DISCUSSION
MORPHO-HISTOLOGY

It is known that L. rohita and C. mrigala, two Indian freshwater major carps are seasonal breeders. They breed only in monsoon season (June to August). Prior to spawn in monsoon, the testes of the two aforesaid fishes undergo preparatory stages during the remaining part of the season. This preparation includes various degrees of morpho-histological and biochemical transformation of the testes in relation to the spermatogenetic activity.

The present study reveals that the testes of L. rohita and C. mrigala, exhibit remarkable seasonal variations in their shape, size and volume. The testes, however, do not display any distinct lobe on the surface except in active spermatogenetic phase. With the initiation of growth phase, they gradually increase in size and volume in both the fishes as also reported by many authors in some other teleosts (Hyder, 1970; Merrett, 1971; Davis, 1977a; Bhatti and Al-Daham, 1978; Chan and Chua, 1980; Hoffmann et al., 1980). On the other hand, the testes with prominent lobes on their outer surface in both the fishes under study attain voluminous in size completely filling the entire body cavity in the late maturation and spawning phases. The formation of lobes on the surface in the testes is probably
imparting the increase of internal surface for accommodating large number of advanced spermatogenetic cells proliferated subsequently in the testicular lobules during the latter phases. However, Swarup (1958) did not report any lobe on the surface of testes even in the breeding phase of the fish, *Gasterosteus aculeatus* while lobular testes have been observed by Sathyanesan (1959) and Rai (1965) in *Mystus seenghala* and *Barbus tor* respectively. Distinct lobes in the testes with the commencement of spawning period but their absence in pre-spawning time in *Limanda limanda* were also observed by Htun-Han (1978). It has been noticed in the present study that the length as well as volume of the testes in both the fishes increases markedly with gradual initiation of spermatogenetic activity. This results the testes to increase their relative weight upto the spawning phase. Therefore, variations in length, volume and surface lobulation of the testes bear a close relationship with the periodical sexual activity of the fishes under study. However, increase in length, volume and weight of the testes in some freshwater Indian teleosts during the breeding season has also been reported by many authors (Nair, 1965; Rai, 1965; Bhatt, 1968; Thakur, 1978; Jayaprakas and Nair, 1981).
Gonadosomatic Index (GSI):

It has observed during the course of present investigations that the GSI value varies greatly during the different months of the year in both the fishes. It remains very low due to the thread-like structure of the testes throughout the entire resting phase when spermatogenetic activity virtually ceases to act. However, because of the active division of the spermatogonial cells mitotically and eventual formation of resultant primary spermatocytes, the testes gradually increase in volume and weight from the growth phase onwards. Further, the formation of advanced stages of spermatogenetic cells by meiotic division is effected in the subsequent phases. Therefore, the highest GSI value in the late maturation and early spawning phases is due to the active proliferation of advanced stages of spermatogenetic cells from spermatagonia resulting the relative increase of the testes weight. Similar changes of GSI value in relation with spermatogenetic activity in the testes of different teleosts have also been observed by various authors (Jaspers, 1972; Bhatti and Al-Daham, 1978; Thakur, 1978; Llewellyn, 1979). But Sanwal and Khanna (1972) failed to record any remarkable seasonal changes in weight and volume of the testes in Channa gachua. However, maximum GSI value during the maturation or pre-spawning phase has been noticed by many workers in different fish species.
Discussion: Morpho-histology

(Ambrose and Brown, 1971; Bhatti and Al-Daham, 1978; Jayaprakas and Nair, 1981). Although very little changes of testes weight in comparison with that of ovaries in *Heteropneustes fossilis* were observed by Bhatt (1968), but he noticed maximum gonad weight in both the sexes during the peak gonadal maturation of the fish studied. Guest (1973), on the other hand, stated that the GSI variation in channel catfish is largely dependent on the changes in size of the fish. But Jaspers (1972), pointed out that the GSI value changes with concomitant increase in the spermatogenetic activity and sperm concentration in channel catfish of other sources which was obtained by Guest mentioned above. In addition to macroscopic and histological evidences, Merrett (1971) considered the relative gonad weight as an index of development and maturity of the fish studied by him.

On the basis of GSI value obtained in the present study, it has been assumed that the maturation process of the testes begins in the month of March in both the fishes and rapidly reaches a peak during the month of May and June when the GSI is recorded maximum. It has been observed that the fishes spawn only after gaining the highest GSI value. However, the reduction of the highest GSI value in *L. rohita* in August but its still persistent in *C. mrigala* indicates the cessation of spawning activity earlier in the former. Therefore, GSI is closely
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related with the maturity of the fishes under study. The highest GSI value in the breeding season and its depletion during the non-breeding season in different fishes were also observed by a number of workers (Rangarajan, 1971; Llewellyn, 1979; Dindo and MacGregor, 1981; Pollock, 1982). Relative abundance of spermatotzoa during the early spawning phase is probably responsible for further increase in GSI value during the aforesaid phase of the two fishes, L. rohita and C. mrigala. Similar observation was made by Bhatti and Al-Daham (1978) in _Esox lucius_. However, maximum GSI value during the active spermatogenesis with full spermatotzoa content in the lobules of testes was observed by Todd (1981b) in hormone injected eels. Relatively low values of GSI during the period in August and September indicates the 'dormant' phase of the testicular activity in L. rohita and C. mrigala respectively. This is in conformity with the finding of Thakur (1978) in _Clarias batrachus_ and Bhatti and Al-Daham (1978) in _E. lucius_.

Cyclical changes and spermatogenesis

_Spermatogenesis_

There are many reports regarding the dormant spermatogonia of the post-spawning period in freshwater teleosts. However, these dormant spermatogonia appear to be responsible for their reconstruction and subsequent formation of spermatogonial cells
occurred during the different periods of the year. With the commencement of growth period of the testes, these cells are divide mitotically to give rise sperm mother cells (Khanna and Pant, 1966; Htun-Han, 1978). Various authors have diversely designated these cells as 'dormant germ cells' in Cottus bairdii (Hann, 1927), 'reserve germ cells' in Placoglossus altivelis (Suzuki, 1939) and 'resting germ cells' in Onchorhynchus nerka (Wiesel, 1943), Placoglossus altivelis (Honma and Tamura, 1962) and Salmo salar (Jones, 1940). The presence of 'dormant nests of spermatogonia' in the testicular lobules during the late resting phase is the characteristic features of L. rohita and G. mrigala. Activation of 'dormant spermatogonial cells' results new spermatogonial cells by mitotic division during growth phase. Therefore, in the aforesaid phase, relative abundance of spermatogonial cells is noticed in the present study of both the fishes. Similar type of 'dormant spermatogonia' has also been reported in some other teleosts by different authors (Khanna and Pant, 1966; Htun-Han, 1978; Sinha and Mondal, 1981, 1982). But contradictory statement regarding the formation of new spermatogonia was given by Foley (1926) who suggested that new spermatogonia are formed from the migratory cells located outside the testes of the fish, Umbra limi. Reports are also available in the literature regarding the origin of germ cells from the resting sperm mother cells
within the testicular lobules of different fishes (Bullough, 1939; Sathyanesan, 1959; Rai, 1965). These germ cells, on the approach of growth period, become active, start dividing and are transformed into primary spermatogonia (Sanwal and Khanna, 1972). However, development of the various spermatogonial cells of both the fishes under study proceeds within the cysts located in the tubular compartments, the lobule. The lobule boundary cells are present in the lobule wall of the testes throughout the different phases in L. rohita and C. mrigala. The presence of aforesaid cells in various fish testes have also been described earlier by many workers (Lofts and Marshall, 1957; Henderson, 1962; Yaron, 1966; Sanwal and Khanna, 1972; Nicholls and Graham, 1972; Gresik et al., 1973; Belsare, 1973; Grier, 1975, 1976).

The event of spermatogenesis has been divided by various authors into different stages viz., into five stages in dab, L. limanda (Htun-Han, 1978), in pike, E. lucius (Hoffmann et al., 1980), in Colisa fasciata (Mondal, 1980), in C. punctatus and A. testudineus (Sinha and Mondal, 1981, 1982); into six stages in C. gachua (Sanwal and Khanna, 1972), in some other teleosts (Upadhyay and Guraya, 1973), in T. tandanus (Davis, 1977a), in B. lutus (Bhatti and Al-Daham, 1978), in L. subviridis (Chan and Chua, 1980). However, all of them described the entire
spermatogenetic event mainly into 1) spermatogonia, 2) primary spermatocytes, 3) secondary spermatocytes, 4) spermatids and 5) spermatozoa in the teleosts reported by them. However, some authors identified two categories of spermatogonial cells—primary and secondary. The cellular boundary of the latter is more prominent than that of the former (Sanwal and Khanna, 1972; Upadhyay and Guraya, 1973; Bhatti and Al-Daham, 1978). Throughout the present investigations, the aforesaid five stages of the spermatogenetic cells have been identified in both the fishes on the basis of the cell size and volume of chromatin material present in the cell concerned. During the course of differentiation of spermatogonia into spermatozoa through the subsequent intermediate stages, the cytoplasm and nuclei of spermatogonia progressively decrease in size and volume. Chromatin material in the nuclei of latter stages of spermatogenetic cells (viz., secondary spermatocytes, spermatids and spermatozoa) are relatively more condensed when compared with those of spermatogonia and primary spermatocytes. These identifying characters of various male germ cells of different teleosts have also been followed by many authors (Russo and Pisano, 1973; Htun-Han, 1978; Hoffmann et al., 1980; Sinha and Mondal, 1981, 1982).

Spermatogonial cells (stage 1) are the largest cells with maximum amount of cytoplasm and comparatively larger but
darkly stained nuclei in the testicular lobules in the two fishes studied. These cells are found throughout the entire period of the season. This corresponds with the findings of earlier authors in a variety of teleosts (Hann, 1927; Stenger, 1959; Rodoni, 1969; Sanwal and Khanna, 1972; Htun-Han, 1978; Sinha and Mondal, 1981; 1982). Majority of the spermatogonial cells undergo mitotic division as evidenced by the presence of mitotic figures detected in the present study. This event increases the relative abundance of primary spermatocytes (stage 2) which appear to be smaller in size than the spermatogonia and are located just beneath the spermatogonial cell layer in both the fishes reported here. Secondary spermatocytes (stage 3) which are formed by the meiotic division of primary ones, are characterized by the presence of condensed and thick chromatin material. These cells in both the fishes are further reduce in size and are lodged at the near proximity of the lumen of the testicular lobules. This observation on the size difference as well as on the other characters between the spermatogonia and spermatocytes resembles with the findings of Stephen et al. (1975), Davis (1977a), Bhatti and Al-Daham (1978), Sinha and Mondal (1981, 1982). In the present study, it has been observed that the spermatids (stage 4) having oval or almost rounded nuclei with highly condensed chromatin material, are smaller than the
secondary spermatocytes and are finally metamorphosed into spermatozoa (stage 5). The close similarity between the spermatids and spermatozoa except the presence of a sperm tail of the latter ones in O. gachua was pointed out by Sanwal and Khanna (1972). However, further reduction in size of mature spermatozoa has been reported by some authors (Khanna and Pant, 1966; Davis, 1977a; Bhatti and Al-Daham, 1978; Sinha and Mondal, 1981, 1982). Reduction in the size of spermatozoa, particularly in mature ones in both the fishes under study confirms the above findings. Jaspers et al. (1976) in their ultrastructural study, emphasized that the spermatozoon of channel catfish comprises of a rounded but darkly stained head having dense chromatin material, a collar-like mid-piece and a long flagellum. Similar observations were also made by Caloianu-iordachel and Sicoe (1976) in Stizostedion lucioperca and Cyprinus carpio and Gardiner (1978) in Cymatogaster aggregata. But Brusle (1981) pointed out that the mature spermatozoon of the teleosts, Liza aurata comprises of a round nucleus and a pseudomid-piece. However, in the present light microscopic study it has not been possible to distinguish clearly in between head- and mid-piece of a spermatozoon except a distinct posterior-most portion of the former from which the
flagellum originates, observed in some squash preparations. Darkly stained as well as highly condensed and specialized nuclei on the other hand, are only seen in the histological preparations. But in the squash preparations, arrow-shaped and/or elongated nuclei with long tails (flagellum) of spermatozoa have been easily identified. Further, a few biflagellar spermatozoa morphologically identical in appearance with those of single flagellar ones have been detected during the spermiogenesis of both the fishes studied. The volume of the sperm head gradually reduces at the time of flagellar elongation. Therefore, a close relationship exists between the volume of sperm-head and the length of flagellum during their course of differentiation in both the fishes. The occurrence of biflagellar spermatozoa in certain teleosts has been reported by some authors (Stanley, 1965; Mattei, 1970; Yasumumi, 1971; Jaspers et al., 1976). The significance of the biflagellar spermatozoa and its advantages over fish life is not clearly understood. However, in the present study it is presumed that exflagellation of one flagellum of a biflagellar spermatozoon during its transformation through the sperm duct, may have certain advantages for maintaining its motility.
Cyclical changes in relation to spermatogenesis:

In some higher vertebrates, particularly in mammals, such as man, rat, etc., the spermatogenetic cycle has been reported to continue at a constant rate throughout the year while in some other vertebrates like the monkey, squirrel, and bird and turkey (Dalrymple et al., 1968), this cycle undergoes seasonal variations in parallelism with seasonal breeding (Conaway and Sade, 1965; Vandenbergh and Vassey, 1968; Shivasankar and Prasad, 1968). The physiological property and spermatogenetic activity of testicular tissue of house lizard undergo seasonal variations (Sanyal and Prasad, 1965) whereas Atherton (1974) also noticed a similar type of seasonal spermatogenetic cycle in the toad.

In fishes, however, the spermatogenetic activity is known to begin at different times of the year following a regular cyclical change (Turner, 1919; Bullough, 1939; Nair, 1965; Rai, 1965; Moiseeva and Ponomareva, 1975). A great majority of freshwater teleosts exhibits spermatogenetic activity for a relatively shorter duration (about 2-3 months) in a particular period of every year. But the period of intense spermiogenetic activity is variable in different fishes. As for example, in a hag fish, Eptatretus burgeri (Patzner, 1977), spawning season begins from October and the spermatocytes, which appear first in January
and attains their maximum number in March. Development of spermatids, however, starts in April and proceeds till June. Sasaki and Igarashi (1974) reported that in *Sebastes vulpes*, the spermatogonia begin to appear in the seminiferous tubules from middle of March and extends upto June, the entire period being known as the preparatory period. The spermatozoa are detected for the first time in the middle of November, which is defined as the period of spermatogenesis. In the discharged period, a maximum number of spermatozoa is observed by them. In *Liza dumerill* (Van der Horst and Barssus, 1978), the testis resumes to mature in early spring, reaching a peak during December and January. Spawning season is represented by January and February. The entire testicular cycle have been divided into four distinct phases by some workers on the basis of morpho-histological changes and spermatogenetic activity of the testes in many teleosts (Brylinska and Dlugosz, 1973; Stephen et al., 1975; Htun-Han, 1978; Hoffmann et al., 1980; Sinha and Mondal, 1981, 1982). But, Hyder (1970) distinguished the testicular cycle of *Tilapia leucosticta* into three phases on the basis of histological findings. Seven arbitrary stages identified on the basis of the macroscopic appearance of gonad during the testicular cycle were reported by Davis (1977a). Similar types of divisions, depending on both the macroscopic and microscopic observations on the testes in *Liza subviridis*, have been classified
by Chan and Chua (1980). Testicular cycle in the fish, *Barbus luteus* (Bhatti and Al-Daham, 1978) has been grouped into five distinct stages (viz., resting, maturing, pre-spawning, spawning and post-spawning stages) depending on the gonadal volume, gonadosomatic index and histological changes of the testis.

In Indian teleosts, various authors reported the occurrence of spermatogenetic cycle during the different months of the year. Ghosh and Kar (1952) observed that in *Heteropneustes fossilis*, the spermatogenesis continues throughout the year with constant rate. In *Glyptosternum pectinopterum* (Khanna and Pant, 1966), however, the lumen of the testicular tubule is completely filled up with spermatogonia during the resting period (November to January) and spermatogenesis progresses actively from January, eventually attaining its peak between April and June with a concurrent decrease in the number of spermatogonia to a bare minimum though other advanced stages viz., spermatocytes, spermatids and spermatozoa outnumber. Spermatids and spermatozoa fill up the cysts of lobule during July, August and September in this fish and complete interruption of spermatogenesis occurs late of this period. Shreatha and Khanna (1976) observed the two spawning periods, one in September and the other in March, and a distinct dormant period during winter season in the fish, *Schizothorax plagiostomus* was noticed. They (1978) further reported the presence of a seasonal spermatogenetic cycle in the fish, *Garra gotyla* and pointed out that the
spermatogenesis in this fish begins in October and reaches its peak in April and May. The spawning period (June to July) is followed by a brief rest when spermiation appears to be intermittent. Sanwal and Khanna (1972) divided the spermatogenetic activity in *Channa gachua* into four distinct phases where spermiation continues throughout the breeding season (June to August) only. On the basis of meiotic and spermiogenetic activity, the annual reproductive cycles of *C. punctatus* and *A. testudineus* have been divided into four phases viz., growth, maturation, spawning or discharge, and resting phases (Sinha and Mondal, 1981, 1982).

According to the spermatogenetic activity of testicular lobules and variations in GSI values obtained in the present study the entire period of the season has been divided into four distinct phases viz., I) Growth phase (December to February), II) Maturation phase (March to May), III) Spawning or discharge phase (June and July in *L. rohita*, June to August in *C. mrigala*), and IV) Resting or post-spawning phase (August to November in *L. rohita*, September to November in *C. mrigala*). It has been observed in the present study that in both the fishes, active spermatogenesis sets in from the early growth phase onwards and continues further at a higher rate reaching a peak during maturation and early spawning phases in the testicular lobules. But from August onwards reduce spermatogenetic activity leads to an
abrupt reduction of various male germ cells in quantity. This is evidenced by the occurrence of frequency percentage of various spermatogenetic cells obtained in the present study. This corresponds with the findings of Sehgal (1971) in Labeo calbasu, Pantic and Lovern (1973) in Serrenus scriba and Sinha and Mondal (1981, 1982) in C. punctatus and A. testudineus.

Growth phase (phase I) with increased GSI value is characterised by the presence of maximum number of spermatogonial cells which arise by the active division of dormant spermatogonial cells of resting phase in the present study. Therefore, the testis becomes activated with the onset of growth phase. Few number of residual spermatozoa, however, still persist in the testicular lobule of C. mrigala while they are completely absent in the case of L. rohitu during growth phase. In the late growth phase, the frequency percentage of primary spermatocytes increases gradually reaching maximum at the early part of the maturation phase (Phase II) due to the active division of spermatogonia. High cellular activity is found to be associated with the testicular follicles during its later part which in turn is characterized by an increase activity in the conversion of spermatogonia to primary spermatocytes. Decrease in the number of spermatogonia is due to an accelerated mitotic activity which brings forth a multitude of spermatocytes. However, during the late maturation period, enormous number of spermatids and
spermatozoa almost completely fill up the entire lumen of the testicular lobules. The GSI value also increases rapidly during this entire phase reaching a peak at the late maturation and early part of spawning phase (Phase III) in L. rohita and C. mrigala respectively. The high GSI values in both the fishes also persist throughout the spawning phase when the males exhibit maximum sexual activity. Early spawning phase is characterized by the presence of maximum number of spermatozoa and moderate quantity of spermatids in the testicular lobules in L. rohita and C. mrigala. This is due to the transformation of a great number of spermatids into mature spermatozoa during the said phase. In order to maintain the meiotic and spermiogenetic activity in the testicular lobules for the production of an enormous number of mature spermatozoa within the tenure of short breeding cycle, which lasts for only 2 to 3 months some spermatogonia and spermatocytes are also encountered during the spawning period in both the fishes reported. However, higher percentage of spermatozoa in the lumen of testicular lobules are noticed during the spawning period from June to August in C. mrigala when compared to that of L. rohita. Marked reduction in GSI value and in relative abundance of spermatozoa are encountered from August to November and September to November in L. rohita and C. mrigala respectively. Therefore, the spermatogenetic activity decreases sharply following the
regressive period and the testes finally enters into the resting phase (August to November in \textit{L. rohita} and September to November in \textit{C. mrigala}). This phase (phase IV) is characterized by the presence of majority empty lobules containing few degenerating spermatids and spermatozoa designated as 'residual' spermatids and spermatozoa respectively. The presence of 'residual' spermatogenetic cells has also been reported by Davis (1977b), Sinha and Mondal (1981, 1982) in some teleosts during the resting phase. But Htun-Han (1978) defined them as 'remnant spermatozoa' which are noticeably inactive. Such inactive sperms are readily recognised in the two fish species reported by the presence of straight and rigid tails without any undulating characteristics commonly found in the spawning period. This is in conformity with the findings of Htun-Han (1978), Sinha and Mondal (1981, 1982). However, the lowest GSI values throughout the resting phase reveal the fact that there is no significant cellular activity occurring in the testes. Decrease of GSI value during this phase is probably due to the degeneration of spermatids and unexpelled spermatozoa. Therefore, it is confirmed that the GSI value and relative abundance of various spermatogenetic cells are closely related to the maturity stages of the testes during the annual cyclical changes of \textit{L. rohita} and \textit{C. mrigala}. 

Discussion: Morpho-histology
Discussion: Histochemistry

HISTOCHEMISTRY

It has been reported by various workers that the occurrence of many substances such as enzymes, protein, lipid, carbohydrate, nucleic acids, etc., in the testes are subject to variation in the different spermatogenetic cells during the various periods of maturity and biochemical differentiation (Butskaya, 1965; Upadhyay and Guraya, 1973; Sinha and Mondal, 1981, 1982). The variations in the occurrence of these substances stated above are mainly related either to the biochemical differentiation and/or to the various cellular activities of the different types of cells viz., spermatogonia, spermatocytes, spermatids and spermatozoa present in the testicular lobules in different stages of the testis maturation. It has also been reported that the meiotic and spermiogenetic activities in the testicular follicles in different vertebrates do not proceed with equal speed either for an entire period of their life-span or for the different periods of the year. Apart from these biochemical changes, there are sufficient data to testify a great deal of variations in cellular physiology occurred in the testicular cells. Majority of the freshwater teleostean fishes, however,

Phosphatases:

It has been emphasized by different authors that the localization of various phosphatases in the testes of different animals varies greatly in regard to the development and maturity of testicular lobules. As for example, the enzyme ALPase is active in the peritubular zone of testes in postnatal rats and in the limiting membrane of seminiferous tubules in the testes of rats aged 1, 7, 14, 21 and 42 days (Morii et al., 1975). Somani and Guraya (1971) reported the gradual and well organized increase and shift of the loci of alkaline phosphatase activity from the periphery of the seminiferous tubules to their centre forming a gradient in the spermatogenic wave. The maximal ALPase activity was noticed in the spermatids and sperm heads of the Indian desert gerbil and the house shrew (Singh and Mathur, 1968). Reports are also available regarding the occurrence and localization of various phosphatases (viz., ALPase, ACPase and ATPase) in the testes and accessory sex organs in different
vertebrate species (Gomori, 1939; Manheimer and Seligman, 1948; Osman et al., 1976; Latalski and Obuchowska, 1978; Silverin, 1978; Haider and Passia, 1981; Guha and Vanhatalo, 1983).

It is known that phosphatases play a major role in the biochemical differentiation of the male germ line cells during the various phases of the testicular development in freshwater teleosts (Sinha and Mondal, 1981, 1982). The occurrence, distribution and localization of some phosphatases (viz., ALPase, ACPase and ATPase) in the different spermatogenetic cells of *L. rohita* and *C. mrigala* vary greatly during their annual reproductive cycle. Their variations, however, indicate a close relationship with the spermatogenetic activity during the different phases of testicular development. Various degrees of activities of these enzymes are noticed in the cytoplasm and nuclei of the different testicular cells in both the fishes studied. The cell membrane as well as nuclear membrane of the various testicular cells is also positive for these enzyme. These enzyme activities, however, in the different regions of various spermatogenetic cells in *L. rohita* and *C. mrigala* are mainly concerned with various biochemical and physiological processes which are essential for their further transformation.
into the next advanced type. Seasonal changes of some phosphatases in relation to spermatogenetic activity in the testes have also been reported in few vertebrates \textit{viz.}, in sparrow (Somani and Guraya, 1971), in pied flycatcher (Silverin, 1978), and in \textit{Rana temporaria} (Juelich et al., 1982).

High level of ALPase, ACPase and ATPase activity has, however, been observed in almost all the spermatogenetic cells of both the fishes studied. Intense activities of these enzymes in the nuclei of the various testicular cells account for the active meiosis and spermiogenesis which set in during the early part of the maturation phase. Besides, active mitosis also occurs in the majority of the spermatogonial cells during the growth phase as evidenced by the presence of mitotic figures in the latter cell type in two species described. Prior to form spermatozoa, the last spermatogonial cells originated by mitotic division, divide to produce primary spermatocytes, secondary spermatocytes and spermatids in sequence. Each of the cell type formed undergoes biochemical maturation before passes on to its immediate next stage. The active participation of the aforesaid phosphatases, however, brings forth the completion of such biochemical maturation in the various male germ line cells.
during the early part of the maturation phase. Hence, the increased activity of these phosphatases is mainly associated with the nucleus while moderate activity of the same is observed in cytoplasm of the various spermatogenetic cells throughout the entire period of maturation and spawning phases in *L. rohita* and *C. mrigala*. It is known that the completion of spermatogenesis necessitates a huge amount of energy which is generally produced from the breakdown of glycogen, lipid and phospholipid materials localized in the testis. Remarkable increase of glycogen and lipid level during the growth and maturation phases following a gradual depletion towards the spawning phase in the testes of giant scallop, *Plecopterus magellanicus*, has been reported by Thompson (1977). Further, Silverin (1978) confirmed that the ATPase activity increases concurrently with the rise of glutamic dehydrogenase (GDH), glycogen and phospholipid content in the testes of pied flycatcher. Therefore, exhaustion of these substances due presumably for releasing energy by the activities of various phosphatases for the completion of spermatogenesis has been observed in the present study.

It is known that the nucleus plays a primary role for the synthesis of many biochemical substances which are
eventually utilized in the subsequent maturation of a particular testicular cell and probably help the same to divide. Therefore, strong phosphatases activities associated with the nuclei of the various germ line cells are significant in the present study. On the other hand, ACPase activity in the nuclei of various spermatogenetic cells during the different phases probably helps to maintain the metabolic status of the cell concerned during the whole spermatogenetic process of L. rohita and G. mrigala. However, the role of ACPase activity in the changes of cellular composition of the testes during its differentiation was emphasized by Guha and Vanha-Perttula (1983).

Many of the biochemical substances synthesized in the nuclei of the particular spermatogenetic cells require to pass through the nuclear membrane to the cytoplasm of the respective cell for their proper functioning. Phosphatases play a key role in the membrane transport of these substances in the aforesaid cells by various ways. As for example, the energy required for such active transport through the nuclear membrane is produced by ATP-splitting enzymes like phosphatases. Therefore, the activities of ALPase, ACPase and ATPase in the nuclear membrane of various spermatogenetic cells particularly in the spermatogonia and spermatocytes are justified
and confirmed in the present study. Moreover, the role of transportation of various chemicals and nutrients through the cell membrane including their involvement in the permeability process has been reported in various vertebrate tissues by different workers (Melani et al., 1967; Holdsworth, 1970; Norman et al., 1970; Motzok et al., 1971; Moog and Glazier, 1972). Juelich et al. (1982) noticed the role of ATPase activity in maintaining the permeability of the various membrane in the different testicular cells as well as in the release of sperms of R. temporalis. Intense phosphatases activities in the cell membrane of various spermatogenetic cells during the different phases of reproductive cycle of the two fishes under study probably facilitate the exchange of various biochemical substances, metabolites and electrolytes between the cell interior and exterior while the aforesaid functions are performed by lobule boundary membrane with the help of these enzymes in order to maintain the equilibrium of the metabolic status in between the various testicular lobules. However, the ALPase activity in the basement membrane of seminiferous epithelium in some mammalian testes was confirmed by some workers (Gomori, 1939; Gogate and Insamder, 1970; Morii et al., 1974; Morii et al., 1975). Morii et al. (1975) on the other hand, pointed out that this enzyme activity helps in maintaining the structural and nutritional status of the tubules. Silverin (1978) confirmed that intense
ATPase activity in the nuclear and cell membranes of the testicular cells accelerates the increased transfer of metabolites and electrolytes across the nuclear and cell membranes in pied flycatcher. Further Haider and Passia (1981) detected the intense ALPase activity in the follicular cells and lamina propria of the seminiferous tubules of *Rana temporaria*. The functional significance of these enzymes in the transport system of several cellular substances across the cyst walls of the seminiferous tubules of *R. temporaria* was also confirmed by the latter authors.

Maximum ATPase activity in the spermatids and spermatozoa during the maturation and spawning phases is presumably connected with the production of more energy by splitting ATP with the help of this enzyme. This is probably the main source of energy for active transport of sperms in the ducts of testes in both the fishes under study. This corresponds with the findings of Gravis et al. (1976) in syrian hamster, Sosa et al. (1979) in human and Storey and Kayne (1980) in rabbit. However, spermatogenetic activity of the testes in *L. rohita* and *C. mrigala* ceases partially in the resting phase. Therefore, the activities of various phosphatases become comparatively lower during this period of the year in both the fishes studied.
Protein:

Variations in protein localization are also noticed in the different spermatogenetic cells during the four phases of reproductive cycle in *L. rohita* and *C. mrigala*. Intense localization of protein material is observed in the nuclei of all the spermatogenetic cells in comparison with that of cytoplasm. This is in conformity with the findings of Upadhyay and Guraya (1973) in some freshwater teleosts. However, in the present study, the reaction intensity for protein in the nuclei as well as in cytoplasm of the various spermatogenetic cells and lobule boundary wall increases gradually from the growth phase onwards and reaches maximum during the maturation and early spawning phases when the GSI and spermatogenetic activity of the testes are also maintained at higher level. During the latter two periods, it has been observed that the majority of the testicular lobules are mainly packed with spermatids, spermatozoa and with some secondary spermatocytes. Therefore, the nuclei of the latter spermatogenetic cells of the aforesaid phases are rich in protein which is mainly nucleo-protein in nature. This nucleo-protein, however, helps in maintaining the highly complex structure of the cell concerned. On the other hand, intense deposition of protein material in the lobule boundary membrane and cells probably acts as a source of energy substrate for the
development of testicular lobules as well as for the proliferation of various spermatogenetic cells in the present study. Further, during the course of progressive spermatogenesis with concomitant increase in protein, the chromatin material of earlier spermatogenetic cells condenses gradually resulting the formation of highly differentiated and more advanced types—spermatids and spermatocytes. Dixon (1972), however, evaluated the major changes in basic protein of the nuclei during the condensation of chromatin in spermatid of trout testis. The major proteins forming an essential constituent of chromatin are histone (Davis and Langford, 1970) which is chemically attached to the deoxyribonucleic acid molecules thus forming the outer texture of chromosomes (Zubay and Doty, 1959). This attachment is essential for cell division and also for controlling genetic expression (Davis and Langford, 1970). Chromatin body of various spermatogenetic cells comprises with DNA and protein (Upadhyaya and Guraya 1973). Therefore, the variations of protein content in various male germ line cells in the present study are probably associated with the physiochemical changes of the cell concerned during its biochemical differentiation. Maximum localization of protein during maturation and early spawning phases when phosphatases activities as well as spermatogenetic activity also go high, probably indicates its major role as a substrate for the
source of energy which is essential for the growth, differentiation and maturation of various male germ line cells in L. rohita and C. mrigala. On the other hand, comparatively low reaction intensity at the resting phase in the present study may be due to the minimum cellular activity encountered. However, depletion of basic as well as non-histone proteins in the nuclei of later stages of spermatogenetic cells during spermiogenesis was also reported by Mezquita and Teng (1977) in the maturing testes of rooster.

**Bound Lipid:**

The occurrence and localization of bound lipid in the lobule boundary wall and cells as well as in the cytoplasm and nuclei of various spermatogenetic cells in the testes of L. rohita and C. mrigala have been confirmed. However, the presence of relatively higher amount of lipid material in all the spermatogenetic cells of different vertebrates has been reported by many authors (Melampy and Cavazos, 1954; Guraya, 1961, 1962; 1971a; Georgiev, 1967; Dokov et al., 1968; Kornblatt et al., 1974). On the other hand, Upadhyay and Guraya (1973) noted comparatively higher quantity of Sudanophilic lipid material in the cytoplasm of spermatogonia but its negative and weak reactions were observed by them in the nuclei and nucleoli of various spermatogenetic cells respectively in some freshwater teleosts.
Discussion: Histochemistry

Low amount of lipid material has been recorded in the lobule boundary wall and cells detected during the resting phase while the highest quantity of the same has been observed at the growth and early maturation phases in the present study of both the fishes. It is interesting to note that these stored lipid material rapidly decline throughout the late maturation and spawning phases. Similar observations were also reported by Lofts and Marshall (1957) in the lobule boundary cells and Hoffmann et al. (1980) in Sertoli cells in *Esox lucius*. The latter author reiterated the opinion that the appearance of Sudanophilic material may be a sign of hormonal activity and probably help in the maturation of spermatozoa. There are also some other reports regarding the accumulation of lipid material in the Sertoli cells as well as in the basement membrane of seminiferous tubules of higher vertebrate species (Moore, 1924; Lynch and Scott, 1951; Shivanandappa and Sarkar, 1979; Dhingra and Sharma, 1980). It seems probable that the accumulated lipid material in the lobule boundary wall as well as in cells in the present study probably transfers to the various spermatogenetic cells during the growth and maturation phases and provide nutrition for the growth and biochemical maturation of the cell concerned.

*L. rohita* and *C. mrigala* show more or less similar type of variation during the different periods of the season in
regards to the bound lipid localization in the different cell types of testicular follicles. During the resting phase, when the testis is in almost inactive condition, deposition of lipid material in the testicular cells particularly in the cytoplasm of dormant spermatogonia is accelerated. The maximum deposition of lipid is observed in the nuclei of earlier spermatogenetic cells (spermatogonia and primary spermatocytes) during the growth and early maturation phases where maximum biochemical differentiation and cellular activity takes place. Peak meiotic activity is observed in the testicular follicules during the maturation phase which, indeed requires maximum energy to complete the entire process. Therefore, glycogen and lipid, being the principal source of energy supply needed for active cell division and sperm transportation, display maximum deposition during these phases. Lipid material in the cell membrane of early spermatogenetic cells probably represents lipoprotein, a principal constituent of all the cell membranes. However, with the commencement of late maturation and spawning phases when the spermatogenetic activity becomes maximum and the male individuals of both the species studied show maximum sexual activity, the overall reaction intensity for lipid material reduces sharply. Similar type of lipid cycle was observed by some authors in various vertebrates (Marshall and Woolf, 1957; Lofts and Boswell, 1960; Lofts et al., 1966; Reddy and Prasad, 1971). They reported however, that the
concentration of total lipid in the testis of the animal studied by them is low during the spermatogenetically active season and attains a peak when the testes are in regress condition. Further, disappearance of lipid material from the male germ-line cells was reported by Guraya (1961, 1962, 1971a) during the late period of spermatogenesis. On the other hand, lipid cycle in the testicular cells of different teleosts in relation to the spermatogenetic activity has been studied by few authors (Lofts and Marshall, 1957; Upadhyay and Guraya, 1973; Sinha and Mondal, 1981, 1982). However, Robertson (1958) and Rodoni (1969) did not find any change in fatty material in the testes of rainbow trout and contradicted the above findings. Deposited lipid material in the previous phases gets depletion during the late maturation and spawning phases probably for maintaining the high sexual activity of *L. rohita* and *C. mrigala*. However, subsequent disappearance of lipid material from the testes during the aforesaid phases in the present study is probably due to its utilization as an endogenous energy source for the growth and maturation of the different spermatogenetic cells at the active period of spermatogenesis. Shivanadappa and Sarkar (1979) evaluated this disappearance of lipid material from the testes of *Mabuya carinata* during its preparatory phase due to its rapid mobilization. Therefore, it may be concluded in the present study
that the accumulation of lipid material starts in the spermatogenetically inactive testes (i.e., in the resting phase) and reaches maximum during the growth and early maturation phases when proliferation and subsequent growth of the early male germ line cells occur but it decreases rapidly later in the spermatogenetically fully active testes in the maturation and spawning phases.

The role of lipid material as a source of endogenous energy including its involvement in the motility of spermatozoa for fertilization in aquatic medium has been reported by Upadhyay and Guraya (1973). Sinha and Mondal (1981, 1982) also noted the role of lipid as one of the principal sources of energy which is essential for the completion of spermatogenesis and also for sperm transportation in G. punctatus and A. testudineus. In the present study, however, retaining of lipid material in the spermatids and spermatozoa probably has an important functional significance in metabolism and maturation process of spermatids and spermatozoa. This may also act as one of the endogenous nutrients required for the motility of sperms and help in subsequent sperm transportation through the ducts of the testes. Georgiev (1967) observed higher content of phospholipid in both the spermatozoa and plasma of ejaculates. The report of Dokov et al. (1968) reiterates the presence of
lipids in very small amount in the bodies of spermatozoa. They noticed a great similarity in the localization of lipid in the spermatozoa of cocks, turkeys and ducks. Guraya and Sidhu (1977) reported that post-nuclear cap and mid-piece of the buffalo spermatozoa are argentophilic to their phospholipid content.

PAS-positive carbohydrate:

During the course of present investigation, it has been observed that the deposition of PAS-reactive carbohydrate starts in the wall of testicular lobules during the resting phase and attains maximum at the late growth phase of the testes in both the fishes studied. Deposition of this material in the basement membrane of seminiferous tubules of bluegill fish (Gavazos and Melampy, 1954) and in the connective tissue as well as in the basement membrane of the testes in some higher vertebrates (Gavazos and Melampy, 1954; Baillie, 1962; Osman et al., 1976) has been reported. The depletion of PAS-positive substances in the lobule boundary wall throughout the maturation and spawning phases in both the fishes indicates its preferential utilization for growth and biochemical differentiation of various spermatogenic cells. Maximum localization of PAS-positive carbohydrate in the spermatogonia and primary spermatocytes particularly in the cytoplasm of the aforesaid cells is observed during the
growth phase when the spermatogonia actively divide mitotically to give rise primary spermatocytes. Nuclei of secondary spermatocytes, spermatids and spermatozoa also exhibit moderate reaction intensity for PAS-positive carbohydrate during the late growth and maturation phases where the peak mitotic and meiotic events are encountered. However, energy required for the completion of these events is being supplemented by this PAS-positive carbohydrates during the said phases. Reports relating maximum localization of PAS-positive substances in the various spermatogenetic cells particularly in the spermatogonia and primary spermatocytes during the growth and early maturation phases of few freshwater teleosts are also on record (Sinha and Mondal 1981, 1982). The latter authors confirmed the functional significance of PAS-positive substances in the spermatogenetic activity. On the other hand, Cavazos and Melampy (1954) as well as Upadhyay and Guraya (1973) failed to detect any PAS-positive acrosomal granules or vacuoles in the spermatogenetic cells of some teleosts. However, there are reports regarding PAS-positive material deposited in the acrosome of sperm head in the higher vertebrates (Leblond and Clermont, 1952; Nath, 1965; Upadhyay and Guraya, 1970; Guraya and Sidhu, 1977; Singh and Bharadwaj, 1978). Although in ordinary preparations, it has not been possible to detect the acrosomal cap in the spermatozoa in both the species studied, but positive PAS reaction in the spermatids
Discussion: Histochemistry

and spermatozoa in the discharge phase reveals the presence of the PAS-positive substances in these cell types parallel to the other vertebrates, particularly in mammals, reported so far. However, low deposition of these substances in the different spermatogenetic cells during the resting phase of both the fishes described, is due to the virtual cessation of cellular activity.

Glycogen:

Some workers have observed the occurrence and localization of glycogen, like lipid material in the various cell types present in the testicular follicles and successfully correlated it with the spermatogenetic activity in higher vertebrates (Ambadkar and George, 1964; Leiderman and Mancini, 1964). During the course of the present investigation, it has been noticed that the various degrees of glycogen localization occur in the cytoplasm and nuclei of all the spermatogenetic cells during the different periods of the reproductive cycle in *L. rohita* and *C. mrigala*. However, both the cytoplasm and nuclei of spermatogonia, spermatocytes and nuclei of spermatids exhibit maximum glycogen deposition during maturation phase while spermatozoa as a whole show its moderate deposition. Similar type of glycogen cycle in the testes of *G. punctatus* and *A. testudineus* has been observed by Sinha and Mondal.
Discussion: Histochemistry

Glycogen deposition in the spermatogonia and spermatocytes in other vertebrate species has also been studied (Long and Engle, 1952; Montagna, 1952; Cavazos and Melampy, 1954; Weaker, 1978). But Nicander (1957) did not find any glycogen localization in the spermatogonia of mouse, rat and dog. Ambadkar and George (1964) on the other hand, pointed out that the variations of glycogen localization from peripheral spermatogonial cells to the spermatozoa located in the centre of testicular lumen are due to the variable activities of oxidative enzymes in the different germ cells. Disappearance of glycogen from the spermatids and spermatozoa during the active spermatogenesis is probably associated with its initiation and completion (Leiderman and Mancini, 1964). Therefore, glycogen in various male germ cells, like lipid material may also act as a substrate for the source of energy required for the growth and differentiation of the aforesaid cells as well as the completion of meiosis and spermiogenesis in both the fishes under study.

Besides, glycogen like lipid in spermatozoa during maturation and spawning phases in L. rohita and C. mrigala may help in maintaining their metabolic status as well as provide energy so essential for sustaining their motility in the sperm duct. Anderson and Personne (1970), however, confirmed the spermatozoal glycogen as an endogenous energy source during the
fertilization commenced in the water medium. Low reaction intensity of glycogen in the various testicular cells during the resting phase in the present study may account for the minimum cellular activity as well as for partial cessation of meiotic activity.

**Nucleic acids (DNA and RNA):**

Variations in the nucleic acids (DNA and RNA) in different spermatogenetic cells during meiosis and spermiogenesis have been reported in different vertebrate species (Doust and Clermont, 1955; Monesi, 1962, 1964, 1965a,b; Hilscher, 1964; Courot et al., 1970; Clarke et al., 1980) although it is known that the quantity of DNA content in a particular cell remains constant throughout its entire period of life-span. These variations are probably associated with the complex physiochemical changes in the different spermatogenetic cells (Viola-Magni, 1978; Clarke et al., 1980). Further Noeske (1969) pointed out that the chemical and biophysical alterations of chromatin in the cellular development occur during the different functional states where various DNA values are found to be present in cell nuclei although the nuclei contained the same amount of DNA.

In the present study, the moderate reaction intensity for Feulgen in the nuclei of spermatogonia is due to their
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diffused nature of chromatin material during the growth phase. The reaction intensity for Feulgen material in the nuclei of different spermatogenetic cells gradually increases concomitantly with the condensation of chromatin material and with the decrease of nuclear volume during differentiation and maturation of various male germ line cells in both the fishes studied. This corresponds with the findings of Upadhyay and Guraya (1973) and Sinha and Mondal (1981, 1982) in some freshwater teleosts. Monesi (1962), however, reported that the duration of DNA synthesis is significantly higher in primary spermatocytes than that of A-type spermatogonia in mouse. Similar observations were also made by Clermont et al. (1959) as well as Hilscher (1964) in rat and Hochereau (1967) in bull. A late spermatocyte contains a tetraploid amount of DNA (Swift, 1950; Swift and Kleinfeld, 1953) and this amount of DNA is sufficient for the requirement of two subsequent maturation divisions (Gledhill, 1970). But Butskaya (1965) observed weak reaction intensity for DNA in the nuclei of primary spermatocytes in the fishes studied by him. However, in the present study, maximum reaction intensity for Feulgen noticed during maturation phase, is due to the presence of higher percentage of primary and secondary spermatocytes when peak spermatogenetic activity is effected in the testes of L. rohita and C. mrigala. Therefore, the
intensive activity of Feulgen reaction during these periods may be related to the biochemical differentiation of the various cells (stage 1, 2, 3 and 4) in the testicular follicles of the two species under study. On the other hand, relative abundance of spermatids and spermatozoa which contain highly condensed nuclei imparts overall intense Feulgen reaction during the spawning phase of both the fishes.

The occurrence and localization of RNA in the various spermatogenetic cells during the four phases of testicular cycle as revealed by the present histochemical tests are also variable. As for example, the cytoplasm and nucleoli of spermatogonia exhibit intense reaction for RNA indicating its presence of relatively higher amount in the spermatogonial cells while detectable amount of the same by the present histochemical tests is not found in the nuclei of latter cell types (stage 3, 4, 5) in both the fishes described. This is in conformity with the findings of several other workers in higher vertebrates (Douet and Clermont, 1955; Monesi, 1964, 1965a,b; Utakoji, 1966) and also in few teleosts (Butskaya, 1965; Upadhyay and Guraya, 1973). Kalt (1980), however, opined that the spermatogonia are the most efficient cells regarding the synthesis of RNA which declines gradually to a bare minimum in spermatozoa in *Xenopus laevis* while Bertout (1983) concluded
that meiotic differentiation of spermatogenetic cells in *Nereis diversicolor*, is accompanied by the increased RNA synthesis. In the present study, the deposition of relatively higher amount of RNA in the nucleoli and cytoplasm of spermatogonial cells unequivocally suggests its important role in active participation in the synthesis of protein which is essential for growth and maturation of the different spermatogenetic cells. Söderstrom (1976) however, suggested that a portion of RNA synthesized by pachytene spermatocytes, is utilized during spermatogenesis.

Overall low reaction for Feulgen has been observed during the resting phase in the present study. This is probably related to the degeneration of spermatids and spermatozoa in the said phase as well as the expulsion of a great majority of spermatozoa formed during spawning phase. Besides, dormant spermatogonia, the major cell types during this phase contain diffuse chromatin material. Cessation of spermatogenetic activity during this phase may be one of the causes for the presence of low amount of RNA detected during the aforesaid phase. But moderate amount of RNA in the lobule boundary wall is presumably related to the growth and maturation of various male germ cells to be produced in the subsequent phase in *L. rohita* and *C. mrigala*. 
BIOCHEMISTRY

Quantitative changes of various nutrients viz., protein, lipid, carbohydrate and glycogen occurring in the testes of *L. rohita* and *C. mrigala* during the different phases of their maturity confirm the earlier histochemical findings of the present study. The results derived from both the studies, histochemical as well as biochemical investigations on the occurrence and localization of the aforesaid nutrients show their significant role in spermatogenesis during the various phases of testicular cycle in the two species under study already discussed in the 'Histoch emical' chapter.

**Total Protein:**

It is known that the stored nutrients (viz., protein, lipid, carbohydrate and glycogen) in the liver and body muscle are deposited in the gonad and subsequently utilized as a source of energy for metabolism as well as gonadal growth and maturation (Love, 1957; Idler and Bitners, 1958, 1960; Jangaard *et al*., 1967; Blay Jr. and Eyeson, 1982; Eiissen and Vahl, 1982a,b). In *L. rohita* and *C. mrigala*, the period between the early growth and late maturation phase may be designated as the protein storage phase of the liver and body muscle. The stored
protein of the liver and body muscle, however, in the later phase is being transferred to the testes of both the fishes reported. This eventually results the increase in protein content of the testes during the late maturation and spawning phases of *L. rohita* and *C. mrigala*. However, the shifting of protein from the liver and body muscle to the testes plays a significant role in the synthesis of nucleo-protein content during the formation of spermatids and spermatozoa abundantly occurred in the late maturation and spawning phases. Therefore, the maximum protein content as revealed by the present biochemical investigations during the aforesaid phases represents mainly nucleo-protein fraction of spermatids and spermatozoa. Further, the stored protein may also serve as the source of energy to be utilized in metabolism, gonadal maturation and spawning activity of *L. rohita* and *C. mrigala*. This corresponds with the finding of Love (1970), Ochiai and Tanaka (1980).

Further, the stored protein in liver and body muscle in the growth and maturation phases is found to be exhausted in the spent fishes of both the species studied. Shreni (1980) also observed the highest values of protein in the liver and body muscle in mature fish, *Heteropneustes fossilis* while its depletion in both the tissues was noticed just before the spawning. He, therefore, concluded that the advancement of
gonadal maturation in the aforesaid fish is intimately associated with the deposited protein in liver and body muscle. Love (1970), however, pointed out that dietary proteins seem to be insufficient for their huge demands by the sex organs when eggs and sperms tend to be matured. Therefore, he concluded that the gonadal growth is accomplished by the expense of muscle protein reserves. Mac Bride et al. (1960) also emphasized that much of the gonadal tissue proliferates by utilizing proteins derived from the musculature in Clupea pallasi.

**Total lipid:**

Like protein material, the lipids are also deposited during the period between the resting and growth phase in the liver and body muscle of L. rohita and C. mrigala. However, this stored lipids in the liver and body muscle are presumably transferred to the testes for its eventual maturation and also for maintaining the spermatogenetic wave during the subsequent phases. This phenomenon of lipid cycle in the testis, liver and body muscle was also reported by different authors in a variety of teleosts viz., in Esox lucius (Medford and Mackay, 1978), in Nibea mitsukurii (Ochiai and Tanaka, 1980) and in yellowtail (Ochiai et al., 1980). The transfer of lipids from the liver to the female gonad through the blood serum is accomplished in response to estradiol injections in
Atlantic cod, *Gadus morhua* (Plack et al., 1971) and in winterflounder (Campbell and Idler, 1976). Furthermore, mobilization of lipids from adipose tissues to the muscle and testes was confirmed by Chester Jones et al. (1972) in some non-mammalian species and by Wingfield and Grimm (1978) in plaice, *Pleuronectes platessa*. The maximum deposition of lipid in the testes during their inactive phase of spermatogenesis and the lowest value of the same at the pre-breeding and breeding season in some higher vertebrates were also observed by some workers (Cavazos and Feagans, 1960; Tahiri-Zagret and Clerc, 1976; Ambadkar and Chauhan, 1979). But Bilaspuri and Guraya (1981) did not find any significant change in the lipid material of the testes in ram during the different periods of the season. Medford and Mackay (1978), however, emphasized that the endogenous protein is more important than the endogenous lipid regarding the gonadal maturation and spawning activity in *E. lucius*. Dindo and Mac Gregor (1981) calculated the monthly values of GSI, serum gonadal steroids and serum lipids in *Mugil cephalus*. They pointed out that the rapid deposition of body lipid is effected prior to gonadal development but its subsequent mobilization occurs during the maturation of gonad as well as during spawning activity.

**Carbohydrate and glycogen:**

Storage phase of carbohydrate and glycogen in the liver and body muscle has been found during the period between late
spawning and early maturation phase in the present study of both the fishes. It has been observed that the total carbohydrate and glycogen content in the testes and body muscle is comparatively low when compared to total protein and lipid content in the aforesaid regions of both the fishes studied. But the quantitative values of carbohydrate and glycogen in the liver of both the fishes throughout the season are relatively high which indicates that the latter organ is mainly responsible for synthesizing the aforesaid nutrients. After synthesizing in the liver they are, however, subsequently transferred to the target organ-testis according to its need. Therefore, the high level of carbohydrate and glycogen in the testes during the late growth phase is being utilised in subsequent phases viz., pre-spawning and spawning probably to meet the energy demand for spermatogenetic activity in L. rohita and C. mrigala. Thompson (1977) observed maximum carbohydrate level in the testes of giant scallop, Placopecten magellanicus during the growth and maturation phase but according to him, it gradually diminishes towards the spawning phase. Free (1970) on the other hand, found that the testes, excluding some species of reptiles and amphibians, contain minimum quantity of reserve carbohydrate and at the same time he concluded that a continuous supply of carbohydrate is probably essential for the proper functioning of the testes. Transfer
of the stored body glycogen to the ovary during its maturation phase has also been reported by many authors (Greene, 1926, Fontaine and Hatey, 1953; Yanni, 1961).

From the foregoing literature it is clear that the endogenous nutrients of liver and body muscle are transferred to the testes during the growth, maturation and early spawning phases. These endogenous nutrients reaching in the testes may play a significant role for supplying necessary energy for spawning activity besides the gonadal growth and maturation as well as metabolism of both the fishes, L. rohita and C. mrigala. Moreover, decrease in feeding intensity prior to spawning in many teleosts has been reported by various authors. Therefore, the necessary energy needed for proliferation, development, growth and maturation of various spermatogenetic cells and lastly for the successful spawning must be derived from the endogenous nutrients after their eventual transfer from the liver and body muscle to the target organs—testes. However, Frost (1954), Medford and Mackay (1978) reported that pike, Esox lucius must depend on endogenous nutrients at a time when considerable energy is required for growth, maturation of the gonad and spawning activity during its suspension of the feeding activity. Cushing (1966), Rae (1967) and Waterman (1968) also observed
that north sea cod consumes little or no food prior to and/or during spawning. Therefore, it is hypothesized that the excess energy required during metabolism, gonadal growth, maturation and spawning activity is supplied by the reserves of the body resulting in the observed change in biochemical composition of the muscle (Damberg, 1964; Love, 1970; Bano, 1978) or of the liver, muscle and gonad of sexually mature fish (Shevchenko, 1972; Ochiai and Tanaka, 1980; Ochiai et al., 1980; Eliassen and Vahl, 1982a,b).

**Cholesterol:**

It has been observed in the present study that the cyclical changes of cholesterol runs parallel with that of lipid content in the testes of *L. rohita* and *C. mrigala*. However, slow accumulation of cholesterol is noticed during the resting phase when the fishes are in spent condition with partial cessation of spermatogenetic activity. This is probably related to the low spermatogenetic activity during this period of both the species reported. High cholesterol level of the testes at the mid maturation phase and its subsequent depletion during the late maturation and spawning phases have been recorded in the present study in both the fishes under study. Similar types of cholesterol cycle was also reported
in some higher vertebrates by many workers (Cavazos and Feagans, 1960; Hoffmann, 1960; Hilton, 1961; Sanyal and Prasad, 1965; Ambadkar and Chauhan, 1979; Chand et al., 1979). All of them unequivocally suggested its functional significance relating to the testicular growth, maturation and spermatogenetic activity. But few authors made a correlation between the cholesterol content of the testes and the endocrine function of the seminiferous tubules (Lofts and Marshall, 1959; Hoffmann, 1960; Chand et al., 1979). It is known that the liver is the main site for cholesterol synthesis. But Srere et al. (1950) pointed out that not only the liver but the testis also synthesizes cholesterol effectively. Similar report was also made by Morris and Chaikoff (1959). Further, Jafri and Shreni (1976) confirmed that the advancement of gonadal maturation is effected by the depletion of cholesterol reserves in liver of *Cirrhinus mrigala*. Therefore, in the present study, high cholesterol content in the testes in growth and early maturation phases of *L. rohita* and *G. mrigala* and its depletion towards the late maturation and spawning phases probably indicate its positive role in metabolism, growth and maturation of the testes.