CHAPTER-5

EFFECT OF SPAYING AND SUBSEQUENT HORMONE REPLACEMENT ON RAT LIVER LIPIDS

Interest in aspects of biochemistry and physiological actions of ovarian hormones has been increasing in importance. Several synthetic steroids are being widely used as oral contraceptives by ever increasing human female population all round the world. Since the liver is a well known primary organ involved in processes of biotransformation and inactivation of hormones, it is possible that before these synthetic hormonal preparations are biotransformed by the liver they may induce metabolic actions which may influence directly or indirectly the homeostatic function of liver.

The work of Aizawa and Mueller (1961) demonstrated that increased lipid synthesis is one of the earliest and most dramatic responses of rat uterus to administration of estrogenic hormones. It is known that both endogenous and exogenous sex steroids are capable of altering cholesterol, triglyceride and lipoprotein levels in the serum (Bradley *et al.*, 1978; Knopp *et al.*, 1982; Powell *et al.*, 1984). It has been shown that effects of sex steroids on plasma lipid concentrations reflect partly corresponding alterations in the rate of hepatic biosynthesis of triglyceride-rich very low density lipoproteins (VLDL) (Ockner *et al.*, 1978). Way back in 1934, Okey *et al.* reported that lipids in the liver of female rats exhibit consistently higher levels as compared to those of males. However, the total cholesterol levels were found to be higher in males. The results obtained by Okey *et al.* (1934) were later confirmed by Barnes *et al.* (1941). The latter have further noted that the higher lipid values in the females were confined to acetone-soluble fraction only and were not observed in the phospholipid fraction.

Numerous reports are available from the literature concerning sex-dependent differences in patterns of lipid metabolism of the rats. It has been observed that female rats have higher plasma cholesterol but lower liver cholesterol levels than do the male rats (Aftergood *et al.*, 1957; Fillios, 1957). Later, Fillios *et al.* (1958) observed that female animals have higher rates of hepatic cholesterol synthesis, and that, administration of estradiol to males induces increase in biosynthesis of cholesterol by the liver. Further Coleman *et al.* (1958) reported that the rate of hepatic cholesterol biosynthesis is lowered after gonadectomy of female rats. Among other aspects of sex hormones on lipid metabolism it has been shown that mitochondria from intact female rats oxidized cholesterol to a greater

extent than those from intact males (Kritchevsky *et al.*, 1963). The fatty acid composition of liver lipids has also been shown to differ between the two sexes (Okey *et al.*, 1961, Okey *et al.* 1962).

Letterie *et al.*, (1988) have studied the effect of oral contraceptives on hepatic cholesterol metabolism, specifically on 3-hydroxy 3-methyl glutaryl co-enzyme A (HMG) reductase. It was found by these authors that hepatic HMG reductase activity was increased significantly with combination dose of ethenyl estradiol and norgestrel. According to them, these results demonstrate that estrogenic component may be related to specific induction of HMG reductase activity in liver and subsequently alter the rate of cellular cholesterol synthesis or metabolism. Very recently Kose *et al.* (1993a) have reported that the synthetic oral contraceptive steroids, affect hepatic cholesterol metabolism. They have shown different doses and durations increased HMG-CoA synth ase and Ac.Ac-CoA thiolase enzyme activities. Short term treatment may increase total cholesterol levels and this with lapse of time may lead to a suppression of the same enzyme activities, through an accumulatory influence. The long period treatments with same hormonal compounds were observed by these authors either not to influence the hepatic cholesterol synthesis or to increase the same.

Taking into consideration the foregoing account about reports regarding the effect of sex hormones on hepatic lipid metabolism, it can be seen that most of the studies were carried out to investigate long term effects of gonadectomy and prolonged/repeated exogenous hormone therapy on hepatic lipids. Earlier work carried out in this laboratory on male rats has shown that effect of castration on hepatic lipid metabolism could be ascertained during 24 to 72 h and that majority of alterations were manifested by 48 h post-operatively (Ambadkar and Gangaramani, 1980). Further, it was also shown that influence of androgen replacement becomes evident after 24 h of treatment (Ambadkar and Gangaramani, 1981). Further, these studies brought out that the spigelian lobe of liver exhibited differential sensitivity to experimental manipulation of hormones that as compared the other lobes. The latter among themselves were not found to differ significantly. Hence, the present investigation was undertaken to know whether sex-dependent differences are existing in hepatic lipids. In addition to this effects of ovariectomy at the short intervals of 24, 48 and 72 h and rapid effect of estrogen and estrogen plus progesterone treatment on total lipids and cholesterol concentration of liver of female rat were studied.

MATERIAL AND METHODS

The adult female albino rats (140 $\pm$ 20g B.W) were used as experimental animals. The experimental setup for the present study was similar to that described in Chapter-2.

The total lipid (gravimetrically) and cholesterol concentration were measured in both the liver lobes (Spigelian and right lobe), according to methods described in Chapter-1.

RESULTS

Ovariectomy:- Table-5.1 showed the values obtained 24, 48 and 72 h after ovariectomy. Values obtained from sham-operated females are not given as the sham-operation was found not to affect the normal 4-day estrous cycle.

The total lipid concentration of spigelian lobe was lowered non significantly by 24 h, but thereafter there was remarkable but graded increase upto 72 h. On the other hand, the right lobe was not observed to vary significantly at 24 and 48 h intervals but did show a significant increase after 72 h (Fig.37). The cholesterol level, expressed as percentage of total lipids, registered remarkable rise by 24 h interval in both lobes. At later two interval the cholesterol percentage of both the liver lobes showed sudden lowering. However, such decrease lead to normalization in the case of right lobe but that of the spigelian lobe was seen to step down below normal level.

OVX + E_2 treated animals:- At all post injection intervals of administration of D_1 it was noticed that there was a general tendency towards significant lowering of total lipid levels whereas there was a corresponding reverse influence on cholesterol percentage (Fig.38).

With regard to total lipid concentration versus cholesterol in liver it can be seen that D_2 replacement further substantiated the decrease in total lipid and increase in cholesterol percentage; particularly so at the 2 h interval. However, cholesterol concentration showed signs of coming off the E_2 influence by 4 h interval, but that was not apparent as far as total lipid levels were concerned (Fig.38 & Fig.39).

Table-5.1 Depicting effects of ovariectomy and subsequent estradiol replacements in 48 h OVX females on total lipid and cholesterol levels.

EXPERIMENTAL TREATMENTS	Total lipid mg/100 mg tissue		Total cholesterol mg/ 100 mg tissue		Cholesterol % of total lipid	
	SP	R	SP	R	SP	R
INTACT FEMALE DURING DIESTROUS	9.161 ± 0.847	9.022 ± 0.694	1.392 ± 0.053	1.149 ± 0.103	14.511 ± 0.415	12.746 ± 0.571
24 h OVX	8.035 ± 0.280	9.134 ± 0.171	1.894**** ± 0.091	1.836**** ± 0.045	20.020**** ± 0.591	19.897**** ± 0.493
48 h OVX	11.944**** ± 0.448	8.115 ± 0.346	1.570**** ± 0.062	0.973**** ± 0.072	11.766**** ± 0.481	12.173 ± 0.795
72 h OVX	13.627**** ± 0.933	11.704**** ± 0.290	1.210 ± 0.104	1.961**** ± 0.064	11.831**** ± 0.532	12.316 ± 0.421
48 h OVX + 5 µg E_2 sacrificed after 1 h	8.883**** ± 0.193	8.516 ± 0.283	1.256**** ± 0.041	1.191**** ± 0.019	14.945**** ± 0.745	14.550**** ± 0.514
48 h OVX + 5 µg E_2 sacrificed after 2 h	5.233**** ± 0.196	6.400**** ± 0.258	1.030**** ± 0.100	0.940 ± 0.045	20.139**** ± 0.902	14.350 ± 1.007
48 h OVX + 5 µg E_2 sacrificed after 4 h	4.456**** ± 0.365	7.240* ± 0.302	1.104**** ± 0.027	1.025 ± 0.080	18.433**** ± 0.955	17.413**** ± 1.015
48 h OVX + 10 µg E_2 sacrificed after 1 h	6.941**** ± 0.367	10.587**** ± 0.392	0.891**** ± 0.045	0.974 ± 0.042	12.468 ± 0.874	9.193**** ± 0.398
48 h OVX + 10 µg E_2 sacrificed after 2 h	4.061**** ± 0.352	5.007**** ± 0.148	1.271**** ± 0.049	1.501**** ± 0.102	39.471**** ± 0.587	33.212**** ± 1.071
48 h OVX + 10 µg E_2 sacrificed after 4 h	4.930**** ± 0.323	6.339**** ± 0.281	0.939**** ± 0.032	1.048 ± 0.042	19.737**** ± 0.703	14.629 ± 1.249
48 h OVX + 15 µg E_2 sacrificed after 1 h	7.716**** ± 0.214	7.675 ± 0.283	1.245**** ± 0.072	1.164* ± 0.046	15.005**** ± 0.734	14.487* ± 0.530
48 h OVX + 15 µg E_2 sacrificed after 2 h	9.840**** ± 0.397	10.819**** ± 0.418	1.711 ± 0.165	2.313**** ± 0.088	19.143**** ± 0.596	20.865**** ± 0.927
48 h OVX + 15 µg E_2 sacrificed after 4 h	7.598**** ± 0.296	7.431 ± 0.356	0.841**** ± 0.071	1.171* ± 0.040	11.570 ± 0.484	14.513* ± 0.806

Each value is mean ± SE of eight animals. SP-Spigelian lobe R-Right lobe

* $p < 0.05$ ** $p < 0.025$ *** $p < 0.01$ **** $p < 0.005$.

Table-5.2 Influence of simultaneously administered constant dose of 2 mg P with each of the three doses of E_2 to 48 h OVX females on total lipid and cholesterol levels.

EXPERIMENTAL TREATMENTS	Total lipid mg/100 mg tissue		Total cholesterol mg/ 100 mg tissue		Cholesterol % of total lipid	
	SP	R	SP	R	SP	R
INTACT FEMALE DURING DIESTROUS	9.161 ± 0.847	9.022 ± 0.694	1.392 ± 0.053	1.149 ± 0.103	14.511 ± 0.415	12.746 ± 0.571
48 h OVX	11.944**** ± 0.448	8.115 ± 0.346	1.570**** ± 0.062	0.973**** ± 0.072	11.766**** ± 0.481	12.173 ± 0.795
48 h OVX + 5 µg E_2 2 mg P sacrificed after 2 h	7.381**** ± 0.230	9.908** ± 0.640	1.183**** ± 0.058	1.266* ± 0.105	12.622 ± 0.688	10.633* ± 0.700
48 h OVX + 10 µg E_2 2 mg P sacrificed after 2 h	4.402**** ± 0.326	7.400 ± 0.765	0.784**** ± 0.050	1.032 ± 0.034	14.316**** ± 0.757	15.233* ± 1.115
48 h OVX + 15 µg E_2 2 mg P sacrificed after 2 h	7.954**** ± 0.275	8.814 ± 0.341	1.026**** ± 0.062	1.052 ± 0.045	11.938 ± 0.436	11.955 ± 0.618

Each value is mean ± SE of eight animals. SP-Spigelian lobe R-Right lobe

* $p < 0.05$ ** $p < 0.025$ *** $p < 0.01$ **** $p < 0.005$.

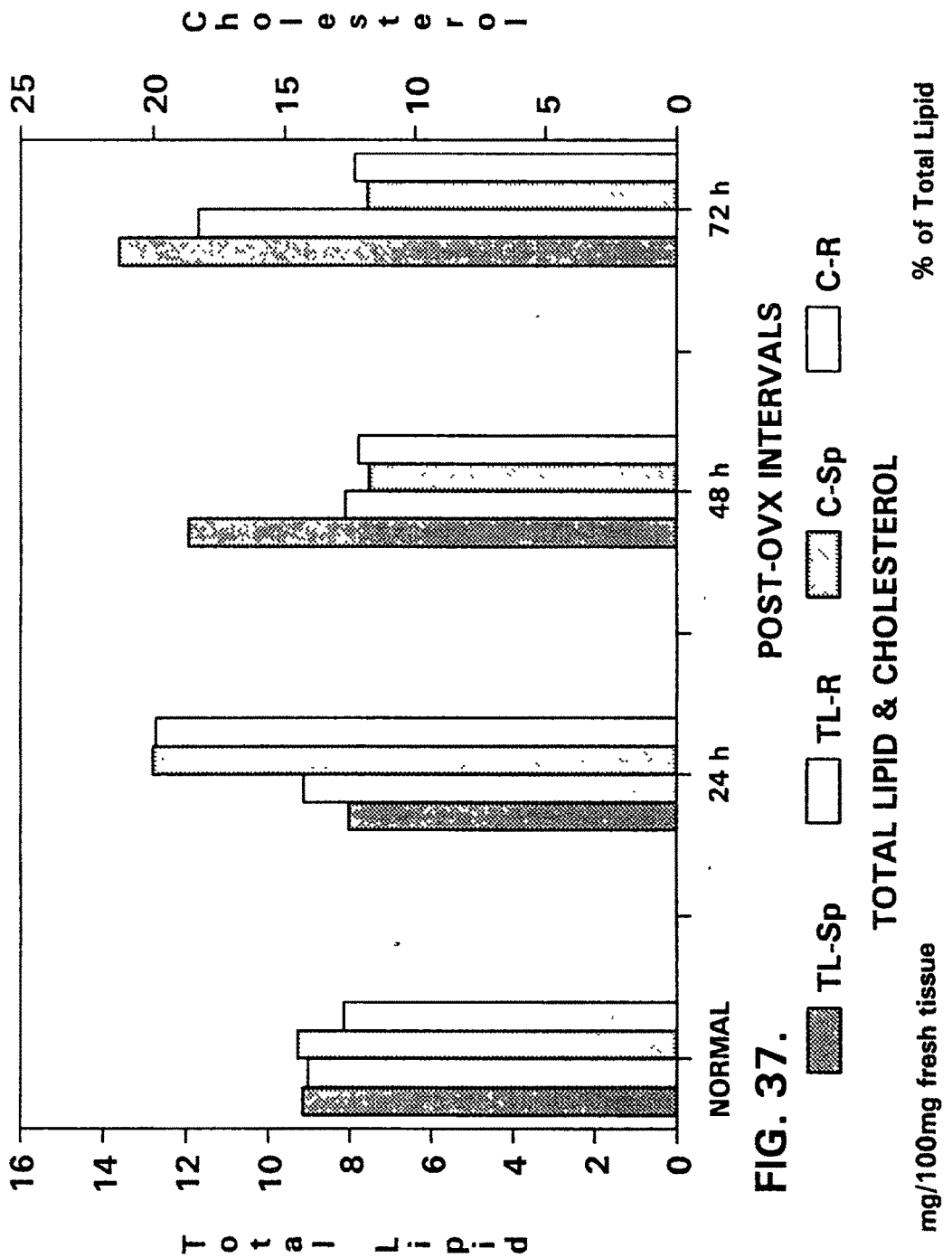
Total Lipid
Cholesterol
16
14
12
10
8
6
4
2
0
25
20
15
10
5
0
NORMAL
24 h
48 h
72 h
POST-OVX INTERVALS
TL-Sp
TL-R
C-Sp
C-R
TOTAL LIPID & CHOLESTEROL
mg/100mg fresh tissue
% of Total Lipid

FIG. 37.

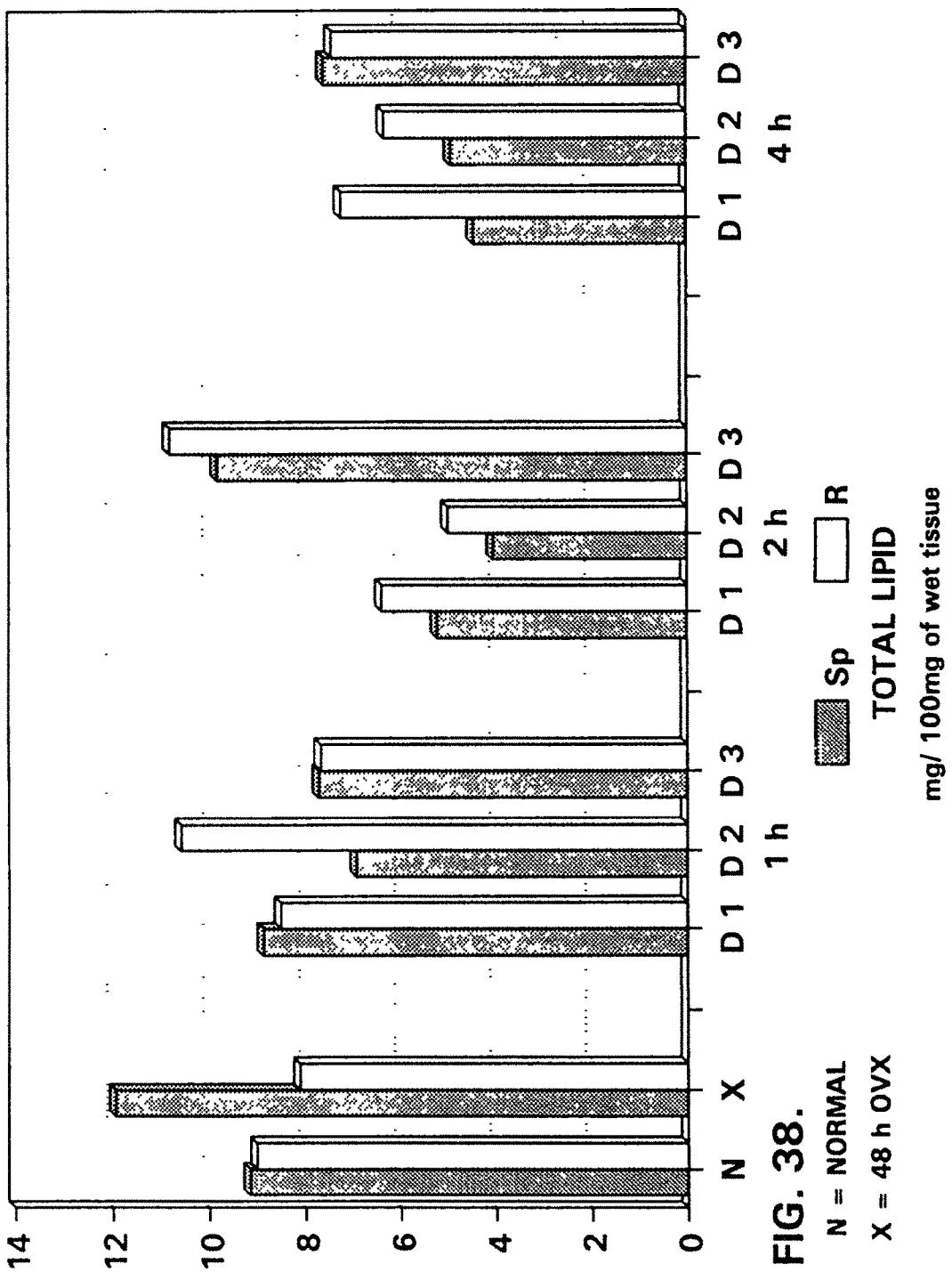

FIG. 38.

N = NORMAL

X = 48 h OVX

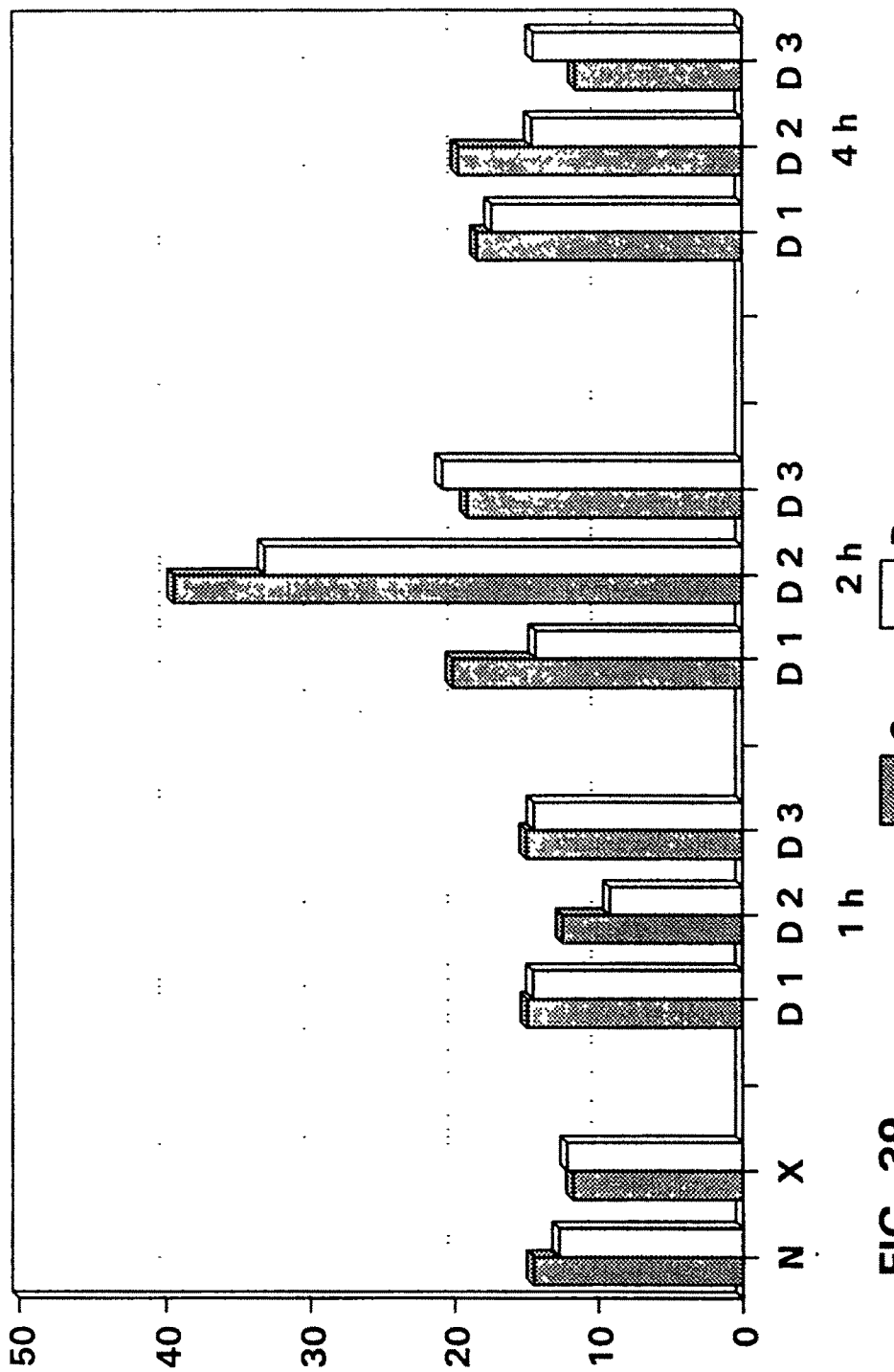
50
40
30
20
10
0
N
X
D 1
D 2
D 3
1 h
D 1
D 2
D 3
2 h
D 1
D 2
D 3
4 h
Sp
R
CHOLESTEROL
% of Total Lipid

FIG. 39.

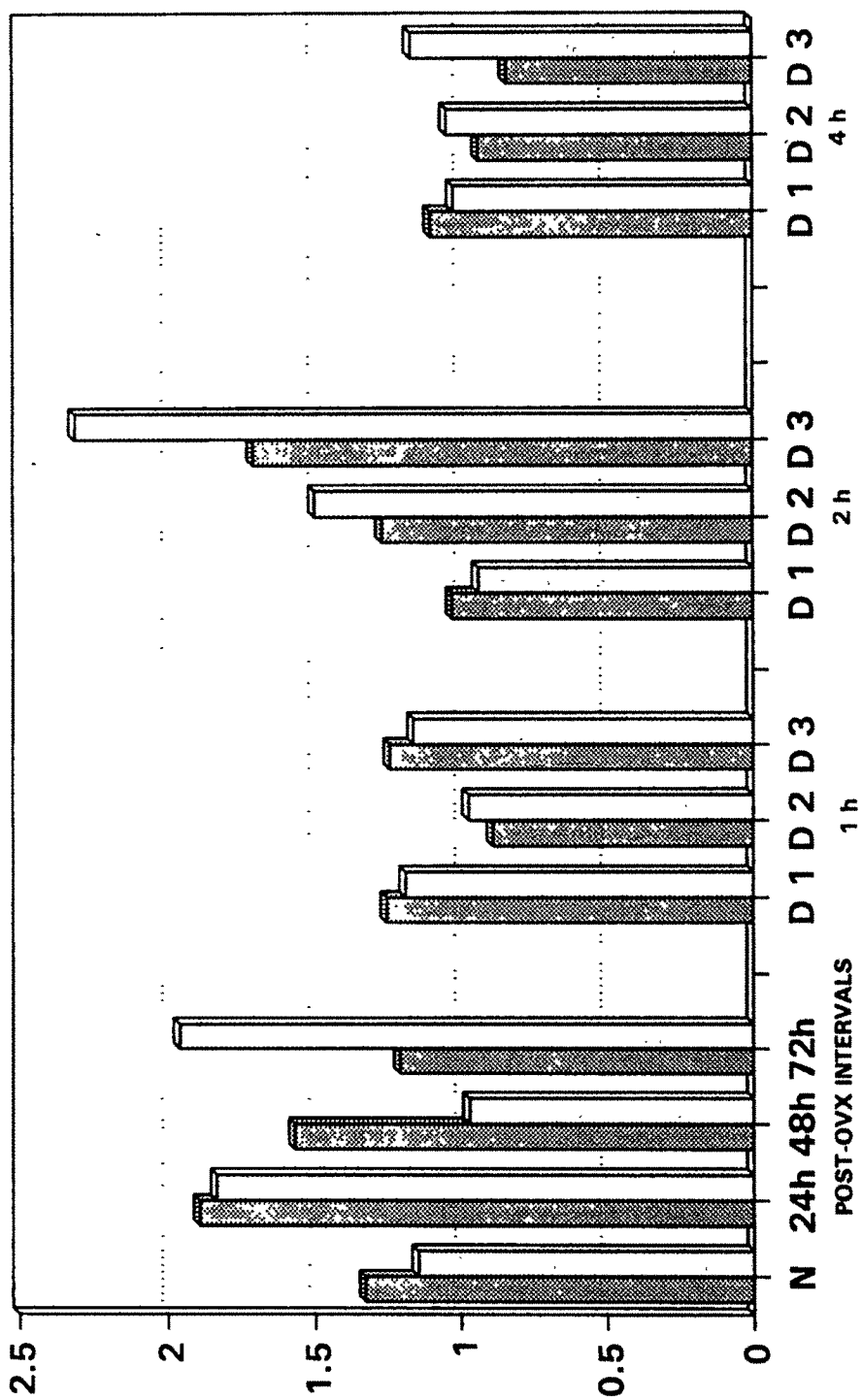
2.5
2
1.5
1
0.5
0
N 24h 48h 72h D 1 D 2 D 3 D 1 D 2 D 3 D 1 D 2 D 3 D 1 D 2 D 3
POST-OVX INTERVALS
1 h
2 h
4 h
Sp
R
TOTAL CHOLESTEROL
mg /100 mg fresh tissue

FIG. 40.

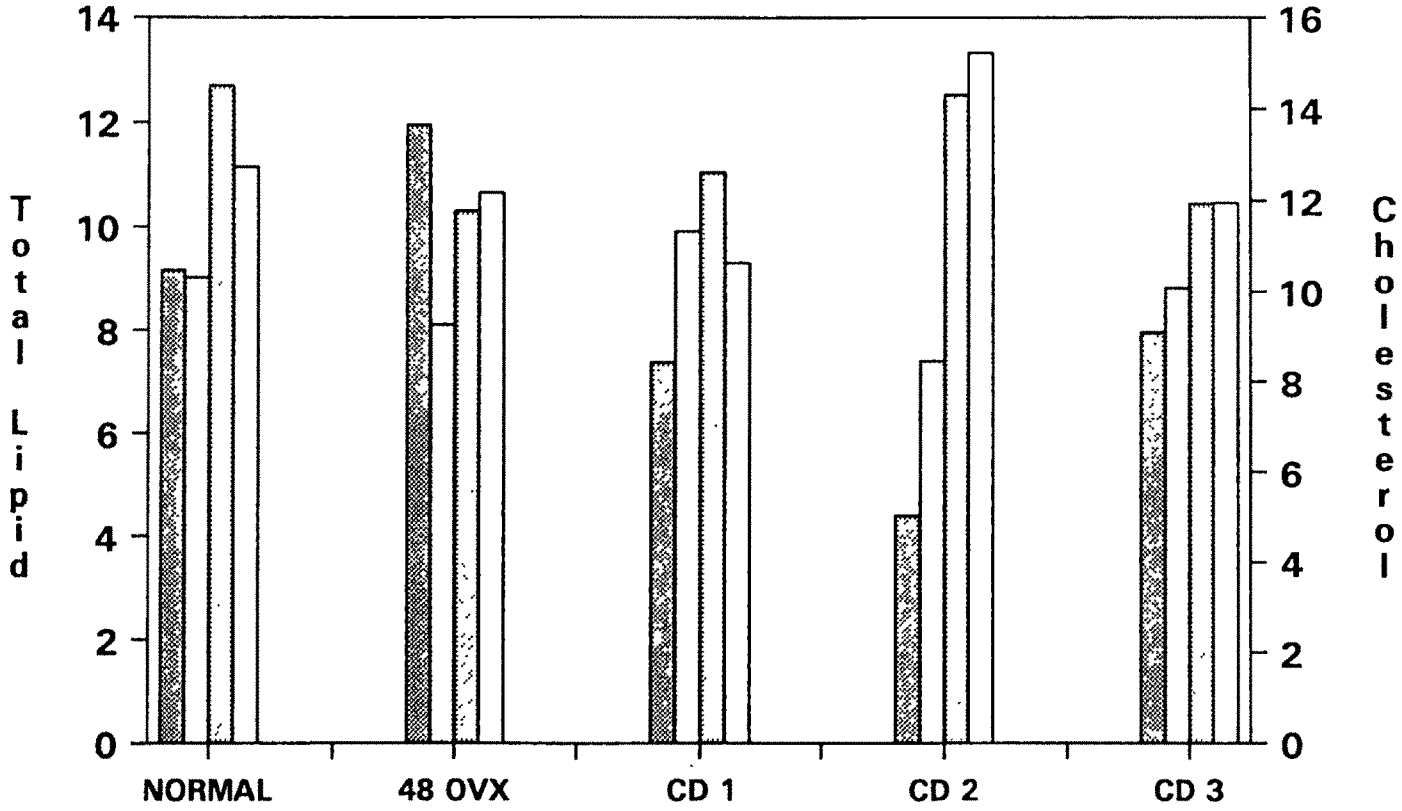
14
12
10
8
6
4
2
0
Total Lipid
16
14
12
10
8
6
4
2
0
Cholesterol
NORMAL
48 OVX
CD 1
CD 2
CD 3
KILLED AFTER 2 h OF INJECTION
TL-Sp
TL-R
C-Sp
C-R
TOTAL LIPID AND CHOLESTEROL
mg/100mg fresh tissue
% of Total Lipid

FIG. 41.

With D_3 it was seen that the patterns of variation of total lipid and cholesterol concentration were more or less similar to that observed with D_2, however the degrees of variation were less intense. Changes at 2 h interval were more obvious.

Simultaneous administration of 2 mg progesterone with 5(CD_1), 10(CD_2) and 15(CD_3) μg of estradiol-17B:- Table-5.5 shows values obtained for lipid and cholesterol concentration after combination dose of E_2 + P except the vehicle treated animals as these values did not differ from those of 48 h post OVX rats.

The total lipid and cholesterol concentration were brought close to normal intact levels after administration of combination doses of CD_1 and CD_3. CD_2 dose was not so effective in restoring either the influence of ovariectomy or altering the effect of E_2 alone. Thus it was seen that the influence of CD_1 and CD_3, was evidently much better than that obtained with E_2 alone (Fig.41).

DISCUSSION

Investigation of the role of the gonadal hormones in lipid metabolism has been receiving increasing attention because of possible involvement of these hormones in coronary atherosclerosis and cardic disorders. Ockner *et al.* (1978) have shown that influence of sex steroids on plasma lipid concentrations and manifestations of actions at the level of hepatic production of triglyceride rich very low density lipoproteins (VLDL). The influence of gonadal hormones on the lipid metabolism in liver of avian species has been extensively studied due to its direct relationship with process of vitelllogenesis (Lorenz, 1954; Raney and Chaikoff, 1951; Pearce, 1977). The activity of hepatic lipogenic enzyme system increases during the onset of sexual maturity, and this specific activity is greater in laying hens than in mature cockrels (Pearce & Brown, 1971). It has been reported (Aftergood and Alfin-Slater, 1965) that ovariectomy leads to significant reduction in hepatic cholesterol synthesis and estradiol replacement restores it. In obvious contrast, present short term observations indicated significant rise in hepatic cholesterol within 24 h of OVX. This could possibly be explained as the immediate influence of lack of normal levels of ovarian hormones. However, the cholesterol level tended to decline thereafter in conformity with reported long term effects (Aftergood and Alfin-Slater, 1965).

Biswas and Mukherjea (1973) have reported heavy increase in cholesterol, total lipids and triglycerides levels after 3-4 weeks of OVX in rats. However, the initial gradual rise in hepatic total lipids after ovariectomy reported herein are therefore of preparatory nature to the report cited above. Thus, it is

apparent that abrupt withdrawal of ovarian sex steroids prepared the biochemical environment facilitating gradual accumulation of total lipid on liver.

In this connection it would be worth considering the suggestion of Mode and Norstedt (1982) that in laboratory rats a kind of hypothalamo-pituitary-liver axis gets entrained during early life due to the presence of circulating female sex hormones in such a way that the liver of female rats comes to possess a metabolic responsivity that is characteristic of female sex. It is, therefore, likely that the alterations in hepatic total lipid and cholesterol concentrations reported here are reflections of influence of lack of ovarian hormones; leading to accumulation of total lipids with decrease in cholesterol in the liver of female rats. It, therefore, appears that there could be a two way influence of OVX on liver lipid profiles. One could be a direct effect on the liver itself,and the other probably acting via hypothalamo-pituitary-liver axis.

As it is of primary importance to know possible side effects of different contraceptive steroid preparations in common use by normal women several authors have concentrated their attention on the influence of exogenously administered ovarian steroids on intact experimental female animals with respect to hepatic lipid metabolism. As opposed to this, scattered attempts have been made on the influence of replacement therapy in gonadectomized animals. In this context the observations presented here deserve due attention. During the course of present work it was observed that E_2 administration to 48 h ovariectomized rats could reverse the trend of lipid accumulation through a lipid metabolising effect on liver. Though the E_2 regimes employed here over different intervals were capable of reversing the effect of ovariectomy it did not lead to normalization but resulted in over suppressive effect leading to levels below normal. When progesterone was administered alongwith E_2 it was seen to have a balancing influence such that the extra effect of E_2 administration alone was reduced. This amounts to say that as would be expected, E_2 as well as P act in concert in normalizing the patterns of hepatic lipid metabolism in female rats.

It is evident from the report published by several workers that there exist sex specific differences in respect of hepatic lipid metabolism. It can be seen that the total lipid (Ockner *et al.*, 1978; Ockner *et al.*, 1980; Patsch *et al.*, 1980) It can be seen that total lipid and cholesterol concentrations in liver of normal intact females are higher (Chapter-2) than that of males (Ambadkar and Gangaramani, 1980). Gonadectomy in both sexes was found to lead to increase in liver lipids. Whereas administration of estrogen to gonadectomized females was observed to decrease and androgen in males was found to induce tendencies for restoration to normal lipid levels. In female rat the combination dose of E_2 + P was noticed to act in much better way in reversing the effects of spaying.

Another note worthy fact that emerged is concerned with differential response to OVX by the spigelian lobe of liver as compared to other lobes represented here by the right lobe. Though this observation was similar to that in case of spigelian lobe of male rats (Ambadkar and Gangaramani, 1980, 1981) the pattern of responses in two sexes appeared to be at variance (see the data in preceding Chapters also). The present observations on female rats therefore, corroborate the findings of Ambadkar and Gangaramani regarding the suggestion that the spigelian lobe of rat liver may prove to be a better indicator of hepatic responses to alterations of gonadal hormones. The present study has also brought forth evidence to indicate that maximum alterations occured in the functional status of liver by 48 h of ovariectomy.