GENERAL DISCUSSION
The role of prolactin, hypophyseal gonadotropic hormone in mammalian male reproduction is not yet clearly understood. The presence of this hormone in human semen (Sheth et al., 1975) indicated its possible role in male reproduction. The role of PRL in male reproduction was worked out and reported to increase fertility and spermatogenesis (Bartke, 1966; Bartke, 1967; Bartke and Lloyd, 1970). However, role played by PRL in immature animals was not clearly understood. Hence the present study has been undertaken to analyse the possible role played by PRL on the puberty of male rats. The present investigation was aimed at to understand the influence of PRL on the onset of maturation processes of reproductive tissues and its consequent effect on the non-reproductive tissues of the immature male albino rats.

Immature male albino rats (21 days old) have been selected for the study, for, they represent the immature stage of the rats, besides having the advantage of least endogenous PRL secretion and also known to express maximum response to extraneous gonadotropin administration (Shikata and Hall, 1967 a, b; Sendler and Hall, 1968; Bartke, 1976).

PRL administration elevated the tissue somatic indices (TSI) of all the tissues studied over the control animals suggesting higher rate of growth in these tissues than the
body growth in response to PRL. Since PRL mimics growth hormone (Grayhack, et al, 1955; Grayhack and Lebowitz, 1967) and its administration increases testosterone production (Hafeiz et al., 1972 a, b) and testosterone also exerts anabolic influence on the tissue (Nyden and Williams Ashman, 1953; Thomas and Nanandhar, 1975; Keenan and Thomas, 1975; Yamanaka et al., 1975; Walvoord et al., 1976; Cameo et al., 1976; Thomas et al., 1976) the observed tissue growth might be due to the direct influence of PRL or through androgen mediation. The elevation of TSI was more in reproductive tissues than the non-reproductive tissues, suggesting the specific gonadotropic influence of this hormone. Even among the reproductive tissues elevation in TSI was higher in sex accessories than the testis, suggesting the onset of a higher rate of functioning by sex accessories than the testis, which might be a pre-requisite for providing optimal conditions for the maturation and maintenance of spermatozoa. The observed increase in tissue weights of immature rats is in agreement with earlier workers where they reported the similar increase in the weight of testis and sex accessories in adult animals in response to PRL administration (Bartke and Lloyd, 1970; Dorfman, 1972; Fransworth, 1972; Horrobin, 1973; Negro-Vilar, 1972; Bartke, 1974; Sheriff and Govindarajulu, 1975; Keenan and Thomas, 1975).
When compared to the growth rate pattern of the tissues of normal rats, PRL treated rat tissues showed the weights, existing in higher age group rats even during early days. In PRL treated immature 26 day old rats, testis, prostate gland, epididymis and seminal vesicles showed the weights equal to those found in 28, 30, 35 and 45 days old normal rat tissues respectively. Hence it was suggested that PRL could probably advance the age of functioning of reproductive system in male immature albino rats. Since the growth of the sex accessories was more advanced, it was suggested that the sex accessories were more responsive towards PRL administration in male immature rats. The non-reproductive tissues also showed such a possibility of advancing the growth, in that, brain and kidney showed the weights equal to the weights of these organs found in 28 days old rats while liver and muscle showed those of 30 days old rats in PRL treated 26 day old rats, suggesting the overall anabolic effect of PRL on these tissues. The elevated tissue weights could be due to increased synthetic activities or due to accumulation of water, since PRL was known to influence the tissue osmotic properties (Ogawa, 1974; Johnson et al., 1974; Nainoya, 1975; Ogawa, 1975; Holt and Perks, 1975; Chan et al., 1975; Odulaye, 1976; Doneen, 1976). Hence water content and dry matter of all the tissues were estimated and found that both were elevated in response to
PRL. These observations suggested the net growth and initiation of functioning of sex accessories, testis and non-reproductive tissues of PRL treated animals.

Since AchE activity was correlated towards the operation of tissue protein biosynthetic activities (Krupa and Hellenbrand, 1974) and known to indicate tissue responsiveness and alertness (Nechamson, 1959; Venkatachari and Muralikrishna Dass, 1968; Muralimohan and Muralikrishna Dass, 1969) its activity levels were estimated in these tissues. AchE level was elevated in these tissues in response to PRL administration in comparison to controls and hence it was suggested that the PRL treated animal tissues were more responsive, alert and had accelerated protein-biosynthetic activities. This enzyme activity was also reported to be elevated by the sex steroids (James and Kanungo, 1978). Hence elevated AchE activity observed in PRL treated tissues might suggest that the tissues were under the influence of sex steroids on PRL administration. This suggestion correlates well with the observations reporting the androgen production by PRL administration (Hafiez et al., 1972 a, b).

Since the tissue growth pattern indicated the possible onset of synthetic activities in response to PRL administra-
tion, some aspects of protein, carbohydrate, lipid and nucleic acid metabolism were studied in reproductive and non-reproductive tissues of control and PRL treated tissues of rats.

The total protein content of all the tissues except brain was elevated in PRL treated animals over controls, suggesting the accumulation of protein in these tissues. Further analysis of tissue proteins like soluble and structural fractions indicated the accumulation of more structural proteins in sex accessories than the testis which might have been responsible for more increase in the weights of sex accessories than the testis as observed in the present study. The non-reproductive tissues were accumulating more structural proteins than soluble proteins, suggesting the elevation of tissue structural organization in response to PRL. The observed accumulation of tissue proteins in response to PRL might be due to the accelerated synthesis of proteins or decreased tissue lytic activities. The levels of DNA and RNA were estimated which represent synthetic phase; protease activity and free amino acids content to represent lytic activities in the tissues of PRL treated rats. In general PRL treatment increased nucleic acids content, suggesting the occurrence of active tissue
Proliferations and protein-biosynthetic activities. Besides the elevation in synthetic activities, the lytic activities were inhibited as represented by decreased protease activity consequently free amino acids content. Thus PRL could elevate tissue protein content of reproductive and non-reproductive tissues probably with accelerated biosynthetic activities with inhibited lytic activities. Such PRL induced tissue protein metabolic modulations might explain the enhanced growth of the tissues.

Since carbohydrate metabolism is intimately associated with spermatogenesis, maturation and seminal plasma formation, any hormone which influences the spermatogenesis and fertility might necessarily influence the carbohydrate metabolism. Hence an attempt was made to understand the PRL induced modulations on tissue carbohydrate metabolism in order to clearly elucidate the metabolic alterations which led to the increased fertility.

The testicular carbohydrate content was decreased in response to PRL administration, which was correlated to its utilization in the initiation and maintenance of spermatogenesis. Since PRL increased spermatogenesis and fertility (Bartke, 1966, 1967; Bartke and Lloyd, 1970) and carbohydrates are maximally utilized during spermatogenesis (Hinwich Nahum, 1929; Dickens and Greville, 1933; Elliot et al., 1937;...
McClymont and Setchell, 1956), the observed decrease in PRL treated testicular carbohydrate content was correlated to the possible utilization in the initiation of spermatogenesis in the immature testis. In view of decreased carbohydrates and operation of least glycolysis the possibility of carbohydrate degradations through alternative pathways like HMP pathway was envisaged. Since G-6-PD, an important enzyme of HMP pathway was elevated by PRL treatment (Methur et al, 1976) such possibility of the operation of HMP shunt in the testis was suggested. Moreover, the operation of this pathway might also be possible in view of increase levels of nucleic acids in the PRL treated tissues because it is the only pathway producing pentose sugars (Harper, 1973). The elevated levels of phosphatases also suggested the mobilization of inorganic phosphate for nucleic acids synthesis. In view of lesser glycolytic mobilization of carbohydrates, increased SDH activity was due to active mobilization of amino acids and phospholipids to the citric acid cycle as evidenced by enhanced SDH activity, PRL treated testis. Thus in testis carbohydrates seemed to have diverted towards the formation of pentose sugars while amino acids and lipids seem to be utilized for the energy release.

In contrast to the situation in testis, all the sex
accessories had increased level of carbohydrates in response to PRL administration, indicating the possibility of a slight change from testis in carbohydrate metabolism in these tissues. Prostate gland recorded maximum total carbohydrate content. Since prostate gland is a major site for seminal plasma formation in rats (Humphrey and Mann, 1948; 49a, b) and plasma glucose forms precursor for seminal fructose (Mann and Parsons, 1956; Mc Clymont and Scottell, 1956) increased carbohydrate content of prostate gland might suggest the accumulation of the same from the blood and suggest the possibility of initiation and seminal plasma formation. ACP and ALP activities which were essential for seminal fructose formation were also elevated on PRL treatment. Hence it can be suggested that prostate gland might have been involved in accelerated fructose formation needed for seminal plasma in response to PRL. Since ACP forms an index of androgenicity (Gutman and Gutman, 1938) its increase in prostate gland might suggest the circulation of androgens in the plasma if immature PRL treated rats. The elevated levels of SDH and G6DH activities in PRL treated tissues might suggest the possibility of increased TCA cycle operation and mobilization of amino acids.

Seminal vesicles accumulated lesser carbohydrates than prostate gland. NAD dependent LDH activity was
decreased suggesting lesser mobilization of carbohydrates and thereby leading to carbohydrates accumulation. Since ALP and ACP activities which are essential for seminal fructose formation were decreased the possibility of least involvement of seminal vesicles in the seminal fructose formation was suggested which was in agreement with earlier workers (Humphrey and Mann, 1948, 1949 a, b). Elevated SDH activity in the presence of decreased NAD dependent LDH activity and G6DH activity might suggest the participation of lipids in the energy release of this tissue. Decreased phospholipid content in the tissues of PRL treated animals suggest such participation of lipid components in the energy metabolism of seminal vesicles under the influence of PRL.

Epididymis recorded least elevation in carbohydrate level in response to PRL. ACP and ALP activities were elevated suggesting the accumulation of fructose in PRL treated rats which might be essential for providing congenial atmosphere for the maturation and maintenance of spermatogenesis. In view of elevated LDH, G6DH and SDH activities the active mobilization of carbohydrates and amino acids into TCA cycle was envisaged.

The carbohydrate metabolism of non-reproductive tissues in PRL treated rats was studied in order to understand
the inter-relationship between reproductive and non-reproductive tissues. In view of drastic decrease in liver carbohydrate level with simultaneous rise in blood carbohydrate content in response to PRL and administration, the possibility of mobilization of carbohydrates from this tissue into blood was suggested. In view of elevated LDH activity and active mobilization of amino acids through GDH activity, least rise in SDH activity of liver in response to PRL, suggested the operation of gluconeogenesis. Since liver is concerned with regulation of blood glucose content and blood glucose is actively utilized by reproductive tissues, there might have been a close inter-relationship between liver and reproductive tissues as far as glucose requirements were concerned. Hence in the light of these evidences dependency of reproductive tissues on liver for glucose requirements was suggested. The carbohydrate metabolism of other non-reproductive tissues suggested the orientation of metabolism towards carbohydrates preservation. Thus the information available on carbohydrate metabolism in response to PRL has suggested the possibility of initiation of spermatogenesis in the testis and seminal plasma formation in sex accessories. The possibility of the liver meeting the glucose requirements of reproductive tissues has been suggested. In view of increased level of increased level of ACP activity, PRL induced androgen
release in immature rats was substantiated.

The proliferation and maturation of spermatogonia are energy requiring processes (Mann, 1964) and seminal plasma contains considerable quantities of neutral lipids, glycolipids, phospholipids, glycosides and fatty aldehydes (Prevost and Duma, 1924; Pittard, 1952; Scott, 1945; Jain and Anand, 1976a, b) and hence the mammalian reproduction seem to be dependent on the lipid metabolism. The cholesterol forms raw material for sex steroids and has a major role in the regulation of reproductive activities. Since PRL releases androgens (Hafiez et al., 1972a, b) it might induce regulatory influence on the tissue lipid metabolism. Hence in the present study an attempt has been made to understand the probable modulatory influence of PRL on lipid constituents and its impact on the proliferation and maturation of spermatogonia.

PRL administration resulted in an overall depletion of total lipid content of all the reproductive tissues with an accumulation in non-reproductive tissues. These lipids might have been utilized towards PRL induced activities in reproductive tissues. Testis recorded maximum drop in total lipid content and testicular lipids are known to be depleted during spermatogenesis and maturation processes (Kar and Roy, 1955; Wing et al., 1974; Wing et al., 1966;
Johnson, 1967; Johnson, 1970; Kornblatt, 1974). Hence in the present study the depleted total lipid level in immature testis might suggest the initiation of testicular functioning in response to PRL. Similarly there was considerable depletion in phospholipids and cholesterol contents in testis on PRL administration, suggesting the onset of function like proliferation and androgenesis.

All the sex accessories studied, had depleted total lipid content suggesting their functional significance in response to PRL. However phospholipid content showed differential trend in these tissues, while prostate gland had depleted level epididymis and seminal vesicles accumulated. The study of carbohydrate metabolism in prostate gland suggested the active utilisation of carbohydrates towards seminal fructose formation, while the carbohydrates in seminal vesicles and epididymis were diverted to energy metabolism. Consequently prostate gland might have been utilising phospholipids as energy source while epididymis and seminal vesicles were accumulating the same. Hence the depleted phospholipid content in prostate gland and elevated content in seminal vesicles and epididymis were dependent on energy metabolism of the tissue. The total cholesterol content was found to be elevated in the sex accessories. Since cholesterol is one of the components of
semenal plasma (Scott, 1945) its accumulation by sex accesor-
sories might suggest the induction of congenital condi-
tions for the onset of seminal plasma formation.

The total lipid content of non-reproductive tissues was elevated in response to PRL. Liver and kidney are known to be the centres of lipogenesis (Harper, 1973) and liver synthesizes high quantities of triglycerides and mobilizes into blood during maturation process (Wallace and Lascelles, 1964; Gatchet, 1970). The observed elevation of lipid content in these tissues might suggest the onset of maturation processes in immature reproductive system with a consequent stepped up lipogenesis in non-reproductive tissues. Since PRL administration results in the release of testo-
sterone and PRL itself mimics the growth hormone, the accumu-
lated lipid content in non-reproductive tissues might suggest the onset of anabolic phase in them.

Phospholipid content was elevated in these tissues except brain, suggesting their *de novo* synthesis in liver and kidney and consequent accumulation in muscle. However brain had depleted phospholipid content. Since maturation process brought about a decrease in brain phospholipids content (Fries and Chaikoff, 1941) the observed decrease in the phospholipid content of brain might also suggest
the initiation of maturation processes in the reproductive system of immature rats in response to PRL.

The cholesterol content of liver and kidney was elevated while that of brain and muscle had decreased levels. Since liver and kidney are the active sites of cholesterol synthesis (Harper, 1973) the observed elevation of cholesterol in these tissues might suggest its synthesis in them in response to PRL.

PRL administration enhances plasma testosterone levels (Hafiez et al., 1972 a, b) the androgens in turn enhances cellular respiratory rate with an increase in mitochondrial number (Harper, 1973) and the administration of gonadotropins elevated tissue dehydrogenase activities (Bathur et al., 1976), the dehydrogenase system might also be subjected to modulation in response to PRL. These studies on oxidative metabolism of tissues in response to PRL administration suggested the active mobilization of amino acids into TCA cycle through elevated GDH activity. Glutamic acid content was decreased in response to sex hormone treatment (Subba Rao and Gupta, 1965). Hence PRL either directly or through androgen mediation might be exerting modulatory influence on the molecular pattern of GDH. The study on kinetic parameters of GDH in the presence of PRL was undertaken in order to analyse the possible mechanism of molecular
modulations of the hormone on the enzymes.

Since GH activity levels were elevated in the tissues in response to PRL, there might have been changes in enzyme-substrate affinity. In order to find out such changes, Michaelis-Menten constant (Km) of the enzyme in response to PRL was studied in the tissues. Km was decreased in PRL treated animal tissues suggesting increased affinity of the enzyme with its substrate with consequent increase in catalytic potential of the enzyme. The enzyme-substrate affinity is dependent on enzyme-co-factor affinity and ionization of active sites and secondary specificity sites of the enzyme. Since enzyme-substrate affinity was elevated in response to PRL, the enzyme co-factor affinity was analysed. Km value of NAD-enzyme was decreased in PRL treated tissues. If Km was to decrease, it indicates increased affinity between enzyme and co-factor. Such an increase in enzyme-co-factor affinity might also enhance the enzyme-substrate affinity thereby leading to elevated catalytic potential of the enzyme was observed in the present study. Since both enzyme sub-substrate and enzyme-co-factor affinities were elevated in the PRL treated tissues, there might have been modulations at either active site or secondary specificity site of the enzyme. The enzyme molecules possess active site for
combination with the substrate and secondary specificity site for the proper ionization of the enzyme molecule. The studies on active site ionization revealed slight changes in the pH values of the groups involved in the active sites in FRL treated tissues. However, the groups of active sites were not altered indicating a slight change at the level of active site ionization of the enzyme. The studies on pH dependency of the enzyme also revealed slight changes at the secondary specificity sites indicating the possibility of shifts in the optimum pH of the enzyme in some tissues. These changes at the primary and secondary specificity sites of the enzyme molecule might have exerted a modulatory influence on the enzyme - co-factor and enzyme - substrate affinities. Thus FRL was exerting modulatory influence on the primary and secondary specificity sites of GM, thereby elevating the enzyme - co-factor and enzyme - substrate affinities and ultimately leading to elevated catalytic potential of the enzyme. Such an increase in GM activity level was warranted in the tissues in view of active feeding of amino acids into the energy metabolism which is a pre-requisite for the establishment of maturation process.

The results of the present study revealed the possibility of initiation of spermatogenesis formation of seminal
plasma, increased androgenesis and accelerated anabolic phase in tissue metabolism.

In general, the present study on PRL administration revealed the initiation of functioning of the reproductive system during immature stages and thereby the possibility of PRL induced advancement of puberty can be envisaged.

These present findings are in close consonance with the earlier reports where PRL induced accelerated spermatogenesis and increased fertility in adult male rats (Bartke, 1966; Bartke, 1967; Bartke and Lloyd, 1970).