-water, drying with a cloth and dissecting on filter papers under a stereoscopic microscope. This method enabled slime-free incisions to be made. The cilia of internal organs, and the activity of spermatozoa, were unimpaired.

For routine gonad histology the fixatives of Helly and Zenker (with or without acetic acid) were found to give good results. Bouin, Susa, Perényi and Lewitsky-saline resulted in inferior sections of the ovotestis. For other parts of the genital system, however, Lewitsky-saline was an excellent fixative, while Susa and Perényi gave best results with the embryonic

stages. Material for sectioning was cleared in amyl acetate, embedded in Hance's rubber wax (Gurr), and sectioned at 5 to 10 $\mu$m. Stains employed included Heidenhain's azan and alum haematoxylin, and Mayer's haemalum counterstained with eosin and alcian blue 8GS (Steedman 1950). Drawings were made with the aid of a Swift camera lucida. A Zeiss photomicroscope was available for photomicrography.

Material for the Akashi electron microscope was either dried on to Formvar-coated copper grids in osmic vapour, or fixed with 2% osmic acid (Malhotra 1962), embedded in Araldite, sectioned on an LKB ultrotome, dried, and treated with saturated uranyl acetate in 50% alcohol.

## 3. Functioning of the mature reproductive organs

### A. *Light microscope observations*

Accounts have been given of the gross anatomy of the reproductive organs by Alder & Hancock (1845–55), Hancock & Embleton (1852), Eliot (1910), and Lloyd (1952). I have verified these descriptions, to which the reader is referred for excellent illustrations of the appearance of the reproductive organs in dissections.

Figure 1 shows the relations and histology of the various parts. For comparison, the reproductive tract of another dorid nudibranch, *Onchidoris fusca*, is shown in figure 2. The state of development of the ovotestis of *Archidoris* at various times of the year is shown in figure 5, and in plate 75, while plate 72 shows in addition sections through certain parts of the conducting system. The nomenclature of the various regions followed in the present paper is for the most part orthodox, but there has been so much confusion about the naming of some parts (Pruvot-Fol 1960) that it is necessary to state the following. (1) Spermatozoa derived originally from the testis of the animal in which they are found are referred to as autosperms. (2) Spermatozoa which have been exchanged at copulation are termed allosperms. (3) The sac which in dorid nudibranchs first receives the allosperms is called here the bursa copulatrix. (4) The sac in which the allosperms are stored and nourished before utilization is called here the receptaculum seminis. (5) The old name for the initial glandular region of the oviduct, the albumen-gland, is here dropped and the term capsule-gland employed in its stead. This gives a more accurate idea of the function of its secretion.

The mature hermaphrodite gonad (plate 75*A*; figure 5*E*) of *Archidoris pseudoargus* forms a layer overlying the digestive gland. It consists of groups of female acini opening into the male acini, each of which opens in turn into a ciliated hermaphrodite ductule. These ductules are external to the gonad and lie between that organ and the overlying kidney. (It is noteworthy that the ovotestis of *A. montereyensis* is said by McGowan & Pratt (1954) to be very different in organization, with an anterior zone which is exclusively male, and a posterior, entirely female, area, but see Post-script on p. 374.) In virgin adults all the ripe oocytes are within the female follicles of the ovotestis, while the ripe autosperms are stored both in the gonad and to a greater extent in the wide region of the hermaphrodite duct (plate 72*D*; figures 1*A*, *C*) which functions as a vesicula seminalis. Spermatozoa from these regions are almost invariably non-motile and non-orientated.

During copulation the penis of each partner is placed in the bursa copulatrix of the other,

and spermatozoa are forced by muscular contractions of the hermaphrodite duct (figures 1*A*, *C*) and vas deferens (figures 1*A*, *F*) through the penis, receiving en route the prostatic secretions. These allosperms are found at first in the bursa (figures 1*A*, *G*) but soon pass to the receptaculum seminis (plate 72*A*; figures 1*A*, *I*), where they are continuously motile

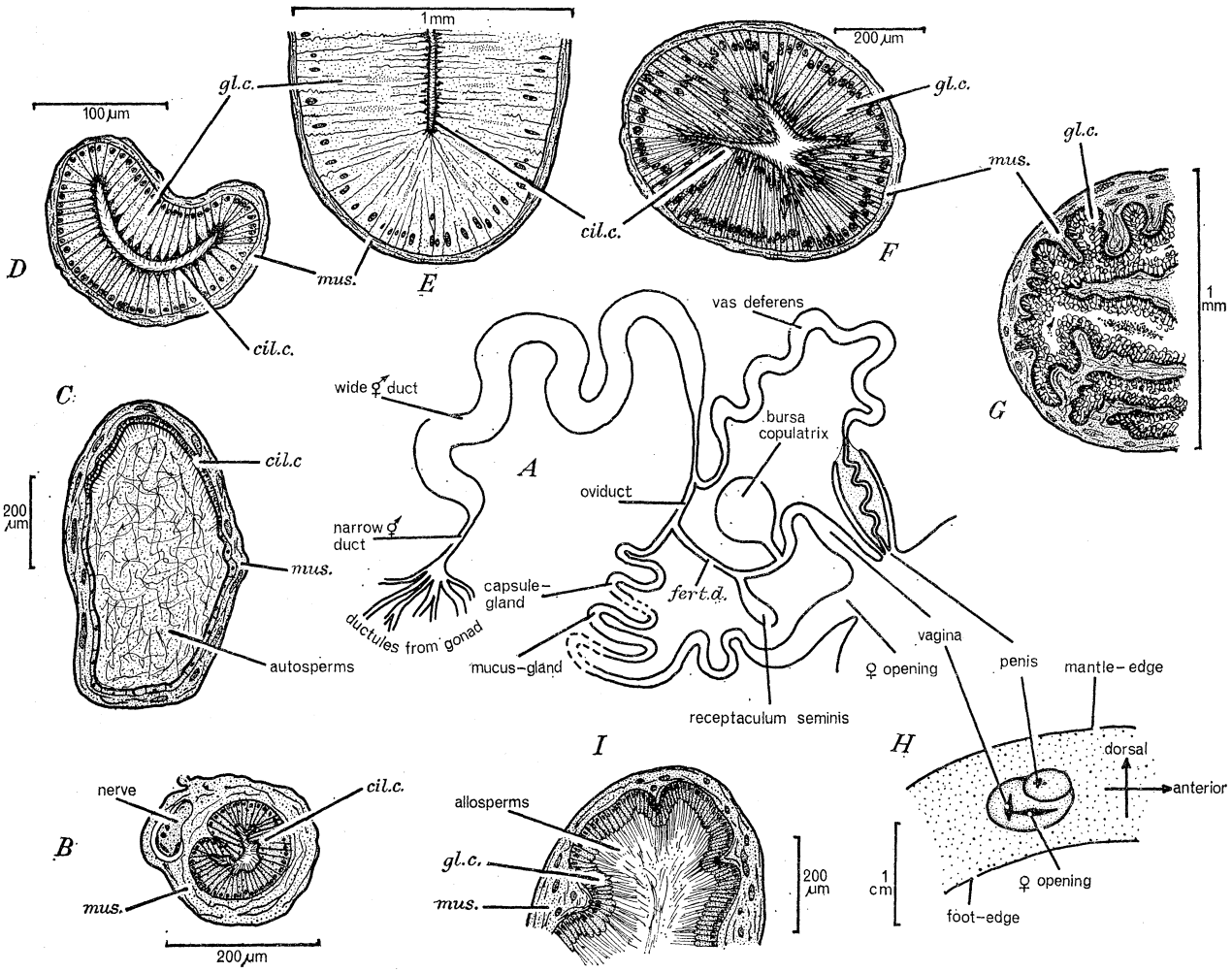



FIGURE 1. Reproductive organs of mature, mated, *Archidoris pseudoargus*. *A*, Diagrammatic representation of the tract. *B*, Transverse section through the initial narrow region of the hermaphrodite duct. *C*, Transverse section through the wide region of the hermaphrodite duct, the vesicula seminalis. *D*, Transverse section through the capsule-gland region of the oviduct. *E*, Transverse section through the mucus-gland region of the oviduct. *F*, Transverse section through the vas deferens, showing the prostatic gland cells. *G*, Portion of a transverse section through the bursa copulatrix of a spawning adult, killed April 1962. *H*, External genital apertures of a mature adult. *I*, Portion of a transverse section through the receptaculum seminis of a spawning adult, killed April 1962. *cil.c.*, Ciliated endothelial cells; *gl.c.*, glandular endothelial cells; *mus.*, coat of mixed circular and longitudinal muscle fibre bundles; *fert.d.*, fertilization duct.

and orientated radially with their heads facing the endothelium. Here they are stored. In sections synchrony of phase of sperm-tail movement at the time of death gives rise to a striated appearance in sections (plate 72*C*) similar to that found in allosperms within the bursa copulatrix of *Tritonia* (Thompson 1961*a*). As in insects (Hinton 1964) it appears

highly unlikely that any intrinsic motility of the spermatozoa plays a part in their translocation either within the male tract or over their initial route to the receptaculum in the female tract of the partner.

Neither the stimulus for, nor the mode of control of, oviposition is known. As at a signal, oocytes begin to travel from the female acini along the hermaphrodite ductules towards the main efferent trunk, the hermaphrodite duct. They are transported along the ductules by the endothelial cilia of this collecting system. During the early stages in oviposition oocytes reach the major collecting ductules faster than the narrow hermaphrodite duct (figures 1 *A*, *B*) can pass them and congestion results. Oocytes in the ductules are irregularly shaped and rather flaccid cells, but if removed to sea water they round off and assume a more turgid appearance. At no time do the eggs have any power of independent movement.

After passing by endothelial ciliary action through the narrow hermaphrodite duct (figures 1 *A*, *B*), the oocytes enter the vesicula seminalis, which is swollen with autosperms. These are non-motile, physiologically immature male gametes and self-fertilization does not occur. Passage of the oocytes through this duct is accomplished in the way described for *Tritonia hombergi* (Thompson 1961 *a*). A ciliated tract (figures 1 *A*, *C*; plate 72 *D*, *cil.c.*), forming a longitudinal endothelial strip through the wide hermaphrodite duct, conducts the outgoing oocytes through the densely packed spermatozoa (plate 72 *D*). Transport of oocytes can be seen through the translucent wall of the duct in vivisected ovipositing specimens. The ciliated tract in *Tritonia* is rather narrow but in *Archidoris* it occupies about one-third of the wall of the duct. How the oocytes are made to remain in contact with this longitudinal tract is unknown, although it is verifiable that they *do* do so, and that this is to a great extent unrelated to the pull of gravity. Sectioned material is somewhat unhelpful, because the oocytes fall away from the ciliated tract after fixation (plate 72 *D*), and may appear in sections anywhere within the hermaphrodite duct.

At the bifurcation (illustrated by Pelseneer 1892, p. 107) of the hermaphrodite duct into its male and female components, co-ordinated muscular contractions direct the stream of oocytes into the ciliated oviduct (figure 1 *A*). The oocytes at this stage are still naked, unfertilized, and flaccid. McGowan & Pratt (1954) state that fertilization in *Archidoris montereyensis* occurs during the passage of the oocytes through the vesicula seminalis; this is certainly not the case in *A. pseudoargus*, where the oocytes, upon entering the capsule-gland, meet and are fertilized by allosperms which have in the meantime travelled through the fertilization duct (figure 1 *A*, *fert.d.*) from the receptaculum. Probably this last lap of their journey the allosperms accomplish by their own efforts. As the fertilized ova pass slowly through the ciliated capsule-gland, ovoid egg-cases are secreted around them, each case surrounding a single ovum, or two, or more ova. Occasional supernumerary allosperms are included in the egg-case and such may often be detected in samples of the egg-stream removed from the next part of the oviduct, the mucus-gland (figures 1 *A*, *E*). The motile life of a spermatozoon is short, after it has left or been removed from the receptaculum, maximally 30 to 50 min *in vitro*, several hours *in vivo* at 10 to 14 °C, and supernumerary sperms in egg-cases taken from the mucus-gland were usually moribund. These must normally be broken down quickly in some way, because they were never found in *Archidoris* eggs after oviposition. This is in contrast to the situation in *Favorinus branchialis*, where sperms may be occasionally found in the complete egg-mass (Trinchese 1881), and in *Tritonia hombergi*,

where numerous motile allosperms may be seen for some time in each egg-case after spawning (Thompson 1961 *a*).

As the egg-stream passes through the mucus-gland, guided by the endothelial cilia, layers of jelly are added (the resulting stratification being visible in the completed spawn-mass), and the eggs are arranged into spiral strings. The completed spawn-mass (plate 76) is slowly extruded through the oviducal aperture in the form of a flattened strip, the edge of which is attached to the substratum under the pressure and guidance of the edges of the mantle and foot. Descriptions and a drawing of the spawn were given by Alder & Hancock (1845–55) who, however, incorrectly speak of the ova as being arranged in transverse rows (as do many more recent authors). In fact the strings of ova form spirals through the jelly.

In order to investigate valve-mechanisms in the dorid reproductive system a mature spawning *Onchidoris fusca* (from the Menai Strait) was narcotized with magnesium chloride, partially dissected and then sectioned, to reconstruct the natural relations of the tract. A number of valve-mechanisms were found which clearly guide the gametes at various stages, and these structures are shown in figure 2. This figure shows the reproductive system of *O. fusca* to be different in important respects from the diagrams, based on dissections alone, published by Hancock & Embleton (1852), Bergh (1880), and Marcus (1961). My investigation of *O. fusca* is mentioned here because it provided confirmation of a number of important particulars relating to *Archidoris*, but *Onchidoris* was found to differ in that the stream of oocytes at oviposition is conducted into and through the active allosperms of the receptaculum (figure 2, *rec.sem.*), whereas in *A. pseudoargus* the allosperms must pass from the receptaculum along the fertilization duct into the capsule-gland to fertilize the oocytes.

Estimates of the number of eggs per spawn-mass have varied, and it is certainly true that the gross dimensions of the egg-ribbon vary widely. Alder & Hancock (1845–55) found one ribbon to contain about 50 000 eggs, and this figure is given also by Pelseneer (1899) and by Fretter & Graham (1964). Miller (1958) records a figure of 300 000 eggs per egg-ribbon, while Colgan (1914) calculated that a very large ribbon contained 654 000 ova. My own observations at Port Erin and in the Menai Strait give a maximum number of ova per spawn-mass of about 120 000; the maximum number of ova per 1 mm length of the ribbon was 554. The colour of the ova varied from white to cream-yellow. The size of the spherical ova varied in observations on spawn-masses in different years from 0·140 to 0·170 mm, but the usual size-range was 0·142 to 0·165 mm. The proportion of multiple embryos (i.e. more than one embryo per egg-case) varies greatly in different egg-masses. Some egg-masses contain only single embryos, whereas in other masses up to 45 % of all the egg-cases may contain more than one ovum. Three, four and five embryos per case, in order of increasing rarity, have all been observed on occasion. Such multiple embryos are apparently at no disadvantage compared with their single siblings. The egg-cases are always ovoid in shape and vary in length from 0·240 to 0·300 mm (single embryos) and 0·320 to 0·400 mm (multiple embryos). A variable number of deformed, empty egg-cases are usually present at the beginning and at the end of each egg-ribbon.

To summarize what is known about gamete-transport in *Archidoris*: it is certain that female gametes are translocated solely by ciliary action (although ovulation may perhaps be facilitated by the contraction of the muscular sheath of the ovotesticular acini, as Chambers (1934) claimed for *Embletonia*), whereas spermatozoa are translocated by

peristaltic muscular action except over the short distance from the receptaculum to meet the stream of outgoing oocytes at oviposition, when it is probable that motility of the allosperm is essential.

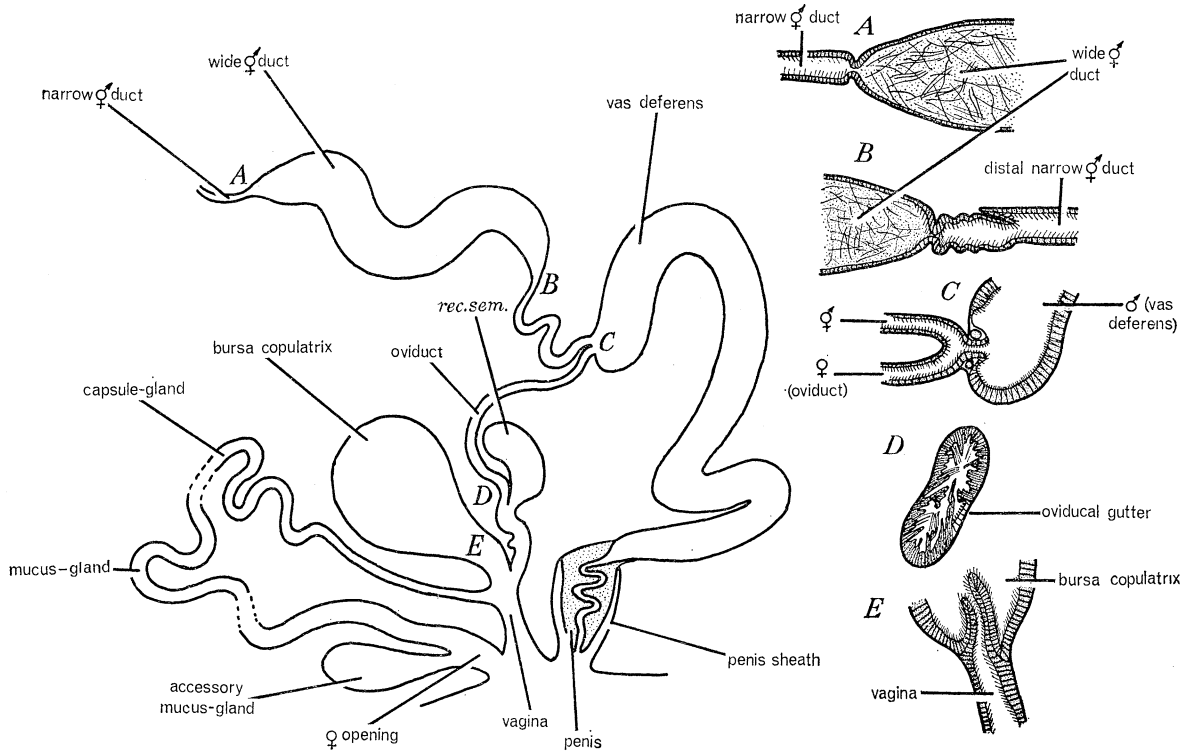
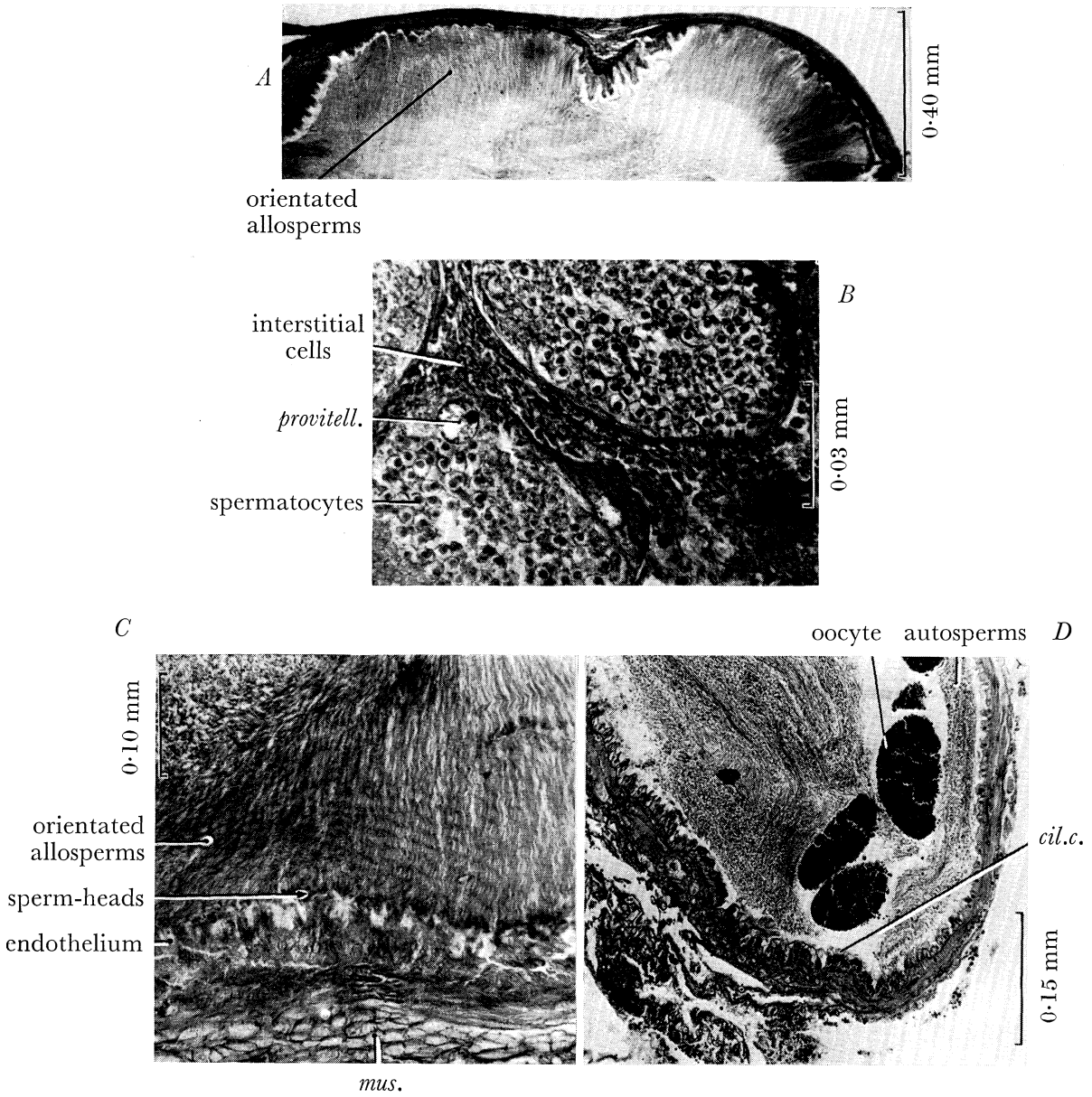



FIGURE 2. Reproductive system of mature, mated *Onchidoris fusca*. Left, diagrammatic representation of the tract. Right, *A* to *C* show semi-diagrammatically valve-like devices located in the indicated sectors of the reproductive tract. *A*, Sphincter presumed to prevent back-flow of autosperms from the wide hermaphrodite duct during copulatory ejaculation. *B*, Sphincter presumed to retain autosperms in the vesicula seminalis, except during copulatory ejaculation. *C*, Hermaphrodite junction where the divergence of path followed by autosperms at ejaculation, and by oocytes at oviposition, is controlled. *D*, Receptaculum seminis, with its internal ciliated gutter, serving to conduct oocytes during oviposition through the active allosperms towards the capsule-gland. *E*, Bursa copulatrix, with valve which possibly prevents uncontrolled back-flow of allosperms after copulation. *rec.sem.*, Receptaculum seminis.

### B. *Other observations*

Mature autosperms are relatively inactive and orientated apparently at random within the vesicula seminalis, whereas allosperms are orientated radially with their heads facing the endothelial lining of the receptaculum seminis (plate 72*A*, *C*) and, if examined in a drop of sea water, are seen to be active. More accurately, the difference between autosperm and allosperm suspensions is such that in the former only a small minority of individuals move and the movement is intermittent and sluggish, whereas in the latter nearly all the individuals exhibit a sustained and vigorous motility, which may continue in sea water for up to 50 min at 10 to 14 °C.

It was desirable to ascertain whether allosperms are stored in an active state *in vivo*, or are inhibited within the receptaculum as apparently occurs inside the spermatophore of




Photomicrographs of sections through the reproductive organs of *Archidoris*.

*A*, Portion of a longitudinal section through the receptaculum seminis of a mated adult, showing allosperms facing the endothelium; Menai Strait, April 1962. Lewitsky-saline and azan.

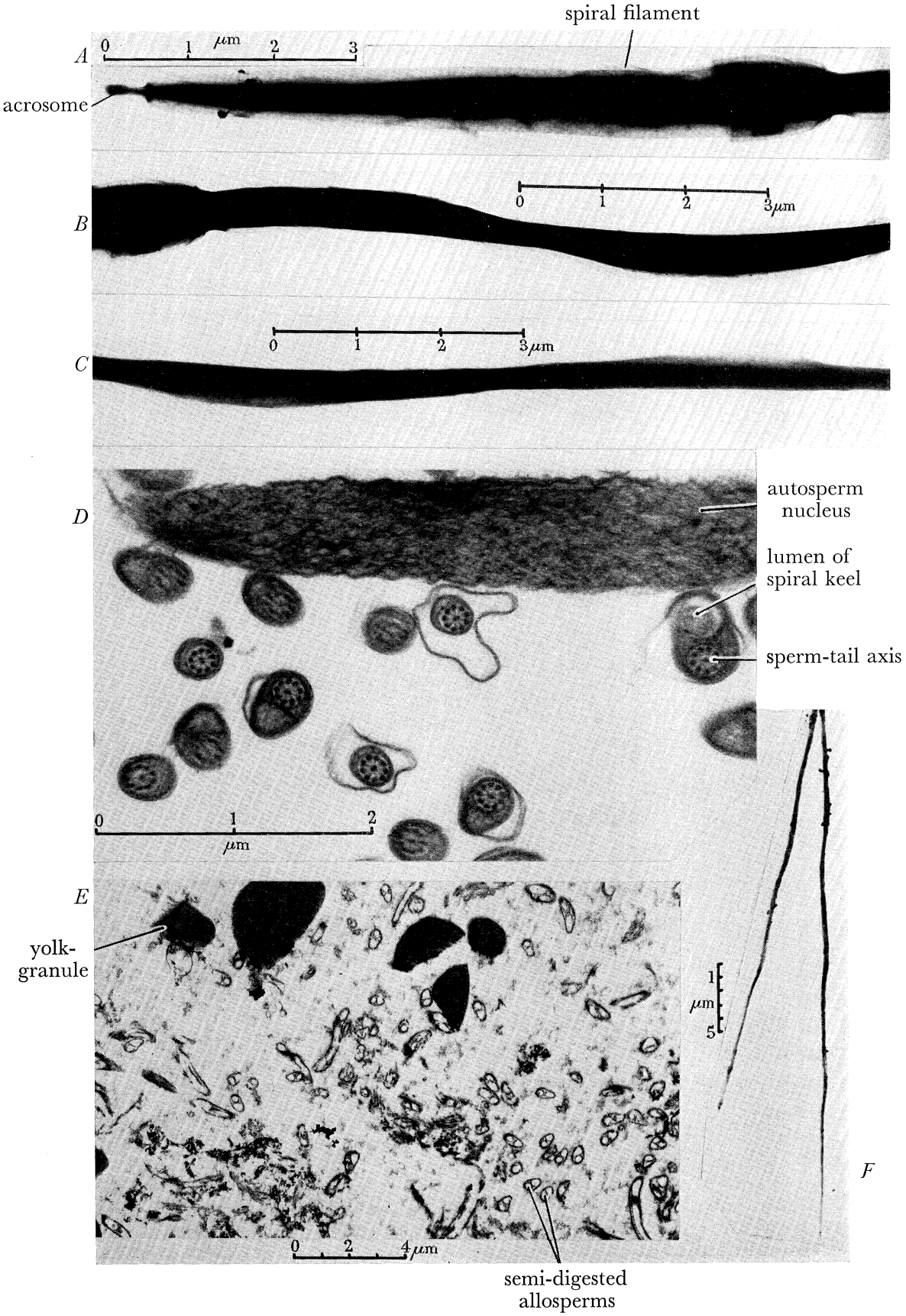
*B*, Section through the gonad of a specimen 38 mm in length, showing early stages in gametogenesis in two of the ovotesticular acini; captured 15 September, killed 29 September 1958, Traie Meanagh, Isle of Man. Perényi and azan.

*C*, Portion of a longitudinal section through the receptaculum seminis of a mated adult, showing the coincidence of phase of the tail helices of adjacent allosperms, and the intimate relationship between the allosperm heads and the receptacular endothelium; Menai Strait, April 1962. Lewitsky-saline and azan.

*D*, Portion of a transverse section through the wide hermaphrodite duct of an adult killed during oviposition, showing outgoing ripe oocytes passing through masses of non-orientated autosperms; Menai Strait, April 1962. Zenker-without-acetic and azan.

*cil.c.*, Ciliated endothelial cells; *mus.*, coat of mixed circular and longitudinal muscle fibre bundles; *provitell.*, oocyte in a stage of provitellogenesis.

(*Facing p.* 350)




Electron micrographs of spermatozoa of *Archidoris*, Cornwall, April 1965.

*A*, Head with acrosome, spiral filament, and posterior dilation; whole autosperm fixed on Formvar film.

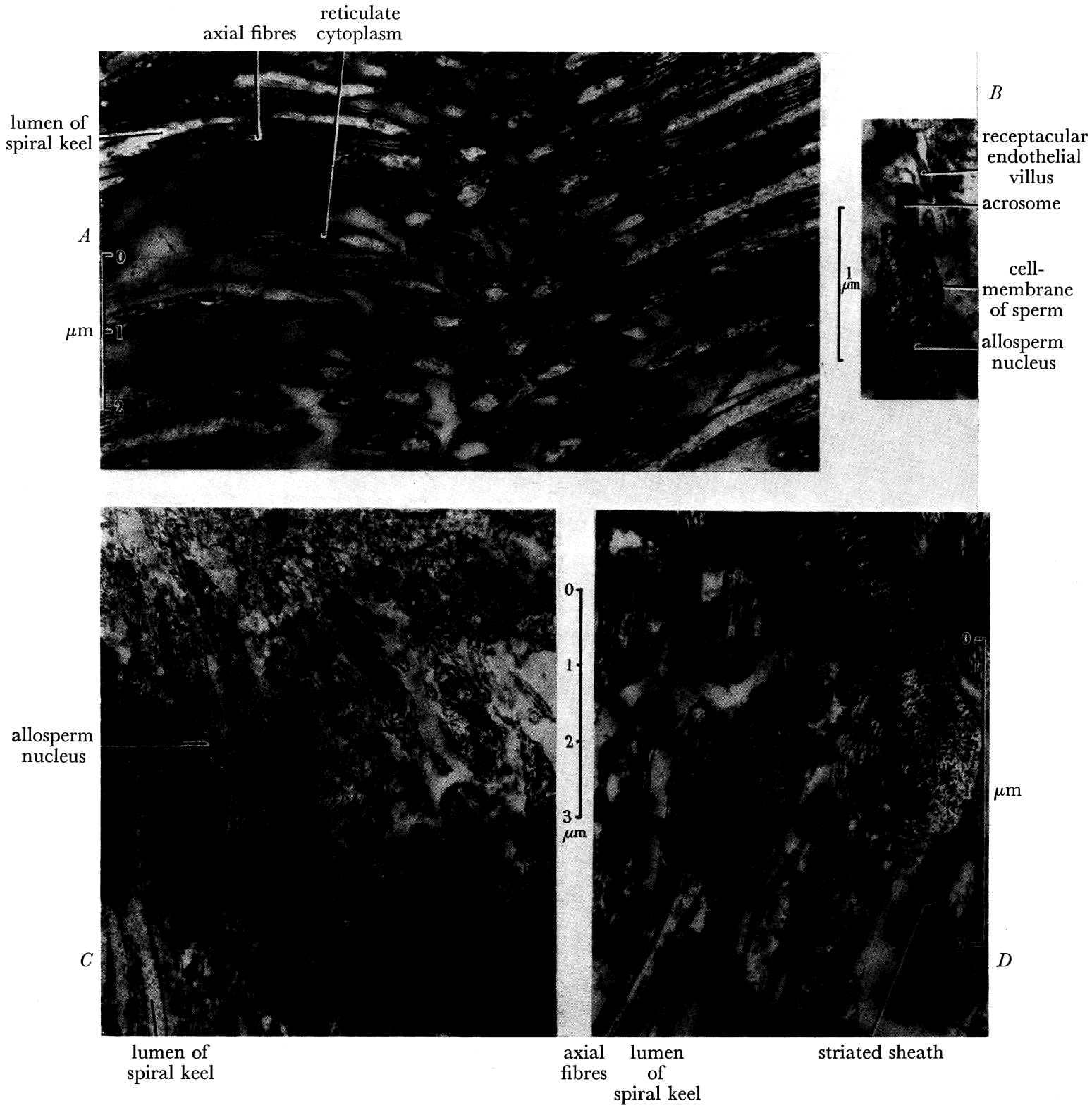
*B*, Initial region of the tail of the same specimen.

*C*, Region of the tail approximately 50 $\mu$m behind the head.

*D*, Section through a mass of autosperms from the vesicula seminalis.

*E*, Section through the bursa copulatrix of a mated adult, showing fragments of stray eggs and the hollow appearance of 'waste' allosperms.

*F*, Rear extremities of allosperms from the receptaculum seminis of a mated adult, showing the posterior persistence of the spiral keel; whole specimens fixed on Formvar film.



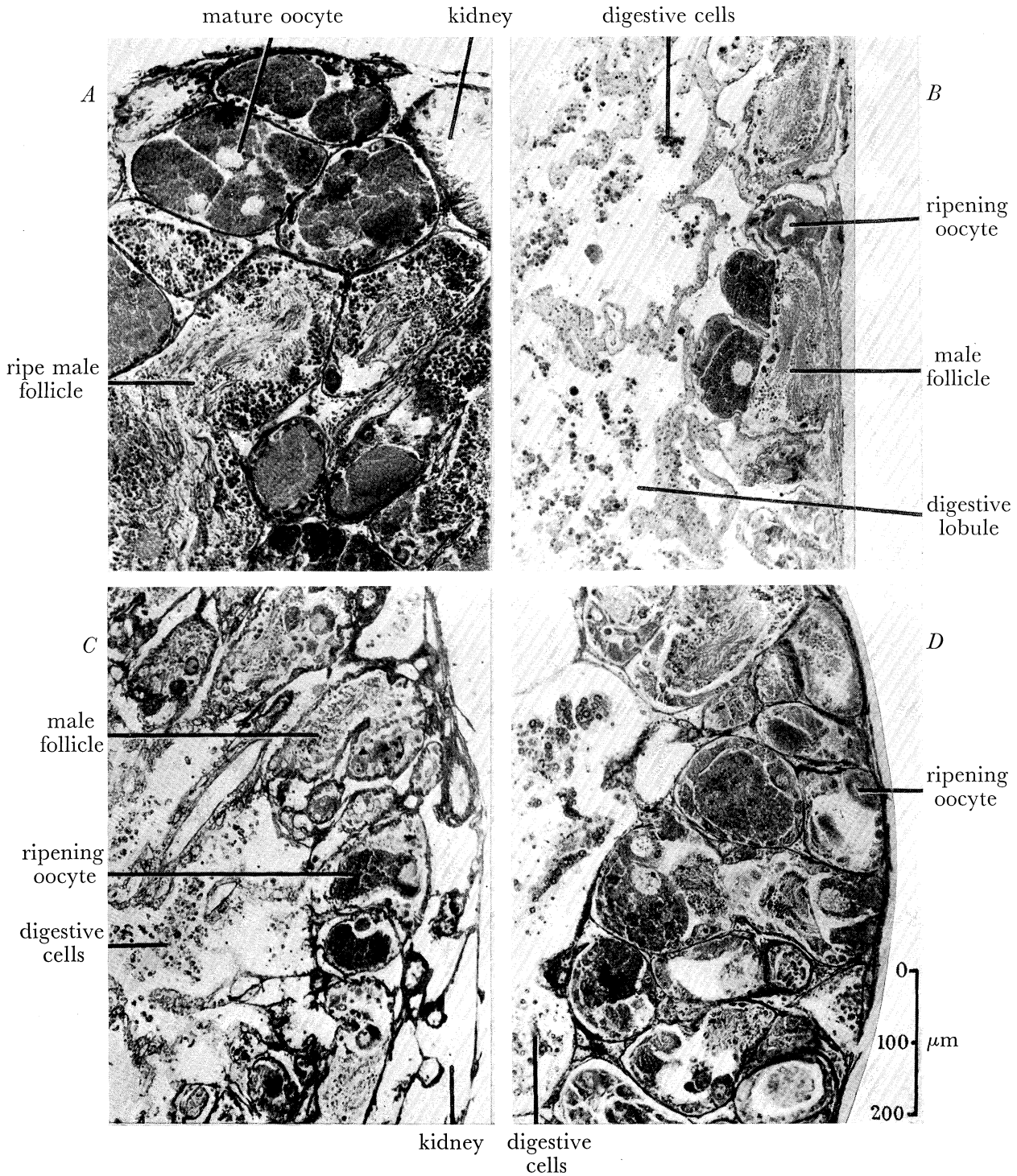

Electron micrographs of receptaculum seminis allosperms of mated *Archidoris*, Cornwall, April 1965.

*A*, Section through the contents of the receptaculum, illustrating the synchrony and identity of orientation of allosperm tails (compare with plate 72*C*).

*B*, Longitudinal section through the allosperm head, showing the intimate association between the spermatozoon and the receptacular endothelium.

*C*, Longitudinal section through numerous allosperm heads, showing their association with the receptacular endothelium.

*D*, Oblique longitudinal section through the neck-region of several allosperms.




Histology of the ovotestis in *Archidoris* from Traie Meanagh, Isle of Man.

*A*, Section through the gonad of a 70 mm specimen, collected 24 November, killed 25 November 1958. Mature yolk-laden oocytes are common and the male follicles show all stages in spermatogenesis. Zenker-without-acetic and azan.

*B*, Section through the gonad of a 40 mm specimen, collected 5 May, killed 6 May 1959. The animal was feeble when found and proved on dissection to have a reduced digestive gland and few ripe eggs. The section shows spermatocytes to be uncommon, although abundant ripe autosperms are present, and that a fresh batch of oocytes was beginning to ripen. In the digestive gland the digestive cells have shrunken, rounded off, and become detached from the basement membrane; the kidney also shows degeneration. Zenker and azan. (*Continued on opposite page*)

Adult *Archidoris* with their spawn, Cornwall, April 1965. The photograph was taken by Heather H. Angel during tidal emersion.

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*Legend to plate* 75 *continued.*

*C*, Section through the gonad of a 35 mm specimen, collected 20 May in a moribund condition, fixed 21 May 1959. On dissection the digestive gland was found to be reduced, and there were only very few eggs visible in the ovotestis. The section shows that breakdown of the digestive and renal cells is advanced; spermatozoa are abundant (but no spermatocytes), while such oocytes as are present are mainly immature. Zenker and azan.

*D*, Section through the gonad of a 35 mm specimen, collected 6 May 1959 and fixed immediately. On dissection the digestive gland was found to be very small, and there were few ripe eggs in the ovotestis. The section shows that spermatozoa and early ripening oocytes are abundant, but that the digestive cells have shrunken, rounded off, and lost contact with the lobular walls. Zenker and azan.

*Loligo* (Austin, Lutwak-Mann & Mann 1964); whether ultrastructural changes accompanied sperm-activation; whether allosperms become embedded in the cells of the receptacular endothelium, as claimed by Lloyd (1952) for *Archidoris pseudoargus*, by McGowan & Pratt (1954) for *A. montereyensis*, and by Fretter & Graham (1962, 1964) for those prosobranch gastropods which copulate; and whether digestion and breakdown of allosperms occurred in the bursa copulatrix, as indicated by conventional histological methods applied to *Archidoris*, and to *Planorbarius* (Alaphilippe 1955), *Oncomelania* (Roth 1960), *Oxychilus* (Rigby 1963), and *Succinea* (Rigby 1965). Furthermore, it became of interest to investigate the functional morphology of the archidorid spermatozoon. Although light microscope investigations of invertebrate sperms have yielded a great deal of information (Retzius 1912; Tuzet 1950; Franzén 1956), few studies have been made with the electron microscope. Earlier molluscan workers in this field confined themselves to observations on whole spermatozoa on collodion or other films (Hanson, Randall & Bayley 1952; Selman & Waddington 1953; Dan & Wada 1955), and the only published accounts of work with thin sections of molluscan spermatozoa noted are those of Bradfield (1955) on *Arion* and *Deroceras*, Grassé, Carasso & Favard (1956) on *Helix*, Rebhun (1957) on *Otala*, Galtsoff & Philpott (1960) on *Crassostrea*, and Gall (1961) on *Viviparus*.

(i) *The morphologically mature spermatozoon* (plates 73, 74; figures 3, 4)

No ultrastructural differences could be detected between autosperms and allosperms. The description which follows applies to both.

With a light microscope of conventional design, the spermatozoon has the appearance shown in figure 3*A*. The overall length is 208 to 210 $\mu$m (205 $\mu$m in *A. montereyensis*, McGowan & Pratt 1954), of which the somewhat flattened head takes up 8 to 9 $\mu$m. The tail appears to be a spiral structure with coils of constant wavelength but diminishing amplitude. It is exceedingly difficult with the light microscope to be sure whether or not the spirality persists to the rear extremity. With the phase-contrast microscope, Franzén (1955, as *A. tuberculata*) discerned three additional features. First, that the head is twisted like a corkscrew; secondly, that a minute filament spirals along the head; and, thirdly, that the tail consists of a relatively straight axis around which runs a closely apposed spiral keel. It will be seen that in all these particulars Franzén was admirably perceptive. I was probably in error (through faulty microscopy) in claiming (Thompson 1961*a*) for *Tritonia* that the central axis of the sperm-tail had a spiral form.

*The head.* Plate 73*A* shows the head in 'side' view after fixation upon a Formvar film; sections through various parts of the head are shown in plates 73*D*, 74*B* to *D*. The slight spirality of the head has disappeared on death. At the anterior tip is the blunt acrosome. Samples of autosperms and allosperms were subjected to a variety of tests using preparations of egg-water (with eggs from the ovotestis) and controls in sea water. In no case could any morphological change in the acrosome be induced; in electron micrographs this organelle invariably presented the appearance shown in plate 73*A*. This contrasts with results obtained in similar tests using sea-urchin material (Afzelius & Murray 1957) and bivalve molluscs (Dan & Wada 1955) when spectacular changes occur on bringing ripe spermatozoa into contact with eggs or egg-water of the correct species. Perhaps acrosomal extension occurs only in those animals which have external fertilization.

The bulk of the sperm-head is occupied by the nucleus, whose contents are coarsely striated, the long axes of most of the striae being coincident with that of the sperm. The nucleus is bounded by a nuclear membrane and the whole head ensheathed by the stout cell-membrane, continuous with that of the tail. This cell-membrane is raised to form the spiral filament of the head (plate 73*A*). It also forms an annular dilation around the posterior region of the head (plate 73*A*).

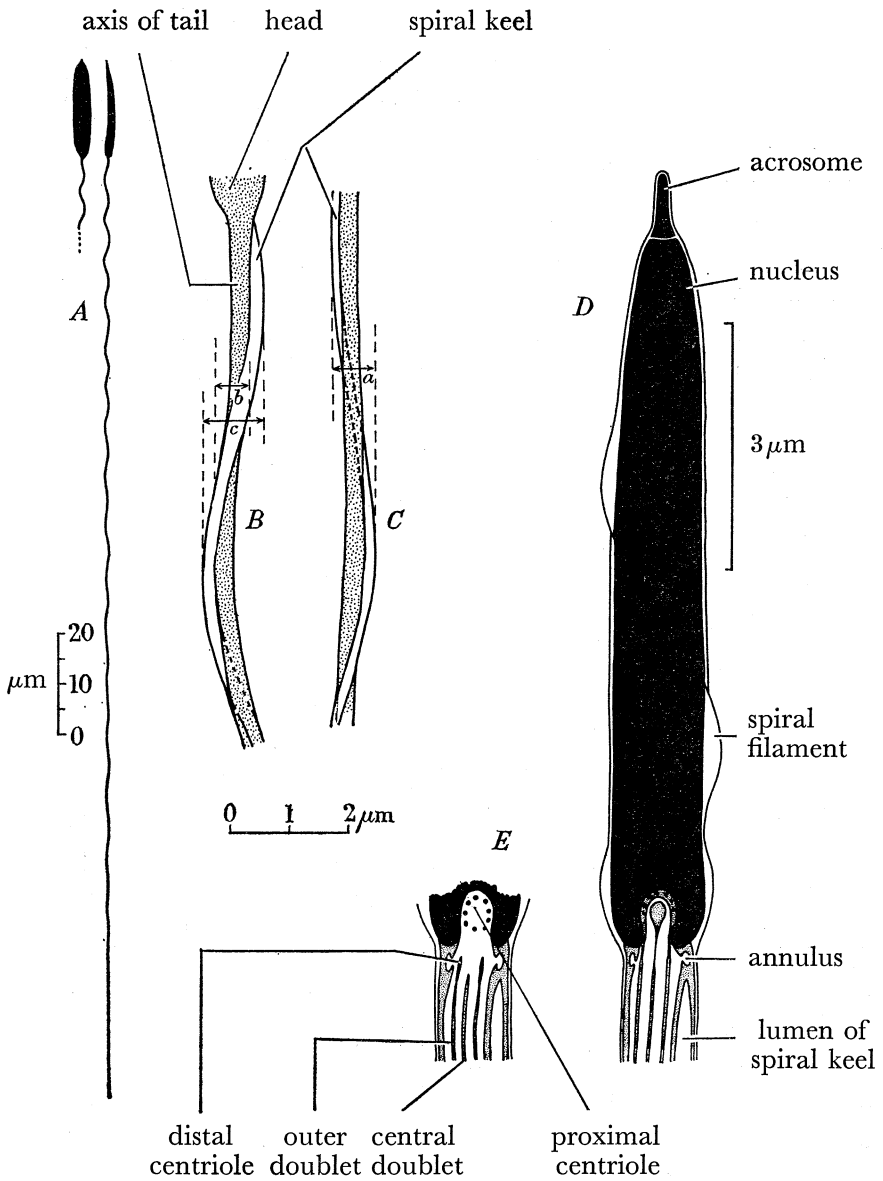


FIGURE 3. Mature spermatozoa of mated *Archidoris*, Cornwall, April 1965. *A* is based upon light microscope observations, *B* to *E* upon electron micrographs, some of which are presented in plates 73 and 74. *A*, Gross morphology of an autosperm; head shown from below and from the side. *B*, The spiral keel, immediately behind the autosperm head; $b = 0{\cdot}84\mu m$, $c = 1{\cdot}0\mu m$. *C*, The spiral keel, approximately $50\mu m$ back from the head; $a = 0{\cdot}5\mu m$. *D*, Semi-diagrammatic reconstruction of the autosperm head, showing the main features visible in sections. The sperm is depicted as if cut in longitudinal section. *E*, Possible interpretation, suggested by M. A. Sleigh, of features of the neck (see text for fuller explanation).

*The neck.* Plate 74*C, D* shows longitudinal sections through this region, while figure 3*D* shows semi-diagrammatically my tentative interpretation of the centriolar apparatus. This was based on electron microscope observations which appeared to show the outer nine fibre-doublets of the tail joining a hollow cone of electron-dense, granular material nestling in a large depression in the rear face of the nucleus. There is also evidence that, as is shown in figure 3*D*, the central fibre-doublet of the tail axis runs to an approximately pear-shaped structure within the cone mentioned above. Dr Michael Sleigh has kindly examined my material and suggests that the geography of the neck may resemble more the arrangement shown in figure 3*E*. This arrangement is not inconsistent with the micrographs and is less unorthodox than figure 3*D*, but my preparations do not at present permit further clarification of this issue. One further feature worthy of remark is an electron-translucent annulus in the neck (figure 3*D*, annulus), whose functions and homologies are uncertain, but which it is suggested may become a fracture-line at some stage in the fertilization process.

*The tail.* The initial region of the tail is shown in plates 73*B, C*, 74*D*, and in figures 3*B, D, E*, while other parts are illustrated in plates 73*C, D, F*; 74*A*, and in figure 4. There is no distinction between a middle-piece and a principal-piece (using the terminology of Fawcett 1958), because the mitochondria in Opisthobranchia lose their affinity for janus green and break up, spreading out along the whole tail during late spermiogenesis (Franzén 1955). This has been reported also for *Otala* by Rebhun (1957), but is rather different from the situation in the eupyrene spermatozoon of *Viviparus* (Gall 1961), and in *Crassostrea* (Galtsoff & Philpott 1960), where the mitochondria of the spermatid fuse to form four nebenkerne of large size and with regular internal lamellae. Mitochondria could not with certainty be identified in mature sperms of *Archidoris*. It may be that the cytoplasmic reticulum (plate 74*A*) of the tail represents the dispersed mitochondrial material mentioned by Franzén (1955). Each mesh of this reticulum measures maximally 0·09 $\mu$m. The cytoplasm of the tail is bounded inside and out by a fine spirally striated sheath, readily visible in electron micrographs (plate 74*D*).

The axis of the tail consists of a central fibre-doublet with a ring of nine peripheral doublets. The diameter of the fibre-bundle in sections is 0·17 $\mu$m. The axis is bilaterally symmetrical. The plane of axial bilateral symmetry remains unaltered along the length of the tail. An unusual feature is the presence of radial 'spokes' of electron-dense material perhaps connecting the central fibres with the peripheral doublets. Such spokes have been reported elsewhere, e.g. in Man (Ånberg 1957), in *Crassostrea* (Galtsoff & Philpott 1960), and in *Psammechinus* (Afzelius 1959). Regular undulations in the tail axis are detectable in fixed material (plate 73*B, C*; figure 3*B*), and have a wavelength identical with that of the spiral keel, but are of smaller amplitude (figure 3*B*). It is possible that these axial undulations are artifacts.

The spiral keel of the tail is its most unusual and interesting feature. Keels or 'undulating membranes' have been described for toad sperms (Burgos & Fawcett 1956), but these are possibly locomotor organelles. The ribs or fins running down each side of the mammalian sperm-tail (Bradfield 1955) are rather similar. I can find no explicit reference to a spiral keel in papers dealing with electron microscopy of invertebrate spermatozoa, although Rebhun (1957, pl. 167, fig. 5) shows a section through a sperm-tail of *Otala lactea* which

indicates that such a keel may be present. Selman & Waddington (1953) illustrate helical fibres on the middle-piece and the tail of the spermatozoon of *Limnaea* but these are probably of a different character. So too are the helical strands surrounding the middle-piece of the *Viviparus* sperm; Hanson *et al.* (1952) suggest that these may be derived from the mitochondria of the spermatid.

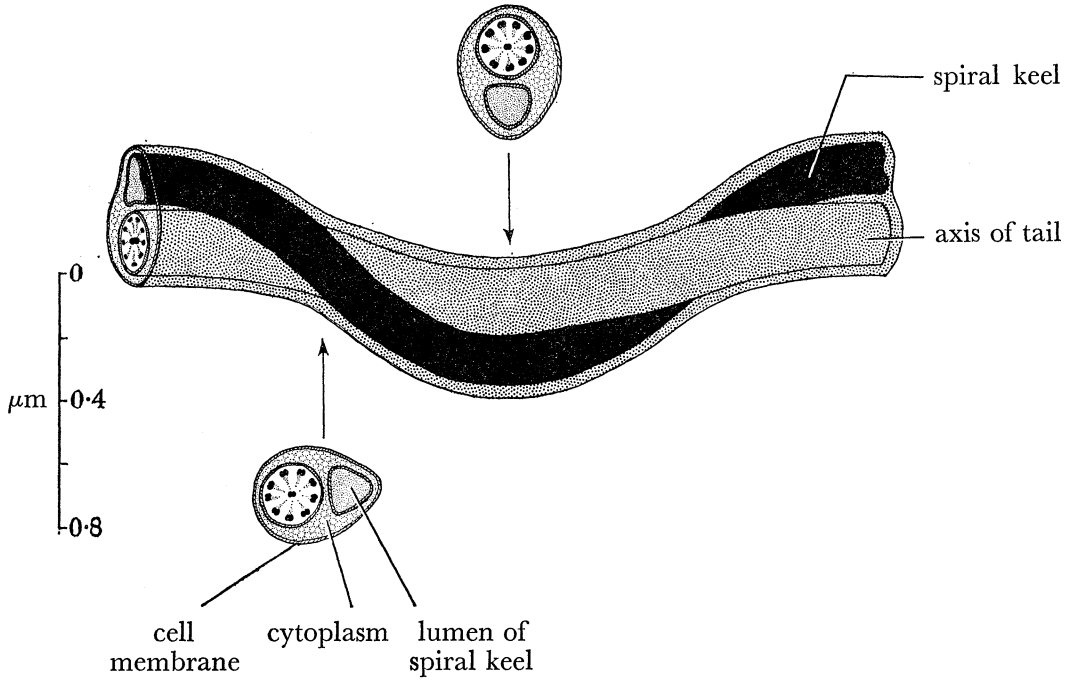


FIGURE 4. The tail of the mature spermatozoon of *Archidoris*, Cornwall, April 1965. Semi-diagrammatic reconstruction based upon electron micrographs, some of which are presented in plates 73 and 74, of sections and of whole spermatozoa fixed on Formvar films. Inset are transverse sections at the levels indicated.

In *Archidoris* the keel consists of a ridge commencing just behind the head and spiralling (in a clockwise direction when viewed from the front) to the tip of the tail (plate 73*F*). The wavelength of the spiral varies from 5 to 11 μm after different methods of preparation for electron microscopy, but is constant along any individual sperm-tail. The crest to trough amplitude also shows individual variation; but superimposed upon this is a diminution in amplitude from neck to rear. Figure 3*B*, *C* shows amplitude measurements in a single spermatozöon at different levels. The spiral keel possesses a lumen (plates 73*D*, 74*A*, *C*, *D*), 0·25 μm wide at its maximum, which is filled after fixation with a loosely coagulated material. The keel does not connect with any organelle of the sperm-neck, so far as could be ascertained.

(ii) *Locomotion of spermatozoa*

Observations on sperm movement were hampered by the high speed of active allosperms in sea water, and by the fact that each spermatozoon spins rapidly in progression. Although many difficulties of interpretation remain unresolved, some insight was gained using ciné-photomicrographic apparatus under the guidance of Dr M. A. Sleigh. It became clear, when viewing film of normal active *Archidoris* sperms at various speeds of projection, that

thrust is provided by flagellation of the kind described by Gray (1955, 1958) in echinoid and mammalian spermatozoa, with a series of propagated waves originating at the neck and progressing along the tail. The spiral path pursued by the active echinoid spermatozoon is, in the opinion of Bishop (1962), attributable to wave vectors of the flagellum itself and not to the known asymmetry of the head.

So far as could be ascertained, the waves originating in the sperm-neck are uniplanar and approximately symmetrical on the two sides. (In abnormally moving individual spermatozoa, encountered occasionally, the waves originate at the rear and pass forwards, resulting in sperm-progression backwards.) The length of the sinusoidal wave is approximately 80 $\mu$m; this does not alter markedly as it passes distally. In a semi-active spermatozoon the amplitude of the waves appears greater than in a fully active individual: the optical envelope of the spermatozoon narrows as it gains speed.

As normal *Archidoris* allosperms progress forwards, they spin in a clockwise direction when viewed from the front. This spinning may exceed 8 rev/s. During spinning the acrosome describes a circle approximately 10 $\mu$m in diameter. As a sperm moves forward the spinning helical keel gives rise to the appearance of short-period waves passing backwards along the tail (similar to the waves apparent on a rotating barber's pole). In the abnormal individuals referred to above this is reversed.

It seemed likely that the spinning of motile spermatozoa of *Archidoris* is brought about by the spiral keel, the spiral head filament, and the spiral shape of the head itself, through their differential alteration of the moving spermatozoon's resistance to torque. To test whether such a keel could function in this way a preliminary glass model was constructed to scale and towed in water. This was found to spin so long as forward motion continued and, in short, reproduced some of the features of natural spermatozoon motility. This is of indefinable significance because of the disparity between the Reynolds numbers of the spermatozoon/seminal fluid and the glass model/tap-water systems. To obtain further evidence a number of models were constructed using nylon thread and glass (permitting greater accuracy). These were towed at 5 cm/h through glycerol at temperatures ranging from 5 to 19 °C. In the experiment the Reynolds number at 15 °C was calculated to be 0·0030, a value acceptably close to that calculated for the natural system (spermatozoon moving at 20 $\mu$m/s in sea water of 35‰ salinity), namely, 0·0036. In the trials, the sperm model when in motion rotated (up to $1\frac{1}{2}$ spins/h) upon its long axis, in a clockwise direction when viewed from the front. This gives experimental support for the hypothesis advanced above. The advantages of spinning progression are uncertain. It may allow faster progression in the female tract; it may even facilitate oocyte-penetration during the process of fertilization.

(iii) *Activation of spermatozoa*

It seemed possible that prostatic secretions of the vas deferens endothelium are responsible for the activation of spermatozoa which have been ejaculated and exchanged during copulation. This was tested in experiments using sea-water extracts of macerated vasa deferentia, with controls in sea water alone. No evidence was obtained of activation brought about by the prostate. Normal sperm-activation occurs in the female tract of the recipient slug. Whether the bursal secretion or that of the receptaculum is responsible can only be tested using virgin bursae and receptaculum extracts from individuals reared in

isolation; contamination from already active allosperms rendered inconclusive tests carried out to date.

Experiments on activation of suspensions of autosperms and allosperms were hampered by the discovery that sea water and *Archidoris* haemolymph stimulate sperm activity to a marked degree. This seems to be a physical effect; in their normal, 'dry', stored state, the sperms are packed together so tightly that their movements are hampered. The possibility arises that oxygen-lack may also play a part in this inhibition. The possibility that secretions of the endothelia of the vesicula seminalis and of the receptaculum might depress sperm activity was tested using extracts of these organs in haemolymph and in sea water. No reliable evidence was obtained of such an effect. Spermatozoa from the ovotesticular acini yielded results similar in every respect to those obtained using autosperms from the vesicula seminalis.

(iv) *Digestion of spermatozoa in the bursa copulatrix* (plate 73*E*)

Electron micrographs of bursal contents were prepared to ascertain the fate of spermatozoa (presumably allosperms) which go astray during and after copulation, and either remain in, or are brought to, the bursa copulatrix. My sections show clearly that such spermatozoa are in an advanced state of breakdown, with the axial fibres lacking. The micrographs also show stages in the breakdown of stray oocytes. As Rigby (1965) puts it, 'all evidence points to the gradual breakdown and resorption of sperm in the bursa' in pulmonates and opisthobranchs.

(v) *Storage of spermatozoa in the receptaculum seminis* (plates 72*A*, *C*; 74*B* to *D*)

Paraffin sections show (plate 72*C*) that the allosperm heads are intimately associated with the endothelial lining of the receptaculum. This led Lloyd (1952) and McGowan & Pratt (1954) to claim for, respectively, *Archidoris pseudoargus* and *A. montereyensis*, that the heads were actually embedded in these cells. Ultrotome sections (plate 74*B*, *C*) through the receptaculum of a mated adult *A. pseudoargus* show that this is erroneous. The allosperm heads in fact lie in close contact with villi of the endothelial cells, but no penetration occurs.

While it is certain that intracellular storage of *autosperms* occurs in some gastropods, for instance the tectibranch *Retusa obtusa* (Smith 1965), my observations cast some doubt on records of intracellular storage of *allosperms*.

## 4. Development up to hatching (28 days at 10 °C)

The major landmarks in development will now be outlined. It is not proposed to deal here with cleavage and early organogenesis in any detail, because these have been adequately described for other nudibranchs (Casteel 1904; Pelseneer 1911; Thompson 1958*a*, 1962), and *Archidoris* differs only in minor particulars.

Table 1 lists a number of readily identifiable stages in development, in a constant temperature bath maintained at 10 °C. These stages are numbered in an arbitrary way and the time-intervals between them are not constant. The duration of the embryonic period varies with temperature. In cultures which fluctuated between 7·7 and 10·4 °C the embryonic period was 36 days, whereas in others, which fluctuated between 9·7 and 12·3 °C, it was 24 days. Field temperatures are recorded by the Port Erin Laboratory staff, at a station in

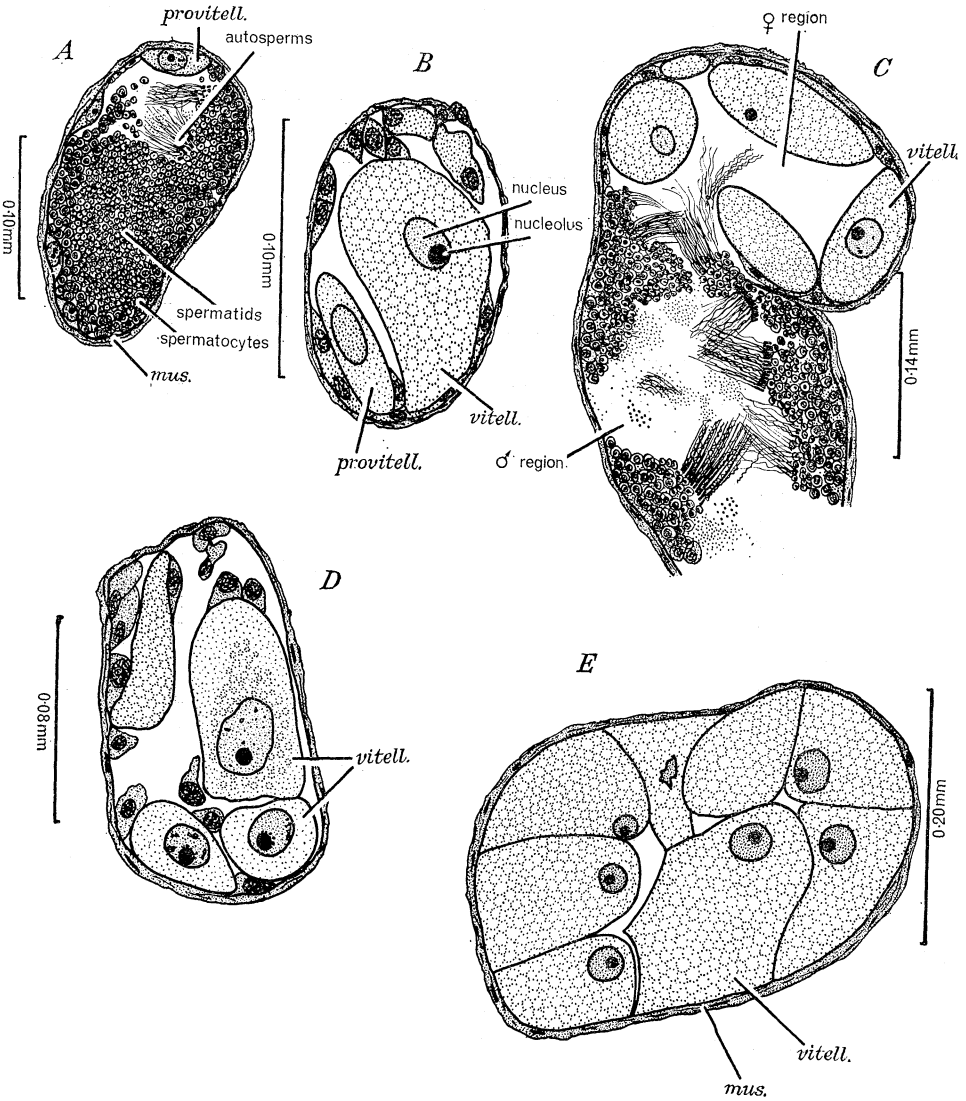


FIGURE 5. Histology of the ovotestis of *Archidoris* from Traie Meanagh, Isle of Man. *A*, Specimen 38 mm in length, collected 13 September 1958, fixed Perényi 19 September, stained azan. Oocytes at an early stage in provitellogenesis; spermatozoa in production with abundant spermatocytes and spermatids. *B*, Specimen 55 mm in length, collected 13 September 1958, fixed Bouin 16 September, stained haemalum. Many oocytes beginning vitellogenesis; the plane of this section does not pass through any male gametes. *C*, Specimen 60 mm in length, collected 11 October 1958, fixed Zenker 14 October, stained azan. The section passes through both male and female regions of an ovotesticular follicle, and shows gametes of both sexes in all stages of ripening. *D*, Specimen 75 mm in length, collected 23 March 1959, fixed Zenker-without-acetic 26 March, stained Heidenhain's alum haematoxylin. The section passes through a female acinus and shows the ripening of a fresh crop of oocytes in this individual (which had spawned at an earlier date). *E*, Specimen 70 mm in length, collected 23 December 1958, fixed Zenker-without-acetic 24 December, stained azan. The section shows the female region of a mature acinus, with yolk-laden full-sized oocytes. The gaps between the cells are artifacts. *mus.*, Coat of mixed circular and longitudinal muscle fibre bundles; *provitell.*, oocyte in a stage of provitellogenesis; *vitell.*, oocyte in a stage of vitellogenesis.

Port Erin Bay. During the natural breeding period of *Archidoris* in 1958 the weekly means varied from 8·6 to 11·8 °C; in 1959 the figures were 7·4 to 8·5 °C. While these measurements ignore fluctuations in air temperature (of importance for a littoral animal and its eggs), they show that my culture temperatures were of the correct order.

TABLE 1. STAGES IN DEVELOPMENT OF *ARCHIDORIS PSEUDOARGUS* (10 °C)

| stage | time in days |
|---|---|
| 1. Oviposition | 0 |
| 2. 4-cells | 1 |
| 3. Beginning of gastrulation (vegetal concavity) | 6 |
| 4. Greatest size and depth of blastopore | 7 |
| 5. Apparent closure of blastopore | 10 |
| 6. Appearance of shell-gland | 10 |
| 7. Appearance of mouth | 12 |
| 8. Appearance of foot | 12 |
| 9. Appearance of cilia | 13 |
| 10. Appearance of anal cells | 13 |
| 11. Appearance of operculum | 17 |
| 12. Metachronism affects velar locomotor cilia | 17 |
| 13. Velar locomotor cilia can be halted | 18 |
| 14. Addition of alcohol brings about violent breakage of connexion between the mantle and the shell-mouth | 19 |
| 15. Hatching | 28 |

Six days after oviposition the flattened, heavily yolked stereoblastulae (figure 6*A*, *B*) begin to gastrulate by epiboly, the blastopore being at first wide and shallow (figure 6*C*, *D*), later becoming more restricted. With the appearance of the blastopore the antero-posterior axis of the organism is revealed (arrow on figure 6). The blastopore closes briefly on the tenth day (figure 6*G*), but the stomodaeum soon makes its appearance at the same spot (figure 6*I*). The stomodaeum makes contact with the endodermal midgut whose lumina begin to develop on the 10th day and whose various regions separate off from one another during the ensuing week. The veliger shape is slowly assumed, the distinctions between the embryonic head, foot, and visceral mass becoming more marked (figure 6*I* to *L*). The ciliated velum or prototroch anterior to the mouth makes its appearance on the 13th day (figure 6*K*, *L*) and later becomes raised on a bilobed velar disk. The velar cilia are initially short and beat continuously and apparently at random; it is not until the 17th day that metachronism is detectable and a further day elapses before the velar locomotor cilia are sufficiently under nervous control for them to be stopped and started at intervals. In *Tritonia hombergi* (Thompson 1962) this ability to control the velar cilia developed soon after the appearance of cerebral ganglia. I could not with certainty confirm this in *Archidoris*.

The shell-gland invagination is at first detectable postero-dorsally on the 10th day (figure 6*G*, *H*), following which its cells spread over the visceral mass (figure 6*J* to *L*), laying down the shell, which is at first cap-shaped (figure 6*L*), later cup-shaped. The leading edge of the spreading shell-gland forms the mantle fold (figure 7*G*). The anal cells appear on the 13th day, in a ventro-lateral position on the right side (figure 6*L*). These cannot be observed to migrate or in any other way alter their position so that, as in *Adalaria* (Thompson 1958*a*), torsion in the veliger of *Archidoris* is not detectable as a mechanical process; all the relevant organs appear in the post-torsional positions.

During the remainder of the embryonic period, most of the yolk, which initially loaded all the blastomeres, is utilized and the positions of the operculum (figure 7*A*), stomach (figure 7*C*), left and right midgut diverticula (figure 7*C*, *E*), larval kidney (immediately beneath the anal cells, figure 7*E*), larval retractor muscle (figure 7*G*), and otocysts (figure 8*C*), become clear. The mode of development of these structures is similar to that of *Adalaria*

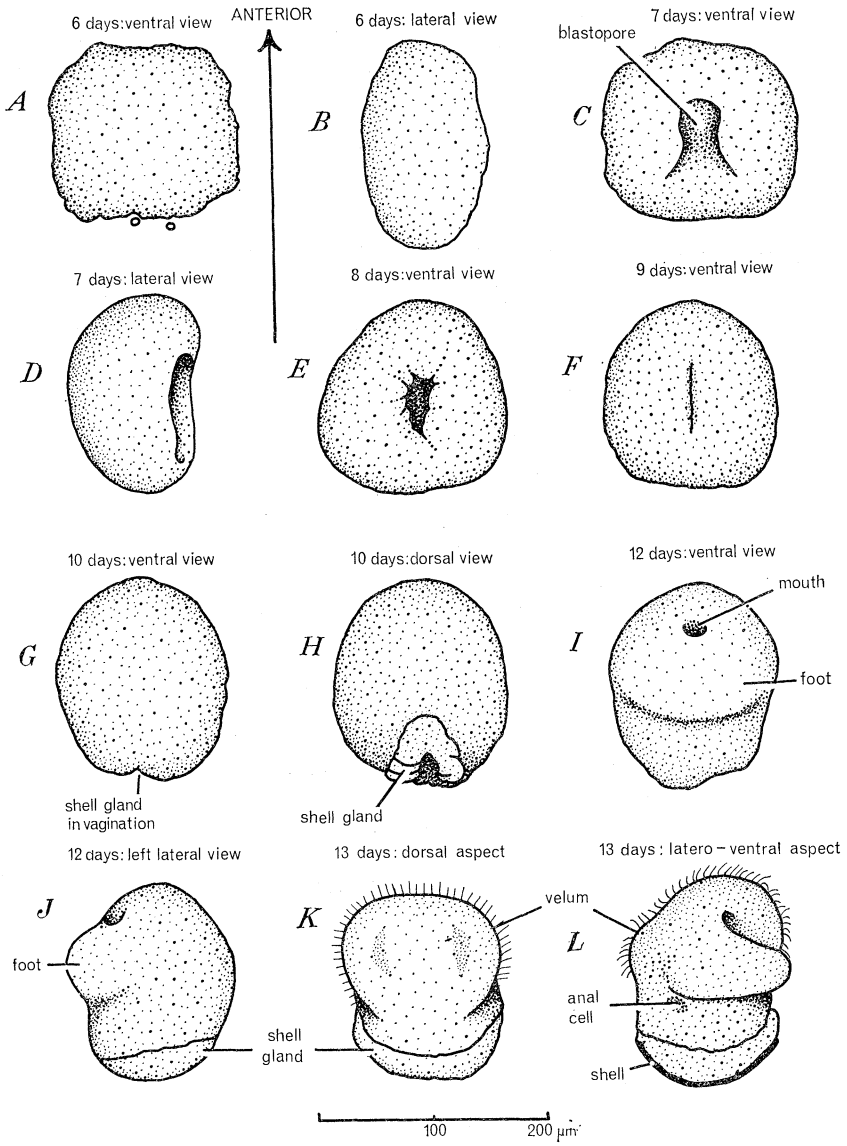


FIGURE 6. Embryonic development of *Archidoris*. All are camera lucida drawings from life. Embryonic membranes are not shown.

(Thompson 1958*a*). One important difference is, however, that a pair of nephrocysts (figure 8*A*, *neph.*, and Thompson 1959, fig. 3), believed to be ectodermal embryonic and larval excretory organs, develop dorso-laterally behind the velum. Nephrocysts were not found in either *Adalaria* or *Tritonia*.

Muscle fibres differentiate in the velum (figure 8*A*) and in the mantle, and may be observed to twitch from time to time. The larval retractor muscle is at first unable to effect

retraction of the whole embryo into the shell because the mantle fold/shell mouth connexion is too intimate, but by the 19th day it may be observed that the addition of a poison such as alcohol is a sufficiently violent stimulus to cause its breakage. This is a readily recognizable landmark in the development of any species of opisthobranch.

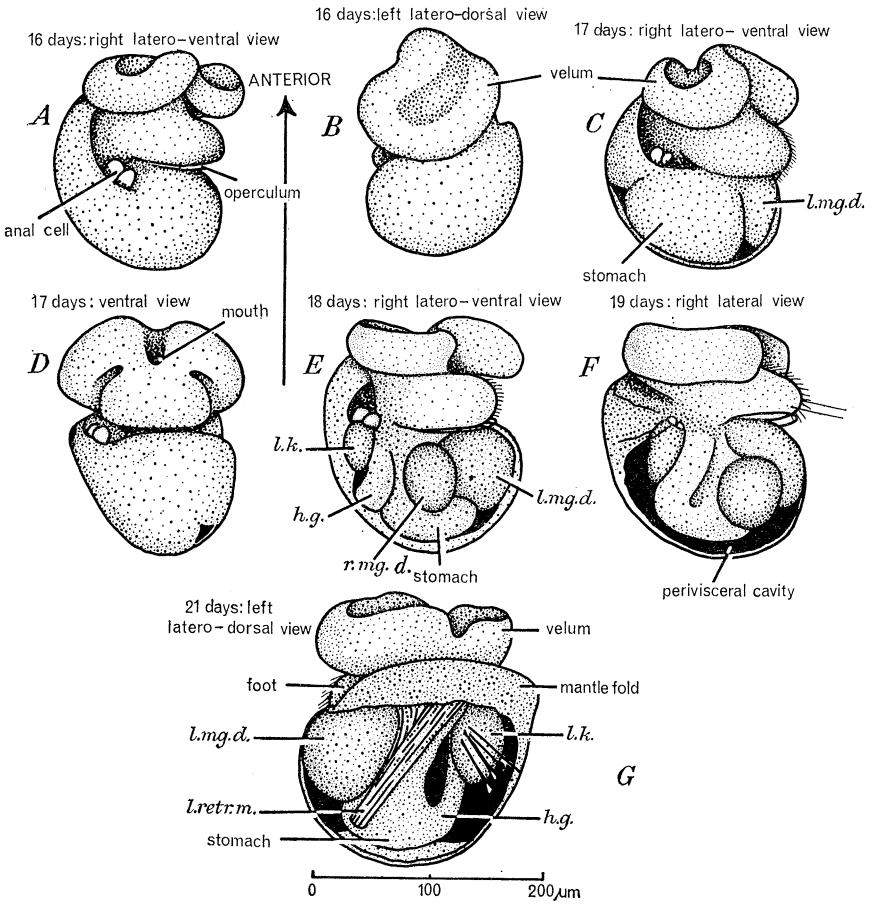


FIGURE 7. Embryonic development of *Archidoris*. All are camera lucida drawings from life. Embryonic membranes are not shown, nor are the velar locomotor cilia. *h.g.*, Hindgut; *l.k.*, larval kidney; *l.mg.d.*, left midgut diverticulum; *l.retr.m.*, larval retractor muscle; *r.mg.d.*, right midgut diverticulum.

Hatching occurs on the 28th day (figure 8*A*). Considerable food-reserves remain (in the form of cytoplasmic yolk granules), notably in the right midgut diverticulum and the stomach, with a little in the left midgut diverticulum. This left diverticulum is larger than the right (figure 8*A*, *l.mg.d.*, *r.mg.d.*), and differs from it also in consisting of a large number of small, relatively yolk-free cells, surrounding a capacious lumen. A full description of the anatomy and histology of a dorid nudibranch veliger has been published for *Adalaria proxima* (Thompson 1958*a*), and it is necessary now to do no more than list the points of difference exhibited by *Archidoris*.

(i) No propodium-rudiment is present at hatching in *Archidoris*. The foot of *A. pseudoargus* at this stage corresponds only to the metapodium-rudiment of the free veliger of *Adalaria*. Metapodial glands are present and the propodial and accessory mucus-glands of the hatching *Adalaria* are as yet undetectable in *Archidoris*.

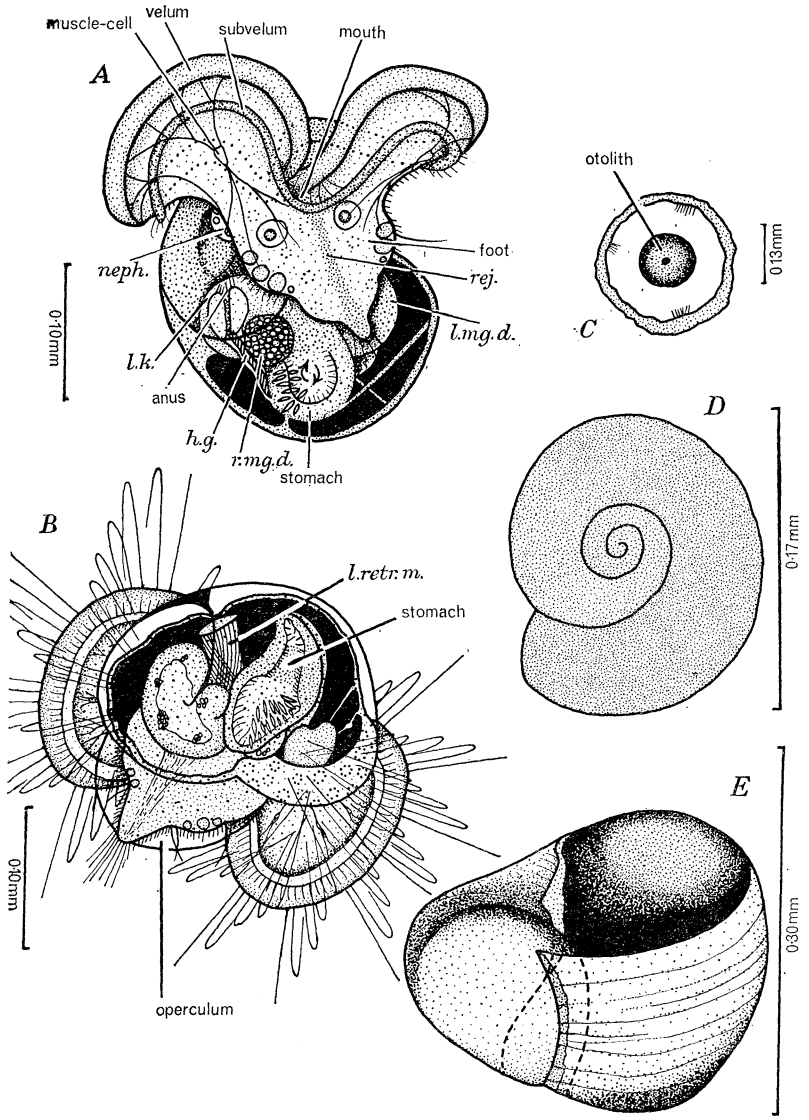


FIGURE 8. Larval *Archidoris*. Camera lucida drawings. *A*, Newly hatched veliger, right latero-ventral aspect (velar locomotor cilia omitted), modified after Thompson (1959, fig. 2); *B*, eyed larva, 2 to 3 days after hatching, posterior aspect; *C*, squash-preparation of a larval otolith in optical section; *D*, detached operculum viewed from the inside; *E*, shell, left lateral aspect, of a veliger which died more than 3 days after hatching, showing lines of growth upon the shell. *h.g.*, Hindgut; *l.k.*, larval kidney; *l.mg.d.*, left midgut diverticulum; *l.retr.m.*, larval retractor muscle; *neph.*, nephrocyst; *rej.*, pedal ciliary rejection tract; *r.mg.d.*, right midgut diverticulum.

(ii) Secretion of the shell, complete at hatching in *Adalaria*, continues during the early part of the planktonic phase in *Archidoris*.

The outer edge of the flared mantle fold is connected intimately with the shell mouth and is withdrawn only during defensive retraction into the shell. No histological differentiation of the mantle fold into the layered arrangement of *Adalaria* is evident in sections of hatching *Archidoris*.

(iii) The radular sac is detectable only as an early rudiment in hatching *Archidoris*, whereas a muscular radula with up to six pairs of teeth was found in hatching *Adalaria*. The

enlargement of the left midgut diverticulum known to precede the stomach rotation described in late larval *Adalaria* is not yet evident in hatching *Archidoris*.

(iv) The nervous system of *Archidoris* consists of only the otocysts, cerebral ganglia and first rudiments of the pedal and optic ganglia; buccal and pleural ganglia are absent and there is no pigment in the eyes. Histological differentiation of the ganglionic masses is at an early stage. The greatly advanced nervous system of hatching *Adalaria* has been illustrated (Thompson 1958*a*, p. 30).

(v) The adult kidney rudiment, detectable in sections of hatching *Adalaria*, is absent in *Archidoris* at this stage. The anal cells, which in *Adalaria* disappear some days before hatching, are in *Archidoris* larvae still detectable in sections and in life. Nephrocysts are, as already mentioned, present in hatching *Archidoris*.

In summary, these features of the foot, mantle, viscera, nervous system, and renal systems, are all evidences of the relative precocity of the hatching larvae of *Archidoris pseudoargus*. They must be balanced against the fact that the larvae of *Archidoris* and of *Adalaria proxima* are about the same size (290 to 310 $\mu$m in the former, 280 to 300 $\mu$m in the latter, the criterion employed being the maximal dimension of the shell in lateral aspect).

## 5. Larval life

After hatching the larvae swim upwards and may become trapped in the surface film of the water. While swimming, the velar lobes are held uppermost, the shell down. Normally periods of swimming, at a speed of up to $7\frac{1}{2}$ cm/min, alternate with short pauses, during which passive falling occurs at from 6 to 10 cm/min. During these pauses the velar locomotor cilia are halted, but the cephalopedal mass is not retracted into the shell, so that the immobile velar lobes and cilia act as a parachute, braking descent. These pauses in velar locomotor activity are not dependent upon the anatomical integrity of the larva; a single velar lobe when excised exhibits such bursts of ciliary activity and full metachronal control of the locomotor cilia. While the larvae of many dorid nudibranchs have been observed to spiral as they swim, this is not the case in *Archidoris*. The negative geotaxis of newly hatched *Archidoris* veligers is so strong that the faint negative phototaxis which they possess is difficult to demonstrate, except in a horizontally held vessel, shaded unilaterally. This negative phototaxis is detectable in advance of the development of black pigment in the eyes.

Retraction of the cephalopedal mass into the shell cavity is brought about, if the larva is violently disturbed, by the coordinated contraction of the intrinsic musculature of the velum (figure 8*A*) and of the larval retractor muscle (figure 8*B*, *l.retr.m.*). The mantle fold/shell mouth connexion is broken temporarily to allow retraction but is speedily re-established on the resumption of normal swimming activity. It is thus clear that the cephalopedal mass is retracted into the shell cavity and not into the mantle cavity.

Some experiments were carried out to see if *Archidoris* veligers were able to resist a number of marine carnivores. In cases where veligers were placed upon the tentacles of *Actinia equina*, *Tubularia larynx*, *Alcyonium digitatum*, *Alcyonidium polyoum*, and *Polydora* sp., the larvae in many cases did not even pause to retract, but merely swam away from the potential enemy. In other cases, a brief phase of retraction occurred, but escape was invariably made. Dr D. I. Williamson attempted to feed some of these *Archidoris* larvae to larvae of *Crangon vulgaris* and *Pandalus montagui*; the former ignored the veligers, but a *Pandalus* was

seen to seize a veliger and attack it for some moments before dropping it. After a minute the veliger opened up and swam away apparently unharmed. Probably the chief danger to these operculate veligers comes from barnacles (Hadfield 1963), and from suspension-feeding organisms of much larger size than those tested.

The feeding behaviour of *Archidoris* larvae has already been described (Thompson 1959). Although the larvae ingested freely in the laboratory such food as *Phaeodactylum tricornutum*, *Isochrysis galbana*, and *Chlamydomonas* sp., and although food vacuoles were subsequently detected in the digestive gland, larvae could not be kept alive for more than a week after hatching (contrary to the experience of Allen & Nelson 1911). During this period, upward-swimming behaviour continued; optic pigment appeared in the intravelar invaginations which form the eyes and the optic ganglia; and the mantle fold withdrew irreversibly from the mouth of the shell. Only a very little shell growth occurred after hatching (figure 8*E*). Culture vessels of various patterns and dimensions were used, stagnant or with filtered inflow and outflow, with or without artificial aeration; all without success. Experiments were attempted of the kind which gave successful metamorphosis of the veligers of *Adalaria proxima* (Thompson 1958*a*), *Tritonia hombergi* (Thompson 1962), and *Eubranchus* sp. (Tardy 1962), where a small sample of the adult nudibranch's natural diet proved a trigger for post-veliger development. Colonies of *Halichondria panicea*, in isolation, on glass slides, or on natural substrata, were suspended at various depths in various types of culture vessel; all expedients failed to promote larval metamorphosis.

Rearing *Archidoris* veligers through metamorphosis will be possible in the laboratory only when the normal larval diet can be provided. Unfortunately, no dorid nudibranch larva has as yet been identified in a plankton sample, and nothing is known of the natural diet. Personal fortnightly search through fine-net plankton from the Menai Strait throughout the 1955 breeding seasons of the local nudibranchs failed to reveal a single dorid veliger.

## 6. Life cycle in the Isle of Man

### A. *Collecting records*

In the Isle of Man *Archidoris* is found on the lower shore, usually in sheltered rock-clefts, overhung by *Fucus serratus* or *Laminaria*. Little is known about the habits of the individuals occasionally taken sublittorally. In my experience *Archidoris* in the field is always found on or near (often actually feeding upon) *Halichondria panicea* or *Hymeniacidon perleve*, especially the former. The feeding mechanism involves the distension under fluid pressure of the oral veil, the extrusion of the lips to form a cup-shaped protuberance applied to the surface of the sponge, while the radular membrane is protruded through the mouth on its muscular odontophore. The movement of the extruded radula is forwards and upwards, during which the teeth present their most effective surfaces, but the movement includes also the bringing together of the two halves of the radula as the whole cycle concludes with the withdrawal of the radular membrane into the buccal cavity together with such food as has been removed from the substratum.

Field observations on *Archidoris* collected in various localities around the south of the Isle of Man are shown in figure 9*B*. The picture is somewhat confused and differing interpretations of these data are possible. It is my conviction that this confusion arises because of the

asynchrony of breeding and growth of *Archidoris* populations in different parts of the coast. To obtain meaningful results it was clearly necessary to concentrate attention upon the dorid population of a single more circumscribed locality. Traie Meanagh in Port Erin Bay, an area of rocky shore, rich in *Halichondria panicea*, was selected for closer study. This is near the area named Baths on chart D of the Manx Fauna (Bruce, Colman & Jones 1963). The

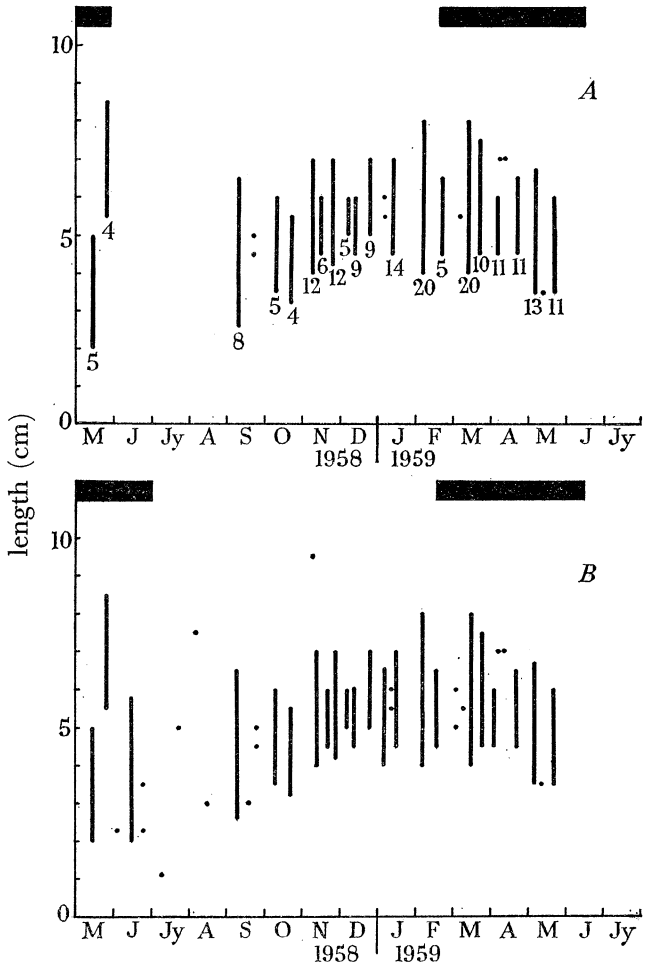


FIGURE 9. Life history of *Archidoris* in the Isle of Man; graphic representation of collecting records. Black rectangles denote periods when spawn was found in the field. *A*, Records from Traie Meanagh, Port Erin Bay. The size-range encountered on each occasion, and the number of individuals comprised in each sample are shown. Circles denote single specimens. *B*, Pooled records from a variety of localities around the south of the Isle of Man (conventions as for *A*, above). It can be seen that pooling of collecting data renders more difficult the interpretation of the field observations.

shore was visited fortnightly and *Archidoris* brought back to the laboratory for measuring and for the dissection and investigation of gonad histology of subsamples. Undamaged specimens were returned to the same shore on the following day. In this way undue depletion of the population was avoided. The striking colour-patterns of the mantle of these dorids enabled in many cases the recognition of individual specimens recurring in the samples for long periods. It was also possible to ascertain that these animals moved about

very little when food was abundant. Specimens which had wandered more than 1 m between fortnightly samples were unusual. Aggregative movements were observed to have occurred during the spring breeding period. Field notes were made of breeding condition and behaviour. Spawn of *A. pseudoargus* can be distinguished from that of other large nudibranchs by the size of the ova which in *Archidoris* measure 0·140 to 0·170 mm when uncleaved, in comparison with those of, say, *Jorunna tomentosa*, which measure 0·073 to 0·090 mm at the same stage.

The data collected at Traie Meanagh are presented in figure 9 *A. A. pseudoargus* in this locality clearly has an annual life-cycle, growth occurring during the autumn and winter months, while a lengthy spawning season occupies the spring, followed by death. Juveniles begin to appear on the shore again in the late summer and the cycle is repeated. In order to seek confirmation of this interpretation of the field data, investigations were made into the anatomy and histology of the reproductive and other organs of subsamples.

### B. *Dissections and histology*

Table 2 summarizes observations on subsamples killed at various times between May 1958 and May 1959. A distinction is made in this table between mature and immature eggs found in sections through the gonad; a word is required in explanation of the criterion employed. Fully mature oocytes visible in sections are rarely circular in shape, and it is

TABLE 2. OBSERVATIONS ON OVOTESTIS HISTOLOGY IN *ARCHIDORIS PSEUDOARGUS* FROM TRAIE MEANAGH, ISLE OF MAN, 1958–59

(A, Abundant; F, few.)

| date of capture | date of dissection | size (length in mm) | immature oocytes | mature eggs in gonad | spermatocytes and spermatids in gonad | tailed sperms in gonad | remarks |
|---|---|---|---|---|---|---|---|
| 2. v. 58 | 13. v. 58 | 50 | F | A | F | A | died in laboratory 13. v. 58 |
| 2. v. 58 | 15. v. 58 | 50 | F | A | F | F | died in laboratory 15. vi. 58 |
| 13. ix. 58 | 16. ix. 58 | 55 | A | none | A | A | — |
| 13. ix. 58 | 29. ix. 58 | 38 | A | none | A | F | — |
| 11. x. 58 | 14. x. 58 | 60 | A | A | A | A | — |
| 13. ix. 58 | 24. x. 58 | 40 | A | A | A | A | seen to copulate in laboratory 10. x. 58 |
| 25. x. 58 | 27. x. 58 | 55 | A | A | A | A | — |
| 11. xi. 58 | 17. xi. 58 | 60 | A | A | A | A | seen to copulate in laboratory |
| 24. xi. 58 | 25. xi. 58 | 70 | A | A | A | A | — |
| 23. xii. 58 | 24. xii. 58 | 70 | F | A | A | A | — |
| 6. iii. 59 | 6. iii. 59 | 55 | A | A | A | A | seen to spawn in the field 6. iii. 59 |
| 23. iii. 59 | 26. iii. 59 | 75 | F | A | F | A | — |
| 21. iv. 59 | 23. iv. 59 | 45 | F | none | F | A | — |
| 5. v. 59 | 15. v. 59 | 40 | F | none | F | A | — |
| 6. v. 59 | 6. v. 59 | 35 | A | F | F | A | dead on capture |
| 20. v. 59 | 21. v. 59 | 40 | F | A | F | F | moribund in field, died in laboratory 21. v. 59 |
| 20. v. 59 | 21. v. 59 | 35 | F | F | F | F | moribund in field, died in laboratory 21. v. 59 |

difficult to standardize the measuring procedure. A maximal dimension of 120 $\mu$m was somewhat arbitrarily chosen as a lower limit above which oocytes of this species may be judged in sectioned gonads to be mature.

The smallest juveniles found on the shore at Traie Meanagh on 13 September 1958, and killed for examination immediately, proved to have gonads showing no stages in either oogenesis or spermatogenesis. One specimen (38 mm in length) was retained in the laboratory with abundant food and killed for examination on 29 September, when the gonad was found to be more advanced, exhibiting countless spermatocytes and spermatids (but relatively few tailed spermatozoa), while the female primordia were small but distinct (figure 5*A*; plate 72*B*). A 55 mm specimen from the same sample presented a still more advanced picture, with numerous sperms in the male acini and oocytes up to 0·099 mm in diameter; some of the oocytes were commencing to condense reserves into cytoplasmic yolk granules, but the great majority were still yolk-free (figure 5*B*). At this stage, the organs of the anterior genital mass, although small, had a superficial appearance of maturity, and the penis was capable of extrusion.

Growth of the oocytes was surprisingly rapid. A specimen collected 11 October 1958 and killed for examination on 14 October contained large numbers of apparently fully mature yolk-filled oocytes up to diameters of 0·160 mm, while the male acini showed that active spermatogenesis was continuing (figure 5*C*). Autosperms were accumulating in the wide hermaphrodite duct, which had now taken on the characteristic greyish distended appearance. Sections through the male acini of the ovotestis show the spermatozoa there to lie free, but they were sometimes orientated radially with their heads facing the wall of an acinus.

In laboratory conditions some individuals as small as 40 mm (collected at Traie Meanagh on 13 September 1958 and retained for 6 weeks before dissection) were observed to copulate in aquaria on 10 October. This was not observed in the field during that period and it is believed that what occurred in the laboratory was an abnormal happening. Laboratory copulation was observed also in late November specimens.

Samples collected after the beginning of October 1958, until the natural breeding season in the spring of 1959, invariably possessed ovotestes which in sections gave the appearance of full maturity (figure 5*E*; plate 75*A*). This could be correlated with the naked eye observations during dissections. A yellow or cream coloured gonad always proved to contain mature, yolky eggs on histological examination.

During December 1958 and in the early months of 1959, the number of ripe eggs in the gonad increased, correlated with a marked diminution in the number of immature, small oocytes in sections through the ovotestis (figure 5*E*). Similarly, the proportion of tailed sperms increased in the male acini through this period, so that it became more difficult to detect spermatocyte and spermatid stages in the gonad.

In the field, copulation was seen sporadically in December 1958 and in January 1959, and many pairs were found *in copula* on 5 February 1959, but it was not until mid-February that spawn was produced in the field. Copulation is nearly always reciprocal, but occasional pairs may be discovered in which one partner is acting as a male while the other is inert. Mating usually continues for several hours on any occasion, but may be greatly prolonged as Fretter & Graham (1964) mention. Individuals are promiscuous. Specimens of the well known variety *flammea* were observed to mate indiscriminately with other colour-

varieties. Spawn ribbons were found on each fortnightly visit until 18 June 1959; the last of the adults had died and disappeared from the sampling area four weeks before this date.

During the breeding season in 1959, the sampled gonads were rather variable. This is to be expected since the state of the gonad must depend to a great extent on the spawning history of the individual slug. Some ovotestes on examination proved to contain great numbers of ripe yolky eggs; others few or none. Some such gonads exhibited stages in the growth of a new wave of oocytes. Growing oocytes are surrounded by small nurse-cells. Condensation of reserves into granular form occurs first in the neighbourhood of the oocyte nucleus (figure 5*D*) and only later is evident peripherally. The granular reserves of the cytoplasm of mature eggs all appear brilliant red after treatment with azan, whereas after Heidenhain's alum haematoxylin a considerable number of the granules remain unstained. The nucleolus of the immature oocyte disappears as the egg attains full maturity.

Sometimes the production of a spawn mass exhausted the gonad (so that sections of the ovotestis showed few remaining yolk-laden eggs), but in other individuals the production of a spawn mass 10 to 12 cm in length left the gonad still crowded with ripe eggs. Copulation, spawning, and gonad regeneration continued throughout the breeding season. The adults are rarely seen to feed at this time of year. This was not correlated with a scarcity of their natural food. This fact was established by observations on *Halichondria* at Triae Meanagh, and by maintaining adult *Archidoris* in the laboratory in the presence of healthy sponge-incrustations. Unfortunately it was not possible to sacrifice sufficient specimens to investigate the question of decreased radular wear such as was found to occur during the breeding period of *Adalaria proxima* (Thompson 1958*c*). It is, however, certain that the bulk of the digestive gland decreases as the breeding season progresses. Weakened, moribund specimens were first found in the field on 5 May 1959, and the last one observed on 20 May. These were characterized externally by their immobility, weak powers of adhesion to the rocks, and extrusion of penis and buccal mass. One specimen (35 mm in length, collected 6 May 1959) was certainly dead when found; microscopic examination of receptaculum allosperms and of stomach cilia proved this. Others were moribund when discovered in the field, but did not die for some hours. The gonads of these senescent adults (plate 75, *B* to *D*) were rather variable. Sometimes they contained only a few eggs, sometimes a great number; these eggs were often immature, but sometimes they had the appearance of full maturity. Post-mortem examination revealed no marked atrophy of the nervous, circulatory, or muscular systems; the organs of the anterior genital mass, the buccal mass, the stomach, the hindgut, and the 'blood-gland' also had the appearance of normality. This was true at both the histological and morphological levels of investigation. The kidney, on the other hand, usually showed a degree of degeneration in senescent specimens. But the greatest abnormality was the invariable shrinkage and histological breakdown of the digestive gland. Sections showed (plate 75*B* to *D*) that the digestive cells were small, rounded, and detached from the basement membrane of the digestive lobules.

### C. *Conclusions*

There is no doubt that at Traie Meanagh *Archidoris* has an annual life cycle, with a maximal life span of approximately one year. Young individuals with undifferentiated gonads come into the samples in early autumn. They grow rapidly and the ripening of the

ovotestis begins. Within a month some of the oocytes have ripened to apparent maturity and the production of tailed spermatozoa is under way. During the winter months more and more oocytes are brought to maturity, and these are stored in the female acini of the gonad. Ripe autosperms pass to the vesicula seminalis. During November and December specimens may be induced to copulate in the laboratory, but this did not happen naturally at Traie Meanagh. Adults reach their maximal size in the spring when spawning begins. Each individual is functionally hermaphrodite, and copulation and oviposition take place time and again, each mating providing ample allosperms to fertilize several egg masses. Feeding declines, and the adults begin to subsist on the reserves of the digestive gland. New waves of ripening oocytes replenish the gonad as long as food reserves remain, but finally death occurs when the digestive gland has shrunken past a level beyond which the digestive cells round off and drift away from the basement membrane of each digestive lobule. Other organ systems still appear normal. This contrasts with the situation in the onchidorid *Adalaria* (Thompson 1958*a*) in which senescent post-reproductive adults retain some spark of life even after extensive decay has come to affect much of the body.

*A. pseudoargus* survives at least part of the summer in the form of planktonic veliger larvae. These are liberated from the egg mass in 36 days at 7·7 to 10·4 °C, or 28 days at 10 °C in the laboratory. These temperatures were close to those recorded in the field at the same time of the year. The duration of the obligatory phase of planktonic life is unknown, but it has been established (Allen & Nelson 1911) that the larvae may survive for up to three months under favourable culture-conditions. The natural stimulus to settlement is not known, nor has progressive development (for instance, mantle fold reflexion, or propodium proliferation) been successfully induced by any worker on these larvae. Their natural diet and growth rate are unknown. This is the greatest gap in our knowledge about *Archidoris*; all that is known is that immature juveniles make their appearance on the shore in late summer and the cycle is repeated.

## 7. Life cycle in other localities

Breeding activities of *Archidoris pseudoargus* have been recorded in different months of different years in different localities. The time of spawning may vary from year to year in the same locality, perhaps related in a fairly simple way to temperature, as was found in *Onchidoris muricata* (Thompson 1961*b*) and in *Adalaria proxima* (Thompson 1958*a*). It certainly varies from locality to locality in any one year: one month after the last of the current Traie Meanagh generation had died in 1958 and in 1959, adult *Archidoris* with spawn were captured in numerous other Manx localities (Fleshwick, Calf Sound, Port Erin breakwater, and others). These observations illustrate the danger of pooling growth and breeding data from a number of localities. Such pooling led Miller (1962) to the probably erroneous conclusion that Manx *Archidoris* have a normal life span of 2 to $2\frac{1}{4}$ years.

All the facts presented in the present paper point to the conclusion that in the south of the Isle of Man, *Archidoris* has an annual life cycle, with a life span of approximately 1 year. It is equally certain that the life cycle is different in important particulars in certain other areas. In the Isle of Bute, Renouf (1915) found evidence of a second, late autumnal, minor spawning period. On the east coast of England spawning of *Archidoris* was observed prolifically during December 1958 in the neighbourhood of Blyth, Northumberland. Furthermore

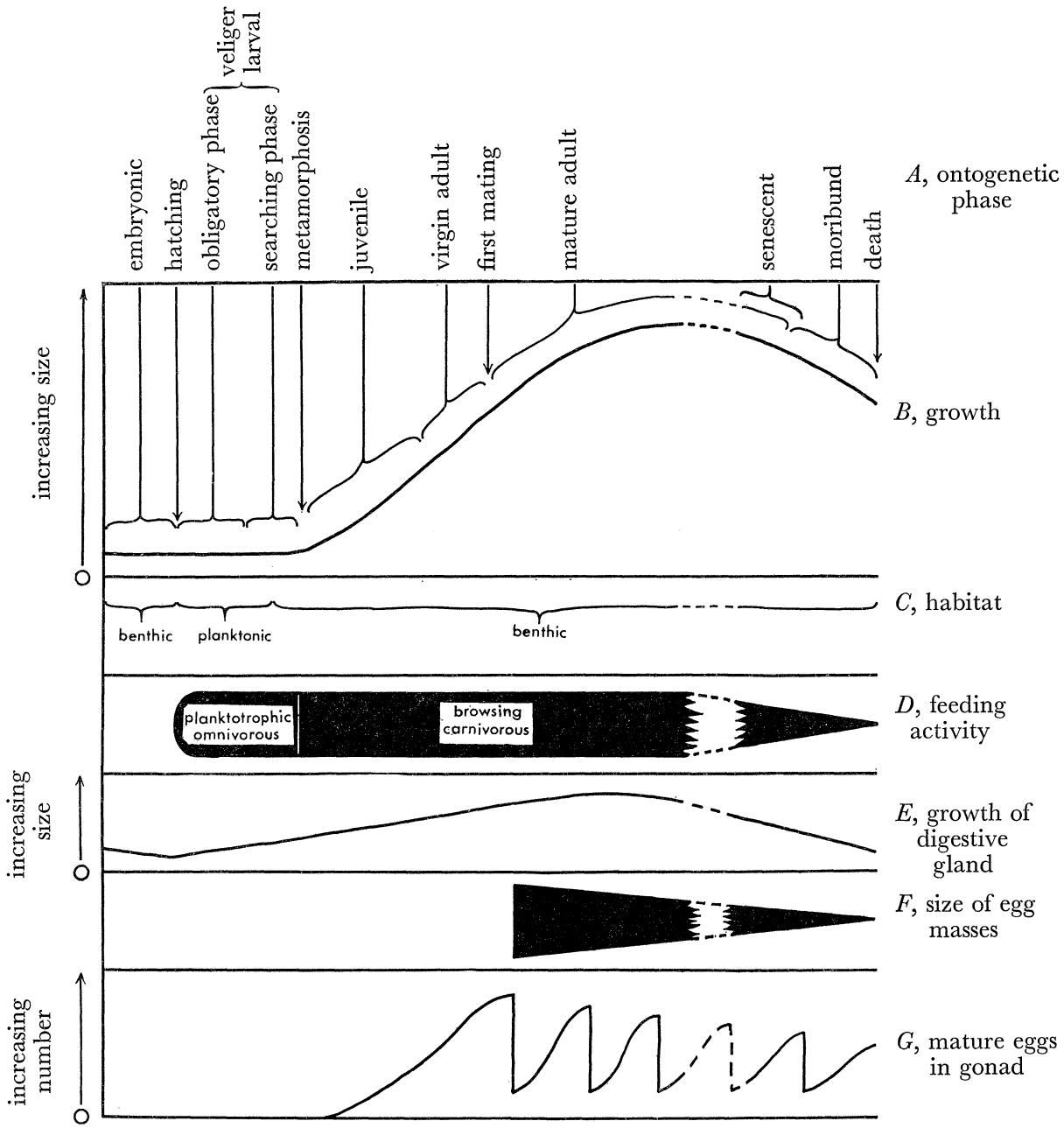


FIGURE 10. Diagram showing features of the life cycles of dorid nudibranchs on British coasts, compiled from observations on *Archidoris*, and on *Adalaria proxima*, *Onchidoris muricata*, and *Goniodoris nodosa*. The full extent of the horizontal axis represents approximately 1 year.

two Northumberland specimens, 70 mm in length, were brought by air to the Port Erin Laboratory on 18 September 1958; these were given ample food, kept in aquaria and copulated in captivity on 28 September, producing egg masses in late November, almost synchronously with undisturbed specimens in the Dove Marine Laboratory at Cullercoats.

Confirmatory evidence that the life history may vary in different localities takes the form of records of the maximal size of adult *A. pseudoargus*. Alder & Hancock (1845–55, North Wales), Dalyell (1853, Havre), Garstang (1889, Plymouth), and Odhner (1939, Norway),

all record specimens up to a length of 12 cm, whereas the maximum attained by the Traie Meanagh specimens was 8·5 cm. There is no evidence, however, that in any locality the maximal life span greatly exceeds 1 year.

## 8. Discussion

Knowledge concerning the life cycles of dorid nudibranchs is fragmentary. Different aspects have been investigated in different species. This has come about because larval metamorphosis, for example, can be studied with ease in *Adalaria proxima*, but cannot as yet be induced in any other species. The histology of senescent adults is, however, more easily investigated in the larger, more conspicuous *Archidoris*; while yet other species are more common or more easy to maintain in the laboratory.

In order to understand the dorid life cycle, it is therefore necessary to pool data derived from the study of a number of species. Figure 10 summarizes much of what is known about the life history of British dorids, and is based in the main on *Archidoris pseudoargus*, with data drawn also from *Adalaria proxima* (Thompson 1958*a*), *Onchidoris muricata* (Thompson 1961*b*), and *Goniodoris nodosa* (unpublished work). Pooling has prevented my showing in figure 10 that the time-relations of the various phases differ in these species. The free veliger phase, for instance, may last for up to 2 weeks in *A. proxima*, but may be prolonged for months in *Archidoris* (Allen & Nelson 1911). Similarly, the embryonic period at 10 °C varies from 14 days in *O. muricata*, to 36 to 42 days in *A. proxima*. Despite its defects, the diagram does present a basis from which further work may be planned. Greatest ignorance at present surrounds the possible influence of diet upon senescent changes in post-reproductive adults, and the neuro-humoral control of the ovotestis and the functioning of the other genital organs. Further work is planned in these fields.

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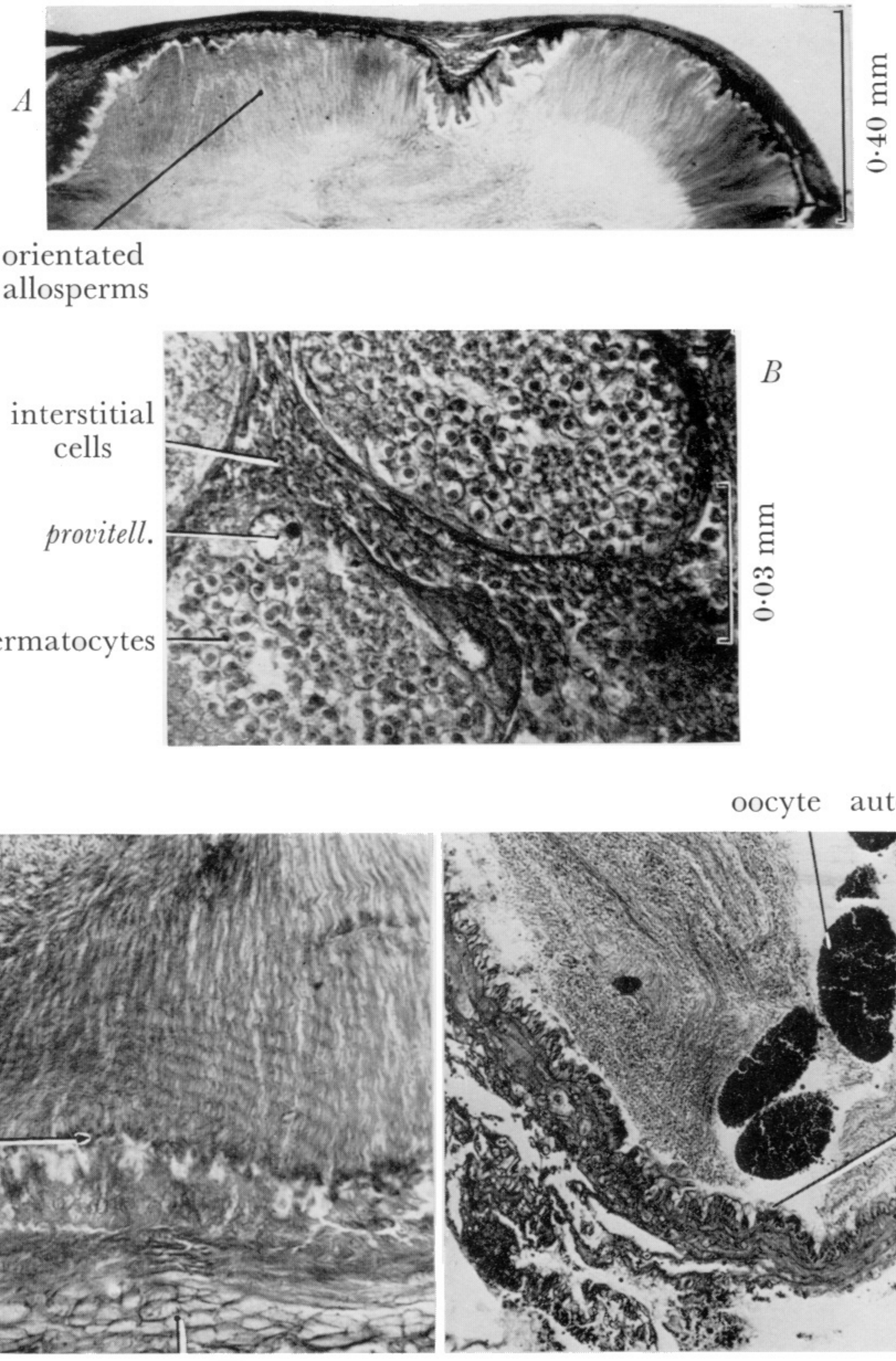
*Post-script* (4 July 1966).

McGowan & Pratt's (1954) assertion that the gonad of *Archidoris montereyensis* consists of an anterior, exclusively male, region, grading into a posterior, chiefly female zone, has now been checked in sections of Bouin-fixed specimens. No such division of sexual functions was found, the ovotestis resembling in all essential features that of *A. pseudoargus*.

Thanks are due to Dr G. F. Gwilliam (Oregon) for sending specimens, and to Mr N. F. Ablett (Bristol) for technical assistance.




Photomicrographs of sections through the reproductive organs of *Archidoris*.

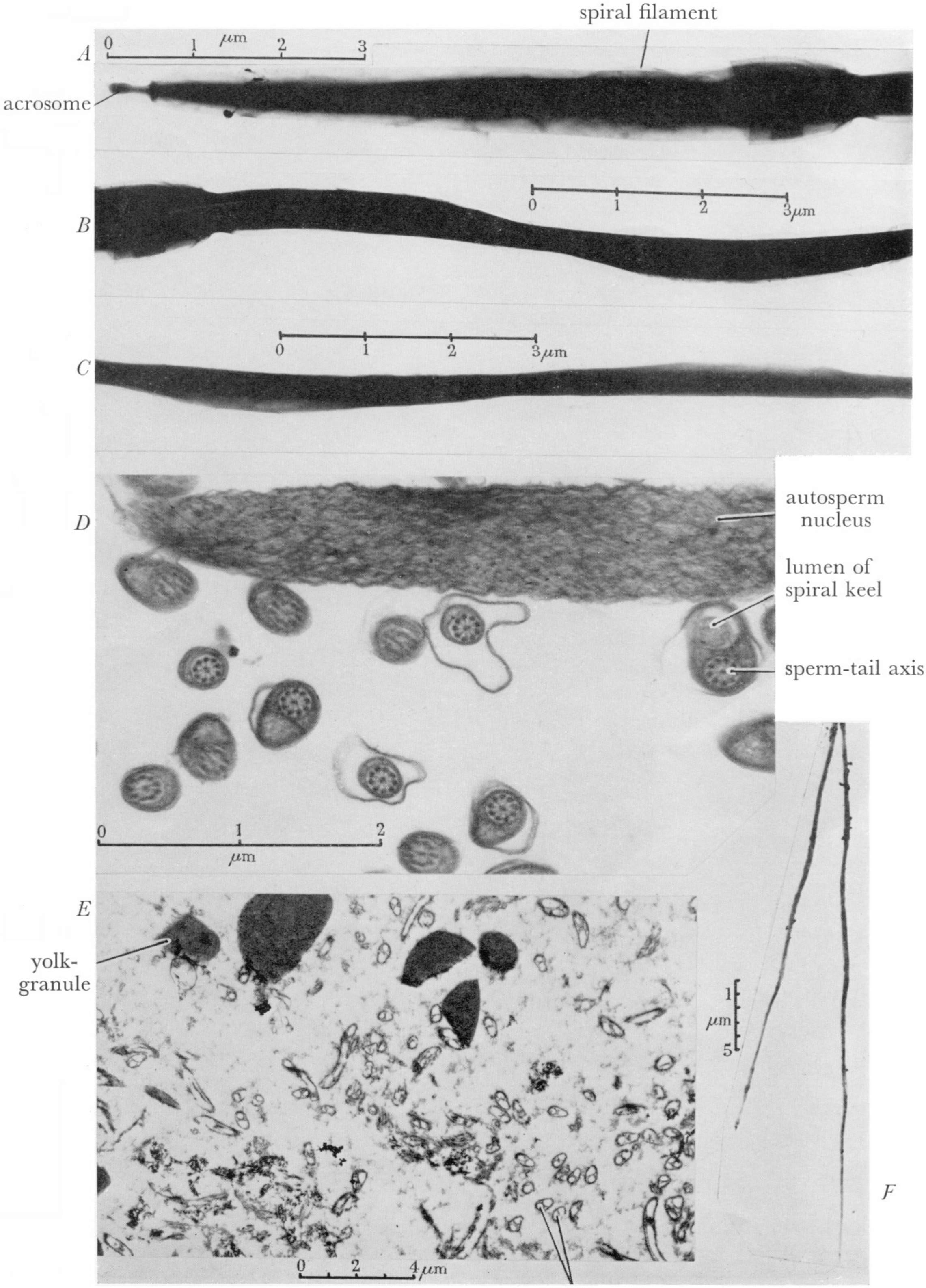
*A*, Portion of a longitudinal section through the receptaculum seminis of a mated adult, showing allosperms facing the endothelium; Menai Strait, April 1962. Lewitsky-saline and azan.

*B*, Section through the gonad of a specimen 38 mm in length, showing early stages in gametogenesis in two of the ovotesticular acini; captured 15 September, killed 29 September 1958, Traie Meanagh, Isle of Man. Perényi and azan.

*C*, Portion of a longitudinal section through the receptaculum seminis of a mated adult, showing the coincidence of phase of the tail helices of adjacent allosperms, and the intimate relationship between the allosperm heads and the receptacular endothelium; Menai Strait, April 1962. Lewitsky-saline and azan.

*D*, Portion of a transverse section through the wide hermaphrodite duct of an adult killed during oviposition, showing outgoing ripe oocytes passing through masses of non-orientated autosperms; Menai Strait, April 1962. Zenker-without-acetic and azan.

*cil.c.*, Ciliated endothelial cells; *mus.*, coat of mixed circular and longitudinal muscle fibre bundles; *provitell.*, oocyte in a stage of provitellogenesis.




Electron micrographs of spermatozoa of *Archidoris*, Cornwall, April 1965.

*A*, Head with acrosome, spiral filament, and posterior dilation; whole autosperm fixed on Formvar film.

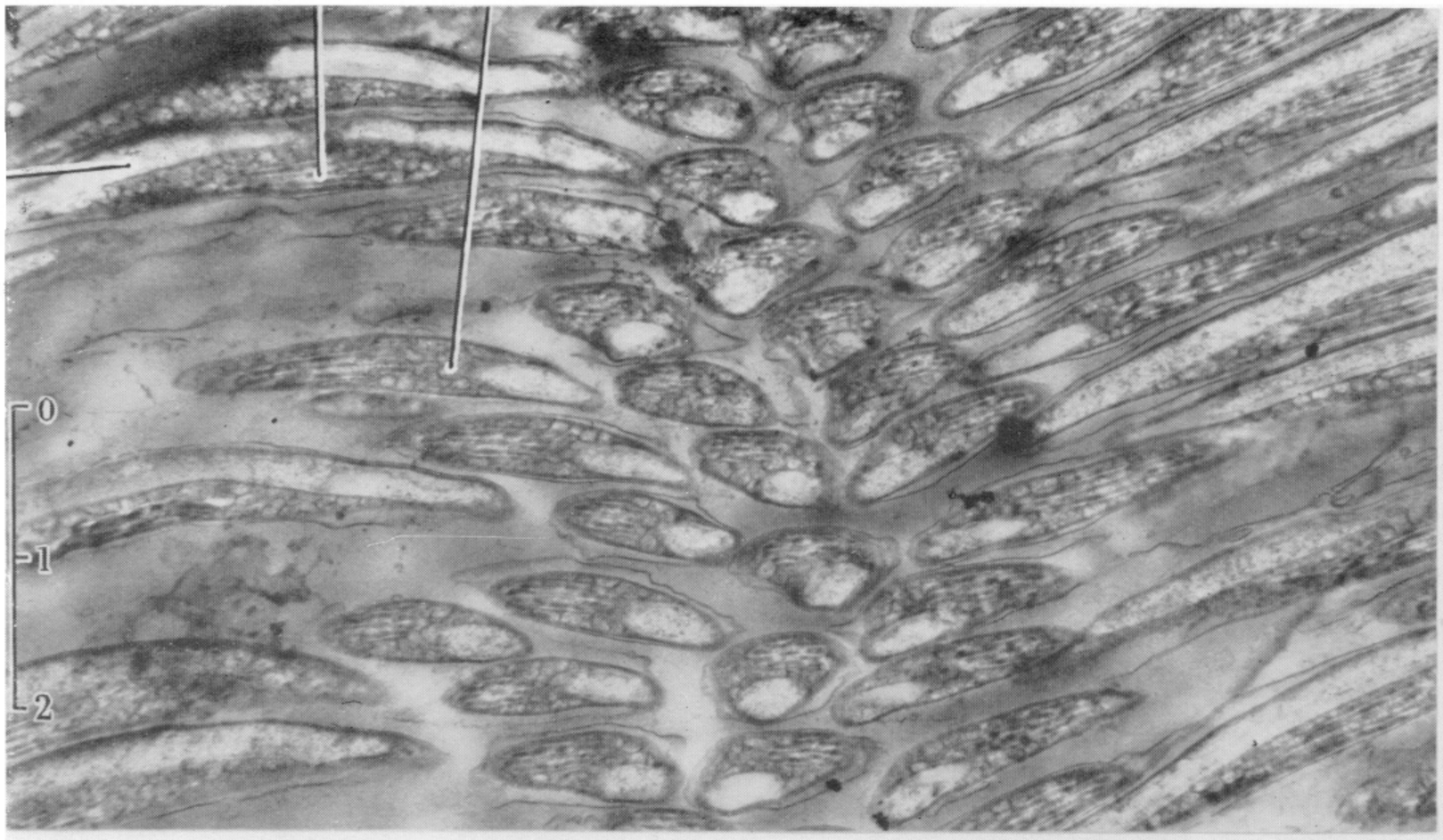
*B*, Initial region of the tail of the same specimen.

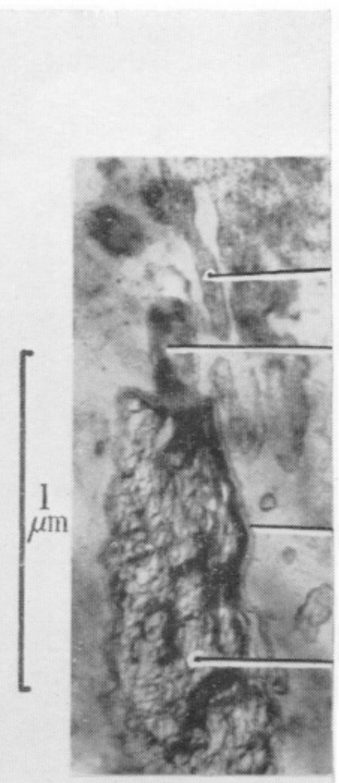
*C*, Region of the tail approximately 50 μm behind the head.

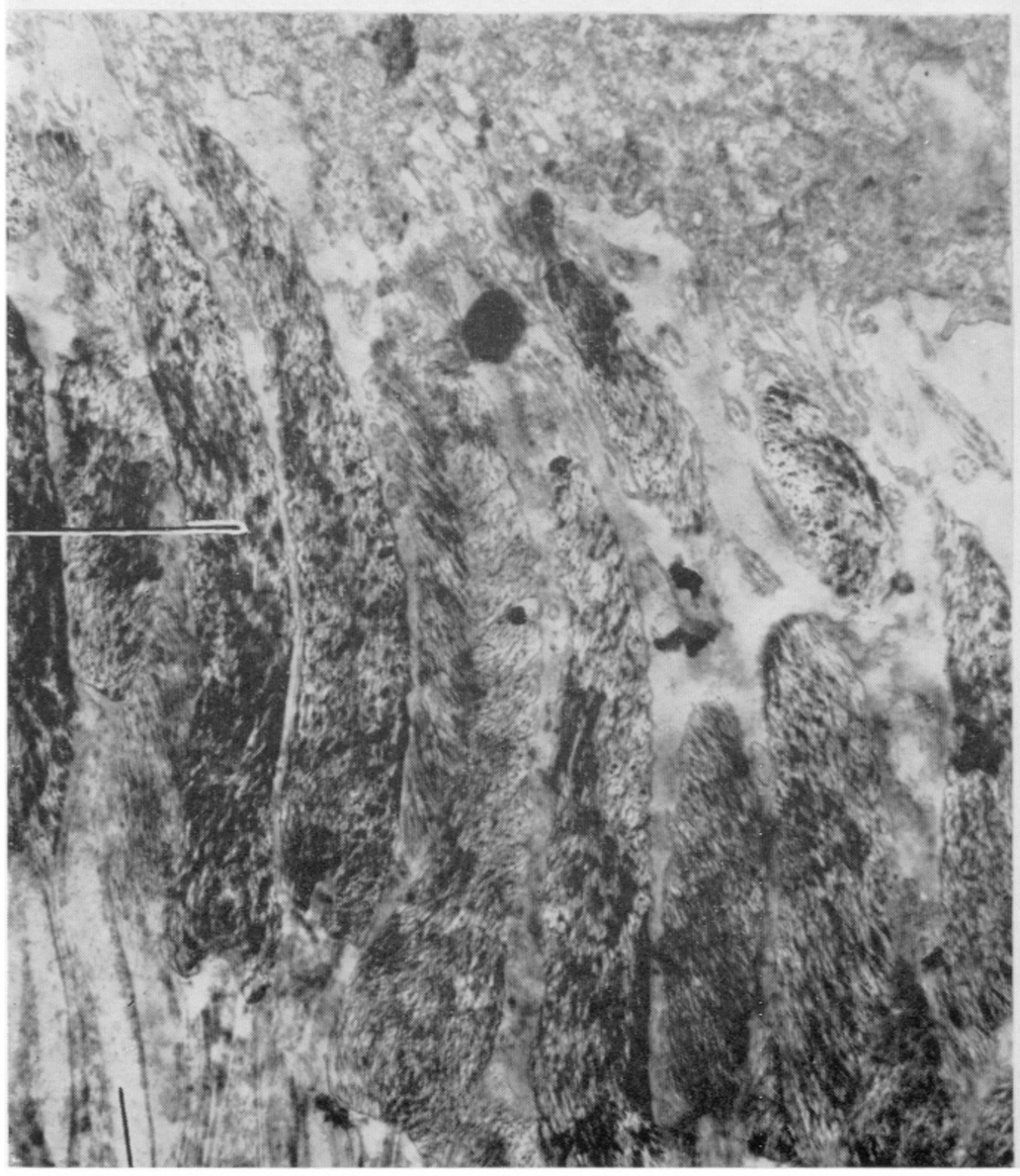
*D*, Section through a mass of autosperms from the vesicula seminalis.

*E*, Section through the bursa copulatrix of a mated adult, showing fragments of stray eggs and the hollow appearance of 'waste' allosperms.

*F*, Rear extremities of allosperms from the receptaculum seminis of a mated adult, showing the posterior persistence of the spiral keel; whole specimens fixed on Formvar film.





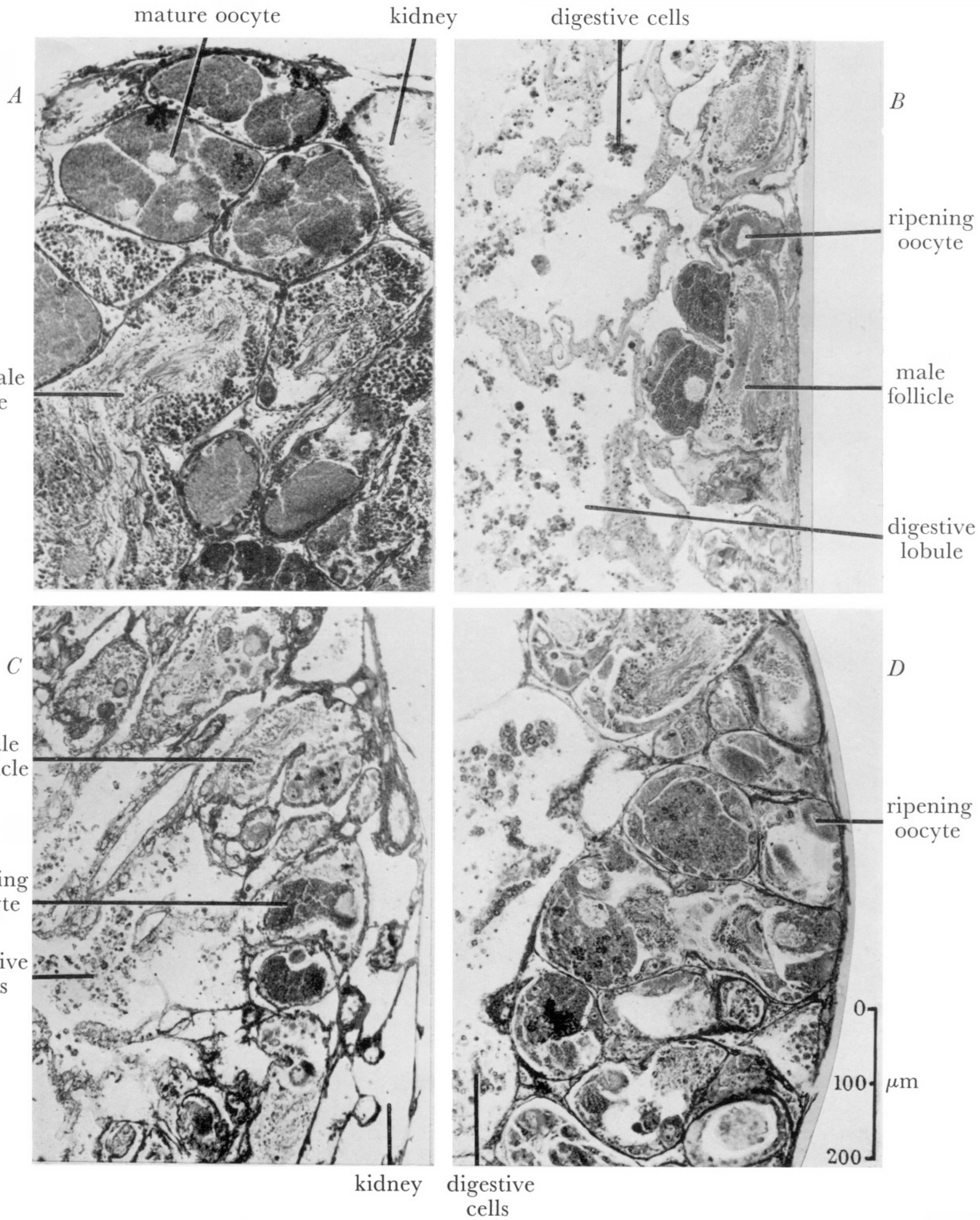
Electron micrographs of receptaculum seminis allosperms of mated *Archidoris*, Cornwall, April 1965.

*A*, Section through the contents of the receptaculum, illustrating the synchrony and identity of orientation of allosperm tails (compare with plate 72*C*).

*B*, Longitudinal section through the allosperm head, showing the intimate association between the spermatozoon and the receptacular endothelium.

*C*, Longitudinal section through numerous allosperm heads, showing their association with the receptacular endothelium.

*D*, Oblique longitudinal section through the neck-region of several allosperms.



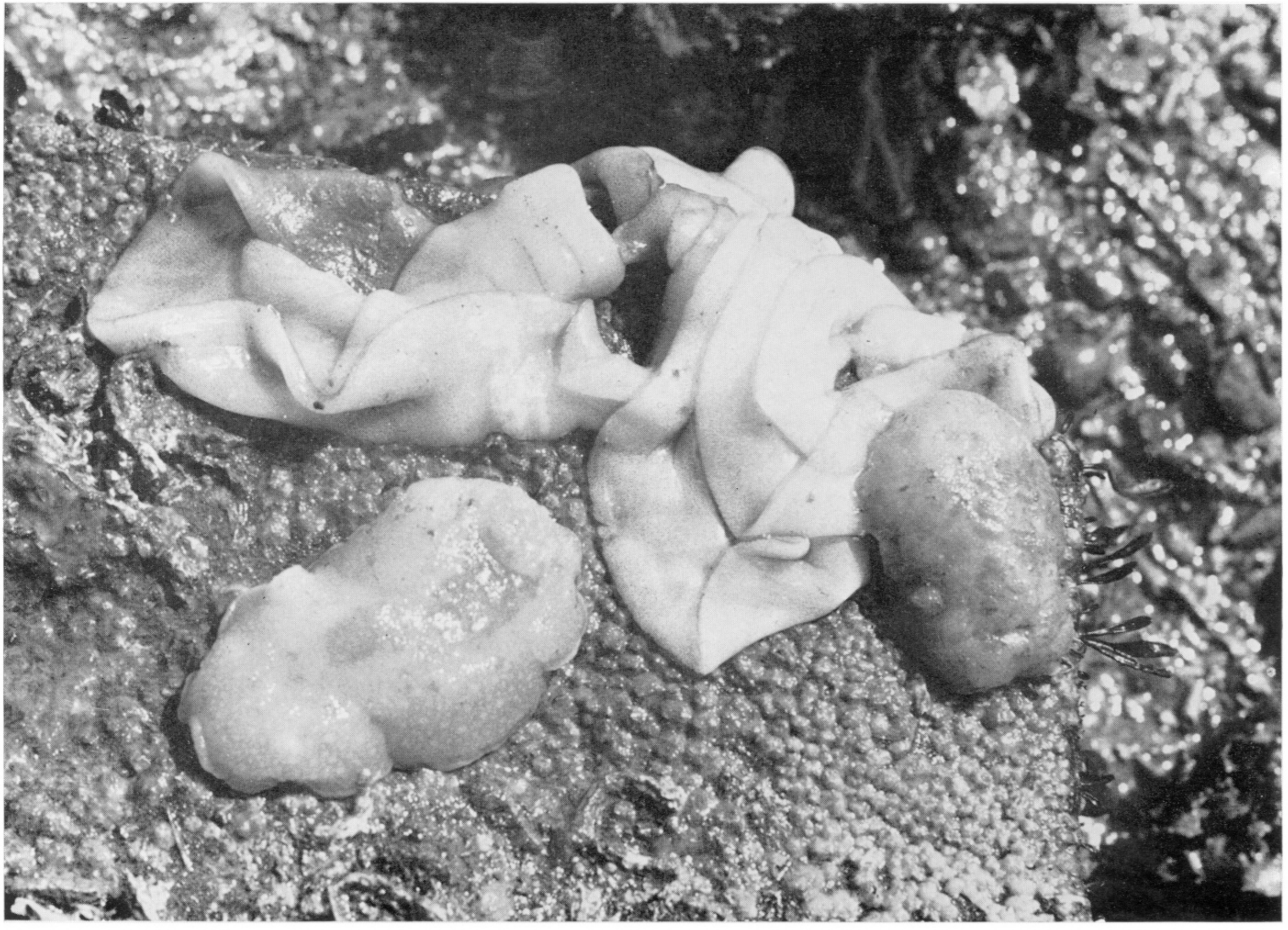

Histology of the ovotestis in *Archidoris* from Traie Meanagh, Isle of Man.

*A*, Section through the gonad of a 70 mm specimen, collected 24 November, killed 25 November 1958. Mature yolk-laden oocytes are common and the male follicles show all stages in spermatogenesis. Zenker-without-acetic and azan.

*B*, Section through the gonad of a 40 mm specimen, collected 5 May, killed 6 May 1959. The animal was feeble when found and proved on dissection to have a reduced digestive gland and few ripe eggs. The section shows spermatocytes to be uncommon, although abundant ripe autosperms are present, and that a fresh batch of oocytes was beginning to ripen. In the digestive gland the digestive cells have shrunken, rounded off, and become detached from the basement membrane; the kidney also shows degeneration. Zenker and azan.

*C*, Section through the gonad of a 35 mm specimen, collected 20 May in a moribund condition, fixed 21 May 1959. On dissection the digestive gland was found to be reduced, and there were only very few eggs visible in the ovotestis. The section shows that breakdown of the digestive and renal cells is advanced; spermatozoa are abundant (but no spermatocytes), while such oocytes as are present are mainly immature. Zenker and azan.

*D*, Section through the gonad of a 35 mm specimen, collected 6 May 1959 and fixed immediately. On dissection the digestive gland was found to be very small, and there were few ripe eggs in the ovotestis. The section shows that spermatozoa and early ripening oocytes are abundant, but that the digestive cells have shrunken, rounded off, and lost contact with the lobular walls. Zenker and azan.



Adult *Archidoris* with their spawn, Cornwall, April 1965. The photograph was taken by Heather H. Angel during tidal emersion.