DISCUSSION

The morphology of the male as well as female reproductive complexes of the helminths, Paradistomoides orientalis, Paramphistomum epiclitum, Oochoristica symmetrica, Senga lucknowensis, Ascaridia galli and Diplotriaena bhamoensis, has been described by Narain and Das (1929), Gupta (1964), Johri, L. N. (1934), Johri, G. N. (1956), Schrank (1788) and Parona (1889) respectively. The present observations concerning the broad morphology of gonads and associated structures in these helminths are in conformity with their respective descriptions.

TESTES

The testes of the helminths under study comprise both germinal as well as non-germinal components. The non-germinal cells in the platyhelminths, Paramphistomum epiclitum, Paradistomoides orientalis, Senga lucknowensis and Oochoristica symmetrica, comprise testicular sheath cells and nutritive or sustentacular cells whereas in the nematodes, Ascaridia galli and Diplotriaena bhamoensis, the non-germinal component is represented by the sheath cells, the central rachis and the apical cells.
The sheath cells are small and comparatively flat in the trematodes (*Paramphistomum* and *Paradistomoides*) and the cestodes (*Senga* and *Oochoristica*) whereas in the nematodes (*Ascaridia* and *Diplostomiaena*), these are quite large. These cells in the trematodes and the cestodes are of non-secretory type. Considering their histological and histochemical nature, it is conjectured that these do not manufacture any nutritive material in their cytoplasm. In the nematode, *Diplostomiaena bhamoensis*, however, the sheath cells have been observed to be quite large and contain in their cytoplasm bodies rich in phospholipids. In addition, these cells in both the nematodes under study are also rich in glycogen and reveal intense alkaline phosphatase activity. The presence of glycogen in the testicular sheath cells of the nematode, *Heterakis gallinarum*, has also been described by Lee (1971). Although Chitwood and Chitwood (1950), Anya (1966), Hulinská (1973) and Shepherd and others (1973) have described epithelial layer investing the testis of the nematodes in general, yet they have not ascribed any special role to these cells. On the basis of the cytochemical study of these cells, it seems likely that these cells absorb the nutritive material from the perienteric fluid and pass that on to the germ cells. Dermadit (1912) and Snodgrass (1935) have also suggested that walls of the testicular tubules serve as trophic intermediaries between the blood and the developing germ cells.
The nutritive cells observed in the peripheral regions of the testes of the trematodes, *Paramphistomum* and *Paradistomoides*, and the cestodes, *Senga* and *Oochoristica*, are very large. Frequently these cells become irregular and have peripheral cytoplasmic processes thrown towards the centre of the testis. Some of these cells reveal darkly staining bodies which are especially prominent in the trematode, *Paradistomoides*. These cells can be compared to the nutritive cells described by Sato and others (1967) and Grant and others (1976) in a variety of trematodes. It is conjectured that these cells, in addition to their functioning as trophic intermediaries, also phagocytose the sloughed off remnants of the developing spermatozoa and perhaps also engulf the degenerating germ cells.

Shedding of cytoplasmic remnants from maturing sperm is a mechanism by which the latter get rid of the excessive cytoplasmic materials. The sperm carry with them the minimal cytoplasm essential to protect the vital genetic material. Shedding and engulfment of such cytoplasmic residual bodies by the non-germinal Sertoli cells of the vertebrate testes is well known (cf. Bishop and Walton, 1960). Lacy (1962) conjectured that the residual bodies seem to act as highly localized messangers and perhaps comprise the precursors to be utilized by the Sertoli cells.
for the synthesis of specific steroids tentatively termed by Lacy as Sertoli cell-hormone (SCH). Could this be not conjectured that these non-germinal cells characteristic of the platyhelminth testes perform the same function and likewise be implicated in the synthesis of 'Sertoli-cell-hormone like material'.

A characteristic feature of the nematode testis is the presence of a central rachis. In both the nematodes, Ascaridia galli and Diplostriaena bhamoensis, the spermatogonia and the spermatocytes are in cytoplasmic continuity with the branched and enucleate rachis. There is difference of opinion among workers regarding the origin of the rachis. Eschricht, as early as 1843, described that the germ cells of Ascaris were not loose within the gonads but were grouped around a central axis termed rachis. Schneider (1866) and Faure-Fremiet (1913) suggested that the rachis comprised the 'residuum of enucleated cytoplasm separated from the germinal syncytium'. Chitwood and Chitwood (1950), on the other hand, conjectured that it was a product and continuation of the terminal cap cell. Recently, it has been suggested that rachis is differentiated from the inter-cellular substance filling the spaces between the germ cells (Ishii and Yanagisawa, 1954). Prestage (1960) has mentioned that the rachis has its origin from the germ cells forming a syncytium after
being initially separated from one another'. Terry and others (1961) reported the presence of a cap cell at the tip of the male reproductive tract of Dipetalonema viteae. McLaren (1973a) described that 'the terminal end of the male reproductive tract of Dipetalonema viteae contains a syncytial mass of cytoplasm which apparently gives rise to the spermatogonia. This syncytium then grows along the length of the testis in the form of rachis, carrying the developing germ cells with it'. A similar multinucleate apical cell has also been observed during present studies on Ascaridia and Diplotriaena. All this supports the original suggestion of Chitwood and Chitwood (1950) who expressed the view that 'cap cell may contain the single nucleus of the otherwise enucleate syncytial rachis'.

The cytoplasm in the central rachis of Diplotriaena and Ascaridia is rich in proteins, 1:2, glycol groups and RNA. In Ascaridia, in addition, there are also observed darkly staining bodies whose lipoidal nature is revealed after various cytochemical tests. Lee (1971) has observed only the mitochondria and rough endoplasmic reticulum in the central rachis of Heterakis gallinarum. McLaren (1973a), however, described the presence of abundant endoplasmic reticulum along with ribosomes, mitochondria and membrane bound vesicles. McLaren (1973a),
therefore, suggested that rachis of *D. viteae* might be contributing substances to the developing germ cells. He has further described that "the germ cells of *D. viteae* have been found to retain their attachment to the rachis whilst they move along the reproductive tract. One can only assume, therefore, that the rachis is a continually elongating structure and that after the germ cells have become detached from it, it breaks down and is resorbed by the epithelial lining cells of the reproductive tract. The rachis would therefore seem to have an important role in maintaining a synchronous maturation of male gametes."

The testes in helminths contain germ cells in various stages of development. In the trematodes, *Paramphistomum* and *Paradistomoides*, and the cestodes, *Senge* and *Oochoristica*, the peripheral regions comprise various generations of spermatogonia only. In *Paramphistomum*, however, the spermatogonial groups are also observed towards the centre of testis. In both the nematodes under study, on the other hand, the testis is telogonic. Nath and others (1961), Favard (1961) and Anya (1966) also reported identical organization of the testis in *Porrocaecum angusticolle*, *Parascaris equorum* and *Aspiculuris tetraptera*. In *Dipetalonema viteae* (McLaren, 1973a), there is present a terminal nucleated syncytial mass. This arrangement is in sharp contrast to the
hologonic testes of some nematodes as described by Eberth (1860) in _Trichinella spiralis_ and _Trichinella trichiura_, Leuckart (1876) in _Dicocotylida renale_, and Neill and Wright (1973) in _Capillaria hepatica_ where the spermatogonial proliferation takes place along the whole length of testis, either all around its perimeter or at definite or limited points along its length. The subsequent stages of development occur, as in the seminiferous tubules of the vertebrates, in deeper layers towards the lumen of the testicular tube. In telogonic testis the arrangement, however, more or less resembles that of the insect testicular tubules where the interior of each tubule is divided into an apical germarium and a series of cysts containing successive stages of developing germ cells (cf. Davey, 1967; Wigglesworth, 1972; de Wilde and DeLoof, 1973).

Unlike the descriptions of Linder (1914) for _Schistosoma haematobium_ and of Severinghaus (1928) for _S. japonicum_, who reported no typical rosette pattern of spermatogenesis, the spermatogonia, spermatocytes and spermatids in _Paramphistomum_, _Paradistomoides_, _Senga_ and _Oochoristica_ are arranged in rosettes with blastophores in the centre. During the course of present studies, it is further observed that the three divisions of the primary spermatogonium result in a rosette of 8 primary spermatocytes. This is followed by a reductional division
(Meiosis I) to give rise to 16 secondary spermatocytes which divide (Meiosis II) equationally and give rise to 32 spermatids. This is in accordance with earlier workers (Dingler, 1910; Cable, 1931; Anderson, 1935; Chen, 1937; Rees, 1939; Markell, 1943; Willmott, 1950; Yosufzai, 1952; Dhingra, 1954a,b, 1955a,b,c; Guilford, 1955, 1961; Franzen, 1956; Gresson, 1957, 1958, 1962a; Dunn, 1959; Sonderson, 1959; Burton, 1960, 1972; Govaert, 1960; Gresson and Perry, 1961; Hendelberg, 1962; Rybicka, 1962, 1964a, 1966b; Douglas, 1963; Silviera and Porter, 1964; Rosario, 1964; Sato and others, 1967; Swiderski, 1968; Guraya and Gupta, 1970a, b; Taneja and Math, 1971; Maamouri and Swiderski, 1975; Grant and others, 1976). In all the species so far considered, the cells of a rosette are arranged around a central cytoplasmic disc or the blastophore. There is seen at light microscope level a continuity of the cytoplasm of blastophore and the cells which project out from it.

Gresson (1962a) described that in the cestode, *Proteocephalus pollanicola*, the rosettes comprising primary spermatocytes and spermatids, like those of trematodes, consist of 8 cells and 32 cells respectively. Rybicka (1964a, 1966b) in *Dipylidium caninum* and *Hymenolepis diminuta*, however, reported that there are 16 cells in the rosette comprising the primary spermatocytes and 64 in those which have the spermatids.
Yosufzai (1952) reported that in the blastophore of *Fasciola hepatica*, there is observed though occasionally, a 'nucleus-like' body in the centre of the blastophore which, he believes, is formed as a result of aggregation of nuclear material which separates from each of the spermatocytes during cell divisions. No such nuclear emissions or aggregation have been observed during the course of present studies on the varied platyhelminths. However, the blastophores, have been found to contain mitochondria and Golgi granules.

In both the nematodes, viz. *Diplostomum* and *Ascaridia* under discussion, the spermatogonia and the spermatocytes are in the cytoplasmic continuity with the enucleate rachis which is branched (*vide supra*). It is conjectured that these blastophores may also have some regulatory role, just like the rachis, in addition to their role as reservoir for the residual material.

The cytoplasm of the spermatogonia and the spermatocytes of all the helminths under study is sufficiently basophilic; the basophilia in the spermatogonia is more than in the spermatocytes. This basophilia is mainly due to RNA. In the spermatids and during subsequent stages of spermateleosis the degree of basophilia is gradually reduced and there is hardly any basophilia left in the late developing spermatids and the spermatozoa. However,
the blastophores/residual cytoplasm/plasma lobes continue to be highly pyroninophilic. Bernhard and others (1952), Sjostrand and Hanzon (1954), Porter (1961), Bourne (1962) and Lima de Faria (1969) observed that basophil areas of the cytoplasm correspond to patches rich in rough ER membranes.

As far as the details of the cytoplasmic organelles or inclusions of the germ cells are concerned, a few workers have studied the mitochondria and other organelles or inclusions in the male germ cells of platyhelminths (cf. Rybicka, 1964a; Silviera and Porter, 1964; Yasuzumi, 1974). Some of these believed that the platyhelminth spermatozoon consist only of nuclear material (Cable, 1931 for Cryptocotyle lingua; Anderson, 1935 for Proterometra macrostoma, Rees, 1939 for Parorchis acanthus; Markell, 1943 for Probolitrema californiense; Willmott, 1950 for Gigantocotyle bathycotyle; Guilford, 1955 for Heronimus chelydrae).

Even those workers who studied these inclusions have differences of opinion regarding the participation of mitochondria in the mature sperm. Dhingra (1954b, 1955a,b,c) while working on the spermatogenesis of Cyclocoelium elongatum, Gastrothylax crumenifer and Asymphylodra sp., Gresson (1957) on Fasciola hepatica, Rosario (1964) on Hymenolepis nana and Hymenolepis diminuta
and von Bonsdorff and Telkka (1965) on *Diphyllobothrium latum* reported granular mitochondria in the early male germ cells. During the maturation of the spermatozoon, the mitochondria are left behind in the residual cytoplasm.

Gresson and Perry (1961) confirmed the earlier investigations of Gresson (1957) with electron microscopy. They stated, 'the spermatozoon of *Fasciola hepatica* consists of an elongated head and a flagellum. We found no evidence that the mitochondria enter into the composition of the flagellum or that the sperm tail possesses a region comparable to the middle-piece of mammalian sperm'.

On the contrary, in *Fasciola hepatica*, Yosufzai (1952a) described filamentous mitochondria in the young spermatocytes. In the spermatid, these mitochondria are transferred into irregular globules which are distributed throughout the cytoplasm. During spermateleosis, according to this worker, some of these mitochondria form a ring around the nucleus. As the process of spermateleosis advances further, all the mitochondria are passed into tail region which Yosufzai (1952a) termed 'middle-piece'.

Sato and others (1967) while studying spermatogenesis in *Paragonimus miyazakii* after employing electron microscope observed that the mitochondria not only surround the axial filament in the tail region but also come to lie in the head region, just ventral to the nucleus.
Taneja and Nath (1971) described that though the bulk of mitochondrial material passes into the tail region of the mature sperm, some of these come to lie inbetween the nucleus and cell membrane. They have further mentioned that the mitochondria present near the blastophore are ultimately discarded along with the residual cytoplasm.

During the course of present studies on Paradistomoides, Paramphistomum, Oochoristica and Senga, the spermatogonia show granular as well as filamentous mitochondria somewhat aggregated in the juxta-nuclear region. In the primary spermatocytes, the mitochondria are dispersed in the cytoplasm though not uniformly. In the spermatid cluster, majority of mitochondria move into the blastophore and are left behind in the residual cytoplasm when the spermatozoa wriggle out—hardly any mitochondria are retained by the spermatozoa. Similarly, Burton (1960) has also described that only very few mitochondria are retained in the tail region of the sperm—bulk of these are left behind in the residual cytoplasm.

In the nematodes, Ascaridia galli and Diplostrephena bhamoensis, the granular as well as filamentous mitochondria, which are concentrated in the juxta-nuclear regions of the spermatogonia, get more or less uniformly dispersed in the
spermatocytes. In the spermatids some of these coalesce to form larger bodies. Lee and Anya (1967) described that the tail in the sperm of *Aspiculuris tetraptera* is constituted by a single mitochondrion formed as a result of fusion of several mitochondria during spermatid stage. However, the vesicular mitochondria of *Ascaridia* and *Diplostoma* spermatozoa do not form any structure but remain as such even after their transference to female duct (cf Lee, 1971; McLaren, 1973a).

Favard (1961), Beams and Sekhon (1972) and Neill and Wright (1973), during their studies with electron microscope on *Parascaris equorum*, *Rhabditis pellio* and *Capillaria hepatica* have described that the mitochondria are closely associated with the nucleus till condensation of nuclear material is complete whereafter these disperse throughout the sperm. Similar nuclear-mitochondria association in the male germ cells of *Ascaris lumbricoides*, *Gnathostoma* sp. and *Dirofilaria immitis* has also been described by Poor (1970).

The mitochondria in these helminths are stained supervitally with Janus green B. Marston (1928) suggested that the reaction of Janus green B is due to the presence of proteolytic enzymes in the mitochondria which form a proteolytically active green precipitate with it. Lazarow and Cooperstein (1953) advocated that the supervital
staining of mitochondria with Janus green B depends upon a delicate balance between the concentration of Janus green B used and the reducing capacity of the cell organelles and the factors which reorganize the Leuco-JG-B formed. They concluded that when the stain penetrates into the cell, the same is reduced to pink form by DPN-dehydrogenase (flavoprotein system) which is the immediate reactant. The reduced dye is then reoxidized by oxidized flavoprotein to blue form. The oxidation of flavoprotein is brought about by the cytochrome C-cytochrome oxidase system which resides only in mitochondria.

The mitochondria in all the helminths under discussion are stained blue with Sudan black B, acid haematein and Nile blue sulphate thus confirming the presence of phospholipids in them. The usual presence of phospholipids in the mitochondria including those of the male germ cells has been reported by a number of workers (cf. Nath, 1965). Besides the lipids, mitochondria also possess proteins. The lipids and proteins occur as lipoprotein complexes. The phosphatide group of lipoprotein plays a vital role in the enzymatic processes either as structural framework or electron acceptor (Gresson, 1957, 1958).

Nath and others (1961) and Taneja and Nath (1971) have also reported polysaccharides in the mitochondria of the nematode, *Porrocaecum angusticolle*, and the trematode,
During the course of present investigations on various helminths, however, the mitochondria could not be stained with periodic acid-Schiff at any stage of spermateleosis. The mitochondria in all the species under investigation do not react with varied tests employed for histochemical localization of DNA and RNA.

During the course of present studies on the trematodes, *P. orientalis* and *P. epiclitum*, and the cestodes, *O. symmetrica* and *O. lucknowensis*, there are observed 2-3 Golgi elements in each of the late spermatogonia which during subsequent stages are seen lodged in the narrow ends of the pear-shaped spermatocytes and spermatids. Ultimately, these shift to the blastophore and are sloughed off along with it.

Yosufzai (1952a), while working on the spermatogenesis of *Fasciola hepatica*, reported that the Golgi elements in primary spermatogonia are present in the form of an aggregated pack of individual elements situated at one pole of the nucleus. In the secondary spermatogonia, these elements are seen as 2-6 masses of Golgi clumps consisting of short rods and granules and these lie around the nuclear membranes widely separated from one another. In the primary spermatocytes there are fewer Golgi elements because some of these pass into the blastophore. In the spermatids the number of
these elements does not increase and at the time of elongation of the spermatid during final stages of spermatelosis, the Golgi bodies are segregated into 2 groups, each occupying the either tip of the elongating cell. As the cytoplasm does not appear to be eliminated, it is probable that the Golgi bodies are retained even in spermatozoon. Sato and others (1967) too reported in the spermatids of Paragonimus miyazakii, the Golgi apparatus lying towards the end of the nucleus facing the blastophore where it later develops into a spherical or 'lens-like' moderately dense body. These workers, however, have not followed the fate of this dense body till the mature spermatozoon is formed.

In conformity with the present observations, Dhingra (1954a) in Isoparorchis eurytremum, Gresson (1957) and Gresson and Perry (1961) in Fasciola hepatica, Mez and Short (1957) in Schistosomum douthi, Guilford (1961) in Halipegus eccentricus, Gresson (1962a) in Proteocephalus pollanicola, Rosario (1964) in Hymenolepis nana and Hymenolepis diminuta and Taneja (1973) in Ophyrotyloides corvorum also reported Golgi dictyosomes in the male germ cells only up to the spermatid stage whereafter these are reportedly sloughed off-there being no acrosome in the mature spermatozoon.

Nath (1965) also concluded in his monograph that the
Golgi elements of the platyhelminths do not form any sperm component.

The Golgi bodies in the male germ cells of the nematodes, Diplotritinae bhamoensis and Ascaridia galli, are found in the form of granules or duplex spheroids up to the spermatocytes whereafter refringent granules start differentiating in their interna. After the formation of the refringent granules, the Golgi remnants or bits get dissociated from them and seem to be resorbed in the cytoplasm since these have never been sighted during late stages of spermatogenesis.

Hirschler (1913), Sturdivant (1934) and Collier (1936) while working on Ascaris canis, A. megalcephala and A. suilla respectively described crescent or ring shaped Golgi bodies in the cytoplasm of spermatogonia and spermatocytes. According to them, most of these after the formation of refringent granules are cast off with the plasma lobe. Hirschler (1913) further reported that 'the Golgi left-overs are seen in the caudal region of the sperm'.

Nath and Singh (1956) and Singh (1957) described granular and spheroidal Golgi bodies in the early spermatocytes of Porrocecum angusticolle, Ascaris suilla and Polydelphis sp. which reportedly transform directly into refringent granules. Later on Nath and others (1961),
while working on Porrocaecum angusticolle observed that
the refringent bodies are formed in close association of
the phospholipid granules (Golgi bodies) which ultimately
are sloughed off along with plasma lobes.

Jamear (1966) and Lee and Anya (1967) during their
studies on Nippostrongylus brasiliensis and Aspicularis
tetranterata did not observe any well-developed Golgi area
although they spotted a few smooth walled vesicles
and cisternae during spermatocyte growth.

Lee (1971), Neill and Wright (1973) and Shepherd
and others (1973) during their, electron microscope studies
on the male germ cells of various nematodes, have described
the presence of Golgi complexes in the spermatogonia and
spermatocytes. Lee (1971) further observed an increase in
the number of Golgi complexes as the spermatocytes
descended in the testis.

The Golgi granules and externa of the duplex spheroids
of all the helminths under study are cytochemically alike,
\[ \text{i.e. Hg-DBP}^+\text{ive, SMB}^+\text{ive, Al}^+\text{ive, indicating the} \]
\[ \text{presence of lipids and proteins in them. In both the} \]
\[ \text{nematodes, however, the interna of these Golgi spheroids} \]
\[ \text{reveal PAS}^+\text{ive material in addition to some proteins and} \]
\[ \text{lipids. Nath and others (1961) in case of Porrocaecum} \]
\[ \text{angusticolle and many other cytologists (Schrader and} \]
Leuchtenberger, 1951; Moriber, 1956; Clayton and others, 1958; Gupta and others, 1960a,b,c; Bawa, 1960; Guraya, 1966a; Mathur, 1962; Bedi, 1962a,b, 1963; Nath, 1965) who worked on the male germ cells of a variety of other animals have reported a similar nature of the Golgi elements of these cells.

Refringent granules constitute a very characteristic feature of the male germ cells of the nematodes. The term refringent granules to describe these conspicuous cytoplasmic inclusions was used because of the high refringence they show when studied under the phase contrast microscope. These granules, though reported earlier by Reichert (1847) in a nematode of frog, were first described in detail by van Beneden and Julin (1884) and were termed 'les granulations protoplasmiques'. These workers, however, did not study their behaviour, participation and ultimate fate during sperm formation nor commented upon their chemistry. These highly refringent granules have been named variously by different workers viz. 'yolk granules' (Wildman, 1913; Sturdivant, 1934), 'ascaridine granules' (Favard, 1958, 1959), 'proacrosomal granules' (Bowen, 1925) but frequently light microscopists called them 'refringent granules' (Collier, 1936; Nath and Singh, 1957; Singh, 1957; Nath and others, 1961). Recent workers while studying nematode germ cells under electron microscope have termed these bodies
'proacrosomal bodies' (Favard, 1961; Clark and others, 1967), 'mitochondria-like inclusions' (Jamuar, 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). These different terms undoubtedly arose because these structures exhibit a wide variety of forms in different animals and also during different developmental stages of spermatogenesis.

During present studies on Ascaridia galli and Diplotriaena bhamoensis, the refringent bodies are homogeneous and spherical in the spermatocytes though these become ellipsoidal in the later stages. However, in A. galli, these ellipsoidal refringent bodies reveal duplex nature. These sizable cytoplasmic components orientate themselves in concentric rows in the late spermatocytes and spermatids. The refringent granules, seem to originate in the chromophobe medullae of the duplex Golgi bodies in the early spermatocytes. With the increase in the number and size of the refringent bodies, there is witnessed a concomitant depletion of
the Golgi elements. Spermatocytes and spermatids, when filled with refringent material hardly show any organized Golgi material in their cytoplasm.

Since van Beneden and Julin (1884) described the existence of the refringent granules in the cytoplasm of *Ascaris megalcephala* spermatocytes, repeated attempts were made to understand their mode of formation. Faure-Fremiet (1911, 1913) precisely described the chemical nature of these granules and demonstrated that they are composed of special protein, which he purified and named 'ascaridine'. The origin of refringent bodies is still disputed despite serious attempts by many notable workers in this direction. The optical and the electron microscopy, each having its own advantages and limitations, have failed to bring unanimity in views pertaining to the exact mode of origin of these granules in the nematode germ cells. Widely divergent views have been expressed with respect to the origin and fate of these inclusions.

The hypothesis suggesting the nuclear origin of these refringent granules was put forth by Scheben (1905) and Wildman (1913) which stands refuted now.

Tretjakoff (1905), Mayer (1908) and Romieu (1911) think that these refringent granules arise from the 'points of condensation of cytoplasm' but they do not exclude the
possible role played by mitochondria. Hirschler (1913) pointed out that the refringent granules owe their origin to mitochondria. Fauré-Fremiet (1913) and Filhol (1937), on the other hand, did not find any direct mitochondrial participation in the development of refringent granules.

Bowen (1925) after studying Hirschler's figures concluded that 'the refringent granules arise as differentiation product of the Golgi apparatus in a manner essentially comparable to that by which normally the acrosome in sperm takes its origin'. Sturdivant (1934) too has emphasized that the refringent bodies are the differentiation products of the Golgi material. Collier (1936), while supporting Sturdivant, has remarked that any apparent relation of these granules with the mitochondria is topographical.

Nath and Singh (1956) and Singh (1957) demonstrated that the Golgi spheroids undergo a radical change in their chemical composition and are then directly transformed into the refringent granules. Nath and others (1961) described that refringent granules arise in association with the Golgi bodies. Nath and others (1961) describe formation of the refringent bodies as follows:

"As the process of spermatogenesis advances and the spermatocyte enters the growth period, small vacuoles or spaces appear within the localized aggregations of phospholipid granules; the latter become attached to the surface of former. These
vacuoles or spaces become quickly filled up with the basophil material (ribonucleoproteins). Gradually the ribonucleoprotein sphere grows; ultimately it gets rid of its phospholipid satellites and forms a refringent body. When the primary spermatocyte approaches the prometaphase stage, its cytoplasm becomes choked with these refringent bodies, obliterating the mitochondria from view. The phospholipids show an obvious decrease during these stages. However, it may be that the phospholipid granules do not directly contribute any substance in the formation of refringent bodies; perhaps they help in the segregation of the dispersed basophil material by forming lipid membranes.

Following this work a number of other workers reported the participation of endoplasmic reticulum in addition to Golgi bodies in the formation of refringent granules (Favard, 1961; Clark and others, 1967, 1972; Lee, 1971; Beams and Sekhon, 1972; Pasternak and Samoiloff, 1972; McLaren, 1973a; Anya, 1976). Favard (1961) in an ultrastructural study on *Ascaris megaloccephala* reported that 'granules d' ascaridine' are synthesized by the endoplasmic reticulum and that these fuse with the crystalloids produced by the Golgi bodies to form the proacrosomal bodies (= refringent bodies of the present study).

Beams and Sekhon (1972), while working on *Ehabditis pellio*, have described that 'as a part of the maturation of the spermatocyte, the smooth-surfaced endoplasmic reticulum of these cells comes into intimate relationship with Golgi dictyosome saccules, a dilation of the terminal saccule is observed, and a fibrous body appears within the dilation'.
According to Anya (1976) this fibrous body is nothing else but crystalline body (c-body) of Pasternak and Samoiloff (1972) or the 'battonet' of Favard (1961). He (Anya, 1976) further stated that occasionally, a dense lipid-like body may be associated with the fibrous body (Lee, 1971; Beams and Sekhon, 1972; Pasternak and Samoiloff, 1972) and a mass of small tubular elements may be observed. When the fibrous body is fully formed, it will separate from the endoplasmic reticulum-dictyosome complex. In the spermatid, the fibrous bodies with their associated dictyosome-derived membranes are arranged in a circle round the circum nuclear mass of mitochondria. With the loss of the residual body in spermateleosis, there is a contraction, folding and separation of the dictyosome derived membranes from the fibrous bodies. These membranes will then reform into vesicular bodies with characteristic fold in which some secretory material is often evident; the vesicular bodies will migrate to the periphery (below the plasma membrane) and are now the characteristic membrane organelles (Lee, 1971; McLaren, 1973a).

Neill and Wright (1973) described the formation of membranous organelles in a slightly different way. According to them, in the spermatocytes, cisternae develop within the endoplasmic reticulum in which accumulation of
dense materials can be discerned. These cisternae presumably contribute to the formation of characteristic tubules which are found in the early spermatid. The membrane organelles are formed from these tubules when the latter collapse on themselves to give rise to concave vesicles. The vesicles, so formed may close and fuse with adjacent units, giving rise to double membrane loops in which cytoplasmic material is often enclosed.

From these descriptions, Anya (1976) concluded that in the formation process of the membrane organelles two sequential 'secretory' cycles are involved: in the first cycle a fibrous, presumably structural protein material, possibly an enzyme, is secreted, associated with PAS-reactive polysaccharide material (Clark and others, 1967, 1972; Lee, 1971). The secretory cycle often involves the reorganization of the membrane component of the organelles (McLaren, 1973a; Neill and Wright, 1973; Wright and others, 1973). The latter stage in the formation of membrane organelles is characterized by the development of pronounced, membrane folds (Beams and Sekhon, 1972).

Attempts to study the cytochemistry of the refringent bodies of nematodes were made long back (Faure-Fremiet, 1913; Filhol, 1937). Bowen (1925) tried to compare refringent body of the nematode sperm with the acrosome which has been described to contain invariably PAS positive substance
During the course of present observations on the male germ cells of the nematodes, *Ascaridia galli* and *Diplotritisena bhamoensis*, it is observed that the refringent bodies are not stained pink after PAS or Himes and Moribé techniques at any stage. Anja (1976), while reviewing the reproduction in the nematodes, too has mentioned that "the refringent bodies of all nematode spermatozoa in which these have been studied are not PAS positive.

During the course of present studies on *Ascaridia* and *Diplotritisena*, it has further been observed that the refringent bodies are composed of proteins rich in -NH₂ groups and tyrosine and RNA. The presence of proteins and RNA in the refringent bodies has also been described by a number of other workers (Sturdivant, 1934; Faure-Frémiéét and Filhol, 1937; Pasteels, 1948; Panijal and Pasteels, 1951; Nath and others, 1961). This further supports the assumption that the major contribution of the refringent body to the fertilized ovum, before the fusion of the maternal and paternal nuclear materials, is to provide the necessary material which later on is utilized in the formation of polyribosomes (Foor, 1970).

In the trematodes, *Paramphistomum* and *Paradistomoides*...
and the cestodes, Oochoristica and Senga, two axial filaments have been observed in the maturing spermatids which later on twine around each other to give the appearance of a single flagellum. Yosufzai (1952a), Dhingra (1954a,b, 1955a,b,c), Gresson (1957, 1962a), Hendelberg (1962) during their light microscope studies described only a single flagellum in each spermatozoon in a variety of trematodes and cestodes. However, during re-examination with the electron microscope, it was observed that the tail of the mature spermatozoon in fact contained two axial units (Burton, 1960, 1972; Gresson, and Perry, 1961; Hendelberg, 1965, 1970; Von Bonsdorff and Telka, 1965; Sato and others, 1967; Maamouri and Swiderski, 1975; Grant and others, 1976). According to Yasuzumi (1974) spermatozoa of flatworms (Platyhelminthes) can be divided into three distinct groups on the basis of ultrastructural organization of their flagella. According to him, the tail may either have 9 + 2 or 9 + 0 or no axial units at all.

The spermatozoa of the nematodes, Diplotriaena and Ascaridia are non-flagellate. Foor (1970) in his review on spermatozoon morphology and zygote formation in the nematodes has described the absence of flagellum in the spermatozoa of all nematodes. He further states: "Even in species in which spermatozoa have a distinct head and tail,
the resemblance to flagellated spermatozoa is purely superficial since critical studies have shown that the tail is usually occupied by nuclear material and appears to be incapable of movement (Jamuar, 1966; Lee and Anya, 1967)."

The mature spermatozoa of all the helminths under discussion viz. *Paramphistomum, Paradistomoides, Oochoristica, Senga, Diplotrisena* and *Ascaridia*, lack an acrosome. Except Burton (1960) who has described the presence of acrosome in the trematode, *Haematoloechus medioplexus*, absence of acrosome in this group in general has been reported by a number of workers (cf. Rybicka, 1964a; Silviera and Porter, 1964; Bacetti, 1969; Foor, 1970; Yasuzumi, 1974; Anya, 1976).

Burton (1967b) during his studies on the penetration of the ovum by a spermatozoon in a frog lung fluke, *Haematoloechus medioplexus*, has mentioned that the ova in these trematodes may either have a very thin or no protective coat at all. According to him, the fertilization may occur directly by the fusion of the plasmalemma of the sperm and the ovum. Similar explanation can be held true for the cestodes which also have a very thin oolemma.

As far as the nematodes are concerned, numerous attempts have been made to homologise refringent body
(Bowen, 1925; Sturdivant, 1934) or the membranous organelles (Favard, 1961; Clark and others, 1967; Beams and Sekhon, 1972; McLaren, 1973a) with the vertebrate acrosome. According to these workers, refringent bodies/membranous organelles release substances which may possibly help in fertilization. During the course of present observations on Diplostriataena and Ascaridia, however, it is observed that the refringent bodies are PAS negative. On the other hand, some dense bodies are formed in the maturing spermatozoa which are PAS +ive and also reveal acid phosphatase activity. It is therefore, conjectured that this material may be helping to remove the surface coat investing the oocyte in case of nematodes.

VAS DEFERENS AND SEMINAL VESICLE

The vas deferens or the sperm duct of the trematodes, Paramphistomum epiclitum and Paradistomoides orientalis, and the cestodes, Senga lucknowensis and Oochoristica symmetrica, is lined by a layer of cuboidal/flat epithelial cells. Unlike the nematodes, insects and other animals (cf. Anya, 1966; Davey, 1967; Wigglesworth, 1972), the cells lining the platyhelminths sperm duct are nonsecretory. There is hardly any work dealing with the cytochemistry of the cells lining the vas deferens in the trematodes and cestodes.
The seminal vesicle in these animals is a simple enlargement of the vas deferens where the spermatozoa are densely packed and stored for a long time. This is thus comparable with the seminal vesicles of insects (Davey, 1967; Wigglesworth, 1972) or the epididymis of vertebrates (Hamilton, 1972). The cells lining the seminal vesicle are also flat and nonsecretory type. Threadgold (1975a) and Grant and others (1976) have given some details of the structure of seminal vesicle of Fasciola hepatica and Pharyngostomoides procyonis respectively. According to Threadgold (1975a), 'the epithelium of the seminal vesicle has three features of interest; first, the polymorphic surface projections and numerous thin surface lamellae with bulbous ends which may enclose spermatozoa; secondly, the deeply penetrating tube-like depressions of the apical plasma membrane within which are one or more spermatozoa of normal morphology and third, the lucid secretory vesicles derived from Golgi complex.' He (Threadgold, 1975a) has ascribed either a destructive or a nutritive function to this close association of spermatozoa and epithelium. No such association of sperm and epithelium was observed in any part of male duct of the platyhelminths under study. Grant and others (1976), too, have not referred to any association of spermatozoa and wall cells though they have described the presence of spermatozoa.
having elongate nuclei with fibrillar strands of electron dense chromatin in the seminal vesicle of Pharyngostomoides procynonis.

The vas deferens of each of the nematodes, *Ascaridia galli* and *Diplotriaena bhamoensis*, is a long tube showing regional differentiation into an anterior glandular and a posterior non-glandular region (cf. Chitwood and Chitwood, 1950; Anya, 1966; Bird, 1971). In both the nematodes, the secretion product in the form of granules and globules reveals the presence of proteins (-NH$_2$ and -SH groups) and polysaccharides with 1:2, glycol groups.

Chitwood and Chitwood (1950) have reported granules in the cells lining the vas deferens in *Ascaris lumbricoides* which these workers considered to be the secretory product.

Anya (1966) during his studies on *Aspiculuris tetrapertera* differentiated three regions of the vas deferens each of which releases one or more substances into the lumen of the male system and thus contributes to the composition of the semen. According to him, the granular secretion characteristic of the proximal part is composed of proteins rich in tryptophan but with very little tyrosyl or sulphydryl groups. Masses of secretory material found in the medial vas deferens stain very intensely for basic proteins and also for phospholipids. Finally in the distal region of
vas deferens, there are witnessed two types of secretory bodies both of which have proteins (rich in tyrosine) but differ in so far as one type shows intense staining for phospholipids whereas the other is devoid of any lipid material.

Hulinská (1973) has also distinguished three regions in the vas deferens of Enterobius vermicularis. According to him, "the secretory granules of the proximal region were mostly proteinaceous, of the medial section mainly lipoidal and of the distal portions lipoproteinaceous, pyroninophilic and also gave sufficiently positive reaction for alkaline phosphatase." He further observed that the "spherical cells" mentioned by Leuckart (1876) are obviously granules of different arrangement and difficult histochemical composition occupying the cylindrical epithelium of vas deferens.

Various views have been put forward regarding the function of the secretory products of vas deferens. Anya (1966) described an oxytocic function of the secretory products of vas deferens. Recently, serotonin (5-HT) has been identified as one of the secretory products in the vas deferens. As a result, the suggestion has been made that the function of the secretion is to aid sperm ascent by initiating motility of the spermatozoa as well as contractions of the female system in a manner that aids sperm
ascent (Anya, 1973). His suggestion has received experimental support inasmuch as it has been observed in *Ascaris* sp. and *Brugia* sp. that homogenate of the vas deferens when introduced into the male system of the living nematodes initiated pseudopod formation in the spermatozoa as well as other changes reminiscent of fully mature (ready for fertilization) spermatozoa of the female tract (Poor and McMohan, 1973; Poor, 1974). Taking all this into consideration, it is conjectured that vas deferens secretion of the nematodes under study, in addition to being nutritive, may also be vital for the proper maturation of the spermatozoa before these are transferred to the female tract and are ready for fertilization.

In *Diplotrisena bhamoensis*, the vas deferens reveals in addition to the secretion product large irregular phospholipid bodies. As these cells have been considered to have a phagocytic function, it is inferred that the dark bodies represent phagocytosed degenerating remnants of the residual cytoplasm. Anya (1966) described that such bodies in the distal part of vas deferens are extruded in the lumen and subsequently released with the semen. Similar phagocytosis of the sperm cytoplasmic remnants by wall cells of the vas deferens in the nematodes has been described by Pasteels (1948), Kanwar and Anand (1971) and Shepherd and others (1973). Bairati (1968), during his studies with
election microscope on Drosophila melanogaster, has conjectured a special function of the last portion of the male duct. He writes, 'considering the epithelium structure, it appears that the cells are prevalently characterized by the presence of cytoplasmic organelles somehow connected with digestive functions, for bulky residual bodies and lysosomes are found in them'. Anya (1966) has also described the presence of acid phosphatase in the distal part of vas deferens of Aspiculuris tetraptera. It is well established that the acid phosphatase activity is mainly confined to the lysosomes in the cells and these organelle are mostly concerned with the digestive function (de Duve, 1959, 1963a,b; Novikoff, 1961, 1963; Lima de Faria, 1969).

**Ejaculatory Duct**

The ejaculatory duct in the helminths is represented by the terminal tubular portion of the male duct. The ejaculatory duct in case of the trematodes, Paramphistomum and Paradistomoides, and the cestodes, Senga and Oochoristica, is lined by epithelial cells which are basically non-secretory type. The PAS positive material observed in Paradistomoides in the apical regions of these cells perhaps represents the secretion of the prostate gland. Threadgold (1975a) too has described that "the ejaculatory
duct epithelium in Fasciola hepatica is composed of cuboidal to columnar cells between or through which project the terminal parts of the ducts of the unicellular prostate glands. The apical surfaces of the epithelia are extended into triangular or filiform projections having thin sinuous lamellae. The cytoplasm contains granular ER cisternae and Golgi dictyosomes which synthesize a dense ovoid secretion.

As far as the ejaculatory duct in the nematodes under study is concerned, it could not be demarcated in Diplotriaena bhamoensis. In Ascaridia, however, the ejaculatory duct is represented by a straight tube with two pouches at its anterior end. The pouches as well as the duct itself are lined by cuboidal to columnar epithelial cells filled with secretory granules comprising proteins and polysaccharides (1:2, glycol groups) and the globules that remain unstained in all other preparations except those employed for detection of the proteins. Looss (1905) and Chitwood and Chitwood (1950) thought that the secretion of ejaculatory or cement gland in various nematodes acts as an adhesive material which helps the male to hold the female during copulation. However, Sommerville and Weinstein (1964) were not able to obtain any evidence to justify the term 'cement gland' in Nematospiroides dubius where worms in copula were always readily separated.
In addition, the ejaculatory duct cells of *Ascaridia* reveal the presence of irregular phospholipid bodies representing the phagocytosed sperm remnants or degenerated spermatozoa. As also described earlier, this phagocytosed material may be acting as a precursor for some vital synthetic material.

**Prostate Gland**

Surrounding the ejaculatory ducts in the trematodes, *Paramphistomum* and *Paradistomoides*, and the cestodes, *Oochoristica* and *Senga*, are observed a number of pear-shaped cells constituting the prostate glands. Their morphology and relationship with the ejaculatory duct resembles that of Mehlis' gland (Smyth and Clegg, 1959; Rybicka, 1966b; Threadgold and Irwin, 1970 and Threadgold, 1975b). Threadgold (1975b), during his studies with electron microscope on the prostate glands of *Fasciola hepatica*, has also described similar cells and has observed that these are separated from the adjacent parenchymal cells by a thin regular layer of interstitial cells.

Except for Threadgold (1975b), no other worker seems to have described in detail the cells comprising the prostate and in platyhelminths. However, there are a few reports which discuss the prostate glands in other lower invertebrates (cf. Kugler, 1965; Smith, 1965; Kanwar and Bhandal, 1976).
The prostate gland cells of the helminths under discussion reveal two types of secretion products, viz. (i) the granular which is cytochemically complex and contain the lipids—predominantly phospholipids, proteins and polysaccharides with \textit{1:2}, glycol groups and (ii) the larger globular containing phospholipids alone. The amount of secretion in the cells and its cytochemical nature tempts one to conjecture that this secretion increases the volume of the seminal plasma. In addition, it may help in sperm maturation or ripening.

Threadgold (1975b), during his studies on \textit{Fasciola hepatica}, has described that the prostate gland secretion is PAS positive (non-acidic) and mercuric bromphenol blue negative. He further suggested that the secretion bodies are composed of either a non-acidic polysaccharide or of a glycoprotein, the latter having only traces of proteins.

**OVARY**

The unpaired ovary in the trematodes, \textit{Paramphistomum epiclitum} and \textit{Paradistomoides orientalis}, is simple and more or less rounded whereas in the cestodes, \textit{Oochoristica symmetrica} and \textit{Senga lucknowensis}, it is bilobed, each lobe being further lobulated. The oogonia, in the trematodes and cestodes, under discussion, are comparatively small and are present either towards the anterior end of the
ovary or towards the periphery of each lobe while more mature cells (oocytes) increase in size and are present either towards the posterior end of ovary or towards the centre of each lobe, from where the oviduct starts (cf. Dawes, 1956; Bjorkman and Thorsell, 1964; Rybicka, 1966b; Erasmus, 1973). The ovary in both the nematodes, viz. Diplotriaena bhamoensis and Ascaridia galli, on the other hand, is telogenic in contrast to the hologenic ovary found in some nematodes (Crofton, 1968; Jenkins, 1975) and can be divided into two regions (i) anterior germinal zone where the rapid divisions of the oogonia take place and (ii) the growth zone where oocytes simply grow and accumulate the nutritive as well as the shell-forming material.

The ovary of each of the helminths under discussion is bounded by an outer sheath of muscle fibres, inner to which is present an epithelial layer of flat or cuboidal cells (cf. Gutwood and Chitwood, 1950; Sasser and Jerkins, 1960; Bjorkman and Thorsell, 1964; Anya, 1964b, 1976; Bird, 1971; Erasmus, 1973). As far as the platyhelminths are concerned, the cells are more prominent in Paramphistomum and Paradistomoides as compared to Senge and Oochoristica. Cytochemically, these cells are less pyroninophilic as compared to the germ cells in their immediate vicinity. The structure of these cells in platyhelminths suggests that these themselves are non-secretory and simply help in
the diffusion of nutritive material from the surrounding parenchymatous tissue into the ovary. Erasmus (1973), during his studies on Schistosoma mansoni, too described that the ovary is enclosed by a thin layer of circular muscles attached to a fibrous basement layer supporting the basal epithelium.

The sheath cells of ovary in both the nematodes under discussion, viz. Diplotrisena bhamoensis and Ascaridia galli, comprise elongated/cuboidal cells which reveal in their cytoplasm granules or spheres rich in polysaccharides (1:2, glycol groups). In addition, the cytoplasm of these cells also contain some phospholipid bodies. The ground cytoplasm of these cells reveals the presence of proteins with -NH₂ groups, small amount of glycogen and also some alkaline phosphatase activity.

Prestage (1960), during his studies on Ascaris lumbricoides, described that the epithelium of the ovary consist of a single layer of cells which in the upper region of the growth zone contain 'secretory vesicles having glycogen'. He also reported the presence of glycogen granules, endoplasmic reticulum and large number of mitochondria scattered in the cytoplasm of these cells.

Kochhar (1960) reported abundance of mitochondria in the follicular epithelial cells of Parrocaecum angusticolle.
He further stated: 'the lipoprotein granules take their origin in the follicular epithelium during early stages of growth period. Heavy infiltration of these from the follicular epithelium to the oocyte is observed during subsequent stages'.

Lee and Lestan (1971) described that the cells of the ovarian epithelium are elongate and flattened, each with a large nucleus, many large mitochondria with many long cristae, numerous ribosomes, a well developed granular endoplasmic reticulum, a few Golgi dictyosomes, small amount of lipids and large deposits of glycogen. They further stated that the amount of glycogen in cells adjacent to the mature oocytes is much less as compared to those adjacent to the maturing oocytes and oogonia.

Raven (1961), Nath (1968) and Engelmann (1970) in a variety of animal groups have reported synthetic activity in the ovarian follicular cells. According to Raven (1961), 'the auxiliary cells play an important part in the nutrition of the oocyte, presumably taking up substances of low molecular weight from the environment, synthesizing them into higher compounds which are then transmitted to the egg cells'. In the light of these observations of Raven (1961), it is conjectured that the epithelial cells in the nematodes under discussion, Diplotriaena and Ascaridia, too,
have similar function to perform, i.e. these absorb nutritive material from the perienteric fluid to be passed, on to the oogonia and oocytes after further processing. This view is substantiated because of the presence of alkaline phosphatase activity in these cells. This enzyme is well known to perform vital role in the transferance of materials across the barrier (cf. Lima de Faria, 1969).

According to Anya (1976), 'The ovary in the nematodes with its investing epithelial monolayer of cells is suspended in the body cavity filled with the perienteric fluid (Chitwood and Chitwood, 1950). Chemical analyses of the perienteric fluid of *Ascaris lumbricoides* and *Anisakis physeteris* indicate that this fluid is a complex mixture of proteins including free amino acids, carbohydrates including glucose and trehalose, fats and inorganic ions (Hobson, 1948; Hobson and others, 1952a, b; Pollak and Fairbairn, 1955; Fairbairn, 1960; Viglierchio and Cortz, 1972). The proteins consist mainly of albumins and globulins, and in *Ascaris* and some other nematodes include traces of haemoglobin (Lee and Smith, 1965). The developing oocytes would thus find in the perienteric fluid a rich pool of metabolites for the active synthesis of proteins necessary for their full development. In *Anisakis physeteris*, Viglierchio and Cortz (1972) identified 17 amino acids in the ovarian tissue as compared to 28 in the
perienteric fluid. Obviously, the investing epithelial cell layer does exert a selective effect on the entry of amino-acids into the ovarian tissue. However, the ultrastructural features of the epithelial cells do not indicate an active synthetic role (Poor, 1967; Lee and Lestan, 1971), even though McLaren (1973b) has suggested that the surface coat of spherical granular units, found on the developing oocytes of Dipetalonema viteae, and which are obviously analogous to the precursors of the dense granules of Ascaris (Poor, 1967) are secreted by the epithelial cells. Indeed, Kochhar (1960) maintains that "the follicular epithelium contributes lipoprotein bodies and glycogen to the oocytes. The lipoprotein bodies are essential for the production of hyaline spheres (protein yolk)." Thus while the perienteric fluid is the obvious source of ovarian metabolites, some part of vitellogenesis involves the ovarian epithelial cells. It is possible, however, that some of the smaller protein molecules could be synthesized in the alimentary cells, secreted into the perienteric fluid and subsequently abstracted therefrom by the epithelial cells (Poor, 1968a).

In both the nematodes, Ascaridia galli and Diplotriaena phaeoensis, the female germ cells are grouped around an enucleate rachis which is branched in the anterior region and reveals the presence of lipid bodies.
In *Ascaridia*, in addition to lipid bodies, are present some PAS +ive inclusions either scattered in the cytoplasm of the rachis or forming small aggregations. Prestage (1960), Poor (1960a), Lee and Løstan (1971) and McLaren (1973b) during their studies on various nematodes, have also described the presence of lipid droplets, glycogen, riosomes, dense bodies and microtubules in the central rachis. The germ cells remain attached to the rachis until freed in the distal portion of the ovary. Further, Prestage (1960), Poor (1967, 1968a) and McLaren (1973b) described the presence of narrow cytoplasmic bridges connecting the germ cells with the rachis which often contain fibres and elements of endoplasmic reticulum.

Varied functions have been assigned to rachis. Bütschli (1873) and Mayer (1908) compared the rachis with the Verson's cell of lepidopterans. Mayer (1908) further expressed the opinion that 'as there are no equivalents of the follicle cells of vertebrate ovarian tissue or the nurse cells of insects and other invertebrate ovary in nematodes, the rachis may have a nutritive function in relation to the developing oocytes'. Chitwood and Chitwood (1950) asserted that the rachis, to which the oocytes are attached, is not nucleated and hence cannot subserve nutritive function. Seurat (1920) stated categorically that rachis 'does not supply yolk to the eggs'. On the
other hand, Prestage (1960) suggested two alternative functions for the rachis, viz. (i) to serve as a reserve store of excess materials, synthesized originally by the oocytes and sequestered by the rachis until needed (this view is supported by the observations of Von Kemnitz, 1912 that glycogen appeared in the rachis of Ascaris only after this substance has become the chief stored product of the ovary); (ii) alternatively, it serves as an absorptive and synthetic "tank" from which nutrients may be drawn by the oocyte as and when required. Anya (1976) further stated that 'the presence of intracellular microtubules connecting the developing oocyte and the rachis as well as certain dense bodies of an apparently protein nature, with periodic repeating substructure support and strengthen the second suggestion'.

Poor (1968a) has drawn attention to the fact that lack of a well defined nucleus and organelle system in the rachis would suggest only a limited ability for the synthesis of ovarian yolk or their precursors. During present studies, there was observed a gradual increase of cytoplasmic inclusions in the rachis from the anterior to the posterior end and the same is true of the oocytes. So it is reasonable to suggest that the oocytes themselves incorporate and assimilate nutritive materials and the inclusions within the rachis are observed only after their
initial appearance in the oocytes. In addition, Foor (1968a) and McLaren (1973b) have ascribed to the rachis the role of synchronization of the oocyte development. The available evidence thus seems to suggest that the oogonia and the oocytes during vitellogenesis depend for their nutrient precursors on the perienteric fluid rather than on the rachis.

Towards the inner side of the outer sheath of the ovary of Paramphistomum epiclitum are observed a few nurse cells which throw cytoplasmic processes into the lumen of ovary. The cytoplasm of these cells reveals certain globules rich in the carbohydrates (1:2, glycol groups) and the phospholipids. The close association of the nurse cells with the germ cells and the cytochemistry of their globules suggest their nutritive role. Such cells have also been described by Bjorkman and Thorsell (1964), Kanwar and Agrawal (1973a) and Kanwar and others (1976) in Fasciola hepatica, Diplodiscus amphichrus and Gastrothylax crumenifer respectively. Bjorkman and Thorsell (1964) have further described that these cells have 'a tight endoplasmic reticulum with narrow tubular lumen'. No such cells were observed in the other helminths under discussion.

The ovary of all the helminths under discussion contain only the oogonia and the primary oocytes; maturation divisions take place only in the female duct, more precisely
in the proximal part of uterus. As also reported by Burton (1960) in *Haematoeloechus medioplexus*, 'the meiotic process typically begins in the ovary. The primary oocyte increases in size and preliminary reorganization of chromosomal material occurs'. According to Burton (1960), this is the so called "diffuse state which persists until the oocyte passes out from the ovary". Burton has clearly stated that the maturation (beginning of Meiosis I) takes place after the oocyte has left the ovary and the shell has been formed. Rybicka (1966b), while describing embryogenesis in the cestodes has also described that only the onset of oogenesis takes place in the ovary. Primary oocytes so formed grow rapidly and leave the ovary before the first maturation division takes place. Similarly, according to Bird (1971), the maturation of the oocytes in the nematodes too takes place when these pass from the ovary to the spermatheca or the proximal part of the uterus.

The nuclei of the oogonia as well as the oocytes of all the helminths under study are spherical, each with a conspicuous nucleolus. The size of the nucleus increases during interphase but growth is more pronounced in the cytoplasm. The nucleolus in each oocyte becomes duplex revealing an outer darkly staining rim and a lighter medulla.

The cytoplasm of the female germ cells of all the
helminths under discussion is highly basophilic-rich in RNA, as is revealed by methyl green/pyronin G method accompanied by usual perchloric acid extraction control. This basophilia corresponds with the abundance of ribosomes as reported by Bjorkman and Thorsell (1964) and Erasmus (1973) in these cells while working on *Fasciola hepatica* and *Schistosoma mansoni* respectively. Bjorkman and Thorsell (1964) further state: "since the endoplasmic reticulum is poorly developed (though there is abundance of ribosomes) a considerable proteinous secretory activity is unlikely".

In addition to diffuse pyroninophilia, a large number of basophilic granules are observed in *Paramphistomum epiclitum* oocytes scattered in the cytoplasm whereas in the oocytes of *Paradistomoides orientalis*, there is present a distinct sizable basophilic cytoplasmic body. It is conjectured that these represent the extruded nucleolar material. Though earlier workers studied these bodies, due to lack proper techniques, they described these under different names. Katheriner (1904) reported such structures in the cytoplasm of the oocytes of *F. hepatica* and called them 'yolk spheres'. Schubmann (1905) Schellenberg (1911), Gillie (1914) and Anderson (1935) considered these basophilic granules as degenerating products of oogonial cells which have been ingested by the oocytes. Cable (1931) observed
that the presence of these bodies in the oocytes of Cryptocotyle lingua and considered them to be centrosomal in origin.

For the first time, Yosufzai (1952b) discussed in detail the phenomenon of nucleolar extrusions in platyhelminths. Further, he described two types of nucleolar extrusions; (i) when an entire nucleolus passes through the nuclear membrane and comes to lie in the cytoplasm where it breaks up into small bits and (ii) when only small fragments of the nucleolus migrate into the cytoplasm. According to Yosufzai (1952b), the first process takes place in those oocytes which possess two nucleoli each.

Dendy (1914), Hilton (1931), Gresson (1933), Subramaniam (1937) and Bretschneider and Raven (1951) all are of the view that the nucleolar extrusions/emissions pass into the cytoplasm as a result of diffusion through the nuclear membrane.

As is well known, the submicroscopic organization of the nuclear membrane reveals the presence of a number of pores in it (cf. De Robertis and others, 1969; Buch, 1974). It is suggested that the passage of at least the bulk of nucleolar material into the cytoplasm takes place through the nuclear pores only (Anderson and Beams, 1956; Kemp, 1956; King and Devine, 1958; Merriam, 1959; Takamoto, 1966; Buch, 1974).
Koulish (1965) and Burton (1967b), while working on the oocytes of Gorgoderina attenuata and Fasciola hepatica respectively, reported the presence of a sizable body in the cytoplasm which stains like the nucleolus and hence designated it as "nucleolus-like body". Hanumantha Rao and Madhavi (1966), Sexena (1973) and Kanwar and others (1976) have also observed RNA rich bodies in the oocytes of various trematodes.

Koulish (1965) and Burton (1967b), however, are not sure about the origin of this nucleolus-like body. They conjecture that either it is formed as a result of coalescence of nucleolar extrusions or may be a portion of the nucleolus is pinched off and comes to lie in the cytoplasm. The first possibility looks more plausible.

Burton (1960), while studying the female germ cells of Haematoloechus medioplexus, has described certain bodies which are different from nucleolar extrusions. Gresson (1964) has also asserted that in Fasciola hepatica and possibly some other digeneans the large bodies seen under the optical microscope are not nucleolar extrusions. In reality these represent some other kind of cytoplasmic inclusions.

Kanwar and Agrawal (1973a,b), during their studies on the female germ cells of Diplodiscus amphichirus, have
described two types of cytoplasmic inclusions. According to them, the nucleolar emissions are observed only during early stages of the oocyte growth. During the late growth phases, however, they describe the presence of uniformly dispersed prominent globules rich in polysaccharides and proteins. Kanwar and Agrawal (1973b) have compared these with the compound yolk reported in the oocytes of a wide variety of other animals (cf. Kanwar and Agrawal, 1973a).

During the course of present investigations, the oocytes of Paradistomoides and Paramphistomum reveal some PAS +ive material either scattered in the cytoplasm or concentrated in the circum-nuclear area. This can be compared to the nutritive globules described by Kanwar and Agrawal (1973b). Rao (1974) also described the presence of PAS +ive granules uniformly distributed in the ooplasm of Ganea tigrinum and Mehrorchis ranarum. Kanwar and others (1976), however, described the presence of a juxta-nuclear patch, rich in polysaccharides, lipids and proteins, in the oocytes of Gastrothylax crumenifer and Ozylocotyle dawesi.

During the course of present studies there were observed in the oocytes of the cestode, Oochoristica symmetraca, 5-8 vitelline bodies. This vitelline material can be compared with the 'yolky' substance described by Rybicka (1966b) in the oocytes of a variety of cestodes. Different workers, however, described this material in the
late oocytes either in the form of small (Ogren, 1957a, b) in Oochoristica symmetrica and Rybicka, 1964a in Diphylidium caninum) or large granules (St. Remy, 1900 in Anoplocephala mammillana; Bona, 1957 in Paricterotaenia porosa; Ogren, 1955 in Hymenolepis nana; Ogren, 1956a in Mesocestoides corti and Rybicka, 1964b in Moniezia expansa). In Taenia serrata (Janicki, 1907), Multiceps symthi (Johri, 1957), Dilepis undula (Ogren, 1962), Ophryocotyloides corvorum (Taneja, 1971) and Raillietina sp. (Kanwar and Agrawal, 1974a), the granules aggregate in a single large yolky mass just before or soon after the oocyte enters the female duct.

Unfortunately, very little is known about the chemical composition of this vitelline material in the tape-worms. Johri (1957) reported acidophil proteins in this material in the oocytes of Multiceps symthi. Pavlova (1963) noticed that the yolky granules of Taenia rhynchus saginatus resemble cytochemically to the 'yolky lipids' found in the oocytes of other animals. During present studies on Oochoristica, the vitelline bodies were found to be predominantly proteinous in nature with -NH$_2$ groups. In addition to proteins, these contained RNA also. Taneja (1971) and Kanwar and Agrawal (1973b), during their studies on Ophryocotyloides corvorum and Raillietina sp. respectively have also described the vitelline bodies to be rich in the proteins and RNA.
As far as the nematodes, *Diplostomina bhamoensis* and *Ascaridia galli*, are concerned, the nutritive material comprises the lipid yolk and the PAS+ive bodies/hyaline spheres. In addition, ooplasm in these nematodes also reveals the presence of glycogen. The lipid yolk globules which are formed from the phospholipid bodies of earlier stages are comprised of pure triglycerides. The hyaline spheres of *Ascaridia*, however, are composed of proteins, RNA and acid mucopolysaccharides. These can thus be safely compared to the compound yolk of other animals (cf. Raven, 1961; Nath, 1968) whereas the PAS+ive bodies of *Diplostomina* oocytes are comparable to the carbohydrate yolk (cf. Nath, 1968). In addition to these inclusions, there are also observed, in the oocytes of *Ascaridia* and *Diplostomina*, the phospholipid bodies and lipoprotein granules.

A number of other workers have also observed similar structures in the oocytes of a variety of nematodes but they have used different terminology for their description. van Beneden (1883a,b), Faure-Fremiet (1913), Wottage (1937), Yanagisawa (1955), Monné (1962) all agree that there exist within the mature oocytes two types of inclusions. These are (i) the hyaline spheres, described first by van Beneden (1883a) which reportedly contribute to the formation of hard shell and (ii) the refringent
bodies of a lipid nature which form the innermost layer of the egg shell— the so called vitelline membrane.

Prestage (1960) described that the primary oocytes accumulate large quantities of nutritive inclusions which are concentrated mostly in the basal portions of the cells. The larger lipid droplets average around 2.25 microns in diameter, whereas the larger yolk platelets which appear to be composed of numerous glycogen particles average around 2.5 microns in diameter.

Kochhar (1960) during his studies on the oocytes of *Porrocaecum angusticolle* described two types of yolks, viz. the lipid yolk and the protein yolk (also called hyaline spheres). According to Kochhar (1960), the lipid yolk consists of triglycerides and is formed by the transformation of phospholipid bodies of the early oocytes. The hyaline spheres, on the other hand, consist of ribonucleic acids derived from the nucleus and the proteins and carbohydrates from the cytoplasm. These are synthesized in close association with the lipoprotein granules contributed to the oocyte by the follicular epithelium.

Anya (1964b) while studying the structure of female reproductive system and egg-shell formation in *Aspiculuris tetraptera* described that the cytoplasm of the mature oocytes, in addition to large amount of glycogen (Anya,
1964a), contains large refringent spheres and hyaline granules. The hyaline granules contain predominantly proteins with which phospholipids may also be associated, whereas the refringent bodies contain very little, if any, proteins. Fouquey and others (1958) have shown that in Ascaris, these refringent spheres consist of glycosides which have so far been found only in nematodes.

Foor (1967), while working on Ascaris lumbricoides, described that the oogonia in the short germinal region of the ovary contained no large inclusions other than lipid droplets. In the young oocytes, however, refringent granules and lipid droplets were numerous. Foor (1972) in the oocytes of Ascaris lumbricoides described another type of inclusion bodies in addition to the refringent granules, viz. the dense granules which are perhaps the same as hyaline granules of earlier workers (Anya, 1976).

Lee and Lestan (1971) reported that the oocytes of Heterakis gallinarum contain two types of granules, viz. the refringent granules which give rise to the ascaroside layer of the egg shell and another kind (type 2 granules) which appear to be a kind of yolk for the developing egg. The refringent granules, according to them, are larger of the two and are formed by the coalescence of several granules which are apparently formed with the participation
of Golgi complex in association with granular endoplasmic reticulum of the oocyte.

Hulinská and Hulinský (1973) discovered two types of granules in the oocytes of Enterobius vermicularis, i.e. small darkly staining lipoprotein granules and somewhat large protein granules. According to them, the first were present on the periphery of the oocyte just near to the thickened vitelline membrane whereas the second category of inclusions first appeared in the centre of the oocyte from where these moved outward and finally are placed next to the thin membrane bounding the plasma of the eggs.

Jenkins (1975) in the hologonic ovary of Trichiuris suis described that the spherical oocytes contained at least two types of granules— the electron dense spherical granules and the reticulate granules which displayed some resemblance to the refringent granules recorded in other nematodes.

Contrary to the views of some of these workers, it is observed during the course of present studies on Ascaridia and Diplostreptus that the lipid globules and the hyaline spheres/PAS positive bodies provide the nutritive material whereas the cytoplasmic glycogen and the lipoprotein granules seem to contribute in the formation of the egg shell.
Henneguy (1906) reported for the first time the presence of long filamentous mitochondria in the oocytes of *Distomum hepaticum*. According to Yosufzai (1952b), the granular mitochondria in the oogonia and early oocytes of *Fasciola hepatica* are aggregated either in a single mass or in two smaller masses lying opposite to one another across the nucleus. As the oocyte grows and gradually migrates towards the central region of the ovary, the mitochondria rearrange themselves and form a perinuclear ring. In the fully mature oocytes, the mitochondria are seen uniformly distributed throughout the cytoplasm. Gresson (1958) and Burton (1960) have also reported identical pattern of mitochondrial distribution in *Sphaerostoma bromeae* and *Haematoloechus medioplexus* respectively. Bjorkman and Thorsell (1964) and Gresson (1964) have described dense granular mitochondria in the oocytes of *Fasciola hepatica*.

During the course of present studies, it is observed that mitochondria in the oogonia and early oocytes of *Paramphistomum* and *Paradistomoides* are aggregated on one side of nucleus whereas in the later stages, these get evenly dispersed in the cytoplasm. However, in the cestodes, *Senga* and *Oochoristica*, the mitochondria are uniformly scattered in the cytoplasm right from the oogonal stages. In *Oochoristica*, before the formation of the vitelline
bodies, the mitochondria are seen specially aggregated at certain points where later on the vitelline bodies develop. After the formation of the vitelline bodies, the mitochondria again get evenly scattered in the cytoplasm. This special arrangement of the mitochondria and the developing vitelline bodies is interesting and is suggestive of some vital participation of the former in the synthesis of the latter.

In both the nematodes, viz. *Diplostrehe bhamoensis* and *Ascaridia galli*, the mitochondria in the oogonia and early oocytes are seen arranged in a juxta-nuclear clump but with the growth of the oocytes, these get dispersed uniformly. Their fate in the fully mature oocytes could not be ascertained as the egg cytoplasm becomes oocluded with the nutritive as well as the egg shell forming material, both of which completely eclipse the mitochondria.

Prestage (1960) described that the mitochondria are randomly disposed in the oocytes of *Ascaris lumbricoides var suum*. Lee and Løstam (1971) referred that the cytoplasm of the oogonia contains small (about 0.4 mm in width), round or slightly elongate mitochondria which possess 'a medium number of short plate-like cristae'. The mitochondria in later stages are no doubt larger (0.7 mm in diameter) but there is reported no corresponding increase in the number of cristae present.
The mitochondria in the female germ cells of all the helminths under discussion, viz. Paramphistomum, Paradistomoides, Oochoriotica, Senga, Diplotriaena and Ascaridia, as usual were found to be rich in proteins and phospholipids. These observations are in conformity with the findings of a number of earlier workers who studied a variety of female germ cells (Chopra, 1958; Guraya, 1959, 1960a; Raven, 1961; Nath, 1968).

The electron microscopic and biochemical studies have established that the mitochondria are double membraned structures, the inner of which is thrown into 'cristae'. The outer membrane reportedly contains monoamino-oxidase and NAD cytochrome C reductase. The inner membrane and cristae contain cytochromes, ubiquinones, dehydrogenases and ATP-synthetase. Bjorkman and Thorsell (1964), while studying the ovarian cells of F. hepatica, have described mitochondria with very few cristae. This reduction in the number of cristae has been correlated with the anaerobic conditions under which these parasites live (Bjorkman and Thorsell, 1962, 1963).

Opinions differ regarding the presence of the Golgi bodies in the oocytes of trematodes and cestodes. Yosufzai (1952b) and Gresson (1962b) have described the presence of Golgi apparatus in the oocytes of F. hepatica. Bjorkman and Thorsell (1964), in the female germ cells of Fasciola
hepatica, denied the presence of any structure comparable with the Golgi apparatus of the vertebrate cells though they reported 'some parallel bundles of saccules with dilated ends'. During the course of present investigations also, the Golgi elements could not be identified in the female germ cells of the trematodes, *Paraecephaloma* and *Paradistomoides*, and the cestodes, *Sengis* and *Oochoristica*. It is likely that these occur in the present material but are somewhat undistinguishable under the optical microscope. Burton (1960), who worked with optical microscope on the female germ cells of *H. medioplexus* remained silent about the presence of the Golgi material but when he extended his work on the same material but with the electron microscope (Burton, 1967b), he did describe the Golgi material in the oocytes.

As far as the nematodes are concerned, most of the workers have not reported any Golgi material in the female germ cells. Lee and Lestan (1971), however, described in the early oocytes of *Heterakis gallinarum*, a few Golgi bodies which reportedly decrease in number as these oocytes mature. Further, Lee and Lestan (1971) have conjectured that the decrease in number of Golgi elements is related with the formation of refringent granules and the yolk granules.

The lipoprotein granules observed in the oocytes of
Ascaridia and Diplotriaena, during present investigations, are comparable with the Golgi bodies of Lee and Løsten (1971). In the early oocytes, these seem to be participating in the formation of hyaline spheres (vide supra). However, in the late stages, these bodies move towards the periphery and seem to be involved in the formation of innermost shell layer (vide infra).

VITELLINE GLANDS

The vitellaria in both the trematodes, Paramphistomum epiclitum and Paradistomoides orientalis, and in the pseudophyllidean cestode, Senga lucknowensis, occur as numerous follicles specially clustered in the lateral regions of the body. In contrast to this, the vitellarium of the cestode, Oochoristica symmetrica, is in the form of a single, more or less compact, slightly lobed mass which lies behind the ovary. This condition is characteristic of cyclophyllidean cestodes in general (Hyman, 1951).

Each vitelline follicle/vitelline gland of cyclophyllid appears as a cell aggregate held together by a definite surrounding membrane. The young vitelline cells undergo a process of maturation during which these grow and become filled with shell-forming as well as nutritive materials.
Markell (1943) and Stephenson (1947) have observed mitotic figures in the immature or the peripheral vitelline cells of Probolitrama californense and Fasciola hepatica respectively. Irwin and Threadgold (1970, 1972), however, failed to find division stages in the vitelline follicles of Fasciola hepatica. During the course of present investigations also, these mitotic figures could not be observed despite repeated attempts. As also conjectured by Irwin and Threadgold (1970) 'although no mitoses were observed, these must be inferred because of the continuous production and release of mature cells from the follicles'.

The immature vitelline cells of the helminths under study have highly basophilic (RNA+) cytoplasm. This basophilia, however, goes on decreasing in the later stages so much so that the fully mature cells reveal hardly any cytoplasmic basophilia. These observations are in conformity with the findings of Guraya (1970) and Irwin and Threadgold (1970), who too have described abundant basophilia-ergastoplasm rich in RNA in these cells. The presence of intense basophilia under the light microscope and abundance of granular endoplasmic reticulum under electron microscope are considered characteristic of cells engaged in the protein synthesis (cf. Caro and Palade, 1964; DeRobertis and others, 1970).

Since at the time of emergence of vitelline granules
within the vitelline cells, hardly any other structure is seen in the cytoplasm, it is easy to observe and conjecture that the vitelline granules arise in definite association with the basophilic regions of the cytoplasm. Guraya (1961b, 1970), during his histochemical and electron microscopic studies on the vitelline cells of Fasciola indica and Paramphistomum bathycotyle has also described the emergence of the vitelline granules in the ergastoplasm. Guraya (1970) states: "The lipid bodies (so called Golgi bodies) and mitochondria do not play any visible role in the process of secretion and continue to exist as such among the secretory globules which, however, distort the shape of the lipid spheres whose participation in the secretion can easily be eliminated by the fact that they have not been observed at the time of appearance of secretory vacuoles". Kanwar and others (1974) and Kanwar and Agrawal (1976a), during their studies on Gastrothylax crumenifer, Ceylonocotyle dawesi and Diplodiscus amphichrus have also described the origin of the vitelline globules in association with the cytoplasmic basophilia. According to Irwin and Threadgold (1970): 'The protein is presumably synthesized in the extensive endoplasmic cisternae and transferred to the Golgi complex'. Irwin and Threadgold (1970) also remarked, 'The protein is concentrated in the Golgi complexes, which are very indistinct, perhaps indicating a rapid membrane turnover and globule production'. Many other workers (Tullock and Shapiro, 1957; Guilford,
1961; Burton, 1963; Lal and Johri, 1967), who studied the vitelline cells do not comment on the origin of the vitelline granules.

In the trematode, Paradistomoides orientalis, it is observed that each vitelline globule of the mature vitelline cell appears as an aggregation of many small granules. Irwin and Threadgold (1970) have also described that the protein in spherical membrane bound packages migrates to the cell periphery, where the large clusters accumulate. According to these workers, 'The individual globules within the cluster are large, compared with the solitary migrating ones, and this may indicate a certain amount of fusion of globules within a cluster'.

The vitelline globules/mass in Paramphistomum epiclitum and Senga lucknowensis appear duplex. Similar nature of the vitelline globules has also been described by Kanwar and others (1974) and Kanwar and Agrawal (1976a) in Gastrothylax crumenifer and Ceylonocotyle dawesi and Diplodiscus amphichrus respectively.

The vitelline cells of the cyclophyllidean cestode, Oochoristica symmetrtea, are very small as compared to those of the trematodes and the pseudophyllidean cestode and are peculiar in the sense that the nucleus surrounded by a thin layer of cytoplasm is placed peripherally on a large vacuole/vesicle. Similar structure of the vitelline
cell has been described in some Anocephalidae by Bischoff (1913) and Rybicka (1964b); in some Taenidae by Janicki (1907) and Johri (1957); in Dipylidium caninum by Venard (1938) and Rybicka (1964a); in Mesocestoides corti by Ogren (1956); in Oochoeristica symmetrica by Ogren (1957a) and in Raillietina sp. by Kanwar and Agrawal (1974c). The vesicle, however, was not reported by Ogren (1959) and Rybicka (1960, 1961). The vesicle during the present studies contained the vitelline material. Rybicka (1966b) has also described similar accumulation of the vitelline material within the vesicle.

The vitelline material of all the four helminths under discussion, is stained blue after mercuric bromphenol blue revealing its proteinous nature which has been further confirmed by ninhydrin-Schiff and ferric-ferricyanide tests. Presence of tyrosine in the vitelline globules of Paradistomoides and Senga is suggested by the pink colouration observed after Millon's reaction. Presence of proteins in the vitelline globules of a variety of platyhelminths has been reported by a large number of workers like Stephenson (1947), Smyth (1951, 1954), Smyth and Clegg (1959), Guraya (1961b, 1970), Lal and Johri (1967), Madhavi (1968), Nollen (1968, 1971), Irwin and Threadgold (1970, 1972), Ramalingam (1971, 1972, 1973), Kanwar and others (1974) and Kanwar and Agrawal (1976a).
In addition, the vitelline material of both the cestodes, viz. Oochoristica and Senga, reveals the presence of RNA. This observation is in conformity with the findings of Smyth (1951), Hanumantha Rao (1959), Guraya (1961b), Bjorkman and Thorsell (1963), Kanwar and others (1974) and Kanwar and Agrawal (1976a), all of whom have described 'basophilic' nature of the vitelline globules.

The vitelline material of the cestode, Oochoristica, also reveals polysaccharides (1:2, glycol groups). This observation is supported by Ogren (1956, 1957a) who has also referred to the presence of PAS positive material in the vitelline cells of some cyclophyllidean cestodes. Kanwar and others (1974) too reported PAS positivity of the vitelline globules of Gastrothylax crumenifer and Oeylonocotyle dawesi.

During the course of present studies, a high tyrosinase (phenolase) activity was observed in the vitellaria of Paradistomoides and Senga which produce tanned eggs. However, no tyrosinase activity was revealed by the vitelline-material of Paramphistomum and Oochoristica. Burton (1963) has also described the presence of DOPA-oxidase in the vitelline globules of Haematoloechus medioplexus. Nollen (1971) detected phenolase in Haematoloechus medioplexus and Philophthalmus megalus but failed to get positive reaction in Gorgoderina attenuata and Megalodiscus temperatus.
Symth and Clegg (1959) reported the absence of phenolase in case of Gorgoderina sp., Bucephaloides sp. and G. manosoni. Freeman and Llewellyn (1958) reported the same in Proctoaces subtenius. Madhavi (1966) investigated several members of Paramphistomatiidae that produce non-tanned egg shells. By using histochemical tests, she could not demonstrate phenolase or phenols in the shell globules of these animals.

In addition, the vitellaria of all the helminths reveal a high alkaline phosphatase activity. Alkaline phosphatase activity in the vitelline cells has also been described by Guraya (1970) in Paramphistomum bathycotyle and Fasciola hepatica. Guraya (1970) associated the alkaline phosphatase activity in the vitelline gland cells with the uptake and transport of the raw materials for secretion production. Phosphatases in various organs of platyhelminths in general have similarly been related with active transport of metabolites (Erasmus, 1957; Halton, 1967; Threadgold, 1968; John and others, 1971).

The vitelline cells of Senga lucknowensis and Paramphistomum epiclitum, in addition to vitelline material also reveal some sudanophilic globules which also pick up blue stain after acid haematein and Nile blue sulphate establishing their phospholipid nature. Guraya (1970) and Kanwar and others (1974), too have referred to the presence
of some lipid spheroids in the vitelline cells of various trematodes. Swiderski and Mokhtar (1974) and Maamouri and Swiderski (1976), while studying vitellogenesis in the cestodes, Bothriocephalus clavibothrium and Echinobothrium beauchampi respectively, have also described the presence of lipid droplets in addition to the shell globules.

The vitelline follicles of *Paramphistomum epiclitum* contain, in addition to the developing vitelline cells, a varying number of cells (nurse cells) with elliptical nuclei and scanty cytoplasm. This observation is supported by Irwin and Threadgold (1970) and Kanwar and others (1974) who too have reported nurse cells in the vitelline follicles of *Fasciola hepatica*, *Gastrothylax crumenifer* and *Oeylonocotyle dawesi*. Further, Irwin and Threadgold (1970) expressed the view that the nurse cells might have a role in the transport of nutritive material from the parenchyma to the developing vitelline cells. Threadgold and Gallagher (1966) and Gallagher and Threadgold (1967) have suggested that the parenchyma in *F. hepatica* acts as a transport system, and the process of translocation is facilitated by the functional complexes which occur between the parenchymal cells and the cells of the other organ systems. These workers have studied in detail the functional complexes between parenchyma and nurse cells and between nurse cells and developing vitelline cells.
MEHLIS' GLAND

Mehlis' gland in both the trematodes, Paradistomoides and Paramphistomum, and the cestodes, Oochoristica and Senega, reveal the same basic structural organization as is characteristic of the platyhelminths in general (cf. Hyman, 1951). Each gland is composed of a cluster of unicellular glands surrounding the ootype, and opening into it by fine ducts.

There is difference of opinion among various workers with regard to the composition of Mehlis' gland. Ujiie (1936a), on the basis of differential chromacity, described two types of cells in the Mehlis' gland of Clonorchis sinensis in haematoxylin/eosin preparation. Gonnert (1962) also described two types of secretory cells in the Mehlis' gland of Fasciola hepatica and termed them as 'serosen' and 'mucosen' cells. Further Ebrahimzadeh (1966) pointed out that the two types of secretory cells occur in many digenetic and monogene tic trematodes, though their distribution in relation to each other varies from species to species. DelConte (1970) too have reported two types of cells, i.e., cyanophilic and amphiphilic, in the Mehlis' gland of Corpopyrum sp.

During the present studies too, two distinct types of cells ($S_1$ and $S_2$) have been recorded in the Mehlis' gland of Paradistomoides orientalis. The $S_1$ cells of the present studies correspond to the 'mucus' cells and the $S_2$ cells to
the 'serous' cells of Gonnert (1962).

Burton (1967a), during his studies with electron microscope on the Mehlis' gland of a frog lung fluke, has described, in addition to two types of secretory cells, a third type—the interstitial cells. According to him, one type of these secretory cells (DB-cells) produce dense bodies (=S₂ cells of present studies) which appear to dissociate after entering into the lumen of the ootype, while the other type (=S₁ cells of the present studies)—the more abundant of the two (MB-cells)—produce membranous bodies. The third type—the interstitial cells are, however, non-secretory. Bogitsh (1970), Irwin (1970) and Throagold and Irwin (1970) have also described three categories of cells which comprise the Mehlis' gland in the various trematodes.

In the Mehlis' gland of *Paramphistomum*, *Senga* and *Oochoristica*, however, no distinction into different cell types, detailed above, could be made except that the cells nearer to the ootype were smaller than those which were away from it.

Johri (1957) has also differentiated two regions in the Mehlis' gland of the cyclophyllidean cestode, *Multiceps myrti*. According to him, the peripheral region consists of enormously elongated cells with prominent nuclei. Each
cell is connected with a tapering process opening into the ootype in the central region. He has further described that in the central region is present an aggregation of small enucleated cells which form a jumbled mass with the processes of the larger cells. During the course of present observations, the nuclei in the central smaller cells could be easily observed. These smaller cells of Johri seem to represent the early stages of the secretory cells which grow, get filled with secretory material and finally become elongated cells of Johri (1957). The present observations are in conformity with the findings of Dawes (1940), Stephenson (1947), Rennison (1953), Erasmus (1973), Kanwar and Agrawal (1974b, c) and Kanwar and others (1975a), all of whom have described only one type of cells in the Mehlis' gland of various platyhelminths.

The histochemical studies have revealed that the secretion of Mehlis' gland cells of all the four helminths under discussion is PAS positive. This is in conformity with the observations in the trematodes by Hanumantha Rao (1959), Del Conte (1970) and Kanwar and Agrawal (1974b), in pseudophyllideans by Smyth (1956), Pavlova (1963) and Hanumantha Rao (1960) and in cyclophyllideans by Johri (1957), Hedrick and Daugherty (1957), Rybicka (1960, 1964a, b), and Kanwar and Agrawal (1974c).
The Mehlis' gland secretion of Paramphistomum, Senga, Oochoristica and S₂ cells of Paradistomoides is also rich in phospholipids as is confirmed by Sudan black B and acid haematein tests. The presence of the phospholipids has been described in the Mehlis' gland secretion of the trematodes by Hanumantha Rao (1959), Burton (1960), Kanwar and Agrawal (1974b) and Kanwar and others (1975a) and also in the cestodes by Hanumantha Rao (1960) and Kanwar and Agrawal (1974c).

Proteinous nature of the Mehlis' gland secretion, observed during present studies, is supported by Johri (1957), Burton (1963), Smyth (1966), Kanwar and Agrawal (1974b,c) and Kanwar and others (1975a).

In addition to this, a high acid phosphatase activity was observed in the Mehlis' gland cells of the platyhelminths under discussion. Existence of acid phosphatase activity has been demonstrated by Bogitsh (1970) and Kanwar and others (1975a).

Various theories have been advanced with respect to the role of Mehlis' gland in the reproductive process. The original view that the Mehlis' gland secreted the egg shell was discredited on histological evidence long before histochemical techniques were applied to the problem (Smyth and Clegg, 1959). However, Yosufzai (1953) holds the view that
the bulk of egg shell was secreted by the Mehlis' gland.

Although it is more or less established now that the Mehlis' gland does not secrete the egg shell, the location of this gland around the ootype suggests its role in the formation of shell and many views have been expressed in this regard.

Leuckart (1886) and Ujiie (1936a) suggested that the secretion from the Mehlis' gland 'hardened the newly formed egg shell'. This view is difficult to accept when we know that the hardening is due to quinone-tanning of egg shell protein (Smyth, 1956; Smyth and Clegg, 1959; Burton, 1963) or by autotanning (Smyth and Clegg, 1959). Smyth and Clegg (1959) however state: "Although the precursors of the quinone-tanning system are derived from the shell globules, it is possible that Mehlis' gland secretes a fluid which effects the tanning process in some way (pH, redox potential, etc.) but secretion is clearly not primarily responsible for the hardening of the shell". Threadgold and Irwin (1970) have also hinted that 'one or other of the secretions plays a part in the actual tanning process, which results in a highly resistant egg shell, either by triggering off the enzymatic process that results in tanning or adding some essential co-factor for the process'.

The observations that the egg shell is formed in the
ootype led Tyzzer (1918) and Ujiie (1936a) to the conclusion that the secretion of the Mehlis' gland caused the release of shell globules from the vitelline cells. Dawes (1940) and Gonnert (1955), however, do not support this view. According to them, the distortion of the vitelline cells, as they pass into the ootype, is sufficient to precipitate the release of the shell globules.

Kouri and Nauss (1938), on the basis of similarity of the cells of Mehlis' gland and the prostate gland in Fasciola, suggested that the passage of eggs along the uterus may be assisted by a lubricating secretion of the Mehlis' gland. Still another function attributed to the Mehlis' gland is to activate the spermatozoa (Stephenson, 1947; Threadgold and Irwin, 1970).

Ibrahimzadeh (1966) stated that the 'serosen' cells probably secrete a substance that is involved in the confluence of shell granules produced by the vitelline cells whereas the secretion of 'mukosen' cells is implicated in the formation of a basic shell membrane. Chemical analysis of the extracts of Mehlis' gland in Fasciola hepatica indicate considerable amount of lipoproteins and some phospholipids and microscopic studies reveal that the lipoprotein granules migrate from the gland cells into the ootype (Clegg, 1965). Based on histochemical and in vitro studies of isolated Mehlis' glands of Haematoloechus...
medioplexus, Burton (1963) reaffirmed Dawes (1940) theory that the function of the gland is to secrete a substance that forms a basic capsule membrane enclosing an oocyte and several vitelline cells. According to this hypothesis, 'enclosed vitelline cells than release their globules which coalesce upon the membrane to build the definite egg shell'. Irwin and Threadgold (1970) and Kanwar and others (1975a) have also described lipoproteinous sheath around the group of cells in various trematodes. Gonnert (1955), who worked on Schistosoma mansoni, favours this hypothesis but believes that the membrane is formed by the secretory epithelium of the ootype.

During the course of present investigations, it is observed that the Mehlis' gland secretion is lipoproteinous in nature. The shell as it is being formed is also in contact with the ootype epithelium. It is suggested that the Mehlis' gland secretion first forms a thin membrane and then the shell is formed within this membrane.

OVIDUCT, SEMINAL RECEPTACLE AND UTERUS

The mature oocytes from the ovary of each of the helminths under study pass through the small oviduct to reach the next region of the female tract, the uterus. As described by Anya (1964, 1976), Erasmus (1973) and Hope (1974), the oviduct in these animals extends from the termination of ovary to the beginning of the uterus.
Geraert (1976) defined the oviduct in Tylenchida as few cells that form the constriction between the spermatheca or uterus and the ovary. The oviduct may occasionally be set off from the uterus either by a conical/funnel-shaped extension of the wall as in Anticoma sp. and Euchromandore sp. (de Man, 1886), which some authors have implied may function as sphincters regulating the passage of oocytes (Rauther, 1918) or by a slight dilation which serves as a seminal receptacle (Any, 1964b).

The wall of the oviduct in the helminths under study appears to be a continuation of the ovarian wall and comprises same layers, i.e., outer muscular layer and inner layer of epithelial cells which may be flat, cuboidal or columnar. Hope (1974) has described that the oviduct in the nematodes consists of spindle shaped-cells whose axes are generally at right angles to the longitudinal axis of the genital tube.

The epithelial cells lining the oviducts in Paradistomoides, Faramphistomum, Oochoristica, Senga, Diplotriaena and Ascaridia do not reveal any secretory activity. Anya (1964b) described that the oviduct in Aspiculuris tetraptera is lined by cuboidal epithelial cells which neither appear to be secretory nor contain any appreciable stores of reserve material. According to
him, their main function is of moulding the eggs to acquire characteristic shape and to provide the right environment in the lumen for the initiation of certain processes prior to and after fertilization.

In the trematodes, _Paramphistomum_ and _Paradistomoides_, it is observed that the proximal region of the duct is lined by a ciliated epithelium. The latter has, however, not been observed in the cestodes, _Oochoristica_ and _Senga_. The presence of cilia in the cells lining the oviduct has also been described by Erasmus (1973) in _Schistosoma mansoni_. According to him, the ciliary lining and the muscular wall assist movement of material in the duct. Erasmus (1973) has further reported that in _S. mansoni_, 'the posterior part of the oviduct is lamellate and functions as an area where sperm are localized, and exhibit the characteristics of intracellular digestion'. Lee and Lestan (1971) described that in _Heterakis gallinarum_, the spermatozoa from the male are stored in the oviduct, the cells of which are columnar with microvilli on their luminar surface. They further described that the spermatozoa stored in this region of the reproductive tract are often embedded in the wall of the oviduct and are surrounded by a fibrillar material which appears to be mucoproteinous in nature (Lee, 1971). No such condition/character has been observed in the oviducts of the trematodes, cestodes or
nematodes under discussion. The sperm in these animals, the other hand, are normally stored in the seminal receptacle which opens either into the ootype or directly in the uterus. In Ascaridia, however, the spermatozoa are stored in the proximal portion of the uterus.

In the trematodes and the cestodes, under discussion, it is observed that the seminal receptacle is lined by thin and flat cells which do not reveal any secretory activity. It is further observed that in these animals the spermatozoa do not reveal any special association with the wall cells. However, in both the nematodes under discussion, viz. Diplostomum and Ascaridia, the seminal receptacle/anterior region of uterus is lined by cuboidal epithelial cells having pseudopodia-like cytoplasmic processes projecting in the lumen. These cells are secretory in nature, the secretion product being rich in polysaccharides with 1:2, glycol groups and phospholipids. The close association of the spermatozoa with these wall cells suggests the nutritive function of the latter. Additionally, this region serves to phagocytose the degenerating germ cells.

Anya (1964b) described the wall of the seminal receptacle of Aspicularis tetragona to be composed of cuboidal cells with their free surfaces often rather rounded or hemispherical. According to him (Anya, 1964b),
this whole arrangement excludes the possibility of much dilatation of the lumen.

Foor (1968b) during his studies on the zygote formation in *Ascaris lumbricoides* has described that the cells lining the uterus reveal indentations in their plasma membranes, where the sperm are lodged. According to him, 'this relationship between the sperm and uterine cells is associated with the retention of sperm in the female reproductive tract. Possibly these areas of sperm "storage" are comparable to the previously described seminal receptacle'.

According to Erasmus (1973), 'the association of sperm with the female duct wall represents a very consistent arrangement and must have some biological significance. A similar retention of the sperm in the female ducts has also been reported in various nematodes by Lee and Anya (1967) and Foor and others (1971). It is possible that the sperm stored between the lamellae of oviduct receive nutrients from the female and this association may be significant in deciding the period for which the sperm are viable in the oviduct, and thus the frequency of insemination is necessary to maintain a viable stock of the sperm in this position. A number of morphological changes have been described in the sperm of *Dipetalonema viteae* stored in the uterus of female (Poor and others, 1971) and it is possible that a
similar process may be necessary in *S. mansoni*.

The role of intracellular digestion in the female duct wall might have considerable significance in a number of processes. The morphology of the phagosomes suggest that the material digested is intraovarian debris and/or nuclear material. This nuclear debris will be derived from degenerating ova, polar bodies or sperm nuclei. The process may be simply one of eliminating degenerating products but may also play a part in a feedback system regulating the release of ova from the ovary, regulating the supply of vitelline cells, providing the necessary substances for the maturation of sperm or providing a chemical gradient along which sperm will migrate from the anterior end of oviduct (Erasmus, 1973).

The oviduct in case of the trematodes, *Paradistomoides* and *Paramphistomum* and the cestodes, *Senga* and *Oochoristica*, before joining the uterus, enlarges to form a chamber, the ootype, which is lined by cuboidal epithelial cells and is surrounded by Mehlis' gland cells. The latter pierce through the epithelial cells to pour their secretion into the ootype lumen. The diameter of the ootype varies according to the luminal contents.

Erasmus (1973) during his electron microscope studies on the female reproductive system of the trematode,
Schistosoma mansoni, has described that the cuboidal epithelium lining the ootype is of different height in different regions. He has further described that the luminal plasma surface at the anterior and mid-regions of the ootype is elevated to form blunt, stumpy finger-like folds. Towards the posterior end of the ootype and particularly in the region of the chamber which receives the ducts of Mehlis' gland, these folds becomes finer, more leaf-like and form a very distinct type of microvillous surface. Some of the microvilli are bifurcate and are oval in cross section. The villi are approximately 1 mm long and it was though that there might be a correlation between the microvilli and the microspines on the surface of the egg. However, there is a considerable size difference between these two structures. Such villi, however, were not observed in the present material when examined under the light microscope. It was further observed in Paradistomoides, Paramphistomum, Oochoristica and Senga, that the cells lining the ootype do not resemble the secretory cells in any way. Erasmus (1973), on the other hand, has described that in Schistosoma mansoni, the cytoplasm of the ootype cells contain a nucleus with finely granular content and an excentrically placed nucleolus, mitochondria and a few Golgi complexes. The granular endoplasmic reticulum is not abundant and tends to be basal and perinuclear in its distribution. In the region of the
Golgi complexes are numerous small vesicles filled with granular material and these may also be observed in close association with the luminal plasma membrane. It is possible that these vesicles may represent a vehicle for the transport of materials synthesized within the cells to the lumen of ootype. The cytoplasm also contains small, dense bodies oval in outline.

The uterus in all the platyhelminths under discussion, viz. Paramphistomum, Paradistomoides, Oochoristica and Senga is a continuation of the ootype. In Paradistomoides, Senga and Oochoristica, this is lined by a layer of thin flat epithelial cells. However, in Paramphistomum, the epithelial lining comprises cuboidal cells covered over (on the luminal side) by a cytoplasmic layer.

Erasmus (1973) while studying the female reproductive system of Schistosoma mansoni has described that 'the structure of the uterus closely resembles that of the tegument and comprises an external cytoplasmic layer containing a variety of bodies and mitochondria which is connected by slender cytoplasmic strands to cell bodies with nuclei, endoplasmic reticulum, Golgi complexes and tegumentary bodies'. This layer is covered over with a granular layer which, according to Burton (1966), Erasmus (1967) and Oaks and Lumsden (1971) is produced by some of the tegumentary bodies and is concerned with providing
resistance to digestion by the host enzymes or of participating in ionic regulation. Such a granular layer could not be encountered under the light microscope in any of the platyhelminths under study.

Coil (1963, 1965) has described that cells of the uterine lining participate to some extent in the formation of egg capsules in Syncocelium spathulatum and Hydrophitrema giganticum. No such activity could be discerned during the course of present investigations on Paradistomoides, Paramphistomum, Cochoristica and Senga.

The uterine lining of the nematodes under study, viz. Ascaridia and Diplotriasena, comprise large cuboidal cells which at places project into the lumen revealing their association with the eggs. These cells are normally filled with lipid material which in case of Diplotriasena is in the form of large spheres, whereas in Ascaridia this is granular.

Anya (1964b) and Hope (1974) described the uterine wall of various nematodes to be formed of polyhedral or squamous epithelial cells apart from the investing muscle cells. Anya (1964b) and Lee and Lestan (1971) have further described the secretory nature of these uterine wall cells which secrete the outer lipoproteinous layer of the shell.

Foor (1967) described that the structure of the uterine cells in Ascaris with respect to rough endoplasmic
reticulum, Golgi complexes and secretory vesicles resembles that of other secretory cells. He further described that the dense sticky material secreted from the uterine cells adheres to the outer surface of the egg and gradually forms the fourth layer of the shell— the uterine layer.

During the course of present investigation, however, it is observed that secretion of the cells lining the uterus does not seem to participate in the egg shell formation. It appears to have a nutritive function in these animals.

The egg shell formation in the platyhelminths, under study, commences in the region of the ootype. This is a complicated process and has been the subject of investigation of various workers. Earlier investigators agreed that in the oviparous trematodes and cestodes, the vitelline cells provide the food material for the egg while the material for the egg shell formation is provided by the unicellular glands (=Mehlis' gland) which surround the dilated portion of the oviduct (Sommer, 1880; Looss, 1885 and Schubmann, 1905). Although the nutritive role of vitelline cells has not been questioned, doubts have been expressed pertaining to the capacity of the Mehlis' gland to provide all the material for the formation of the egg shell. The small size of the Mehlis' gland in most of the trematodes and its absence in others led investigators to seek elsewhere the source of shell material.
Long back, Leuckart (1886), Henneguy (1906) and Goldschmidt (1909) described that the granules which developed in the vitelline cells eventually gave rise to the egg shell. Since then many workers have confirmed on the basis of histological observations, that the vitelline cells contain, in addition to yolk for the developing embryo, large globules which form the bulk, if not whole, of the shell (Ujiie, 1936b; Kouri and Nauss, 1938; Rees, 1939; Dawes, 1940; Markell, 1943 and Stephenson, 1947). The work of more recent authors such as Smyth (1951, 1954), Gonnert (1955), Smyth and Clegg (1959), Bogomolova and Pavlova (1961), Burton (1963, 1967a), Coil (1965, 1966), Madhavi (1968, 1971), Irwin (1970), Irwin and Threadgold (1970), Guraya (1970), Nollen (1971), Ramalingam (1972, 1973), Kanwar and others (1975b) and Kanwar and Agrawal (1976b) also indicates that the egg shell is the product of the confluence of the shell globules produced by the vitelline glands.

Although nearly all workers have concluded that the contribution of the Mehlis' gland in the formation of the egg-shell is marginal, Yosufzai (1953) has tried to revive the older concept that the Mehlis' gland secretes most of the shell material. This view was mainly based on the argentophilic nature of the egg shell of Fasciola after classical Golgi silver techniques (Cajal, DeFano, Aoyama) on one hand and the secretion product of the
Mehlis' gland on the other.

During the course of the present studies on *Paramphistomum*, *Paradistomoides* and *Senga*, it is observed that a number of vitelline cells surround the ovum in the region of the ootype and release the vitelline material which seems to form the shell. During this process, the lumen of the ootype gets enlarged and the shell in the process of its formation is always in close approximation to the ootype epithelium.

There is not much of agreement regarding the specific region/site in the female reproductive tract where the vitelline secretions are released from the vitelline cells. Sommer (1880) reported that in *Distomum hepaticum*, the shell material is released from the vitelline cells long before they reach the vitelline reservoir. Rees (1939) in *Parorchis acanthus*; Stephenson (1947), Smyth and Clegg (1959) and Irwin and Threadgold (1970) in *Fasciola hepatica* and Hanumantha Rao (1960) in *Pentrocephalus ganapatti* reported the release of vitelline globules in the proximal part of the uterus. Yosufzai (1953) is of the view that the vitelline globules are released close to the opening of the oviduct. Tulloch and Shapiro (1957), on the other hand, have concluded that the activation of the vitelline cells is by the stimulation of the whole reproductive tract and that the granular release, which is the final
stage in the ripening process of vitelline cells, is independent of the cell migration to another site. Guilford (1961) states that due to the contraction of the ovovitelline duct, a group of vitelline cells pass to the ootype where the shell material is suddenly released from the cells.

During the course of present investigations on *Paramphistomum*, *Paradistomoides* and *Senga*, however, the release of vitelline material from the cells is noticed in the region of the ootype and that too in the presence of the oocyte. Willmott (1950), Dawes (1956), Kanwar and others (1975b) and Kanwar and Agrawal (1976b) also hold a similar view after studying egg shell formation in various trematodes.

In the cyclophyllidean cestode, viz. *Oochoristica symmetrica*, single vitelline cell becomes associated with each ovum around which then is formed a thin shell. A similar thin membranous shell around a single vitelline cell and the associated ovum has also been described in other cestodes (Janicki, 1907; Spätlich, 1925; Venard, 1938; Ogren, 1955, 1956, 1957a; Bona, 1957; Rybicka, 1964a, b).

A number of workers including Tyzzer (1918), Dawes (1940), Burton (1963) and Coil and Kuntz (1963) have
suggested that the Mehlis' gland produces a thin membrane on the outer surface of the egg shell. Clegg (1965) and Clegg and Morgan (1966), who described the lipoproteinous nature of the Mehlis' gland secretion in *F. hepatica* also demonstrated lipoproteinous membrane on the inner as well as the outer surface of the egg shell. Likewise, Irwin and Threadgold (1972) and Kanwar and others (1975b), too have reported lipoproteinous membrane on the outer surface of the egg shell.

During the course of present investigations, it is observed that the developing egg is always in contact with the ootype epithelium. In addition, the secretion of the Mehlis' gland is also observed in the ootype region which suggests that this material also participates in the egg shell formation.

A number of studies have shown that the egg shells of the trematodes and cestodes, although appearing apparently identical during formation, do show variations in their chemical nature. In most of the species so far studied, the amber-coloured egg shells, whether thin or thick, are composed of quinone-tanned protein-sclerotin (Stephenson, 1947; Smyth and Clegg, 1959; Burton, 1963; Coil, 1965, 1966; Wilson, 1967; Kanwar and others, 1975b). This tanned inelastic and highly resistant protein is formed from the precursors such as basic proteins, proteins
rich in tyrosine and phenols which in turn are produced in the shell globules of the vitelline cells. Evidence indicates that the phenolic compounds are oxidized by the enzyme phenolase (polyphenol oxidase, tyrosinase) to ortho-quinones which then react with free amino or sulphydryl groups on the adjacent protein molecule to bind the shell material together (Smyth and Clegg, 1959). As all the precursors for sclerotization of proteins, viz. proteins rich in -NH₂ groups and tyrosine and the enzyme tyrosinase have been identified in the vitelline material of Paradistomoides and Senga (vide supra), it is conjectured that the egg shell in these helminths is formed by quinone tanning. As also described by Clegg and Smyth (1968), it is observed that the egg shells in early stages of their formation exhibit all the cytochemical properties of the vitelline globules. However, when the egg capsule hardens and darkens, the shell remains unstained in various cytochemical tests.

In Paramphistomum epiclitum, tyrosinase activity could not be detected in the vitelline globules (vide supra) which on the other hand, revealed the presence of keratin after aldehyde fuchsin and alcian blue (after permanganate oxidation) methods of Madhavi (1971). Similar staining characteristics are also the revealed by the egg shells of this trematode even in the distal parts of the
uterus. From all this it can be inferred that the egg shells are of keratin-type. Madhavi (1966, 1968, 1971) and Kanwar and Agrawal (1976b) have also described the presence of keratin-type of shells in a number of digenetic trematodes that produce transparent and non-tanned eggs.

As far as the nematodes are concerned, the developing eggs are covered with the shell material as these pass through the uterus. Nelson (1852) was perhaps the earliest worker to study this egg shell formation in the nematode Toxascara cati (= Ascaris mystax). This was followed by a number of studies which have been ably reviewed by Christenson (1950), Fairbairn (1957), Rogers (1962), Bird (1971) and Anya (1976).

During the course of present investigations on Ascaridia galli and Diplostomum phemoensis, it was observed that as an immediate consequence of fertilization (which takes place in the anterior part of the uterus/seminal receptacle), the oolemma separates out to form the outermost layer leaving a structureless zone which eventually gets filled with the chitinous layer. It is conjectured that glycogen material of the egg serves as a precursor for this chitinous material. This is substantiated by the observations of Fairbairn (1957) who reported that as an immediate response to the stimulus of sperm...
penetration, almost one half of the glycogen reserves of the egg is mobilized and converted into the N-acetyl-glucosamine units of chitin, utilizing the rich pool of glutamine and acetate in the oocyte. Similarly Kochhar (1960) has also discussed the involvement of glycogen in the formation of chitin comprising the middle layer of the egg shell.

Yanagisawa (1955) and Anya (1964b) suggested that in *Ascaris lumbricoides* and *Aspicularis tetraptera*, the protein moiety of the chitinous layer was derived from the pre-existent proteinaceous hyaline granules of the oocyte. Further studies on the egg shell formation in *Porroecaecum angusticolle*, *Ascaris lumbricoides* and *Heterakis gallinarum* by Kochhar (1960), Poor (1967) and Lee and Løsten (1971) respectively have shown, however, that the hyaline granules do not contribute any material in the formation of the egg shell. During the course of present studies on *A. galli* also it is observed that the hyaline spheres remain intact even up to quite late stages and do not seem to participate in the formation of the chitinous portion of the shell.

In *Ascaridia* and *Diplogastera*, inner to this chitinous layer is laid the third layer—the lipoprotein layer which is formed as a result of the extrusion and fusion of the lipoprotein granules from the oocytes. This
layer can be compared to the ascaroside layer described by some workers (cf. Anya, 1976). As discussed by Anya (1976), 'this layer arises from the extrusion and subsequent coalescence of the ascaroside content of the refringent granules (lipoprotein granules of present studies). These ascarosides have been described to account for 75% of the material of the ascaroside layer, the other 25% being protein (Foor, 1967; Jezyk and Fairbairn, 1967). As the ascorosides exist in the refringent granules as esters (Jezyk and Fairbairn, 1967; Tarr and Fairbairn, 1973), it is further conjectured that these esters must first be converted into the free ascorosides before incorporation into ascaroside layer and the conversion process must take place in the refringent granules before extrusion or in the forming layer immediately on extrusion'.

Foor (1967), during his electron microscope studies on *Ascaris lumbricoides* has described that the material of the refringent granules comprises a dense core of particulate material embedded in a less dense but homogeneous matrix with the same consistancy as the ascaroside layer. He (Foor, 1967) further adds that the protein material of the ascaroside layer may be provided by the dense core material of the refringent granules while the homogeneous matrix consists of the ascaroside esters.
The presence of these three layers in the egg shells of *Ascaridia galli* and *Diplostrephae bhamoensis* is in conformity with the observations of Christenson (1950), Fairbairn (1955), Fairbairn and Passey (1955), Rogers (1956, 1962), Kochhar (1960), Anya (1964b), Hulinský and Hulinsky (1973), who too have described similar 3 layers in the nematode egg shells.

Lee (1961) and Monné (1962) described that in the ascaroids and the oxyuroids, the outermost lipoprotein layer may be invested with an additional and external deposit of jelly-like material which may consist of mucoprotein as in *Ascaris lumbricoides*, *Parascaris equorum* and *Ascaris suum* or acid mucopolysaccharides as in *Thelestoma* sp. Poor (1967) described this fourth layer of the shell as a product of the secretion of the uterine cells. No such layer, however, has been observed in the egg shells of *Ascaridia galli* and *Diplostrephae bhamoensis* during the course of present investigations.