DISCUSSION
Alloxan, a B-cell destructive agent has been widely used to induce diabetic or hyperglycemic state in different animal models (Kerup, 1970; see Dulin et al., 1982; Lernmark, 1985). As expected, in the present study also, single injection of alloxan induced the diabetic state by registering about five-fold increase in blood glucose level, which was sustained throughout the experiment. Besides, the classical symptoms of diabetes, such as weight loss, polydipsia and polyuria were also noticed in the experimental animals confirming the severity of the disease.

In the present study, alloxan-induced diabetes caused a significant decrease in body weight, accessory sex organs' weight and serum testosterone. Similar results have also been reported previously in streptozotocin-diabetic mature rats (Oksanen, 1975; Howland and Zebrowsky, 1976; Paz et al., 1970; Murray et al., 1981; Hendrix and Niewenhuis, 1982).

In general, the observed decrease in serum testosterone could be either due to decreased synthesis or increased metabolic clearance rate or both.
Testosterone synthesis in mammalian testes is largely under the control of LH (de Kretser et al., 1971; see Eik-Nes, 1975; Purvis et al., 1981). LH binds with specific receptors in the Leydig cell membrane (Dufau et al., 1971; Catt and Dufau, 1973) and initiates a series of biochemical events which lead to increase in testosterone synthesis and secretion (see Eik-Nes, 1975). LH is responsible for the maintenance of many of the testicular steroidogenic enzymes, like the enzyme complex concerned with the cleavage of cholesterol side-chain (Tan and Robinson, 1977; Wiebe, 1973), as well as 3β-hydroxy steroid dehydrogenase (3β-HSD) (Shaw et al., 1979). Prolactin also favors testosterone synthesis by enhancing binding of LH to its receptors in Leydig cells in mice and rat (see Bartke et al., 1985) and by increasing the number of LH receptors in Leydig cells (Purvis et al., 1979). Insulin synergizes with LH in the stimulation of testosterone synthesis (Adashi et al., 1982). The activity of at least one of the regulatory enzymes (3β-HSD), of the biochemical pathway leading from cholesterol to androgens was shown to be affected by insulin (see Eik-Nes, 1970).
Streptozotocin-induced diabetes has been shown to cause significant decrease in the serum levels of gonadotropins (Howland and Zebrowsky, 1976; Paz and Homonnai, 1979; Hutson et al., 1983) as well as prolactin (Prl) (Hutson et al., 1983) in rats. Lowered LH and FSH levels have also been observed in diabetic patients (Distiller et al., 1975). Hence the lowered serum testosterone observed in the present study might be due to the decreased gonadotropins, Prl and insulin levels. Furthermore, it is suggested that the decreased serum testosterone may partly be contributed by peripheral conversion of testosterone to estradiol, as increased estradiol was observed in diabetic human males (Guoliang et al., 1987). The possible contribution of elevated metabolic clearance of testosterone seems to be unlikely, since the urinary excretion of androgen metabolites were found to be lowered in diabetic condition (Schoffling et al., 1963). However, these aspects deserve further experimentation to confirm the same.

Androgens are the major regulators of the structure and functions of male accessory sex organs. The weight of accessory sex organs serve as an index
of androgenic status (see Mann, 1964; see Brandes 1974; see Cazares, 1975; Brooks, 1979). In addition to the possible direct effect, Prl has also been found to potentiate the action of testosterone on male accessory sex organs (Grayhack, 1963). Hence, the decrease in weights of seminal vesicle and ventral prostate of the diabetic rat seen in the present study, might be due to the combined effect of decreased serum testosterone and Prl (Hutson et al., 1983). Nevertheless, the possible contribution of insulin binding sites have been reported in accessory sex organs of male rat (Lauzier et al., 1991).

In the present study, unlike the accessory sex organs' weight, the testicular weight was not significantly decreased in the diabetic animals. In agreement with the present study, similar observations were also made by other investigators in mature rats treated with alloxan and streptozotocin (Blanco et al., 1931; Ford and Hamilton, 1934; Jackson and Hutson, 1934). However, testicular atrophy has been reported in juvenile rats made diabetic with streptozotocin (Paz et al., 1973; Rossi and Rastelli, 1981). The effect of diabetes on the testis is more severe when the disease is induced
before or during puberty (Paz et al., 1973; Muckey and Hamilton, 1981). Therefore, the lack of any significant change in testis weight in the present study could be explained on the basis that the testis is more sensitive to diabetes in prepubertal and pubertal animals, but less so in adults.

The initiation of spermatogenesis in testis is dependent on the presence of FSH and testosterone. The maintenance of spermatogenesis is largely obtained in the presence of testosterone which regulates the formation of spermatogonia and the second meiotic divisions (Steinberger, 1971; see Eik-Nes, 1975; see Fritz, 1973). The Sertoli cell is the primary target for FSH, where it binds to specific receptors and initiates a variety of biochemical events (Cistaro et al., 1970). The main action of FSH on the Sertoli cell involves the stimulation of AWP production (see Findall et al., 1974; Fakunding et al., 1975). AWP plays an important role in spermatogenesis, by causing intratesticular retention of testosterone (Means et al., 1976). Completion of spermatogenesis and formation of spermatozoa also requires FSH (Steinberger and Duckett, 1967).
Along with reduced serum testosterone, as observed in the present study, low levels of FSH, LH, Prl and testicular ABP have been reported in streptozotocin-diabetic adult rats (Hutson et al., 1983). Based on the information gained from these studies, it is suggested that the alloxan induced diabetes in the present study could lead to hypospermatogenesis. The decrease in testicular germ cell-specific LDH observed in the present study is indicative of hypospermatogenesis. In addition to this, it is further confirmed by the observation of Sivasharmugam (1969) from this laboratory who recorded a marked reduction in sperm content in the epididymal segments of alloxan-diabetic adult rats.

Proper functioning of the testis requires a constant supply of carbohydrate, especially glucose (see Free, 1970). Pyruvate kinase (PK) is one of the rate-limiting enzymes of the glycolytic pathway which is involved in the phosphorylation of ADP (see White et al., 1973; see Mayes, 1988). Activity of testicular PK depends on LH (Brown et al., 1966; Sosa et al., 1972). Since diabetes causes a decrease in serum LH levels (Howland and Zebrowsky, 1976), the observed reduction
in the activity of PK might be due to decreased LH stimulation. The reduction in activity of PK may indicate decreased production of pyruvate. Since this enzyme is involved in the key position of shifting pyruvate for mitochondrial respiration and lipogenesis (see Mayes, 1988), the decrease in PK activity may lead to decreased mitochondrial respiration and lipogenesis and subsequently it may affect testicular function.

Pyruvate is thought to be one of the major products which act to maintain a high rate of protein synthesis in spermatocytes and spermatids (Jutte et al., 1983). Pyruvate production in Sertoli cells has been reported to be markedly stimulated by FSH (Jutte et al., 1983). Hence, the lowering of FSH level due to diabetes may result in decreased pyruvate production and subsequent impairment in spermatogenesis.

3-6-PDH, an enzyme of the pentose phosphate pathway is involved in the supply of coenzyme NADPH needed for lipogenesis and steroidogenesis in the testis (see Free, 1970; see Banks et al., 1979). Despite the decrease in gonadotropins due to diabetes (Hutson et al., 1983), the increased testicular 3-6-PDH activity is
quite intriguing. It is well known that the 6-6-PDH along with other enzymes such as IIDH and malic enzyme, provides NADPH necessary for lipogenesis and steroidogenesis in the testis (see Free, 1970; see Banks et al., 1979). Data on serum testosterone of the present study as well as other studies pertaining to the testicular steroidogenic enzymes of the diabetic animals (Howland and Zebrowsky, 1976) establish the diminished testosterone synthesis. In general, these observations imply that the increased NADPH supply associated with stimulated 6-6-PDH activity may not be utilized for steroidogenesis, instead it may be utilized for lipogenesis. Under these circumstances, it should be remembered that the supply of NADPH by 6-6-PDH for steroidogenesis is not the unique controlling factor of steroidogenesis in the gonads. Therefore, the shunting of elevated NADPH, associated with increased 6-6-PDH activity, towards lipogenesis seems to be more probable. Contrary to the present observation, Calvo et al. (1979), have reported low level of Leydig cellular 6-6-PDH in streptozotocin-diabetic rats. Such discrepancy may very well be attributed to differences in duration of the experiment.
and severity of the diabetic state. Further, it should be noted that in these studies the NADPH-generating enzymes were studied in the isolated Leydig cells, while in the present study 3-6-PDH was estimated in the whole testicular tissue which comprises of Leydig, Sertoli and germ cell populations. In general it appears that studies on the isolated cell types would provide a better insight to understand the unexplained events associated with diabetes.

Testicular hexokinase (HK), phosphofructokinase (PFK) and LDH have been reported to be under the control of gonadotropins and testosterone (Brown et al., 1966; Blackshaw and Samisoni, 1966; Rosa et al., 1972). Though diabetes has been shown to decrease serum gonadotropin levels (Howland and Zebrowski, 1976) in the present study, activities of HK, PFK and LDH were unaltered in the diabetic rat. This might suggest that these enzymes of the glycolytic pathway are resistant to diabetes and the associated changes in hormonal profiles.

LDH isozymes are known markers for the type of metabolism in any tissue (Lakshmi and Ramakrishnan, 1933). LDH exists in multiple molecular forms (LDH-isoenzymes),
which are presumed to be tetramer molecules built up from two parent sub-units of muscle (M) and heart (H) types. Each isozyme differs in many properties, including the metabolic significance (Lodja and Frie, 1970). While the M subunits represent the anaerobic metabolism, the H subunits represent aerobic metabolism (Clausen, 1970). In the present study, diabetes induced a significant increase in specific activities of LDH₃ and LDH₅ and resulted in elevation of total M subunits. Under normal condition, the testicular tissue shows an aerobic pattern of respiration. However, alloxan diabetes caused a metabolic shift showing equal distribution of H and M subunits.

LDHₓ is a testis-specific isozyme of LDH, which is reported to be a marker for active spermatogenesis (Blackshaw and Samisoni, 1967). LDHₓ is associated with specialized mitochondria found only in primary spermatocytes, spermatids and spermatocytes (Sarkar et al., 1973). In the present study, the activity of LDHₓ was found to be significantly decreased due to diabetes. The decrease in sperm-specific LDHₓ fraction may be due to partial arrest of spermatogenesis, since it is specific to germ cells.
Such a decrease in LDH$_x$ has also been observed in other instances where there is hypospermatogenesis (Zinkham et al., 1964). Lactate, the terminal product of glycolysis diffuses into mitochondria, where it is converted to pyruvate by LDH$_x$, with a production of NADH. LDH$_x$ is believed to transfer the reducing equivalent from the cytoplasm via a lactate-pyruvate shuttle in the mitochondrial electron transport chain for ATP generation (Dawson, 1979). Thus, the diabetes induced inhibition of LDH$_x$ may lower the reducing equivalents in the mitochondria and thus the ATP generation.

ATPases are surface membrane enzymes involved in the active transport of ions across the cell membranes (Kobinson and Flashner, 1979). The alteration in Na$^+$ - K$^+$ dependent ATPase is found to have a profound influence on the synthesis of macromolecules (Kuchler, 1967; Lubin, 1967). The incorporation of precursors into DNA, RNA and proteins depends on the intracellular potassium and sodium balance (Kuchler, 1967). It is therefore likely that the alloxan-diabetes associated changes in Na$^+$ - K$^+$ dependent and Mg$^{2+}$ dependent ATPases may have an impact on the incorporation of precursors.
into nucleic acids and proteins, by altering the membrane transport. Considering the hormonal control of ATPases, the decreased Na\(^+\) - K\(^+\) and Mg\(^{2+}\) dependent ATPases may be attributed to low levels of gonadotropins, Prl (Hutson et al., 1983) and testosterone. Nevertheless, it may also be due to the consequence of insulin loss, as specific binding sites for insulin have been reported in rat testis (Saucier et al., 1981).

In summary, it is obvious from the present study that alloxan-diabetes has a definite role to play in the regulation of testicular glycolytic enzymes and ATPases. The observed changes were found to be mediated by decreased serum gonadotropins, Prl and testosterone.