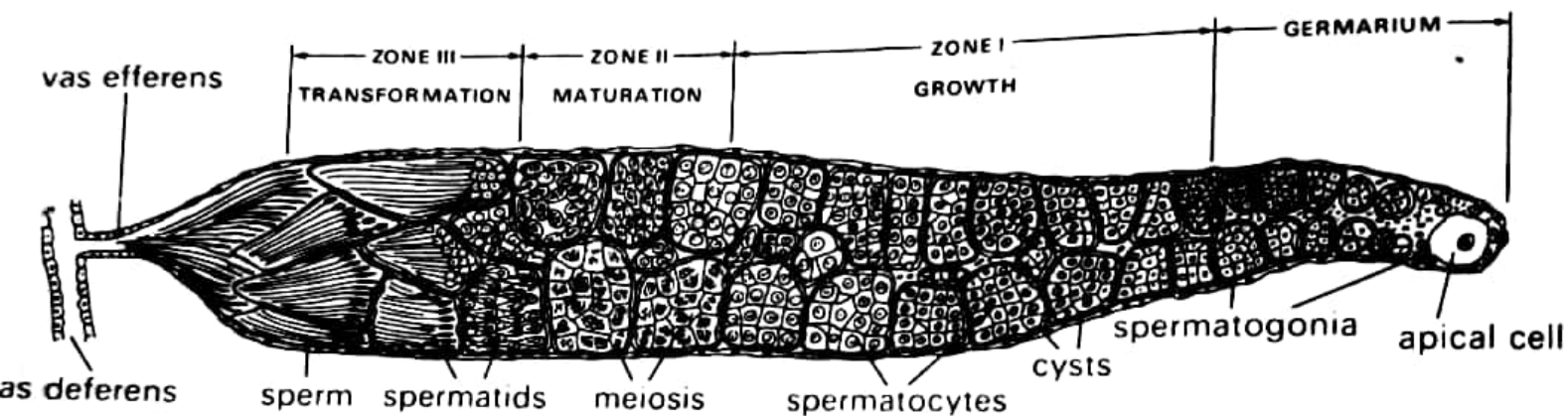


value for the female (p. 375), or they may accer---

## 15.2 Spermatogenesis

At the distal end of each testis follicle is the germarium, in which the germ cells divide to produce spermatogonia (Fig. 212). In Orthoptera, Dictyoptera, Homoptera and Lepidoptera, the spermatogonia probably obtain nutriment from a large apical cell with which they have cytoplasmic connections, while in Diptera and Heteroptera (Bonhag and Wick, 1953) there is a comparable apical complex consisting of a syncytium with numerous nuclei. In Diptera the transfer of mitochondria from this complex to the spermatogonia has been observed (Carson, 1945).

These apical connections are soon lost and the spermatogonia associate with other cells which form a cyst around them (Fig. 212). One, or sometimes more, spermatogonia are enclosed in each cyst and, in *Prionoplus*, there are initially two cyst-cells round each spermatogonium. The cyst-cells may be spermatogonia which lack adequate nutrition and therefore fail to continue their normal development. They may supply nutriment to the developing sperm and, in *Popillia* (Coleoptera), the sperm at one stage



**Fig. 212** Diagram of a testis follicle showing the stages of development of the sperm (from Wigglesworth, 1965)

have their heads embedded in the cyst-cells, this perhaps facilitating the transfer of nutrients (J. Anderson, 1950). In Heteroptera large cells with irregular nuclei, called trophocytes, are scattered amongst the cysts.

As more spermatogonia are produced they push those which have developed earlier down the follicle; a range of development is therefore present in each follicle with the earliest stages distally in the germarium and the oldest in the proximal part of the follicle adjacent to the vas deferens. Three zones of development are commonly recognised below the germarium (Fig. 212):

I a zone of growth, in which the primary spermatogonia, enclosed in cysts, divide and increase in size to form spermatocytes

II a zone of maturation and reduction, in which each spermatocyte undergoes the two meiotic divisions to produce spermatids

III a zone of transformation, in which the spermatids develop into spermatozoa, a process known as spermiogenesis.

Since, in general, all the cells in a cyst are derived from a single primary spermatogonium they remain synchronised in their subsequent development. The number of sperm which a cyst ultimately produces depends on the number of spermatogonial divisions which occur and this is fairly constant for a species. In Acrididae there are between five and eight spermatogonial divisions and *Melanoplus*, which typically has seven divisions before meiosis, usually has 512 sperm per cyst. Normally four spermatozoa are produced from each spermatocyte, but in many coccids the spermatids which possess heterochromatic chromosomes degenerate so that only two sperm are formed from each spermatocyte and 32 are present in each cyst (Nur, 1962). In *Sciara* (Diptera) only one spermatid is formed from each spermatocyte because of the unequal distribution of chromosomes and cytoplasm which occurs at the meiotic divisions (Phillips, 1966).

Biochemical changes occur in the course of spermatogenesis. The repeated cell divisions entail the synthesis of large amounts of DNA and RNA, but the synthesis of DNA stops before meiosis occurs, while RNA synthesis continues into the early spermatid. Subsequently no further synthesis occurs and the RNA is eliminated first from the nucleus and then from the cell as the nucleus elongates. The reduction in RNA synthesis is associated with a rise in the production of an arginine-rich histone which forms a complex with DNA stopping it from acting as a primer for RNA synthesis. It is suggested that this mechanism insulates the genetic material during transit from one generation to the next (Bloch and Brack, 1964; Das, *et al.*, 1964; Muckenthaler, 1964).

The time taken for the completion of spermatogenesis varies, but in *Melanoplus* the period is about 28 days, the spermatogonial divisions occupying eight or nine days and spermiogenesis ten (Muckenthaler, 1964). In most insects meiosis is complete before the final moult and in insects which do not feed as adults spermatogenesis may be complete before the adult emerges (Fig. 213).

### 15.2.1 Structure of mature spermatozoa

The mature sperm of most insects are filamentous in form, often about 300  $\mu\text{m}$  long and less than a micron in diameter, while the sperm of *Drosophila* (Diptera) may be as much as 1.7 mm long. The head and tail of the sperm are of approximately the same diameter (Fig. 214). The cell wall of the sperm is a typical three-layered membrane, but in some

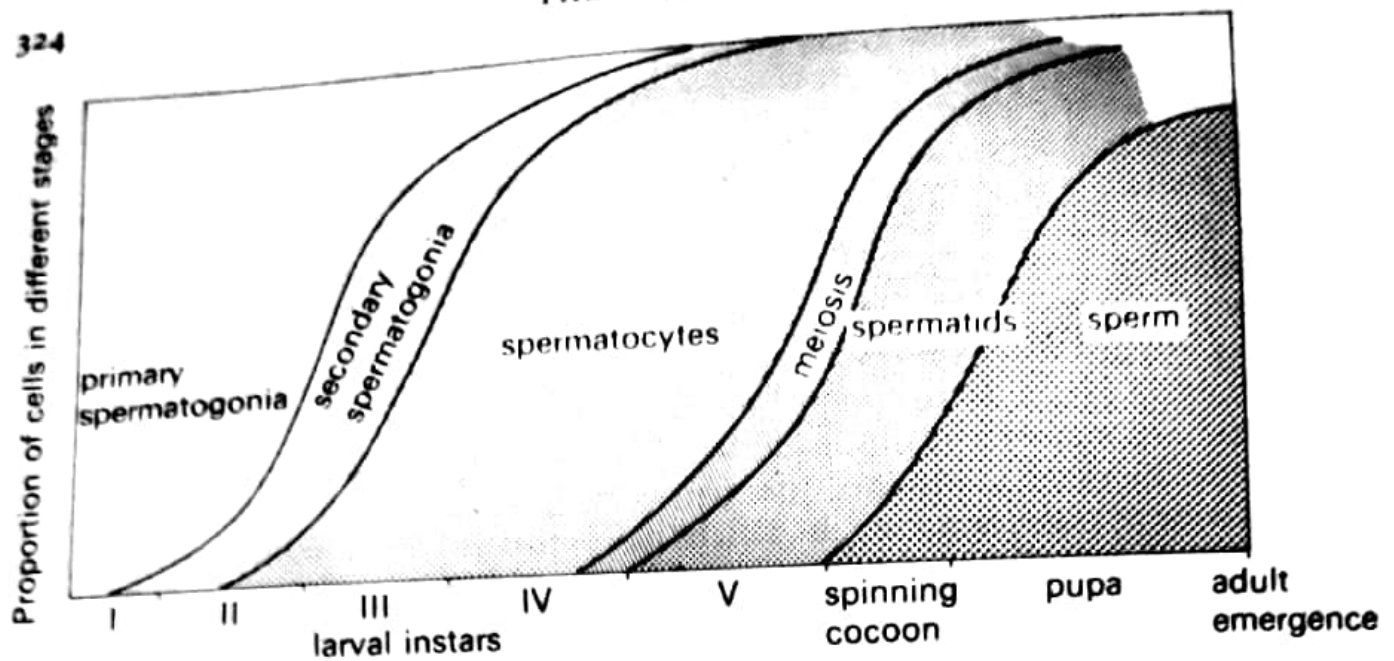
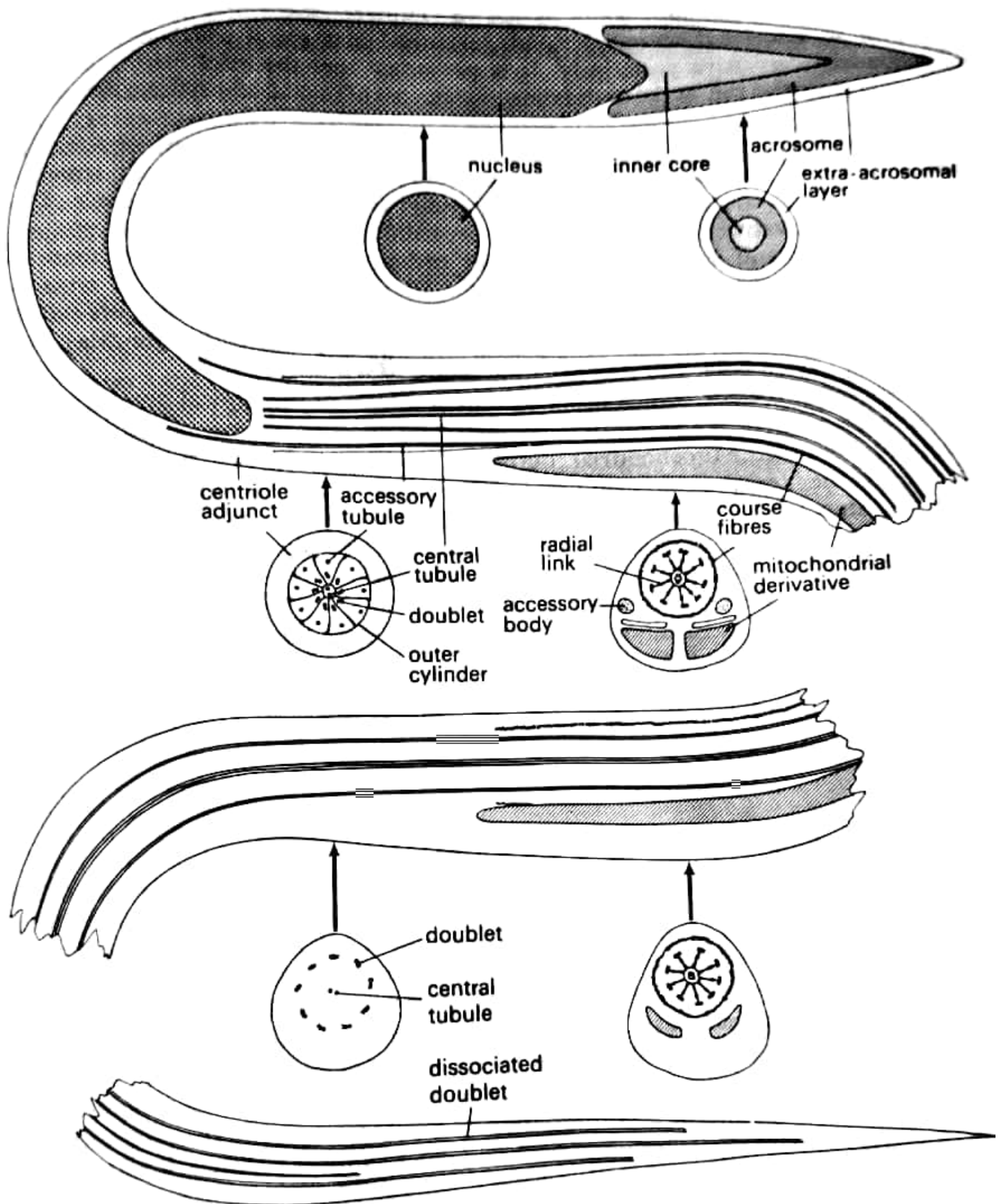


Fig. 213 Spermatogenesis in *Bombyx* showing the proportion of germ cells in each stage at different periods during the development of the insect (after Engelmann, 1970)

species it is coated on the outside by a layer of glycoprotein known as the glycocalyx. In fleas this is about 13 nm thick, and in grasshoppers about 30 nm; the cell membrane proper is about 10 nm thick. The glycocalyx is made up of rods at right angles to the surface of the sperm. Lepidopteran sperm have a series of projections running along their length. These projections are made up of thin laminae stacked parallel with the surface membrane. They become rearranged in the ejaculatory duct to form a complete coating all round the sperm (see Baccetti, 1972).

The greater part of the head region is occupied by the nucleus (Fig. 214). In the mature sperm of most species the nucleus is homogeneous in appearance, but sometimes, as in the grasshopper *Chortophaga*, it has a honeycomb appearance. The DNA is apparently arranged in strands parallel with the long axis of the sperm. In front of the nucleus is the acrosome. This is a membrane bound structure of glycoprotein with, in most insects, a granular extra-acrosomal layer and an inner rod or cone. Sperm of Neuroptera have no acrosome and occasional species with no acrosome occur in other orders. The acrosome is probably concerned with attachment of the sperm to the egg and possibly also with lysis of the egg membrane, thus permitting sperm entry. Mature sperm do not have a centriole, although this is present during spermiogenesis, but most insect sperm do have a centriole adjunct. This consists of a mass of ribonucleoprotein, usually in granular form, which develops at the back end of the nucleus. It is best developed in the spermatid and progressively declines in extent as development proceeds.

Immediately behind the nucleus the axial filament, or axoneme, arises. In most cases this consists of two central tubules with a ring of nine doublets and nine accessory tubules on the outside (Fig. 214). The central tubules are surrounded by a sheath and are linked radially to the doublets. Some unusual exceptions to this 9 + 9 + 2 arrangement occur. Protura have 12 or 14 doublets with no other tubules, while accessory tubules are lacking in Collembola, Japygidae, Mecoptera and Siphonaptera. Psocoptera, Mallophaga, Anoplura, Thysanoptera and many bugs have two axial



**Fig. 214** Diagram showing the structure of a sperm in longitudinal section with representative transverse sections at the points shown. The anterior end shown in the upper half of the figure comprises only a small proportion of the total length



filaments in the sperm tail, while in *Sciara* (Diptera) there are 70–90 doublets, each with an associated accessory tubule, arranged in a spiral which encloses the mitochondrial derivative posteriorly. It is presumed that the axial filament or the equivalent structure causes the undulating movements of the tail which drive the sperm forwards.

The sperm of Pterygota have two mitochondrial derivatives (p. 329) which flank the axial filament. Within these the cristae become arranged as a series of lamellae projecting in from one side of the derivative and at right angles to its long axis. The matrix of the derivative is occupied by a paracrystalline material. Sperm of Mecoptera and Trichoptera and species of some other orders have only one mitochondrial derivative, while phasmids have none at all. In this case respiration is entirely anaerobic. More or less normal mitochondria persist in the sperm of Apterygota except that they fuse together and become elongated. There are three such mitochondria in the sperm tail of Collembola, and two in Diplura and Machilidae.

The sperm of coccids, which occur in bundles, lack all the typical organelles. In *Parlatoria* the nucleus is apparently represented by an electron opaque core with no limiting membrane. Mitochondrial derivatives are absent, but Robison (1966) suggests that the homogeneous cytoplasm of the sperm is a mitochondrial product and serves as a store of energy so that mitochondria themselves are not necessary. Each sperm has 45–50 microtubules about 20 nm in diameter in a spiral round a central mass of chromatin. These run the whole length of the sperm and may be concerned with its mobility, replacing the typical axial filament.

In Kalotermitidae and Rhinotermitidae there is no flagellum at all. The sperm of *Reticulitermes* is spherical with no acrosome, but it has a few normal mitochondria. There are also two short axial filaments, but these do not protrude from the body of the sperm. It is presumed that this sperm is non-motile. Non-motile sperm also occur in the dipteran family Psychodidae and in *Eosentomon* (Protura).

### Sperm bundles

In a number of insects sperm are grouped together in bundles for at least some part of their existence and sometimes the bundles persist even after transference of the sperm to the female. The sperm of *Thermobia* normally occur in pairs, the two individuals being twisted round each other, and although their plasma membranes remain distinct an electron opaque substance is visible between them where they are close together. In addition, a continuous membrane appears to be present round both spermatozoa in some places (Bawa, 1964). Pairs of sperm also occur in some Coleoptera.

Coccids have much more specialised sperm bundles. In these insects each cyst commonly produces 32 sperm and these may become separated into two bundles of about 16 sperms. Each bundle becomes enclosed in a membranous sheath and the cyst wall degenerates. The bundles of *Pseudococcus* are much longer than the sperm, which occupy only the middle region, and the head-end of the bundle has a corkscrew-like form, which may be involved in locomotion (Nur, 1962). The sperm bundles of *Parlatoria*, on the other hand, are only the same length as the sperm, which are all orientated in the same direction within the bundle. Movement of the bundle results from the combined activity of the sperm within (Robison, 1966).

In some Orthoptera and Odonata different types of sperm bundle, known as

spermatodesms, are formed. The spermatodesms of tettigoniids comprise about ten sperm anchored together by their acrosomes. These bundles are released from the testis and the sperm heads then become enclosed in a muff of mucopolysaccharide secreted by the gland cells of the duct. In acridids the whole structure is completed within the testis cyst and may include all the sperm within it. The spermatids come to lie with their heads orientated towards a cyst cell and extracellular granular material round the acrosome of each coalesces to form a cap in which the heads of all the sperm are embedded (Szöllösi, 1974). The spermatodesms of Acrididae persist until they are transferred to the female.

### 15.2.2 Sperm capacitation

In a number of species sperm undergo changes after they are transferred to the female spermatheca and in some cases, at least, these changes are essential before the sperm can fertilise an egg. This process of maturation of sperm within the female is known as capacitation. In *Sciara* part of the mitochondrial derivative is sloughed off and at this time the sperm becomes motile. In coccids the sperm, which were previously in bundles, become separated, while in Lepidoptera and other groups changes occur in the glycocalyx.

### 15.2.3 Spermiogenesis

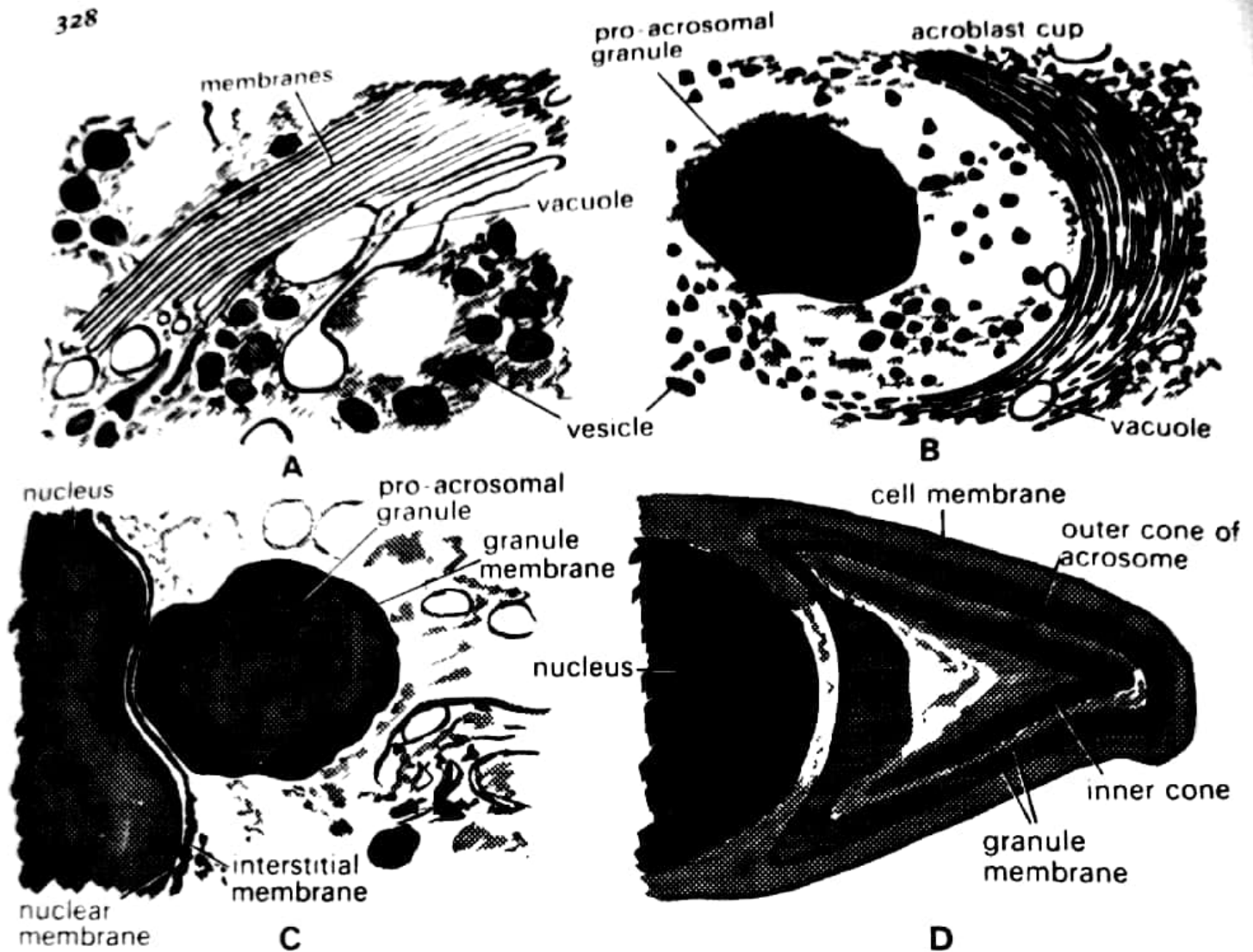
The spermatid which is formed after meiosis is typically a rounded cell containing the normal cell organelles. Subsequently it becomes modified to form the sperm and this process of spermiogenesis entails a complete reorganisation of the cell. It is convenient to consider separately each organelle of the mature sperm.

#### *Acrosome*

The acrosome is derived, at least in part, from Golgi material, which in spermatocytes is scattered through the cytoplasm in the form of dictyosomes. There may be 30 or 40 of these in the cell and they consist of several pairs of parallel membranes with characteristic vacuoles and vesicles (Fig. 215A). After the second meiotic division the dictyosomes in *Acheta* fuse to a single body called the acroblast, which consists of 6–10 membranes forming a cup with vacuoles and vesicles both inside and out (Fig. 215B).

In the later spermatid a granule, called the pro-acrosomal granule, appears in the cup of the acroblast and increases in size. The acroblast migrates so that the open side faces the nucleus and then the granule, associated with a newly developed membrane, the interstitial membrane, moves towards the nucleus and becomes attached to it (Fig. 215C). As the cell elongates the acroblast membranes migrate to the posterior end of the spermatid and are sloughed off together with much of the cytoplasm and various other cell inclusions. The pro-acrosomal granule then forms the acrosome, becoming cone-shaped and developing a cavity in which an inner cone is formed (Fig. 215D) (Kaye, 1962).

In *Gelastocoris* (Heteroptera) the pro-acrosome is formed from the fusion of granules in the scattered Golgi apparatus and no acroblast is formed. This may also be the case in Acrididae (F. Payne, 1966).



**Fig. 215** Development of the acrosome of *Acheta*. A. A dictyosome. B. Acroblast with pro-acrosomal granule. C. Pro-acrosomal granule attached to the nucleus. D. Acrosome of a mature spermatozoon. Not all to same scale (after Kaye, 1962)

### Nucleus

In the early spermatid of grasshoppers the nucleus appears to have a typical interphase structure with the chromosome fibrils unorientated. In *Chorthippus* each of the fibrils, which constitute the basic morphological units of the chromosomes, is about 20 nm in diameter and is made up of two subunits about 10 nm in diameter. The nucleus becomes very long and narrow and as it does so the chromosome fibrils become aligned more or less parallel with its long axis. The 10 nm microfibrils appear to separate into 4 nm fibrils and at the same time the non-histone protein, which in *Acheta* is largely in granules in the nucleoplasm, disappears from the nucleus (see above). The 4 nm fibrils appear to form an anastomosing network when the nucleus is seen in cross-section and as the nucleus elongates and narrows the nucleoplasm between them is progressively reduced until finally the whole of the nucleus appears to consist of a uniformly dense material (Dass and Ris, 1958). A similar linear arrangement of the chromosomes occurs in other groups but in several species the fibrils become thicker rather than thinner. In *Acheta* Kaye and McMaster-Kaye (1966) suggest that the nucleus at this time does not

consist wholly of chromatin, but also contains thin fibres containing non-histone protein.

### *Mitochondria*

In the spermatid the mitochondria fuse to form a single large body, the *nebenkern*, which consists of an outer limiting membrane and a central pool of mitochondrial components. The *nebenkern* separates into two mitochondrial derivatives which are associated with the developing axial filament immediately behind the nucleus and which elongate to form a pair of ribbon-like structures. At the same time the internal structure of the mitochondrion is reorganised so that the cristae form a series of parallel lamellae along one side and the matrix is replaced by paracrystalline material.

### *Centriole and axial filament*

Young spermatids contain two centrioles composed, as in most cells, of nine triplets of tubules and orientated at right angles to each other. One gives rise to the axial filament, but ultimately both disappear. The tubules of the axial filament grow out from the centriole and are initially enclosed within a cup-shaped vesicle. They elongate to extend finally throughout the length of the tail of the sperm. The accessory tubules arise from tubule doublets, appearing first as side arms, which become C-shaped and then separate off and close up to form cylinders (Phillips, 1970).

## 15.2.4 Control of spermatogenesis

The factors which regulate spermatogenesis are not well understood (see review by Dumser, 1980). There is no strong evidence to indicate that hormones are generally involved (Engelmann, 1970), but in some moths the moulting hormone facilitates the process by increasing the permeability of the wall of the testis to some macromolecular factor (de Wilde and de Loof, 1973b). The corpora allata do, however, regulate maturation of the accessory glands and may affect other aspects of maturation and reproductive behaviour (see Chapter XXXIV).

## 15.3 Transfer of sperm to the seminal vesicle

In some Heteroptera, in *Chortophaga* (Orthoptera), and possibly in other insects, the sperm make a complex circuit of the testis follicle before they leave the testis, moving in a spiral path to the region of the secondary spermatocytes and then turning back and passing into the vas deferens. In *Chortophaga* the movement occurs after the spermatodesm is released from the cyst, but in the heteropteran *Leptocoris* the sperm are still enclosed in the cyst. In this case the displacement starts while the spermatids are still differentiating and is at least partly due to the elongation of the cyst which occurs during the development of the sperm (M. Payne, 1934).

The fate of the cyst-cells is variable. In *Prionoplus* they break down in the testis (Edwards, 1961), but in *Popillia*, although the sperm escape from the cysts as they leave the testis, the cyst-cells accompany the sperm in the seminal fluid into the bursa of the female. Here they finally break down and it is suggested that they release glycogen which is used in the maintenance of the sperm (J. Anderson, 1950).