# CHAPTER-2

## INFLUENCE OF ESTROUS CYCLICITY ON SOME ASPECTS OF HEPATIC METABOLISM

Biochemical and physiological changes that occur in female reproductive organs during normal ovarian cycles are brought about due to functioning of hypothalamo-hypophysial-gonadal axis (Yoshinaga, 1973). The interrelation between gonadotropins and ovarian hormones is a well documented fact (Major and Vaughn, 1966; Caligaris *et al.*, 1968; Mc-Clintock and Schawrtz, 1968; Niswender *et al.*, 1976; Rajaniemi *et al.*, 1977; Sanchez *et al.*, 1993).

As has been already reviewed in detail by Mueller (1960 and 1965), He.chter and Halkerston (1964) and Jensen and DeSombre (1971), the estrogenic hormones exerts influence at different metabolic levels of many cellular components in target tissues. Although most of these effects are probably secondary phenomena resulting from initial stimulation of some biochemical processes it is quite likely that estrogens may exert influence on more than one primary sites in target cells.

It is known that blood titers of sex hormones vary in phase with normal cycle (Butcher *et al.*, 1974). Influence of varying levels of ovarian hormones on uterine metabolism through the phases of estrous cycle, are well documented (Davis and Alden, 1959; Goswami *et al.*, 1963; Biswas and Mukherjea, 1973; Katzenellenbogen, 1975; Muthu and Vijayan 1994). Only sparse reports have focused attention on influence of variations of endogenous sex hormones due to estrous cycle on hepatic metabolic patterns in laboratory rats (Biswas and Mukherjea, 1973; Sladek, 1974). Literature cited here to fore indicated that regulatory role of estrogens is not restricted to altering different metabolic patterns of classical target tissues alone but several other tissues may respond to ovarian hormones.

Of recent, the mammalian liver has come to be recognized as the target tissue for sex steroids (Thompson and Lucier, 1983; Eagon *et al.*, 1985; Francavilla *et al.*, 1986). Hence, it was thought desirable to study the possible effects of variations in levels of circulating sex hormones on liver during normal estrous cycle.

The balance between processes involving release of glucose by the liver and its uptake by various tissues is intricately brought about by multiple hormonal

influences prevailing at any given time. Such hormonal profiles govern various enzyme activities of the liver on one hand and sensitive tissue cells on the other, thus effecting homeostasis. Studies on mechanism of hormonal control of metabolic processes of reproductive organs have shown that female sex hormones are capable of inducing synthesis of several important enzymes involved in carbohydrate metabolism (Singhal *et al.*, 1967 and 1969; Singhal and Valadares, 1970). However, as only scanty information is available on action of sex hormones on hepatic carbohydrate metabolism, attention was directed towards study of certain enzymes that are rate limiting. An attempt has, therefore been made to study alteration in hepatic glycogen concentration and concerned enzyme activities viz.- Glycogen phosphorylase and G-6-Pase along with plasma glucose level during the 4 phases of normal estrous cycle.

Significance of ATPase activity as an indication of the active transport mechanism has been suggested by several investigators (Scholefield, 1964; Wolfe, 1964; Stein, 1967). One of the primary mechanisms underlying the influence of steroid hormones on their respective target tissue involves alteration of rate of transport of substances across the plasma membranes, hence investigation of alterations in ATPase activity was carried out.

Androgens have been shown to facilitate activities of several enzymes of carbohydrate metabolism of the seminal vesicle and ventral prostrate gland by increasing the intracellular levels of cAMP (Singhal and Valadares, 1968; Singhal *et al.*, 1968; Mangan *et al.*, 1973). Similar enzymic stimulation is also known to occur in the uterus by administration of estrogens (Barker and Warren, 1966; Eckstein and Villee, 1966; Hilf *et al.*, 1972). A very specific cellular enzyme activity cyclic AMP- phosphodiesterase (cAMP)-PDE converts cAMP to 5'AMP. Garnovskaya *et al* (1982) reported that 17B-estradiol inhibits the cAMP_PDE activity of uterine preparation from mature rat. It is therefore possible that cAMP-PDE (or PDE) represents a controlling step in the process of hormonal modulation of cellular functions. Hence a study was conducted on the variations of PDE activity to ascertain possible implications of PDE in respect of hepatic metabolism. This may provide some information regarding probable significance of intracellular concentration of cAMP with respect to mechanism of influence of female sex hormones on the hepatic tissue.

Influence of estrogen on protein metabolism has been extensively reviewed by Aschkenassy-lelu and Aschkenassy (1959). They pointed out that the action of these hormones on nitrogen balance of rat varies with dosages. Low dose levels of estrogen exert a mild anabolic action but higher doses are distinctly catabolic. It is a well established fact that nucleic acids are associated with protein biosynthesis and growth (Riddiford, 1960; Osteen and Walker, 1961). It has been

shown that estrogens induce changes in ribonucleic acid concentration in the rat uterus (Sergeev *et al.*, 1973; Catelli and Baulieu, 1977). Hence to gain some understanding about the sensitivity of liver to cyclic variations in female sex hormones the protein and nucleic acid levels were measured.

Variations in uterine lipids during estrous cycle have been reported in a number of species-rabbit (Gilbert, 1942 and Vaes and Van Ypersele, 1960), Guinea pig (Nicol and Snell, 1954) and human (Boutsellis *et al.*, 1963). All these reports are based on histological studies. Only a few reports are available on biochemical assessment of lipids in different tissues of rat or other species (Biswas and Mukherjea, 1973). Moreover, Davis and Alden (1959) reported about certain differences between histological and biochemical findings regarding endometrial lipids as influenced by female sex hormones. The present investigation was therefore, undertaken to study the influence of estrous cyclicity on lipid and cholesterol concentrations in the hepatic tissue of rats.

## MATERIALS AND METHODS

Adult female *albino* rats (140 $\pm$ 20 g B.W) maintained on balanced diet and water *ad-libitum* served as experimental animals.

All the four phases of estrous cycle were determined by examining vaginal lavages under light microscope daily around 9.00 A.M.

Blood samples were collected at each phase by nictitating membrane puncturing with slightly jagged end of anticoagulant coated capillary tubes for plasma glucose estimation. Later, the animals were killed under mild ether anaesthesia.

The spigelian and right lobes were dissected out quickly, freed of adherent connective tissue, blotted free of blood and processed separately for different estimations. Following parameters were estimated/assayed by the methods described in Chapter-1.

1.. Glycogen

2.. Total Protein

3.. Total Lipid

4.. Cholesterol

5.. DNA

6.. RNA

7.. Glycogen phosphorylase enzyme activity.

8.. G-6-Pase

9.. SDH

10.cAMP-PDE

11.Total ATPase

12.5'-Nucleotidase

## RESULTS

Values obtained for various parameters during four phases of normal estrous cycles are given in Table-2.1 and 2.2.

Fluctuating pattern of plasma glucose was observed during different phases of estrous cycle (Fig.1). Glucose level was lowest at proestrous and a remarkable spurt was noted during estrous phase, which was lowered at later two phases of estrous cycle.

Hepatic glycogen concentration was highest at diestrous phase and decreasing pattern was seen from proestrous to metestrous phase. Phosphorylase enzyme activity was higher at metestrous and diestrous phase but at the later two phases it declined (Fig.2). G-6-Pase activity exhibited a pattern parallel to that of plasma glucose (Fig.3). The phase at which glucose level was highest the activity of G-6-Pase was also highest.

SDH activity showed significant alterations (Fig.4). It was lowest at proestrous but increased levels were noted during rest of the phases. Gradual decreasing pattern of cAMP-PDE activity in both liver lobes was observable from proestrous to diestrous phase, but that of right lobe stopped decreasing metestrous (Fig.5). As far as ATPase activity is concerned it was observed that the spigelian lobe of liver exhibited higher activity levels than that of right lobe at any phase of

Table 2.1 Biochemical variations during different phases of 4-day estrous cycles in rat.

| PARAMETERS | PROESTROUS | | ESTROUS | | METESTROUS | | DIESTROUS | |
|---|---|---|---|---|---|---|---|---|
| Plasma Glucose mg/100 ml plasma | 84.600 ± 1.189 | | 139.500 ± 1.929 | | 101.251 ± 0. 871 | | 112.500 ± 1.742 | |
| | Sp | R | Sp | R | Sp | R | Sp | R |
| Glycogen mg/100 mg fresh tissue | 3.719 ± 0.088 | 3.973 ± 0.050 | 3.082 ± 0.059 | 3.162 ± 0.084 | 2.702 ± 0.172 | 2.105 ± 0.094 | 3.810 ± 0.425 | 4.193 ± 0.620 |
| Phosphorylase μg $PO_4$ released/mg protein /60 min at 37°C | 94.544 ± 1.694 | 80.148 ± 1.576 | 127.824 ± 2.244 | 118.000 ± 2.344 | 169.100 ± 1.344 | 169.932 ± 3.276 | 183.884 ± 2.152 | 165.628 ± 1.336 |
| G-6-Pase μmole $PO_4$ released/mg protein/60 min at 37°C | 0.680 ± 0.016 | 0.524 ± 0.036 | 1.508 ± 0.032 | 1.584 ± 0.004 | 0.920 ± 0.024 | 1.060 ± 0.032 | 1.039 ± 0.060 | 0.984 ± 0.036 |
| SDH μg Formozan formed/mg protein /60 min at 37°C | 18.324 ± 0.462 | 14.852 ± 0.356 | 23.106 ± 0.934 | 27.352 ± 1.112 | 24.332 ± 0.712 | 26.070 ± 0.636 | 24.534 ± 1.046 | 27.148 ± 0.706 |
| cAMP-PDE μg $PO_4$ released/mg protein /60 min at 37°C | 3.676 ± 0.134 | 3.450 ± 0.062 | 2.918 ± 0.220 | 3.090 ± 0.172 | 2.646 ± 0.110 | 2.618 ± 0.102 | 1.972 ± 0.192 | 2.632 ± 0.192 |
| Total ATPase μg $PO_4$ released/mg protein /60 min at 37°C | 938.940 ± 31.986 | 811.470 ± 30.624 | 736.740 ± 9.630 | 610.056 ± 19.086 | 822.300 ± 35.160 | 801.180 ± 38.590 | 947.160 ± 19.370 | 754.970 ± 18.960 |

Each value is mean ± SE of at least eight animals. Sp-Spigelian lobe; R-Right lobe.

Table-2.2 Biochemical variations during different phases of 4-day estrous cycles in rat.

| PARAMETERS | PROESTROUS | | ESTROUS | | METESTROUS | | DIESTROUS | |
|---|---|---|---|---|---|---|---|---|
| | Sp | R | Sp | R | Sp | R | Sp | R |
| Total protein mg/100 mg of fresh tissue | 21.265 ± 0.394 | 21.691 ± 0.564 | 18.990 ± 0.384 | 19.998 ± 0.481 | 26.134 ± 0.217 | 26.190 ± 0.443 | 21.516 ± 0.936 | 23.556 ± 0.699 |
| 5'-nucleotidase μg $PO_4$ released/mg protein /1 h at 37°C | 17.143 ± 0.428 | 17.147 ± 0.317 | 17.401 ± 0.494 | 16.163 ± 0.634 | 22.881 ± 0.199 | 15.547 ± 0.476 | 23.474 ± 0.309 | 23.953 ± 0.574 |
| Deoxyribonucleic acid (DNA) μg/mg tissue | 0.341 ± 0.005 | 0.324 ± 0.004 | 0.324 ± 0.007 | 0.350 ± 0.004 | 0.427 ± 0.020 | 0.427 ± 0.017 | 0.302 ± 0.014 | 0.351 ± 0.008 |
| Ribonucleic acid (RNA) μg/mg tissue | 0.207 ± 0.002 | 0.237 ± 0.007 | 0.138 ± 0.008 | 0.176 ± 0.008 | 0.209 ± 0.008 | 0.295 ± 0.015 | 0.214 ± 0.014 | 0.217 ± 0.009 |
| Total lipid mg/100 mg tissue | 5.074 ± 0.176 | 5.523 ± 0.287 | 5.324 ± 0.409 | 5.532 ± 0.326 | 7.841 ± 0.312 | 8.518 ± 0.356 | 9.161 ± 0.847 | 9.022 ± 0.694 |
| Total cholesterol mg/100 mg tissue | 0.460 ± 0.011 | 0.563 ± 0.012 | 0.483 ± 0.007 | 0.459 ± 0.007 | 0.669 ± 0.012 | 0.692 ± 0.019 | 1.329 ± 0.053 | 1.149 ± 0.103 |
| Cholesterol % of total lipid | 9.068 ± 0.236 | 10.558 ± 0.281 | 9.076 ± 0.133 | 8.322 ± 0.151 | 8.567 ± 0.165 | 8.131 ± 0.225 | 14.511 ± 0.415 | 12.746 ± 0.571 |

Each value is mean ± SE of at least eight animals. Sp-Spigelian lobe; R-Right lobe.

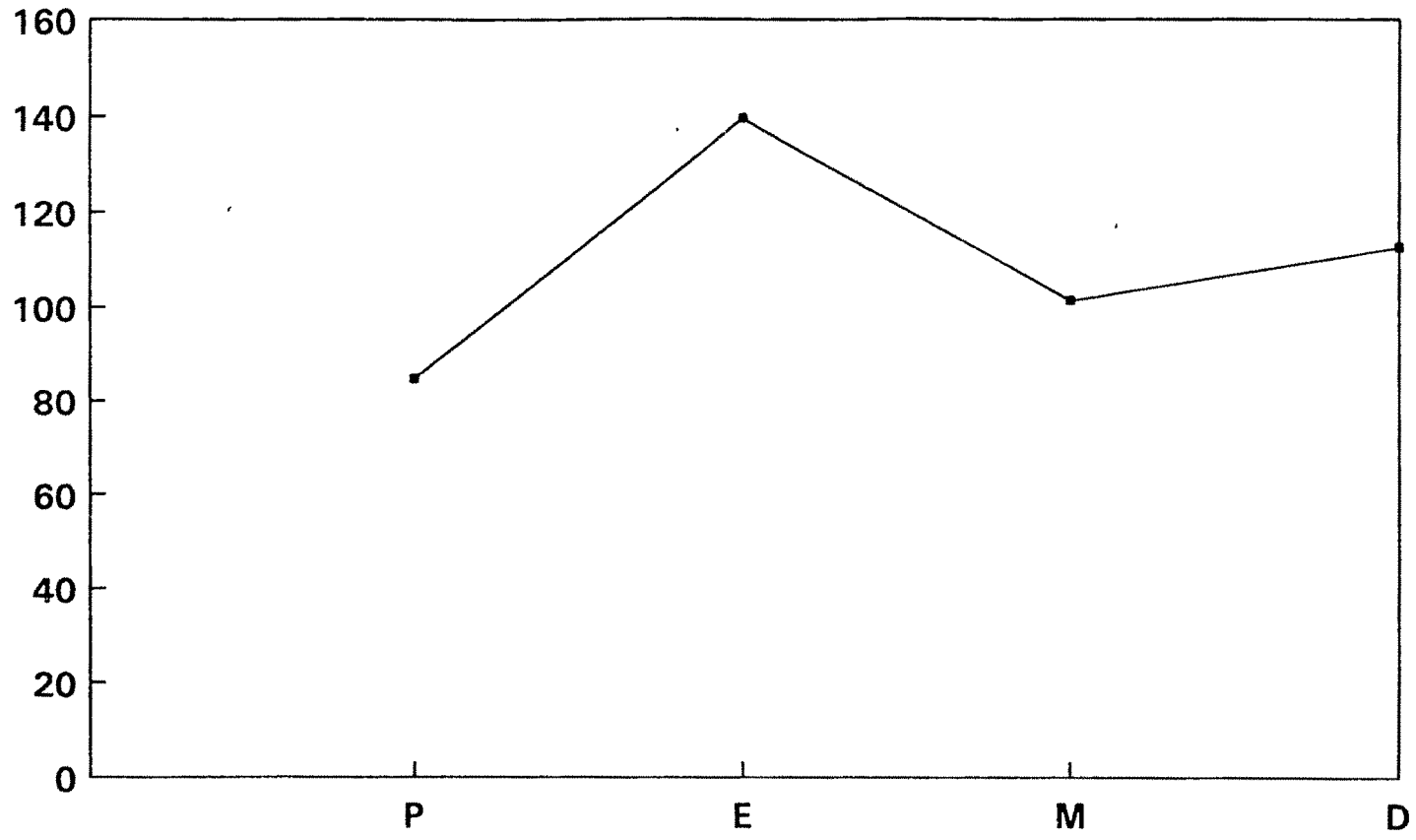


FIG. 1.

PLASMA GLUCOSE

mg/100ml of plasma

P - PROESTROUS E - ESTROUS M - METESTROUS D - DISTROUS

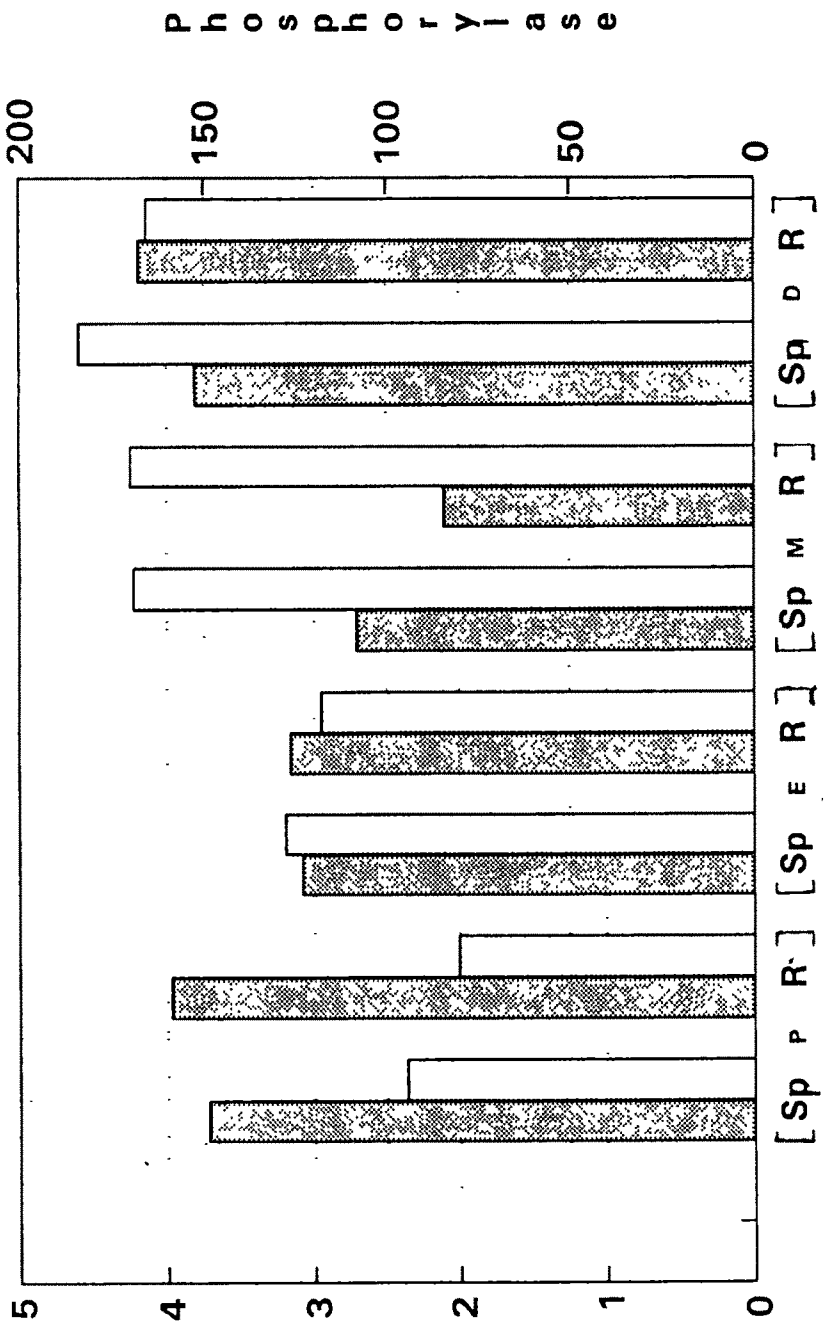
Phosphorylase
200
150
100
50
0
Glycogen
5
4
3
2
1
0
[Sp P R]
[Sp E R]
[Sp M R]
[Sp D R]
GLYCOGEN
PHOSPHORYLASE
mg/100 mg fresh tissue
µg PO4 released /mg protein / 60 min at 37°C

FIG. 2.

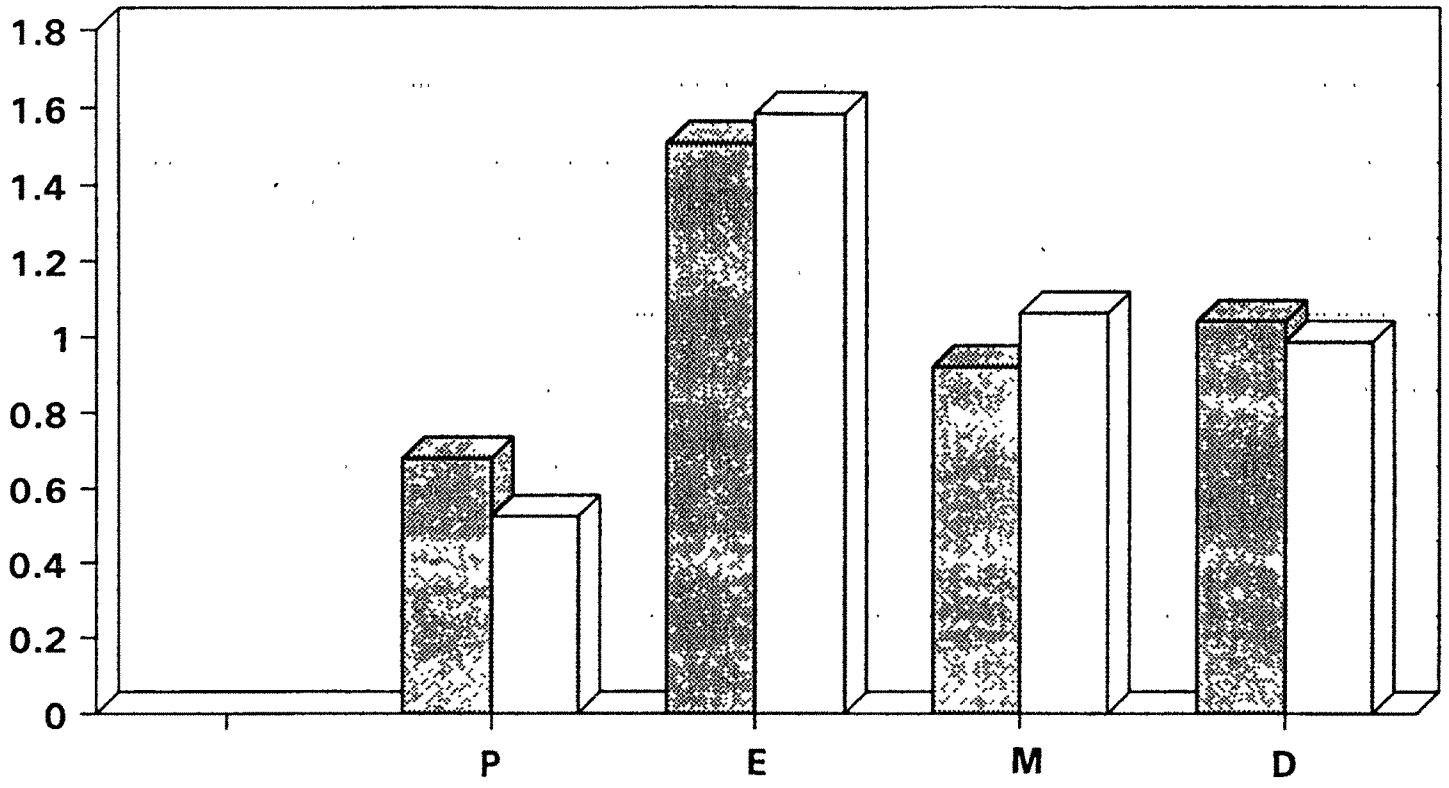
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
P
E
M
D
G-6-Pase
Sp
R
µmole PO4 released / mg protein / 60 min at 37°C

FIG. 3.

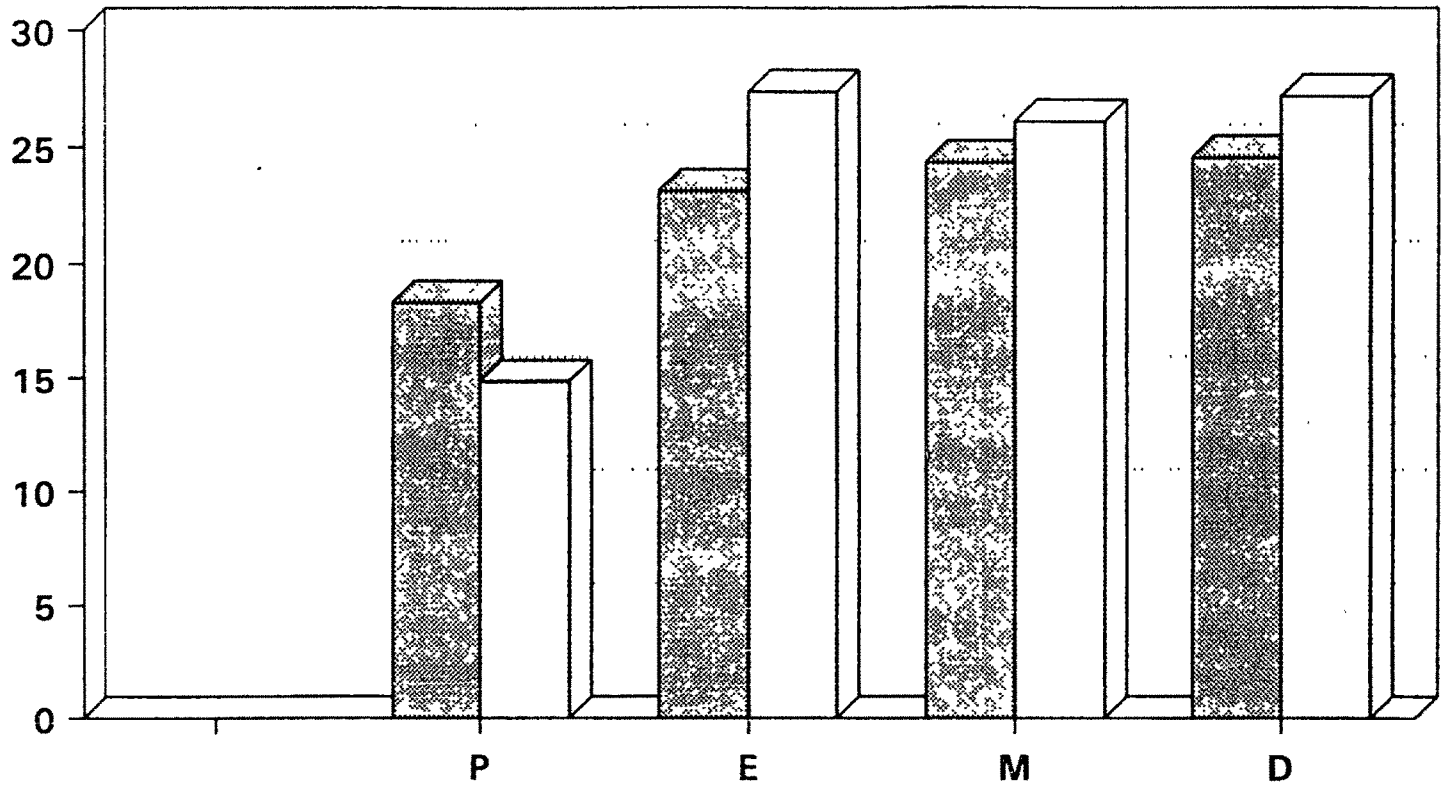


FIG. 4. SUCCINATE DEHYDROGENASE

Sp R

µg formozan formed /mg protein / 60 min at 37°C

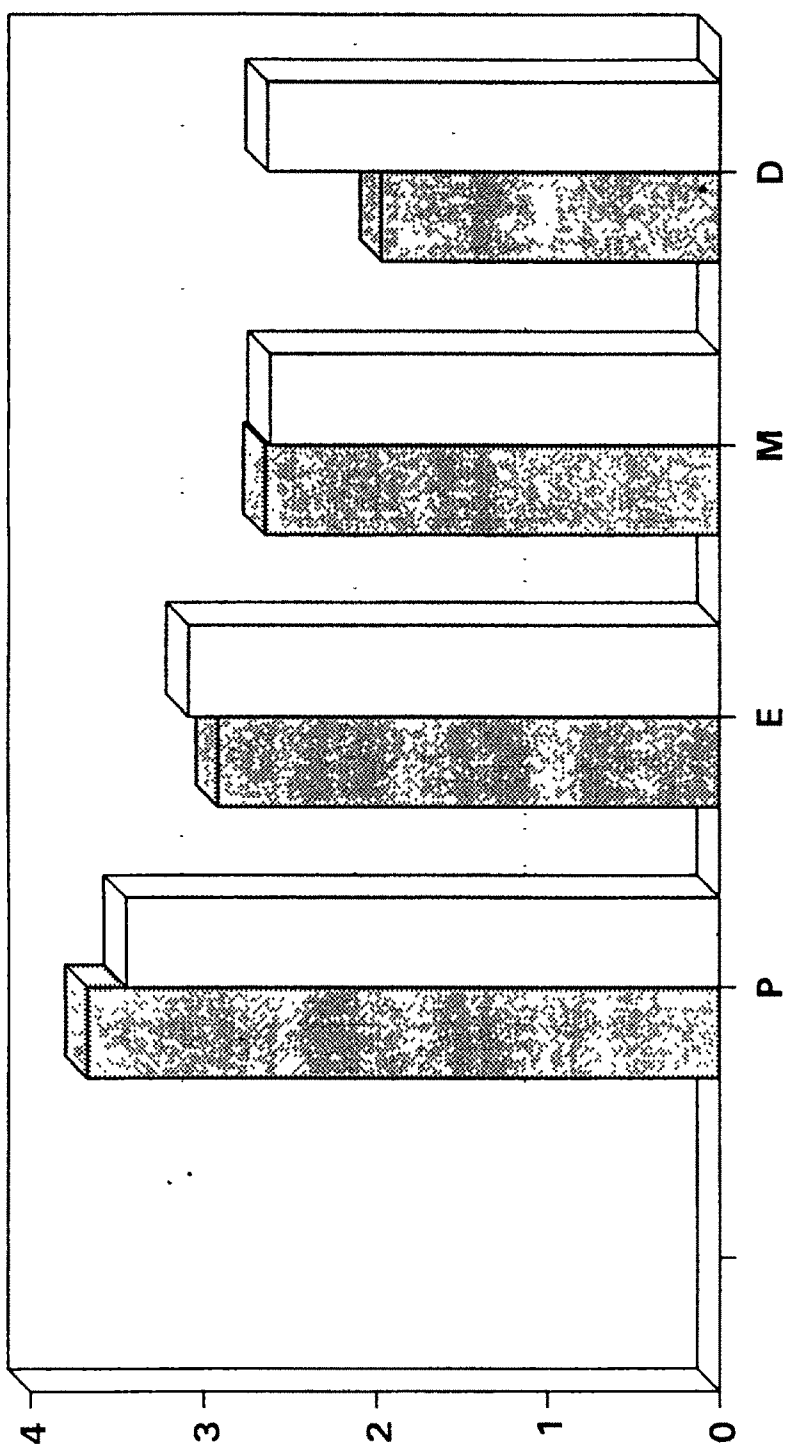
4
3
2
1
0
P
E
M
D
Sp
R
cAMP-PDE
µg phosphate released /mg protein/60 min at 37°C

FIG. 5.

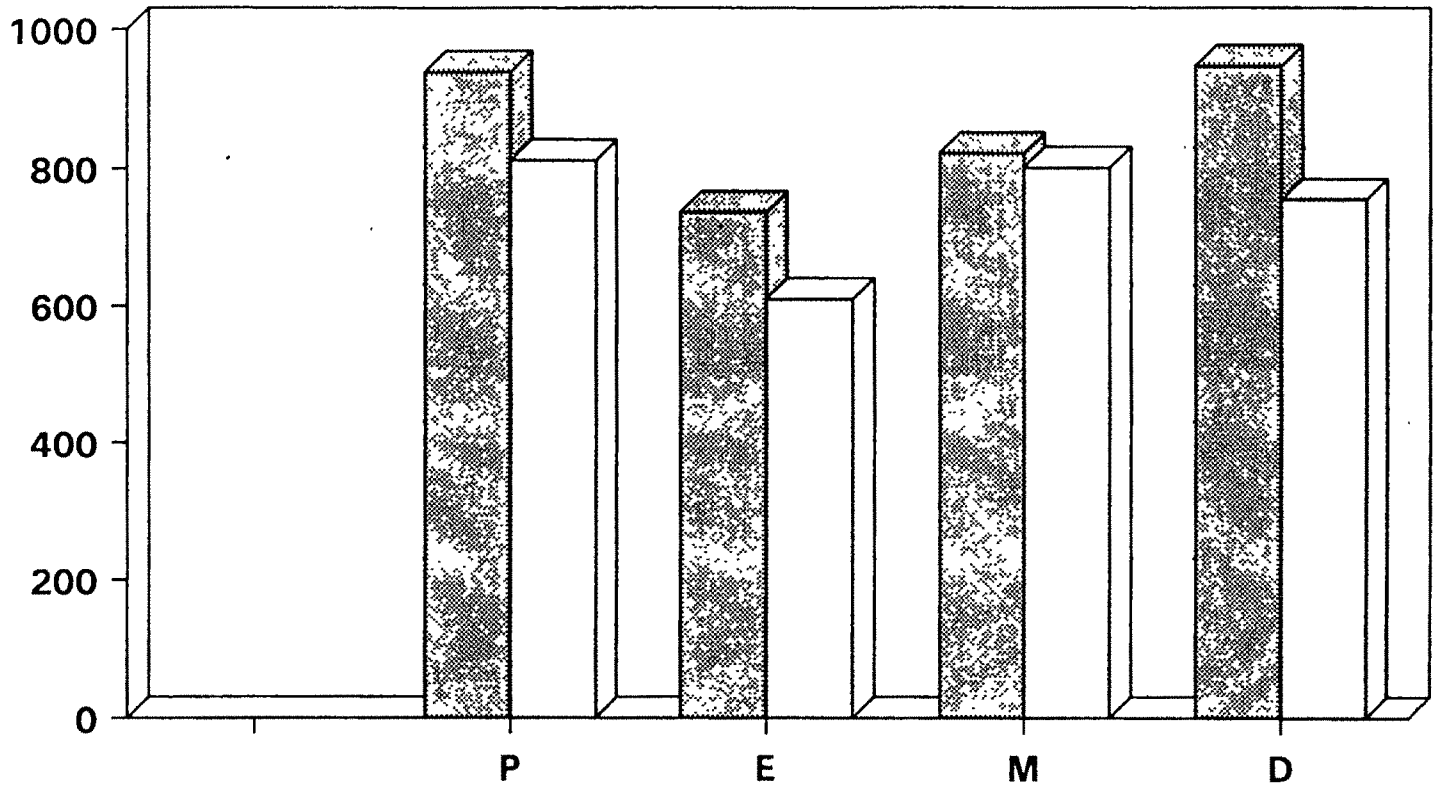
1000
800
600
400
200
0
P
E
M
D
TOTAL ATPase
Sp
R
µg PO4 released / mg protein / 60 min at 37°C

FIG. 6.

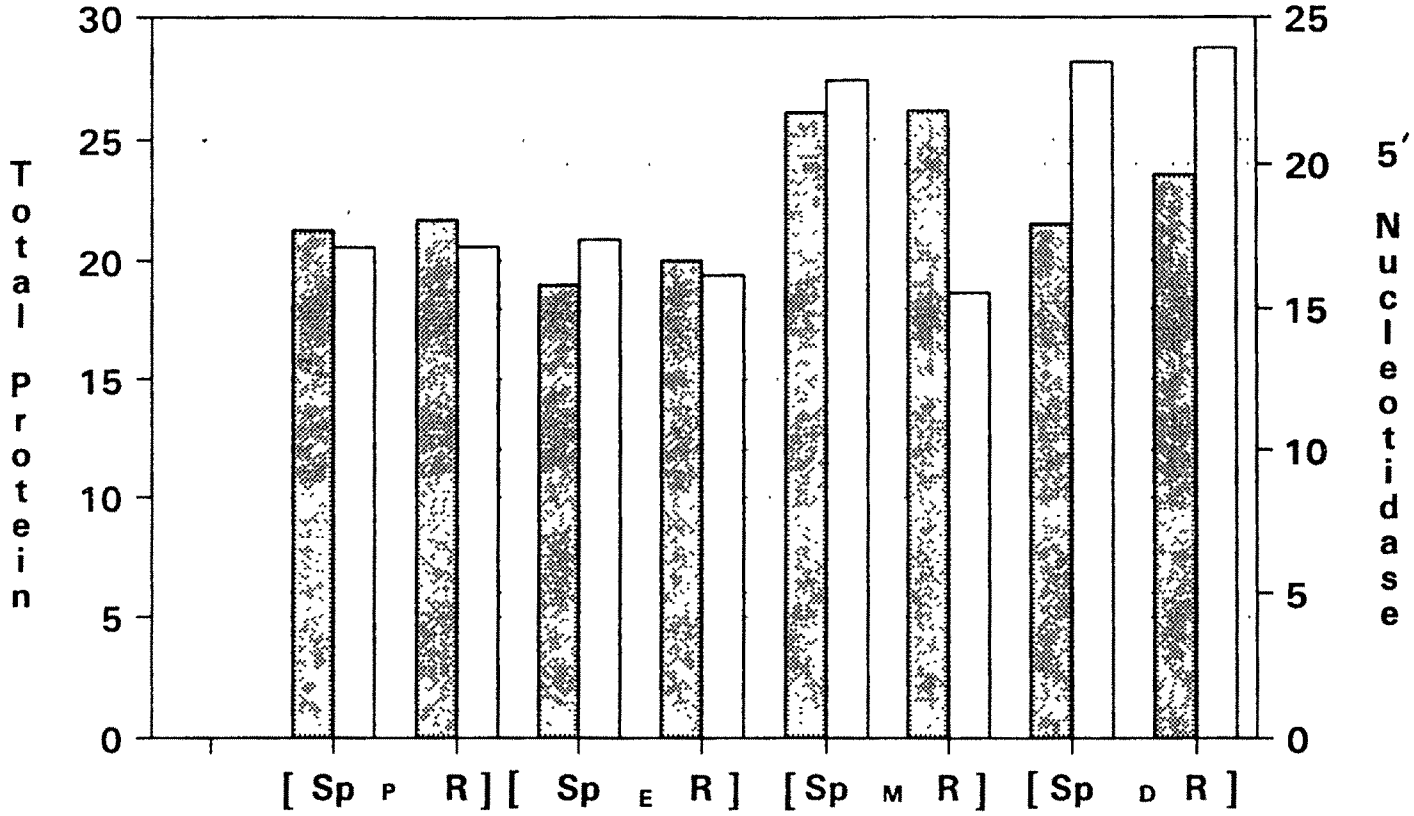


FIG. 7. TOTAL PROTEIN 5'-NUCLEOTIDASE

mg /100mg fresh tissue

μg PO4 released / mg protein / 60 min at 37°C

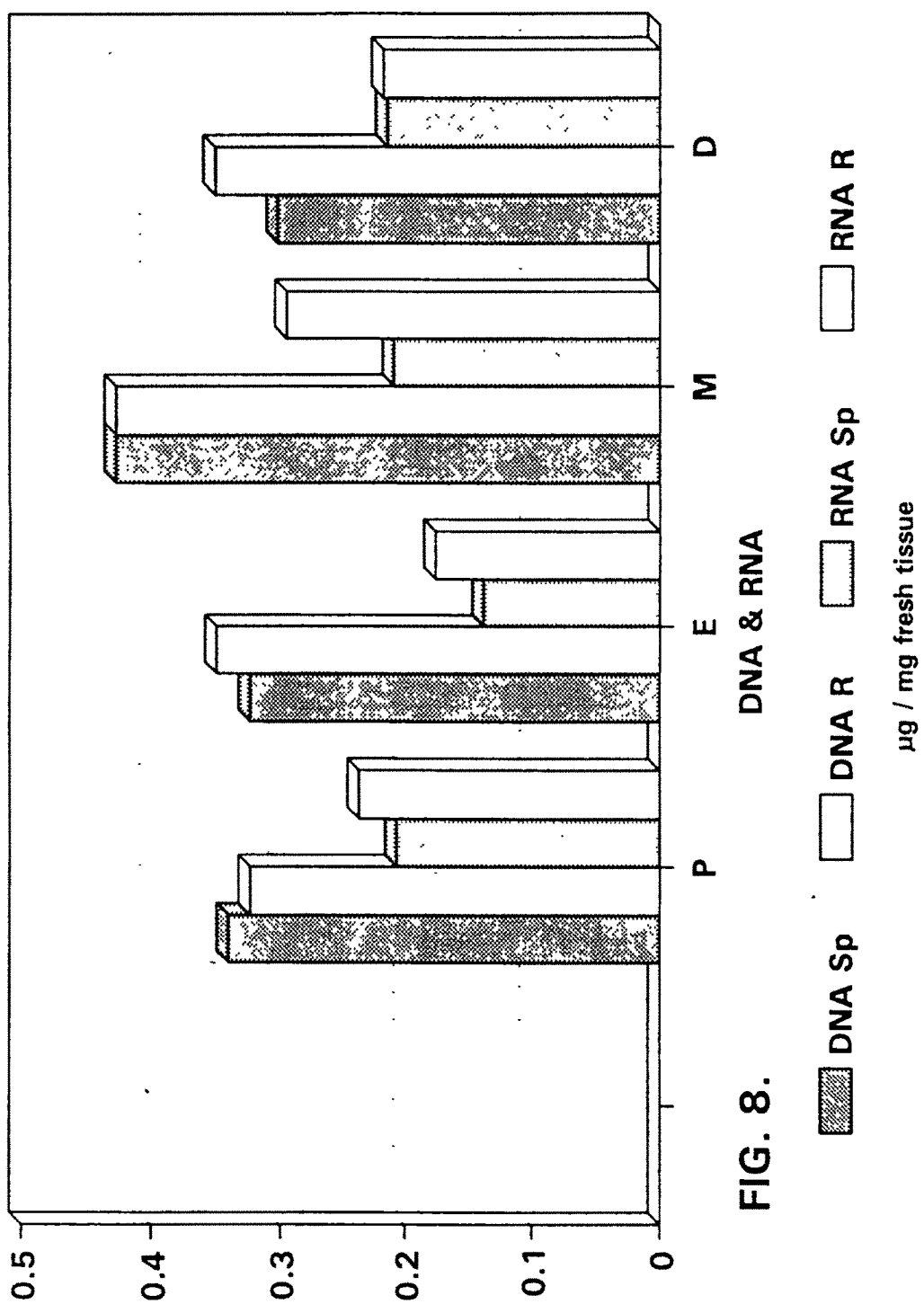
0.5
0.4
0.3
0.2
0.1
0
P
E
M
D
DNA & RNA
µg / mg fresh tissue
DNA Sp
DNA R
RNA Sp
RNA R

FIG. 8.

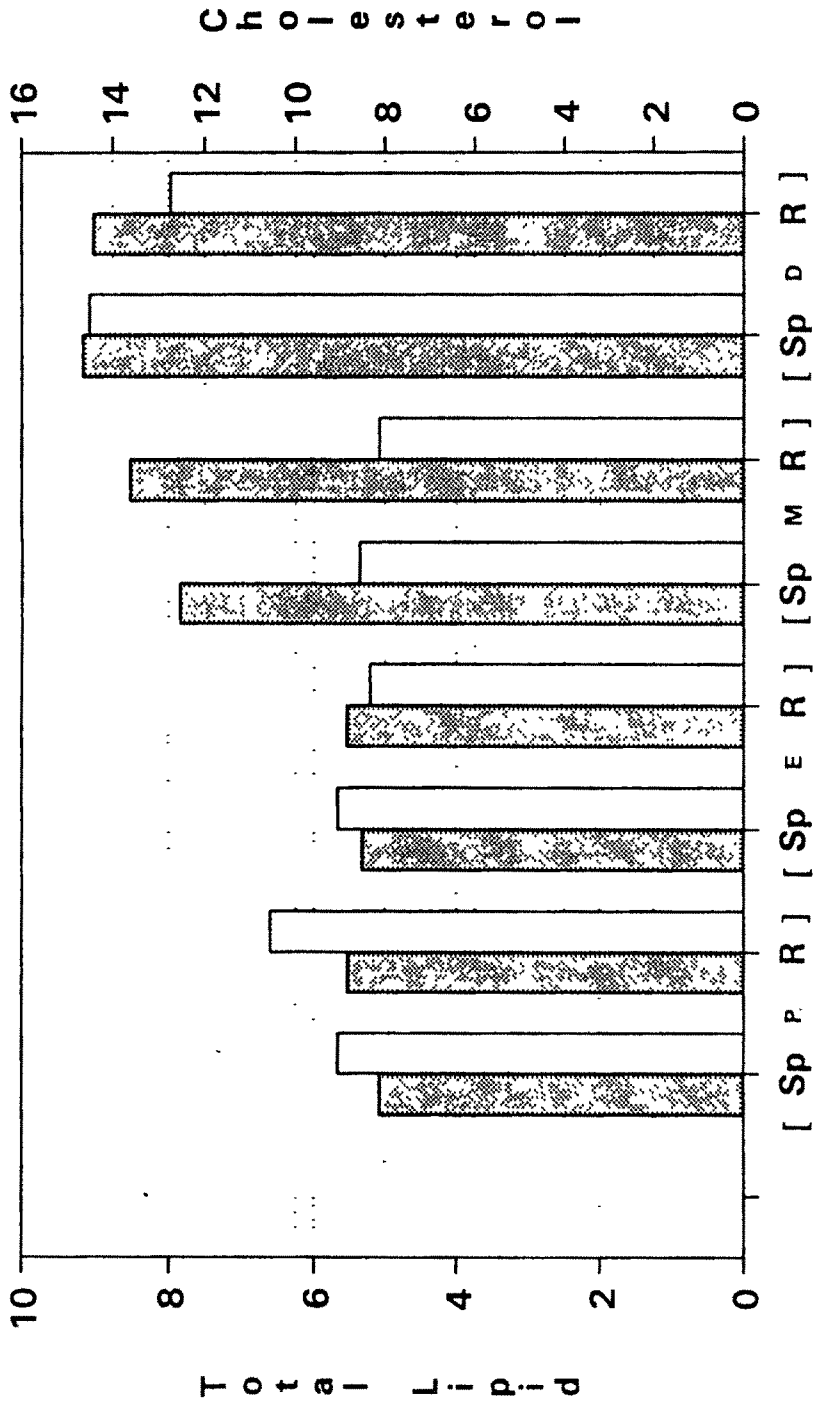
Cholesterol
16
14
12
10
8
6
4
2
0
Total Lipid
10
8
6
4
2
0
[ Sp P R ]
[ Sp E R ]
[ Sp M R ]
[ Sp D R ]
TOTAL LIPID
mg/100mg Fresh Tissue
CHOLESTEROL
mg/100mg lipid

FIG. 9.

the cycle (Fig.6). This enzyme activity was higher during diestrous as well as proestrous than at other two phases.

Hepatic protein concentration was highest at metestrous and showed gradual reduction from diestrous to estrous phase when it was at its lowest (Fig.7). DNA level too, was highest at metestrous, during the rest of the phases no significant variation was noted. RNA concentration in right lobe of the liver was always more than that of spigelian (Fig.8). The lowest concentration in both the liver lobes was observed during estrous phase. 5'-nucleotidase activity was highest in both liver lobes at diestrous phase and a successively decreasing trend was observable during the proestrous and estrous phase but only spigelian lobe showed increase in the enzyme activity at metestrous (Fig.7).

Total lipid concentration was highest at diestrous phase. Marked reduction was observed during proestrous and estrous phase, but then a rise was noted at metestrous. Cholesterol concentration was highest at diestrous and gradual decrease was observed during proestrous and estrous but the levels started rising during metestrous phase (Fig.9).

## DISCUSSION

The present investigation demonstrated marked alterations in hepatic metabolic patterns during 4-day estrous cycle of rat. Variation in plasma glucose level and hepatic glycogen along with enzyme activities viz- glycogen phosphorylase, G-6-Pase, c-AMP-PDE, SDH and ATPase during different phases of estrous cycle clearly demonstrated influence of varying profiles of ovarian steroids through estrous cycle on hepatic carbohydrate metabolism. Sladek (1974) has reported that the fluctuations in plasma levels of the ovarian steroids during estrous cycle are sufficient to cause alterations in gluconeogenesis and hepatic glycogen metabolism. He has also demonstrated that the cyclic alterations of gonadal hormones may bring about observed changes due to their influence on pancreatic as well as adrenocortical secretions.

Variations in plasma glucose level and those in G-6-Pase activity in liver are obviously interdependent. This was evident from increased G-6-Pase activity at estrous, metestrous and diestrous phases as reflected in increased plasma glucose, and lowering of both these parameters during proestrous. Phosphorylase is also known to be catabolic enzyme for hepatic glycogen. Gradually decreasing hepatic glycogen concentration from proestrous to metestrous accompanied by, as would be expected, gradually rising level of phosphorylase enzyme activity indicated the influence of normal cyclic titers of female sex

hormones acting in concert. However, during the diestrous phase both these parameters registered higher levels. This might have probably been due to minimal levels of sex hormones during diestrous phase.

Variation in cAMP-PDE activity are known to have direct bearing on the intracellular level of cAMP, as has been stated by Rindi (1971). Further, he has also shown that intracellular cAMP stimulates glycogen phosphorylase activation and thereby altering carbohydrate metabolism in liver. Further Higazi and Kvinsland (1974) have already shown that estradiol-17B promotes the cAMP-PDE activity in the cervicovaginal epithelium of neonatal mice. In the light of these observations it can be said that present results are in conformity with these observations, and the corresponding variations in cAMP-PDE activity during different phases of estrous cycle indicate concomitant increase or decrease in ovarian hormones and cAMP levels and therefore phosphorylase activity and hepatic glycogen variations. For example, the highest cAMP-PDE activity during proestrous is promoted by higher level of plasma estradiol and hence the subsequent changes.

As far as succinate dehydrogenase activity is concerned it becomes obvious that comparatively high level of estrogenic hormones, during proestrous phase, apparently suppresses this enzyme activity at least in liver. This is in contrast with reported stimulation of SDH activity by estradiol in the uterine tissue (Eckstein and Villee, 1966). This is, in all probability, an example of differential organ-specific response. It may be added here that, during the rest of the phases when estrogen titers are lower, the SDH activity was at its normal without any noticeable variations. Spigelian lobe showed higher ATPase activity than that of right lobe during all the phases. Lobe wise differential response to varying ovarian hormones was obvious only during proestrous phase with respect to total ATPase activity, that of right lobe being enhanced.

From the values obtained during normal estrous cyclicity it was evident that initial action of high estrogenic level at proestrous get belatedly manifested as depletion of protein during estrous phase. This finds further support in lower concentration of nucleic acids, particularly that of RNA, during this phase of the cycle. In a different context, it was suggested by Aschkenassy-lelu and Aschkenassy (1959) that high levels of estrogens are generally catabolic in their effect. Further, it could be seen during metestrous phase that an already estrogen- primed tissue coming under increasing influence of progesterone exhibited rise in hepatic protein concentration. The increased DNA and RNA levels during metestrous could be said to lend support to rising hepatic protein.

It would be pertinent at this stage of discussion to briefly review the recent literature on mechanism of stimulation of protein biosynthesis by estrogens. $E_2$ has been shown to regulate synthesis of uterine protein at transcriptional and translational levels (Rasmussen *et al.*, 1988). Further, these authors stated that $E_2$ induces an increase in specific amino acid accepting activities of uterine RNA's, with the largest increase seen for proline, glycine and methionine. The synthesis of three types of protein, that are rich in proline and glycine viz:- estrogen receptor, progesterone receptor and glucose-6-phosphate dehydrogenase, is induced by $E_2$ (Rasmussen *et al.*, 1988). However, influence of endogenous ovarian steroids on liver could differ in some manner from uterine tissue. This supposition finds corroboration in the work of Mataradze and Gontar, (1986). These authors have demonstrated some organ-specific peculiarities in respect of the process of translocation of estrogen receptors from cytosol to nuclei in both hepatic and uterine tissues under the influence of $E_2$. Low levels of hepatic lipid during proestrous and estrous phases indicates the possibility of lipid mobilizing action of $E_2$ which is in conformity with result reported by Aftergood and Alfin-Slater, (1965) and Biswas and Mukerjea (1973). Probably with waning titers of gonadal hormones from metestrous to diestrous phase favour accumulation of hepatic lipids; exhibiting maximum effect of such an influence when the levels of sex hormones in the blood are reported to be lowest (Butcher *et al* ., 1974). The pattern of variation of cholesterol was almost parallel to that of lipids. From this it could be said that ratio of these two component is seemingly maintained, irrespective of slight variations, by circulating ovarian hormones.

By way of summary, two points can be mentioned. One, most of the parameters under investigation are sensitive to cyclic variations of ovarians hormones. Two, there exists a difference in responsiveness between the right and spigelian lobe of the rat liver, the latter being more sensitive.