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INFLUENCE OF PROLACTIN ON THE EPIDIDYMAL LIPID PROFILES OF MALE ALBINO RATS

A THESIS

submitted in fulfilment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

By

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"Of the epididymis: This body may be considered as an appendix to the testis, and it's name is derived from it's being placed upon this organ, as the testes were anciently called didymi.

It is of a crescenti form; it's upper edge is rounded, it's lower edge is thin. It's anterior and upper extremity is called it's caput, the middle part it's body, and the lower part it's cauda."

Sir Astley Cooper
1830

DEDICATED:

To my parents ...

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in this thesis entitled, ' INFLUENCE OF PROLACTIN ON THE EPIDIDYMAL LIPID PROFILES OF MALE ALBINO RATS' in fulfilment of the requirement for the award of the Degree of Doctor of Philosophy in Department of Biosciences & Biotechnology, University of Roorkee, Roorkee is an authentic record of my own work carried out during a period from September 1986 to June 1990, under the supervision of Dr. Ben M.J. Pereira.

The matter embodied in this thesis has not been submitted by me for the award of any other Degree.

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This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

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ABSTRACT

(ABSTRACT)

Over the past few decades there has been an accumulation of reports concerning fertility disorders in man with disturbed levels of serum prolactin. One such fertility disorder is defective sperm maturation which is associated with the epididymis. The epididymis is unique, in the sense, it facilitates sperm maturation at its proximal end and stores them in a quiescent yet viable form at the distal region. Thus, the epididymis by virtue of performing two contrasting functions has puzzled many an andrologist. In addition, the identification of binding sites for prolactin in the epididymis has drawn the attention of many investigators at the present time. Thus, an attempt has been made to investigate the role of prolactin in the epididymis.

As a means of achieving this objective, experiments have been designed so that other factors that influence epididymal function are delineated and the precise action of prolactin are exposed. The approach has been to perform the experiments on castrated animals so that the interference from spermatozoa, testicular fluid and androgens could be minimized. Further, prolactin from an exogenous source is injected at different doses (50,100,150 and 200 μg of o-PRL/100 g body weight, daily, subcutaneously for 7 days) to simulate clinical conditions where reproductive disorders have been observed. Incidentally, hyperprolactinemia

is also associated with low levels of androgens and this also fits in very well with the experimental design. One group of animals has also been treated with bromocryptine (an ergot alkaloid, which significantly reduces the levels of circulating prolactin at a dose of 0.3 mg/100 g body weight, for a similar period of 7 days) to see the response of the epididymis when serum prolactin levels are low. The response of the male reproductive tract to prolactin was initially monitored in terms of changes in weight of these tissues.

It was now felt necessary to establish how long the exogenously administered prolactin remained in circulation. Hence, a time course study was done and the serum levels of prolactin monitored at intervals of 15 min, 30 min, 2h, 6h and 24 h after the last of the series of 7 injections. Since interest was in the biological activity of the heterologous prolactin present in samples, this hormone was measured by the 'local micro' pigeon crop sac bioassay. From the results, it is clear that the exogenously administered prolactin has a short half life and is not detectable at 15 min.

The action of prolactin on the functional status of the epididymis also needed to be known. In this connection, it was thought that measurement of glycerophosphocholine (GPC) could throw more light, since it has been used as an

index for this purpose by other investigators in the past. Many compounds are known to interfere with the determination of GPC. Thus, in order to make an accurate analysis, GPC was isolated and purified by a rapid procedure using Amberlite CG 400 'semi micro' columns and then quantified. Since the function of the epididymis change along its course, the analysis was done in segments that could form gross points of reference (caput, corpus and cauda). Prolactin was found to generally increase GPC levels in all segments of the epididymis but to a varying degree. Of the three regions, the action of prolactin on GPC levels of the caput seems to be more pronounced. The composition of epididymal fluid of which GPC forms a part is crucial for sperm maturation and storage. Therefore, it is suggested that any change in the level of this compound is bound to have the repercussions on this process.

Lipids are important molecules that have multiple functions in the epididymis. The acyl glycerols provide fatty acid side chains, the metabolism of which results in the release of energy much needed by sperm during their transit through the epididymis. Phospholipids are also known to be important sources of oxidizable substrate, in addition to their role in maintaining the stability and permeability characteristics of membranes. Like other macromolecules, the epididymal lipid composition is under the influence of hormones, predominantly androgens. A number of other

hormones as well have been implicated, but the action of prolactin remains to be elucidated.

In the present study total lipids were extracted in chloroform: methanol (2: 1 V/V) and quantified by gravimetric methods. The individual lipid subclasses from these extracts were separated using thin layer chromatographic techniques and quantified spectrophotometrically. Recent reviews on this subject have suggested that measurement of the pool size of individual lipid classes (as done in the present study) rather than estimation of the enzymes involved in lipid metabolism truly reflect the situation in vivo.

Prolactin increased total lipids which could be attributed to the general rise in total glyceride glycerol, total cholesterol and total phospholipids. A reduction in the level of diacylglycerol was observed with a concomitant rise in triacylglycerols, indicating a tendency for lipid storage. Prolactin also ensured the conversion of cholesterol from the free to the esterified form. Among the phospholipids, phosphatidyl serine, sphingomyelin and phosphatidic acid were reduced while phosphatidyl inositol, phosphatidyl choline and phosphatidyl ethanolamine showed a rise. The results enumerated above are prominent in the 100 μg O-PRL treated group which seems to be the dose at which maximum changes are effected in the androgen starved condition. Thus, it could be deduced that prolactin treat-

ment leads to an accumulation of triacylglycerol, phosphatidyl inositol, phosphatidyl ethanolamine and phosphatidyl choline. Based on these results, it has been inferred that prolactin selectively favours metabolism through specific pathways. The implications of these results are discussed.

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1.0 INTRODUCTION

In recent times, studies on male reproduction have assumed great impetus in India, primarily because of the exponential rise in human population. With the limited options for contraception in males and the willingness of this sex to participate in family planning programmes, there is not only need to develop newer and safer methods but also to improve the existing ones. While research from this angle is gaining importance, we are faced with situations when reproductive disorders arising from hormonal disturbances need to be corrected. Moreover, there is also a feeling among animal breeders that the fertility of selected, domesticated and farm species should be enhanced through improvement in sperm quality to make this planet a better place to live in. A deeper understanding of the physiology and factors that regulate the effective functioning of organs involved in reproduction would alone help in achieving this goal.

In view of the dynamic role of the epididymis in the development and maintenance of active spermatozoa, current emphasis is on understanding the basic physiology of epididymal tissue, hormones controlling the epididymis and the contribution of this organ to sperm maturation. Many excellent reviews on the epididymis and their function have been published recently (Cooper, 1986; Amann, 1987; Robaire and Hermo, 1988). In most species, the proximal regions

of this duct are involved in selective resorption of material and fluid originating from the testis and also in the synthesis and secretion of unique compounds that are essential for sperm maturation. The distal segments of the epididymis are responsible for storing sperm in a viable, fertile but quiescent form. How exactly the epididymis manages to carry on two complementary phases of action (1) promoting the ability of spermatozoa to interact with the oocyte and (2) preventing the premature expression of these abilities within the male tract is still a wonder.

Due to basic differences in the functional activity and structure, there are regional variations in the secretory and absorptive characteristics of the epididymis. The epididymis is not merely a passive conduit for sperm, but infact, provides a favourable milieu for sperm to acquire forward progressive motility and the capacity to fertilize ova. If this general consensus were to be true, then one means by which these events are brought about would be through alterations in the permeability of the epididymal epithelium. In this context, the phospholipids would be important. There seems to be no doubt that specific luminal macromoleculus are needed by sperm to meet their energy demands during their sojourn in the epididymis. One such macromolecule is lipid which is a rich source of oxidizable substrate(Voglmayr, 1975; Brooks, 1981a).

The functions of the epididymis are known to be controlled by a number of factors including hormones (Orgebin Crist et al., 1975; Brooks, 1984). One such hormone that has been implicated is prolactin, on account of the presence of binding sites in this organ (Aragona and Triesen, 1975; Orgebin-Crist and Jiane, 1979). Besides, alterations in circulating levels of prolactin have been associated with a number of reproductive disorders in males (Fluckiger et al., 1982a). The nature of these disorders once again point to the action of prolactin at the level of the epididymis.

Despite these significant observations, no substantial effort has been made to experimentally elucidate a role for prolactin in the epididymis. Thus, a set of experiments have been performed with the following objectives in mind :

1) To simulate a condition in the epididymis where interference from spermatozoa, testicular fluid and androgens are kept minimal by surgical castration.

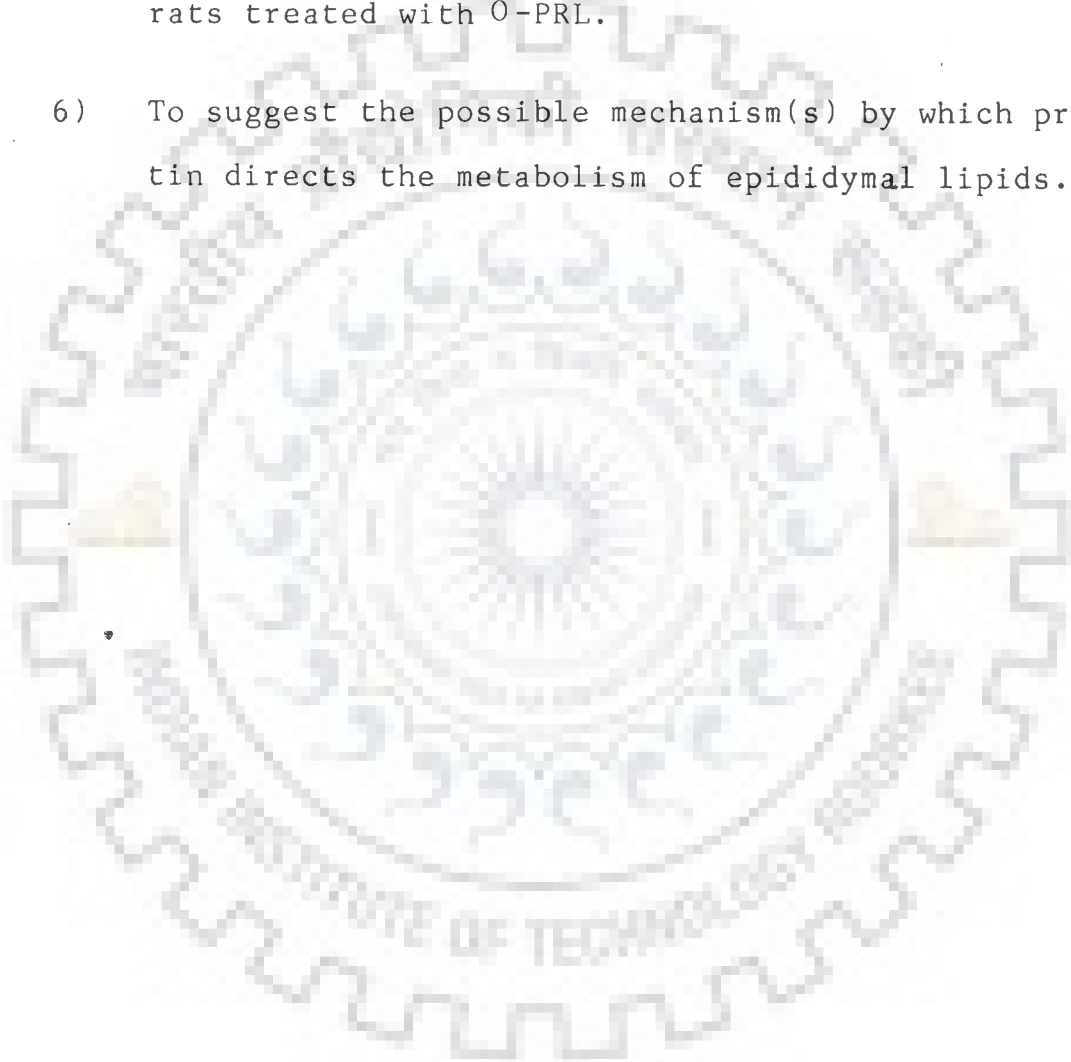
2) To pin point the action of prolactin on the epididymis of castrated rats by exogenously administering prolactin at graded doses.

3) To examine the circardian pattern of circulating prolactin bioactivity in castrated rats given ovine prolactin (O-PRL) treatment.

4) To monitor the epididymal function of these rats, by quantifying glycerophosphocholine (GPC) an unique secretory product in the epididymis.

5) To analyse the epididymal lipid profiles in castrated rats treated with O-PRL.

6) To suggest the possible mechanism(s) by which prolactin directs the metabolism of epididymal lipids.



2.0 LITERATURE REVIEW

The epididymis is a very dynamic organ which not only acts as a transient passage for the spermatozoa from the testis to the ejaculatory duct, but also provides them with a favourable internal milieu for acquiring the capacity to fertilize mature ova. In the spermatozoa, the acquisition of fertilizing ability parallels several morphological, biochemical and physiological changes which collectively is referred to as 'sperm maturation' (Hamilton, 1975). By virtue of its synthetic, secretory, resorptive and absorptive nature, the epididymis is actively involved in sperm maturation and storage of the same in a viable form (Jones and Glover, 1975; Orgebin-Crist, 1981; Mann and Mann, 1981; Cooper, 1986 ; Jones, 1989). The regulation of these epididymal functions and complex nature of inter-relationships between the epididymis and sperm pose challenging problems in the study of the process of reproduction. The present chapter surveys the current state of knowledge on the epididymis and identifies such areas which are worth exploring.

2.1. THE MAMMALIAN EPIDIDYMIS:

Cellular differentiation and development of what arises as the embryonic wolffian duct leads to the formation of an elongated coiled tube, the epididymis (Ortiz, 1945; Flickinger, 1969; Alexander, 1972). In adult testicond rodents, the epididymis is located in the scrotum, adjacent

and loosely adherent to the testis by tunica albugenia and embedded in a highly vascular fat pad. Further, this duct connects cranially with the ductuli efferentes and caudally with ductus deferens.

Based on characteristics like tubular diameter, height of epithelial cells, histological appearance, ultra structure of main cell types and function, several terminologies have been used to refer to specific regions of the epididymis (Reid and Cleland, 1957; Glover and Nicander, 1971; Hamilton, 1972; Zuncke and Grossrau, 1981). However the terminology used for one animal species does not hold good for another (Nicander and Glover, 1973; Hoffer and Greenberg, 1978; Fawcett and Hoffer, 1979; Nicander et al., 1983), since differences in the structures of epididymis are known to exist. Even in the same species as the rat, the regions of the epididymis have been demarcated very differently (Reid and Cleland, 1957; Hamilton, 1975). However, to make possible comparison of data obtained in laboratories, the world over, it is generally agreed that at least in the rat, the epididymis is discernable into three gross points of reference, the caput, the corpus and the cauda (Hamilton, 1975).

There are 4 physiologically interdependent compartments within the epididymis - (a) spermatozoa, (b) epithelium, (c) luminal plasma, (d) the blood lymph channels and

interstitial spaces filled with fluid (Hamilton, 1972). Besides, the different regions of the epididymal duct possess a unique distribution of musculature, vasculature and innervation and suited specifically to the functions of these parts (Baumgarten _et al_., 1971; Gunn and Gould, 1975; Chubb and Desjardins, 1982).

The histoarchitecture of the epididymis in the rats mainly comprise of principal cells (59 percent), basal cells (27 percent) and other cell types like clear cells (10 percent) (Yeung and Cooper, 1982). The existence of halo cells and apical cells is also reported (Hamilton, 1975). The principal cells play a major role in transport, mediating secretion and resorption across the epididymal epithelium. They aid in the removal of proteins from the lumen by multivesicular bodies (Moore and Bedford, 1979a,b; Yeung and Cooper, 1982). Besides, they show lipid droplets in their ultra structure (Hamilton, 1975). There is also a suggestion of their involvement in synthetic activities (Hamilton, 1972; Flickinger _et al_.,1984; Flickinger, 1985). More recently, an endocrinerole for principal cells is also postulated (Abe _et al_., 1983; Goyal, 1985). The basal cells, so named by their location in the epididymal canal, were thought to be holocrine (Martan and Risley, 1963). This concept has now been disregarded (Hamilton, 1972), although as yet a definite role has not been ascribed. It has also been demonstrated that apicalcells give rise to principal

cells(Moore and Bedford 1979a). While the apical cells are normally involved in absorption (Moore and Bedford, 1979a), the clear cells are thought to serve a secretory function as well (Prakash et al., 1979; Anand Kumar et al., 1980). They are capable of synthesizing protein (Dadoune et al., 1985). The nature of its vacuolar contents are lipid in the caput and corpus and glycoprotein in the cauda (Kreth, 1965; Anand Kumar et al., 1980). Halo cells are now visualized as lymphocytes (Dym and Romrell, 1975; Miller and Killian, 1983) believed to be transformed by conditions arising from leaving circulation and entering the epididymal epithelium (Hoffer et al., 1973). Thus, the epididymis apart from effecting absorption, resorption, secretion and transport, also serve as a safe repository for sperm. At this juncture, it is proper to point out that while spermatozoa acquires the ability of hyperactivation in the proximal portion of the epididymis, they become quiscent without losing their fertilising ability in the distal part (Cooper, 1986; Amann, 1987). It is amazing that the same organ strikes a balance between these two diverse functions.

It is now well accepted that the unique epididymal milieu partly results from the selective absorption of molecules of testicular origin. Ninety percent of the fluid from the testis is reportedly resorbed in the caput of bull, boar and rats (Crabo, 1965; Turner, 1984), thus increasing the concentration of secreted impermeant compounds (Setchell

and Hinton, 1981; Turner, 1984). Structural details of the resorptive process is similar to that which occurs in other cells by micro pinocytosis (Nicander, 1965; Hamilton, 1972). An intercellular route of chanelling of water from the lumen through the formation of osmotic gradient has been postulated in the rat caput and cauda (Wong _et al_., 1978a; Wong _et al_., 1978b, 1979; Cooper and Yeung, 1980; Yeung and Cooper, 1982). In rat and rabbit, the epididymal epithelium has been shown, to besides transferring fluid from the lumen into the interstitium (Wagenseil, 1928; Young, 1933; Mason and Shaver, 1952), absorb and digest particulate matter as well (Shaver, 1954; McMillan, 1957; Nicander, 1965; English, 1979). In this connection, fluid phase and receptor mediated mechanism have been proposed (Djakiew _et al_., 1984, 1985).

In addition to the resorptive and absorptive nature of the epididymis, the synthesis and secretion of a variety of macro-molecules contribute significantly to the composition of the epididymal fluid. These macromolecules are either produced by the epididymal epithelium (Wang _et al_., 1981; Flickinger, 1983) or may be derived from the blood by a selective concentrating mechanism (Brooks _et al_., 1973; Hinton and Howards, 1981; 1982). The synthetic ability of the epididymis has largely been undisputed, although regional variations in rates of synthesis of particular compounds are reported (Yeung _et al_., 1980; Brooks, 1981b; Flickinger, 1981). Both _in vitro_ and _in vivo_ studies have provided subs-

tantial evidence for the incorporation of amino acids into proteins (del Rio, 1979; Brooks and Tiver, 1984). The high concentrations of certain enzymes like galactosyltransferase (Fleischer et al., 1969), sialyltransferase (Bernals et al., 1980), glycosyltransferase (Tadolini et al., 1977) and α-lactalbumin (Byers et al., 1984) when viewed together with the ability of the epididymis to synthesize dolichols (Wenstrom and Hamilton, 1980), suggest that post-translational modifications occur in the epididymis. There is adequate evidence for the interaction of these luminal proteins with spermatozoa which are supposedly associated with events that take place in this organ (Brooks and Tiver, 1983; Kǒṕecny et al., 1984; Klinefelter and Hamilton, 1985). The interactions could be in the form of membrane surface changes which may influence permeability thereby controlling sperm motility and fertilizing ability (Voglmayr et al., 1980). Other roles for proteins have also been proposed. For example, transferrin and caeruloplasmin by their ability to prevent oxidation of tissue have a general protective effect on sperm (Papkoff, 1966; Al-Timiniand D' ormandy, 1977). Similarly, the presence of forward motility protein (Hoskins et al., 1979), immobilin (Usselman and Cone, 1983) and sperm survival factors (Morton and Chang, 1973) in the epididymis are also worth mentioning.

2.1.1. LIPIDS:

Lipids are important constituents of epididymal secretion (Voglmayr, 1975; Brooks, 1979a). The occrrence of high

levels of lipids in the epididymis and their synthesis *in-situ* may have important implications for maintaining its functional activity and for maturation and survival of spermatozoa in this organ (Voglmayr, 1975). They are probably secreted into the epididymal lumen for utilization by the spermatozoa (Voglymayr *et al*. 1977; Brooks, 1979a). It is believed that the oxidation of fatty acid side chains from both neutral and phospholipids provide the necessary energy for metabolism (Voglmayr, 1975; Evans and Setchell, 1979). The presence of free fatty acids have been reported in the epididymal fluid from a number of species (Scott *et al*., 1961; Brooks *et al*. 1974). However, they seem to arise from either epididymal synthesis (Brooks, 1979b), by transport from blood stream or breakdown of glycerolipids (Voglmayr *et al*., 1977; Evans and Setchell 1979) thus making their origin debatable.

From the calculations of *in vitro* lipid utilization rates, it has been suggested by Brooks (1979a), that there is insufficient amount of fatty acid in sperm cells to last them their entire sojourn through the epididymis. This makes it necessary for the epididymis to make them available to the sperm. In this context, the glycerolipids are important. *In vitro* studies have indicated that ejaculated sperm can utilize phospholipids in a substrate free media (Lardy and Philips, 1941a, b; Hartree and Mann, 1959, 1961). This proves that sperm can metabolize glycerolipids derived from sources other than their own.

The neutral lipids specifically the acyl glycerols stored in cells can probably satisfy the energy requirements of maturing spermatozoa to a much greater extent than the phospholipids, which are principally membrane components (Evans and Setchell, 1979). Histochemical and biochemical studies show that the epididymis can also synthesize cholesterol (Hamilton et al., 1969; Hamilton and Fawcett, 1970; Hamilton, 1971). This has been confirmed through in vitro studies where formation of cholesterol from acetate has been demonstrated (Hamilton and Fawcett, 1970). Cholesterol, besides being a mother for most steroids (Hamilton and Fawcett, 1970; Hamilton, 1971), also promotes close packing of phospholipids on membranes(Davis, 1976, 1980, 1981). As a result, such characteristics as fluidity, permeability (Go and Wolf, 1983) and resistance to cold shock are altered (Quinn and White, 1967; Davis and Byrne, 1980).

Regional variations in the concentrations of epididymal neutral and phospholipids have been reported (Riar et al., 1973; Rajalakshmi et al., 1973; Gupta et al., 1974a). The distribution of these lipids have, however, never conformed to any definite pattern, even in the same experimental animal (Terner and Johnson, 1971). It must be emphasized that the epididymis is made up of three intricate compartments - the epithelium, the luminal fluid and spermatazoa. Due to technical difficulties faced in the separation of these compartments, most earlier workers have analysed the epididymis as a whole. This is one of the main-

causes for inconsistent results that have been documented over the years. It is relatively simpler to isolate sperm from epididymal segments, and hence research workers prefer to concentrate on changes occurring in sperm during the transit through the epididymis. The changes specifically pertaining to lipids are summarized below :

a) A general decrease in the level of total lipid, total cholesterol and total phospholipids (Dawson and Scott , 1964 ; Grogan _et al_., 1966; Quinn and White, 1967; Scott _et al_., 1967, Lavon _et al_., 1970, Johnson _et al_., 1972; Scott, 1973; Poulos _et al_., 1973; Teichmann et al., 1974; Poulos _et al_., 1975; Dacheux, 1977; Evans and Setchell, 1979).

b) Among the phospholipid classes, phosphatidyl choline, phosphatidyl elthanolamine, phosphatidyl inositol and phosphatidyl serine were found to be appreciably lowered (Mann and Mann, 1981; Jones, 1989).

c) The acyl ester content of both neutral and phospholipids went down with a corresponding elevation in the activity of triacylglycerol lipases. (Terner _et al_., 1975; Adams and Johnson, 1977).

Phospholipids are known to control the physiological functioning of membranes (van Deenan, 1965). Besides regulating fluidity (Chapman, 1975), permeability (DeGier _et al_., 1968; Cohen, 1975) and viscosity (Shinitzky and Inbar, 1976), the dynamic changes in phospholipids within the mem-

branes aid in modulating the activity of membrane bound enzymes (Fuorcans and Jain, 1974; Kimelberg, 1977; Cooke, 1989). Further, differences in polar head groups are responsible for the opening and closing of gated channels for specific ions. For example, it has been observed that the H^+ and Cl^- movement is altered by one negative and one positive charge on phosphatidyl choline, sphingomyelin and phosphatidyl ethanolmine, two negative and one positive charge on phosphatidyl serine and a single negative charge on phosphatidyl inositol (Robertson and Thompson, 1977). Besides these physiological and structural roles, currently great importance is attached to phosphatidyl choline, phosphatidyl inositol and diacylglycerol in cell signalling (Tijburg et al., 1989; Exton, 1990). The breakdown of phosphatidyl inositol and phosphatidyl choline generate diacyglycerols and phosphatidic acid. These two, now serve as intracellular secondary messengers and activate protein kinase C, thus triggering messenger mediated mechanisms (Tijburg et al., 1989; Exton, 1990).

The oxidation of particularly phosphatidyl choline in the normal state is reflected in the formation of glycerophosphocholine (GPC) as a secondary metabolite (Brooks, 1979a). Ample evidence is available to substantiate this view in rats (Dawson and Rowlands, 1959), rabbits (Scott et al., 1963b) and ram (Dawson, 1958). It has also been

proposed by Infante and Huszagh (1985) that synthesis of GPC occurs via transfer of choline from phosphatidyl choline to glycerol-3-phosphate. The exact site of GPC synthesis along the epididymal duct has however been a subject of dispute. In the case of rat, it is reported that both head and tail regions of the epididymis can synthesize GPC (Dawson and Rowlands, 1959; Scott et al., 1963a,b) but the head is generally more active in secreting the same (Hinton and Setchell, 1980). It has also been demonstrated that the concentrations of GPC, did not vary in the rat caput and cauda(Riar et al. 1973; Brooks et al., 1974; Brown-Woodmann et al., 1980). Several other scientists estimated slightly higher levels in the cauda (Dacheux et al., 1970; Setty et al. 1979). Despite the lack of common opinion, it is generally agreed that the levels of GPC found in the semen is the best indicator for epididymal secretory activity (Mann, 1964). The role of GPC is not particularly understood. Nonetheless, it has been suggested that owing to its water soluble properties the osmotic pressure of epididymal fluids may be affected (Levine and Marsh, 1971). Like most epididymal functions, the synthesis and secretion of GPC is controlled to a large extent by the presence of spermatozoa, flow of epididymal fluid (Scott et al., 1963a) and the concentration of androgens (Brooks et al., 1974; Bjerve and Reitan, 1978; Beck, 1980).

2.1.2 EPIDIDYMIS AND HORMONES

A number of hormones have been known to profoundly influence the epididymis. The differentiation and development of epididymis from the wolffian duct is brought about mainly by androgens early in fetal life (Bishop, 1961; Flickinger, 1969; Alexander, 1972). The influence of androgens continues through puberty until the functional stability of this organ is attained in adult hood (Orgebin Crist et al., 1975). Drastic changes in the histology of the epididymis marked by a decrease in tubular diameter, epithelial cell height and length of stereocilia are seen in androgen deprived animals (Maneely, 1958; Orgebin Crist et al., 1975). Castration also results in the reduced synthesis and secretion of a number of compounds that are directly needed for sperm maturation and survival in this organ (Allen and Slater, 1957; Rajalakshmi and Prasad, 1968; Prasad et al., 1972 1973; Gupta, et al., 1974; Orgebin Crist, 1975; Brooks, 1984). Other physiological events like absorption and resorption that are known to occur in the epididymis are also severely hampered when the primary source of androgen is removed (Wong and Yeung, 1977). These changes are dramatically reversed when androgen replacement is given to these animals (Orgebin-Crist et al., 1975). Thus the androgen dependency of the epididymis is clearly undisputed.

The androgens that control epididymal function are derived either from blood or through the testicular fluid (Aafjes and Vreeburg, 1972; Turner et al., 1981). The epi-

didymis is also capable of synthesizing steroids (Hamilton and Fawcett, 1970). Besides, the epididymis also possesses such enzymes that are required for their interconversion (Hammerstedt and Amann, 1976), thus making available a variety of steroids in the epididymis. Among the steroids, androgens (Hamilton, 1971), estrogens (Lubicz-Nawrocki et al., 1973), aldosterone (Hinton and Keefer, 1985), progestins (Lubicz-Nawrocki, 1973) and corticoids (Mc Gadey et al., 1966) have been investigated for their possible influence on the epididymis. Strong evidence for the action of these hormones in the epididymis comes from the detection of cytoplasmic receptors (Danzo and Eller, 1985; Cooper, 1986) and other related binding proteins (Danzo and Eller, 1985). The role of thyroid hormones in the control of epididymal function has been extensively worked out (Pereira et al., 1983, 1984).

The hormones secreted from the pituitary are of late gaining much importance particularly in view of the fact that hypophysectomy disturbs epididymal function (Bishop, 1961; Mann, 1964). One such hormone is prolactin (PRL), receptors for which have been demonstrated in the epididymis (Aragona and Friesen, 1975; Barkey et al., 1977; Orgebin-Crist and Jiane, 1979).

Prolactin is a major glycoprotein hormone produced by acidophilic cells of anterior pituitary (Friedgood and Dawson, 1940). Analysis of the amino acid sequence of pro-

lactin reveals molecular heterogenity in a number of species including rat under basic (Wallis *et al.*, 1980), stressful and/or pharmacological conditions (Lawson *et al.*, 1980), There is a wealth of information on the regulation of peripheral levels of prolactin under various conditions (Bartke, 1980; Fluckiger *et al.*, 1982b). The peripheral level of prolactin in male mammals is comparable to that of non pregnant nonlactating females (Meites,1973). The release of prolactin is influenced by a number of factors of hypothalamic origin, collectively termed as prolactin inhibiting factors (Talwalker *et al.*, 1963). Besides, prolactin is known to control its own secretion by an autoregulatory or short loop feed back mechanism (Herbert *et al.*, 1979). It is also shown that transplantation of pituitary to an extra pituitary site in the same animal causes increased prolactin secretion (Everett, 1954; Chen *et al.*, 1970). This has been attributed to the removal of inhibition from central nervous system caused by disconnecting the pituitary from the hypothalamus (Chen *et al.*, 1970). The neuronal control of prolactin secretion involves not only classic neurotransmitters, like acetyl choline, nor adrenaline, serotonin and dopamine (Clemens, 1976) but also other polypeptides, gamma amine butyric acid, histamine, enkephalins (Mc Cann *et al.*, 1978) and possibly prostaglandins (Ojeda *et al.*, 1978). Serotonergic control is of importance as they are known to alter the circulating prolactin levels

(Koeing *et al.*, 1979). Another overtopping means of control is through the tubero-infundibular dopaminergic system (Fuxe *et al.*, 1970). This pathway is highly relevant, since ergot alkaloids or dopamine agonist like bromocryptine which act by interferring with dopamine function are extensively used to control circulating levels of prolactin (Fluckiger, 1978). Bromocryptine is the drug of choice because at therapeutic doses, rapid and prolonged inhibition of prolactin is brought about (Fluckiger and Wagner, 1968). This ergot alkaloid acts at the level of hypothalamus and also on dopamine receptors of pituitary lactotrophs (Fluckiger, 1978; Fluckiger and Vigouret, 1979). It thus reduces circulating levels by inhibiting transcription and translation of this hormone (Kinch, 1980; Weinstein *et al.*, 1981).

The levels of most hormones are interdependent and happen to be finely tuned and delicately balanced. Prolactin is no exception. Thyroid releasing hormone (TRH) produced in the hypothalamus enhances prolactin release, thereby implicating a stimulatory effect of the central nervous system (Jacobs *et al.*, 1971). Estrogen by acting at both the hypothalamic and pituitary level is considered an important modulating factor in the control of prolactin secretion (Dufy *et al.*, 1979). Testosterone is able to increase peripheral prolactin levels (Grosser and Robaire, 1987) while castration produces the opposite effects (Grosser and Robaire, 1987).

Like other hormones a rhythmic release of prolactin has been observed in many species (Clark and Baker, 1964; Koch _et al._, 1971). In male rats, a diurnal rythm in circardian periodicity is seen with maximum release of prolactin at 16 h and a minimum at 22 h (Koch _et al._, 1971). In man, however, a circardian release has been demonstrated with levels reaching a maximum at night (Parker _et al._, 1973). The half life for circulating prolactin vary greatly, probably depending on the experimental situation. Grosvenor (1967) reported a half life of three minutes for porcine and bovine prolactin in the serum of adult female castrated rats. For ovine prolactin, the half life was measured to be 10-12 mins. Using ether as a stimulus to release endogenous prolactin in intact and castrated male rats, Chi and Shin (1978) found a disappearance rate with a half life of 7 mins. After prolonged treatment of rats with ovine prolactin, Diamond _et al._ (1980) observed a decrease in the heterologous prolactin clearance rate with the initial short half life of 6-12 mins being maintained and a new slow half life of 40→200 mins becoming prominent. This agrees with the conclusion of vander Gugten and Kwa (1970) that prolactin clearance is not a single constant process and this phenomenon may not be relevant for endogenous prolactin levels.

Alterations in serum prolactin titres have been known to effect bodily functions in many ways. Of the 85 biological functions known for prolactin, their role in male

reproduction is not insignificant (Nicoll, 1980). In the human species, increased circulating levels of prolactin results in failure to enter puberty (Koeing *et al.*, 1977), oligoaspermia (Roulier *et al.*, 1976; Francks *et al.*, 1978), subfertility (Boucher *et al.*, 1977), hypogonadism (Carter *et al.*, 1978) and defective sperm maturation (Francks *et al.*, 1978). Signs of androgen failure, low androgen production, positive pituitary and testicular response to exogenous stimulation have also been noticed (Fluckiger *et al.*, 1982). Low circulating level of prolactin has also been reported to delay puberty (Cohen *et al.*, 1988), hamper seminal quality and alter serum testosterone (Gonzales *et al.*, 1989).

Prolactin has been known to cross the blood tissue barrier and find its way into semen (Sheth *et al.*, 1975, Biswas *et al.*, 1978). On a number of occasions, attempts have been made to correlate prolactin concentration with semen quality (Segal *et al.*, 1978). The outcome of this has been conflicting. More recently, Sueldo *et al.* (1985) in an attempt to resolve this issue, have provided sufficient proof to infer that high seminal prolactin levels have a negative impact on sperm functional capacity. The biochemical effects of prolactin from seminal plasma are seen in the form of increased cyclic AMP (Shah *et al.*, 1976), fructose utilization (Shah *et al.*, 1976), glucose oxidation (Pedron and Ginner, 1978) and ATPase activity (Shah and Sheth, 1979).

In rats too, hyperprolactinemia is found to be associated with testicular atrophy which ultimately disturbs their copulatory behaviour (Fang *et al*., 1974; Bartke, 1980). Despite the innumerable male reproductive disorders cited above, the mechanisms of prolactin action in male reproductive tissues have remained poorly understood. Several attempts have been made to know the mode of prolactin action in male accessory sex glands(Klezdick *et al*., 1976; Aragona *et al*., 1977). Under normal circumstances receptors mediate the action of hormones in tissues. The demonstration of receptors for prolactin in seminal vescile, ventral prostrate and epididymis (Aragona and Friesen, 1975; Barkey *et al*., 1977; Orgebin Crist and Jiane JD, 1979) have rejuvenated interest in getting to know the implications of the same. Prolactin is reported to stimulate the growth of testis and other accessory sex glands (Bartke and Llyod, 1970; Negro-Vilar *et al*., 1977). It also brings about an accumulation of important secretory products like fructose in the seminal vesicle (Shah *et al*., 1976) and citric acid in the prostate (Grayhack *et al*., 1967). Several enzymes like ornithine decarboxylase (Rui and Purvis, 1987) and adenylate cyclase (Golder *et al*., 1972) are activated. Involvement of this hormone in steroidogeneis is evident from the stimulation of enzymes associated with the steroid biosynthetic pathway (Bartke, 1976). In all these respects, synergism with androgens is noticed (Grayhack *et al*., 1954; Prins, 1987).

It is suggested that prolactin functions by increasing the receptor mediated uptake of androgens in these tissues (Farnsworth, 1972; Baker et al., 1977). However, much less is known about the action of prolactin in the epididymis.

Thus, from the foregoing account, it is clear that the epididymis is a dynamic organ where sperm gain the property of forward progressive movement and the ability to fertilize ova. It is also in this organ that sperm are stored in a viable form for varying periods of time before they are finally ejaculated.By performing such roles, the epididymis has now come to be recognized as an important and vulnerable site for contraception in males. In order to achieve this goal, it is imperative to have a greater understanding of the events that take place in this organ together with factors that are capable of modulating them. In view of the disturbances in reproductive function associated with altered levels of circulating prolactin, it would be interesting to investigate the role of this hormone in reproductive tissues. In this context, the epididymis is one organ which has been largely neglected and much work remains to be done before clearer concepts emerge.

3.0 MATERIALS AND METHODS

3.1 CHEMICALS:

All reagents were of analytical grade and obtained from several Indian commercial firms (Merck, BDH, Sisco, Glaxo, CDH, LOBA etc.). Ovine prolactin (O-PRL-18 NIADDK-NIH biopotency 30 I.U./mg) was generously provided by the National Pituitary Agency, NIADDK NIH, Bethesda, Maryland, USA.Bromocryptine mesylate was also a gift from Messrs. Sandoz Chemical Company, Switzerland. Authentic reference standards for lipids and the ion exchange resin, Amberlite CG 400 were from Messrs. Sigma Chemical Company, (U.S.A.). Cholesterol, however was obtained from BDH (England).

3.2 ANIMALS:

Adult male albino rats of Wistar strain (150-250g) used for the experiments were bought from the Haryana Agricultural University, Hissar.Male pigeons used in bioassays were purchased from local commercial sources.

3.2.1 MAINTENANCE OF ANIMALS:

Colony bred adult male albino rats originally derived from the Wistar strain were maintained in a well ventilated animal room with 12 h L and 12 h D schedule. They were fed a standard pelleted diet (Lipton, India) and water was made available ad libitum. Essential cleanliness and sterile conditions were maintained.

3.2.2 SURGICAL PROCEDURE:

The epididymis, is greatly influenced by the presence of spermatozoa, luminal fluid and androgen levels (Brooks, 1979b;Nicander et al. 1983). To delineate the possible interference from these factors and to pinpoint the actions of prolactin, the experiments were performed on castrated animals. Surgical castration was performed bilaterally through the scrotal route under mild ether anesthesia. The procedure involves removal of the testis after ligating the vasa efferentia along with the testicular blood vessels, leaving the epididymis and its blood supply intact.

3.2.3 PREPARATION OF INJECTIONS USED IN EXPERIMENTS:

The procedure followed in reconstituting O-PRL was the same as that suggested by the National Institutes of Health, U.S.A. Ovine prolactin (Biopotency 30 I.U./mg) was prepared initially at a concentration of 2.5 mg/ml in 0.03 M $NaHCO_3$ in 0.15 M NaCl (pH 10.8) with very gentle agitation. After solubilization was effected, the pH was lowered to 7.0 by dropwise addition of 2N HCl. This stock solution was diluted appropriately to match the dose of prolactin to be injected. A 50 mg/ml stock solution of Bromocryptine mesylate was prepared in 45% alcohol. This was then diluted as per requirement just before use.

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3.3 EXPERIMENTAL DESIGN:

The experiments were performed on castrated male rats. In all 360 animals were used. They were divided into six groups consisting of 60 animals each. Preliminary histological studies in our laboratory have shown that the epididymis of rats become completely devoid of sperm 15 days after castration(data not presented). Thus, the injections were started fifteen days post-castration as detailed below.

While the first group received vehicle, animals belonging to groups II-V received graded doses of O-PRL 50,100, 150, 200 ug/100 g body weight, respectively, subcutaneously, daily, for 7 days. Group VI was given 0.3 mg Bromocryptine mesylate per 100 g body weight, subcutaneously, daily, for the same period of 7 days. In pilot experiments different doses of bromocryptine were tried (0.15, 0.3, 0.5 mg/100g body weight). From the results of bioassay performed in the serum of these animals, 0.3 mg/100 g body weight was found to be the most effective in reducing the circulating levels of endogenous prolactin. A similar dose has been used, by earlier workers as well, for bringing down the circulating levels of endogenous prolactin in rats. (Lloyd *et al*., 1975).

In all the experiments, certain generalizations were made. The injection volume was kept between 0.2-0.25 ml

and all injections were given between 9.30-10.30 in the morning. The injection schedule were so arranged that a particular animal received the injection exactly at the same time each day for 7 days.

Animals belonging to each group were killed in batches by decapitation at 15 mins, 30 mins, 2 h, 6h and 24 h after the last of 7 injections.Blood was collected immediately on decapitation and kept for 10-14 mins for clotting. The serum was separated and stored at -20°C until used for prolactin bioassay. The animals were then transcardially perfused with physiological saline for about 10 mins until the epididymis turned pale. This indicated that perfusion was complete and the epididymis was free from blood. The epididymis was now excised, cleared of fat and connective tissue and weighed on a torsion balance. The weights recorded were rounded up to the nearest milligram. The tissue was wrapped in aluminium foil and stored at -20°C before it was processed further for GPC and lipid analysis. These biochemical parameters were monitored in the three epididymal segments- the caput, the corpus and the cauda, a classification adopted by Hamilton (1975).

3.4 ASSAY FOR SERUM PROLACTIN BIOACTIVITY:

Prolactin levels in the serum of rats used in the preceeding experiments were estimated through a bioassay. The 'local micromethod', using pigeon hemicrop sac was followed

(Grosvenor and Turner, 1958). Adult common male pigeons weighing 250 - 350 g were obtained from local sources. They were acclimatized to laboratory conditions in an artifically illuminated room (12 h L : 12 h D schedule) which coincided with the natural day and light pattern. They were given a balanced bird feed and water ad libitum.

3.4.1 PROCEDURE:

Serum samples to be tested were injected intradermally in 0.1 ml volumes daily for 4 days over the crop sac at symmetrically opposite sides. The geometrical centre of the crop sac of each side, was permanently marked with a non-toxic dye to ensure the injection of the sample in the same area each day. 24 h after the last injection, each pigeon was killed and the entire crop sac removed. The left and right crop sac were excised and the responding area was identified. A similar protocol was employed for O-PRL-18, which was used as a reference standard.

Prolactin levels were determined by measuring the response of the crop sac biochemically (Turkington et al., 1965).

3.4.2 BIOCHEMICAL ANALYSIS:

The entire hemicrop sac of the pigeon given a particular dose of O-PRL was at first excised and weighed. Each hemicrop was stretched slightly, the mucosal layer of the stimulated areas scraped out and weighed.

The responding mucosa from the hemicrop sac given a known dose of O-PRL-18 was homogenized with alkaline water of pH 7.0-7.5. The homogenate was centrifuged at 2000 xg. The pellet was discarded and the supernatant saved. An aliquot was used for determination of protein (Lowry et al. 1951) and the remaining supernatant was subjected to a dropwise addition of 20% glacial acetic acid till a thick curd white precipitate resulted. The precipitate was then pelleted, washed with distilled H_2O and resuspended in 0.1 NaOH by boiling in a water bath for 15 mins. Protein was estimated in this suspension also. Casein is a major protein in the cells of the responding mucosa which precipitates in acidic media. Thus, the ratio of casein : total protein was plotted against known concentrations of O-PRL-18 injected. The serum prolactin bioactivity in unknown samples were read from this plot.

3.4.3 DETERMINATION OF TOTAL PROTEIN:

Total protein was estimated by the method of Lowry et al (1951) using Folin-Phenol reagent after alkaline copper treatment. The chemistry of estimation have two distinct steps which lead to the final colour with protein : (a) reaction with copper in alkali and (b) reduction of the phosphomolybdic-phosphotungstic reagent by the copper-treated protein.

3.4.3.1 REAGENTS

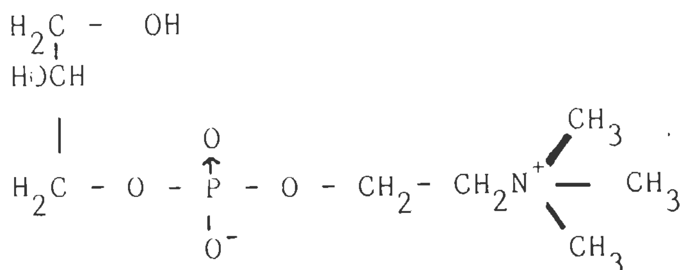
Protein standard		1 mg of Bovine serum albumin is dissolved in 1 ml of distilled water and diluted to 5 ml.
Reagent A	:	2% Na_2CO_3 in 0.1 N NaOH
Reagent B	.	0.5% $CuSO_4$. 5 H_2O in 1% sodium potassium tartrate.
Reagent C	:	Alkaline Copper Solution 50 ml (A) + 1 ml (B)
Reagent E	:	Dilute Folin-Ciocalteu reagent made to 1 N

3.4.3.2 PROCEDURE

0.2 ml of sample was taken and volume was made upto 1 ml with 0.1 N NaOH. To this, 1 ml of reagent C was added and mixed. It was allowed to stand for 10 minutes at room temperature. 0.1 ml of reagent E was added to the tube with immediate mixing. After 30 min. standing at room temperature, the intensity of colour was spectrophtometrically read at 690 nm on a Beckman DU-6 spectrophotometer.

3.5 ESTIMATION OF EPIDIDYMAL GLYCEROPHOSPHOCHOLINE:

Glycerophosphocholine (GPC) is often used as an accurate index to assess the functional status of the epididymis. The structure of GPC is as follows:



GPC is the only phosphate containing compound in the epididymis not bound to an anion exchange resin since

a) Choline and phosphate are present in the water effluent in a 1:1 molar ratio.

b) Choline phosphate and choline containing phospholipid which contain choline and phosphate in this ratio are not eluted by water.

c) Complete recovery of GPC from samples is achieved.

The method employed in the present studies is essentially that of Hammerstedt (1974). The principal advantage of this method is the speed of analysis and applicability to most biological extracts. In the present investigations, the resin Dowex-1-acetate used by Hammerstedt (1974) has been substituted by Amberlite-CG-400.

3.5.1 **PREPARATION OF AMBERLITE CG 400 'SEMI-MICRO' COLUMNS :**

Amberlite-CG-400 chloride was converted to acetate form by a sequential batch treatment with 1N NaOH followed by distilled water, 1 N acetic acid and once again distilled water. The final washing with distilled water was continued until the pH of the effluent was greater the 5. The

resin thus prepared, was packed into a 'semi-micro' column (0.4 x 4 cm).

3.5.2 PROCESSING OF SAMPLES:

The epididymal tissue was homogenized in a known volume of deionized water (30 mg/ml). The extract thus obtained, was centrifuged at 2000 x g and 0.2 ml of the supernatant was loaded on the Amberlite-CG-400 column just prepared. The column was flushed with distilled water.

1 ml fractions were collected and the molar concentration of glycerol and phosphorous determined. Trials performed on various tissue extracts showed that the molar ratio of glycerol : phosphorus was around 1.0 in the first 9 ml of the elute. Therefore, while routinely estimating the GPC in epididymal extracts the first 9 ml of the elute were pooled and the phosphorous levels determined. GPC was expressed as a direct measure of this phosphorus content.

3.5.3 ESTIMATION OF PHOSPHORUS:

Phosphorus was estimated by the method of Fiske and Subbarow (1925) as modified by Hammerstedt (1974). The organic phosphorus compounds are converted into orthophosphate by oxidation with perchloric acid, which forms a colour complex with molybdate and reducing reagent.

3.5.3.1 REAGENTS

1. Perchloric acid: 72%
2. Ammonium molybdate:0.0405 M in H_2O
3. Reducing reagent:
   Sodium bisulfite 1.15 M, sodium meta bisulfite 0.191 M and 0.081 M 1, 2,4, Amino napthol sulfonic acid in H_2O
   Stock standard : K H_2PO_4(1 μ mole/ml)

3.5.3.2 PROCEDURE

Suitable aliquots were evaporated to dryness in a sand bath and digested for 20 mins. with 0.8 ml perchloric acid. After the sample was cooled to room temperature, 6 ml of water, followed by 0.5 ml of ammonium molybdate reagent and 0.4 ml of reducing solution were added and mixed well. The solution was made to 10 ml with H_2O and once again mixed thoroughly. After 20 mins the optical density were read at 660 nm. KH_2PO_4 solution (1μmole/ml) was used as a standard.

3.5.4 ESTIMATION OF GLYCEROL:

Glycerol was estimated by the procedure of White (1959), adopted to a microlitre scale (Crabo, 1965). Glycerophosphocholine is determined as glycerol which is oxdised to formaldehyde by periodate and the excess periodate destroyed by arsenite. The formaldehyde forms a coloured complex with chromotropic acid which is estimated colorimetrically.

3.5.4. 1 REAGENTS

1. Barium hydroxide: 0.3 N p.a.
2. Zinc sulphate [Zn SO_4. 7 H_2O]: p.a. 5 g/100 ml
3. Sulfuric acid: 2 N
4. Potassium metaperiodate: 0.1 M p.a.
5. Sodium arsenite: p.a. 13.13 g/100 ml
6. Chromotropic acid reagent:- 1.08 g of salt of chromotropic acid in 100 ml distilled water + 450 ml, 65% (Vol/vol) sulphuric acid
7. Stock standard - 400 mg glycerol/100 ml.

3.5.4.2 PROCEDURE

To 10 µl of epididymal tissue extract were added 20 µl distilled water, 10 µl barium hydroxide solution and 10 µl zinc sulphate solution. The mixture was mechanically shaken for 10 secs. and centrifuged for one min. 20 microlitres of supernatant was transferred to a clean teflon tube and 20 µl sulphuric acid and 20 ul potassium periodate were added. After 10 secs. mixing, the tests were left for exactly 5 mins. where upon 20 µl sodium arsenite was added. 10 mins. thereafter 2.0 ml chromotropic acid was added to 40 µl of solution. The mixture was then placed in a boiling water bath for exactly 30 mins. Cooling of the solution was followed by colorimetric determination at a wavelength of 580 nm, against a blank consisting of 10 µl distilled water treated in the same way as the tests. The results were evaluated from a standard curve based on a number of dilu-

tions from the stock standard treated exactly as the unknown tests. The resulting value in millimoles glycerol per litre was multiplied by 2.

3.6 LIPID ANALYSIS:

3.6.1 EXTRACTION OF TOTAL LIPIDS

Total lipids were extracted as per the method of Folch et al (1957). The tissue was homogenized with 10 volumes of chloroform : methanol (2 : 1 v/v) containing 0.01 percent Butylated hydroxy toluene (BHT) as an antioxidant. The homogenate was centrifuged to obtain a clear extract. This extraction process was repeated thrice, the resulting supernatants were cooled and evaporated to complete dryness at 40-45°C under a stream of nitrogen.

3.6.2 BREAKING OF PROTEOLIPIDS

The residue was resuspended in 10 ml of chlorofom : methanol (2 : 1 v/v)containing 4 percent H_2O with 0.01 percent BHT to break up the proteolipids. The mixture was evaporated under nitrogen at 45°C once more. This process was repeated thrice.

3.6.3 REMOVAL OF GLYCOLIPIDS

The final residue was redissolved in 10 ml of chloroform : methanol (2 : 1 v /v) with 0.01 percent BHT and equilibrated with 0.9 percent NaCl (every 10 ml of chloroform; methanol is equilibrated with 2 ml of normal saline)

in a separating funnel. This was swirled gently and allowed to stand for 7-8 hours, in which time the organic and inorganic phases separate. The upper inorganic phase removes the glycolipids from the lower organic phase. On completion of a separation, the lower organic phase was collected and evaporated. The residue of the total lipid thus extracted was dissolved in 3 ml of chloroform and used almost immediately for further analysis.

3.6.4 ESTIMATION OF TOTAL LIPIDS

A classical gravimetric method of Kates (1972) was used to quantify total lipids. 0.5 ml of lipid extract was added to preweighed glass planchets. The planchets were then placed in a dessicator until a constant weight was obtained. The difference in weights represented the total lipids which was finally expressed as mg/g of tissue.

3.6.5 DETERMINATION OF TOTAL CHOLESTEROL

Total cholesterol was determined by the method of Hanel and Dam (1955). This is a rapid and sensitive method of estimation based on the reaction of acetic anhydride.

3.6.5.1 REAGENTS:

1. Chloroform
2. Zinc chlride reagent : 40 g of Zn Cl_2 dissolved in 153 ml of glacial acetic acid.
3. Acetyl chloride : 98 percent
4. Stock solution of cholesterol (100 µg/ml).

3.6.5.2 PROCEDURE:

Suitable aliquots of the lipid extract were taken and evaporated. The residue was dissolved in 2.0 ml of chloroform then 1.0 ml of zinc chloride reagent and 1.0 ml of acetyl chloride were added and the contents were mixed well. The tubes were shaken carefully (the reaction is exothermic). The tubes were kept in a water bath at 60°C for 10 mins. and the color formed was read spectrophotometrically at 528 nm against a reagent blank. Separate standards were run along with the samples on every occasion.

3.6.6 QUANTIFICATION OF TOTAL GLYCERIDE GLYCEROL

A spectrophotometric procedure was followed to estimate glyceride glycerol (van Handel and Zilversmit, 1957). The method is based on the amount of formaldehyde-colour complex formed. Saponification of glycerides and oxidation of glycerols releases formaldehyde which forms a color complex with chromotropic acid.

3.6.6.1 REAGENTS:

1. Potassium hydroxide : 0.1 N made in ethanol
2. Sulfuric acid: 0.4 N
3. Sodium metaperiodate: 0.05 M, freshly prepared.
4. Sodium sulfite : 20%, freshly prepared.

5. Chromotropic acid reagent : 0.5 gm of chromotropic acid was dissolved in 10 ml of water and 250 ml of 66% H_2SO_4 (v/v) was added. This reagent is stable and is stored in a brown bottle.
6. Standard Tripalmitin:stock solution (100 µg/ml).

3.6.6.2 PROCEDURE:

Suitable aliquots were evaporated to dryness and saponified with 0.5 ml of ethanolic KOH at 70°C for 20 mins. 0.2 ml of H_2SO_4 was added to liberate glycerol. The oxidation of liberated glycerol was brought about by adding 0.1 ml of freshly prepared sodium meta periodate. The tubes were left at room temperature for 10 mins. The reaction was arrested by adding 0.2 ml of freshly prepared sodium sulfite. Then, 8 ml of chromotropic acid reagent was added. The mixture was shaken and boiled in a boiling water bath for exactly 30 mins. The color developed was read at 570 nm on a Beckman DU-6 spectrophotometer. Tripalmitin was used as standard from which the glyceride glycerol in unknown samples were calculated.

3.6.7 ESTIMATION OF TOTAL PHOSPHOLIPID PHOSPHORUS

The original method of Bartlett (1959) as modified by Marinetti (1962) was used. This method is based on the principle that orthophosphoric acid forms a complex with molybdate and on reduction with reducing agent produces a blue color.

3.6.7.1 REAGENTS:

1. Perchloric acid (60%)
2. Ammonium molybolate (2.5 %)
3. Reducing reagent : 30 g of sodium bisulfite, 6g of sodium metabisulfite and 0.5 g of 1,2,4 Amino Napthol sulfonic acid (ANSA) was mixed thoroughly in a mortar and pestle dissolved in 250 ml of distilled water. It was then diluted 1 : 12 with distilled water. This was made to stand for 3 hours in the dark. The solution was stable for 6-8 weeks.
4. Stock standard (0.05 μmole) of potassium dihydrogen phosphate (KH_2PO_4).

3.6.7.2 PROCEDURE:

Appropriate aliquots of lipid sample were evaporated to dryness. To it was added 1 ml of perchloric acid and the contents of the tube digested in a sand bath (150-200°C) for 15 mins, till fumes subsided. After cooling at room temperature, 7 ml of distilled water was added followed by the addition of 0.5 ml of ammonium molybdate and 0.2 ml of reducing reagent. Contents of the tube were mixed well and placed in a boiling water bath exactly for 7 mins. The tubes were cooled to room temperature for 20 mins. and intensity of color was measured at 830 nm against a reagent blank. Standard KH_2PO_4 was also run concurrently and phosphorus

values obtained were multiplied by 25 to get phospholipid values (Beiri and Prival,1965).

3.6.8 SEPARATION OF NEUTRAL LIPIDS

The term neutral lipids apply to all lipids excluding those containing phosphorus and sugars in their molecules. For the separation of these neutral lipids, a system described by Mangold (1965) has been employed. This is a three step development procedure of adsorption chromatography on plates with silica gel layers. The lipids are separated according to the type and number of functional groups.

3.6.8.1 PREPARATION OF PLATES FOR THIN LAYER CHROMATOGRAPHY

0.25 - 0.275 mm thick silica gel G layers were prepared on glass plates of size 200 mm x 200 mm. To accomplish this, 30 gms of silica gel G (250 mesh) containing calcium sulfate as binder was slurried with 60 ml of distilled water. The slurry was transferred to the applicator as soon as possible and applied to the plates. The whole process was completed in 4-5 mins, since the slurry hardens quickly on account of calcium sulfate. The plates were then run in methanol to remove all contaminants.

3.6.8.2 APPLICATION OF SAMPLES:

The chromatoplates thus prepared were activated by heating in an oven at 110-115°C for 30-60 mins. On cooling, suitable amounts of lipid samples were applied in a row

with micropipettes from side to side, leaving a margin of 2 cms from the bottom edge.

3.6.8.3 DEVELOPMENT OF CHROMATOGRAM

A three step solvent system proposed by Mangold (1965) was used, for the separation of neutral lipids. The composition of solvents used were as follows :

Solvent System I : n-Hexane: Diethyl ether : glacial Acetic acid (60:40:1)

Solvent System II : n-Hexane : Diethyl ether : glacial Acetic acid (90 :10:1)

Solvent System III : n-Hexane : Diethyl ether : glacial Acetic acid (30:70:1)

After the samples were applied, the plates were run in solvent system I upto a height of 7 cms. The plates were then air dried and run in solvent system II upto a height of 15 cms. The plates were air dried once more and placed in an iodine chamber to identify the diacyl glycerol fraction. The solvent system III was now used and run upto a level just below the diacylglycerol fraction.

3.6.8.4 DETECTION OF SPOTS

The neutral lipids separated by the above mentioned procedure was identified by subjecting the plates to vapours of iodine and comparing them with authentic standards. The

spots obtained from the bottom of the plate upwards were identified to be monoacylglycerol, diacylglycerol, free cholesterol, triacylglycerol and esterified cholesterol.

3.6.8.5 ELUTION OF NEUTRAL LIPIDS

The boundary of each spot just identified was marked, scraped and collected into separate vials. 5 ml of cholo-roform was used to elute these lipids from the scrapings. The individual classes of lipids were quantified using sp-ectrophotometric procedures detailed earlier (glyceride gly-cerol, section: 3.6.6; Cholesterol; Section : 3.6.5)

3.6.9 SEPARATION OF PHOSPHOLIPIDS:

This was done by thin layer chromatography, a proce-dure outlined by Abramson and Blecher (1964). The method for the preparation of plates was the same as that descri-bed for neutral lipids. However, for the separation of phos-pho-lipids a solvent system of chloroform : methanol : 7N ammonia (115 : 45 : 7.5) was used. The sequence of separa-tion of phospholipids from the base upwards were phospha-tidyl inositol, phosphatidyl serine, sphingomyelin, phos-phatidyl choline, phosphatidyl ethanolamine and phospha-tidic acid.

3.6.9.1 ELUTION OF SPOTS

The individual classes of phospholipids were identified using iodine vapour and reference standards. Elution was done with a solvent mixture composed of chloroform : methanol : water: formic acid (97:97:4:2) and each class of phospholipid was further quantified by estimating phosphorus colorimetrically as mentioned in section: 3.6.7.

3.7 STATISTICAL ANALYSIS

The data were subjected to statistical analysis and expressed as Mean ± S.E.M. (Sandard error of mean). The S.E.M. was calculated by the following formula :

$$S.E.M. = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n(n-1)}}$$

where x = individual observations

n = number of observations

Students 't' test was used to calculate the degree of significance by the formula given below :

$$t = \frac{x_1 - x_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where

$$S = \sqrt{\frac{\sum x_1^2 - \frac{(\sum x_1)^2}{n_1} + \sum x_2^2 - \frac{(\sum x_2)^2}{n_2}}{n_1 + n_2 - 2}}$$

n_1 and n_2 denote the number of observations in the two classes being compared (Ostle 1954).

Based on the degree of freedom, value of probability was obtained from the standard table given by Fischer and Yates (1948). If the calculated value was more than the table value, it is significant at that probability level.

The following levels of significance were used. $p < 0.001$ to $p < 0.05$ for significant data and $p > 0.05$ for non-significant data.

4.0 RESULT AND DISCUSSION

The purpose of the present study was to investigate the role of prolactin(PRL) in the epididymis. The epididymal functions are to a large extent influenced by the presence of spermatozoa, luminal fluid, and circulating androgens (Abe *et al.*, 1983; Nicander *et al.*, 1983). In clinical conditions, hyperprolactinemia is often associated with reduced levels of androgens (Buvat *et al.*, 1985), which is not related to gonadotropins (Grosser and Robaire, 1987). Therefore, in order to simulate an identical condition, it was felt that the experiments should be conducted on castrated animals. Castration results in emptying of spermatozoa, eliminates the interference from testicular fluid and leads to a reduction in the levels of androgen available for action on the epididymis. Albino rats of wistar strain were chosen as the animal model because of easy availability, simple procedures involved in handling, high resistance to infection following surgery and also because of the abundant literature available that permit comparisons to be made.

4.1 IMPACT OF PROLACTIN ON THE WEIGHT OF ORGANS ASSOCIATED WITH THE MALE REPRODUCTIVE TRACT:

The influence of PRL and Bromocryptine on the accessory sex organs associated with male reproductive tract are presented in Table-1. PRL has a stimulatory influence on the weight of these organs in castrated animals. The

TABLE-1: THE INFLUENCE OF PROLACTIN(O-PRL) AND BROMOCRYPTINE ON THE REPRODUCTIVE TRACT OF CASTRATED MALE ALBINO RATS

Groups Treatment	Epididymal weight mg/100 g b.wt.	Seminal vesicle weight mg/100 g b.wt.	Ventral prostate weight mg/100 g b.wt.
GROUP-I(24) Castrated + vehicle	124.90±5.00	70.00±4.20	11.80±1.05
GROUP-II(25) Castrated +50 µg O-PRL/ 100 g b.wt.	134.90±8.00	78.00±6.00	15.00±1.23
GROUP-III (24) Castrated+100 µg O-PRL/ 100 g b.wt.	150.30±9.50*	102.00±7.70**	16.40±1.42**
GROUP-IV(25) Castrated+150µg O-PRL/ 100 g b.wt.	132.60±7.50	83.00±7.00	14.70±1.50
GROUP-V(25) Castrated+200 µg O-PRL/ 100 g b.wt.	128.10±9.60	80.00±5.00	14.50±1.60
GROUP-VI (20) Castrated + 0.3 mg Bromocryptine/100 g b.wt.	93.10±3.00**	56.00±2.00**	11.40±1.12

The number of animals taken are indicated in parenthesis against each group. Each value represents Mean ± SEM. Statistical analysis is done by students 't' test. All comparisons have been made with the castrated controls given vehicle

* $P < 0.05$, ** $P < 0.01$

effect on epididymis ($p < 0.05$), seminal vesicle ($p < 0.01$) and prostrate ($p < 0.01$) seems to be highly significant in the 100 μg of PRL treated group. Bromocryptine produces the opposite effect by reducing the weights of these organs.

The changes in weight have been used in the past as an index for ascertaining the response of the accessory sex glands to hormones. From this angle, the androgen dependency of the male reproductive tract has been well documented (Orgebin-Crist et al., 1975; Brooks, 1984). Further, the trophic action of prolactin in male sex accessory glands have been consistently reported (Grayhack, et al., 1954; Nicoll, 1974; Bartke, 1980; Fluckiger et al., 1982b). Changes in weight may result from either proliferation of cells or increase in synthetic and secretory activity of organs. Several investigators have shown that prolactin can potentiate the action of exogenous androgen on the growth of seminal vesicle and prostate in castrated animals (Thomas and Keenan, 1976). Earlier, Pasquilini (1953) reported, an increase in secretory activity of the seminal vesicle in castrated rats given Luteotrophic hormone (LTH) but primed with testosterone. Administration of prolactin alone in castrated animals is reported to cause a small but detectable increase in the weight of accessory reproductive glands (Bartke and Lloyd, 1970; Negrovilar et al., 1977). This low responsiveness in the absence of endogenous and exogenous testosterone is attributed to androgen dependency of prolactin binding in these tissues (Klezdik et al., 1976). In the present

studies too, prolactin seems to favour the increase in weight of accessory sex glands, which assumes significant proportions in the 100 μg O-PRL treated group. It appears that the response of the epididymis, seminal vesicle and prostate is dependent on an optimal dose of exogenous prolactin in the androgen deprived condition. It must be mentioned here that details of experimental design, dose and type of prolactin, duration of treatment and responsiveness of tissues are also important. The direct influence of prolactin is further confirmed by the reduction in weights observed in the bromocryptine group.

Thus, the preliminary studies on the weight provide important clues to the action of prolactin on the male reproductive tract. It now became essential to find out how long the exogenously injected prolactin remained in circulation and this, if in some way, influenced the circardian pattern in serum prolactin.

4.2 DIURNAL PATTERN IN SERUM PROLACTIN BIOACTIVITY

It is now known from literature, that immunologically active and biologically active prolactin are not quite the same (Forsyth, 1971). Since prolactin is a proteinaceous hormone, whose structure varies with the animal species in which they are found, separate procedures are generally evolved for dealing with heterologous prolactin. In most experimental studies, the origin of prolactin used for injections is from a species different from the animal used.

Thus, invariably a situation arises when heterologous prolactin is encountered. While immunological procedures are in common use for separately quantifying the molecular species of prolactin, one draws a blank as far as its biological activity is concerned. In experimental studies, where the response of organs to prolactin is of interest, it would be helpful if the biological activity of the circulating prolactin becomes known. Therefore, in the present investigation an attempt has been made to measure the biological activity of prolactin through the local 'micro' pigeon crop sac method.

The serum levels of prolactin as ascertained by pigeon crop sac bioassay using biochemical indices are represented in Figs.1 and 2. From Figure 2 it is apparent that there is a significant rise in prolactin titres 2 h after the last of a series of O-PRL injections given once daily for a period of 7 days. This rise in serum prolactin was more pronounced when low doses of O-PRL were injected. However, when higher doses of O-PRL were administered the amplitude of this surge was found to be low. Thus, it appears that the observed rise in serum prolactin is inversly related to the dose of exogenously administered O-PRL.

In intact male rats, serum prolactin levels have been reported to show a rythmic pattern, with levels reaching an all time high in the late afternoon (Clark and Baker, 1964; Koch et al., 1971). The present experiments were per-

FIG. 1 PIGEON CROP-SAC RESPONSE TO OVINE PROLACTIN (1mg = 30 I.U.) BY 'LOCAL MICRO' METHOD OF ASSAY USING THE COMMON PIGEON AS THE ASSAY ANIMAL

245707

PIGEON CROP SAC MUCOSAL RESPONSE

(CASEIN/PROTIEN RATIO)

60.0

50.0

40.0

30.0

20.0

10.0

0

0.934 $DOSE_1$

1.969 $DOSE_2$

3.733 $DOSE_3$

7.47 $DOSE_4$

O-PROLACTIN DOSES →

(μg)

FIG 2 -- EFFECT OF o-PRL ADMINISTRATION ON SERUM PROLACTIN BIOACTIVITY IN CASTRATED MALE RATS
(Data based on the biochemical response of pigeon crop sac mucosa)

formed on castrated rats and no such pattern was observed. The results are however, comparable to that of Hart (1973) who has also reported a steady basal level of prolactin throughout a 24 h period, in castrated male goats. Studies on male rats have revealed that serum prolactin levels drop right from day 1 post-castration (Goldman et al., 1971). Castration was found to reduce not only mean values but also pulse amplitude and pulse frequency of prolactin (Grosser and Robaire, 1987). Thus, the serum prolactin pattern observed for castrated animals in the present studies, are quite in order.

Earlier reports have proposed a very short half life for exogenously administered O-PRL in rats which is around 6-12 mins. (Grosvenor, 1967; Diamond et al., 1980). In the present experiments too, it appears that most of the injected O-PRL is removed from circulation within the first 15 mins. Because of the brief half life, it is possible that injection of O-PRL brings about only a transient rise in heterologous prolactin which could also suppress the release of endogenous prolactin. As a further support to this contention, it is interesting to recall the work of Damerast et al (1986) who have reported that a transient rise in heterologous prolactin suppresses the release of endogenous prolactin rather rapidly. This is reflected in our studies where serum levels of prolactin at 15 and 30 mins. are low.

It should be noted that the measurements made by bio-assay, reflect a heterologous prolactin in circulation. A significant observation in our results is the elevation in serum levels of prolactin, 2 h after the O-PRL injection. That this increase in prolactin at 2 h is due to O-PRL injection is confirmed by the fact that the castrated control rats where no prolactin was administered did not exhibit this pattern. Further, it seems most likely that the prolactin measured at 2 h is r-PRL since this peak appears to be inversely related to the dose of O-PRL employed.

It can also be inferred from the data that the rise in r-PRL at two hours may be due to a 'rebound' effect of the rat pituitary after a period of suppression brought about by O-PRL injections. In addition, it appears that at low doses this 'rebound' effect is a result of a quick removal of the suppression while at higher doses the suppressive action of exogenous O-PRL is long lasting.

The serum prolactin profiles that have been observed in the present study is not a sporadic or random event, but is a consequence of the dose related exogenous O-PRL administration. Evidence for this statement stems from the fact that the pattern is not a result of a single injection but is the outcome of a series of 7 injections of O-PRL administered at the same time, each day and therefore represents a stabilized picture of the newly acquired circulating prolactin circardian pattern.

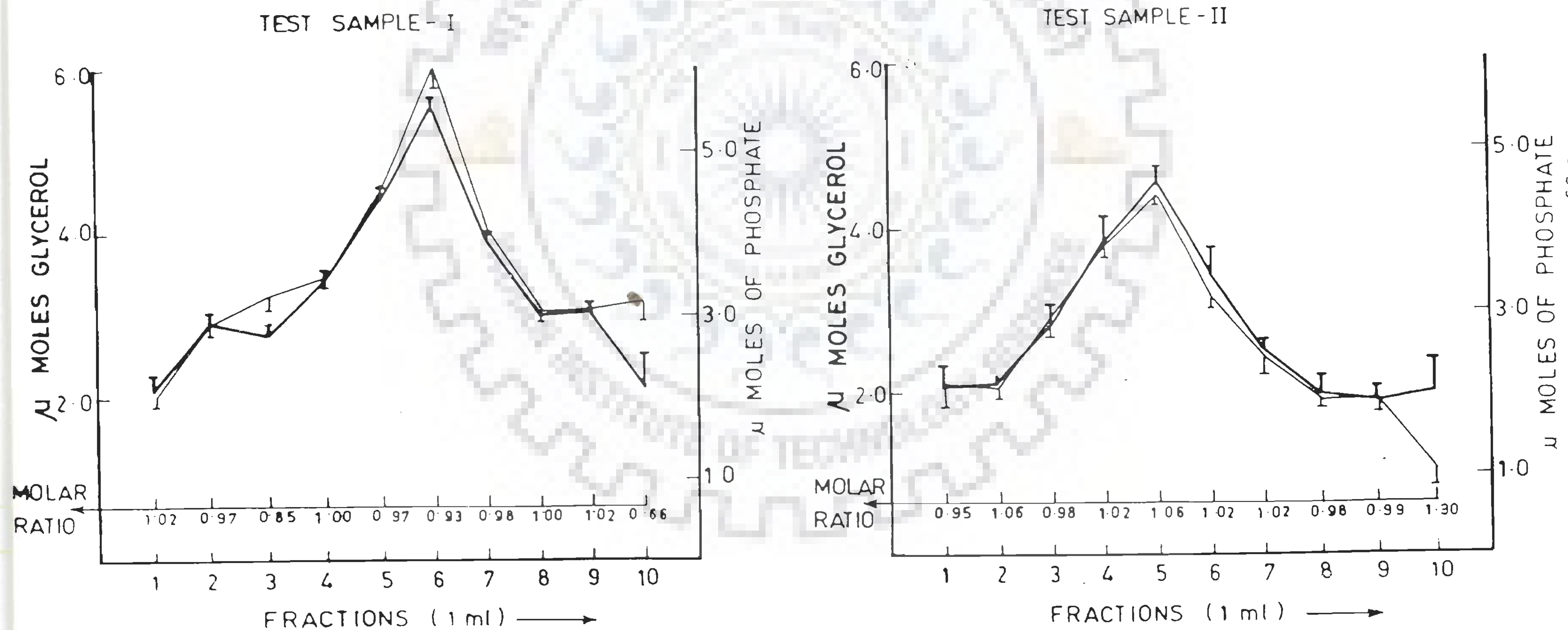
Thus, the profile of prolactin indicates that the exogenous O-PRL is not sustained in circulation for long and what appears as a spike at 2 h is a rebound of native prolactin. This rise is also not persistent and the animals try to reach a steady through inbuilt homeostatic mechanisms.

Because of the presence of binding sites, it is reasonable to expect that prolactin from circulation is captured by the epididymis. The response if any, would now be reflected in the glycerophosphocholine (GPC) level which is reported to be the best indicator of epididymal secretory activity.

4.3 INFLUENCE OF PROLACTIN ON GLYCEROPHOSPHOCHOLINE(GPC):

Of the several methods used to quantify GPC, the method proposed by Hammerstedt (1974) appears to be most suitable. This procedure eliminates the interference from other phosphate containing compounds, by passing samples through anion exchange resins. In the present studies, a similar approach was used, except that Amberlite-CG-400 was substituted for Dowex-1-acetate, other factors remaining the same. Trials were carried out initially with test samples and the elution pattern is presented in Fig.3. What eluted is infact GPC was confirmed by the equimolar concentrations of phosphate and glycerol obtained.

FIG. 3 RECOVERY OF GLYCERO PHOSPHOCHOLINE FROM EPIDIDYMAL TISSUE EXTRACTS USING AMBERLITE CG-400 'SEMI MICRO COLUMNS'



It is relevant to mention that as per the experimental design, animals belonging to each group were killed at 15 mins, 30 mins, 2 h , 6 h and 24 h, after the last of 7 injections. Even though the animals belonging to each group were killed at different times, the changes in the level of GPC in a particular epididymal segment was not satistically significant (Table-2). The absence of sperm in the epididymis of animals chosen in the present studies, may be the reason for the consistent values. Hence, the data obtained from animals belonging to the same group are pooled and the resulting picture represented in Fig.4.

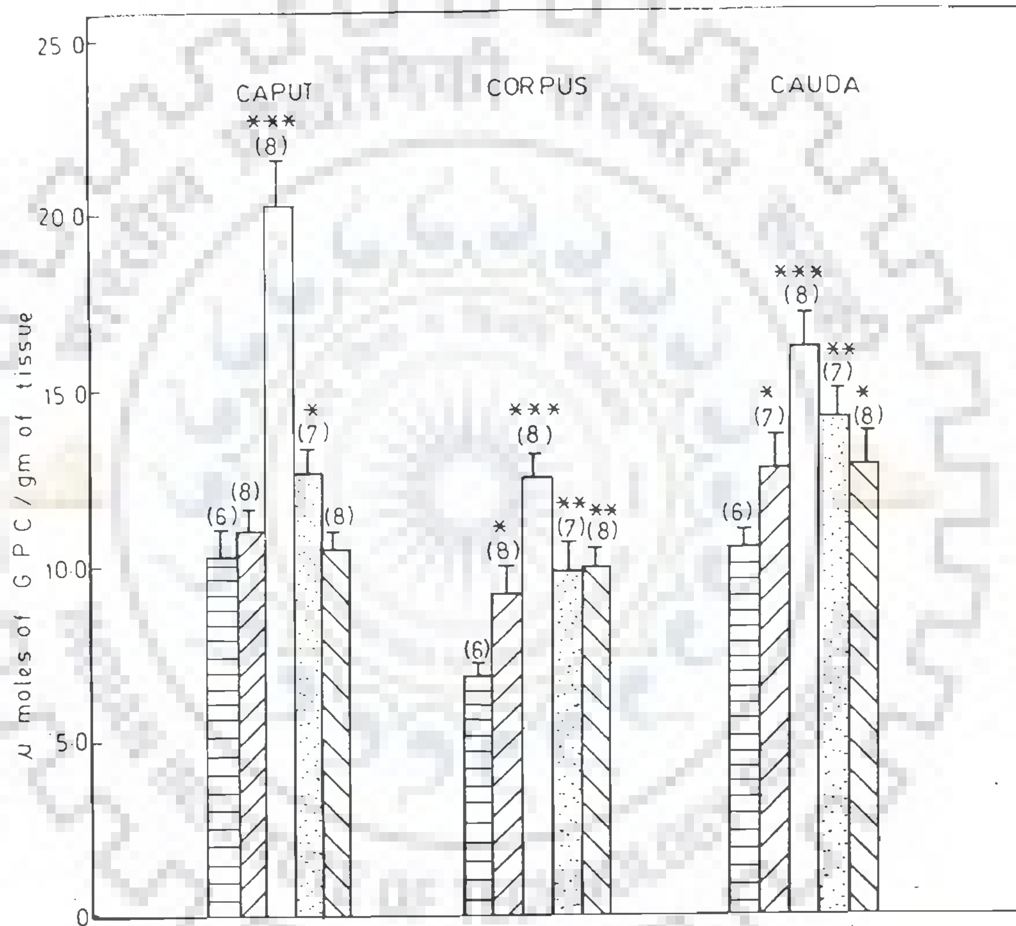
From the results, it is seen that the caput and caudal segments have higher levels of GPC than the corpus. GPC level in the epididymis is androgen dependent (Dawson and Rowlands 1959; Brooks *et al*., 1974) and the distribution that is seen in castrated control animals reflect a pattern caused by androgen deprivation. What is of more concern, is the action of prolactin. Although GPC levels are appreciably elevated in the 100 μg O-PRL treated group (caput $p < 0.001$, corpus $p < 0.001$, cauda $p < 0.001$) when compared to castrated controls, an increase in epididymal GPC is witnessed at all other doses of O-PRL as well. This action of prolactin in the epididymis is not unexpected, since PRL binding sites have been demonstrated in this organ. The stimulation in synthetic and secretory activity of prolactin on other male accessory organs like seminal vesicles

TABLE-2 : THE DISTRIBUTION OF GPC IN THE EPIDIDYMAL SEGMENTS AS PER EXPERIMENTAL DESIGN

TREATMENT	Time at which animal was killed				
	15 mins	30 mins	2 h	6 h	24 h
Caput epididymidis :					
GROUP-I: Castrated +vehicle (6)	10.52±0.64	9.84±0.54	10.37±0.84	10.68±0.63	9.92±0.69
GROUP-II: Castrated+50μgO-PRL/100 g b.wt.(8)	10.86±0.77	11.03±0.84	11.05±0.94	11.02±0.84	11.04±0.91
GROUP-III:Castrated+100μgO-PRL/100 g b.wt.(8)	20.26±1.24	20.66±1.43	19.27±1.20	20.94±1.52	20.04±1.25
GROUP-IV:Castrated+150μgO-PRL/100 g b.wt.(7)	12.65±1.01	12.80±0.99	13.08±1.07	12.49±1.01	12.61±1.00
GROUP-V:Castrated+200 μgO-PRL/100 g b.wt.(8)	10.25±0.68	10.67±0.83	10.48±0.85	10.54±0.99	10.39±0.85
Corpus epididymidis:					
GROUP-I:Castrated +vehicle (6)	7.00±0.58	6.74±0.49	6.87±0.50	6.92±0.58	7.00±0.84
GROUP-II:Castrated+50 μgO-PRL/100 g b.wt(8)	9.26±0.94	9.28±0.72	8.90±0.68	9.00±0.70	9.01±0.72
GROUP-III:Castrated+100 μgO-PRL/100 g b.wt.(8)	12.27±1.01	12.40±1.06	12.30±1.20	12.48±1.30	12.28±1.01
GROUP-IV:Castrated+150μgO-PRL/100 g b.wt.(7)	9.82±0.94	9.90±1.00	10.00±1.10	9.90±1.00	9.79±1.00
GROUP-V:Castrated+200 μgO-PRL/100 g b.wt.(8)	9.90±0.99	9.85±1.00	10.01±1.20	10.00±1.10	9.74±1.00
Cauda epididymidis:					
GROUP-I: Castrated+vehicle (6)	10.46±0.78	10.40±0.99	10.36±0.84	10.40±0.70	10.20±0.88
GROUP-II:Castrated+50μgO-PRL/100g b.wt.(8)	12.86±0.99	12.79±1.01	12.90±1.00	12.78±0.89	12.89±1.01
GROUP-III:Castrated+100 μgO-PRL/100 g b.wt.(8)	16.00±1.00	16.09±1.01	16.08±1.00	15.99±0.99	16.10±1.40
GROUP-IV: Castrated+150 μgO-PRL/100 g b.wt.(7)	14.50±0.84	14.39±0.90	14.58±1.00	14.45±0.98	14.57±0.86
GROUP-V:Castrated+200 μgO-PRL/100 g b.wt. (8)	12.90±0.97	12.70±0.99	12.96±0.94	12.88±0.86	12.90±0.98

Statistical analysis by students 't' test reveal that GPC levels in a particular epididymal segment were not very different for the same group irrespective of the time of killing.

FIG. 4 EFFECT OF PROLACTIN (O-PRL) ON EPIDIDYMAL GLYCEROPHOSPHOCHOLINE (GPC) IN CASTRATED RATS



Adult rats (15 days post-castrated) were treated as follows: Vehicle; 50 ug o-PRL/100 g.b.wt.; 100 ug o-PRL/100 g.b.wt.; 150 µg o-PRL/100 g.b.wt.; 200 ug o-PRL/100 g.b.wt. The treatment was in the form of daily injections for a period of 7 days. The results represent mean ± SEM. The number of animals included in each group are given within parenthesis. Statistical analysis of data was done using students 't' test and comparisons made with vehicle treated controls. The levels of significance indicated are * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

and ventral prostate(Golder *et al*., 1972; Thomas and Keenan, 1976) which have receptors for this hormone has been shown (Aragona and Friesen, 1975; Barkey *et al*, 1979). Therefore, it is possible that prolactin has a similar direct action on the epididymis. The epididymal GPC is known to be influenced by the presence of spermatozoa and flow of luminal fluid (Scott *et al*., 1963b). In the present case, the interference from these factors is ruled out because the experiments were done on castrated animals.

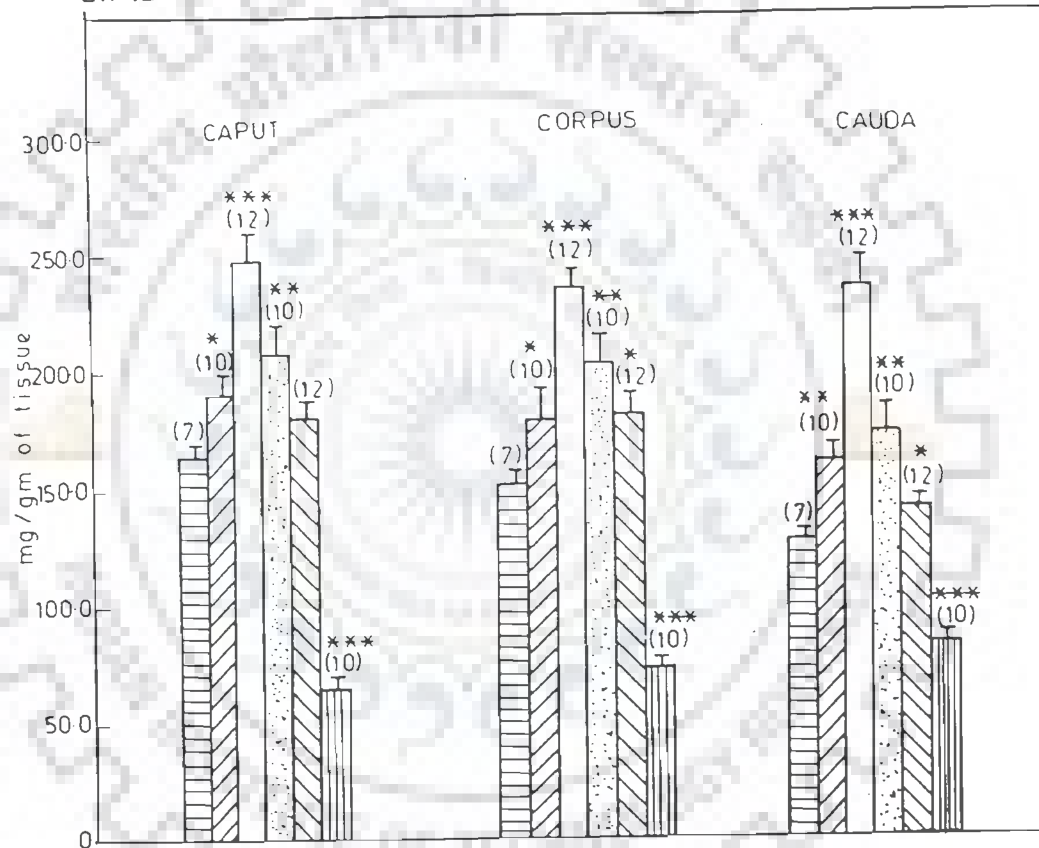
Segmentwise variation in epididymal GPC are also noticed and the caput is seen as being most responsive. It is relevant to recall that the epithelial cells from the caput, contain more GPC than from corpus and cauda (Hoffman and Killian, 1981). Moreover, among the various cells isolated by elutriation from rat caput epididymidis, the principal cells were the richest source of GPC (Killian and Chapman, 1980). The distribution of binding sites for prolactin along the epididymal duct seem also important, data concerning which is not available in literature, more so, in the castrated conditio n. Thus, the distribution of both the receptors for prolactin and cell types that comprise the epididymal epithelium may have a consolidated effect on the regional differences in the amplitude of response observed.

4.4 EFFECT OF PROLACTIN ON EPIDIDYMAL LIPID PROFILES:

Since lipids are known to profoundly influence the events that take place in the epididymis, it was of interest to study the lipid profiles and investigate how prolactin regulates their metabolism. Studies on the regulation of lipid metabolism are usually limited to the determination of the incorporation of radioactively labelled substrates, or to measurement of the activity of enzymes of the individual steps of the pathway. The latter is generally performed under conditions which are optimal in vitro, but do not necessarily reflect the situation in vivo. This is primarily because the enzymes associated with lipid metabolism are highly unstable. The determination of the rate of incorporation of labelled precursors is not free of risks. It presumes homogeneous labelling of biological specimens and the pool sizes of the intermediates are supposed to remain constant during the experimental period, which is not true, in most cases. For these reasons, it has been recommended that the determination of the pool sizes of various lipid classes would be a method of choice to monitor the pathways that are operational in vivo(Tijburg et al., 1989). Therefore, this approach has been followed in the present study.

The effect of prolactin and bromocryptine on the total lipids in the epididymal segments are shown in Fig.5. In the caput, prolactin increased the total lipids which

FIG. 5 EFFECT OF PROLACTIN (O-PRL) AND BROMOCRYPTINE ON TOTAL LIPIDS IN THE EPIDIDYMAL SEGMENTS OF CASTRATED RATS

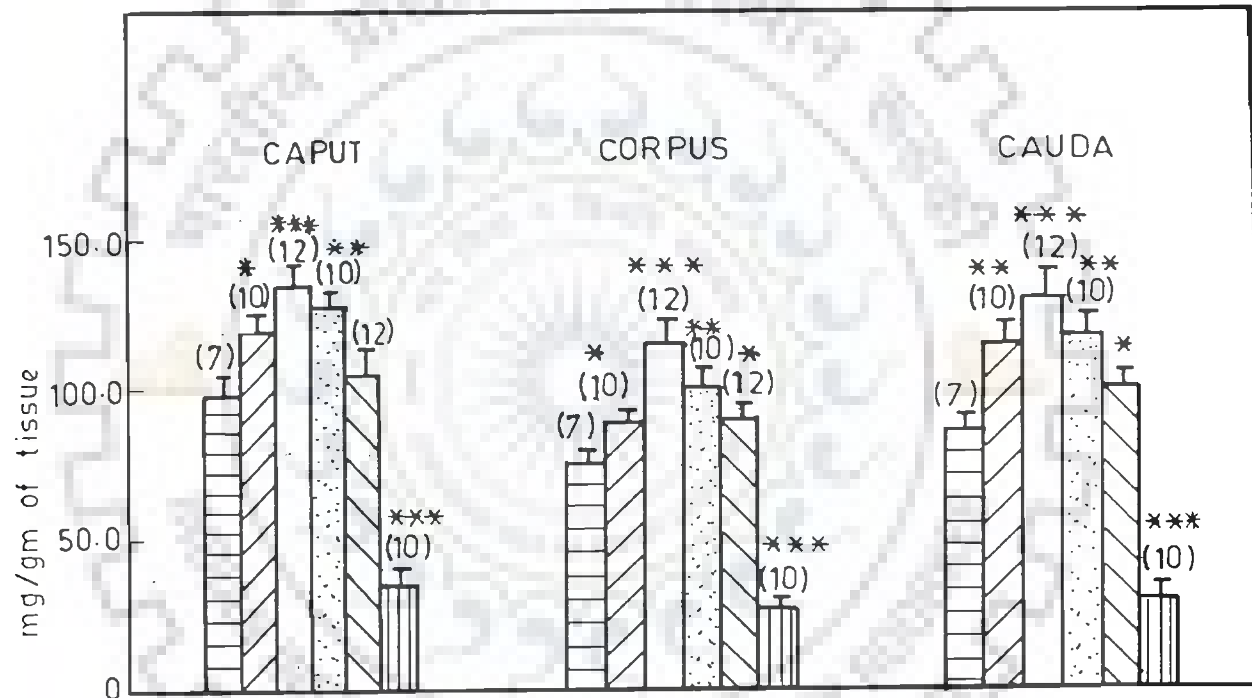


Adult rats (15 days post castrated) were treated as follows: ▤ Vehicle; ▨ 50ug o-PRL/100 g.b.wt.; □ 100 ug o-PRL/100 g.b.wt; ▩ 150µg o-PRL/100 g.b.wt.; ▧ 200 ug o-PRL/100 g.b.wt.; ▥ 0.3 mg bromocryptine/100g.b.wt. The treatment was in the form of daily injections for a period of 7 days. The results represent mean ± S.E.M. The number of animals included in each group are given within parenthesis. Statistical analysis of data was done using students 't' test and comparisons made with vehicle treated controls. The levels of significance indicated are $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

were statistically significant. In other epididymal segments too a similar increase was observed. Bromocryptine, on the other hand, produced opposite effects by substantially reducing the total lipids in the epididymal segments to a value much below the castrated level. These changes are the net outcome, more on account of alterations in total glyceride glycerol (Fig.6), than the total cholesterol (Fig.7) and total phospholipids (Fig.8). The increase in total cholesterol and total phospholipids was statistically significant only in the 100 µg O-PRL treated group all along the duct. The bromocryptine treatment brought about a drastic decrease in all the lipids quantified, irrespective of the epididymal region.

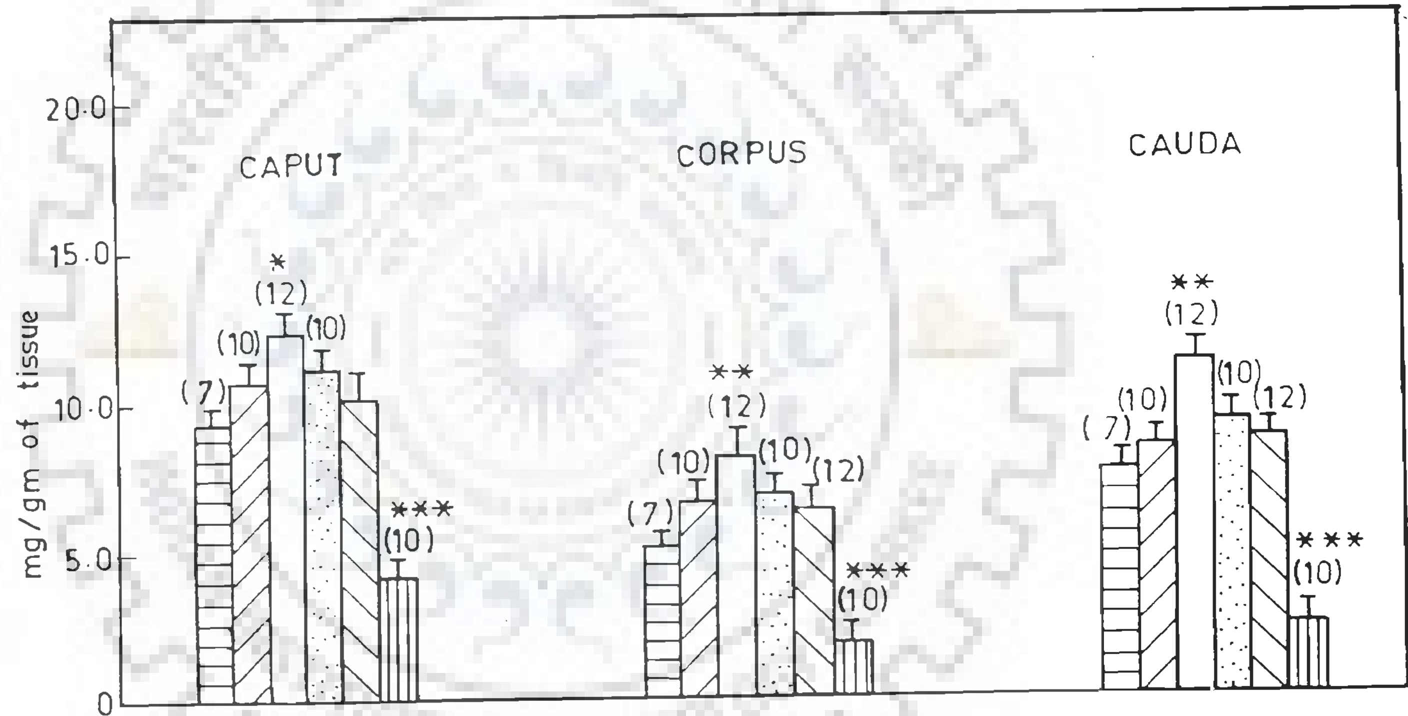
In most tissues where receptors for prolactin have been found, lipids have tended to accumulate when exposed to prolactin (Nicoll and Bern,1971). This holds good for male reproductive tissues like the testis (Sheriff and Govindarajulu, 1976), prostate (Umapathy et al., 1979) and seminal vesicle (Umapathy et al., 1979). In the present studies too a similar situation is encountered for epididymal lipids. Under normal conditions the epididymis harbours spermatozoa and the lipid profiles become more difficult to analyse because of the contribution from testicular fluid as well. One must keep in mind that the present experiments were conducted on castrated animals which means there is no contribution of spermatozoa and testicular fluid to the lipid pools that have been quantified. It appears, there-

FIG. 6 EFFECT OF PROLACTIN (O-PRL) AND BROMOCRYPTINE ON TOTAL GLYCERIDE GLYCEROL IN EPIDIDYMAL SEGMENTS OF CASTRATED RATS



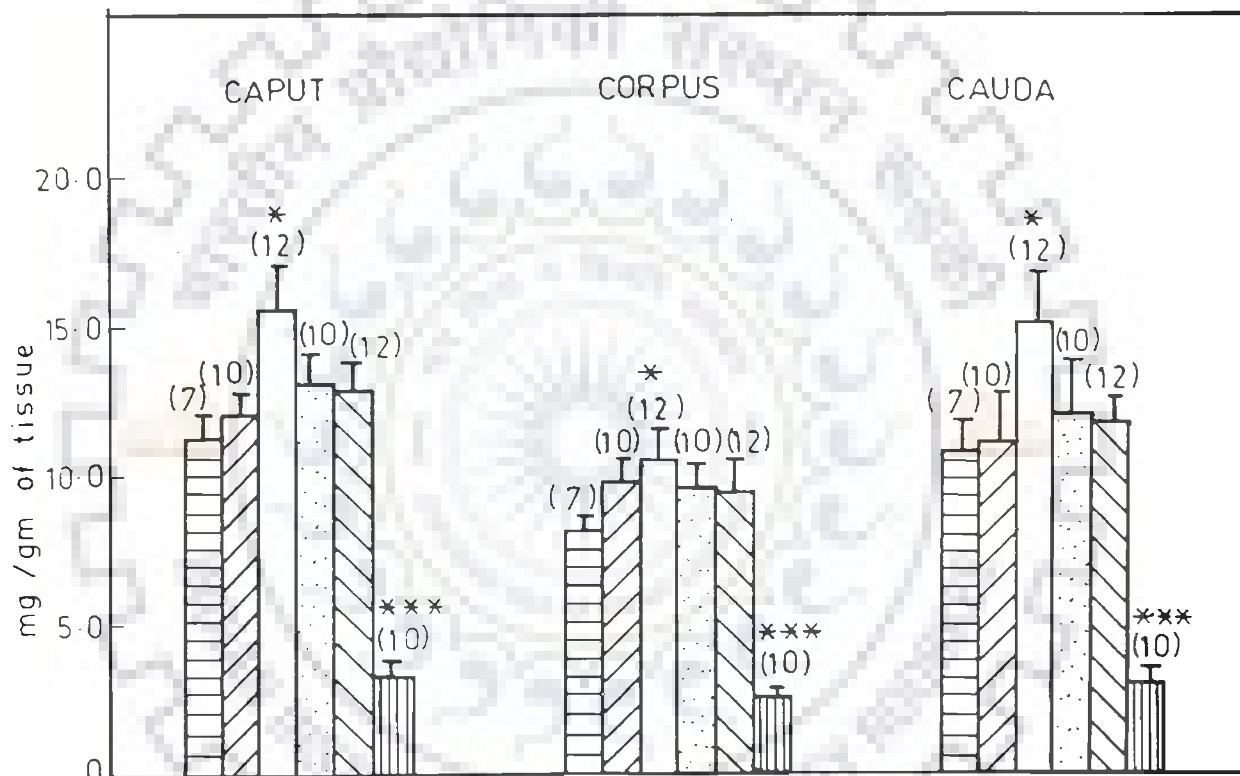
Adult rats (15 days post castrated) were treated as follows: Vehicle; , 50ug o-PRL/100 g.b.wt.; 100 ug o-PRL/100 g.b.wt; 150μg o-PRL/100 g.b.wt.; 200 ug o-PRL/100 g.b.wt.; 0.3 mg bromocryptine/100g.b.wt. The treatment was in the form of daily injections for a period of 7 days. The results represent mean ± S.E.M. The number of animals included in each group are given within parenthesis. Statistical analysis of data was done using students 't' test and comparisons made with vehicle treated controls. The levels of significance indicated are $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

FIG. 7 EFFECT OF PROLACTIN (O-PRL) AND BROMOCRYPTINE ON TOTAL CHOLESTEROL IN EPIDIDYMAL SEGMENTS OF CASTRATED RATS



Adult rats (15 days post castrated) were treated as follows: Vehicle; , 50ug o-PRL/100 g.b.wt.; 100 ug o-PRL/100 g.b.wt, 150μg o-PRL/100 g.b.wt.; 200 ug o-PRL/100 g.b.wt.; 0.3 mg bromocryptine/ 100g.b.wt. The treatment was in the form of daily injections for a period of 7 days. The results represent mean ± S.E.M. The number of animals included in each group are given within parenthesis. Statistical analysis of data was done using students 't' test and comparisons made with vehicle treated controls. The levels of significance indicated are *p <0.05; ** p < [illegible]; ***p < 0.001.

FIG. 8 EFFECT OF PROLACTIN (O-PRL) AND BROMOCRYPTINE ON TOTAL PHOSPHOLIPIDS IN EPIDIDYMAL SEGMENTS OF CASTRATED RATS



Adult rats (15 days post castrated) were treated as follows: Vehicle; 50ug o-PRL/100 g.b.wt.; 100 ug o-PRL/100 g.b.wt; 150µg o-PRL/100 g.b.wt.; 200 ug o-PRL/100 g.b.wt.; 0.3 mg bromocryptine/100g.b.wt. The treatment was in the form of daily injections for a period of 7 days. The results represent mean ± S.E.M. The number of animals included in each group are given within parenthesis. Statistical analysis of data was done using students 't' test and comparisons made with vehicle treated controls. The levels of significance indicated are *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

fore, that the increase in lipids observed has been brought about by the direct action of prolactin on the epididymis. That prolactin has a direct role to play is further confirmed by the depletion in lipids observed in the epididymis of animals given bromocryptine.

The changes in the neutral lipids of the caput epididymis following prolactin and bromocryptine treatment in castrated rats are presented in Table-3. A significant feature is the rise in the level of triacylglycerols and esterified cholesterol in the 50 μg ($p < 0.05$), 100 μg ($p < 0.001$) and 150 μg ($p < 0.01$) O-PRL treated groups. A concomitant decrease in the level of diacylglycerol and free cholesterol was observed which was prominent in the 100 μg treated group ($p < 0.05$). Bromocryptine brought about a reduction in all the neutral lipid ($p < 0.001$). The neutral lipid profile in the corpus and cauda epididymidis of castrated rats given O-PRL and bromocryptine treatment are depicted in Tables 4 and 5. A similar pattern was seen in these two epididymal segments. Triacylglycerols were found to accumulate in the 50 μg ($p < 0.01$), 100 μg ($p < 0.001$), 150 μg ($p < 0.01$) and 200 μg ($p < 0.05$) O-PRL treated groups. Once again, diacylglycerols were found to decrease which is obvious in the group given 100 μg of O-PRL. A striking feature is the accumulation of cholesterol in the esterified form. All neutral lipid kept to a low profile when given bromocryptine treatment when compared to the castrated controls ($p < 0.001$).

TABLE-3 : THE INFLUENCE OF PROLACTIN(O-PRL) AND BROMOCRYPTINE ON THE NEUTRAL LIPID PROFILE OF CAPUT EPIDIDYMIDIS

Groups Treatment	Total Glyceride Glycerol (mg/gm of tissue)	Monoacyl Glycerol (mg/gm of tissue)	Diacyl Glycerol (mg/gm of tissue)	Triacyl Glycerol (mg/gm of tissue)	Total Cholesterol (mg/gm of tissue)	Free Cholesterol (mg/gm of tissue)	Esterified cholesterol (mg/gm of tissue)
Group-I(7) Castrated+vehicle	98.44±5.80	29.01±1.78	26.42±1.78	33.00±2.80	9.21±0.60	1.03±0.14	5.96±0.34
Group-II(10) Castrated+50 µg O-PRL /100g b.wt.	120.35±6.0*	28.23±1.48	25.32±1.67	43.00±3.40*	10.56±0.76	0.92±0.09	7.60±0.64*
Group-III(12) Castrated+100 µg O-PRL/100g b.wt.	134.73±6.60***	27.14±1.64	20.75±1.28*	72.81±4.13***	12.28±0.88*	0.71±0.04*	10.11±0.88***
Group-IV(10) Castrated+150µg O-PRL/100 g b.wt.	128.64±6.44**	28.07±1.55	23.48±1.48	49.57±3.88**	11.00±0.80	0.94±0.08	8.55±0.76**
Group-V(12) Castrated 200µg O-PRL/100 g b.wt.	108.00±5.68	28.56±1.40	24.30±1.54	38.38±3.02	9.99±0.75	0.98±0.085	6.8±0.62
Group-VI(10) Castrated 0.3 mg Bromocryptine/100 g b.wt.	34.60±3.66***	10.90±1.07***	5.19±0.91***	17.39±1.50***	4.16±0.30***	0.44±0.03***	2.08±0.21***

The results represent mean ± S.E.M. The number of animals included are indicated against each group within parenthesis. Statistical analysis of data was done using students 't' test and comparisons are done with vehicle treated controls. The levels of signifiance indicated are:

*p < 0.05, ** P < 0.01, ***p < 0.001

TABLE- 4 : THE INFLUENCE OF PROLACTIN(O-PRL) AND BROMOCRYPTINE ON THE NEUTRAL LIPID PROFILE OF CORPUS EPIDIDYMIDIS

Groups Treatment	Total Glyceride Glycerol (mg/gm of tissue)	Monoacyl Glycerol (mg/gm of tissue)	Diacyl Glycerol (mg/gm of tissue)	Triacyl Glycerol (mg/gm of tissue)	Total cholesterol (mg/gm of tissue)	Free cholesterol (mg/gm of tissue)	Esterified cholesterol (mg/gm of tissue)
Group-I(7) Castrated ± vehicle	76.90±3.43	17.28±1.23	22.57±1.27	26.26±1.37	5.03±0.50	1.15±0.08	2.30±0.40
Group-II(10) Castrated+50µg O-PRL/100 g b.wt.	89.34±3.97*	16.28±0.85	20.11±1.22	30.55±1.44*	6.64±0.67	1.01±0.07	4.96±0.87*
Group-III(12) Castrated+100µg O-PRL/100 g.b.wt.	115.00±5.83***	15.00±0.62	18.71±1.25*	57.14±2.25***	8.00±0.86**	0.92±0.06*	6.68±0.93***
Group-IV (10) Castrated+150µg O-PRL/100 g b.wt.	100.95±5.36**	16.31±0.70	21.54±1.29	35.67±2.05**	6.69±0.78	0.97±0.06	5.65±0.91**
Group-V(12) Castrated+200µg O-PRL/100 g b.wt.	90.02±4.47*	17.09±1.21	21.78±0.86	31.69±1.99*	6.48±0.74	1.03±0.08	5.41±0.86*
Group-VI(10) Castrated+0.3mg Bromocryptine/ 100g b.wt.	26.67±1.87***	7.45±0.78***	4.67±0.48***	10.00±0.98***	1.83±0.32***	0.41±0.04***	0.82±0.16***

The results represent mean ± S.E.M. The number of animals included are indicated against each group within parenthesis. Statistical analysis of data was done using students 't' test and comparisons are done with vehicle treated controls. The levels of significance indicated are:

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE- 5 : THE INFLUENCE OF PROLACTIN(O-PRL) AND BROMOCRYPTINE ON THE NEUTRAL LIPID PROFILE OF CAUDA EPIDIDYMIDIS

Groups Treatment	Total Glyceride Glycerol (mg/gm of tissue)	Monoacyl Glycerol (mg/gm of tissue)	Diacyl Glycerol (mg/gm of tissue)	Triacyl Glycerol (mg/gm of tissue)	Total cholesterol (mg/gm of tissue)	Free Cholesterol (mg/gm of tissue)	Esterified cholesterol (mg/gm of tissue)
Group-I(7) Castrated + vehicle	86.67±4.09	26.00±1.44	22.00±08	30.50±1.64	7.47±0.41	0.87±0.18	3.02±0.30
Group-II(10) Castrated+50µg O-PRL/100 g b.wt.	115.03±7.93**	25.68±1.45	20.07±1.09	38.60±1.74**	8.25±0.50	0.79±0.09	4.10±0.37*
Group-III(12) Castrated+100µg O-PRL/100 g b.wt.	130.06±8.96***	25.06±1.33	19.06±0.84*	68.30±3.34***	11.18±0.97**	0.43±0.05*	6.89±0.53***
Group-IV (10) Castrated+150µg O-PRL/100 g b.wt.	118.94±8.08**	24.64±1.67	20.83±0.88	39.47±1.89**	9.06±0.80	0.70±0.09	4.92±0.42**
Group-V(12) Castrated-200µg O-PRL/100 g b.wt.	100.74±6.50*	25.00±1.71	21.08±1.02	36.42±1.42*	8.92±0.62	0.77±0.09	4.30±0.45*
Group-VI(10) Castrated+0.3mg Bromocryptine/ 100g b.wt.	33.98±3.62***	10.11±0.68***	4.77±0.35***	17.74±1.68***	2.33±0.84***	0.10±0.02***	1.25±0.14***

The results represent mean ± S.E.M. The number of animals included are indicated against each group within parenthesis. Statistical analysis of data was done using students 't' and comparisons are done with vehicle treated controls. The levels of significance indicated are:

$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

The glyceride glycerols are potential sources of energy and thus their function is predominantly metabolic (Tijburg *et al*., 1989). Monoacylglycerols are readily acylated to diacylglycerols and to a large extent considered to be unitary molecules for diacylglycerol and triacylglycerol synthesis (Thompson and McDonald 1978). Diacylglycerols hold a key position in the synthesis of triacylglycerols and phospholipids. In addition, they also play a role in cell signalling (Exton, 1990). Energy derived from metabolism is stored mainly in the form of triacylglycerols in tissues, which are mobilized when need arises (Numa and Yamashita, 1974; O'Doherty, 1978). With this as the background, if we take a look at the influence of prolactin on the epididymal neutral lipid profiles, a clear picture emerges. It appears that prolactin favours storage of lipids since an increase in the pool size of triacylglycerols is observed. The enzyme involved in the conversion of diacylglycerol to triacylglycerol is reported to be active in the epididymis (Brooks, 1981a). It is possible that prolactin stimulates this enzyme. Further support to this view, could be obtained from the bromocryptine treated group where this pathway seems to be inhibited. Had prolactin been injected in intact rats, the energy requirements of spermatozoa present within the epididymis may have led to the depletion of triacylglycerol stores and hence the effect of prolactin would have been shadowed. Since the experiments were

done using castrated rats, it may be inferred that the energy derived through metabolism is channelled to storage by the action of prolactin.

Cholesterol is a parent molecule from which all other steroids are derived. The synthesis of cholesterol from acetate (Hamilton and Fawcett, 1970) and subsequently androgens from cholesterol (Hamilton and Fawcett, 1970; Hamilton, 1971) have been reported in the rat epididymis. Cholesterol when not utilized is often stored in the esterified form (Brown and Goldstein, 1976). The present studies indicate that prolactin facilitates the accumulation of esterified cholesterol. Such an event has been reported in other male reproductive tissues (Bartke, 1976). Reports also show a decrease in the level of testosterone not only at the site of production (Fang *et al.*, 1974), but also in circulation (Fang *et al.*, 1974), when prolactin levels are increased artifically. Laboratory assessment of pathological conditions reveal that hyperprolactinemia is associated with low serum testosterone. If these reports could be taken as evidence, then it is tempting to speculate that prolactin does not promote the synthesis of at least androgens. Thus, in the present experiments, the cholesterol resulting from non-utilization tends to build up and is therefore perhaps converted to the esterified form.

The influence of prolactin and bromocryptine on the individual subclasses of phospholipids in the epididymis of castrated rats are shown in Tables 6,7 and 8. A quick glance at these three tables would reveal that all regions of the epididymis respond to the prolactin treatment alike. However, only the group given 100 µg O-PRL treatment produced results that were statistically significant. A deeper look would also indicate the existence of a common pattern. While there was increase in phosphatidyl inositol, phosphatidyl choline and phosphatidyl ethanolamine, a reduction in the pool size of phosphatidyl serine, sphingomyelin and phosphatidic acid was observed. In the bromocryptine treated group, all the subclasses of phospholipids were uniformly lowered ($p < 0.001$).

The possible avenues used by cells in synthesizing and degrading individual classes of lipids are consolidated and presented in Figure 9. The phospholipids in particular have a continuous turnover and their metabolic flux is often found to vary with the physiological activity of the organs in which they are found (Thompson, 1973).

From the results obtained in the present study, it appears that PRL favours the accumulation of phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl inositol by promoting metabolism through specific pathways. Evidence for this, comes from the very similar pattern obtained in all the three segments of the epididymis. Two meta-

TABLE 6 : THE IMPACT OF PROLACTIN(O-PRL) AND BROMOCRYPTINE ON THE PHOSPHOLIPID PROFILE OF CAPUT EPIDIDYMIDIS

Groups Treatment	Total Phospholipid (mg/gm of tissue)	Phosphatidyl inositol (mg/gm of (tissue)	Phosphatidyl serine (mg/gm of tissue)	Sphingomyelin (mg/gm of tissue)	Phosphatidyl choline (mg/gm of tissue)	Phosphatidyl ethanolamine (mg/gm of tissue)	Phosphatidic acid (mg/gm of tissue)
Group-I(7) Castrated+vehicle	11.32±0.73	0.44±0.03	0.52±0.04	1.10±0.09	3.48±0.17	1.66±0.09	2.32±0.16
Group-II(10) Castrated+50µg O-PRL/100 g b.wt.	12.13±0.72	0.51±0.04	0.45±0.03	1.02±0.08	4.00±0.20	1.70±0.08	1.98±0.11
Group-III(12) Castrated+100µg O-PRL/100 g b.wt.	15.50±1.57*	0.92±0.08***	0.34±0.03**	0.78±0.05**	6.06±0.48***	2.88±0.23***	1.50±0.07***
Group-IV(10) Castrated+150µg O-PRL/100 g b.wt.	13.00±1.02	0.54±0.04	0.48±0.04	1.04±0.08	3.89±0.18	1.76±0.08	2.00±0.12
Group-V(12) Castrated 200µg O-PRL/100 g b.wt.	12.84±1.06	0.56±0.05	0.49±0.04	1.06±0.09	3.60±0.16	1.71±0.07	2.10±0.13
Group-VI(10) Castrated+0.3mg Bromocryptine/ 100 g b.wt.	3.32±0.25***	0.10±0.011***	0.16±0.03***	0.22±0.02***	1.00±0.09***	0.32±0.03***	0.63±0.05***

The results represent mean±S.E.M. The number of animals included are indicated against each group within parenthesis. Statistical analysis of data was done using students't' test and comparisons are done with vehicle treated controls. The levels of significance indicated are:

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE 7 : THE IMPACT OF PROLACTIN (O-PRL) AND BROMOCRYPTINE ON THE PHOSPHOLIPID PROFILE OF CORPUS EPIDIDYMIDIS

Groups Treatment	Total Phospholipid (mg/gm of tissue)	Phosphatidyl inositol (mg/gm of tissue)	Phosphatidyl serine (mg/gm of tissue)	Sphingomyelin (mg/gm of tissue)	Phosphatidyl choline (mg/gm of tissue)	Phosphatidyl ethanolamine (mg/gm of tissue)	Phosphatidic acid (mg/gm of tissue)
Group-I(7) Castrated+vehicle	8.18±0.44	0.54±0.04	0.60±0.05	1.06±0.08	2.36±0.12	1.72±0.09	1.26±0.07
Group-II(10) Castrated+50µg O-PRL/100 g b.wt.	9.71±0.61	0.68±0.06	0.52±0.04	0.96±0.08	2.54±0.16	1.87±0.07	1.08±0.05
Group-III(12) Castrated+100µg O-PRL/100 g b.wt.	10.34±0.90*	0.96±0.08***	0.47±0.03*	0.80±0.07*	4.00±0.20***	2.68±0.10***	0.70±0.06***
Group-IV(10) Castrated-150µg O-PRL/100 g b.wt.	9.54±0.73	0.70±0.07	0.58±0.05	1.00±0.05	2.66±0.14	1.97±0.10	1.08±0.05
Group-V(12) Castrated 200µg O-PRL/100 g b.wt.	9.40±0.88	0.63±0.07	0.59±0.04	1.01±0.11	2.60±0.16	1.90±0.13	1.15±0.07
Group-VI(10) Castrated+0.3mg Bromocryptine/100 g b.wt.	2.47±0.13***	0.13±0.01***	0.21±0.02***	0.35±0.03***	0.92±0.06***	0.41±0.03***	0.39±0.04***

The results represent mean±S.E.M. The number of animals included are indicated against each group within parenthesis. Statistical analysis of data was done using students 't' test and comparisons are done with vehicle treated controls. The levels of significance indicated are:

*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$.

TABLE 8 : THE IMPACT OF PROLACTIN(O -PRL) AND BROMOCRYPTINE ON THE PHOSPHOLIPID PROFILE OF CAUDA EPIDIDYMIDIS

Groups Treatment	Total Phospholipid (mg/gm of tissue)	Phosphatidyl inositol (mg/gm of (tissue)	Phosphatidyl serine (mg/gm of tissue)	Sphingomyelin (mg/gm of tissue)	Phosphatidyl choline (mg/gm of tissue)	Phosphatidyl ethanolamine (mg/gm of tissue)	Phosphatidic acid (mg/gm of tissue)
Group-I(7) Castrated+vehicle	10.78±1.00	0.42±0.03	0.70±0.06	1.18±0.08	3.04±0.13	1.64±0.06	1.75±0.09
Group-II(10) Castrated+50µg O-PRL/100 g b.wt.	11.08±1.77	0.49±0.03	0.68±0.05	1.06±0.07	3.34±0.17	1.75±0.08	1.55±0.07
Group-III(12) Castrated+100µg O-PRL/100 g b.wt.	14.78±1.33*	0.62±0.04**	0.42±0.04**	0.87±0.07*	5.07±0.43***	2.31±0.14***	1.11±0.06***
Group-IV(10) Castrated+150µg O-PRL/100 g b.wt.	12.07±1.81	0.48±0.04	0.66±0.04	0.97±0.08	3.40±0.20	1.81±0.08	1.50±0.08
Group-V(12) Castrated 200µg O-PRL/100 g b.wt.	11.83±1.60	0.44±0.03	0.68±0.05	1.00±0.08	3.36±0.16	1.80±0.08	1.56±0.08
Group-VI(10) Castrated+0.3mg Bromocryptine/ 100 g b.wt.	3.04±0.16***	0.11±0.03***	0.17±0.01***	0.26±0.04***	1.12±0.12***	0.90±0.04***	0.41±0.03***

The results represent mean±S.E.M. The number of animals included are indicated against each group within parenthesis. Statistical analysis of data was done using students't' test and comparisons are done with vehicle treated controls. The levels of significance indicated are:

*p $<$ 0.05, **p $<$ 0.01, ***p $<$ 0.001

FIG. 9 A SUMMARY CHART SHOWING INTERRELATED PATHWAYS OF LIPID METABOLISM IN ANIMAL TISSUES

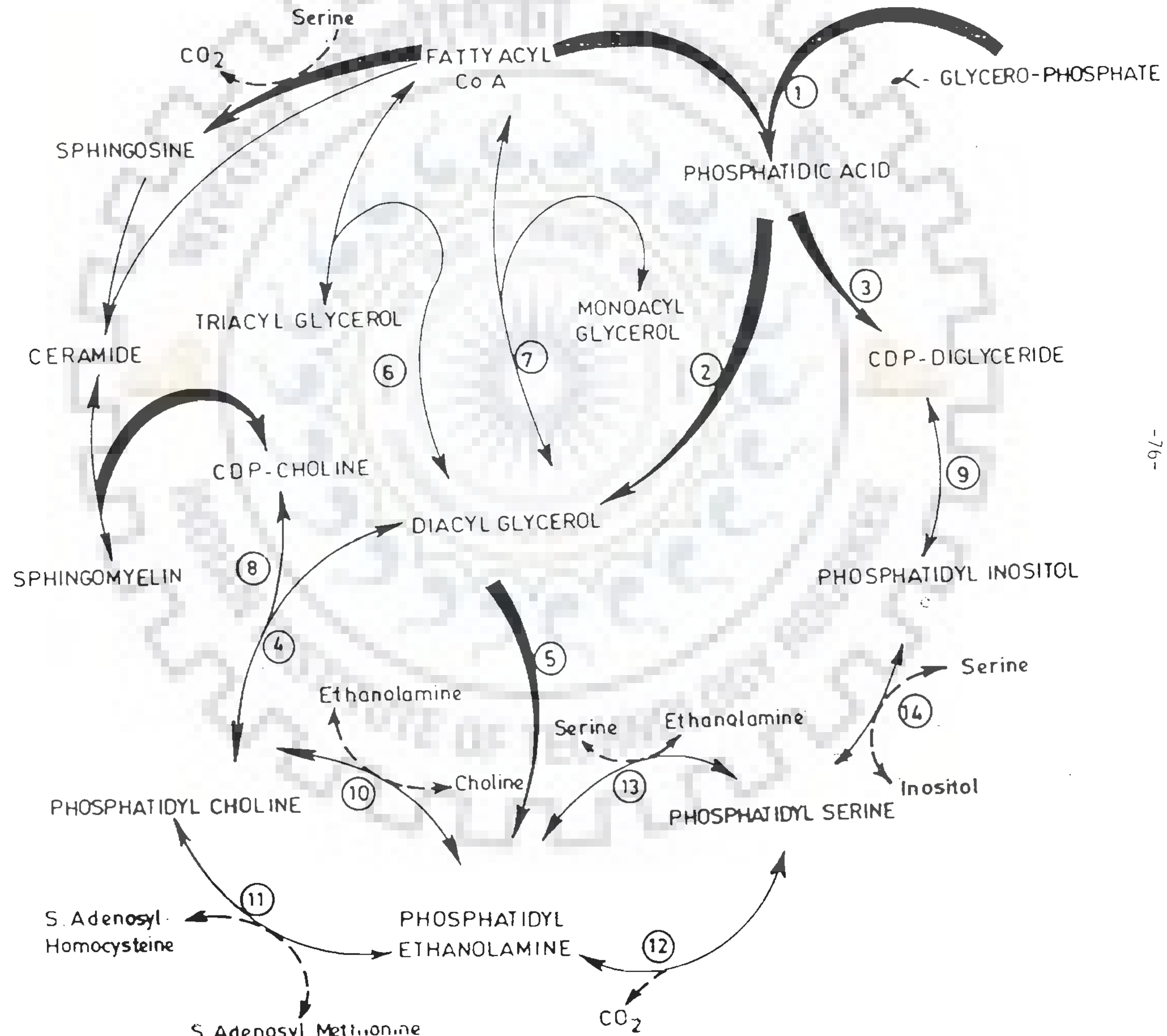


TABLE-9 : LEGEND TO THE SUMMARY CHART ON LIPID METABOLISM (FIGURE-9)

Reaction number	Source of tissue	Reference
1	rat liver, intestine, epididymis, mammary gland, lung	Bremer et al., 1976; Lands and Crawford, 1976; Polheim et al., 1973; Brooks, 1981a; O'Doherty, 1978.
2	Lung, liver	Bell and Coleman, 1980; Lands and Crawford, 1976; Tijburg et al., 1989
3	Liver	Sundler et al., 1974.
4,5	Liver, pancreas, adipose cells, human and bovine, epididymal spermatozoa	Sundler et al., 1974; Hawthorne, 1973; Bell and Coleman 1980; Selivonchik et al., 1980; Jones, 1989.
6	Liver, kidney, adipose, intestinal mucosa ,(in all animal tissues)	O'Doherty, 1978
7	Intestine, mammary glands	O'Doherty, 1978
8	Tumour cells, intestine liver	Stoffel, 1973
9	Brain and other tissue, erythrocytes kidney	Hokin and Hokin,, 1964;
10,11,12,13,14	Brain, liver, all animal tissues.	Kanfer, 1972; Miura and Kanfer, 1976; Tijburg, et al., 1989.
14	In all animal tissues	Tou et al., 1970.

bolic pathways have been known to be operational for the formation of phosphatidyl inositol. They can either be derived from phosphatidic acid (Baker and Thompson, 1972) or from phosphatidyl serine (Kanfer, 1972). In the present investigations, PRL was found to decrease the pool size of both phosphatidic acid and phosphatidyl serine which may account for the rise in phosphatidyl inositol. Similarly, the large pool size of phosphatidyl ethanolamine in the epididymis brought about by prolactin treatment may be related to the decrease in phosphatidyl serine and diacylglycerols that have been registered. The existence of such pathways have been reported in literature (Miura and Kanfer, 1976; Kanoh, 1970). The build up in phosphatidyl choline may have been due to the increased synthesis from diacylglycerol (Kanoh, 1970; Thompson, 1973) and sphingomyelin (Stoffel, 1973). Thus, it is found that prolactin produces a shift in the lipid metabolism and favours the accumulation of selected classes of phospholipids.

The epididymis is an organ in which phospholipids are of immense importance. The process of sperm maturation that is believed to take place here is influenced by the fluid in which they are bathed. The composition of this fluid is further controlled by the secretory and absorptive nature of the epididymis which varies along the duct. Thus, the epididymis plays an important role in a process that culminates in the sperm acquiring the capacity to fertilize ova at its proximal end and storing them in a quiescent, yet

viable form, in the distal region. The phospholipids on account of their amphipathic nature, are important constituents of membranes. Any alterations in the pool size of these compounds is bound to alter the characteristics of membranes like fluidity, rigidity and permeability. The results of the present investigations show that the subclasses of phospholipids are profoundly influenced by prolactin. It is only logical to speculate that changes in characteristics of membranes would hamper the transport of important molecules from the epididymal epithelium into its lumen which are necessary for sperm maturation. This may be the reason why defective sperm maturation has been observed in clinical conditions of hyperprolactinemia.

5.0 CONCLUSION

Prolactin favoured the increase in weight of the organs associated with the male reproductive tract. These changes in weight may be attributed to increased proliferation and growth of cells and/or increased secretory activity in these organs.

Based on the time course study of serum prolactin bioactivity, it has been suggested that the exogenously administered prolactin is removed from circulation within 15 mins.However, a spike in serum prolactin bioactivity appears at 2 h which may be a 'rebound' of native prolactin caused by a transitory suppression of endogenous prolactin synthesis/secretion. The suppressive action may be the result of a feedback brought about by exogenous prolactin.

Prolactin increased glycerophosphocholine in the epididymis, which is indicative of a stimulation in the synthesis of this compound. It is suggested that this action of prolactin may have its repercussion on 'sperm maturational' events.

Measurements of the pool size of total lipids together with their subclasses have projected specific action of prolactin. Total lipids are increased more on account of glyceride glycerols rather than phospholipids. Among the

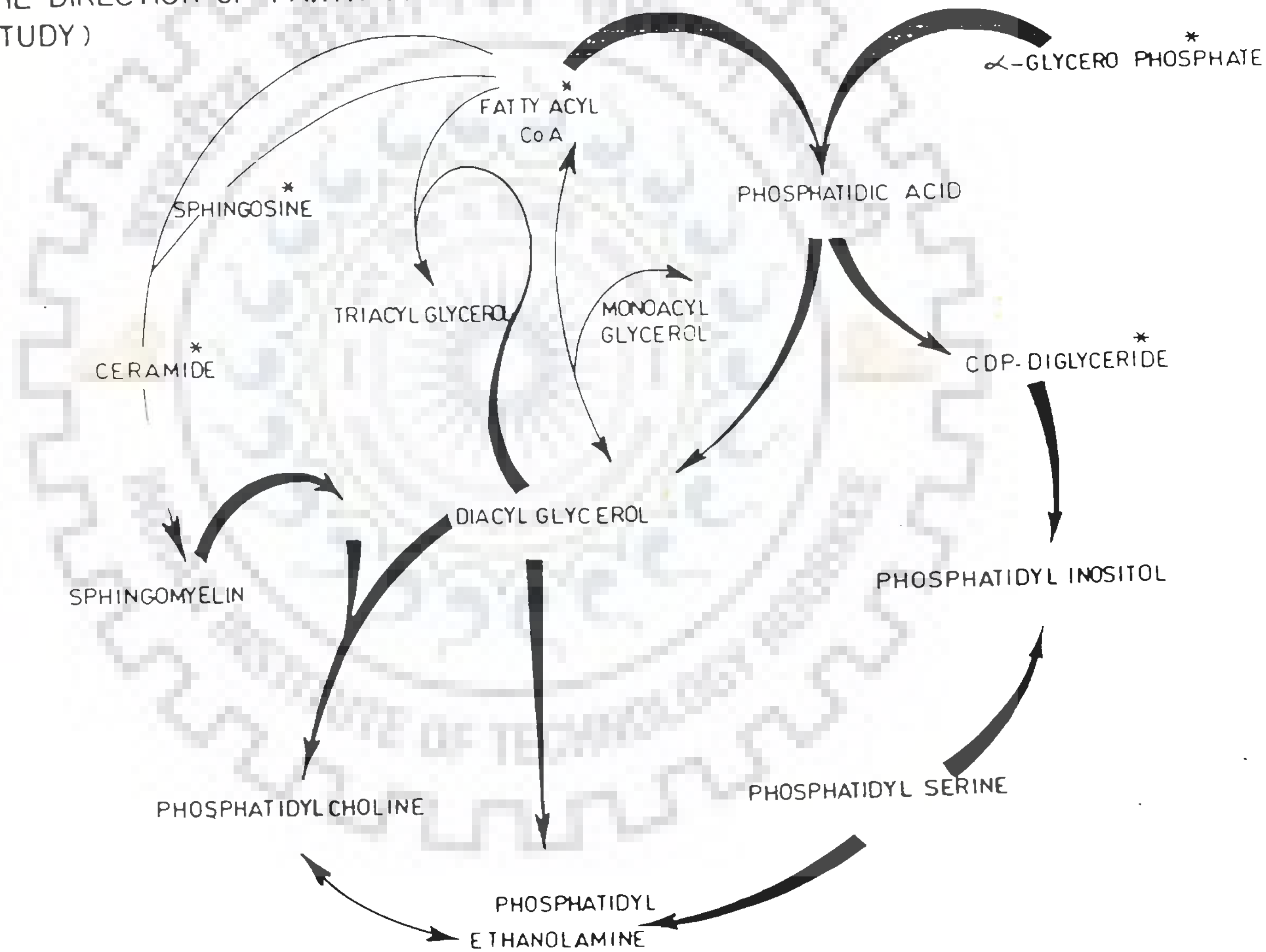
glyceride glycerol, triacylglycerol were found to be substantially raised implicating that there is a tendency for storage of lipids. Prolactin promotes the conversion of free cholesterol into the esterified form.

Analysis of phospholipids have shown that prolactin increases phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl inositol, but correspondingly reduces the pool size of phosphatidicacid, sphingomyelin and phospatidyl serine. Prolactin, thus favours metabolism of lipids through specific pathways. The possible routes of lipid metabolism are summarised in figure-10.

It has been suggested that phosphatidyl inositol is derived from phosphatidic acid and phosphatidyl serine. The rise in phosphatidyl choline has been attributed to the selective stimulation of pathways that help in their formation from diacyl glycerols and sphingomyelin. The high levels of phosphatidyl ethanolamine perhaps arises from the conversion of diacylglycerol and phosphatidyl serine.

Bromocryptine significantly reduces the total lipid content together with all its subclasses much below that found in castrated controls. This supports the contention that minimal levels of prolactin are essential for maintaining the lipid profile of the epididymis.

FIG.10 - SCHEMATIC REPRESENTATION OF PROLACTIN ACTION IN THE CONTROL OF EPIDIDYMAL LIPIDS. (THE DIRECTION OF PATHWAYS INDICATED ARE BASED ON DATA OBTAINED IN THE PRESENT STUDY)



* Not quantified

Finally, it is tempting to make the following remarks. Although the epididymal segments perform very different functions, the general pattern of prolactin action on these segments remain the same. However, the magnitude varies probably depending upon the cellular composition and distribution of prolactin receptors along this duct. Of the four doses of prolactin employed, the 100 μg O-PRL treatment seems to be optimal in terms of the biochemical response observed. Thus, it may be inferred that even in the androgen deprived condition, prolactin forms one part of a complex mechanism that regulates events in the epididymis. Further, the results obtained from the Bromocryptine treated group indicate that certain basal levels of prolactin are essential for maintenance of epididymal function.

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