CHAPTER-4

EFFECTS OF OVARIECTOMY AND SUBSEQUENT OVARIAN HORMONE REPLACEMENT ON HEPATIC PROTEIN AND NUCLEIC ACID LEVELS IN ALBINO RAT.

Sex hormones such as 17B-estradiol and progesterone have been shown to induce significant alterations of mammalian cell functions (Knowler and Beaumount, 1985; O'Malley, 1985). These authors have also shown that the sex hormones are known to initiate intracellular processes involving receptor subtypes leading to direct interaction with deoxyribonucleic acid resulting in ribonucleic acid production and *de novo* protein synthesis.

It has been recognized since many years that estrogen modulates synthesis and secretion of several hepatic proteins (Song *et al.*, 1969; Tamulevicius *et al.*, 1982). It is also a proven fact that both DNA and RNA are essentially associated with protein synthesis as well as growth (Wilson, 1962; Vollmer and Kauffman, 1963; William-Ashman and Shimassuki, 1967; Kurtz *et al.*, 1976).

Alfert and Geschwind (1958) demonstrated that the liver parenchyma of adult rats contain five different cell classes. These are distinguished on the basis varying degrees of ploidy. Leeuwin *et al.* (1973) reported that there exist an apparent sex-dependent difference in the DNA concentration of rat liver and that spaying of female did not alter DNA concentration.

Influence of estrogens on protein metabolism has been extensively reviewed by Aschkenassy-Lelu and Aschkenassy (1959). They pointed out that these hormones affect nitrogen balance of rat that varies with dosage. Low levels of estrogens are significantly anabolic but higher levels are distinctly catabolic. It is obvious from previous studies (Fujii and Villee, 1968; Meyol and Thayer, 1970; Stormshak *et al.*, 1976; Engel *et al.*, 1980) that it is not only the dose levels of hormone that count but relation with time also needs to be studied.

According to Munro (1970) hormones play an important role in the integration of adaptive changes in protein metabolism of the animals. Further, he has suggested that each cell or tissue is competent to synthesize and degrade its own constituent proteins. The extent of such alterations of protein is limited largely by overall metabolic processes occurring within the cells. Munro (1970)

has also suggested that liver proteins, including plasma proteins, synthesized by liver comprise a major part of labile protein reserves. Such turnover of the cellular proteins is faster in liver than any other organ, except the pancreas.

One of the important enzymes that influences metabolism of nucleic acids is the 5'-nucleotidase. Alterations of this enzyme activity have been shown to get reflected in the status of nucleotide pool of seminal visicle of mice (Takuma and Massyoshi, 1977). Hepatic tissue of rat has been shown to possess substantial 5'-nucleotidase activity (Kim Dae Sung, 1982).

Earlier work carried out in this laboratory on male rat to investigate the possible short term effect of castration and subsequent androgen replacement on hepatic protein and nucleic acid metabolism by Ambadkar and Gangaramani (1981) has revealed that maximum responses to different experimental treatments were observable at 48 h post castration interval and with 0.1 mg testosterone propionate replacement. Mean and Hamilton (1966) demonstrated with the single injection of estrogen 40% increase in RNA synthesis occurred as rapidly as within 2 minutes. It was, therefore, thought desirable to investigate early effects of spaying and subsequent ovarian hormone replacement at different dose levels on hepatic protein and nucleic acid metabolism.

MATERIAL AND METHODS

Adult female albino rats (140 $\pm$ 20 g B.W.) were used as experimental animals. Animals were divided into twelve groups as described in Chapter-3.

Hepatic total protein, DNA, RNA concentrations and 5'-nucleotidase enzyme activity were assayed. Other details regarding methods employed are described in Chapter-1.

RESULTS

Table-4.1 showed results obtained after 24, 48 and 72 h of ovariectomy. Values obtained in case of sham-operated females were not found to differ in any significant way from those of normally cycling animals, hence are not given. In other words, sham operation did not affect the normal 4 day estrous cycle.

Ovariectomy lead to decrease in hepatic protein concentration which was more obvious ($P < 0.005$) in the right lobe after 24 h of spaying. The protein levels

Table-4.1 The effect of ovariectomy after 24, 48 and 72 h on hepatic concentrations total protein, nucleic acids and levels of 5'-nucleotidase activity

PARAMETERS	INTACT FEMALES DURING DIESTROUS PHASE		24 h		48 h		72 h	
	SP	R	SP	R	SP	R	SP	R
Total protein mg/100 mg fresh tissue	21.516 ± 0.936	23.556 ± 0.699	21.062 ± 0.636	20.552**** ± 0.495	18.939*** ± 0.210	20.321**** ±0.373	19.050* ± 0.578	22.447 ± 0.423
5'-nucleotidase µg PO_4 released/mg protein/60 min at 37°C	23.474 ± 0.309	23.953 ± 0.514	24.419 ± 0.384	22.347 ± 0.787	26.590**** ± 0.777	27.744**** ± 0.544	23.155 ± 0.976	24.714 ± 0.444
Deoxyribonucleic acid (DNA) µg/mg tissue	0.302 ± 0.014	0.351 ± 0.008	0.543**** ± 0.009	0.552**** ± 0.009	0.570**** ± 0.016	0.593**** ± 0.020	0.543**** ± 0.025	0.499**** ± 0.015
Ribonucleic acid (RNA) µg/mg tissue	0.214 ± 0.014	0.217 ± 0.009	0.299**** ± 0.017	0.455**** ± 0.008	0.201 ± 0.013	0.262* ± 0.014	0.294** ± 0.026	0.202 ± 0.013

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-4.2 Influence of 5 μg E_2 replacement in 48h OVX rats on parameters given under Table 4.1

PARAMETERS	INTACT FEMALES DURING DIESTROUS PHASE		48 h OVX		48 h OVX injected with 5 μg E2 and sacrificed after 1h		2h		4h	
	SP	R	SP	R	SP	R	SP	R	SP	R
Total protein mg/100 mg fresh tissue	21.516 ± 0.936	23.556 ± 0.699	18.939 ± 0.210	20.321 ±0.373	26.911**** ± 0.408	26.372**** ± 0.391	16.511**** ± 0.534	20.696 ± 0.498	23.598**** ± 0.754	24.524**** ± 0.282
5'-nucleotidase μg PO_4 released/mg protein/60 min at 37°C	23.474 ± 0.309	23.953 ± 0.570	26.570 ± 0.749	27.744 ± 0.544	59.856**** ± 1.260	65.516**** ± 0.799	30.815**** ± 0.577	37.160**** ± 0.897	19.710**** ± 0.687	23.487**** ± 1.075
Deoxyribonucleic acid (DNA) μg/mg tissue	0.302 ± 0.014	0.351 ± 0.008	0.570 ± 0.016	0.593 ± 0.020	0.248**** ± 0.004	0.255**** ± 0.014	0.359**** ± 0.014	0.390**** ± 0.014	0.383**** ± 0.009	0.438**** ± 0.009
Ribonucleic acid (RNA) μg/mg tissue	0.214 ± 0.014	0.217 ± 0.009	0.201 ± 0.013	0.262 ± 0.014	0.153**** ± 0.012	0.076**** ± 0.002	0.208 ± 0.014	0.248 ± 0.016	0.224* ± 0.011	0.229* ± 0.008

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-4.3 Influence of 10 µg E_2 replacement in 48 h OVX on parameters given under Table-4.1.

PARAMETERS	INTACT FEMALES DURING DIESTROUS PHASE		48 h OVX		48 h OVX injected with 10 µg E2 and sacrificed after 1h		2h		4h	
	SP	R	SP	R	SP	R	SP	R	SP	R
Total protein mg/100 mg fresh tissue	21.516 ± 0.936	23.556 ± 0.699	18.939 ± 0.210	20.321 ±0.373	22.990**** ± 0.490	27.123**** ± 0.715	17.319 ± 0.332	17.888**** ± 0.322	28.146**** ± 0.867	27.567**** ± 0.296
5'-nucleotidase µg PO_4 released/mg protein/60 min at 37°C	23.474 ± 0.309	23.953 ± 0.570	26.570 ± 0.749	27.744 ± 0.544	45.009**** ± 1.000	42.823**** ± 1.031	40.137**** ± 1.985	30.268**** ± 0.562	36.563**** ± 1.684	37.333**** ± 0.869
Deoxyribonucleic acid (DNA) µg/mg tissue	0.302 ± 0.014	0.351 ± 0.008	0.570 ± 0.016	0.593 ± 0.020	0.341**** ± 0.005	0.359**** ± 0.025	0.420**** ± 0.011	0.464**** ± 0.007	0.315**** ± 0.009	0.328**** ± 0.005
Ribonucleic acid (RNA) µg/mg tissue	0.214 ± 0.014	0.217 ± 0.009	0.201 ± 0.013	0.262 ± 0.014	0.284**** ± 0.021	0.165**** ± 0.008	0.188 ± 0.015	0.183 ± 0.010	0.130**** ± 0.010	0.142*** ± 0.013

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-4.4 Influence of 15 μg E_2 replacement in 48 h OVX rats on parameters given under Table 4.1

PARAMETERS	INTACT FEMALES DURING DIESTROUS PHASE		48 h OVX		48 h OVX injected with 15 μg E2 and sacrificed after					
					1h		2h		4h	
	SP	R	SP	R	SP	R	SP	R	SP	R
Total protein mg/100 mg fresh tissue	21.516 ± 0.936	23.556 ± 0.699	18.939 ± 0.210	20.321 ±0.373	23.206**** ± 0.447	23.092**** ± 0.387	17.406* ± 0.524	15.366**** ± 0.664	22.378**** ± 0.441	20.109 ± 0.846
5'-nucleotidase μg PO_4 released/mg protein/60 min at 37°C	23.474 ± 0.309	23.953 ± 0.570	26.570 ± 0.749	27.744 ± 0.544	46.714**** ± 0.744	53.075**** ±0.763	43.619**** ± 0.842	53.922**** ± 1.195	56.483**** ± 1.085	45.204**** ± 0.882
Deoxyribonucleic acid (DNA) μg/mg tissue	0.302 ± 0.014	0.351 ± 0.008	0.570 ± 0.016	0.593 ± 0.020	0.324**** ± 0.064	0.359**** ± 0.007	0.385**** ± 0.009	0.438**** ± 0.015	0.455**** ± 0.009	0.390**** ± 0.015
Ribonucleic acid (RNA) μg/mg tissue	0.214 ± 0.014	0.217 ± 0.009	0.201 ± 0.013	0.262 ± 0.014	0.178 ± 0.007	0.189**** ± 0.021	0.232* ± 0.012	0.294 ± 0.015	0.432**** ± 0.023	0.353**** ± 0.020

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-4.5 Influence of simultaneous administered constant dose of 2 mg P with each of the three doses of E_2 given under Table 4.2-4.4 on parameters listed therein.

PARAMETERS	INTACT FEMALES DURING DIESTROUS PHASE		48 h OVX		48 h OVX injected with E_2 + P and sacrificed after 2 h					
					5 µg E_2 + 2 mg P		10 µg E_2 + 2 mg P		15 µg E_2 + 2 mg P	
	SP	R	SP	R	SP	R	SP	R	SP	R
Total protein mg/100 mg fresh tissue	21.516 ± 0.936	23.556 ± 0.699	18.939 ± 0.210	20.321 ±0.373	22.438**** ± 0.360	24.720**** ± 0.672	19.551NS ± 0.371	21.578NS ± 0.654	20.656**** ± 0.295	21.251NS ± 0.872
5'-nucleotidase µg PO_4 released/mg protein/60 min at 37°C	23.474 ± 0.309	23.953 ± 0.570	26.570 ± 0.749	27.744 ± 0.544	22.696* ± 1.381	23.600**** ± 0.759	35.131**** ± 0.746	44.751**** ± 0.575	16.487**** ± 0.618	15.619**** ± 0.500
Deoxyribonucleic acid (DNA) µg/mg tissue	0.302 ± 0.014	0.351 ± 0.008	0.570 ± 0.016	0.593 ± 0.020	0.359**** ± 0.021	0.356**** ± 0.024	0.271**** ± 0.017	0.309**** ± 0.019	0.342**** ± 0.040	0.429**** ± 0.005
Ribonucleic acid (RNA) µg/mg tissue	0.214 ± 0.014	0.217 ± 0.009	0.201 ± 0.013	0.262 ± 0.014	0.289** ± 0.033	0.318*** ± 0.013	0.322**** ± 0.038	0.205**** ± 0.009	0.450**** ± 0.036	0.370**** ± 0.029

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

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

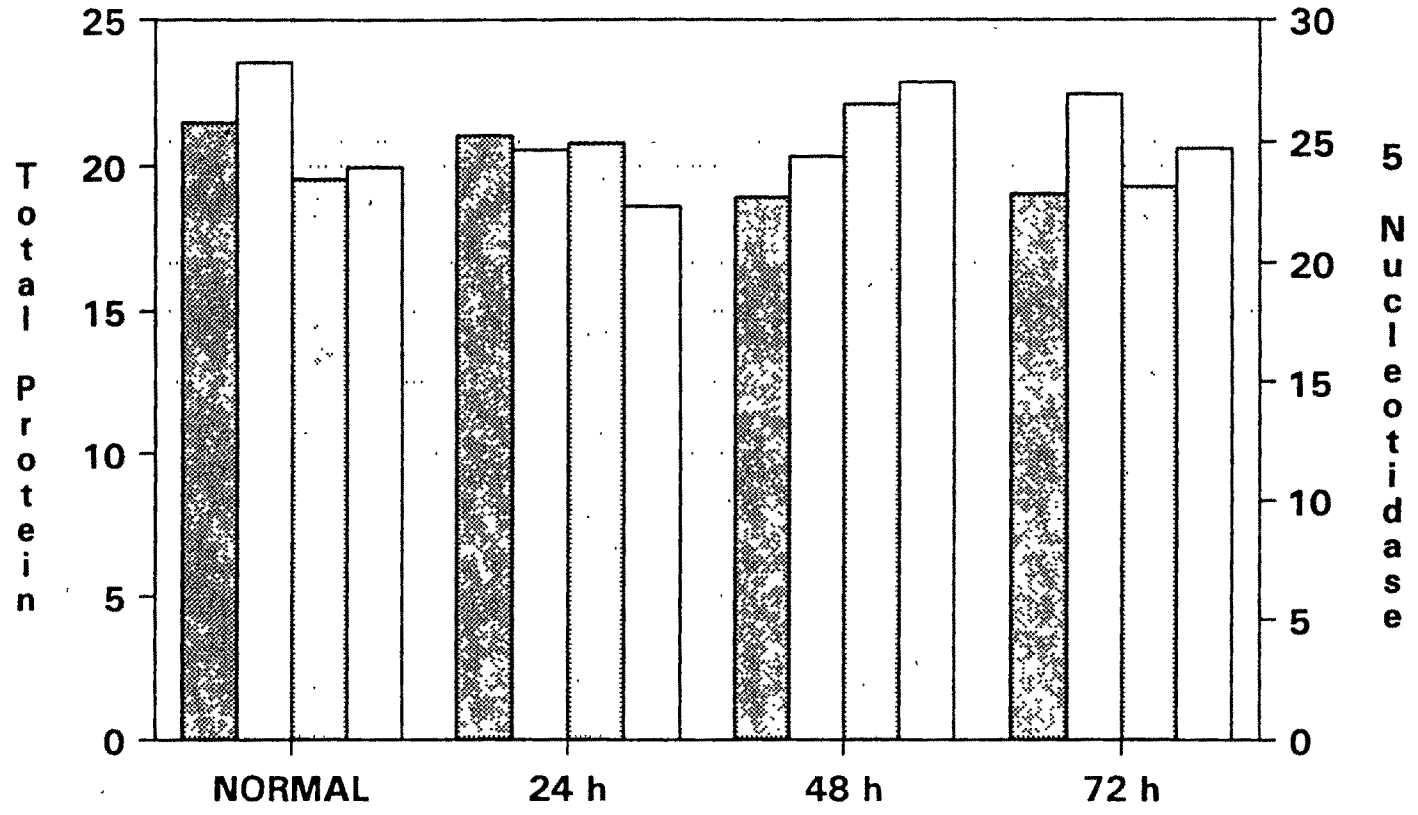

FIG. 29.

TOTAL PROTEIN AND 5'-NUCLEOTIDASE

mg/100mg fresh tissue

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

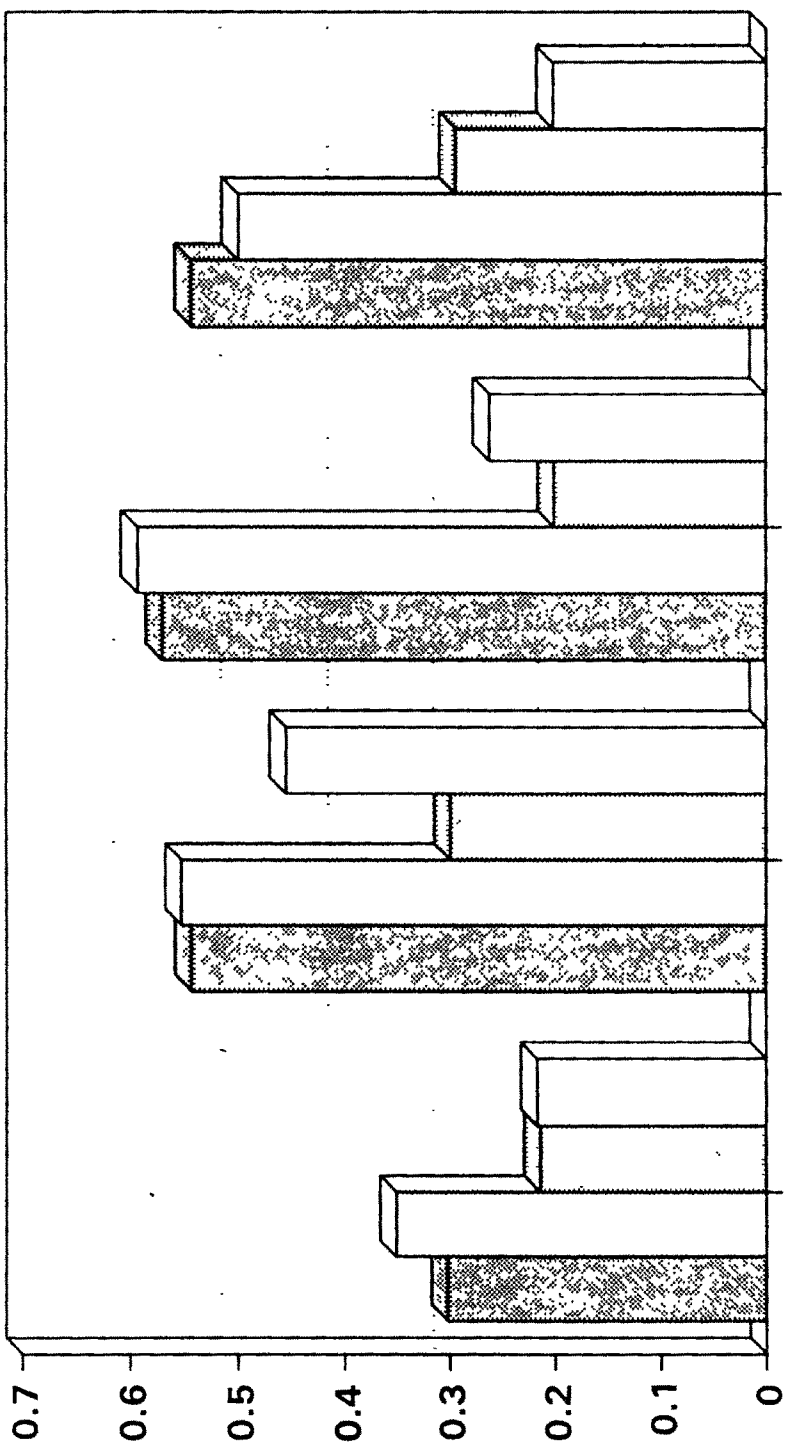
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
NORMAL
24 h
48 h
72 h
POST-OVX INTERVALS
DNA-Sp
DNA-R
RNA-Sp
RNA-R
DNA & RNA
µg/mg fresh tissue

FIG. 30.

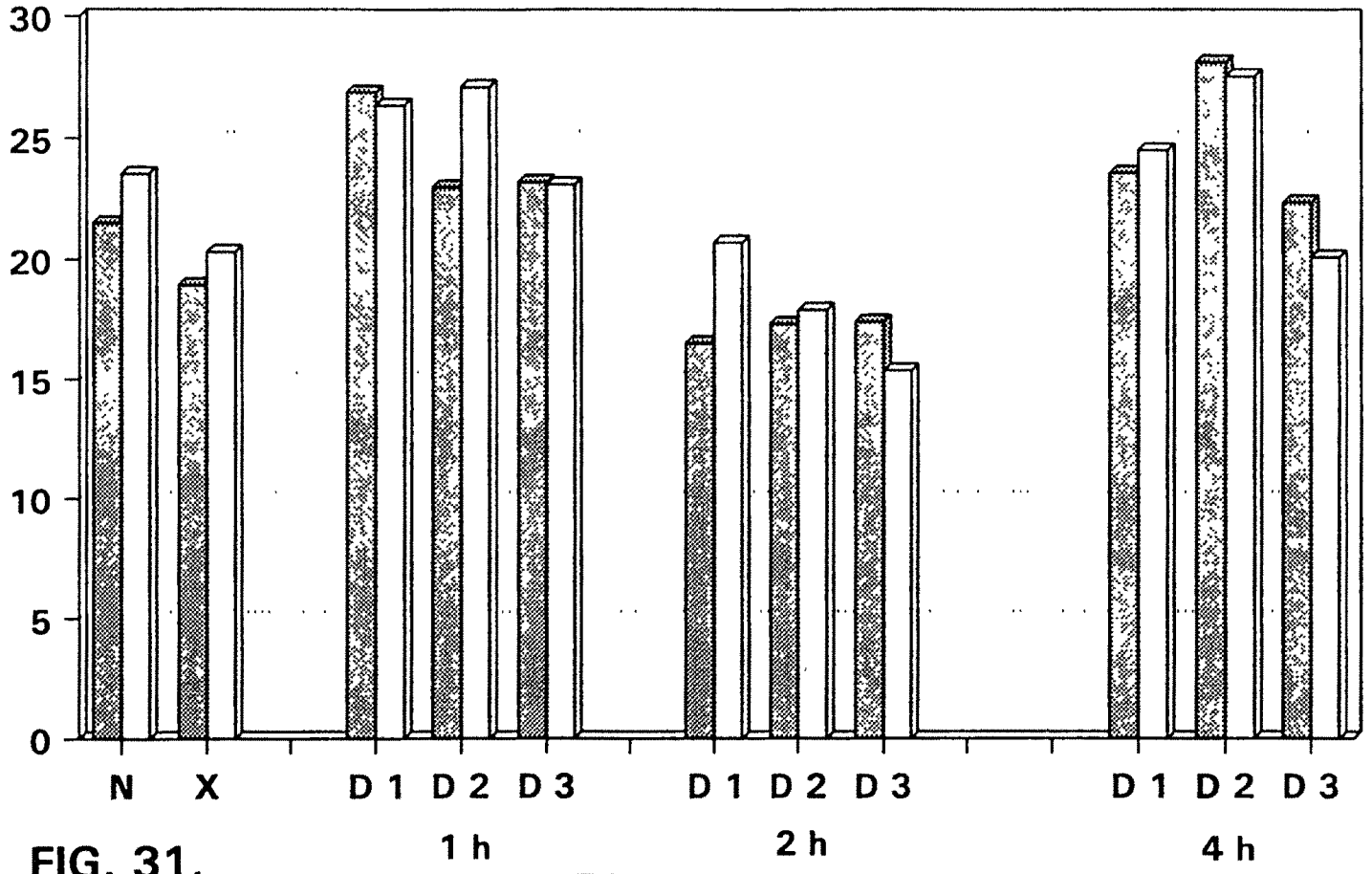

FIG. 31.

N = NORMAL

X = 48 h OVX

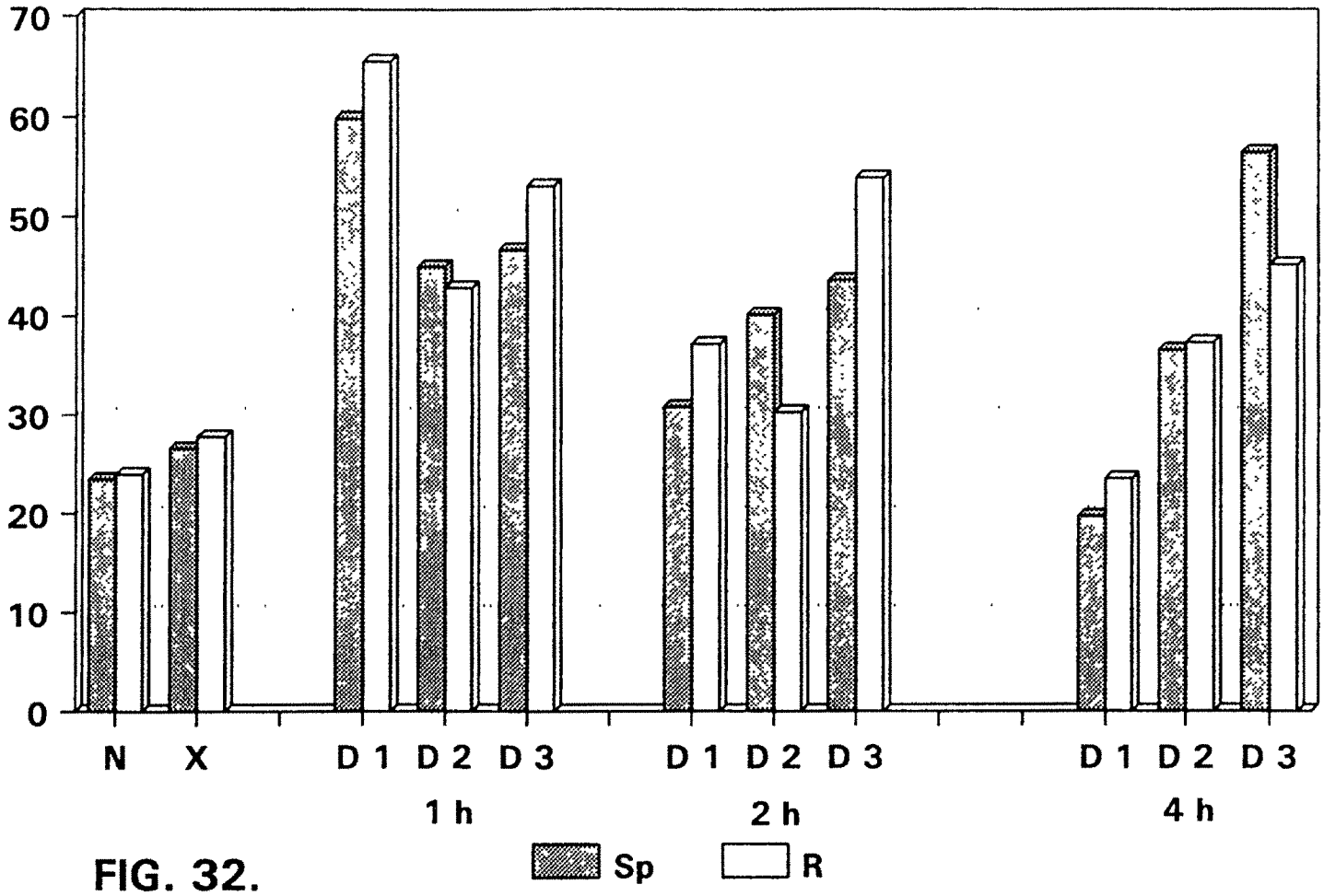
70
60
50
40
30
20
10
0
N
X
D 1
D 2
D 3
1 h
D 1
D 2
D 3
2 h
D 1
D 2
D 3
4 h
Sp
R
5'-NUCLEOTIDASE
µg PO4 released / mg protein/ 60 minute /37°C

FIG. 32.

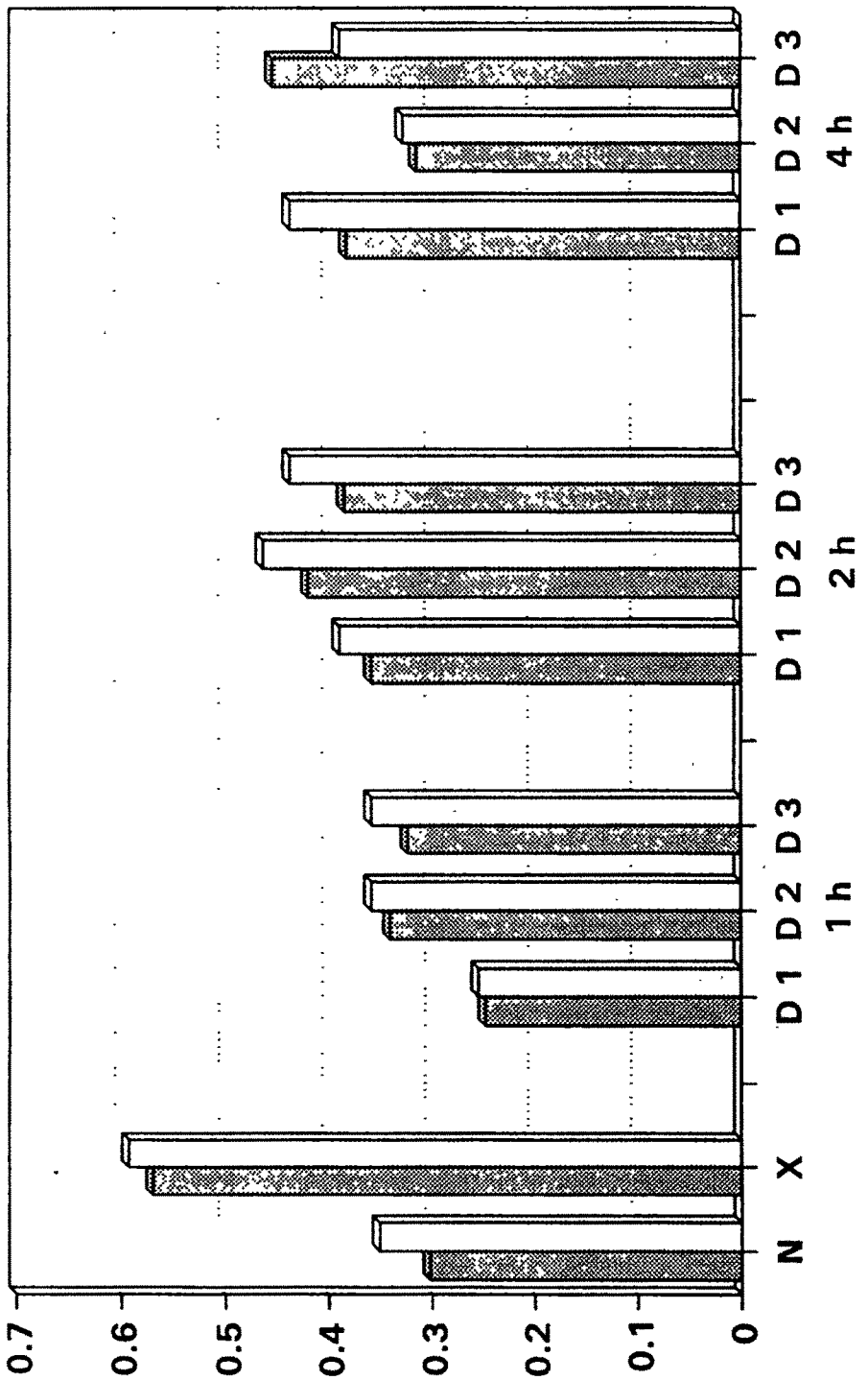
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
N
X
D 1
D 2
D 3
1 h
D 1
D 2
D 3
2 h
D 1
D 2
D 3
4 h
Sp
R
DNA
µg/ mg fresh tissue

FIG. 33.

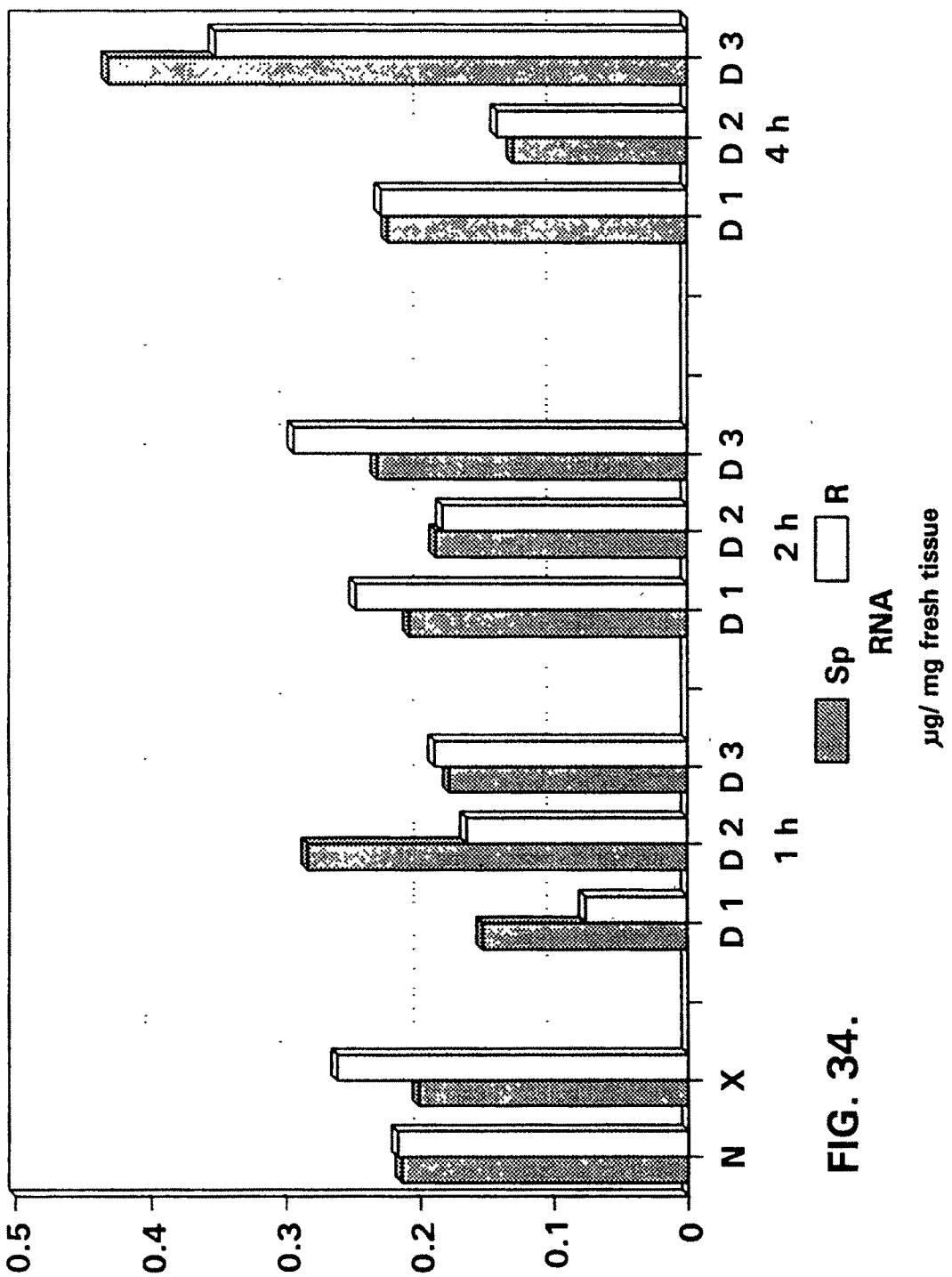
0.5
0.4
0.3
0.2
0.1
0
N
X
D 1
D 2
D 3
1 h
D 1
D 2
D 3
2 h
D 1
D 2
D 3
4 h
Sp
R
RNA
µg/ mg fresh tissue

FIG. 34.

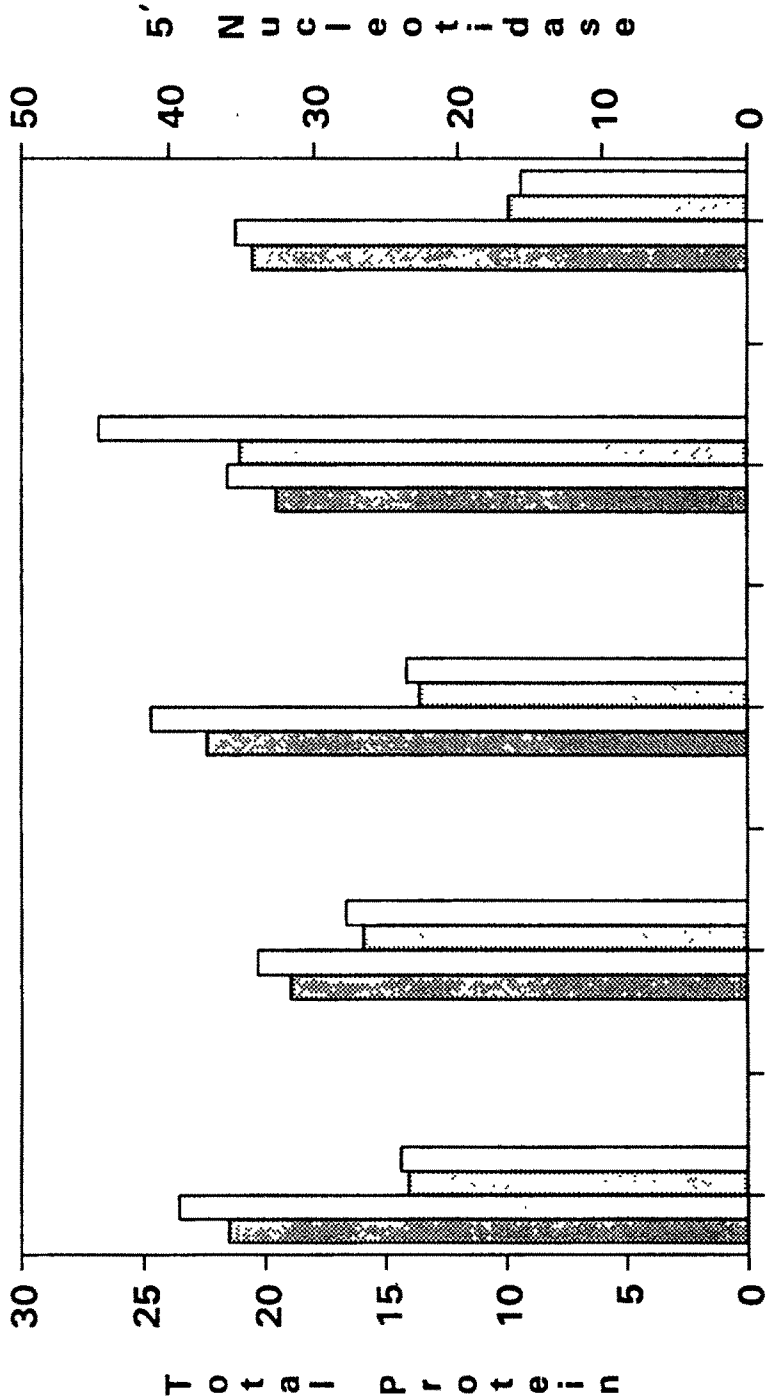
Total Protein
mg / 100mg fresh tissue
0
5
10
15
20
25
30
5' Nucleotidase
µg PO4 released / mg protein / 60 min at 37°C
0
10
20
30
40
50
NORMAL
48 OVX
CD 1
CD 2
CD 3
KILLED AFTER 2 h OF INJECTION
Tp-Sp
TP-R
NU-Sp
NU-R
TOTAL PROTEIN & 5'-NUCLEOTIDASE

FIG. 35

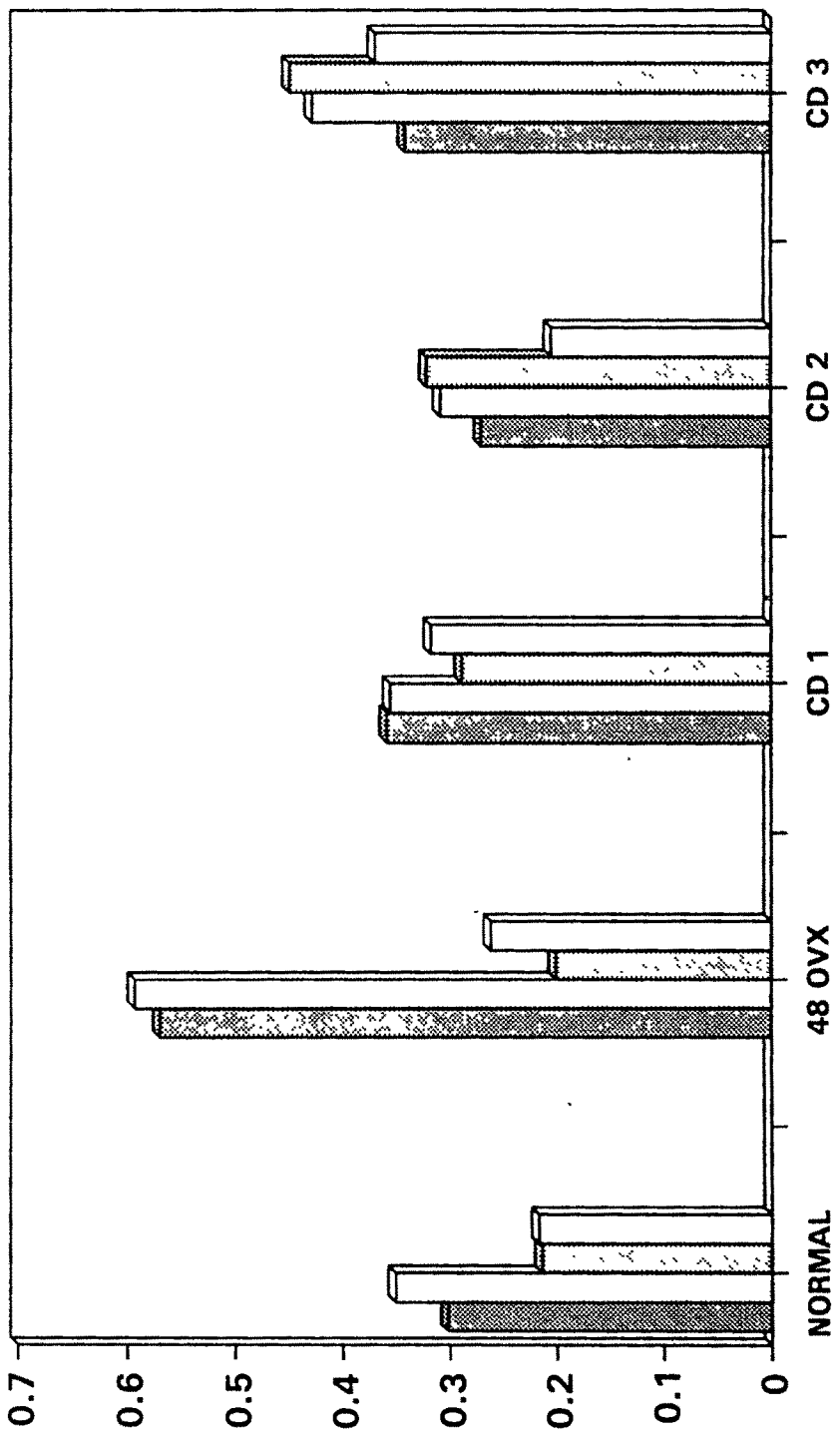
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
NORMAL
48 OVX
CD 1
CD 2
CD 3
KILLED AFTER 2 h OF INJECTION
DNA-Sp
DNA-R
RNA-Sp
RNA-R
DNA AND RNA
µg / mg fresh tissue

FIG. 36.

decreased in both the lobes after 48 h, but it was nearly restored by 72 h.(Fig. 29). 5'-nucleotidase enzyme was apparently not influenced by OVX upto 24 h, however, it increased by 48 h of spaying. This enzyme activity was also found to get restored by 72 h. Increase in DNA concentration in both lobes was evident throughout. On the other hand, though RNA concentration was elevated after 24 h due to spaying it exhibited fluctuations differing in the two lobes depending on time lapse (Fig.30).

Effects of E_2 replacement:- Results are depicted in table-4.2 to 4.4 except those of vehicle treated animals since these were similar to those of 48 h spayed rat.

Under replacement therapy the hepatic protein concentration exhibited significant increase within 60 minutes. However, after this initial rapid increase there was a remarkable reversal of leading to subnormal values by 2 h. Strangely enough, again the protein levels of both the liver lobes were raised a little above normal by 4 h interval. This influence was more obvious after 10 μg injection than with the other two doses (Fig.31).

Marked increase in 5'-nucleotidase activity was observed just after 1 h with all three doses of E_2 administration (Fig.32). at 2 and 4 h intervals However, declining levels were observable with 5 (D_1) and 10 μg(D_2) E_2 injection. An exception was noticeable in case of the right liver lobe at 4 h interval only with D_2. Nevertheless, the enzyme activity was still above the control level. With D_3 also the enzyme activity was raised within 60 minutes but instead of showing declining tendency seen with D_1 and D_2 it remained high upto 4 h.

Hepatic DNA concentration was significantly decreased by 1 h interval but reversal of such depression was noticeable by 2 and 4 h post injection intervals, however the concentrations were still below the control levels. A noticeable fact was that D_2 brought the DNA levels of both the liver lobes to those obtained in intact diestrous rats (Fig.33). D_1 and D_2 brought about suppression of RNA concentration in general, in both of the liver lobes at all post administration intervals (Fig.34). An exceptional response only at 1 h interval with D_2 was shown by the spigelian lobe registering an increase in RNA level. As against this, the D_3 dose was noted to elevate the RNA levels at 2 and 4 h, which were higher than that of normal animals.

Simultaneous administration of a fixed dose of 2 mg progesterone (P) with 17B-estradiol (E_2) doses.

All three combination doses lead to normalization of hepatic protein concentration of both liver lobes at 2 hourly interval (Fig.35). This was more clearly seen in the case of spigelian lobe. 5'-nucleotidase enzyme activity was

reduced to almost normal level with CD_1. CD_2 was found to enhance the enzyme activity above normal level. The third dose i.e. CD_3 was found to suppress remarkably the 5'-nucleotidase activity (Fig.35).

The effect of spaying on DNA concentration, in general, was reversed by CD_1 and CD_3. This treatment was found not just to counter the effect of OVX but was noted to bring it down below normal (Fig.36). In contrast to this the three combination doses were not found to be of restorative nature as far as RNA was concerned, but generally showing enhancement of RNA concentration (Fig.36). These fluctuations indicated lobe wise differential senstivity.

DISCUSSION

Ovariectomy during diestrous seemingly leads to protein breakdown upto 48 h, but this flitting effect wanes off soon and protein concentration get restored by 72 h. Many reports on the long term effects of ovariectomy on uterine protein synthesis have demonstrated that OVX leads to decrease in protein synthesis (Maiskii, 1974; Ekka *et al.*, 1977; De-Hertogh *et al.*, 1977) and estrogen stimulates the same. Konm *et al.* (1985) found two new proteins in the uterus after administration of estradiol to ovariectomized rats. In vitro study by Nicolar *et al.* (1987) on rat hepatocyte showed that ovariectomy caused reduction in the rate of incorporation of ^{14}C-leucine in protein and amino acyl tRNA. Further they have demonstrated either partial or complete compensatory influence of hormonal treatment on this processes. Considering the influence of OVX and replacement therapy, the present results also point to the fact that OVX does reduced hepatic protein concentration; at least after 48 h and E_2 treatment (5 μg/ animal) reverses this effect of OVX in a better way than the 10 and 15 μg doses. Considering the fluctuating patterns in protein concentrations obtained after E_2 administration it could be said that some normalizing influence becomes apparent by 4 h interval particularly with 5 μg dose. This influence of 5 μg dose was observable in a much better way within 2 h when given in combination with 2 mg P.

Significant increase in DNA concentration brought about by OVX (24 h) without any significant changes in protein concentration may point to occurrence of the phenomenon of nuclear polyploidy in hepatic tissue, as has been reported in case of male and female rats due to gonadectomy (Swartz *et al.*, 1960; Konopkova and Nedvidek, 1972). It could be said that increase in 5'-nucleotidase activity at 48 h interval is associated with observed rise in nucleic acid concentrations, particularly that of DNA. Enough reports are available on time and dose dependent influence of estrogen on DNA and RNA synthesis in uterus (Stormshak

et al., 1976; Steplewski and Wlodzimerz, 1973; Water's *et al.*, 1983). Water's *et al.* (1983) demonstrated a biphasic stimulation at transcriptional level by E_2 treatment on rat uterine tissue. According to them first phase comprises of a sharp stimulation of RNA synthesis 2-4 h after hormone administration. It was 7 to 11 time more than unstimulated controls, however, this phase rapidly falls nearly to control level by 48 h. The second phase depicted a broad peak of RNA synthesis typically lasting from 12 to 32 h after hormone administration. In the present study it was seen that hepatic DNA and RNA concentrations were drastically lowered at 1 h interval after D_1, D_2 as well as D_3. However, both parameters were found to rise after 2 h probably indicating a delayed stimulatory influence of the same. Despite such an increase in nucleic acids after 2 h of E_2 administration hepatic protein levels were found to be reduced. It therefore, seems that E_2 administration exerted a noticeable catabolic influence on hepatic proteins. In all probability the rise in DNA is associated more with nuclear polyploidy rather than with general metabolic aspects. However, the catabolic effect of administration of E_2 alone on hepatic protein was counteracted by simultaneously administered progesterone, more obviously with CD_3.

Taking into consideration reversal of the effect of E_2 regime by combination doses it seems to conform to the fact that priming with E_2 facilitates manifestation of biological action of progesterone (Engle and Smith, 1938; Kalra, 1975). Mataradze and Gontar (1986) demonstrated that hepatic and uterine estrogen receptors show some organ-specific peculiarities in respect of intracellular compartmentation processes. Thus it could be suggested that hepatic tissue of female rats also responds to sex hormones in a organo-specific manner. Though not a new finding, the finding presented here indicates that influence of female sex hormones act at different metabolic levels. Such responses of liver qualify for being considered as true target organ for sex steroids. Finally, the results suggest that administration of estradiol alone is not capable of restoring influence of OVX on the hepatic parameters under study. E_2 + P combination apparently helps restore hepatic protein concentration to a good extent. However, hormone replacement regime employed here was not so effective in its restorative function with respect to nucleic acids levels and 5'-nucleotidase activity.

By way of summary it can be said that the hepatic tissue of female rats do show differential sensitivity with reference to spigelian and right lobe as far as variations in sex steroids are concerned. Administration of estrogen alone is not so effective as is the combined estrogen progesterone treatment. It should be admitted that a much wider range of replacement regime should be tried out with inclusion of at least a few more important concerned enzymes for coming to a better understanding about short term influences of sex hormones.