GENERAL CONSIDERATION

Although the mammalian ovary was identified as an endocrine gland before 1900, assessment of the wide spectrum of overall metabolic actions of its secretory products remains a challenge even today. The study of cyclic changes in vaginal exfoliative cytology and the findings of estrogenic activity in follicular fluid during early 1920s led to an enhanced interest in the field of physiology of reproduction.

Research on reproductive hormones, as of now, is not restricted to the classical target tissues alone but an increasing number of non-target tissues are being included. Thus, at times the definition of target tissue seems to need amendment. The hepatic tissue is one such organ which is no longer considered a non-target tissue as far as the steroid hormones are concerned. Further, in more recent years, it has been reported that lack or excess of steroid hormones lead to significant alterations in the normal hepatic metabolic patterns (Konopkova and Nedvidek, 1972; Khandewekar *et al.*, 1973; Pirkko *et al.*, 1975; Pirkko, 1981; Muddeshwar *et al.*, 1984; Ambadkar *et al.*, 1987; Rasmussen *et al.*, 1988; Kose *et al.*, 1993a; Ambadkar and Wagh, 1994).

More than six decades ago it became known that there could be basic differences in patterns of metabolism of the liver of male and female rats (Greisheimer, 1931). Since then many workers (Best *et al.*, 1951; Aftergood *et al.*, 1957; Coleman *et al.*, 1958; Kritchevsky *et al.*, 1963; Patsch *et al.*, 1980; Ambadkar and Wagh, 1993) have reported several sex-dependent differences in various metabolites and metabolic processes of mammalian liver.

It is needless to make a special mention of already existing voluminous literature on effects of gonadectomy and subsequent replacement therapy on varied tissues and organs. A cursory survey of this literature brings forth that almost all these studies reported on the effects observable after few weeks to several weeks of gonadectomy and influence of considerably extended periods of hormonal replacement. Another point of significance worth noting is that very sparse reports are available on the study of influence of estrous cyclicity on hepatic metabolic patterns. Published research work clearly hints at very rapid effects of ovarian hormones on certain fundamental biochemical processes; even those involving nucleic acids and proteins (Liao *et al.*, 1965; Fujii and Villee, 1968; Stormshak *et al.*, 1976; Engel *et al.*, 1980). Considering all these facts it was difficult to ignore the desirability of knowledge regarding immediate effects of spaying and replacement therapy on metabolic adjustments in the mammalian

liver.

It was with this view the present investigation was undertaken. Arbitrary intervals of 1,2 and 3 days were chosen for looking into early effects of spaying. In regard of replacement studies initially it was thought desirable to investigate effects of at least three different doses (5, 10 and 15 μg/animal) of 17ß estradiol (E_2) alone with three time intervals (1, 2 and 4 h) in order to find out minimal requirement of dose and time for restoration. As progesterone (P) also plays important role, a fixed dose of 2 mg of P was given simultaneously with the above three doses of E_2. The influence of combined hormone replacement was assessed after 2 h of injection.

As a part of this investigation (chapter 2-6) many important metabolites viz.-Glycogen, protein, nucleic acid, total lipid, cholesterol and phospholipid and certain important enzyme activities viz.- phosphorylase, G-6-Pase SDH, cAMP-PDE, ATPase and 5'-nucleotidase were studied during the four phases of normal estrous cycle and under the experimental conditions outlined in the preceeding paragraph. Certain parameters *viz-* glucose, total lipid, cholesterol and phospholipid of plasma were also studied. Most of the parameters of liver and plasma showed maximum significant alterations after 48 h of spaying, hence the influence of replacement therapies were studied by injecting the hormones to 48 h spayed rats.

The Spigelian lobe (nomenclature of Green, 1959) of liver of male as well as female rats has been reported to exhibit decidedly different responses to sex hormone deprivation as well as replacement than the remaining lobes (Ambadkar and Gangaramani, 1980, 1981 and 1982; Ambadkar and Wagh, 1993 & 1994). Consequently the primary focus of the entire present study was on separate investigation of the right representative of rest of the liver lobes) and spigelian liver lobes of female rat. The contention of regional differences is understandable as regional functional differences in metabolic responses of different lobes of liver have been already reported (Hems *et al.*, 1972; Krishna Kantha, 1972; Tyagi and Mishra, 1977).

When the observations obtained during different phases of estrous cycle are considered together the following inference can be drawn:-

a) Diestrous is that phase of the cycle when gonadal hormone levels are at their minimum. Under these conditions liver probably discharges one of its usual functions of regulated release of glucose into the blood. Another feature pertains to accumulation of hepatic total lipids and cholesterol contents. Conversely, the total phospholipid content was lowest, containing predominantly by serine and

spingomyelin containing components followed in decreasing order by inositol, ethanolamine and choline. Apparently, it seems that lipid accumulating influences is some how concerned with release of phospholipids by hepatocyte into blood as was apparent from plasma phospholipid profile. It is tempting to suggest that increase of plasma phospholipids probably supplements follicular development during subsequent phases.

Decreased cAMP-PDE activity favour some degree of complementary enhancement of glycogen synthase leading to noticeable increase of hepatic glycogen level. It can also be seen that hepatic protein levels are reduced to a good extent along with rise in 5'nucleotidase and lowered RNA and DNA levels. On the basis of data on hand it is not possible to explain occurrence of such phenomenon. It could, therefore, be suggested that further work only would throw more light on this situation availing during diestrous phase of the cycle.

b) Proesterous phase of the cycle is known to be dominated by follicular development and higher titers of estrogenic hormones. Under the circumstances , it was noticed that there was a clear cut lowering of blood sugar level accompanied by suppression of hepatic phosphorylase, G-6-Pase and SDH activity and noticeable rise in glycogen content and total ATPase activity. Taking into consideration these facts accompanied by lipid mobilizing action it may be surmised that these are manifestations of the influence of rising level of estrogen on the hepatic tissue. Concomittant with reduction in hepatic total lipid there was rise in the same parameter of the plasma. As would be expected this was accompanied by converse changes in the phospholipid moiety in both of these compartments. Among the hepatic phospholipid fractions choline and inositol containing ones were comparatively more. This was probably also due to enhancement the process of methylation of ethanolamine to choline due to higher estrogenic level (Nishigori and Aizawa, 1968b; Young, 1970).

c) As is well known the female rat show spontaneous activity on the day of estrous which would naturally put greater demand for supply of blood glucose and that was borne out clearly from lowered hepatic glycogen and increased blood glucose level. The hepatic lipid also was at its lowest and that of blood at its peak. Regarding the hepatic phospholipid composition it could be seen that the total phospholipid content was highest during this phase of cycle exhibiting maximum levels of choline and inositol containing fractions. Apart from bringing about the said changes, the prevailing titers of ovarian hormones also lead to lowering of hepatic protein concentration that was accompanied by reduced RNA level.

d) Biochemical characterization of metestrous phase comprised of build up of hepatic proteins & lipids and degradation of glycogen. Other related accompany-

ing changes pertain to rise in both DNA and RNA as well as total ATPase activity. As could be expected the hepatic phospholipid level was on the decline with concomitant rise in that of the plasma. From these observations it can be seen that this phase of the cycle is consistent with preparation for anticipated conception.

Throughout the cycle it was evident that there is a direct relationship between the total lipid and cholesterol concentration and an inverse one between total lipid & phospholipid concentration in the liver. These variations were reflected in that manner to a significant extent in the plasma profiles of these parameters. These results also corroborate the findings (Aschekenassy-lelu & Aschekenassy, 1959) that comparatively high levels estrogenic compounds have a protein catabolic influence and lower one are anabolic, in general. Further it may be added that the alterations observed during metestrous are, in all probability those that are due to progestagenic influence subsequent to estrogenic action on hepatic tissue. It is, therefore, obvious that estrous cyclicity certainly influences the metabolic patterns of the hepatic tissue in a well defined manner.

Though the above description so far pertained to general remarks on metabolic alterations through out the estrous cycle; it can not escape the notice that there were enough indication of differences in responses shown by the spigelian lobe and the right lobe at all the phases and in respect of important metabolites as well as enzyme activities under investigation. It is, therefore emphasized that the spigenlian lobe is a better indicator of hormonal influences on patterns of hepatic metabolism.

Diestrous phase is considered as the quiescent period during the estrous cycle when minimum levels of ovarian hormones are detectable in circulating blood. Therefore, it was thought most opportune time for the removal of ovaries during this phase to study effects of ovariectomy. Of recent, it has become amply clear that normal biological half life of various hormones including sex steroids, is a matter of few minutes to a few hours. Earlier studies on effects of gonadectomized and hormonal replacement were invariably conducted over extended periods of few week and repeated replacement regimes. So, it was the primary aim of the present work to find out short term / rapid effects of both treatments.

At the end of 24 h of OVX the plasma glucose level was found to be decreased but that of phospholipid was heightened. The hepatic tissue responded by showing decreased protein and glycogen concentration with simultaneous rise in nucleic acids, cholesterol and phospholipids. This was accompanied by suppression of all the enzymic activities but that of PDE was enhanced. Taking all these immediate alterations into consideration it can be suggested that OVX

induced nuclear polyploidy and modulated general metabolic pattern towards biosynthesis of cholesterol and inositol rich phospholipids by the liver.

48h after OVX there was noticeable alteration of the sequence of events. There was recovery of glucose level in plasma but lipid level dropped further. Changes in hepatic tissue were also apparent. These were, on one hand, favourable for release of glucose into blood as evinced from improvement in phosphorylase and G-6-Pase activities. Higher DNA as well as 5'nucleotidase levels favoured polyploid condition. The lipid profile in liver showed some lobe-wise differential responses. Total lipid and phospholipid got restored in the spigelian lobe but the right lobe did not come out of the influence of OVX. ON the other hand, hepatic metabolism was so altered that phospholipid component *viz.*-choline , serine and inositol decreased and sphingomyelin and ethanolamine were increased. Reduction in choline and simultaneous rise in ethanolamine indicated inhibition of methylation process, which convert PE to PC perhaps due to the absence of estrogen, (Young, 1971).

In all probability it is logical to expect that the ovarian hormones would reach almost negligible levels with further lapse of time after OVX. The integrated picture of different biochemical status of hepatic tissue and blood plasma presented, at the end of third day of OVX, led to further enhancement of glycemic level with increase in hepatic phophorylase and G-6-Pase activity along with glycogen breakdown. However, there was restoration of hepatic protein concentration and 5'nucleotidase activity. This alteration was better represented by the spigelian lobe with higher DNA and RNA levels.

As regards the lipid metabolism, it was clear that there was tendency towards accumulation of total lipid content of liver, as one scans the effects of OVX from 24 to 72 h. This corroborates the report by Biswas and Mukherjea (1973) but the initial response was different, however, ultimately leading to reported long term effects. Here too, the spigelian lobe was found to maintain its status as far as total lipid to phospholipid ratio is concerned unlike the right lobe that could not withstand effect of OVX and indicated lipid accumulation from 48 h onwards.

On an overall level, it could be seen that there was a continuously increasing release of phospholipid into plasma but the components were varied, at least initially during 24 to 72 h. By 72 h of OVX sphingomyelin, choline and serine decreased but the other two increased. Probably at a still later interval these components might settle down to composition noted after longer periods. Similarly immediate rise in cholesterol was found to fall by 72 h and may be in conformity with reported opinion at long term that OVX causes reduction in its

synthesis (Aftergood and Alfin - Slatter, 1965).

It is a time tested approach to ablate the suspected source of particular hormone from the body and then replace with expected hormonal preparations to study endocrine regulation of physiological aspects of the experimental animal. It is logical to expect that after administration of female sex hormones the alterations induced by ovariectromy in laboratory rats should restored to normal. Hence, it was thought desirable to administer three different doses of estradiol to OVX female rats to study possible restorative effects at three different administration intervals. The present attempt was also intended to find out minimal hormonal requirement as well as time necessary for restoration.

From the data obtained after administration of E_2 doses of 5 (D_1), 10 (D_2) and 15 (D_3) μg/ animal at intervals of 1,2 and 4 h, it was seen that the effect of estradiol on hepatic metabolic pattern varied with different doses and time intervals. Following account represents highlights of the findings:-

D_1 dose of estradiol alone was capable of reversing the effect of OVX but very transiently after 1 h as far as glycogen breakdown and release of glucose into blood were concerned. With further time lapse hormonal treatment induced glycogen deposition with drastic lowering of phosphorylase, G-6-Pase, SDH and ATPase activities. Notable exception was only the PDE activity that was noted to rise. Consequently, the plasma glucose was affected accordingly. More or less similar pattern of responses was observed also with D_3 dose. Apparently, increasing PDE activity lead to diminishing intracellular availability of cAMP and hence retardation of noted enzyme activities. As a corollary, glycogen deposition was increased. This replacement regimes of E_2 alone is not satisfactory but for only 1 h interval and that too, to a limited extent.

With D_2 entirely contrary results were obtained. Almost normoglycemic level was reached by 2 h. Alterations in the case of glycogen and the enzyme activities exhibited exactly opposite trend to that obtained with D_1 and D_3. However, overall effect of E_2 did not seem to be beneficial. One of the noticeable facts was that despite numerical variations mutually inverse relationship between glycogen concentration and phosphorylase activity was found to be invariably maintained. Whatever degree of restorative tendencies could be picked out from the data most of these were evident with 10 μg E_2 (D_2) at 2 h interval. To this extent D_2 at 2 h exerted maximal restorative action.

As would be expected, administration of all the three doses of E_2 considerably lowered hepatic DNA and RNA concentration in the case of 48h spayed rats. However, the rise in nucleic acids after 2 h probably indicates waning

influence of D_1 and D_3. Here again, D_2 indicated extended influence upto 4 h by keeping nucleic acids at lower levels with only transient increase at 2 h interval. As far as hepatic protein concentrations were concerned, at 2 h interval E_2 administration apparently induced a catabolic influence. However, these influence of E_2 doses obviously waned off by 4 h except that of D_2. Therefore, it seemed that E_2 administration, in general, exerted a noticeable catabolic influence on hepatic proteins. Taking into consideration levels of hepatic DNA and protein; it could be said that nucleic acid levels are associated more with nuclear polyploidy rather than general metabolic aspects. As was referred to earlier, this contention is tenable only in the light of quick bioinactivation of administered doses of E_2, and thereby, once again leading to spayed effects.

Estradiol administration to 48h spayed rats could revert the trend of lipid accumulation, perhaps through lipid mobilizing effect of E_2 on liver. Though the E_2 regimes were capable of reverting the effects of ovariectomy these did not lead to normalization. On the contrary, some degree of over suppression was observable. All the three doses of E_2 were found to lower the tissue cholesterol *per se*. However, a noticeable rise was noted in the right lobe with D_3 after 2 h and in case of spigelian lobe with D_3 only at 2 h interval. On the other count, if cholesterol values expressed as percentage of total lipid were considered then cholesterol percentage was higher in both the lobes with all the doses at th same interval. This variation (Cholesterol *per se* Vs percentage of total lipid concentration) clearly suggested that the marked rise in cholesterol percentage at 2h of E_2 injection was reversed by 4 h as soon as the tissue total lipid level was enhanced. Here again, quick bioinactivation of E_2 became apparent.

Among the ovarian steroid hormones progesterone is one of the important hormones which plays major role during the post ovulatory period either in a synergistic or antagonistic way depending on what metabolic effects are being considered in which tissue. In order to find out differences between restorative influence of estradiol alone and that with combined estradiol and progesterone (P), a fixed dose of 2 mg of P was administered simultaneously with three doses of E_2 (i.e.CD_1, CD_2 and CD_3). It was observed, that during the E_2 regimes maximum changes occurred with all E_2 doses at 2 h interval. Hence, the effects of combined treatment were considered only at that time interval.

Combined doses 1 & 2 CD_1 & CD_2 at 2 h interval apparently bring about some restorative effects on glycogen phosphorylase, SDH, ATPase and G-6-Pase. However, glycemic level seems to be closer to normal with CD_1 than with CD_2, the latter being some what hyperphysiological in effect. In stark contrast, CD_3 brings about a very different metabolic state leading to significant glycogen build up through suppression of phosphorylase and G-6-Pase and lowering of

plasma glucose.

E_2+P combinations apparently help restore hepatic protein concentration to a good extent. However, hormone replacement regime employed here was not so restorative in its function with respect to nucleic acid levels and 5'-nucleotidase activity. As far as hepatic total lipid and cholesterol concentrations were concerned, spaying lead to accumulation of total lipid and cholesterol. E_2 replacement reduces the total lipid with slight accumulation of cholesterol. When progesterone was administered along with E_2, it was seen to have antagonistic effect to that of E_2 administration. In general, E_2 as well as P act in concert (synergism) in normalizing the pattern of hepatic lipid metabolism in female rat.

From the foregoing account it is obvious that administration of E_2 alone is not effective to satisfactory level in reverting different trends of alteration induced by OVX. Nevertheless, 10 ug of E_2 dose shows better influence only by 2h interval. Of the combination doses of E_2+P the CD_1 and CD_3 doses are better than E_2 alone with regard to protein and lipid respectively. Carbohydrate patterns are not amenable to either the D or CD series and are more susceptible to disturbances due to OVX. Further studies are necessary to understand this dogma. Probably, it being a very labile entity, still rapid effects need to be looked into.

Another, important observation pertains to lobe-wise differences. In several of the cases spigelian lobe exhibited greater sensitivity to OVX operation. as well as E_2 or E_2+P administration (both, dependent on dose level and intervals). It was also apparent that in case of both of the replacement regimes quick bioinactivation of E_2 and P occurs within 2 to 4 h of administration leading to recurrence to OVX pattern. In a few cases the hormones lead to overt actions by 4 h interval e.g. D_3 and CD_3. It should be admitted that presently employed D and CD series are not very satisfactory in counteracting effects of OVX, but among these D_2, CD_1 and CD_3 are better to a certain extent only. Hence, a wider range of doses, combinations and time intervals may help understand the problem to a better extent.