INTRODUCTION

Parasitic helminths have evolved complex reproductive systems which equip them to fight successfully the varied and harsh environmental conditions to which these parasites are subjected. For an effective control of these disease-causing animacules, the study of their reproductive processes, in particular, is a prerequisite. The physiochemical characteristics of the peculiarly varied helminth environment have contributed significantly to the evolution of their complex reproductive systems. Parasitic helminths have tided over the environmental hazards primarily in 2 ways: (i) by evolving a very high rate of reproductive cell turnover and (ii) by acquiring the protective coverings around the eggs. In spite of its tremendous importance, both academic and applied, the biology, including the cytology of helminth reproduction, is poorly understood and the integrated cytochemistry of the gonads and associated reproductive structures of the helminths has not been seriously carried out so far.

The reproductive processes in the different groups of helminths, in general, vary markedly and hence these processes cannot be typified as being either 'invertebrate
type' or 'vertebrate type'. However, similarities in the mechanisms at the cellular levels even among widely different animals cannot be ruled out, as rightly pointed out by Davey (1967), while discussing the comparative physiology and biochemistry of the reproductive processes in general.

The platyhelminths, barring a few, are hermaphroditic and possess highly complex reproductive systems. The morphology of the testes in the trematodes and cestodes has been studied by a number of workers (cf. Hyman, 1951; Dawes, 1956). It has been described that the germ-cells in the testes are enclosed with thin epithelial walls. Not much work has been done on the cytochemistry and functions of these wall cells which perhaps are homologous with the boundary tissue (= basement membrane) enclosing the vertebrate seminiferous tubules to which a vital role has been attributed (cf. Lacy, 1962, 1967). Though the male germ-cells of these parasitic animals have long been a favourable material for cytological work, yet most of the work accomplished so far is mainly karyological (Dingler, 1910; Cable, 1931; Anderson, 1935; Chen, 1937; Rees, 1939; Markell, 1943; Willmott, 1950) or pertains to the study of cytoplasmic organelles and inclusions in these cells (Yosufzai, 1952a; Dhingra, 1954a, b, 1955a,b,c; Gresson, 1957, 1962a; Burton, 1960; Gresson and Perry, 1961;
Guilford, 1961; Rosario, 1964; Sato and others, 1967; Taneja and Nath, 1971). Despite all this work, there is hardly any unanimity in views with regard to the role and the final fate of the various spermatid organelles or inclusions during spermiogenesis.

Earlier workers (Cable, 1931; Anderson, 1935; Rees, 1939; Markell, 1943; Willmott, 1950; Guilford, 1955), except Dingler (1910) and Severinghaus (1928), denied the very presence of mitochondria in the spermatozoa of the platyhelminths. Dhingra (1954a,b, 1955a,b,c), Gresson (1957) and Gresson and Perry (1961), however, concluded that mitochondria, though present in the early stages of spermiogenesis, were left behind in the residual cytoplasm. Yosufzai (1952a) and Burton (1960), on the other hand, stated that the mitochondria seen in the spermatids of Fasciola hepatica and Haematoloechus medioplexus were finally incorporated into the middle region of a spermatozoon. Sato and others (1967), while studying spermatogenesis in the lung fluke, *Paragonimus miyazakii*, reported that mitochondria not only formed the middle piece of a spermatozoon, but also came to lie in the head region. Kitajima and others (1976) too have reported the presence of undifferentiated mitochondria even in the anterior part of the head.

Though Dhingra (1955a) denied the very presence of
the Golgi material in the male germ-cells of *Cotylophoron elongatum*, Golgi dictysomes have been reported by Dhingra (1954a, b), Gresson (1957, 1958, 1962a), Gresson and Perry (1961) and Hendelberg (1965) up to the spermatid stage in different helminths. According to these workers, the Golgi material is subsequently sloughed off and is not represented in the mature spermatozoa. Sato and others (1967) also reported the Golgi apparatus in *Paragonimus miyazakii* lying towards the posterior end of the nucleus facing the blastophore which, later on, developed into a spherical or 'lens-like' moderately dense body. These workers, however, remained silent about the final fate of the dense body in the mature spermatozoon. On the contrary, Yosufzai (1952a) has reported the participation of the Golgi bodies in the formation of mature spermatozoa. Though the presence of an acrosome in the spermatozoa of platyhelminths has been described only by a few workers, e.g. Burton (1960) in *Haematoloechus mediaplexus* and by Henley (1968) in *Childia groenlandica*, the majority of the workers (cf. Rybicka, 1964a; Silviera and Porter, 1964; Yasuzumi, 1974) have failed to identify an acrosome in the spermatozoa of platyhelminths even by using electron microscopy. These conflicting reports pertaining to the role of cytoplasmic organelles during the formation of spermatozoa in platyhelminths, in general, and also even the morphology of
mature spermatozoa in the various groups warrant fresh investigations by using the current cytochemical techniques to settle some of the above-mentioned controversies.

Though the association of the prostate glands with the male reproductive tract has been described in a number of trematodes and cestodes (cf. Hyman, 1951), Threadgold (1975b) perhaps is the pioneer worker to study in detail the cytology of these gland cells. He, too, has not discussed the chemical nature of the secretion nor has he ascribed any definite role to these glands.

The male reproductive tract of the nematodes, in general, is comparatively simple and comprises a long tube, showing a regional differentiation into testis, vas deferens and in certain cases into an ejaculatory duct (Chitwood and Chitwood, 1950). Investigations on the male germ-cells of nematodes were initiated by van Beneden and Julin (1884) on *Ascaris*. Subsequently, this field attracted many noted workers (Bowen, 1925; Sturdivant, 1934; Nath and Singh, 1956; Nath and others, 1961; Favard, 1961;Januar, 1966; Lee and Anya, 1967; Clark and others, 1967, 1972; Beams and Sekhon, 1967, 1972; Kaulenas and Fairbairn, 1968; Poor, 1968b, 1970; Shepherd and others, 1973; McLaren, 1973a; Anya, 1976). Despite these investigations, Januar (1966) states, 'the lack of studies on nematode spermatogenesis has made it difficult to provide a generalized structure of a typical
nematode sperm'. This statement still holds true inasmuch as nematode spermatogenesis presents certain peculiarities which so far have impeded a proper understanding of this phenomenon.

Refringent granules constitute a very characteristic feature of the male germ-cells of nematodes. These granules, though reported earlier by Reichert (1847) in a nematode parasitizing frog, were first described in detail by van Benden and Jullin (1884) and were termed 'les granulations protoplasmiques'. These granules have been subsequently named variously by different workers, viz. 'yolk granules' (Wildman, 1913; Sturdivant, 1934), 'ascaridine granules' (Favard, 1958, 1959), 'proacrosomal granules' (Bowen, 1925), but frequently those using the light microscope called them 're refringent granules' (Collier, 1936; Nath and Singh, 1956; Singh, 1957; Nath and others, 1961). Recent workers, while studying nematode germ-cells by using an electron microscope, have termed these bodies 'proacrosomal bodies' (Favard, 1961; Clark and others, 1967), 'mitochondria-like inclusions' (Jaminar, 1966), 'invaginated pockets' (Beams and Sekhon, 1967), 'ovoid bodies' (Maeda, 1968; Maeda and others, 1970), 'vesicular components' (Foor, 1968b), 'membranous specializations' (Foor, 1970; Neill and Wright, 1973), 'alpha bodies' (Lee, 1971), 'fibrous bodies' (Beams and Sekhon, 1972), 'c-bodies and v-bodies' (Pasternak and
Samoiloff, 1972) and simply 'membranous organelles'
(McLaren, 1973a; Anya, 1976). The optical microscopy and
the electron microscopy, each having its own advantages
and limitations, have failed to bring unanimity in views
pertaining to the origin, chemistry, behaviour and ultimate
fate of these inclusions during the formation of spermatozoa.

Bowen (1925), using the optical microscope, described
the general structure of the Ascaris spermatozoon as a
'blunt, cone-shaped body containing a nucleus surrounded
by mitochondria'. According to Bowen (1925), at the narrow
end of the spermatozoon is lodged the prominent structure—
the refringent body which has been differently described by
various authors as 'Glazkoerper', 'Fettkoerper', 'Kopfkappe'
'Schwanz kappe', 'Corps refringent' and 'Refringent cone'
(cf. Bird, 1971). Studies of the spermatozoa in utero
with the electron microscope indicate in this region the
presence of a refringent body surrounded by a layer of
vesicles made up of characteristic dense invaginated
membranes' (Foor, 1968b). Homologous structures have been
termed specialized mitochondria' by Jamuar (1966) in the
sperm of Nippostrongylus braziliensis.

The reported absence of acrosome in the nematode
spermatozoon has been particularly confusing to investi-
gators and many attempts to establish a functional relationship
between either the peripheral membrane specializations
(Favard, 1961; Clark and others (1967) or between the refringent body of the *Ascaris* spermatozoon (Bowen, 1925) and the acrosome of the flagellated spermatozoon have met with little success.

The vas deferens, which is a prominent portion of the male reproductive system in the nematodes, reveals regional differentiation into sections, each with characteristic secretory cells (Chitwood and Chitwood, 1950; Anya, 1966). More work is needed to establish the chemistry and function of these secretory products.

One peculiarity of the female reproductive system in the platyhelminths is its distinct compartmentalization into (i) the ovaries proper and (ii) the yolk or the vitelline glands (Hyman, 1951). Whereas in other animals, the ovary also provides yolk for the developing eggs, in the majority of platyhelminths (except Acoela, Polycladida and few other Turbellaria), the yolk instead is supplied by the special yolk or vitelline cells. In addition, the female reproductive complex includes a sac— the seminal bursa or the copulatory bursa or the seminal receptacle — for storing spermatozoa, and different glands, primarily associated with the formation of the egg shell, or those engaged in synthesizing adhesive secretions for fastening the eggs after they are laid. There is also present a long conspicuous tubular, often extensively branched uterus, capable of storing a large number of eggs (Hyman, 1951).
In the oocytes of the trematodes and the cestodes, dark and prominent granules or spheres have been described by a number of workers—Schellenberg (1911) in Fasciola hepatica, Cable (1931) in Cryptocotyle lingua, Anderson (1935) in Proterometra macrostoma, Pennypacker (1936) in Pneumocotyle medioplexus, Markell (1943) in Probolitrema californiense, Willey and Godman (1951) in Zygocotyle lunata, Yosufzai (1952b) in Fasciola hepatica, Burton (1960) in Haematoloechus medioplexus, Rybicka (1966a) in Hymenolepis diminuta, Kanwar and Agrawal (1973a) in Diplodiscus amphichrus and Kanwar and others (1976) in Gastrothylax crumenifer and Oeylonocotyle dawesi. The majority of the workers agree that these spheroidal inclusions of varying sizes are nutritive in function, but opinions widely differ with respect to the origin of these sizeable bodies.

Cable long back in 1931 suggested that these bodies were centrosomal in origin. Yosufzai (1952b) reported that these granules were nothing but nucleolar extrusions. Burton (1960) and Gresson (1964), however, are of the view that the large bodies, as seen under the optical microscope, do not seem to represent nucleolar emissions but some other category of the cytoplasmic inclusions. Koulisch (1965) and Burton (1967b) too observed in the oocytes of Gorgoderina attenuata and Haematoloechus medioplexus respectively a sizeable body, which they designated as 'nucleolus-like body'. Kanwar and Agrawal (1973a,b) have described the
presence of some 'cytoplasmic droplets' which they considered to be different from the nucleolar emissions. In addition, Hanumantha Rao and Madhavi (1966) and Kanwar and others (1976) have reported RNA-rich bodies in the ooplasm of at least some of the trematodes.

Rybicka (1966a), while studying the embryogenesis in the cestode, *Hymenolepis diminuta*, has described the presence of vitelline material in the form of granules. Taneja (1971) and Kanwar and Agrwal (1974a), however, reported large cytoplasmic vitelline bodies, rich in nucleoproteins, in the oocytes of *Ophryocotyloides corvorum* and *Raillietina sp.* respectively.

Yosufzai (1952b) and Gresson (1958, 1964) have described an aggregation of the Golgi material, comprising rods and granules on one side of the early oocytes. As the growth proceeds, the Golgi material reportedly forms a dense mass near the nucleus. Burton (1960), who worked on the female germ-cells of *H. medioplexus*, however, is silent about the presence or absence of the Golgi bodies. Burton (1967b) states: "As expected in an undifferentiated cell, the membrane system, such as the endoplasmic reticulum and the Golgi elements are not abundant". Erasmus (1973) has described in unmistakable terms the presence of Golgi elements in the oocytes of *Schistosoma mansoni*. Bjorkman and Thorsell (1964) are of the opinion that in the oocytes
of _F. hepatica_ there is no structure which could be compared with the Golgi apparatus of the vertebrate cells.

As far as the cestodes are concerned, no worker has so far seriously attempted to study in detail the Golgi component in the female germ-cells.

For the first time in the oocytes of platyhelminths, Henneguy (1906) described long filamentous mitochondria. Yosufzai (1952b) in the egg cells of _F. hepatica_, on the other hand, reported granular mitochondria concentrated on one side of the oogonia from where these mitochondria later on got scattered throughout the cytoplasm. Burton (1960), while working on the oocytes of _H. medioplexus_, has described the mitochondria as 'dense cluster of granules located eccentrically in the cytoplasm and these mitochondria appear to be crowded against the nuclear membrane, forming a sort of cap.' Bjorkman and Thorsell (1964) have described mitochondria in the oocytes of _F. hepatica_ as 'dense granules with sparse cristae' scattered in the cytoplasm.

Bjorkman and Thorsell (1964) have described nurse cells in the ovary of _F. hepatica_. According to them, these cells are with pycnotic nuclei and scanty cytoplasm. They have described a 'tight endoplasmic reticulum with narrow tubular lumens' in these cells.
Studies on the oogenesis of parasitic nematodes are somewhat scarce and have been restricted mainly to ascarid species. As early as 1848, Eschricht noted that the female germ cells in *Ascaris* did not lie free in the tubular ovaries but instead were grouped around a central cytoplasmic axis, the rachis, and developed progressively till they were freed from the rachis near the junction of the ovary and the oviduct. Similar observations were also made by van Beneden (1883a, b) and Fauré-Fremiét (1913) in *Ascaris megaloecephala* (= *Parascaris equorum*); by Musso (1930), Ishii and Yanagisawa (1954), Prestage (1960), Foor (1967, 1968a) in *Ascaris lumbricoides* and by McLaren (1973b) in *Dipetalonema viteae*. Certain authors regard the rachis as a residuum of enucleate cytoplasm separated from the germinal syncytium (Schneider, 1886; Fauré-Fremiét, 1913), whereas others (Chitwood and Chitwood, 1950) think it as a continuation and product of the terminal cap cell. The cap cell was reported to occur in the nematodes with telogonic gonads (Musso, 1930; Chitwood and Chitwood, 1950). While describing in detail the cytoplasmic bridges encountered in the oocytes of *A. lumbricoides*, Foor (1968a) was unable to determine the precise role of the rachis except that he suggested a supporting function to it. Although he pointed out that the rachis was apparently a part of the cytoplasmic continuum, no reference was made to the terminal cap cell. The origin and the function of the rachis so far remain undetermined.
In the early oogonia, still lying in the germinal zone, the first cytoplasmic inclusions which appear comprise the lipid droplets (Fauré-Fremié, 1913; Lee, 1960; Anya, 1964a; Foor, 1967). Soon, however, large reserves of glycogen and the characteristic ascaroside esters are accumulated in the developing oocytes (Fauré-Fremié, 1913; Fairbairn, 1957). In addition, certain other characteristic inclusions described first by van Beneden (1883b) become discernible in the cytoplasm. Van Beneden (1883b) described two types of inclusions in the mature oocytes: (i) hyaline spheres, which reportedly contribute to the formation of a hard shell and (ii) the refrigent bodies of a lipid nature which formed the innermost layer of the shell. Subsequently, these cytoplasmic inclusions in the nematode oocytes have been the subject of numerous studies (Fauré-Fremié, 1913; Fauré-Fremié and Filhol, 1937; Panijal and Pasteels, 1951; Fauré-Fremié and others, 1954; Yanagisawa, 1955; Kochhar, 1960; Anya, 1964b; Foor, 1967, 1972; Anya, 1976) but different terminology has been used for these inclusions and more work is needed on the formation process, the chemistry and the fate of these characteristic cytoplasmic inclusions in the nematode oocytes.

The oocytes of platyhelminths, after having been released from the ovary, pass through a small oviduct to
reach the ootype which is a dilation of the oviduct and is surrounded by a large number of club-shaped cells of the Mehlis' gland (cf. Hyman, 1951). So far, there is no unanimity of views pertaining to the structure and function of Mehlis' gland. Ujiie (1936a), Burton (1963), Ebrahimzadeh (1966), Bogitsh (1970) and Threadgold and Irwin (1970) described two types of cells comprising the Mehlis' gland of various platyhelminths. Burton (1967a) and Irwin (1970), after employing the electron microscope, on the other hand, described three types of cells; two of which were secretory, whereas the third category comprised non-secretory interstitial cells. Dawes (1940), Stephenson (1947) and Rennison (1955), however, have described only one type of cells comprising the Mehlis' gland.

Similarly, various functions have been assigned to the secretion of the Mehlis' gland. The original view that the Mehlis' gland secreted the egg shell (Sommer, 1880; Looss, 1885; Schubmann, 1905) was based on the location of this gland around the ootype. Leuckart (1886) and Ujiie (1936b) suggested that the secretion from the Mehlis' gland hardened the newly formed shell, whereas Kouri and Nauss (1938) expressed the view that this gland might be lubricating the eggs facilitating their onward passage along the uterus. Dawes (1940), Burton (1963), Clegg (1965), Irwin (1970), Erasmus (1973),
Kanwar and Agrawal (1974b) and Kanwar and others (1975a), however, suggested that Mehlis' gland was responsible for the secretion of a basal membrane and that the vitelline secretion was reinforced within this membrane during shell formation. Another function ascribed to the secretion of the Mehlis' gland is to initiate the release of the shell globules from the vitelline cells (Tyzzer, 1918; Ujiie, 1936b; Burton, 1967a). Thorsell and Bjorkman (1965) and Bogitsh (1970) further described that Mehlis' gland secretion was rich in acid phosphatase and suggested a lytic role to it.

Most of the trematodes and pseudophyllidean cestodes have extensive vitellaria dispersed laterally. In cyclophyllidean cestodes, however, the vitelline gland is small compact median and is poorly developed. Such a small gland might be producing far less secretion than the well-developed vitellaria of trematodes and pseudophyllids (Smyth and Clegg, 1959).

The mature vitelline cells are filled with vitelline or protein globules. As far as the origin of these globules is concerned, Guraya (1961b, 1970), Irwin and Threadgold (1970), Kanwar and others (1974) and Kanwar and Agrawal (1976a) have expressed the view that they arise in close approximation or association with the cytoplasmic basophilia. Irwin and Threadgold (1970) have further described that the
proteins synthesized in the extensive endoplasmic cisternae are transferred to the Golgi complex for packing into membranous bodies. Guraya (1970), on the other hand, has denied any direct participation of the Golgi bodies in the formation of these secretory bodies. Tulloch and Shapiro (1957), Guilford (1961), Burton (1963), Rybicka (1966b) and Lal and Johri (1967) are, however, silent about the origin of these bodies.

Hardly any attention has been paid to the histology and histochemistry of the female ducts of the helminths in general. A few workers who have studied wall cells of these ducts (Anya, 1964b; Lee and Lestan, 1971; Erasmus, 1973) have done very superficial work; their accounts are rather too brief and do not deal with the nature and function of the secretion products of these duct wall cells.

The formation of the egg shell in the helminths starts in the ootype or in the proximal part of the uterus. The nature of the egg envelope and its formation in the helminths, in general, presents complex problems, many of which warrant fresh investigation. It was originally believed that the Mehlis' gland or the 'shell' gland secreted the egg shell in the trematodes and pseudophyllidean cestodes (Sommer, 1880; Looss, 1885; Schubmann, 1905) but careful observations led Leuckart (1886) to the conclusion
that the shell was formed from the globules of the material contained in the vitelline cells. Since then, many workers have confirmed that the vitelline cells synthesize in addition to yolk for the developing embryo large globules which form the bulk, if not all, of the shell material in the trematodes and pseudophyllids (Smyth and Clegg, 1959; Rybicka, 1966b; Irwin and Threadgold, 1970, 1972; Nollen, 1971; Ramalingam, 1972, 1973; Erasmus, 1973; Kanwar and others, 1975b; Kanwar and Agrawal, 1976b).

Although the egg shells of the trematodes and cestodes appear apparently similar, they show variation in their chemical nature. In most of the species so far studied, the amber coloured egg shell, whether thin or thick, is composed of quinone-tanned protein (Stephenson, 1947; Smyth and Clegg, 1959; Burton, 1963; Coil, 1965, 1966, 1969; Wilson, 1967; Clegg and Smyth, 1968; Kanwar and Agrawal, 1976b). It has further been recognized that not all the species of the trematodes have tanned eggs (Nollen, 1971). The nontanned or transparent egg shells, on the other hand, are described to have either keratin type (Madhavi, 1968) or elastin type of protein (Madhavi and Hanumantha Rao, 1971). Nollen (1971) and Ramalingam (1972, 1973), however, are doubtful about the presence of keratin in the egg shells of non-tanned eggs.

A number of embryological investigations have shown
that in the formation of cyclophyllidean eggs, only one vitelline cell is associated with each ovum (Janicki, 1907; Spatlich, 1925; Venard, 1938; Ogren, 1956; Bona, 1957; Rybicka, 1966b; Kanwar and Agrawal, 1974b). These workers have observed that a thin membranous shell is formed around the single vitelline cell and the ovum, but the work to determine the exact mode of its origin has not received much attention.

The structure and the composition of the eggs of nematodes and of their shells, in particular, have been studied rather extensively (Christenson, 1950; Fairbairn, 1957; Rogers, 1962; Foor, 1967; Kaulen, and Fairbairn, 1968; Bird, 1968), but widely divergent views of these workers have created a lot of confusion which primarily is due to the difficulty in identifying the origin of different layers and because of the paucity of the work on the synthesis of various shell components (cf. Bird, 1971; Anya, 1976). It has been reported that the egg shell consists of three basic layers that are secreted by the egg itself, namely an inner lipid layer, a middle chitinous layer and an outer vitelline layer (Bird, 1971). In some forms, there is also a fourth outermost layer which reportedly is secreted by the uterus and hence appropriately known as the uterine layer (Foor, 1967).
There has been some disagreement on the actual number of layers in the egg shell of the nematodes. In the genus *Ascaris*, for instance, various authors have reported 3-5 layers (cf. Rogers, 1956). The use of inconsistent terminology used to describe these layers has added to the confusion. For instance, the innermost lipid layer has been referred to as a vitelline membrane by many workers (cf. Bird, 1971). However, Foor (1967), while studying the shell formation in *Ascaris lumbricoides* by employing the electron microscope states that the vitelline layer, which is the first layer to be formed, lies on the outer surface of the chitinous layer and is thus the outermost layer of the true layers of the egg shell. Foor refers to the innermost layer as the 'ascaroside layer' and states that "it has hitherto been known, improperly as the vitelline membrane". Bird (1971), however, refers to it as the lipid layer which thus is homologous with the vitelline membrane of earlier workers.

Keeping in view the above-mentioned lacunae in information, the present problem on the gonads and associated structures of some parasitic helminths has been undertaken. During the course of the present investigations, an attempt has been made to study the testes, the ovaries and the associated ducts and glands by employing the morphological and current techniques of
proven cytochemical validity. As many as six species of helminths, viz. Paradistomoides orientalis and Paramphistomum epiclitum (trematodes), Senga lucknowensis and Oochoristica symmetrica (cestodes) and Ascaridia galli and Diplostomia bhamoensis (nematodes) have been chosen and the data of various helminth groups have been correlated and physiological interpretations advanced.