INTRODUCTION

Mammalian liver is metabolically very important gland. It possess a higher degree of structural complexity and diversity of functions. No other organ is involved in so many complex inter-relationships as the liver. This very complex nature makes the liver prone, in that proportion, to a wide spectrum of malfunctions too. Functions of no other single organ have been so extensively and frequently studied from varied scientific points of view. Liver has more often been used as an experimental tissue for both normal and abnormal functions. Its easy, accessibility, relative homogeneity (presumed) and availability in different developmental stages as well as physiological status make it excellent experimental material.

Modulation of metabolic processes evoked by gonadal steroids are extraordinarily diverse. These may occur in almost any tissue of the vertebrate body. Influence of endocrine secretions of male and female gonads on various metabolic aspects could be studied at different levels of regulatory mechanisms.

It is well known that ovarian hormones are responsible for growth and maintenance of female reproductive as well as accessory structures in mammals (William, 1961). These hormones not only significantly influence metabolism of sex specific tissues (Barker and Warren, 1966; Spooner and Gorski, 1972; Steplewski and Wlodzimerz, 1973; Lee and Katzenellebogen, 1993; Bigsby, 1993) but also that of certain non-reproductive tissues viz. muscles (John *et al.*, 1973), Kidney (Van-pilsum *et al.*, 1968, Biswas and Mukerjea, 1972; Pellanda, 1976), heart (Stumpf, *et al.*, 1977) and liver (Aftergood and Alfin-slater, 1965, Biswas and Mukherjea, 1972, Kose *et al.*, 1993a; Kose *et al*; 1993b), intestine (Thomas *et al.*, 1993)

Certain effects of removal of gonads on hepatic tissue of male and female rats have been reported by several authors (Swartz *et al.*, 1960; Fujii and Villee, 1968; Moore *et al.*, 1977; Ambadkar and Ganagaramani, 1980; Rasmussen *et al.*, 1988). All these earlier attempt to unravel influence of gonadal steroids on intermediary metabolism of hepatic tissue have paved the way for detailed studies on this (classically considered) non-target tissue.

Literature concerning effects of ovarian hormones on intermediary metabolic patterns of hepatic tissue is enormous (Jellink and Lucieer, 1965; Fewster *et al.*, 1967; Moore *et al.*, 1977; Ockner *et al.*, 1979; Ockner *et al.*, 1980; Tamulevisius *et al.*, 1982; Nicolar *et al.*, 1987; Kose *et al.*, 1993a; Kose *et al.*, 1993b.)

Recent studies on receptor of sex hormones in liver have provided ample evidence in support of the view that the liver is a target organ for sex hormones (Duffy and Duffy ,1978; Tompson and Lucier, 1983; Mataradze and Gontar, 1986),. Yet, it is presently not possible to weld the available bits and pieces of experimental information into anything approaching coherent account of the action of ovarian hormones on involvement of mammalian liver in overall physiology.

Most of the earlier studies were made on females after few weeks of spaying (Aftergood and Alfin-slater, 1965; Dimitrov *et al.*, 1987) and after prolonged/ repeated administration of female sex hormones. Particularly, such studies did not take in to account the influence of normal cyclic hormonal changes that occur during estrous cycle. On the other hand, of recent it has become amply clear at different tissues respond to hormonal deprivation/ administration within few days (Schechi *et al.*, 1973; Rath and Prasad, 1974; Sutter Dub., 1979) or even few hours (Tapper *et al.*, 1972; Schwartz, 1974; Ishihara *et al.*,1988). Mean and Hamilton (1966) demonstrated that within 2 minutes of a single injection of estrogen significantly increased rapid synthesis of labelled nuclear RNA in rat uterine tissue.

The major aim, therefore, of the present study was to observe the cyclic variation in hepatic metabolites and concerned enzymes during normal estrous cycle. Secondly, the early effects of spaying and rapid effect of replacement therapy with estrogen alone or estrogen and progesterone combination were also studied.

Another aspect was to find out whether there are any lobe wise (regional) differences since such were found to exist in the case of male rats (Ambadkar and Gangaramani, 1981).

As a prerequisite, initially all the different lobes of the liver were tackled separately. This was an attempt to find out evidence, if any, in support of earlier observation about the existence of differential responses exhibited by the spigelian lobe and other lobes of liver in male rat. There are enough reports regarding lobe-wise differences in the hepatic tissue (Hems *et al.*, 1972; Krishnakantha, 1972; Tyagi and Mishra, 1972; Ambadkar and Ganagarmani, 1979; Ambadkar and Derasari, 1987). Hence, it was thought desirable to verify the possibility of functional differences among the different lobes of liver in the female rat. The preliminary findings established the fact that the spigelian lobe of liver in female rats differs significantly from the rest of the lobes with regard to its metabolic aspects. This was borne out further during subsequent studies, Hence, later on only right lobe was chosen as the representative of rest of the liver lobes for

comparison with the spigelian lobe.

The sequence of events which repeats regularly during estrous cycle as a consequence of inter-relationship between gonads and pituitary gland (Moore and Price, 1932). Features of this cycle vary widely in different mammalian species. Estrous cycle in rat last for four or five days according to strains. The day of estrous is ascertained by the presence of cornified cells on the vaginal epithelial surface. The period following this is metestrous and represents the preparatory phase in anticipation of conception. The next phase is called diestrous, characterized by dropping levels of ovarian hormones. This is succeeded by proestrous during which the follicular growth preparatory to ovulation is the obvious feature . Such biochemical and physiological changes occur in female rats during normal ovarian cycles and are brought about due to working of hypothalamo-hypophyseal-gonadal axis (Yoshinaga, 1973). The interaction between gonadotropin and ovarian hormones has been a well documented fact (Major and Vaughn, 1966; Mc-Clintock and Schwartz., 1968; Niswender *et al.*, 1976; Sanchez *et al.*, 1993). It is known that blood titers of ovarian sex hormones vary in phase with estrous cycle (Butcher *et al.*, 1974).

There are numerous reports regarding the alterations during the estrous cycle in responsiveness of pituitary gland, hypothalamus and uterus (Cidlowski and Muldoon, 1976; Zeballous and Mccann, 1975; Kato *et al.*, 1969; Korach and Muldoon, 1973; Muthu and Vijayan, 1994). Only a few reports are available on influence of endogenous sex hormone, on hepatic metabolic patterns in laboratory rat (Biswas and Mukerjea, 1973; Sladek, 1974). In the circumstance an initial attempt was made to investigate possible changes in physiology of liver in relation to estrous cycle which could provide additional information on the relation between cyclic variations of gonadal hormones and hepatic carbohydrate, protein, lipid and nucleic acid metabolism. Variation during normal 4-day estrous cyclicity in the certain metabolites viz:- plasma glucose and hepatic glycogen, protein, lipid, cholesterol, phospholipid, DNA, RNA as well as some enzymes related to the above parameters were assessed (Chapter 2).

Logically changes induced by gonadectomy could be prevented or reversed, atleast to a certain extent, by administration of appropriate sex hormones. To substantiate this understanding the influence of the replacement therapy with estradiol 17ß and progesterone was tried out. Three different doses of estradiol viz:-5 μg (D_1) 10 μg (D_2) and 15 μg (D_3) were administered to 48 h spayed animals.

It is well known that the rate of metabolic turn over is quite rapid. The half-life of progesterone is of 30 minutes; it is converted into the relatively inactive pregnediol which appears in the urine as pregnenodiol glucuronide. Compara-

tively, estradiol has a much longer half-life as long as 24 hours. The liver converts it into estriol (E_3) having less estrogenic activity. Studies with radiolabelled precursors of different hormonal metabolites have revealed the importance of the liver in manifestation of effects of hormones. Balnave and Brown (1967) have shown that in the case of testosterone and progesterone treated birds maximum incorporation of labelled acetate into liver phospholipids occurred within 15 minutes of injection. Similar experiments using radioactive tracers with respects to metabolic processes in several tissues have revealed that the manifestation of hormones occurs within a matter of minutes (Mean and Hamilton, 1966; Szego and Davis, 1967).

With a view to investigate such immediate or rapid effects of the sex hormones the present work was carried out employing varying doses (5, 10 and 15 μg of E_2) at short intervals of 1, 2 and 4 h.

During the course of the study effects of E_2 replacement at different post injection intervals it was realized that the 2 h time interval could bring back the hepatic gland towards normality in a comparatively better way. Hence, only that post injection interval was chosen for studying the effects of combined treatment of estrogen and progesterone.

The central role of the liver in carbohydrate homeostasis is well recognized (Altszuler and Finegold, 1974; Hers; 1976; Hers and Hue, 1987). Its primary function in this regard is to adequately provide glucose via circulating blood to tissues that mainly utilize it as metabolic fuel. Liver glycogen deposited during alimentation, serves as immediate available source of glucose. During extended periods of fasting, this energy supply is supplemented by sustained process of gluconeogenesis. The liver is also known to process other ingested nutrients and transforms them into specific constituents required by the body.

Various metabolic reactions within the hepatic cells that lead to glycogen formation, glycogenolysis, gluconeogenesis and hepatic glucose release are regulated to a large extent by hormones and the availabilty of appropriate substrates. Glycogen is stored in the cytosol of liver cells in granular form. Defects in the enzymes involved in the synthesis or degradation of glycogen may lead to disturbances of glycogen metabolism.

It is a well recognized fact that the enzymes glycogen phosphorylase brings about initial step in the breakdown of glycogen. Further, the phosphorylase enzyme activity catalyzes phosphorolytic cleavage of 1-> 4 glycosidic linkage at the non-reducing end of an outer branch of glycogen molecule. Glucose-6-phosphatase is classically considered as a key enzyme for the release of glucose

into circulating blood by the hepatic cells.

The effects of ovarian sex hormones on carbohydrate metabolism of uterine tissue have been studied by several authors (Bitman *et al*,, 1965; Eckstein and Villee 1966; Bo *et al*; 1973; Demers *et al.*, 1973; Garrison *et al.*, 1973). Only few studies have focused attention on the influence of female sex hormones on hepatic carbohydrate metabolism (Matute and Kolkhoff, 1973).

Great deal of attention has been given to the general problem of energy assisted transport mechanisms across biological membranes against concentration gradient. Assessment of ATPase activity as a measure of such transport of substances has been validated (Schloefield, 1964; Stein, 1967 and Barnabei *et al*, 1973).

Many studies have made it clear that several hormonal influences on general metabolism of the tissue usually involve a mediator like cAMP, and the latter is referred to as a second messenger (Robison, 1973). Its role in activation of phosphokinases and carbohydrate metabolism of liver is also well known (Rindi, 1971). The cAMP-Phosphodiesterase (cAMP-PDE) degrades the cyclic nucleotide to non cyclic form 5'-AMP. Hence any change in this enzyme activity would indicate intracellular cAMP fluctuations. Higazi and Kvinsaland (1974) showed that cAMP degrading enzymes are present in the cervico vaginal epithelium and that the mechanisms of estradiol action involves an effect on the cAMP level regulating system.

Among the dehydrogenases of the Kreb's cycle of mammalian liver the succinate dehyrdogenases (SDH) is more active than any others (Putili na and Eschenko, 1969). Eckstein and villee, (1966) reported, on the basis of their work on the enzymes of Kreb's cycle of the rat uterus, that estradiol stimulates SDH activity. It is therefore, obvious that assessment of SDH would provide good index of the state of oxidative metabolism.

Taking into account glycogen, plasma glucose and enzymes like glycogen phosphorylase, G-6-Pase, SDH, ATPase and cAMP-PDE under different experimental condition (ovariectomy and replacement therapy) were investigated (Chapter-3).

Sex hormones have been implicated in an important way in the integration of adaptive changes in the protein metabolism (Kochakian, 1964). It has been recognized since many years that estrogen modulates synthesis and secretion of several hepatic protein (Song *et al.*, 1969; Tamulevicius *et al* ., 1982). It is also proven fact that both DNA and RNA are essentially associated with protein synthesis as well as growth (Willson, 1962; Vollmer and Kauffman, 1963;

William-Ashman and Shimassuki, 1967; Kurtz *et al.*, 1976).

Leeuwin *et al.* (1975) reported that there exist an apparent sex-dependent difference in the DNA concentration of rat liver and that spaying of females alters DNA concentration. Influence of estrogens on protein metabolism have been extensively reviewed by Aschkenassy-lelu and Aschkenassy (1959). They pointed out that these hormones affect nitrogen balance of rat in dose-dependent manner; such that low levels of estrogens are significantly anabolic but higher levels are distinctly catabolic. Swartz *et al.* (1960) and Konopkova and Nedividek (1972) reported on the phenomenon of nuclear polyploidy of liver cells due to gonadectomy in rats. It is quite possible that induction of such polyploidy in liver cells of female rat may occur due to spaying. In order to confirm such a view hepatic protein, DNA, RNA and enzyme 5'-nucleotidase activity were evaluated after spaying and replacement therapy (Chapter-4).

Investigation pertaining to the role of gonadal hormones in lipid metabolism has recieved attention of several workers for years. (Aftergood and Alfin-Slater, 1965; Nathaniel and Nathaniel, 1966; Kitao Manobue, 1971; Ockner *et al.*, 1980; Kose *et al.*, 1993a). Interest in aspects of biochemical and physiological actions of ovarian hormones has increased since several synthetic steroids are being widely used as oral contraceptives by ever increasing human population all round the world. The liver is a well known primary organ involved in the processes of biotransformation and inactivation of hormones . It is possible that before these synthetic hormonal preparations are biotransformed by the liver they may already have induced metabolic alterations, which may directly or indirectly influence homeostatic functions of liver. It is known that both endogenous and exogenous sex steroids are capable of altering cholesterol, triglyceride and lipoprotein levels of serum (Bradley *et al.*, 1978; Knopp *et al*, 1982; Powell *et al* ., 1984). Effects of sex steroids on plasma lipid concentrations reflect partly corresponding alterations in the rate of hepatic biosynthesis of triglyceride rich very low density liproproteins (Ockner *et al* ; ,1978). In the light of above information a quantitative evaluation of hepatic total lipid and cholesterol was carried out (Chapter 5).

From the results obtained for total lipid and cholesterol level during estrous cycle, spaying and subsequent replacement therapy it was noted that total lipid levels varied during different phases of estrous cycle. Further, a significant increase in the same occurs after spaying and decrease after hormone replacement. From these observations it could be said that such variation may be due to some 'particular' lipid components of the total lipids. Aizawa and Mueller (1961) have demonstrated the importance of estrogens in controlling lipid synthesis in the uterus and they have further suggested that the pace of phospholipid metabolism of the uterus is a sensitive indicator of early estrogen action.

Patsch *et al.* (1980) found significant sex-related differences in the secretion pattern of lipoproteins. They have also shown that changes in plasma lipoprotien concentration were due to the effect of sex hormones on the liver. The most conspicuous change in uterine phospholipids observable during estrous cycle was almost abolished by ovariectomy but was found to be restored by estrogen therapy (Goswami *et al.*, 1963).

. Keeping these finding in view, an attempt was made to observe the possible cyclic variations in different phospholipid components of liver during estrous cycle and after ovariectomy in relation with plasma lipid profiles and circulating sex hormones (Chapter-6).

. On the basis of available literature and experimental evidences an attempt has been made to uncover the role of ovarian steroids in intermediary metabolism of liver while discussing the results obtained in the light of available literature following points were given due consideration:-

.1.. Sex specific differences in hepatic metabolic patterns in normal male and female rats by way of comparison.

.2.. Possible correlations between reported circulating levels of ovarian sex hormones and the hepatic metabolic alterations during normal estrous cycle.

.3.. Desirability of knowing short term effects of ovariectomy, since long term effects have already been known since long.

.4.. In the light of recent information on quick effects of hormones it was thought better to study the rapid effects of hormone replacement on hepatic metabolism.

.5.. Desirability of discussion by way of extrapolation of the role of exogenous hormone administration with endogenously varying sex hormones during estrous cyclicity, with respect to involvement of liver of female rats in functional adjustments.

.6.. To bring out the significance of differential responses shown by spigelian lobe and other lobes of liver to variations in female sex hormones.

FIG : REFLECTED LIVER LOBES OF RAT

(RATTUS NORVEGICUS). REF : GREEN,(1959).