REPRODUCTIVE BIOLOGY
OF INVERTEBRATES

Series edited by

K.G. and RITA G. ADIYODI

Cochin University of Science & Technology, Kochi 682022,
Vatsyayana Centre of Invertebrate Reproduction
Calicut University, Kerala 673635, India

Volume edited by

B.G.M. JAMIESON

Department of Zoology and Entomology, University of Queensland
Brisbane 4072, Queensland, Australia

VOLUME IX, PART C

Progress in Male Gamete Ultrastructure and Phylogeny

8. CEPHALOCHORDATA

BARRIE G. M. JAMIESON

Department of Zoology and Entomology, The University of Queensland,
Brisbane Q4072, Australia

I. INTRODUCTION

Cephalochordates (also termed 'Acrania') are small marine, fish-like animals (up to 8 cm long) (Fig. 1) which lie buried in the substrate with the buccal cirri exposed, feeding by straining minute organisms from the water.

The notochord extends to the anterior end of the body, in front of the brain. A cranium is absent. There is a cerebral vesicle but no true brain. The fibres of the peripheral nerves differ from those of vertebrates in lacking a myelin sheath. In cephalochordates, unlike vertebrates, peripheral muscles send processes to the nerve cord as in echinoderms and nematodes. There are no vertebrae; no cartilage or bone; and no red blood corpuscles. The long dorsal fin and shorter ventral fin are each supported by a fin ray box. The heart is simple, consisting of a contractile vessel. The epidermis has only a single layer of cells. The excretory system is protonephridial, with solenocytes. The alimentary canal has a liver diverticulum and a pharyngeal endostyle which contains iodine-fixing cells and is considered to be the homologue of the vertebrate thyroid. Anteriorly, a series of buccal cirri forms a sieve around the opening of an oral hood. The numerous gill slits open from the pharynx into a chamber, the atrium, which opens to the exterior posteriorly at the atriopore and is covered by lateral folds of the body wall, the metapleural folds. The feeding current is maintained by cilia of a 'wheel organ' in the oral hood; and by cilia of the gill bars, and of the endostyle. Near the centre of the wheel organ opens a groove, Hatschek's pit, which represents the opening of the left, first coelomic sac to the exterior, an opening which also occurs in the oligomerous phyla, including echinoderms. Sensory velar tentacles are present within the hood. The anus is well anterior to the hind end of the body, leaving a definite postanal tail as is typical of chordate metamerism. Sexes are separate (Wickstead, 1975; Young, 1981; Nelson, 1984; Jamieson, 1984, 1991).
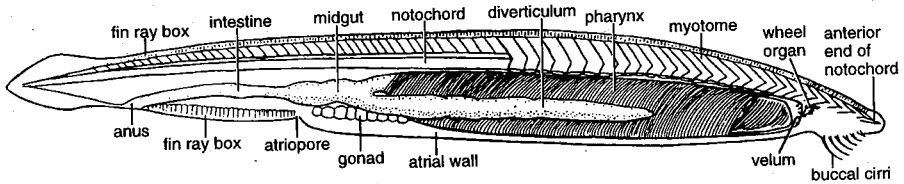
Fig. 1. *Branchiostoma*, the amphioxus or lancelet. The body wall and atrial wall have been removed on the right side to show the pharynx, midgut with 'liver diverticulum', and intestine. The oral hood has been cut away on the right, leaving the buccal cirri, wheel organ and velum. From Young, J.Z. (1981), fig. 2.1.

Cephalochordates and (at least as embryos) vertebrates share attributes indicative of a close relationship: a notochord; a dorsal tubular nerve cord; paired lateral gill slits; a hepatic portal system; and an endostyle or its (i.e. its) homologue, the thyroid.

There is a single order, the Amphioxiformes (lancelets), exclusively marine, in the Atlantic, Indian and Pacific Oceans.

There are two families: Branchiostomidae, with one genus, *Branchiostoma* (Fig. 1) with about 15 species; and Epigonichthyidae, containing *Epigonichthys* (=*Asymmetron*, *Heteropleuron*) with about five species, differing from *Branchiostoma* in having the gonads on the right side only (Nelson, 1984).

II. THE REPRODUCTIVE ORGANS

Cephalochordates are bisexual animals. Their serial ductless gonads bulge into the atria along both sides of the body. The testes and ovaries are composed of non-germinal and germinal cells and are enclosed in complex narrow cavities and envelopes comprising blood vessels and myoepithelia. The male non-germinal cells are single phagocytic and secretory epithelial cells which do not form a blood-testis barrier (Welsch and Fang, 1996). It is presumably these cells which are considered Sertoli cells by Fang (1991) (see below). Spermatogonia, different stages of primary spermatocytes and spermatids, are connected by intercellular bridges (Welsch and Fang, 1996). The spermatozoa are described in Section III. The ovarian non-germinal cells are interconnected by cell junctions, produce secretory granules and presumably also steroids, and have phagocytotic properties. During growth and maturation the oocytes undergo striking changes of nuclear, nucleolar, and cytoplasmic organization. The amount and distribution of nuage material, yolk granules, cortical granules, and precursors of the extracellular vitelline coat mark specific stages of differentiation in the cytoplasm (Welsch and Fang, 1996).

Putative Sertoli cells of *Branchiostoma belcheri* were described at different stages of testis development by Fang (1991). At the early stage of testicular development, these cells adhered to the basement membrane and were located between spermatogonia. The Sertoli cells gradually migrated up from the basement

membrane to the tubular lumen during gondal development and maturation. Abundant rough and smooth endoplasmic reticulum, well-developed Golgi complex, mitochondria and many lysosome and glycogen granules were present in the cytoplasm of supra- and infranuclear portions of the Sertoli cell. The spermatids adhered to the cytoplasm of the Sertoli cell. The fine structure of the latter cell suggested that it was involved in nutrition, phagocytosis and release of sperm (Fang, 1991).

III. ULTRASTRUCTURE OF THE SPERMATOZOON

A. General

Ultrastructural studies on cephalochordate sperm are listed in Table 1.

Table 1

TEM studies on cepaphalochordate spermatozoa

Species	Reference
Branchiostoma lanceolatum	Baccetti *et al.*, 1972; Wickstead, 1975
Branchiostoma belcheri	Chen *et al.*, 1988; Lin *et al.*, 1987; Welsch and Fang, 1996
Branchiostoma floridae	Holland and Holland, 1989
Branchiostoma moretonensis	Jamieson, 1984, 1991

Wickstead (1975), Holland and Holland (1989) and Welsch and Fang (1996) give valuable accounts of reproduction and related morphology in the Cephalochordata. Distribution of nuage material during spermiogenesis is investigated with TEM by Aizenshtadt and Gabaeva (1987).

The spermatozoon of *Branchiostoma* (Figs. 2–4) approximates to the primitive type *sensu* Franzén (Franzén, 1956) or ect-aquasperm of the plesiosperm type (Jamieson, 1986; Rouse and Jamieson, 1987) in having a cap-like acrosome surmounting a compact nucleus; a single mitochondrion incorporated in the head behind the nucleus and, not elongated; two mutually perpendicular centrioles with triplet microtubules; and a long flagellum with the '9+2' configuration of microtubules. But it differs notably from the basic ect-aquasperm in possessing only a single mitochondrion and in the form of this. The head (including the acrosome, nucleus and mitochondrion) is approximately 3 μm long.

B. Acrosome

The acrosome (Figs. 4A-D) in all species consists solely of a bell-shaped acrosome vesicle (0.3–0.4 μm long and maximally, near the posterior rim, 0.7–0.8 μm wide in *Branchiostoma moretonensis*; 0.5 μm long and basally 0.7 μm wide in *B. lanceolatum*) deeply invaginated posteriorly by the acrosomal space so that its
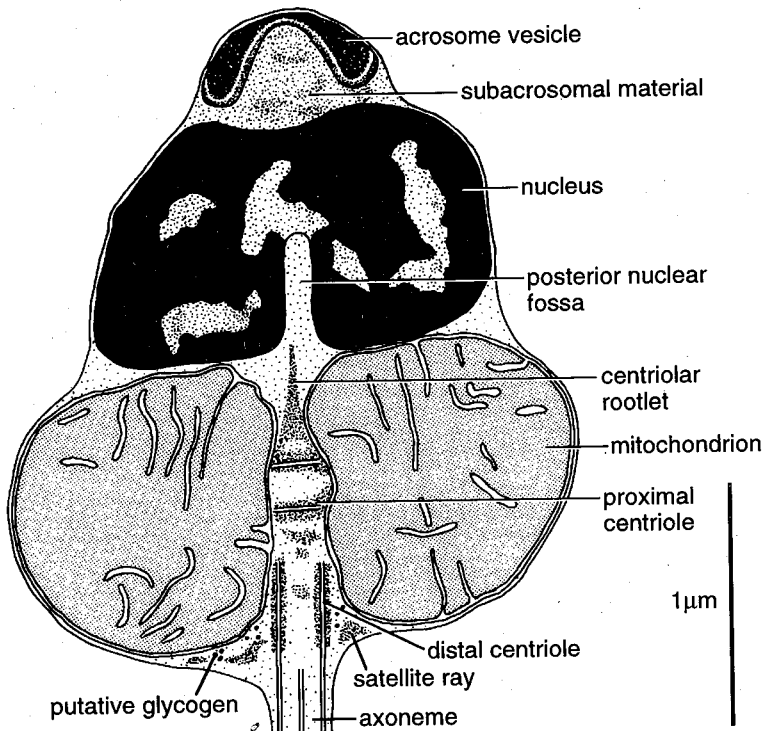
Fig. 2. *Branchiostoma moretonensis.* Semidiagrammatic representation of a longitudinal section through the spermatozoon by TEM. Original.

anterior and posterior bounding membranes are only narrowly separated at the apex, though well separated at the posterior rim.

The subacrosomal invagination (Figs. 4A-D) contains unstructured, somewhat diffuse material which is less electron-dense than the contents of the vesicle. This material extends for about 0.1 µm posteriorly, separating the vesicle from the nucleus. In *Branchiostoma moretonensis* the plasma membrane covering the acrosome projects as a small apical cone or button; this is a common artefact in various phyla, but possibly reflects some underlying differentiation (Jamieson, 1984, 1991). The acrosome vesicle originates, as is normal, from the Golgi apparatus but there is no evidence, in *B. floridae*, for such an origin of the subacrosomal material (Holland and Holland, 1989). The latter authors observed a dual origin of the subacrosomal material: one component originates at the posterior end of the spermatid, and the other at the anterior end. Subsequently, the two components merge into one after the acrosomal vesicle has migrated to its definitive anterior position in the mature spermatozoon.
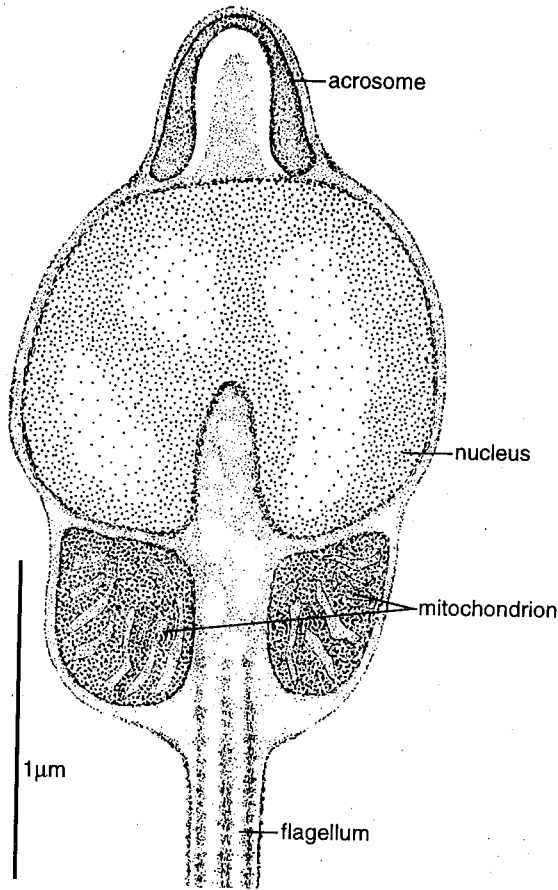
Fig. 3. *Branchiostoma lanceolatum.* Semidiagrammatic longitudinal section of the spermatozoon. From Jamieson (1991, fig. 4.3). After Wickstead, J.H. (1975), fig. 6.

C. Nucleus

The nucleus (Figs. 4A-C), subovoid and wider (1.8 μm) than long (1.0 μm), is shallowly concave anteriorly, and posteriorly is penetrated for about half its length by a narrow, anteriorly tapering, axial nuclear fossa (Figs. 4A-C, F, G). This fossa or canal is circular in cross-section and is about 0.12 μm wide at its midlength. A narrow perinuclear space (cisterna) is present between the inner and outer nuclear membranes and (Figs. 4G and J) expands as a unilateral vesicle in the mitochondrial region. It is possible that this vesicle is at least partly artefactual, however. The contents of the nucleus consist of electron-dense homogeneous material, with some evidence of a flocculent nature, but a considerable proportion of its volume consists also of tortuous lacunae, lacking chromatin, some of which extend to its surface (Figs. 4A-C, G-I) (Jamieson, 1984, 1991).
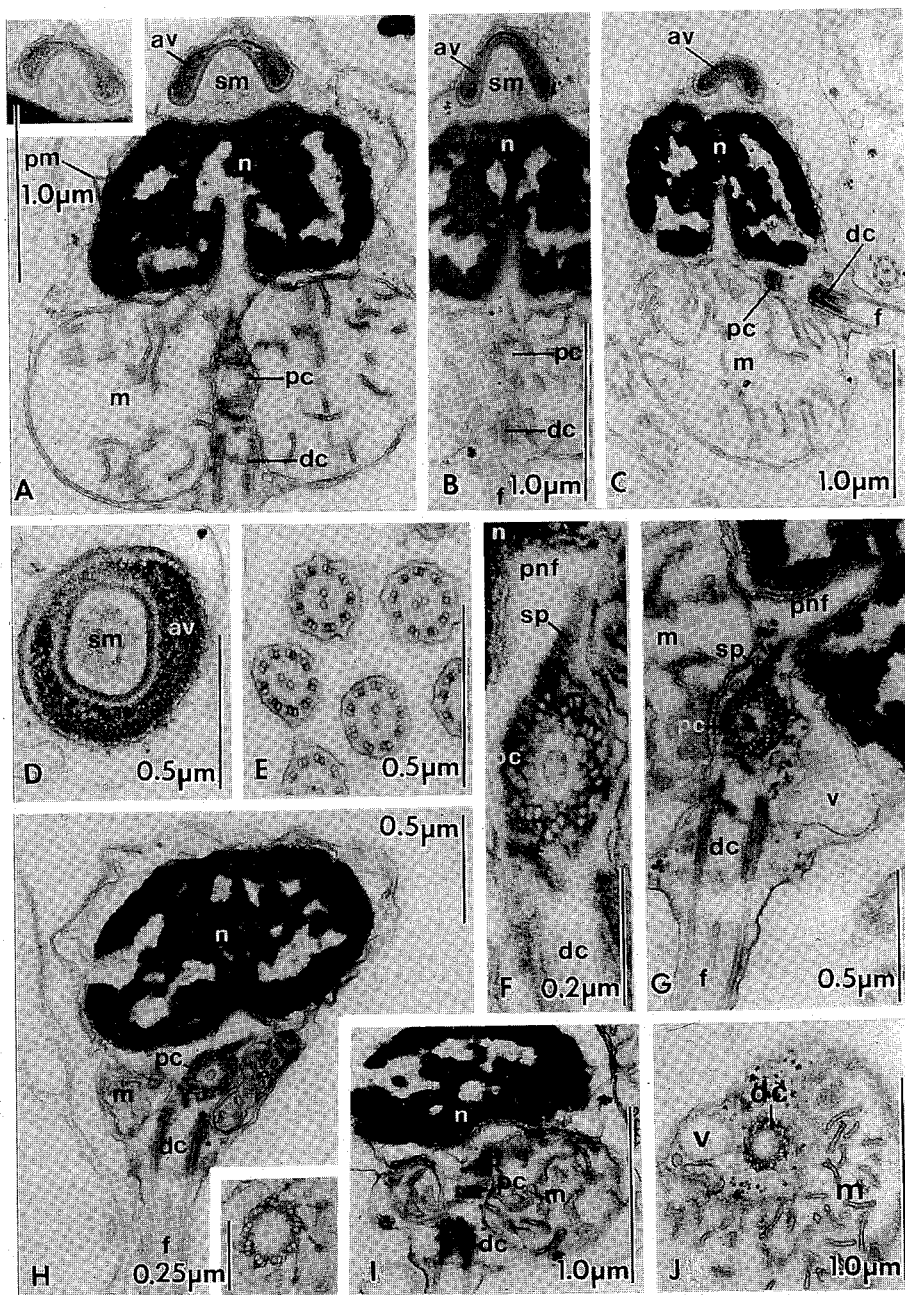
Fig. 4. *Branchiostoma moretonensis*. Spermatozoal ultrastructure. **A,B**: Longitudinal section (LS) of the heads of two spermatozoa, showing the bell-shaped acrosome vesicle (see also inset, Fig. A), subacrosomal material, nucleus with tubular posterior fossa and single mitochondrion enclosing the two mutually

During spermiogenesis in *Branchiostoma lanceolatum,* 'nuage' appears owing to the extensive release of nuclear fibrogranular material. The spermatocyte contain a lesser number of nuage aggregates than does the oocyte, the aggregates are of smaller size, and there is no typical chromatoid body (homologue of nuage). During spermogenesis the nuage is dispersed throughout the cytoplasm (Aizenshtadt and Gabaeva, 1987).

D. Midpiece

The mitochondrion is closely juxtaposed to, but does not indent, the nucleus (Figs. 4A-C, H, I). It has numerous, tortuous cristae, but apart from its shape appears unmodified. In *Branchiostoma moretonensis* and *B. floridae,* the mitochondrion is C-shaped in the transverse plane, with terminally tapering arms extending from a wide central region. The two centrioles are embraced by the arms, the proximal centriole being at midlength, the distal centriole at the posterior end of the mitochondrion (Figs. 4A, B, H, I). The C-shape is seen in cross-section in Fig. 4J, in which the axial distal centriole and a peripheral vesicle of the perinuclear cisterna are shown. It appears that the opening in the C is nearly, but not exactly, opposite one end of the transverse, proximal centriole (Jamieson, 1984, 1991).

The mitochondrion in *Branchiostoma lanceolatum,* though wider on one side, forms a complete ring surrounding the centrioles (Baccetti *et al.,* 1972), as confirmed by Wickstead (1975) in micrographs of cross-sections. It is also shown to be a single mass in *B. belcheri* by Chen *et al.* (1988).

E. Centrioles

Transverse sections of the proximal (Figs. 4F, G) and distal centriole (Fig. 4H inset, J) reveal the normal structure of nine skewed triplets but the proximal centriole, with its long axis transverse to the long axis of the sperm shows unusual

(contd.)

---

perpendicular centrioles. C: Spermatozoon with strongly eccentric axoneme. D: Transverse section (TS) of acrosome. E: TS of the '9+2' flagellar axoneme. F: L.S. centriolar region showing a TS of the proximal and an LS of the distal centriole (basal body) (detail from H). The proximal centriole has a core and a spur (striated rootlet) which extends into the nuclear fossa. G: LS further sperm showing rootlet. Note that in this plane the mitochondrion is seen only on one side of the centrioles, the other side being occupied by the vesicle of the perinuclear cisterna, and that the flagellum is at an angle to the nuclear axis. H: LS sperm, showing the centrioles and narrow arms of the C-shaped mitochondrion. Inset: TS of a distal centriole, showing anchoring apparatus. I: LS centriolar region approximately at right angles to F. J: TS distal centriole, showing the C-shaped mitochondrion, in the opening of which is a portion of the vesicle of the perinuclear cisterna. Abbreviations: av, acrosome vesicle; dc, distal centriole; f, flagellum; m, mitochondrion; n, nucleus; pc, proximal centriole; pm, plasma membrane; pnf, posterior nuclear fossa; sm, subacrosomal material; sp, spur-like process (striated rootlet) of proximal centriole; v, vesicle of perinuclear cisterna. From Jamieson (1991), fig. 4. After Jamieson, B.G.M. (1984), figs. 1–10.

features. Most distinctive in *Branchiostoma moretonensis*, but not apparently in other species, is a discrete central core (Figs. 4F-H) which, though not uncommon in basal bodies of sperm in various animal groups, is perhaps unprecedented in proximal centrioles. Less peculiar, but nevertheless remarkable, is a spur-like process (centriolar rootlet), oblique to the long axis of the sperm, extending from the anterior border of the proximal centriole into the base of the posterior nuclear fossa; the spur shows weak, transverse or slightly oblique striations; the posterior border of the centriole is also fringed by satellite-like structures (Figs. 4F-H) which abut on the distal centriole and there is some suggestion of continuity of the spur with these and with the distal centriole. A projection towards the distal centriole is also seen in *B. floridae*. The pointed anterior tip of this spur appears to attach to the nuclear membrane on one side of the basal region of the posterior nuclear fossa. The distal centriole lying in the long axis of the axoneme, which originates from it, and at right angles to the proximal centriole has satellite rays (presumably nine) radiating from the triplets. Each ray is inclined at about 45° to the radius passing through its triplet and ends peripherally in a swelling, though no terminal branches have been detected (Fig. 4H inset, J). The distal centriole is surrounded by many putative glycogen granules and these are sparsely present between other organelles and under the plasma membrane (Jamieson, 1984, 1991). No equivalent of the core to the proximal centriole has been described for, nor is it visible in published micrographs of, the centriole of *B. lanceolatum*; and it is absent from *B. floridae* (Holland and Holland, 1989) and probably from *B. belcheri*. There is some suggestion of the spur extending from the proximal centriole into the nuclear fossa in *B. lanceolatum* (fig. 2 of Baccetti *et al.*, 1972); clearly visible in micrographs of *B. floridae*, it is probably to be regarded as a characteristic of cephalochordate sperm.

F. Flagellum

The axoneme, arising from the distal centriole, has nine peripheral doublets, each with two dynein arms and two central singlets. All microtubules are hollow. In some transverse profiles a bridge between doublets 5 and 6 is apparent (Fig. 4E). There are no accessory microtubules. In longitudinal sections passing transversely through the proximal centriole (Fig. 4G), the flagellum appears strongly tilted, relative to the axis of the posterior nuclear fossa. This is more evident in advanced spermatids when they are loosely connected in tetrads than in mature sperm, but is sometimes still pronounced in the latter (Fig. 4C) (Jamieson, 1984, 1991). The flagellum is 40 μm long in *Branchiostoma lanceolatum* in which it is tilted at 135° relative to the axis of the endonuclear canal; its last 4 μm is less than 0.1 μm wide owing to loss first of the nine doublets and then of the two central (Baccetti *et al.*, 1972). In the terminal few micrometres of the axoneme there is a reduced number of microtubules (Holland and Holland, 1989).

G. Sperm Proteins

Saperas *et al.* (1994) isolated and characterized the chromosomal proteins from the nucleus of the sperm of *Branchiostoma floridae*. The major protein component of the sperm-chromatin of a lancelet is a highly specialized protamine-like (PL) protein that has structural and compositional features similar to those of PL-III from bivalve molluscs. This contrasted with the chromatin of the sperm of the lamprey which has a structural arrangement and protein composition (histones) very similar to that found in the somatic cells of all eukaryotic organisms. It was found that among the deuterostomes, chromosomal protein variability is considerably greater in representatives of the Phylum Chordata than in echinoderms.

Chiva *et al.* (1995) reviewed studies on sperm nuclear basic proteins (SNBPS) carried out in several groups of deuterostomes (tunicates, cephalochordates, agnathans, chondrichthyans, and osteichthyans). Four general conclusions were drawn: (1) There are two main types of SNBPs: (a) proteins similar to histones but with enhanced basicity (the 'PL', protamine-like, protein noted above) and (b) very specialized proteins, there named 'P'; (2) The 'PL' proteins have appeared independently several times during deuterostome evolution; (3) In some cases, 'P' proteins may have arisen from 'PL' proteins, but other origins cannot be ruled out for a particular P protein; (4) The classical evolutionary point of view about the appearance of protamines (histones to intermediate proteins to protamines) was reinterpreted as histones to PL proteins to P proteins. This transition seems to have repeatedly occurred during the evolution of different groups of deuterostomes. Thus, there does not appear to have been a continuous evolutionary line relating the SNBPs of echinoderms with the bony fish and tetrapod protamines. In cephalochordates, they investigated *Branchiostoma floridae*. They demonstrated a main protein component (Pceph) accompanied by small quantities of residual histones. Pceph has an electrophoretic mobility similar to that of tunicate protein Psty2. Its size, estimated from the electrophoretic behaviour, is of about 120 amino acid residues and its compositional analysis is very simple, consisting of only six different types of amino acid residues, with arginine, lysine, alanine and serine as the most abundant. In size and amino acid composition this protein is more similar to SNBPs of bivalve molluscs than to other deuterostomes. Furthermore, the N-terminal sequence contains three alternating arginine-serine motives and such alternation of basic (R/K) and phosophorylatable residues (S/T) has also been demonstrated for several gastropod molluscs, also in birds and mammals, but is not present in the "typical protamines" of bony fish. In conclusion, *Branchiostoma* Pceph shares some characteristics with typical protamines, such as low amino acid diversity, its basic composition and presence of serine. However, Pceph has distinctive characteristics: lysine and argine present in approximately equal proportions (24.7 and 25.3, per cent, respectively) whereas protamines consist almost exclusively of arginine; secondly, Pceph has an alanine-rich composition (21.7 per cent) not found in protamines; and finally, Pceph is much larger than typical protamines and

contains the repetitive arg-ser motifs unlike bony fish protamines (Chiva *et al.*,1995, and references therein).

IV. CEPHALOCHORDATE SPERM: SUMMARY

Close resemblance of the spermatozoon of *Branchiostoma moretonensis* (Jamieson, 1984, 1991) to that of *B. lanceolatum* (Baccetti *et al.*, 1972; Wickstead, 1975), *B. belcheri* (Lin *et al.*, 1987) and *B. floridae* (Holland and Holland, 1989), permits recognition of a cephalochordate sperm type in so far as this can be established on four species. The characteristics of this are: a slight modification of the pleisosperm ('primitive') form; a bell-shaped acrosome; diffuse subacrosomal material not structured as an acrosome rod; subovoidal nucleus with shallow anterior concavity, deep posterior fossa ('endonuclear canal' of Baccetti *et al.*, 1972), and condensed but lacunate chromatin; single asymmetrical, postnuclear ring-shaped or C-shaped mitochondrion encircling the centrioles; mutually perpendicular proximal and distal centrioles of the triplet type, with the distal forming the basal body of the flagellum; flagellum tilted relative to the longitudinal axis (and endonuclear canal) of the nucleus; a '9+2' axoneme with hollow tubules and both dynein arms present on the doublets; and scattered glycogen granules, numerous around the distal centriole (Jamieson, 1984, 1991).

V. GONADAL HORMONES AND FERTILIZATION

The contents and multiple forms of gonadotropin-releasing hormone (GnRH) in *Branchiostoma belcheri* during the breeding season were studied by Yin *et al.* (1994), using radioimmunoassay and high performance liquid chromatography. The GnRH level in females decreased significantly at 12 h after spawning and then returned to the initial level. No obvious change was observed after spermiation in males. There are two forms of GnRH in amphioxus gonads. Changes of GnRH content during the amphioxus breeding cycle are similar to the changes during the mammalian reproductive process in both sexes. It was suggested that mammalian GnRH played an important role in the development of gonads and the reproductive process, especially in female amphioxus (Yin *et al.*, 1994).

Experimentally administered hypothalamic leutinizing hormone-releasing hormone (LHRH)-A results in obvious changes of spermatogenic cells in the testes of amphioxus. It initiated type A spermatogonia to divide into type B spermatogonia, and primary spermatocyte developed into secondary spermatocyte and spermatids. Furthermore, LHRH-A may also promote the release of spermatozoa (Fang, 1985).

This account of fertilization will be confined to the most recent investigation, by Holland and Holland (1992). The male and female pronuclei meet at 16 min after insemination. In the unfertilized egg, although mitochondria, as well as yolk granules, are evenly distributed (both are absent only from the egg cortex and meiotic spindle), the mitochondria in the animal third have a more electron-lucent

matrix than those elsewhere. The cortex of the unfertilized egg is occupied chiefly by cortical granules, and the subcortical cytoplasm in the vegetal third includes sheets of dense granules interleaved with cisternae of endoplasmic reticulum. Within 45 sec after insemination, (1) the fertilizing sperm enters, in the animal hemisphere, (2) yolk granules become patchily distributed around the newly entered sperm, (3) cortical granule exocytosis occurs, and (4) the sheets of dense granules and associated endoplasmic reticulum aggregate with numerous mitochondria into whorls in a yolk-free zone near the vegetal pole. These whorls are the vegetal pole plasm, which is segregated into a single blastomere at each cleavage and possibly plays a role in germ line determination. Within 2 min after insemination, the zone of cytoplasm near the animal pole with patchily distributed yolk has enlarged, and the male pronucleus has migrated to the vicinity of the vegetal pole and formed an aster, at the centre of which a few mitochondria are aggregated. In cephalochordates, unlike ascidians, there is no obvious widespread ooplasmic segregation or translocation of cytoplasm from animal to vegetal pole accompanying the movement of the sperm. Between 6 and 16 min, (1) the zone of cytoplasm with patchily distributed yolk enlarges to occupy about the animal third of the egg, (2) the female pronucleus forms by fusion of chromosome-containing vesicles and migrates vegetally, leaving a track of yolk-poor cytoplasm, and (3) the male pronucleus, surrounded by increasing numbers of mitochondria, migrates to meet the female pronucleus just above the equator. In contrast to current opinion, lancelets differ from ascidians both in having a vegetal pole plasm and in lacking marked ooplasmic segregation (Holland and Holland, 1992).

VI. SPERM AND LOPHOPHORATE-DEUTEROSTOME EVOLUTION

Jamieson (1991) suggested that a protostome stock ancestral to the lophophorates probably had an ect-aquasperm slightly modified, if at all, from the plesiosperm morphology, with the usual complement of cap-like acrosome, subspheroidal nucleus, several discrete rounded mitochondria, two centrioles, an anchoring satellite apparatus arising from the distal centriole, and a '9+2' flagellum. The Bryozoa have ent-aquasperm little modified from the plesiosperm (lacking nuclear membranes and an acrosome) in Bugula or highly modified in others. Phoronids have highly modified sperm, probably as a modification for expulsion to the exterior in a spermatophore.

Further lophophorates, the brachiopods, retain a primitive fertilization biology, with ect-aquasperm, or (Terebratulina) acquire ent-aquasperm. Their aquasperm foreshadow special features of those of enteropneusts, echinoderms and cephalochordates. These similarities are presumably symparamorphies indicative of close relationships but the possibility exists that brachiopods have played a more central role in the origin of deuterostomes than has been formerly supposed. Certainly sperm structure supports the view that the brachiopods originated close to the branching point between the protostome and deuterostome lines (Afzelius and Ferraguti, 1978). In the inarticulate brachiopods, which as the plesiomorph sister-

group would be structurally nearer this branching, the sperm of *Lingula* shows remarkable deuterostome features: it approaches the cephalochordate *Branchiostoma* in shallow depression of the anterior face of the nucleus and well-developed subacrosomal material, while resembling the enteropneusts (*Saccoglossus, Saxipendium*) in the inflated, roughly heart-shaped form of the acrosome, embedment of this in periacrosomal material (both apomorphies or perhaps symparamorphies) and the plesiomorph retention (in *Saccoglossus*, at least) of separate mitochondria; *Discinisca tenuis* has a basically similar sperm. Another inarticulate, *Crania*, has a cap-like acrosome with abundant subacrosomal material, much as in *Branchiostoma*, but differs from the latter in the independent mitochondria. Finally, *Terebratulina*, in an order of the articulate brachiopods which developed latest in the fossil record of the phylum (Williams and Rowell, 1965), has an ent-aquasperm which appears plesiomorphic in most features, but, as a symparamorphy with the echinoderms and cephalochordates, has a single, annular mitochondrion, seen also in *Kraussina rubra*.

In the enteropneusts, we saw above a sperm which has features foreshadowed in *Lingula*. The heart-shaped acrosome with periacrosomal material well developed laterally but less so subacrosomally, resting on a broad depression of the nucleus is strikingly precursory to echinoderm and appendicularian (larvacean) urochordate sperm with which the separate mitochondria are a plesiomorph contrast. In pterobranchs, which are generally considered representative of the lophophorate ancestry of echinoderms and of protochordates, the only investigated species, *Rhabdopleura normani*, has highly modified sperm (Lester, 1988) which cannot be construed as basic for the lophophorate-deuterostome lineages (see Chapter 4).

In a protoechinoderm derived from a putative hemichordate-like ancestry shared with the protochordates, Jamieson (1991) envisaged a spherical acrosome embedded in periacrosomal material and underlain by (but not enclosed in) an anterior nuclear (subacrosomal) fossa, which was developed as an adaptation primarily for housing the actomere; a slight posterior nuclear (centriolar) fossa may have been present and, as a symparamorphy with some cephalochordates and with *Terebratulina*, an annular mitochondrion. Fusion of mitochondria, though as a C-shape, is also seen in cephalochordates and in the urochordates.

The phyologenetic position of echinoderms in relation to speramtozoal morphology is discussed in Chapter 5.

The hemichordate ancestry (cephalodiscus-like but with simpler sperm) of the echinoderms is envisaged as giving rise to a sister-group of the echinoderms, those deuterostomes with gill slits, including extant enteropneusts and rhabdopleuran pterobranchs, the urochordates and the cephalochordates. Appendicularian sperm resemble echinoderms in embedment of the acrosome (otherwise heart-shaped as in enteropneusts) in the anterior nuclear fossa and similarly have fused the mitochondria, though as a C-shape. A urochordate ancestor has therefore been envisaged with a sperm resembling that of Appendicularia except in the larger nucleus (apomorphically oligopyrene in Appendicularia), shorter centriolar fossa and, as in enteropneusts,

cephalochordates and the ascidiacean *Ciona* (spermatids and apparently spermatozoa), two centrioles. In the Ascidiacea, the mitochondrion has become perinuclear and the acrosome vesicle is reduced (Jamieson, 1991).

Branchiostoma sperm give no clear indications of the commonly accepted origins of the cephalochordates from a stock shared with the urochordates after emergence of the echinoderms. It shows resemblances to the sperm of both the brachiopods and the echinoderms.

In fact, as Jamieson (1991) noted, the cephalochordate sperm falls well within the known variation for brachiopods: having a cap-like acrosome with subacrosomal material (as in *Crania*), and an annular mitochondrion (as in *Terebratulina*). This is in striking contrast with the inflated acrosome, embedded in periacrosomal material, seen in echinoderms, hemichordates and appendicularian urochordates. From a purely spermatological standpoint, it would therefore be tempting to derive cephalochordates from a proto-cephalodiscus below the branching point of the echinoderms. This would necessitate the unorthodox propositions that pharyngotremy in notochordates (cephalochordates and, if monophyletic with them, craniates) is an independent symparamorphy compared with enteropneusts and urochordates or, alternatively, that echinoderms have lost pharyngeal perforations during the profound modifications which they have clearly undergone from the presumed cephalodiscus ancestry (see Jefferies, 1979, 1981). If we accept the conventional view of a close affinity of cephalo- and other chordates with the enteropneust-urochordate assemblage, i.e. monophyly of chordates *sensu lato*, it will be necessary to assume that the inflated, embedded acrosome has occurred in parallel (by symparamorphy) in enteropneusts and appendicularians relative to echinoderms, or that this condition is basic to echinoderms and chordates and that cephalochordate sperm have secondarily approached the plesiosperm condition of a cap-like acrosome. Greater secondary simplification is well demonstrated by the teleosts and the ectoprocts, at least one species of *Bugula* lacking an acrosome, but a functional reason for deflation of the *Branchiostoma* acrosome, if secondary, is elusive (Jamieson, 1991).

Branchiostoma sperm resemble those of some echinoderms in a number of respects beyond mere similarity of 'primitive' sperm. These resemblances include well-developed periacrosomal material, though this is subacrosomal in cephalochordates, as noted by Afzelius (1977); the commonly asymmetrical ring-shaped or sometimes (*Cucumaria lubrica* (Atwood and Chia, 1974), *Branchiostoma moretonensis, B. floridae*) C-shaped single mitochondrion; and the rootlet arising from the proximal centriole and extending into the nuclear fossa (shared with holothuroids) and the often tilted emergence of the flagellum (Jamieson, 1991).

It is noteworthy, in view of the above apomorphies shared between cephalochordate and echinoderm sperm, that Jefferies (1979, 1981) considers that cephalochordates are less closely related to vertebrates than are urochordates. In vertebrates and urochordates, the locomotory muscles are innervated by nerves from the dorsal nerve cord or the brain (a shared derived character according to Jeffries) while in cephalochordates and echinoderms, as we have seen, skeletal

muscles send processes to the dorsal nerve cord or its equivalent. Even if the latter is a symplesiomorphic condition it distances cephalochordates from the urochordate-vertebrate assemblage in this respect.

If urochordates have an especially close relationship with vertebrates we might speculate that the modified sperm typical of basal gnathostomes (only the Actinopterygii having sperm with the 'primitive' sperm morphology) have been inherited from a common urochordate-vertebrate ancestor or that the complex form represents a common genetic and phylogenetic propensity independently realized in each group. Against this proposition is the view, on the evidence of *Oikopleura*, that the ground plan for the urochordate spermatozoon is not complex and that complexity of the sperm in 'lower' fish could not be a retention of a urochordate condition. The spermatozoon of the ancestral urochordate would then be envisaged as having the following plesiomorphies: a fully developed acrosome; a compact, subspheroidal or perhaps slightly cylindrical nucleus; a discrete midpiece, containing the mitochondrial material, situated behind the nucleus and encircling the base of the flagellum; a proximal and distal centriole; and external fertilization. Fusion of the spermatid mitochondria to form a single mitochondrion and its asymmetrical location in the mature sperm lateral to, and partly embracing the flagellum is then seen as an apomorphy of the basal urochordate but, as this condition of the mitochondrion is seen in cephalochordates (and echinoderms), it is conceivable that it was inherited from a common ancestor (Jamieson, 1991).

Alternatively, similarities of the *Branchiostoma* sperm to those of brachiopods and echinoderms may be symparamorphies, parallelisms by virtue of relationship, which would not necessitate a closer relationship of cephalochordates to these groups than to vertebrates (Jamieson, 1991). If also we accept the by no means unequivocal view that the sperm of the ancestral vertebrates were ect-aquasperm of a simple type, we may attempt to reconstruct its morphology and compare this with that of the *Branchiostoma* sperm. Holland and Holland (1989) correctly noted similarities of the *Branchiostoma* acrosome (bell-shaped with granular subacrosomal material) to that of the hagfish (Jespersen, 1975), and Jamieson (1991) suggested that these might be features of an ancestral vertebrate ect-aquasperm. A rounded nucleus, as in *Branchiostoma*, and unlike the elongate nucleus of agnathans, chondrichthyans and Chondrostei could be envisaged. The midpiece could be ascribed several, separate mitochondria, as parsimoniously demanded by the multiple mitochondria of these groups, fusion as a single annulus in, for instance, salmoniforms being a symparamorphy of these with *Branchiostoma* and of this with echinoderms, urochordates and some brachiopods, and not a plesiomorphic state for vertebrate sperm. Two centrioles, as in most fish sperm, and a satellite apparatus which is present at least as a propensity in fish (realized most fully in advanced neopterygians, the atheriniforms) and a simple axoneme were suggested as other attributes of the basic vertebrate sperm. While a plesiosperm was considered likely in ancestral protochordates, it was nevertheless suggested that ancestral fish might have had modified ect-aquasperm or, less likely, internal fertilization (Jamieson, 1991).

ACKNOWLEDGEMENTS

REFERENCES

Afzelius, B. A. (1977). 'Spermatozoa and spermatids of the crinoid *Antedon petasus*, with a note on primitive spermatozoa from deuterostome animals', *Journal of Ultrastructure Research*, **59**, 272–281.

Afzelius, B. A., and Ferraguti, M. (1978). 'Fine structure of brachiopod spermatozoa', *Journal of Ultrastructure Research*, **63**, 308–315.

Aizenshtadt, T. B., and Gabaeva, N. S. (1987). 'Perinuclear bodies (nuage) in the developing germ cells of the lancelet *Branchiostoma lanceolatum*', *Tsitologiya*, **29**, 137–141.

Atwood, D. G., and Chia, F. S. (1974). 'Fine structure of an unusual spermatozoon of a brooding sea cucumber, *Cucumaria lubrica*', *Canadian Journal of Zoology*, **52**, 519–523.

Baccetti, B., Burrini, A. G., and Dallai, R. (1972). 'The spermatozoon of *Branchiostoma lanceolatum* L', *Journal of Morphology*, **136**, 211–226.

Chen, D., Zhao, X., Song, X., and Song, Y. (1988). 'The fine structure of the spermatozoa of the amphioxus (*Branchiostoma belcheri tsingtaoensis*)', *Acta Zoologica Sinica*, **34**, 106–109.

Chiva, M., Saperas, N., Caceres, C., and Ausio, J. (1995). 'Nuclear basic proteins from the sperm of tunicates, cephalochordates, agnathans and fish', in *Advances in Spermatozoal Phylogeny and Taxonomy*, Vol. 166, *Mémoires du Muséum National d'Histoire Naturelle* (Eds. B. G. M. Jamieson, J. Ausio, and J.-L. Justine), pp. 501–514.

Fang, Y. (1985). 'The effects of the hypothalamic LHRH analog on the spermatogenesis of *Amphioxus*: A study of histology and ultrastructure', *Acta Zoologica Sinica*, **31**, 319–323.

Fang, Y. Q. (1991). 'Further study of the ultrastructure of the Sertoli cell of the testis of Amphioxus', *Acta Zoologica Sinica*, **37**, 123–126.

Franzén, Å. (1956). 'On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates', *Zoologiska Bidrag från Uppsala*, **31**, 355–482.

Holland, L. Z., and Holland, N. D., (1992). 'Early development in the lancelet (equals amphioxus) *Branchiostoma floridae* from sperm entry through pronuclear fusion: Presence of vegetal pole plasm and lack of conspicuous ooplasmic segregation', *Biological Bulletin*, **182**, 77–96.

Holland, N. D., and Holland, L. Z. (1989). 'The fine structure of the testis of a lancelet (equals amphioxus), *Branchiostoma floridae* (Phylum Chordata: Subphylum Cephalochordata equals Acrania)', *Acta Zoologica*, **70**, 221–220.

Jamieson, B. G. M. (1984). 'Spermatozoal ultrastructure in *Branchiostoma moretonensis* Kelly, a comparison with *B. lanceolatum* (Cephalochordata) and with other deuterostomes', *Zoologica Scripta*, **13**, 223–229.

Jamieson, B. G. M. (1986). 'Onychophoran-euclitellate relationships: evidence from spermatozoal ultrastructure', *Zoologica Scripta*, **15**, 141–155.

Jamieson, B. G. M. (1991). *Fish Evolution and Taxonomy: Evidence from Spermatozoa*, Cambridge University Press, Cambridge. pp. 319.

Jefferies, R. P. S. (1979). 'The origin of chordates — a methodological essay', in *The Origin of Major Invertebrate Groups*, Vol. Systematics Association Special Volume (Ed. M. R. House), Academic Press, London, New York, pp. 443–477.

Jefferies, R. P. S. (1981). 'In defence of calcichordates', *Zoological Journal of the Linnean Society*, **173**, 351–396.

Jespersen, Å. (1975). 'Fine structure of spermiogenesis in eastern Pacific species of Hagfish (Myxinidae)', *Acta Zoologica (Stockholm)*, **56**, 189–198.

Lester, S. M. (1988). 'Ultrastructure of adult gonads and development and structure of the larva of *Rhabdopleura normani* (Hemichordata: Pterobranchia)', *Acta Zoologica (Stockholm)*, **69**, 95–109.

Lin, J., Chen, X., and Wang, D. (1987). 'Studies on ultrastructure in spermatogenesis of amphioxus (*Branchiostoma belcheri* Gray)', *Oceanologia et Limnologia Sinica*, **18**, 432–436.

Nelson, J. S. (1984). *Fishes of the World*, second edition, John Wiley and Sons, New York.

Rouse, G. W., and Jamieson, B. G. M. (1987). 'An ultrastructural study of the spermatozoa of the polychaetes *Eurythoe complanata* (Amphinomidae), *Clymenella* sp. and *Micromaldane* sp. (Maldanidae), with definition of sperm types in relation to reproductive biology', *Journal of Submicroscopic Cytology*, **19**, 573–584.

Saperas, N., Chiva, M., Ribes, E., Kansinsky, H. E., Rosenberg, E., Youson, J. H., and Ausio, J. (1994). 'Chromosomal proteins of the sperm of a cephalochordate (*Branchiostoma floridae*) and an Agnathan (*Petromyzon marinus*): Compositional variability of the nuclear sperm proteins of deuterostomes', *Biological Bulletin*, **186**, 101–114.

Welsch, U., and Fang, Y. Q. (1996). 'The reproductive organs of *Branchiostoma*', *Israel Journal of Zoology*, **42**, 183–212.

Wickstead, J. H. (1975). 'Chordata: Acrania (Cephalochordata)', in *Reproduction of Marine Invertebrates. II. Ectoprocts and Lesser Coelomates* (Eds. A. C. Giese and J. S. Pearse), Academic Press, New York, pp. 283–319.

Williams, A., and Rowell, A. J. (1965). 'Brachiopod anatomy. Evolution and phylogeny', in *Brachiopoda*, Vol. 1, Part H. *Treatise on Invertebrate Paleontology* (Ed. R. C. Moore), The Geological Society of America, Inc. and the University of Kansas Press, pp. H6-57. H164–199.

Yin, H., Zhang, C. L., Wang, H., and Shen, W. B. (1994). 'Studies on amphioxus GnRH during breeding season', *Acta Zoologica Sinica*, **40**, 63–68.

Young, J. Z. (1981). *The Life of Vertebrates*, third edition, Clarendon Press, Oxford.