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13. SIPUNCULA

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I. INTRODUCTION

A small phylum of unsegmented marine coelomate worms, the Sipuncula, includes approximately 320 species. Distinguishing features of the sipunculans are: division of the body into thickened posterior trunk and narrower anterior introvert; tentacles usually surrounding the mouth at the end of the introvert; 1–4 strong retractor muscles which function to withdraw the introvert into the trunk; spacious, unsegmented coelomic cavity containing coelomocytes and developing gametes; spiralled intestine having descending and ascending portions, the former continuous with the oesophagus and the latter with the rectum and dorsal anus located at or near the base of the introvert; nervous system consisting of median, unpaired ventral nerve cord, circumoesophageal connectives, and supraoesophageal brain; one or two nephridia with nephrostomal opening to the coelom and external nephridioporal opening. Sipunculans are widely distributed in the world's oceans occupying many habitats from intertidal waters to abyssal plains. In the tropics, they are found in abundance in burrows in calcareous rock associated with coral reefs. Some species reside in discarded gastropod shells; others live under rocks or burrow into sand or gravel.

Studies on oogenesis of sipunculans include those of Andrews (1889) on *Phascolopsis gouldi*, Gonse (1956a, b, 1957a, b) on *Golfingia vulgaris*, Sawada *et al.* (1968) on *G. ikedai*, Rice (1974) on *G. pugettensis* and *Phascolosoma agassizi*, and Gibbs (1975) on *G. minuta*. Observations on additional species have been reported in various accounts of reproductive cycles and development (Gerould, 1906; Åkesson, 1958; Towle and Giese, 1967; Green, 1975). The subject is reviewed by Rice (1975a).

II. FEMALE REPRODUCTIVE SYSTEM

A. Ovary: Location and Morphology

The ovary of a sipunculan is a narrow band of tissue, extending from the lateral basal edge of one ventral retractor muscle under the ventral nerve

cord to the lateral edge of the other muscle. In those species with only one muscle, the ovary continues from the base of the muscle upwards along the inner side of the muscle for a short distance. Suspended along its width by a mesentery attached to the base of the retractor muscles or the internal body wall, the ovary is enclosed by peritoneum. It is comprised of numerous fine digitations which increase in thickness and length during seasons of gonadal productivity. The digitations may vary in length from 0.1 to 0.5 mm. In those species with definite annual breeding seasons, the ovary either disappears or is considerably reduced during part of the year. Oocytes develop within the gonad to the diplotene stage of the first meiotic prophase, at which time they are released into the coelom where they undergo vitellogenesis and the remainder of their growth in the coelomic fluid as is common in polychaete annelids (see Olive, this volume). Prior to spawning, the fully grown oocytes are accumulated into the nephridia where they are stored for a short time before expulsion into the surrounding sea-water.

B. Nephridium

Typically, there are two nephridia in sipunculans, although in a few species the number is reduced to one. The nephridium is essentially an elongate sac, having at its proximal end two openings: one is a ciliated funnel or nephrostome opening into the coelomic cavity and the other is the nephridiopore opening externally through the body wall. Before spawning, the advanced oocytes are taken into the nephridium from the coelom by way of the nephrostome. At spawning, they are ejected via the nephridiopore to the exterior. A nephridium is joined to the wall of the coelom by a mesentery along all or part of its length. Covered externally by peritoneum, the nephridial wall is composed of connective tissue and circular and longitudinal muscle fibres. Internally the lumen is lined by elongate epithelial cells. A recent ultrastructural study (Ocharan, 1974) of the nephridium of *Phascolosoma granulatum* reports the epithelial cells of the lumen to be ciliated, and the presence of acidophilic granular cells in the outer layer. The latter cells are assumed to secrete into the coelom, thus Ocharan (1974) suggests a hormonal function for the nephridium.

III. THE EGG

Descriptions of recently spawned, unfertilized eggs are found in several accounts of sipunculan development (cf. Rice, 1975a, b). Eggs are characterized by a multilamellate vitelline envelope which is perforated by fine pores. The number of layers of the envelope varies from 3 to 14 in different species; an additional outer covering of jelly occurs in some species (Fig. 1). Considerable diversity is found in shape, pigmentation, size, and yolk content. The eggs of most species are spherical, but in *Aspidosiphon*, *Paraspidosiphon* and two

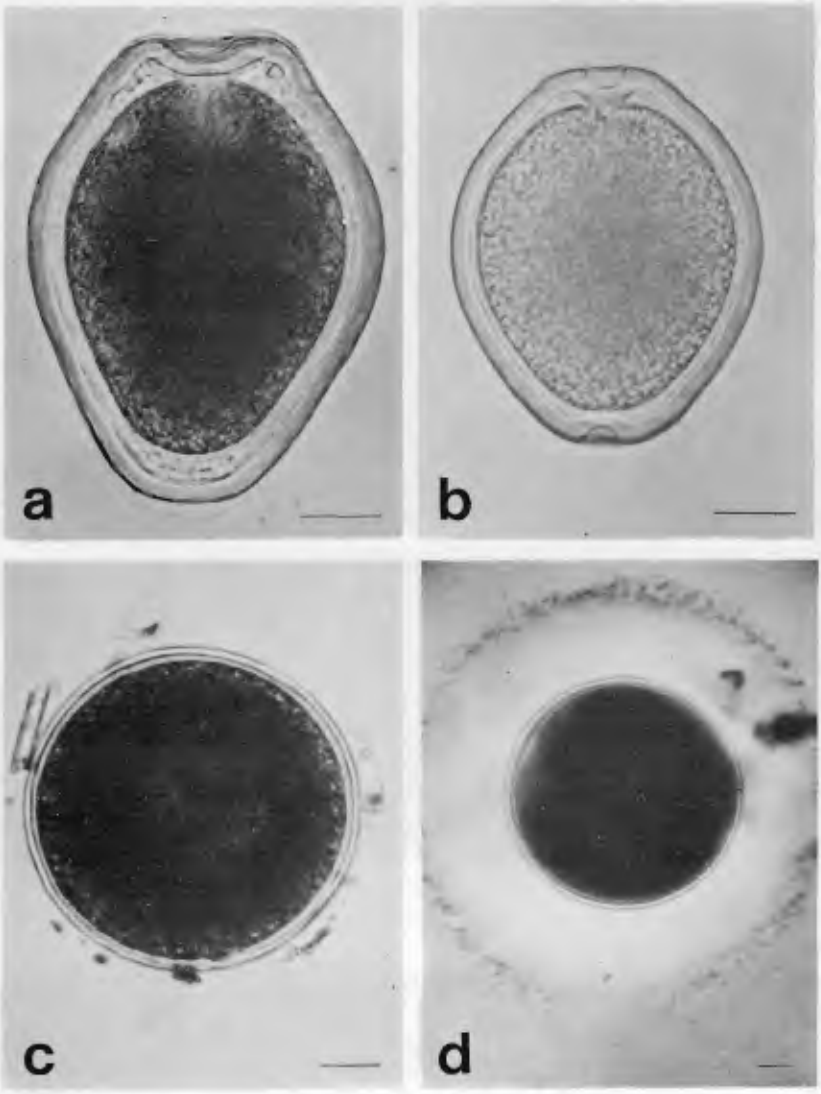

Fig. 1. Photographs of living eggs of sipunculans. Eggs are recently spawned and unfertilized. (a) Egg of *Phascolosoma agassizi*; (b) egg of *Phascolosoma perlucens*; (c) egg of *Phascolion cryptus*; (d) egg of *Themiste pyroides*. Note thick jelly layer. Scale = 25 μm. (From Rice, 1967, reproduced by permission of *Ophelia*; and 1975a, b, reproduced by permission of Academic Press, Inc.)

species of *Golfingia* (*G. minuta* and *G. misakiana*), they are oval. In species of *Phascolosoma*, the eggs are flattened ellipsoids, frequently with depressed apexes at the animal pole or, in the case of *Phascolosoma perlucens*, at both poles. Sipunculan eggs are frequently colourful, varying from shades of red to yellow and orange. Eggs high in yolk content, such as those of *Themiste*, *Phascolion*, and several species of *Golfingia*, are opaque and often white to grey. Eggs of *Sipunculus* and *Siphonosoma* are meagre in yolk, transparent, and colourless. Reports of egg size in the phylum range from $70 \times 88\ \mu m$ for *Golfingia misakiana* (Rice, 1978) to $230 \times 280\ \mu m$ for *G. minuta* (Åkesson, 1958; Gibbs, 1975).

At the time of spawning the germinal vesicle has broken down and eggs are arrested in the first meiotic metaphase. The haploid number of chromosomes has been reported to be 10 for *Golfingia vulgaris*, *Sipunculus nudus* (Gerould, 1906), and *Phascolosoma agassizi* (Rice, 1967).

IV. OOGENESIS

A. Oocyte Differentiation

Definitive evidence on the origin of oocytes in sipunculans is lacking. The resemblance of oogonia to the peritoneal cells surrounding the base of the ovary has led to speculation that oocytes arise from the peritoneum. Andrews (1889) first proposed a peritoneal origin of germ cells and later Hérubel (1908) reiterated this assumption. Gonse (1956a) in studies of oogenesis of *Golfingia vulgaris*, and Rice (1974) in observations on *Phascolosoma agassizi* both noted cytological similarities of the proximal cells in the ovary to the surrounding peritoneal cells. In *P. agassizi*, a seasonal thickening of the peritoneum in the region of the gonad was reported to occur during periods of gonadal growth.

Little is known about primordial germ cells and the embryological derivation of oocytes in sipunculans. In a detailed investigation of the development of *Golfingia vulgaris* and *Phascolopsis gouldi*, Gerould (1906) reported 'reproductive cells' at the base of retractor muscles in larvae at 2–3 weeks of age. No other developmental studies of sipunculans have included observations of developing germ cells.

Differentiation of oocytes within the ovary progresses from the mitotically dividing oogonia in the proximal region through a series of nuclear changes of the first meiotic prophase to the diplotene stage at the distal border; oocytes are released at this stage into the coelom from the distal region of the ovary. Gonse (1956a) has identified seven regions in the ovary of *Golfingia vulgaris* (Fig. 2). The first and most proximal region is comprised of cells with small nuclei, resembling peritoneal cells and undergoing mitotic divisions. The second region is characterized by a progressive despiralization of the chromosomes from the condensed form of the preceding mitotic telophase. The

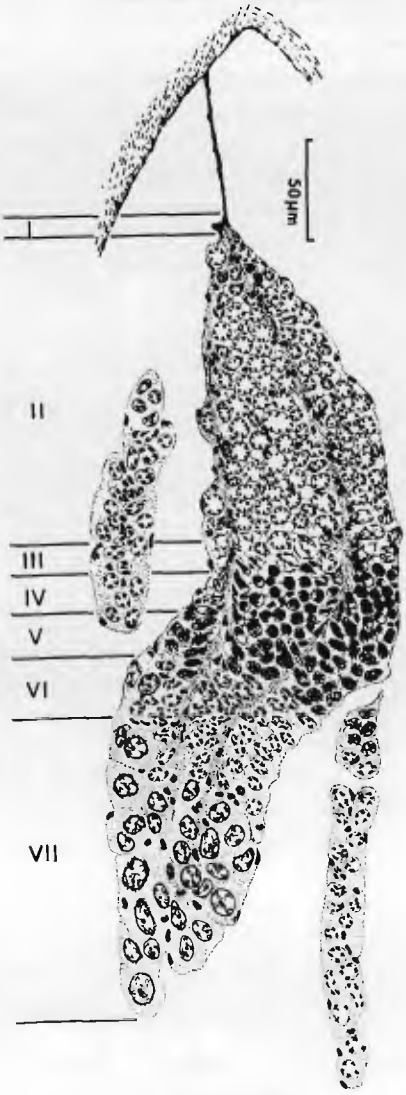

Fig. 2. Longitudinal section of ovary of *Golfingia vulgaris*, suspended by peritoneal mesentery from base of retractor muscle. Seven regions of successive nuclear changes are designated. I, oogonia; II, transitional from oogonia to first meiotic prophase; III, leptotene stage; IV, zygotene stage; V, pachytene stage; VI, diplotene stage; VII, completion of first meiotic prophase, chromosomes despiralized, nuclear and cytoplasmic volumes increased. (From Gonse, 1956a, reproduced by permission of *Acta Zoologica*; and Rice, 1975a, reproduced by permission of Academic Press, Inc.)

nucleus increases in size and is characterized by a polarization of chromosomes at the narrow end and the appearance of a nucleolus. In region 3, the chromosomes advance to the leptotene configuration; the region is narrow, indicating a rapid transition at this stage. In region 4, the nucleoli disappear and the chromosomes reach the zygotene stage of chromosomal synapsis. The pachytene phase is attained in region 5; the thickened but still elongated chromosomes are grouped in a bouquet. Oocytes of regions 6 and 7 are in the diplotene stage of the first meiotic prophase. In region 6, chromosomes pass through a strongly spiralized phase and tetrads are formed. Chromosomes are progressively despiralized in region 7, nucleoli reappear and nuclear and cytoplasmic volumes increase. At the time of release into the coelom, the nucleus has assumed the features of the typical germinal vesicle.

It is in the coelom, as free cells suspended in coelomic fluid, that oocytes undergo vitellogenesis and the major portion of their growth, increasing in volume 200 times or more. Studies on coelomic oogenesis have been carried out in four species: *Golfingia vulgaris* (Gonse, 1956a, b, 1957a, b), *G. ikedai* (Sawada *et al.*, 1968), *G. pugettensis* and *Phascolosoma agassizi* (Rice, 1974). Additional observations on oogenesis in other species are found in various studies on development and reproductive cycles (Gerould, 1906; Åkesson, 1958; Gibbs, 1975; Green, 1975). The most detailed study is that on *G. vulgaris* in which Gonse (1956a, b, 1957a, b) has described cytological, cytochemical, and physiological properties of developing oocytes (Fig. 3). He designated six stages of coelomic oocytes: 0, 1, T, 2, 3, and M. The earliest stage is 0 and consists of clumps of small cells, 20–30 μm in diameter, recently released from the ovary; M is the most mature stage, 160–170 μm in diameter, and is the stage immediately preceding nephridial uptake and spawning. Stages 0, T, and M are transitory, whereas stages 1, 2, and 3 designate the principal periods of growth, each stage corresponding to the elaboration of a particular type of yolk reserve: polysaccharides, lipid, and yolk platelets respectively. Defining features of the various stages of coelomic oogenesis in *G. vulgaris* are summarized in Fig. 3. Similar stages have been proposed for coelomic oocytes of other species, although specific variations are found in such features as size and shape of the oocytes, sequential changes in the nucleus, localization and relative time of appearance of yolk and other cytoplasmic inclusions, occurrence and disappearance of follicular cells, and the formation of the vitelline envelope.

At the time of detachment from the ovary, the oocytes of all species are spherical. In the majority of species, this shape is retained throughout coelomic growth. Species of *Phascolosoma* and *Aspidosiphon* are exceptional in that their oocytes change from spherical to ovoid shape during coelomic oogenesis. In *Phascolosoma agassizi*, the oocytes transform into flattened ellipsoids, thus assuming both polarity and symmetry while developing as free cells in the coelomic fluid. Oocytes of other species, having a final spherical shape, may

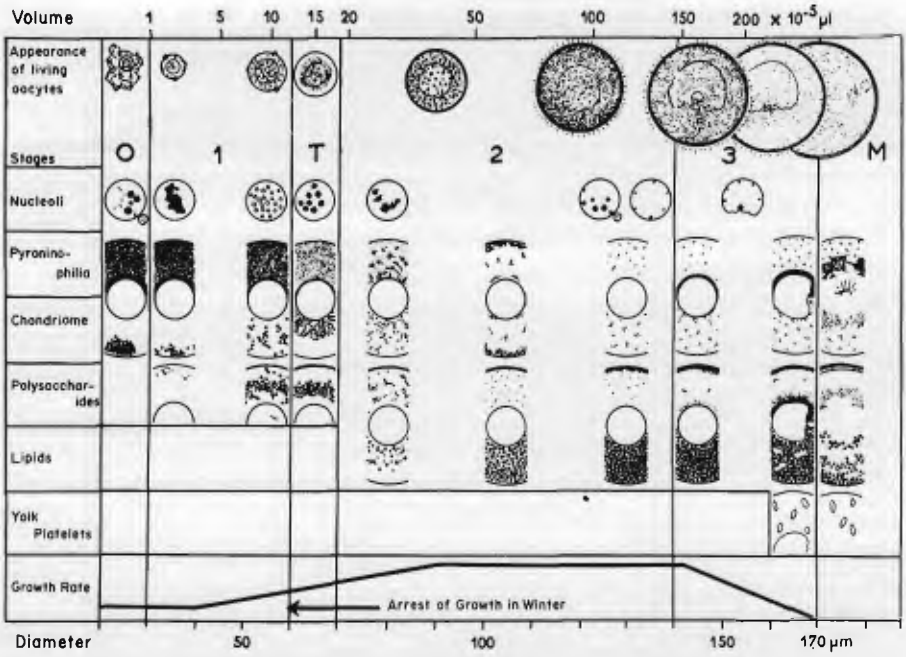

Fig. 3. Differentiation of coelomic oocytes of *Golfingia vulgaris*. Six stages of coelomic differentiation are designated: 0, Oocytes in clumps, recently detached from ovary with annex cells; 1, clumps dispersed as single oocytes with surrounding follicular cells, growth begins; T, transitory stage, follicular cells lost; 2, period of growth; 3, growth decelerated, burst of RNA production, appearance of yolk platelets; M, final or 'mature' stage, dissolution of germinal vesicle. (From Gonse, 1956a, p. 22, reproduced by permission of *Acta Zoologica*; and Rice, 1975a, reproduced by permission of Academic Press, Inc.)

manifest polarity at certain stages by the eccentric location of the nucleus or flattening of one side of the nucleus (Gonse, 1956a).

During coelomic oogenesis the nucleus or germinal vesicle of the oocyte increases in size and undergoes specific changes, such as infolding of the nuclear membrane and modifications in the appearance and number of nucleoli. In early oocytes of all species, the nucleus is spherical. Peripheral infoldings of the nucleus occur in the final stage of oogenesis in *Golfingia vulgaris* and *Phascolosoma agassizi*; in the former, the infoldings are restricted to one side of the nucleus. The nucleus of oocytes of *G. pugettensis* shows peripheral undulations at an early stage when the diameter of the oocyte is about one-half of its mature dimension. Nucleoli are present as numerous small fragments in the early oocytes of *G. pugettensis* and *G. vulgaris*. Later oocytes of *G. pugettensis* have 2–3 discrete nucleoli and, in addition, retain some nucleolar fragments. The nucleolar fragments of *G. vulgaris* are replaced in later stages

by many individual nucleoli, one dominating the others in size, and all arranged at the periphery of the nucleus. Two to five nucleoli are present in all stages of oocytes of *P. agassizi*; in early oocytes, one nucleolus is larger than the others.

Localization of cytoplasmic organelles and components identified cytochemically varies throughout oogenesis. Pyroninophilia, interpreted to signify the presence of RNA, has been identified around the nucleus in the earliest and latest oocytes of *Golfingia vulgaris*. Electron microscope studies of oocytes of *G. ikedai* have revealed a similar earlier perinuclear concentration of ribosome clusters. Mitochondria are first localized in the periphery of the oocytes, later increasing in numbers and appearing throughout the cytoplasm. Ultrastructural observations indicate that mitochondria are fused with peripheral endoplasmic vesicles in early oocytes of *G. ikedai* and later are associated with one of two kinds of yolk granules.

B. Vitellogenesis

In the elaboration of yolk, there is little consistency among species of sipunculans in the sequence in which the carbohydrate, protein, and lipid yolk make their appearance. Also, the form and localization of yolk may vary widely. The most comprehensive study of vitellogenesis in sipunculans is that by Gonse (1956a) on *Golfingia vulgaris* (Fig. 3). He reported the earliest yolk to be carbohydrate. Granules, presumed to be galactogen, appear in the peripheral cytoplasm of oocytes, soon after their detachment from the ovary. As the oocyte grows the granules move towards the nucleus, then move again to the periphery, giving rise in the final growth stage (M) to the cortical granules. Later in oogenesis, a second carbohydrate, identified cytochemically as glycogen, emerges as a diffuse substance around the nucleus. Lipid inclusions appear in stage 2 in association with a mitochondrial mass in the perinuclear zone. Later the lipid disperses throughout the cytoplasm, concentrating in the mature or final stage of growth at the periphery. Protein yolk is formed during the last stages of oogenesis, appearing as a few, relatively large yolk platelets. Two peaks of exogenous respiration have been demonstrated, the first corresponding to the initiation of carbohydrate synthesis, the second to protein, and both corresponding to periods of high concentrations of RNA (Gonse, 1957a, b). Peaks are coupled to metabolism of hexoses and pentoses.

Differing from the carbohydrate yolk of *Golfingia vulgaris*, that of *Phascolosoma agassizi* is associated with protein as a carbohydrate–protein complex in distinctive, heterogeneously staining yolk granules. Appearing around the nucleus in early oocytes, these granules are dispersed through the cytoplasm in mid-oogenesis and move away from the periphery in the latest stages, leaving a clear cortical area. In *G. pugettensis*, carbohydrate yolk occurs in homogeneous granules first obvious midway between nucleus and periphery of the cell. As the granules increase in number they spread through the cytoplasm. No information is available on protein yolk in *G. pugettensis*. Lipid droplets

are more prominent in *G. pugettensis* than in *G. vulgaris*, but the pattern of distribution during coelomic oogenesis is similar. In *P. agassizi*, small granules suspected to be lipid are distributed throughout the cell from the earliest oocyte; large and relatively few lipid droplets occur in later oocytes.

C. Follicular Cells

Oocytes are released from the ovary into the coelom, usually in clumps of 10–20 cells which are soon dispersed as individual oocytes. Interspersed within the clumps are peritoneal cells, derived from the peritoneal covering of the ovary and peritoneal infoldings within the ovary. As the clumps break up, the peritoneal cells remain attached to the oocytes, their cytoplasm spreading out in a thin, tenuous layer around the periphery of the oocyte, forming a covering of follicular cells. Oocytes of *Phascolosoma agassizi* are usually released as single cells from the ovary and follicular cells are absent. Follicular cells of sipunculans, when present, do not remain with the oocyte during its entire development, but are lost at some stage of coelomic growth. The follicular cells of *Golfingia pugettensis* disappear late in coelomic oogenesis when cytoplasmic processes are extended through the pores of the egg envelope; those of *G. vulgaris* are lost at an earlier stage, prior to the formation of cytoplasmic processes. In oocytes of *Themiste pyroides*, follicular cells are elevated away from the egg envelope when a thick jelly coat is formed; they are no longer apparent in late oocyte stages.

Differing from most investigators who have found the follicular cells to arise from the peritoneal covering of the ovary (Gerould, 1906; Åkesson, 1958; Sawada *et al.*, 1968; Rice, 1974), Gonse (1956a) reported the follicular cells of *Golfingia vulgaris* to be abortive oocytes, undergoing meiotic changes in the ovary simultaneously with the ovarian oocytes. He assumed the follicular cells to originate from oogonia in region 2 of the ovary, but was unable to demonstrate transitional stages.

What role, if any, the follicular cells have in coelomic oogenesis has not been determined. In the past, it was suggested that they participated in the formation of the egg envelope (Åkesson, 1958). Because of their small size and more recent information on the paucity of cytoplasmic inclusions (Sawada *et al.*, 1968; Rice, 1974), it now seems unlikely that they perform any significant secretory activity. Moreover, they are entirely absent in at least one species, *Phascolosoma agassizi*, and in other species they are lost during coelomic oogenesis. No information is available on their possible function in the transfer of nutrients from the coelomic fluid to the young stages of coelomic oocytes.

V. FORMATION OF THE EGG ENVELOPE

The fully formed egg envelope of a sipunculan oocyte is comprised of several layers which are perforated by pores through which microvillar extensions of the

cytoplasm protrude. In a few species, the envelope is encompassed by an outer adhesive jelly coat. Formed as a secretion of the oocyte, the envelope has been characterized as a mucoprotein (Gonse, 1956a; Rice, 1974). The number of layers and thickness vary among different species. For example, the envelope of *Golfingia ikedai* has 14 layers and is 2 μm thick, whereas that of *Phascolosoma agassizi* has 3 layers and is 10 μm thick; the latter envelope is approximately one-tenth the width of the entire egg (Sawada *et al.*, 1968; Rice, 1974). Ultrastructural observations of the egg envelope of *P. agassizi* show that the microvilli pass through pores in the inner and middle layers, branching in the relatively thin outer layer. Surrounding the outer layer is a narrow fringe of amorphous material designated as fuzz. In contrast with the inner and middle layers and the surrounding fuzz, all of which are mucoprotein, the outer layer stains as an acid mucopolysaccharide.

A scheme for the formation of the egg envelope of *Golfingia ikedai* has been proposed by Sawada *et al.* (1968) in an electron microscope study of coelomic oogenesis of this species (Fig. 4). The fully differentiated envelope consists

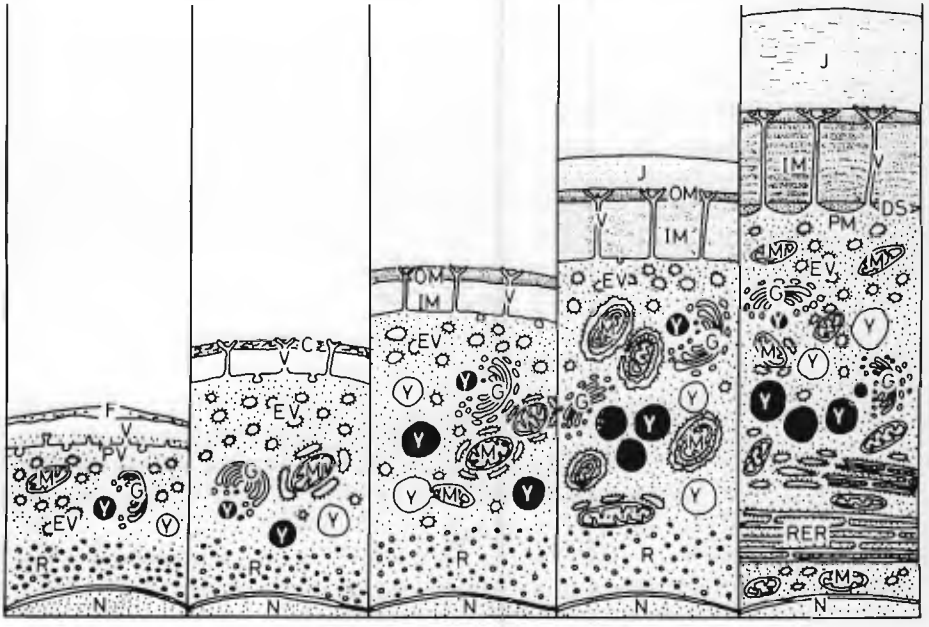

Fig. 4. Diagram showing growth of coelomic oocytes of *Golfingia ikedai*, from electron microscopic observation. C, chorion; DS, diffused substances; EV, endoplasmic vesicles; F, follicular cell; G, Golgi body; IM, inner membrane; J, jelly layer; M, mitochondria; N, nucleus; OM, outer membrane; PM, plasma membrane; PV, pinocytotic vacuole; R, ribosome cluster; RER, rough-surfaced endoplasmic reticulum; V, microvillus; Y, yolk granules. (Reproduced with permission from Sawada *et al.*, 1968, p. 37; and from Rice, 1975a, reproduced by permission of Academic Press, Inc.)

of an outer fibrous layer, a middle multilamellate layer, and an inner diffuse layer. In the earliest stages, prior to the appearance of the egg envelope, microvilli extend from the surface of the oocyte. As the oocyte grows, the diffuse inner layer is secreted around the microvilli from the surface of the cell. This layer, presumably containing precursor substances, condenses to form in succession the numerous middle layers. The formation of the outer fibrous layer as well as the covering of jelly is attributed to secretions from the tips of the microvilli.

VI. SPAWNING

Spawning of sipunculans occurs when gametes in the nephridia are ejected through the nephridiopores into the surrounding sea-water. At the time of spawning, the margins of the nephridiopores are often swollen and the entire body is rigid. Observations of *Phascolosoma agassizi* in the laboratory revealed that, prior to spawning, animals in sand and gravel extend the anterior body above the substratum, thus exposing the nephridiopores and allowing gametes to pass directly into the sea-water (Rice, 1966). Other species in laboratory dishes without substratum have been observed to increase their activity before spawning, frequently extending and retracting the introvert. To achieve fertilization under natural conditions, spawning of males and females has to be synchronized, but little is known of the manner in which this is accomplished. Because gametes must be accumulated by the nephridia before spawning is possible, any consideration of the controlling mechanisms should take into account two events: (1) movement of gametes from the coelom into the nephridia; (2) expulsion of gametes from the nephridia to the exterior. Definitive experimental evidence on the control of these events is lacking; however, observations reported in studies of development and reproductive biology provide the basis for speculation and hypotheses.

During nephridial uptake the most mature oocytes are selected from the smaller oocytes and coelomocytes of the coelomic fluid, passing through the ciliated nephrostome to be stored in the nephridium for a short time before spawning. Gerould (1906) observed the nephridia to be distended, presumably with sea-water, before spawning. He suggested that the largest coelomic eggs are hydrotropic, thus accumulating near the nephrostome of nephridia containing sea-water and being transported into the nephridium by nephrostomal ciliary activity. Åkesson (1958) further suggested that the eggs could secrete a substance that would influence the direction of ciliary movement.

Available data indicate that nephridial uptake may be dependent on initiation of oocyte maturation. Nephridial oocytes, as well as spawned oocytes, of all species studied are arrested in the first meiotic metaphase, in contrast with the majority of coelomic oocytes in which the germinal vesicle is intact. In monthly measurements of coelomic oocytes of *Phascolosoma agassizi*, the

dissolution of the germinal vesicle has been observed only in those specimens having oocytes present in the nephridia (Rice, 1966). Both Gerould (1906) and Gonse (1956a) have reported nuclear breakdown in the largest coelomic oocytes of *Golfingia vulgaris*. Rice (1966) induced nuclear breakdown in coelomic oocytes of *P. agassizi*, *in vitro*, with crude extracts of coelomic sperm, coelomic oocytes, coelomocytes, brain, and muscle, suggesting a possible chemical or hormonal control of maturation and subsequent nephridial uptake.

There have been no observations of spawning of sipunculans in the field, and little data exist on which to base an understanding of the control of spawning in the natural habitat. In the laboratory, spawning can often be triggered, if gametes are present in the nephridia, by a change of sea-water or a sudden change of temperature. Gerould (1906) reported the spawning of *Golfingia vulgaris* to be influenced by light. Spawning in the laboratory occurred only at night and if animals were kept continually in darkness the rhythm was interrupted, resulting in some daytime spawning. *Golfingia elongata* and *Phascolion strombi* have also been reported to spawn at night (Åkesson, 1958, 1961). However, other species, *Phascolosoma agassizi* and *Themiste pyroides*, were found to spawn in the daytime as well as at night (Rice, 1967).

Ultimately, spawning is dependent on gametogenic cycles and the production of large oocytes. Gametogenic cycles of several species of sipunculans have been described (Gonse, 1956a, b; Rice, 1966, 1975a; Towle and Giese, 1967; Gibbs, 1975; Green, 1975), although processes regulating these cycles remain unknown. The literature on reproductive cycles and breeding seasons in sipunculans was reviewed by Rice (1975a). Since that review there has been one additional study by Gibbs (1975) on the reproductive cycle of *Golfingia minuta*.

VII. OOSORPTION

Large oocytes that have not been spawned at the end of a breeding season may be resorbed in the coelomic cavity. Gonse (1956b) reports that large oocytes of *Golfingia vulgaris* occasionally persist in the autumn and winter after the period of spawning. These relics of the previous reproductive period undergo phagocytosis in the coelom. In unpublished observations, the author has noted the degeneration of large oocytes of *Golfingia misakiana*, *G. puget-tensis*, and *Themiste lageniformis* and their subsequent destruction by phagocytic activity of coelomic amoebocytes. Only those oocytes that are swollen and show evidence of cytolysis are attacked by the amoebocytes. Other investigators have mentioned the possibility of resorption of unspawned oocytes without documenting the event (Towle and Giese, 1967; Gibbs, 1975).

VIII. CONCLUSION

A review of the present status of knowledge on sipunculan oogenesis reveals many unanswered questions. Whether oocytes originate by a differentiation of

peritoneal cells or from a line of primordial germ cells is a question requiring further evidence from ultrastructural and developmental studies. As more information becomes available, attention should be also directed to the significance of specific diversity in yolk formation and the relevance of the type and abundance of yolk to developmental patterns. One of the most exciting areas of research yet to be explored in sipunculans involves the controlling mechanisms of oogenesis. Of particular interest are those processes regulating the initiation of maturation in the coelom and nephridial selectivity in the uptake of the advanced coelomic oocytes for storage prior to spawning.

REFERENCES

Åkesson, B. (1958). 'A study of the nervous system of the Sipunculoideae with some remarks on the development of two species, *Phascolion strombi* Montagu and *Golfingia minuta* Keferstein', *Undersökningar över Öresund*, **38**, 1–249.

Åkesson, B. (1961). 'The development of *Golfingia elongata* Keferstein (Sipunculidea) with some remarks on the development of neurosecretory cells in sipunculids', *Ark. Zool.* (Ser. 2), **13**(2), 511–531.

Andrews, E. A. (1889). 'The reproductive organs of *Phascolosoma gouldii*', *Zool. Anz.*, **12**, 140–142.

Gerould, B. (1906). 'Studies on the embryology of the Sipunculidae. II. The development of *Phascolosoma*', *Zool. Jb. Abt. Anat. Ontog. Tiere*, **23**, 77–162.

Gibbs, P. E. (1975). 'Gametogenesis and spawning in a hermaphroditic population of *Golfingia minuta* (Sipuncula)', *J. mar. biol. Ass. U.K.*, **55**, 69–82.

Gonse, P. (1956a). 'L'ovogenèse chez *Phascolosoma vulgare*. I. Définition cytologique des stades de croissance des ovocytes', *Acta Zool.*, **37**, 193–224.

Gonse, P. (1956b). 'L'ovogenèse chez *Phascolosoma vulgare*. II. Recherches biométriques sur les ovocytes', *Acta Zool.*, **37**, 225–233.

Gonse, P. (1957a). 'L'ovogenèse chez *Phascolosoma vulgare*. III. Respiration exogène et endogène de l'ovocyte. Effet de l'eau de mer', *Biochim. Biophys. Acta*, **24**, 267–278.

Gonse, P. (1957b). 'L'ovogenèse chez *Phascolosoma vulgare*. IV. Étude chromatique des sucres du plasma, action de différents substrats et du malonate sur la respiration de l'ovocyte', *Biochim. Biophys. Acta*, **24**, 520–531.

Green, W. (1975). 'The annual reproductive cycle of *Phascolosoma lurco* (Sipuncula)', *Proc. Intern. Symp. Biol. Sipuncula and Echiura*, Naucno Delo Press, Belgrade, pp. 161–168.

Hérubel, M. A. (1908). 'Récherches sur les sipunculides, *Mém. Soc. Zool. Fr.*, **20**, 107–419.

Ocharan, F. J. (1974). 'Sobre los nephridios de *Phascolosoma granulatum* (Sipuncula)', *Revista de la Facultad de Ciencias*', **15**(1), 21–42.

Rice, M. E. (1966). 'Reproductive biology and development in Sipuncula', Doctoral dissertation, University of Washington, Seattle, 322 pp.

Rice, M. E. (1967). 'A comparative study of the development of *Phascolosoma agassizii*, *Golfingia pugettensis*, and *Themiste pyroides* with a discussion of developmental patterns in the Sipuncula', *Ophelia*, 4, 143–171.

Rice, M. E. (1974). 'Gametogenesis in three species of Sipuncula: *Phascolosoma agassizii*, *Golfingia pugettensis*, and *Themiste pyroides*', *Cellule*, **70**(2), 295–313.

Rice, M. E. (1975a). 'Sipuncula', in *Reproduction of Marine Invertebrates*, Vol. II (Eds. A. C. Giese and J. S. Pearse), Ch. 4, Academic Press, New York, pp. 67–127.

Rice, M. E. (1975b). 'Observations on the development of six species of Caribbean Sipun-

cula with a review of development in the phylum', *Proc. Intern. Symp. Biol. Sipuncula and Echiura*, Naucno Delo Press, Belgrade, pp. 141–160.

Rice, M. E. (1978). 'Morphological and behavioral changes at metamorphosis in the Sipuncula', in *Settlement and Metamorphosis of Marine Invertebrate Larvae* (Eds. F. S. Chia and M. E. Rice), Elsevier, New York, pp. 83–102.

Sawada, N., Noda, Y., and Ochi, O. (1968). 'An electron microscope study on the oogenesis of *Golfingia ikedai*'. *Mem. Ehime Univ. Sci.* Ser. **B6**(1), 25–39.

Towle, A., and Giese, A. C. (1967). 'The annual reproductive cycle of the sipunculid *Phascolosoma agassizii*', *Physiol. Zool.*, **40**, 229–237.