CHAPTER 3

EFFECT OF OVARIECTOMY AND OVARIAN SEX HORMONES REPLACEMENT ON HEPATIC CARBOHYDRATE METABOLISM OF RAT

The central role of the liver in carbohydrate homeostasis is well recognized (Altszuler and Finegold, 1974; Hers, 1976; Hers and Hue, 1983). Its primary function in this regard is to provide adequate quantities of glucose to peripheral tissues that mainly utilize it as metabolic fuel. Excess of glucose is normally converted into glycogen or lipids. Glycogen is widely distributed in the animal body but the largest bulk occurs in the muscles and liver. Liver glycogen may be considered as ready source of energy fuel. Defects in enzymes involved in the synthesis or degradation of glycogen may lead to disturbances of carbohydrate metabolism.

The balance between processes involving supply of glucose by the liver and its uptake by various tissues at any particular time, depends, on the prevailing multiple hormonal influences that govern the activities of various enzymes in the liver. These hormones modulate different levels of varied regulatory mechanisms, involved in homeostasis. Studies on metabolic process of reproductive organ have demonstrated that sex hormones induce synthesis of several important enzymes concerned with carbohydrate metabolism (Singhal *et al.*, 1967 and 1969; Singhal and Valadares, 1970). Both stimulatory and inhibitory effects, depending on dose levels and time, of administration of estrogen and progesterone on uterine glycogenesis have been demonstrated (Demers and Jacobs, 1973; Garrisson *et al.*, 1973; Ishihara *et al.*, 1988). Antagonistic effect of progesterone on estradiol induced changes in uterine glycogen content has also been reported (Poteat and Walter, 1977; Tripathi and Krishna, 1985).

Meier and Garner (1987) suggested that stimulation of transmembrane glucose transport by estradiol occurs through an increase in intrinsic activity of the membrane bound transport protein rather than quantitative increase. Only scanty information is available on the actions of female sex hormones on hepatic carbohydrate metabolism (Paul, 1971; Matute and Kalkhoff, 1973; Dahm *et al.*, 1977; Dasmahapatra and Medda, 1982). However, the available information is not adequate enough. Hence, it was thought desirable to pay more attention to the study of influences of sex hormones on certain enzymes concerned with hepatic carbohydrate metabolism.

It is a well recognized fact that the enzyme phosphorylase brings about the initial step in the breakdown of glycogen. Further, the phosphorylase enzyme activity catalyzes the phosphorolytic cleavage of 1-4 glycosidic linkage at the non-reducing end of an outer branch of glycogen molecules. Glucose-6-phosphatase is classicaly considered as a key enzyme for the release of glucose into circulating blood by hepatic cells.

Among the dehydrogenases of the Kreb's cycle of mammalian liver the succinate dehydrogenase (SDH) is more active than the others (Putilina and Eshchenko, 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 stimulated the SDH activity.

The role of cyclic adenosine 3'-5'-monophosphate (cAMP) as second messenger, in respect of actions of hormones, has been well established (Robison *et al.*, 1971; Robison *et al.*, 1973). Its role in activation of phosphorylase and carbohydrate metabolism of liver is also well known (Rindi, 1971). Role of this second messenger in mediation of action of female sex hormones in uterine tissues has been studied by several authors (Szego and Davis, 1967; Pankova *et al.*, 1977; Liu *et al.*, 1976; Salganik *et al.*, 1979; Pansini *et al.*, 1984). The cAMP-specific phosphodiesterase (cAMP-PDE) degrades the cyclic nucleotide to non cyclic form-5'-AMP. Hence, any change in this enzyme activity would indicate fluctuations occurring in the intracellular level of cAMP.

Great deal of attention has been given to the general problem of energy assisted transport mechanisms across biological membrane against concentration gradient. Assessment of Na^+K^+- ATPase activity as a measure of such transport of substances has been validated by some investigators (Scholefield, 1964; Stein, 1967; Barnabei, *et al.*, 1973).

From the forgoing account it is obvious that involvement of these enzymes in hepatic carbohydrate metabolism and their modulation by sex hormones is one of the important aspects of study of functions of liver. In the light of these observations the present study was carried out to asses possible influences of ovariectomy and subsequent replacement with estradiol alone or in combination with progesterone on hepatic carbohydrate metabolism.

MATERIAL AND METHODS

Adult female albino rats weighing between 140 $\pm$ 20 g were maintained under laboratory conditions on a balanced diet and water *ad libitum*. These served as experimental animals.

I. Ovariectomy (OVX):- Of sixty diestrous females, thirty were bilaterally ovariectomized and thirty were sham-operated under mild ether anesthesia. OVX and sham-operated females were divided into three equal groups each of ten rats. These were sacrificed 24, 48 and 72 h after OVX and sham-operation.

II. Replacement with estradiol 17B (E_2):- Each of the 48 h OVX individuals were treated with single i.m. injection of E_2 dissolved in 0.1 ml of propylene glycol. Three different doses viz:- 5,10 and 15 μg of E_2 (considered D_1, D_2 and D_3 respectively) were adminstered to three groups each of ten individuals. Thirty other 48 h OVX rats injected with 0.1 ml vehicle only.

All these animals, including sham-operated and vehicle injected, were sacrificed after 1, 2 and 4 h of injection.

III. Replacement with E_2 and progesterone (P):- Four different groups of 10 rats each comprising of 48 h OVX post-ovariectomy animals were treated as follows.

I group- Single i.m. injection of 0.5 ml propylene glycol containing 5μg E_2+ 2 mg P (CD_1)

II group- injected with 10 μg E_2+ 2 mg P (CD_2)

III group- injected with 15 μg E_2 + 2 mg P (CD_3)

IV group- injected with 0.5 ml vehicle only.

Animals were sacrificed after 2 h of injection

Just prior to killing, blood samples were collected by nictitating membrane puncture with heparin coated glass capillaries. From these plasma samples were prepared for glucose estimation. Immediately after collecting the blood samples; the animals were sacrificed. Pieces of spigelian and the right lobe were dissected out quickly, blotted free of blood and adherent connective tissue and were weighed. Following parameters were assayed:-

1) Plasma glucose

2) Liver glycogen

3) Glycogen phosphorylase

4) G-6-Pase

5) SDH

6) cAMP-PDE

7) Total ATPase

The details of methods are given in Chapter-1.

RESULTS

Effects of ovariectomy (Table 3.1):- Ovariectomy lowered the plasma glucose concentration just after 24 h of operation but thereafter gradual rise in the level was noted upto 72 h, which was then slightly higher than normal (Fig.10). Values obtained in case of sham-operated females were not considered as these exhibited normal cycles. In other words, sham-operation did not affect the normal 4-day cycle.

Significant lobe-wise differential response was noted at 24 h interval with respect to G-6-Pase activity (Fig.12). The spigelian lobe recorded as rise in the enzyme activity whereas the same was found to be decreased in the right lobe. However, later on a gradual rise in enzyme activity in both the lobes was noted from 48 to 72 h. Spaying operation brought about gradual suppression of glycogen concentration upto 72 h, and it got lowered below normal levels (Fig.11). Once again, a distinct lobe wise difference in response was evident in the case of phosphorylase enzyme activity. The right lobe was found to show decreased enzyme activity only at 24 h post-OVX interval but thereafter a gradual increase was noticeable up to 72 h interval. As against this the spigelian lobe exhibited initial decrease, increasing significantly at 48 h to lower once again by 72 h. It may be added here that, irrespective of individual actual value of enzyme activity in both lobes these were always lower than normal diestrous level.

Ovariectomy was found to induce significant decrease in SDH activity by 24 h but thereafter it almost got restored to normal level by 72 h however, the right lobe was comparatively slow in its response (Fig.13). OVX was found to slightly enhance cAMP-PDE activity, at all the post-OVX intervals for significant enhancement only in the spigelian lobe and that too, only at 24 h interval (Fig.14). As far as total ATPase activity is concerned, it can clearly be seen that it followed a pattern of variation parallel to that of glycogen phosphorylase activity inclusive of differential responsivity. Very muck like phosphorylase here also the ATPase

Table-3.1 Effect of ovariectomy on plasma glucose level, hepatic glycogen concentration and some enzyme activities of carbohydrate metabolism.

PARAMETERS	NORMAL INTACT FEMALES DURING DIESTROUS PHASE		POST-OPERATIVE INTERVALS 24 h		48 h		72 h	
Plasma Glucose mg/100 ml plasma	112.500 ± 1.742		84.000**** ± 1.642		100.500**** ± 0.821		120.000*** ± 1.642	
	Sp	R	Sp	R	Sp	R	Sp	R
Glycogen mg/100 mg fresh tissue	3.810 ± 0.425	4.193 ± 0.620	1.985**** ± 0.142	2.551**** ± 0.207	2.039**** ± 0.241	2.018**** ± 0.447	1.453**** ± 0.161	1.408**** ± 0.168
Phosphorylase μg PO_4 released/mg protein /60 min at 37°C	183.884 ± 2.152	165.628 ± 1.336	120.670**** ± 5.092	110.592**** ± 3.716	164.144** ± 7.555	134.388**** ± 3.408	149.816*** ± 7.588	146.584*** ± 4.352
G-6-Pase μmole PO_4 released/mg protein/60 min at 37 C	1.039 ± 0.060	0.984 ± 0.036	1.066**** ± 0.024	0.872** ± 0.016	1.096**** ± 0.036	1.104**** ± 0.028	1.212**** ± 0.028	1.444**** ± 0.024
SDH μg Formozan formed/mg protein /60 min at 37°C	24.534 ± 1.046	27.148 ± 0.706	15.220**** ± 0.214	13.134**** ± 0.450	23.420 ± 1.200	21.038**** ± 0.492	22.650 ± 0.932	23.612**** ± 0.548
cAMP-PDE μg PO_4 released/mg protein /60 min at 37°C	1.972 ± 0.197	2.632 ± 0.192	3.690**** ± 0.072	2.906 ± 0.108	2.282 ± 0.196	2.684 ± 0.062	2.700 ± 0.062	2.250 ± 0.064
Total ATPase μg PO_4 released/mg protein /60 min at 37°C	947.160 ± 19.370	754.970 ± 18.960	451.440**** ± 19.080	333.750**** ± 16.800	513.490**** ± 23.860	337.380**** ± 15.060	414.840**** ± 16.380	384.520**** ± 13.980

Each value is mean + SE of at least eight animals. Sp-Spigelian lobe R-Right lobe.
* $p < 0.05$ ** $p < 0.025$ *** $p < 0.01$ **** $p < 0.005$

Table-3.2 Influence of 5 μg E_2 replacement in 48 h OVX rat on plasma glucose level, hepatic glycogen concentration and some enzyme activities of carbohydrate metabolism.

PARAMETERS	NORMAL INTACT FEMALES DURING DIESTROUS PHASE		48 h OVX FEMALES		48h OVX INJECTED WITH 5μg E_2 AND SACRIFICED AFTER 1 h		2 h		4 h	
Plasma Glucose mg/100 ml plasma	112.500 ± 1.742		100.500 ± 0.821		135.000**** ± 2.846		79.617*** ± 2.758		49.364**** ± 1.379	
	Sp	R	Sp	R	Sp	R	Sp	R	Sp	R
Glycogen mg/100 mg fresh tissue	3.810 ± 0.425	4.193 ± 0.620	2.039 ± 0.241	2.018 ± 0.447	1.621 ± 0.117	1.264 ± 0.111	1.944 ± 0.143	2.545 ± 0.235	3.253**** ± 0.176	3.683**** ± 0.130
Phosphorylase μg PO_4 released/mg protein /60 min at 37°C	183.880 ± 2.150	165.620 ± 1.330	165.140 ± 7.550	134.380 ± 3.400	182.240* ± 5.280	192.820**** ± 2.250	152.840 ± 5.520	173.890 ± 4.650	53.366**** ± 2.852	51.976**** ± 1.728
G-6-Pase μmole PO_4 released/mg protein/60 min at 37°C	1.039 ± 0.060	0.984 ± 0.036	1.096 ± 0.036	1.104 ± 0.028	1.420**** ± 0.032	1.488**** ± 0.040	0.848*** ± 0.080	0.592**** ± 0.028	0.181**** ± 0.012	0.392**** ± 0.032
SDH μg Formozan formed/mg protein /60 min at 37°C	24.534 ± 1.046	27.148 ± 0.706	23.420 ± 1.200	21.038 ± 0.492	9.406**** ± 0.646	9.688**** ± 0.134	17.500**** ± 0.786	15.428**** ± 1.026	12.852**** ± 0.368	12.502**** ± 0.354
cAMP-PDE μg PO_4 released/mg protein /60 min at 37°C	1.972 ± 0.197	2.632 ± 0.192	2.282 ± 0.196	2.684 ± 0.062	3.902**** ± 0.192	4.630**** ± 0.184	4.350**** ± 0.150	5.086**** ± 0.224	10.236**** ± 0.424	12.092**** ± 0.414
Total ATPase μg PO_4 released/mg protein /60 min at 37°C	947.160 ±19.370	754.970 ± 18.960	513.490 ± 23.860	337.380 ± 15.060	588.580**** ± 6.240	465.260**** ± 8.380	369.560**** ± 15.130	291.510**** ± 14.190	221.310**** ± 14.920	201.080**** ± 7.26

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

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

Table-3.3 Influence of 10 μg E_2 replacement in 48h OVX rat on plasma glucose, hepatic glycogen concentration and some enzyme of carbohydrate metabolism.

PARAMETERS	NORMAL INTACT FEMALES DURING DIESTROUS PHASE		48 h OVX FEMALES		48h OVX INJECTED WITH 10μg E_2 AND SACRIFICED AFTER 1 h		2 h		4 h	
Plasma Glucose mg/100 ml plasma	112.500 ± 1.742		100.500 ± 0.821		87.970**** ±2.826		97.451 ± 1.509		86.669**** ± 3.148	
	Sp	R	Sp	R	Sp	R	Sp	R	Sp	R
Glycogen mg/100 mg fresh tissue	3.810 ± 0.425	4.193 ± 0.620	2.039 ± 0.241	2.018 ± 0.447	3.334**** ± 0.146	2.049 ± 0.098	2.940*** ± 0.200	2.155 ± 0.217	0.817**** ± 0.080	1.677 ± 0.164
Phosphorylase μg PO_4 released/mg protein /60 min at 37°C	183.880 ± 2.150	165.620 ± 1.330	164.140 ± 7.550	134.380 ± 3.400	102.500**** ± 2.460	88.760**** ± 5.640	122.604**** ± 4.320	113.550 ± 2.940	218.870**** ± 2.930	205.240**** ± 2.620
G-6-Pase μmole PO_4 released/mg protein/60 min at 37°C	1.039 ± 0.060	0.984 ± 0.036	1.096 ± 0.036	1.104 ± 0.028	0.378**** ± 0.001	0.648**** ± 0.028	0.868*** ± 0.024	0.904 ± 0.112	0.760**** ± 0.072	0.604**** ± 0.040
SDH μg Formozan formed/mg protein /60 min at 37°C	24.534 ± 1.046	27.148 ± 0.706	23.420 ± 1.200	21.038 ± 0.492	17.046 ± 0.528	16.446**** ± 0.614	21.746 ± 0.838	15.526**** ± 0.406	16.110**** ± 0.390	12.778**** ± 0.506
cAMP-PDE μg PO_4 released/mg protein /60 min at 37°C	1.972 ± 0.197	2.632 ± 0.192	2.282 ± 0.196	2.684 ± 0.062	9.236**** ± 0.398	6.620**** ± 0.218	3.862**** ± 0.320	3.862**** ± 0.320	2.758* ± 0.160	3.296**** ± 0.276
Total ATPase μg PO_4 released/mg protein /60 min at 37°C	947.160 ±19.370	754.970 ± 18.960	513.490 ±23.860	337.380 ± 15.060	678.480**** ± 18.720	499.150**** ± 21.980	313.090**** ± 6.960	290.650**** ± 5.270	269.650**** ± 10.670	273.960**** ± 8.16

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

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

Table-3.4 Influence of 15 μgE2 replacement in 48h OVX rat on plasma glucose, hepatic glycogen concentration and some enzyme activities of carbohydrate metabolism.

PARAMETERS	NORMAL INTACT FEMALES DURING DIESTROUS PHASE		48 h OVX FEMALES		48h OVX INJECTED WITH 15μg E2 AND SACRIFICED AFTER 1 h		2 h		4 h	
Plasma Glucose mg/100 ml plasma	112.500 ± 1.742		100.500 ± 0.821		80.987**** ± 1.763		101.924 ± 3.496		90.764**** ± 1.850	
	Sp	R	Sp	R	Sp	R	Sp	R	Sp	R
Glycogen mg/100 mg fresh tissue	3.810 ± 0.425	4.193 ± 0.620	2.039 ± 0.241	2.018 ± 0.447	0.633**** ± 0.067	0.694 ± 0.039	2.816*** ± 0.190	3.903**** ± 0.150	3.131**** ± 0.172	3.941 ± 0.263
Phosphorylase μg PO_4 released/mg protein /60 min at 37°C	183.880 ± 2.150	165.620 ± 1.330	164.140 ± 7.550	134.380 ± 3.400	302.900**** ± 8.760	316.660**** ± 8.650	103.860**** ± 4.020	132.530 ± 4.110	63.140**** ± 0.340	91.180**** ± 4.940
G-6-Pase μmole PO_4 released/mg protein/60 min at 37°C	1.039 ± 0.060	0.984 ± 0.036	1.096 ± 0.036	1.104 ± 0.028	0.388**** ± 0.012	0.460**** ± 0.240	1.004 ± 0.006	1.056 ± 0.024	0.624**** ± 0.024	0.592**** ± 0.012
SDH μg Formozan formed/mg protein /60 min at 37°C	24.534 ± 1.046	27.148 ± 0.706	23.420 ± 1.200	21.038 ± 0.492	15.044**** ± 0.498	14.028**** ± 0.564	14.912**** ± 0.580	14.820**** ± 0.040	9.316**** ± 0.358	14.350**** ± 2.880
cAMP-PDE μg PO_4 released/mg protein /60 min at 37°C	1.972 ± 0.197	2.632 ± 0.192	2.282 ± 0.196	2.684 ± 0.062	2.804* ± 0.094	2.926 ± 0.116	5.928**** ± 0.324	4.084**** ± 0.432	5.220**** ± 0.174	4.942**** ± 0.330
Total ATPase μg PO_4 released/mg protein /60 min at 37°C	947.160 ±19.370	754.970 ± 18.960	513.490 ±23.860	337.380 ± 15.060	418.320**** ± 9.710	387.610**** ± 6.430	278.380**** ± 4.100	214.350**** ± 8.650	195.370**** ± 12.030	176.520**** ± 9.81

Each value is mean ± SE of least eight animals. Sp-Spigelian lobe R-Right lobe.
* $p < 0.05$ ** $p < 0.025$ *** $p < 0.01$ **** $p < 0.005$.

Table-3.5 Influence of simulataneously administered constant dose of 2 mg P with each of the three doses of E_2 given under Table 3.2-3.4 on parameters listed therein.

PARAMETERS	NORMAL INTACT FEMALES DURING DIESTROUS PHASE		48 h OVX FEMALES		48h 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	
Plasma Glucose mg/100 ml plasma	112.500 ± 1.742		100.500 ± 0.821		141.000**** ± 3.000		155.000**** ± 2.570		79.714**** ± 2.636	
	Sp	R	Sp	R	Sp	R	Sp	R	Sp	R
Glycogen mg/100 mg fresh tissue	3.810 ± 0.425	4.193 ± 0.620	2.039 ± 0.241	2.018 ± 0.447	1.599**** ± 0.095	2.883 ± 0.423	1.430*** ± 0.244	1.757 ± 0.126	5.059**** ± 0.142	6.601**** ± 0.289
Phosphorylase µg PO_4 released/mg protein /60 min at 37°C	183.880 ± 2.150	165.620 ± 1.330	164.140 ± 7.550	134.380 ± 3.400	191.864**** ± 5.164	139.328**** ± 2.784	205.004**** ± 4.528	186.836 ± 3.604	57.740**** ± 1.930	56.888**** ± 1.412
G-6-Pase µmole PO_4 released/mg protein/60 min at 37°C	1.039 ± 0.060	0.984 ± 0.036	1.096 ± 0.036	1.104 ± 0.028	0.372**** ± 0.043	0.324**** ± 0.036	0.740**** ± 0.012	0.976 ± 0.028	0.252**** ± 0.028	0.317**** ± 0.008
SDH µg Formozan formed/mg protein /60 min at 37°C	24.534 ± 1.046	27.148 ± 0.706	23.420 ± 1.200	21.038 ± 0.492	36.604**** ± 1.936	35.516**** ± 1.408	25.342 ± 1.690	26.834** ± 2.174	27.206 ± 2.022	23.568**** ± 0.396
cAMP-PDE µg PO_4 released/mg protein /60 min at 37°C	1.972 ± 0.197	2.632 ± 0.192	2.282 ± 0.196	2.684 ± 0.062	3.776**** ± 0.350	3.718 ± 0.122	3.382**** ± 0.114	3.444**** ± 0.082	2.716 ± 0.322	2.196**** ± 0.074
Total ATPase µg PO_4 released/mg protein /60 min at 37°C	947.160 ±19.370	754.970 ± 18.960	513.490 ±23.860	337.380 ± 15.060	606.156**** ± 11.470	443.500**** ± 11.670	534.150**** ± 5.420	686.140**** ± 8.310	558.920* ± 8.400	566.250**** ± 11.56

Each value is mean ± SE of least eight animals. Sp-Spigelian lobe R-Right lobe.
* $p < 0.05$ ** $p < 0.025$ *** $p < 0.01$ **** $p < 0.005$.

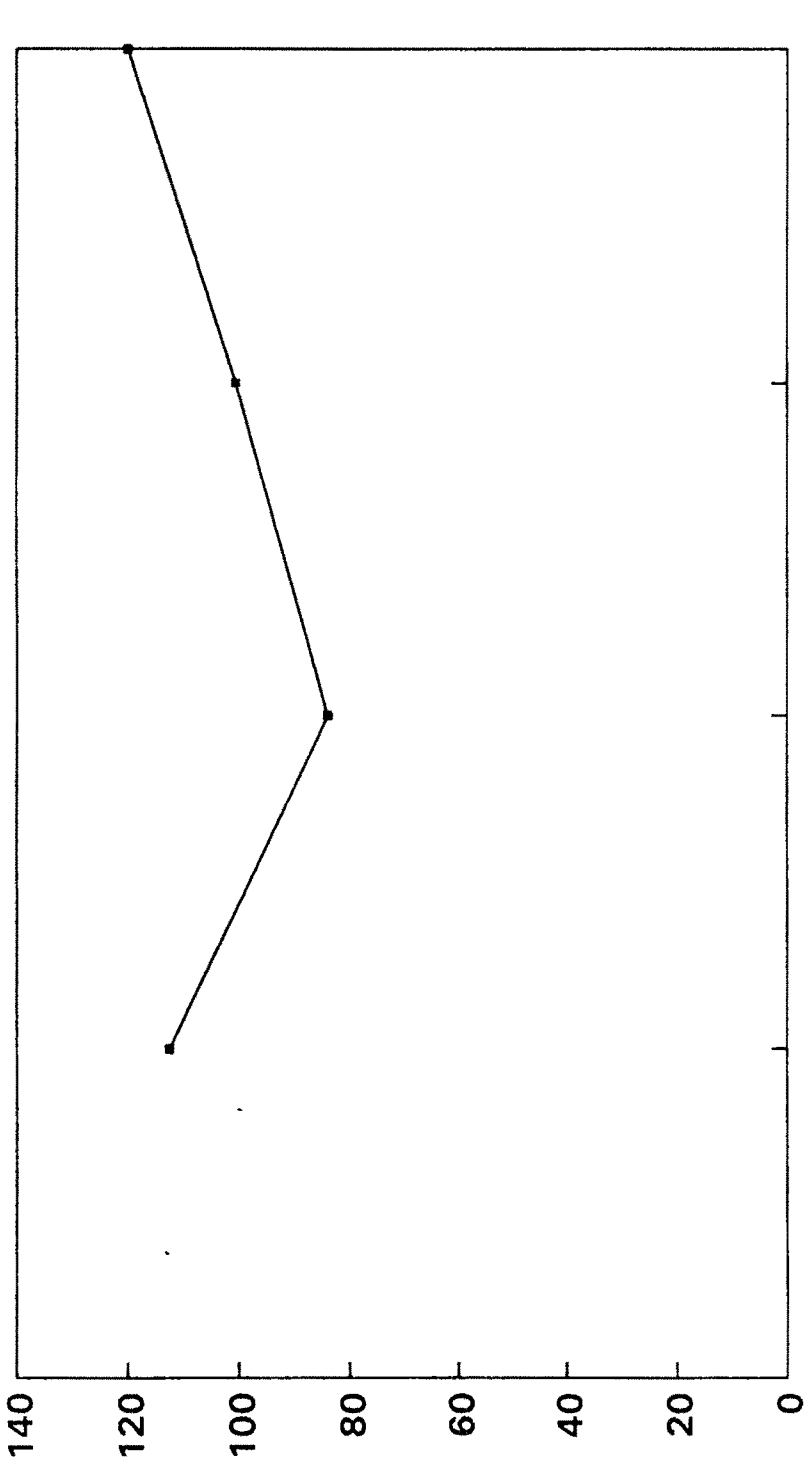

140
120
100
80
60
40
20
0
NORMAL
24 h
48 h
72 h
POST - OVX INTERVALS
PLASMA GLUCOSE
mg / 100 ml plasma

FIG. 10

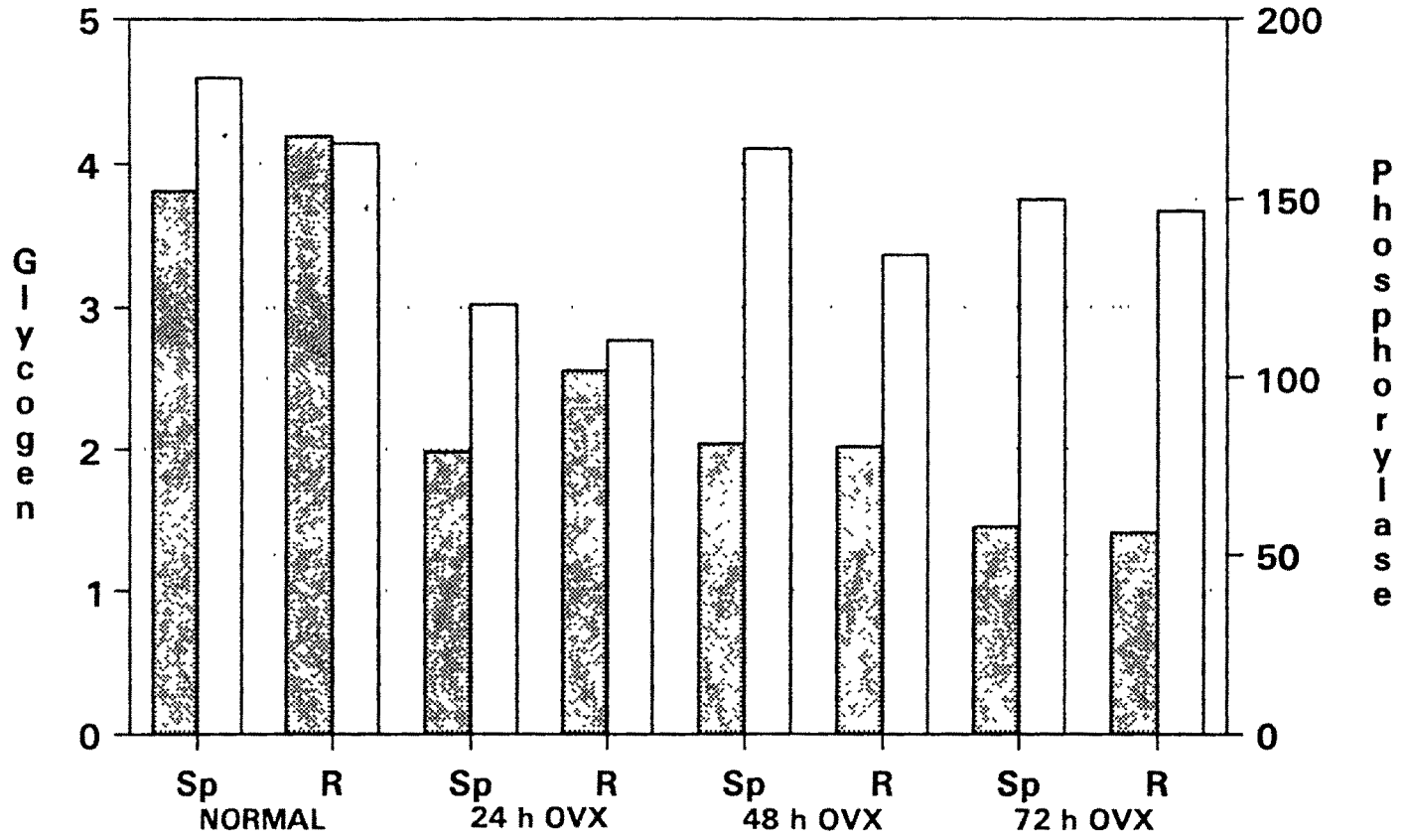


FIG. 11. GLYCOGEN PHOSPHORYLASE

mg / 100mg fresh tissue

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

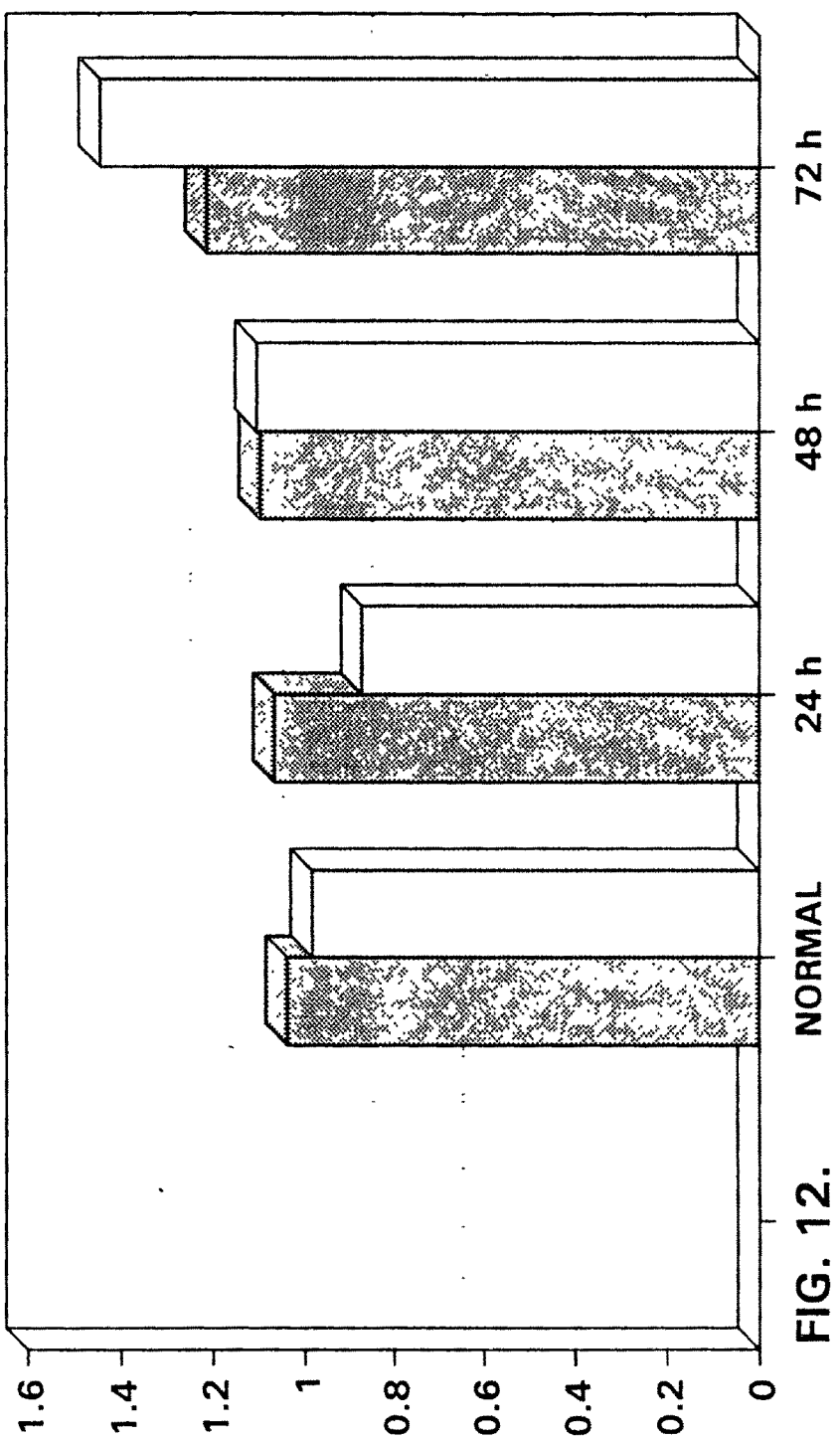

1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
NORMAL
24 h
48 h
72 h
POST-OVX INTERVALS
Sp
R
G-6-Pase
µmole PO4 released / mg protein/ 60 min at 37°C

FIG. 12.

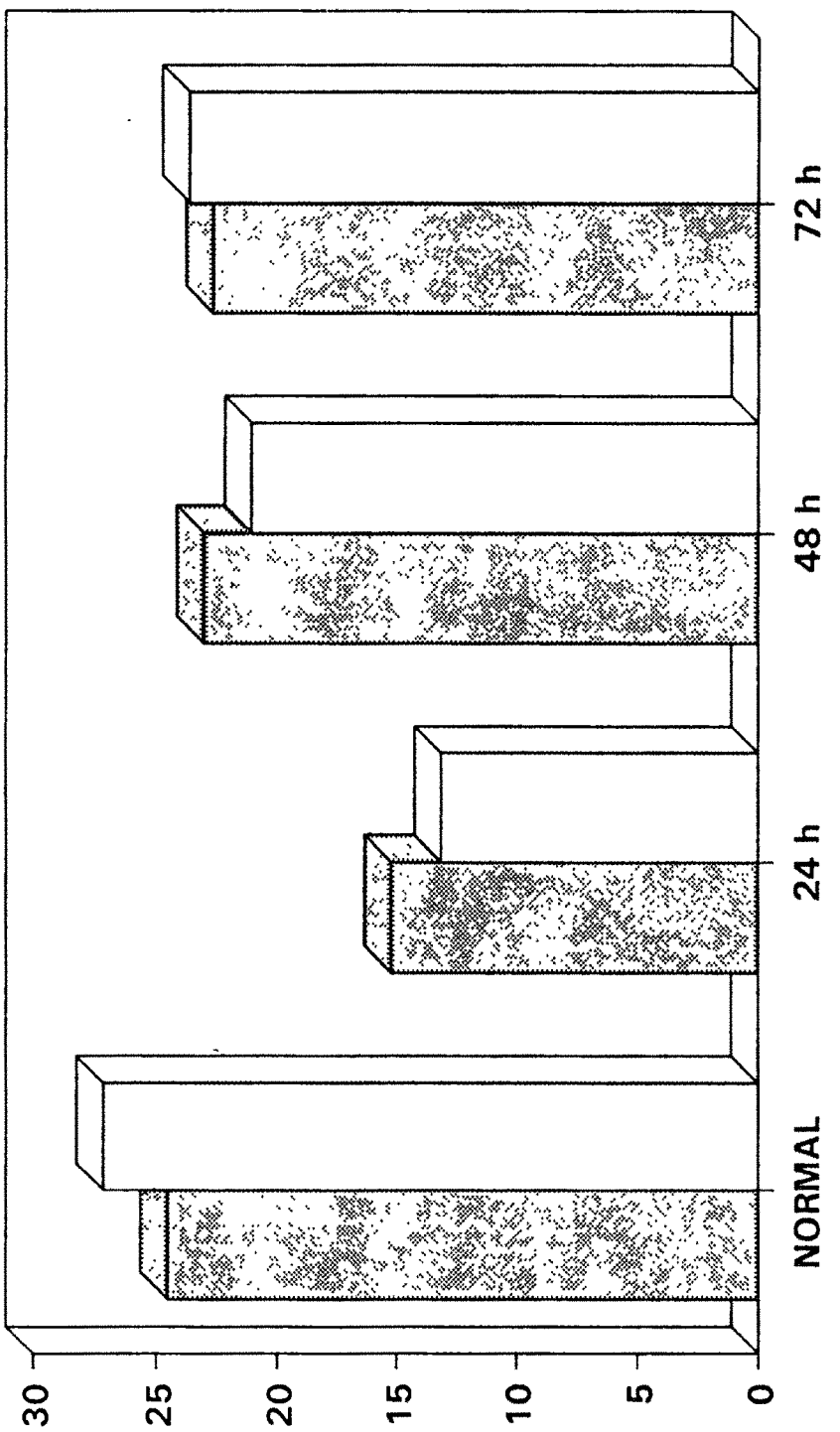

30
25
20
15
10
5
0
NORMAL
24 h
48 h
72 h
POST-OVX INTERVALS
Sp
R
SUCCINATE DEHYDROGENASE
µg Formozan formed/mg protien /60 min at 37°C

FIG. 13.

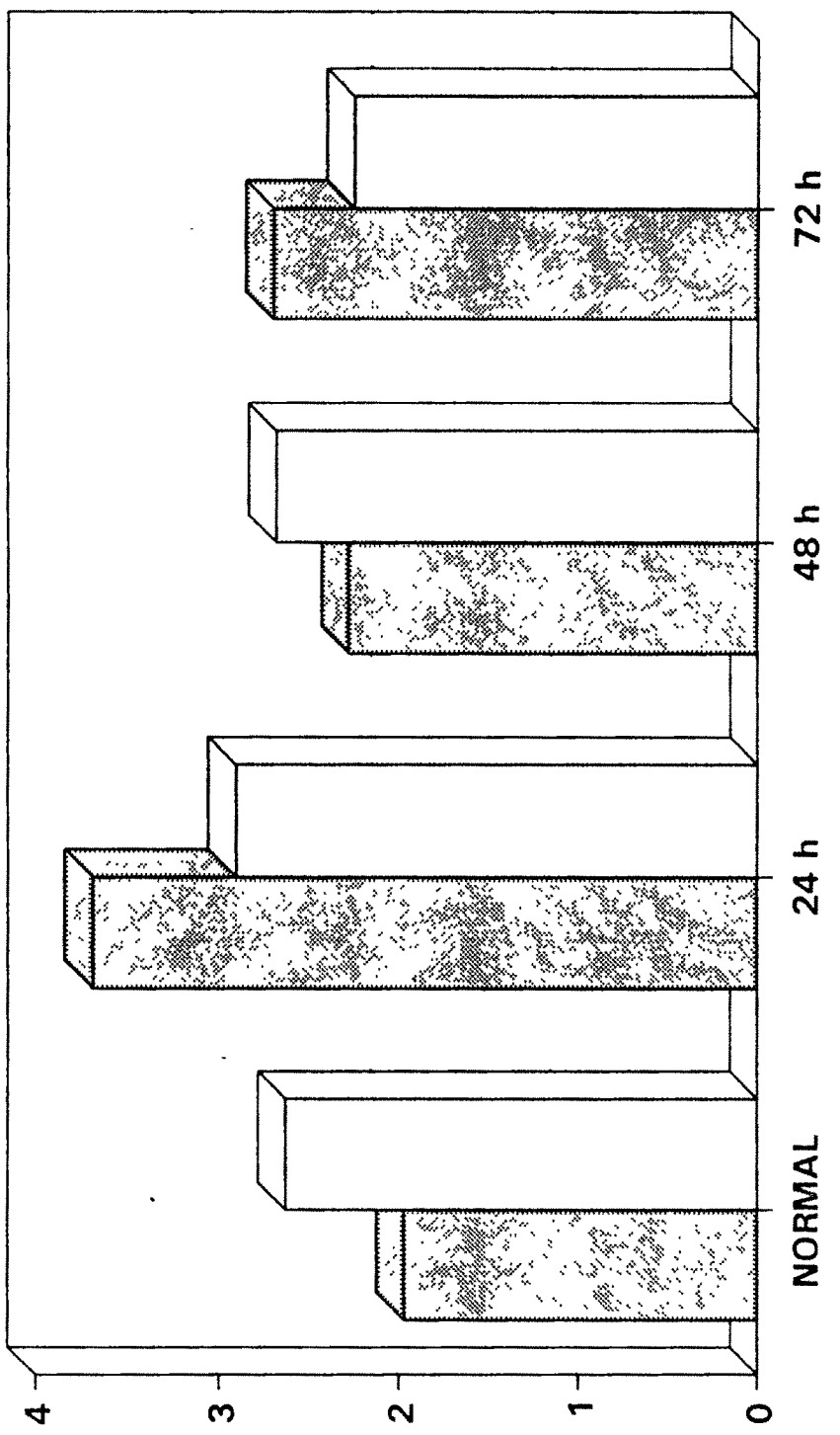

4
3
2
1
0
NORMAL
24 h
48 h
72 h
POST-OVX INTERVALS
Sp
R
cAMP-PDE
µg PO4 released / mg protein /60 min at 37°C

FIG. 14.

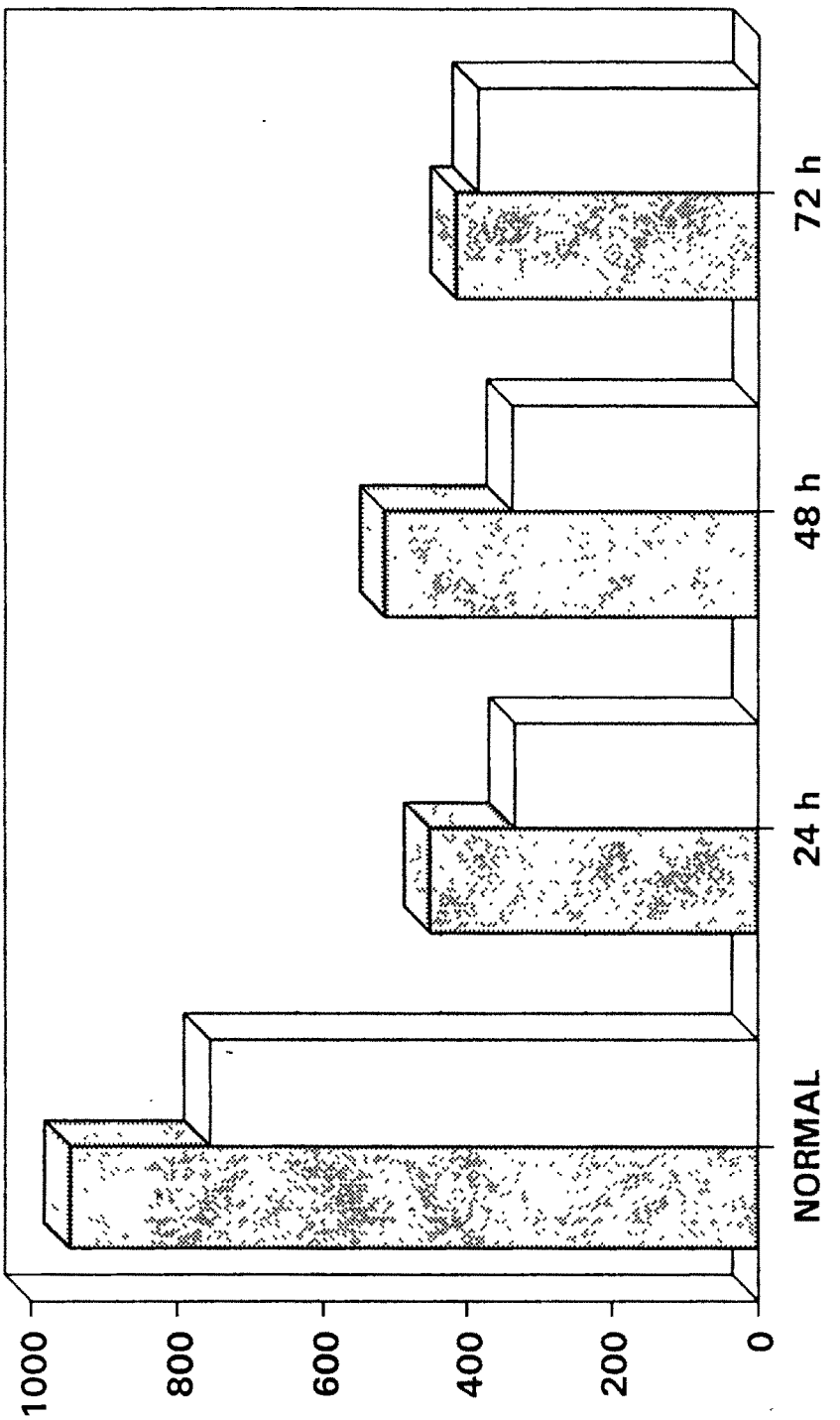

1000
800
600
400
200
0
NORMAL
24 h
48 h
72 h
POST-OVX INTERVALS
Sp
R
TOTAL ATPase
µg PO4 released /mg protein /60 min at 37°C

FIG. 15.

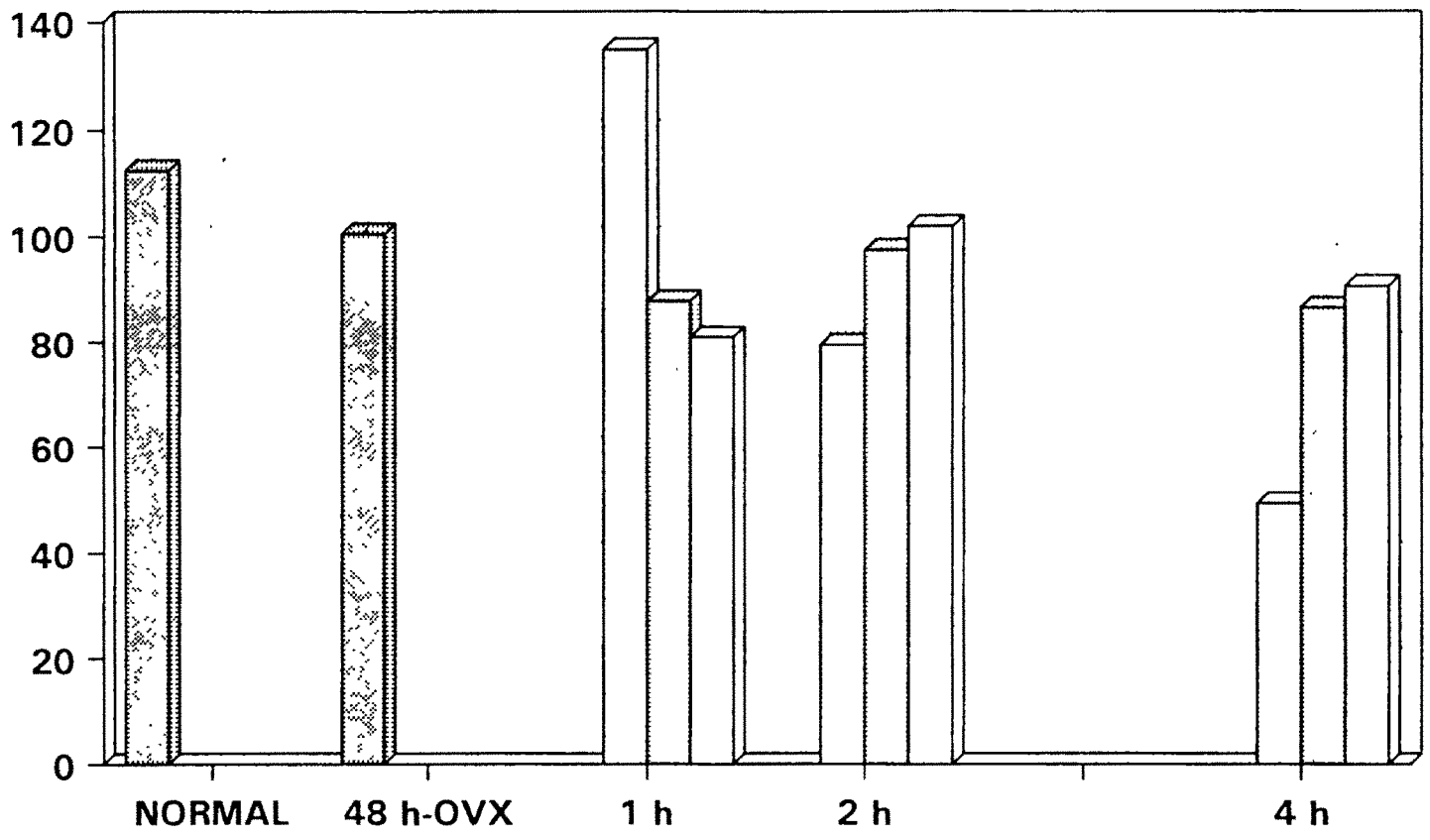
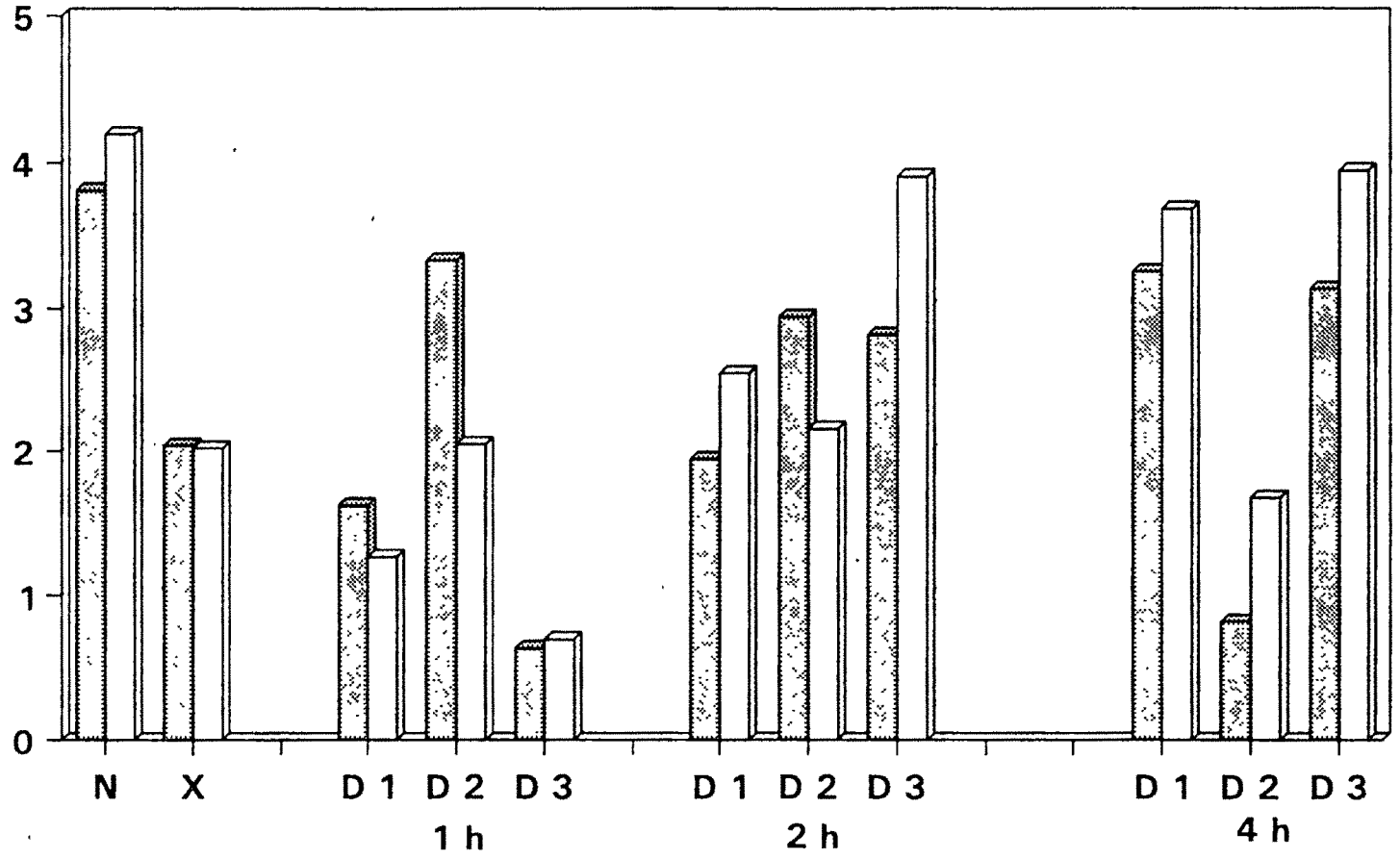

140
120
100
80
60
40
20
0
NORMAL
48 h-OVX
1 h
2 h
4 h
POST INJECTION INTERVALS
D 1
D 2
D 3
PLASMA GLUCOSE
mg/100 ml plasma

FIG. 16.



N = NORMAL

X = 48 h OVX

FIG. 17.

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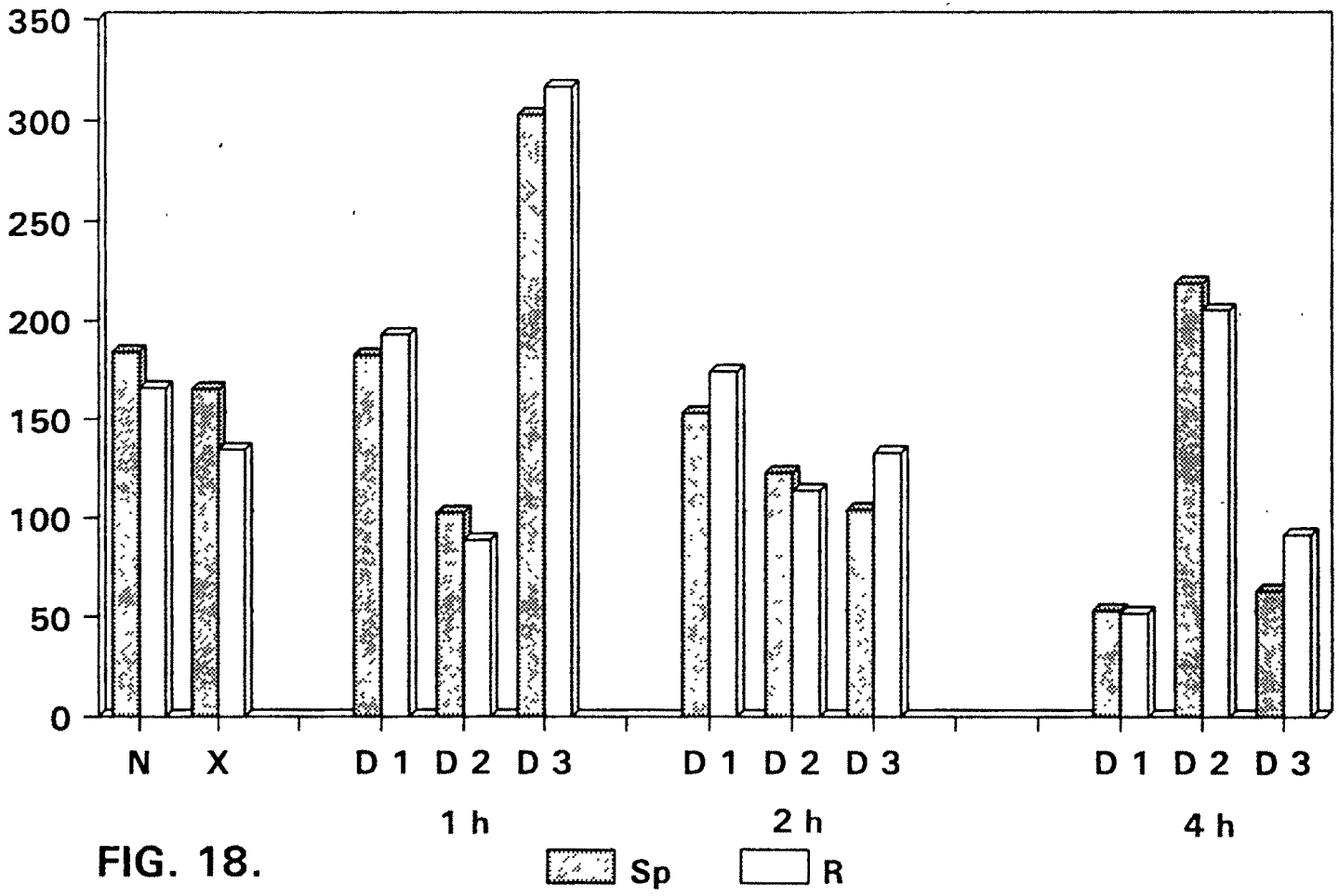
350
300
250
200
150
100
50
0
N
X
D 1
D 2
D 3
1 h
D 1
D 2
D 3
2 h
D 1
D 2
D 3
4 h
Sp
R
PHOSPHORYLASE
µg PO4 released / mg protein / 60 min at 37°C

FIG. 18.

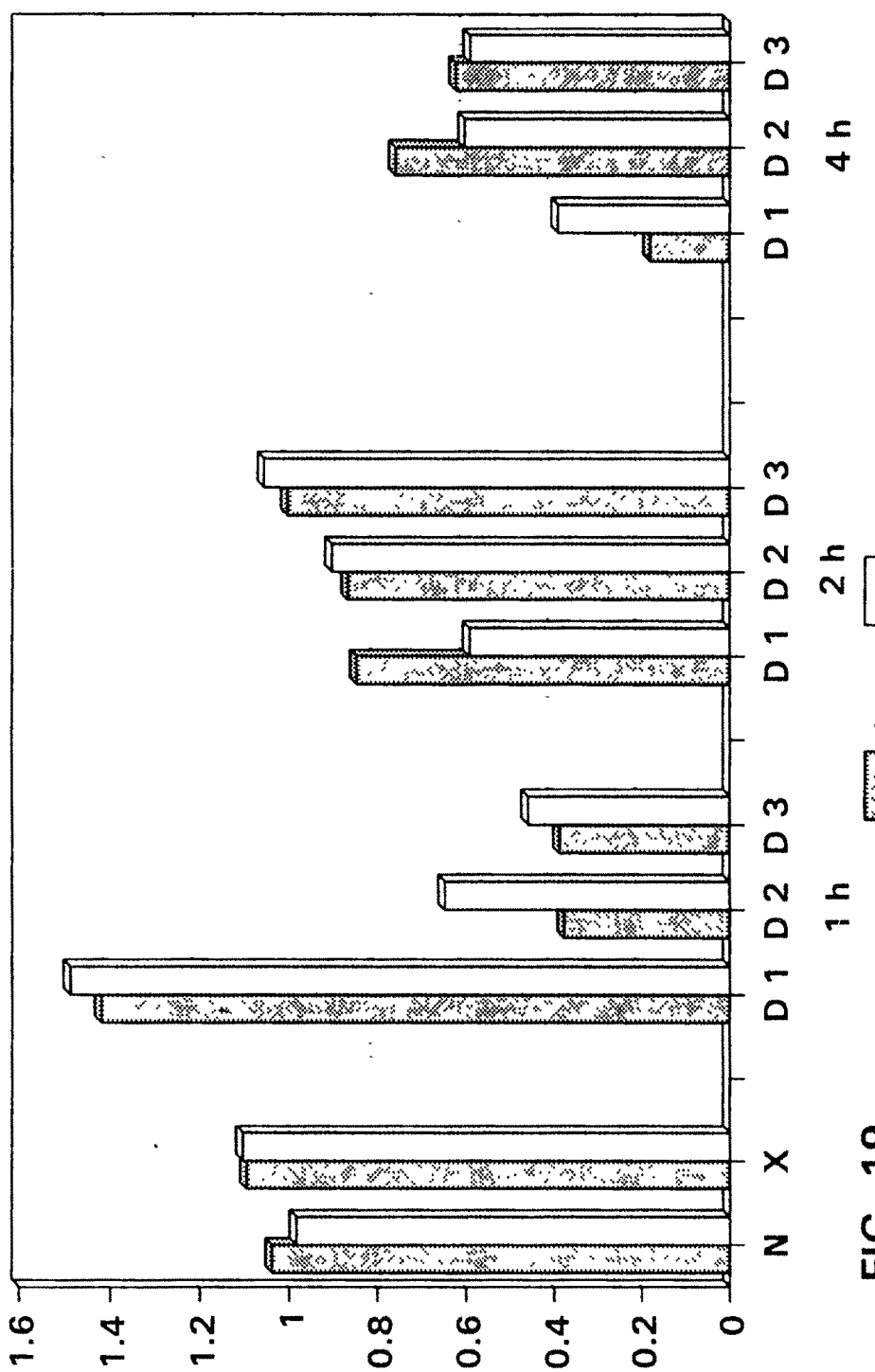
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
N
X
D 1
D 2
D 3
1 h
D 1
D 2
D 3
2 h
D 1
D 2
D 3
4 h
Sp
R
G-6-Pase
µmole PO4 released / mg protein / 60 min at 37°C

FIG. 19.

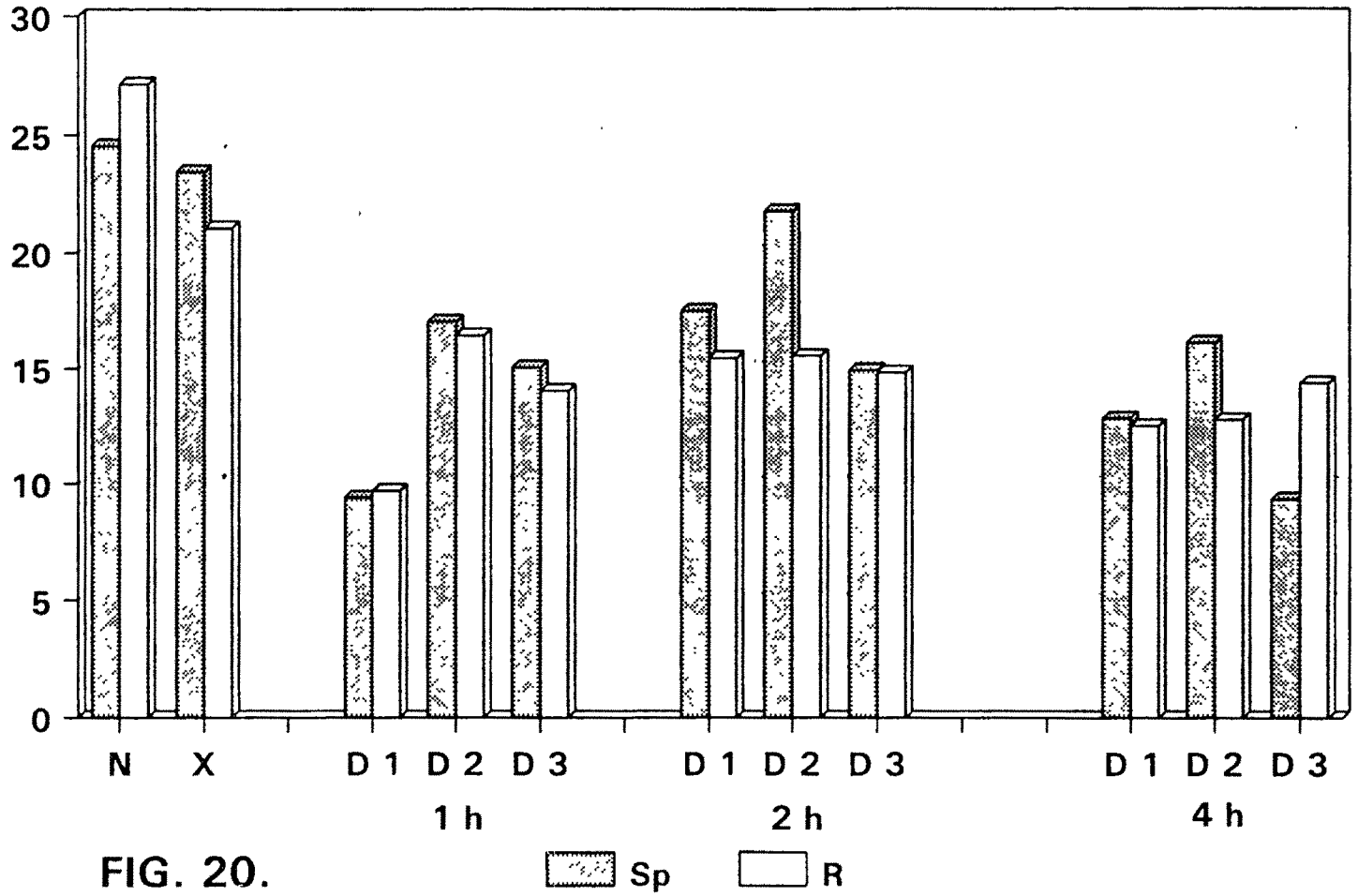
30
25
20
15
10
5
0
N
X
D 1
D 2
D 3
1 h
D 1
D 2
D 3
2 h
D 1
D 2
D 3
4 h
Sp
R
SUCCINATE DEHYDROGENASE
µg Formozan formed / mg protein /60 min at 37°C

FIG. 20.

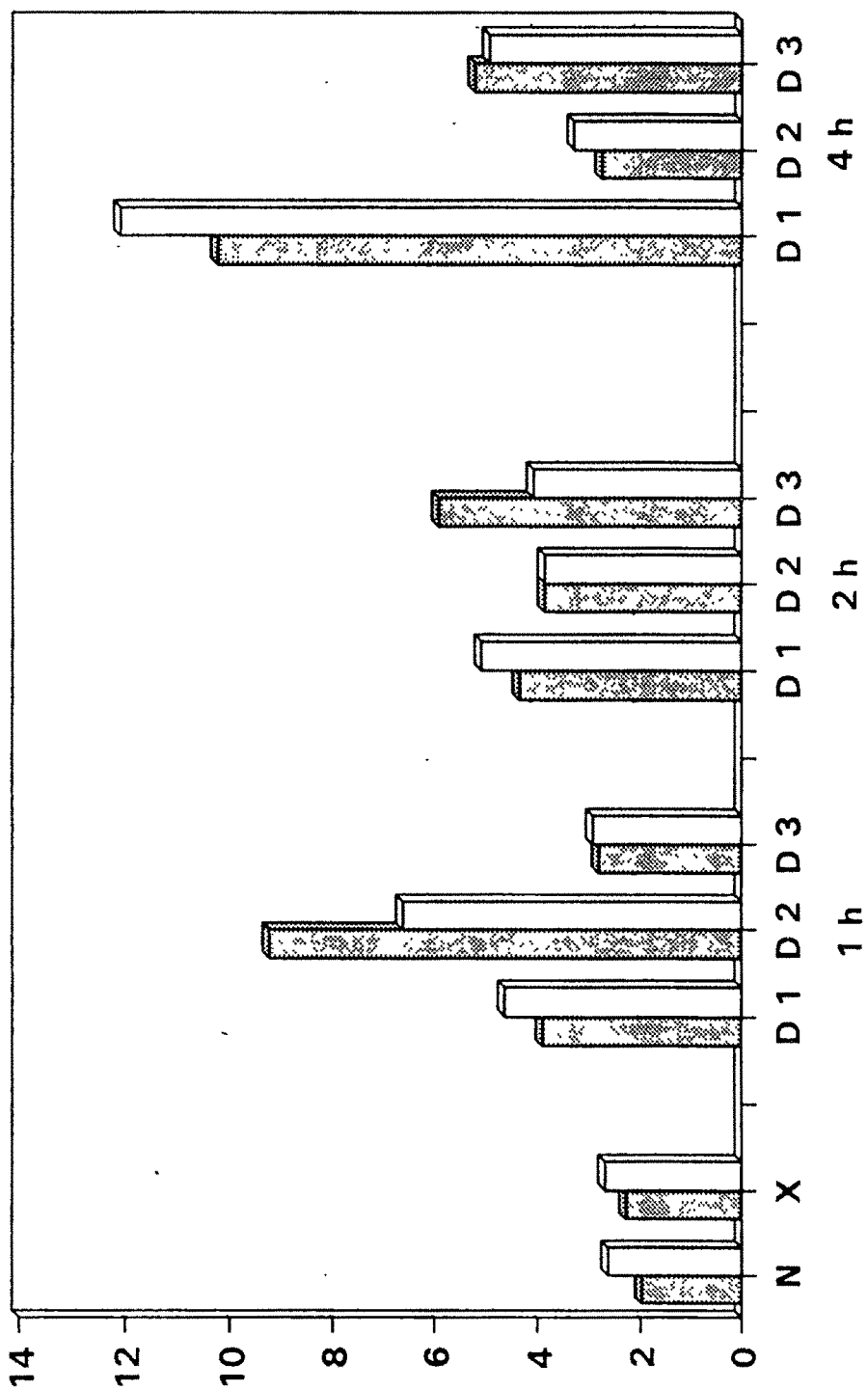
14
12
10
8
6
4
2
0
N
X
D1 D2 D3
1 h
D1 D2 D3
2 h
D1 D2 D3
4 h
Sp
R
cAMP-PDE
µg PO4 released / mg protein / 60 min at 37°C

FIG. 21.

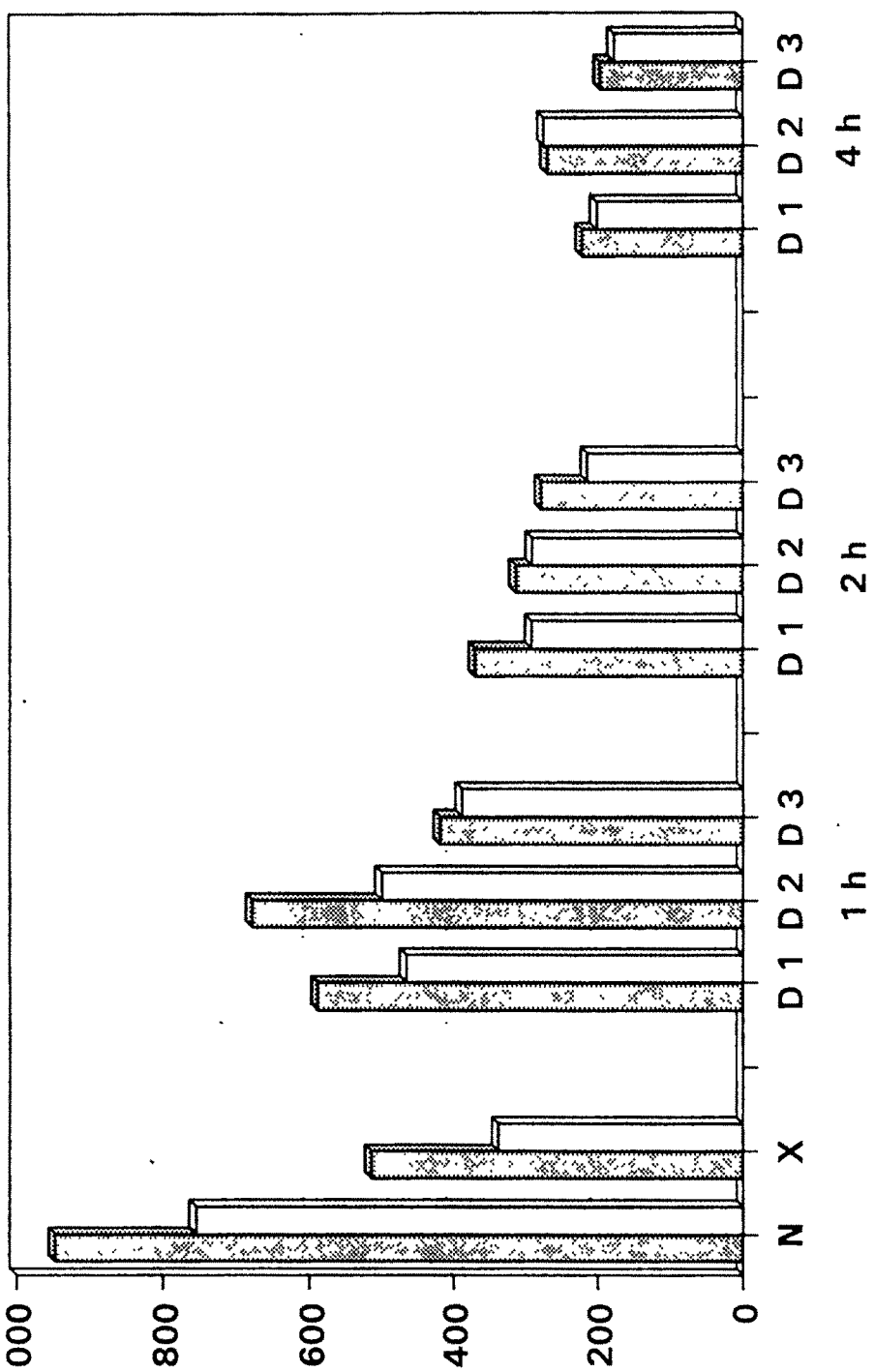
1000
800
600
400
200
0
N
X
D 1 D 2 D 3
1 h
D 1 D 2 D 3
2 h
D 1 D 2 D 3
D 1 D 2 D 3
4 h
Sp
R
TOTAL ATPase
µg PO4 released / mg protein /60 min at 37°C

FIG. 22.

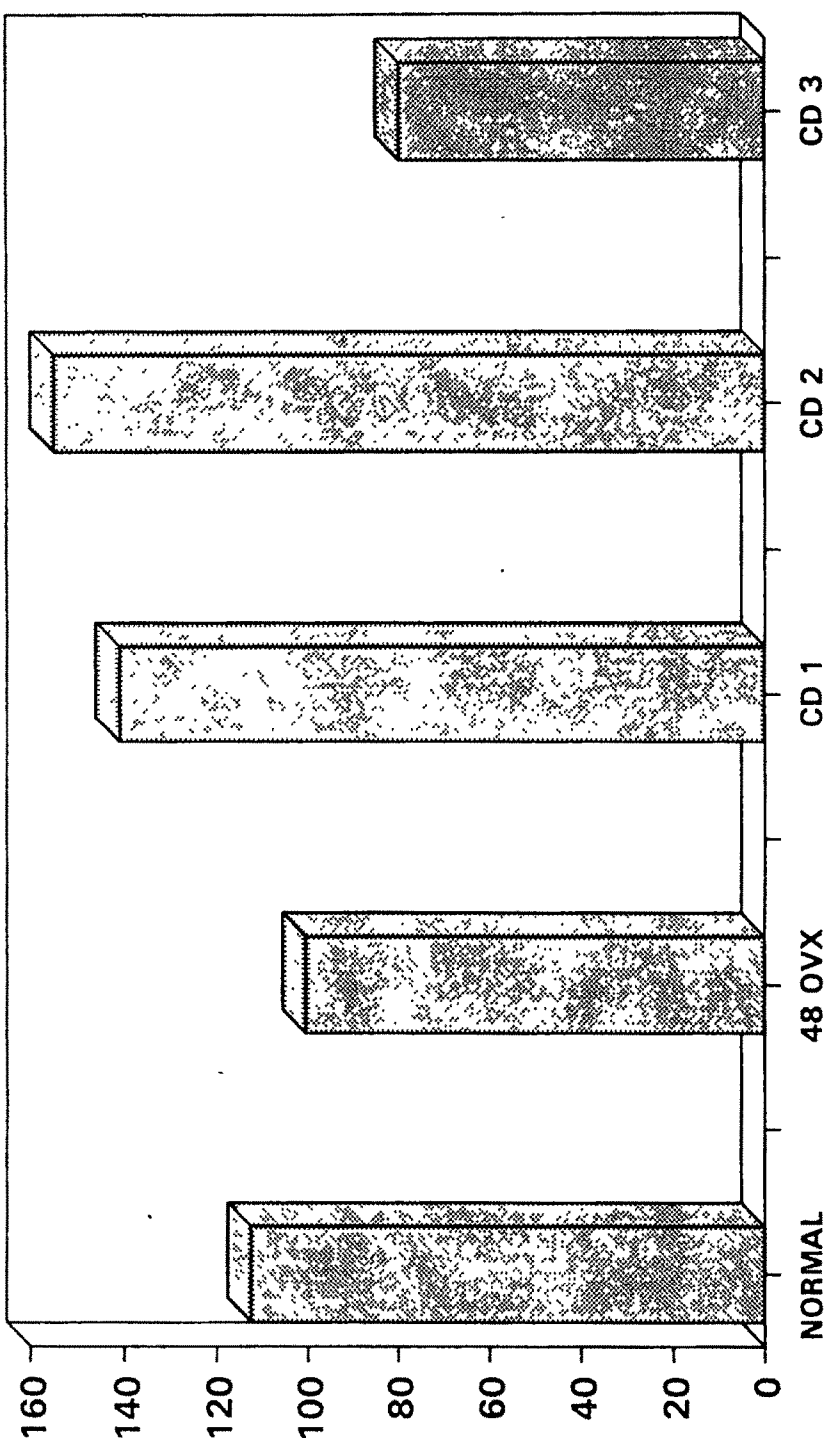
160
140
120
100
80
60
40
20
0
NORMAL
48 OVX
CD 1
CD 2
CD 3
KILLED AFTER 2 h OF INJECTION
PLASMA GLUCOSE
mg/100 ml plasma

FIG. 23

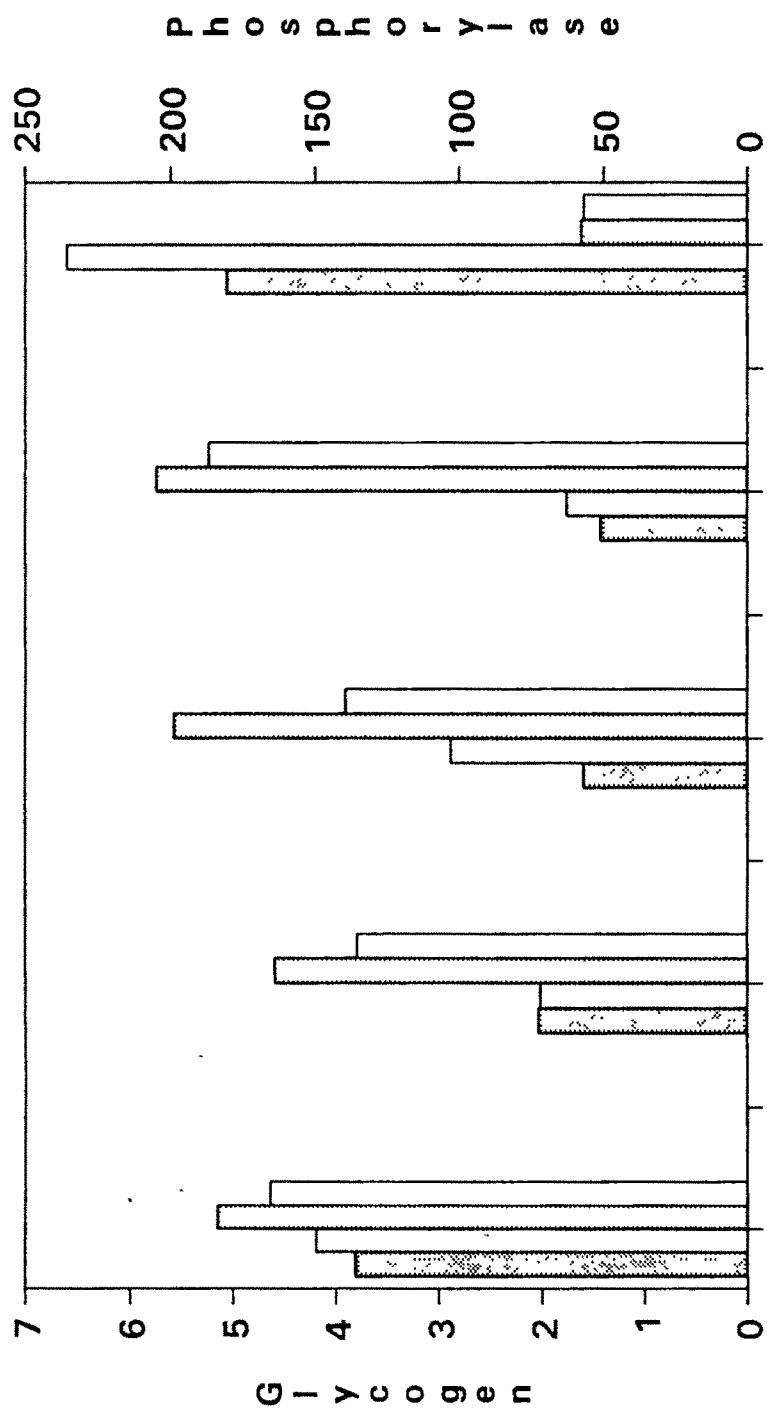
Glycogen
Phosphorylase
0
1
2
3
4
5
6
7
0
50
100
150
200
250
NORMAL
48 OVX
CD 1
CD 2
CD 3
G-Sp
G-R
P-Sp
P-R
KILLED AFTER 2 h OF INJECTION
GLYCOGEN AND PHOSPHORYLASE
mg/100mg fresh tissue
µg PO4 released / mg protein / 60 min at 37°C

FIG. 24.

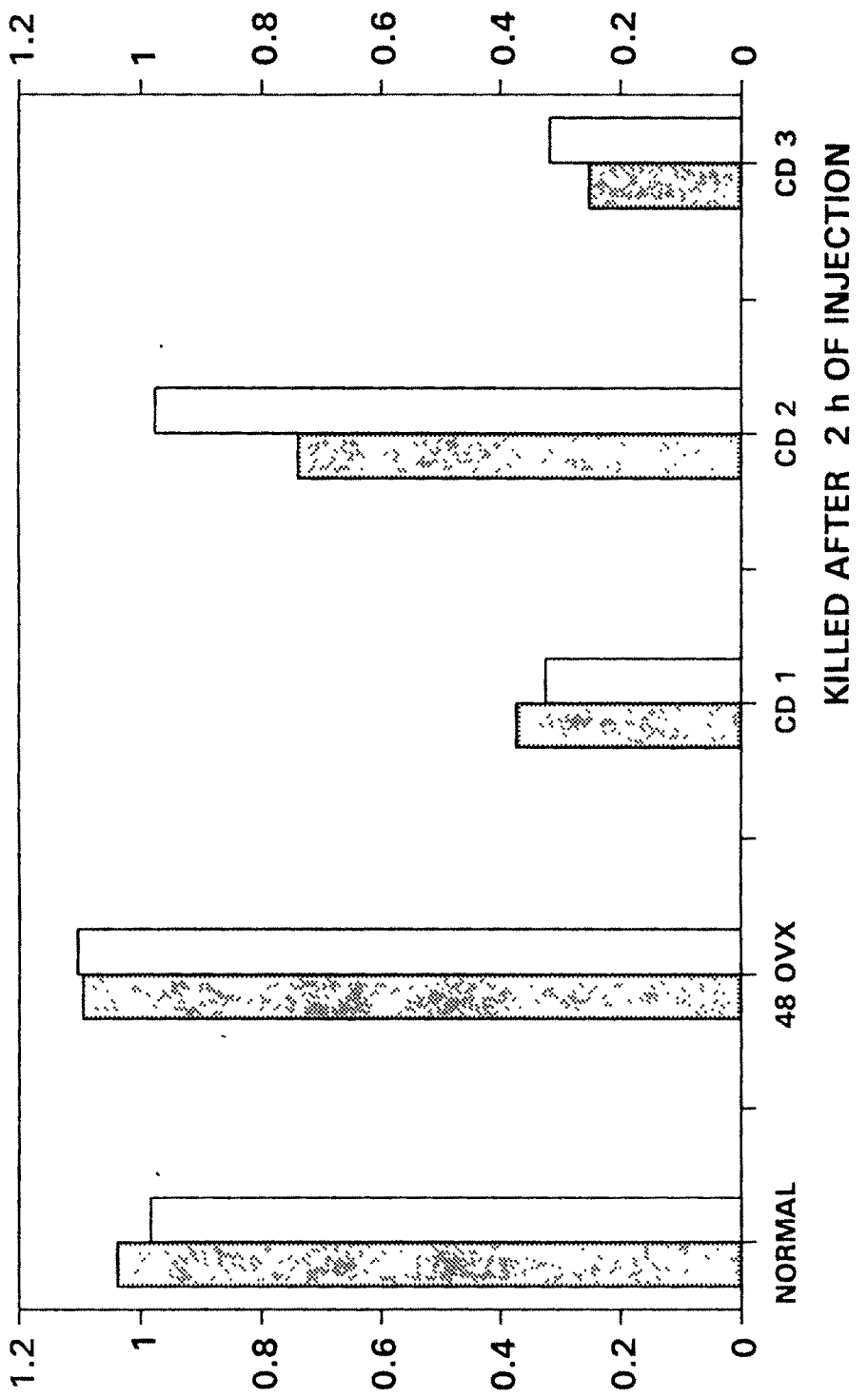
1.2
1
0.8
0.6
0.4
0.2
0
NORMAL
48 OVX
CD 1
CD 2
CD 3
KILLED AFTER 2 h OF INJECTION
Sp
R
G-6-Pase
µmolePO4 released / mg protein / 60 min at 37°C

FIG. 25.

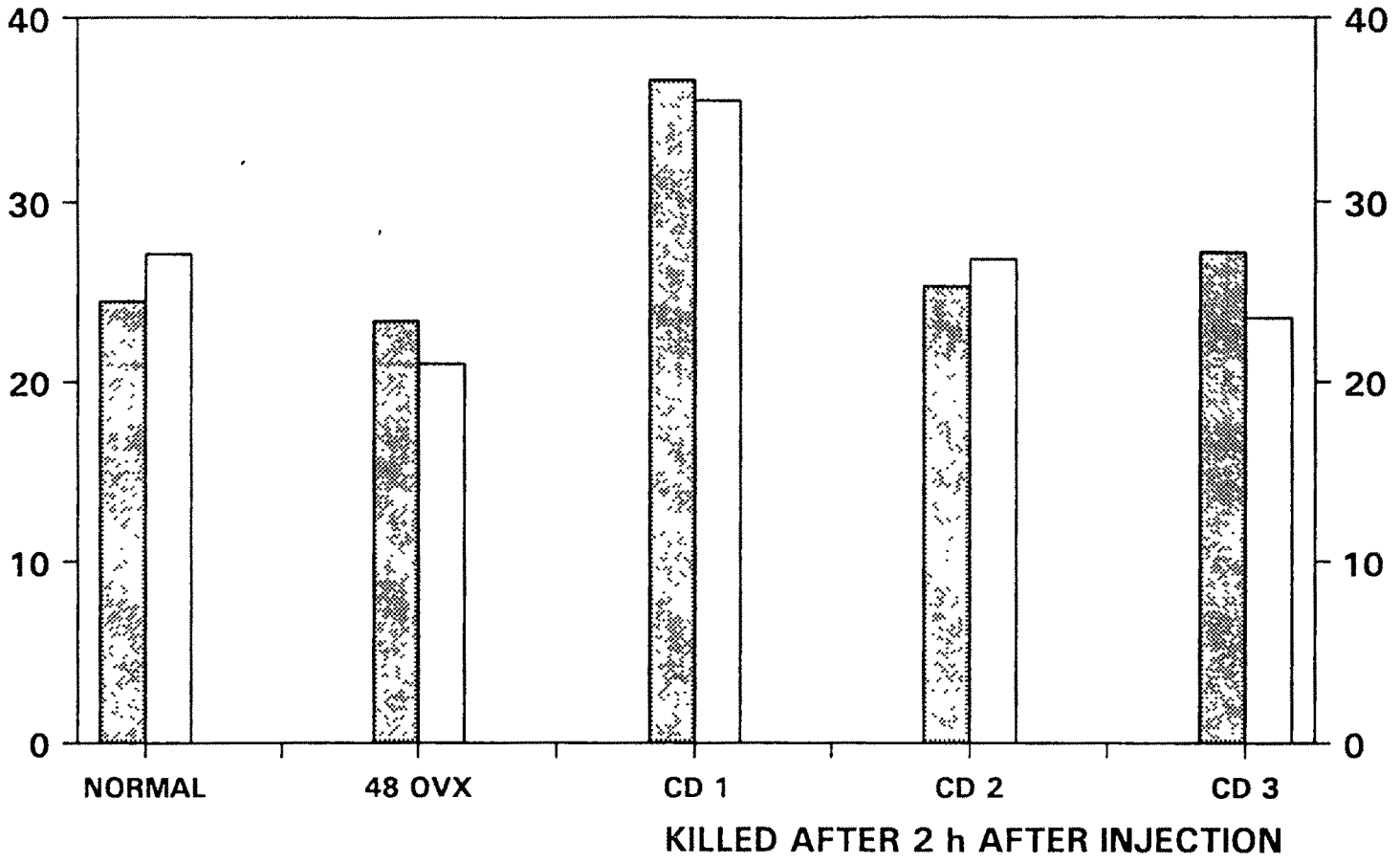
40
30
20
10
0
NORMAL
48 OVX
CD 1
CD 2
CD 3
KILLED AFTER 2 h AFTER INJECTION
Sp
R
SUCCINATE DEHYDROGENASE
µg Formozan formed / mg protein / 60 min at 37°C

FIG. 26.

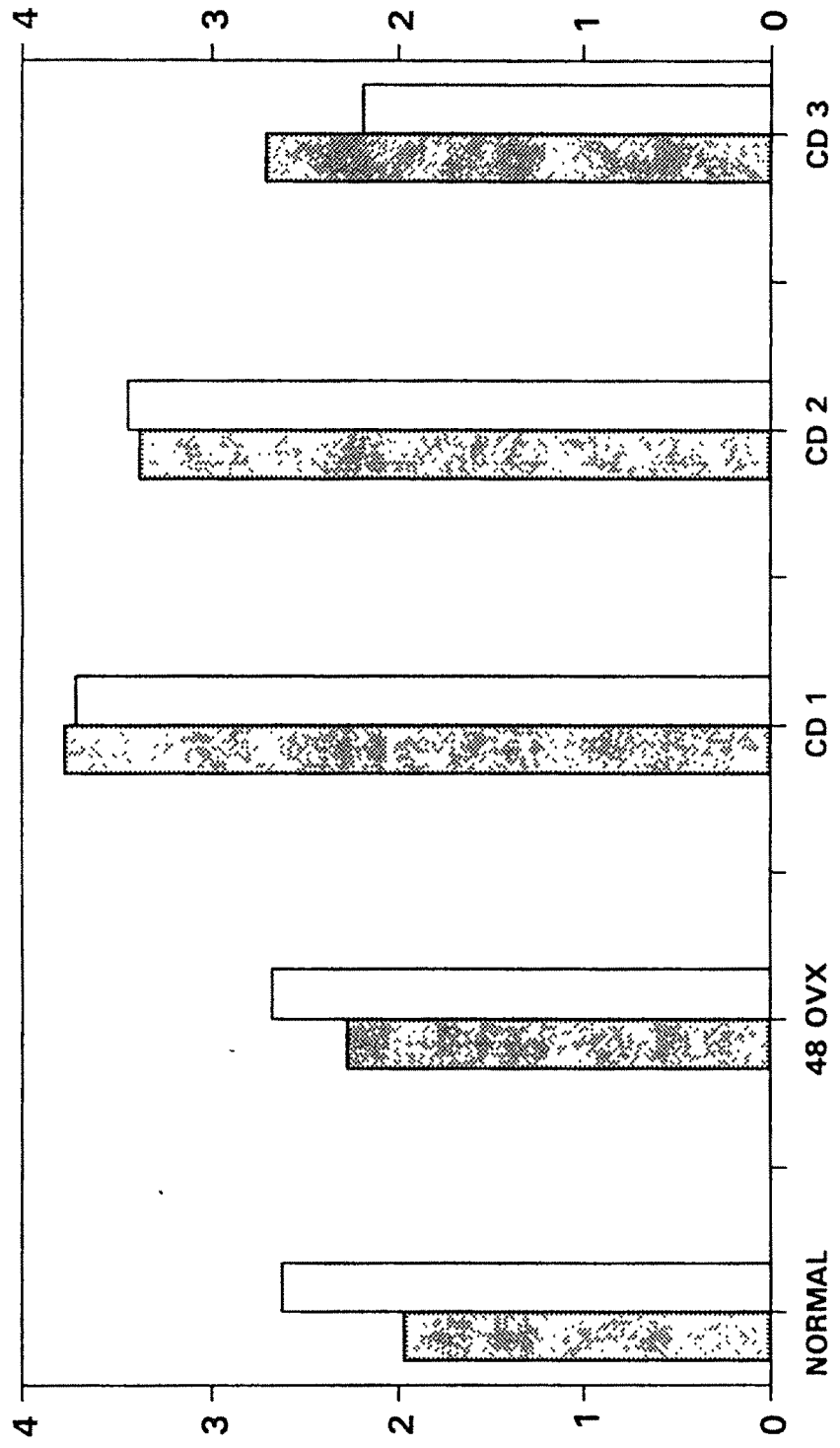
4
3
2
1
0
NORMAL
48 OVX
CD 1
CD 2
CD 3
KILLED AFTER 2 h OF INJECTION
Sp
R
cAMP-PDE
µg PO4 released / mg protein / 60 min at 37°C

FIG. 27.

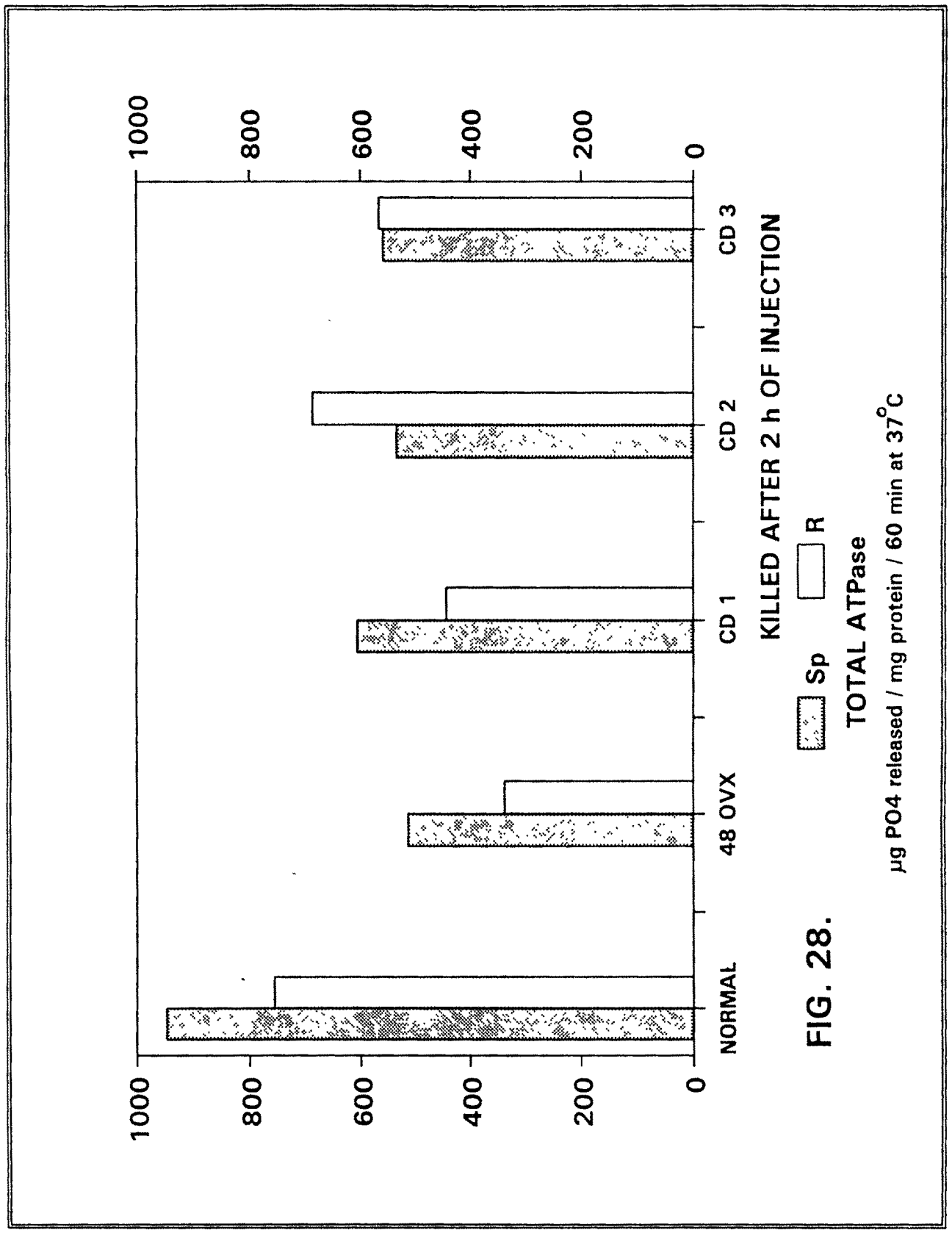
1000
800
600
400
200
0
NORMAL
48 OVX
CD 1
CD 2
CD 3
KILLED AFTER 2 h OF INJECTION
Sp
R
TOTAL ATPase
µg PO4 released / mg protein / 60 min at 37°C

FIG. 28.

activity in both the lobes at any post-OVX interval was decidedly lower than normal (Fig.15).

Replacement therapy with E_2 alone:- With D_1 after 1 h a significant hyperglycemic influence was observable. On the other hand, a distinctly increasing hypoglycemic action of D_1 was recorded with further lapse of time. As compared to this, with D_2 as well as D_3 though hypoglycemic influence was apparent, at all the intervals its intensity and variations were not so significant (Fig.16).

After E_2 administration G-6-Pase activity showed more or less a pattern of variation similar to that of plasma glucose with all the three doses as well as during every interval (Fig.19). The case was different with respect to hepatic glycogen concentration and phosphorylase enzyme activity, which exhibited mutually converse patterns (Fig.17 & 18). The pattern of variation of glycogen phosphorylase activity exhibited noteworthy dose and time dependent differences. D_1 and D_3 both induced an increase in enzyme activity at 1 h interval but at 2 h interval decreasing tendency became apparent to intensify markedly by 4 h interval. A point that should be noted here concerns the higher magnitude was suppressed at 1 h interval but at subsequent intervals the same increased gradually. As stated earlier, generally the variation in hepatic glycogen concentration was antagonistic to that of glycogen phosphorylase activity.

If OVX lowered hepatic SDH activity then E_2 replenishment would be expected to counteract the influence of OVX on this enzyme activity. Contrary to this, in general, the enzyme activity during all E_2 regimes was lower than normal diestrous level as well as that of 48 h OVX animals (Fig.20). With D_1 regime the SDH activity was highly decreased at 1 h interval, it showed increased level at 2 h but decreased once again by 4 h interval. Not considering the individual numerical value of SDH activity with D_2 and D_3 regimed of replacement between these two that of D_2 at 2 h interval seemingly counteracted the influence of OVX to a better extent. Nevertheless, no replacement therapy employed here was capable of completely restoring this enzyme activity.

With respect to cAMP-PDE it can be said that OVX brought about a slight increase in its activity and that none of the E replacement could restore it to normal diestrous level (Fig.21). On the contrary, varying degrees of additional enhancement of the enzyme activity were apparent. Such variations, however, differed in increasing patterns. With D_2 initial enhancement was significant but later it depicted gradual reduction. D_3 was initially without noticeable influence but with further time lapse this dose brought about significant increase in PDE activity.

Though the initial increase in hepatic total ATPase activity was seen by 1 h after D_1 and D_2, dose and time dependent decrease in the same was evident. All E_2 regimes were found to suppress the activity drastically from 2 to 4 h post injection intervals (Fig.22).

Administration of constant dose of 2 mg P in combination with 5 (CD_1), 10 (CD_2) and 15 μg E_2 (CD_3) each:-

40 and 55% increase in plasma glucose concentration was observed giving with first two combination doses. These values were significantly higher than in normal animals. On the other hand, CD_3 reduced the glucose level drastically (Fig.23).

All three combination doses suppressed the hepatic G-6-Pase activity in OVX animals but this effect was more obvious with CD_1 and CD_3 (Fig.25). The first two combination doses induced lowering of glycogen concentration, but marked increase was noted with third one. In keeping with these variations, as would be expected, phosphorylase activity exhihibited inversely related changes (Fig.24). SDH activity was increased by CD_1 restoration was brought about by CD_2 and CD_3 (Fig.26). CD_1 and CD_2 enhanced the PDE activity (Fig.27). ATPase activity was raised by all the three combination doses (Fig.28).

The values obtained from vehicle treated animals for all parameters were almost close to that of 48 h OVX values, hence are not taken into consideration.

DISCUSSION

The homeostatic mechanism of carbohydrate metabolism in mammalian tissue has been a subject of intensive investigation in the past years. The rate - limiting enzyme systems of carbohydrate metabolism in liver, muscle, brain, cardiac and adipose tissue are known to be under the homeostatic control of hormones and cofactor (Larner, 1966 and Leloir, 1967).

The present work showed that lack of ovarian sex hormones (OVX) induced a general glycogenolytic action with concomitant noticeable increase in G-6-Pase activity getting reflected in rising plasma glucose level and later bringing it to normal range by 72 h. Though the variations in concerned supportive enzyme activities like those of glycogen phosphorylase and total ATPase exhibited fluctuating patterns, these, show, in general, were found to be of compli-

mentary nature. However, since the cAMP-PDE activity was on the higher side after OVX it could be surmised that consequent reduction in intracellular cAMP might have got reflected in general lowering of glycogen phosphorylase activity (Hers, 1976; Matschensky, 1990). Simultaneously observed decreasing levels of total ATPase activity may be understood to have been responsible for reduced intracellular utilization of products of glycogen break down but facilitating output of glucose into blood as evidenced by enhansing G-6-Pase activity.

Initial suppression of SDH activity due to OVX and its gradual normalization by 72 h seemingly denotes a transient and hence not so significant influence of lack of ovarian hormones in the context. However, in a latter Chapter-5, accumulation of hepatic lipids due to OVX has been shown, which probably is a reflection of decreased oxidation as indicated by lowered SDH activity.

With respect to E_2 and E_2 + P replacement regimes it is apparent from the data presented here that E_2 alone (D_1) reverses the effect of OVX very transiently only by 1 h interval as far as glycogen breakdown and release of glucose into blood is concerned. However, overall effect of E_2 alone does not seem to be beneficial. With the three doses there is general suppression of SDH activity and enhancement of PDE activity. Further E_2 alone suppressed the total ATPase activity. Considering these three variables it could be said that there is lowering of general metabolic activity. Fluctuations brought about in other parameters understudy do not show normalization. One of the noticeable fact is that, despite numerical variations, mutually inverse relationship between glycogen concentration and glycogen phosphorylase activity is maintained. Secondly whatever degree of restorative tendencies could be picked out from the data many of these were evident with D_2 at 2 h interval.

When one takes into consideration the recorded values with E_2 + P treatments it can be seen that CD_1 and CD_2 at 2 h interval apparently bring about some restorative effects. eg. 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 somewhat on the hyperphysiological side. In stark contrast, CD_3 brings about a very different metabolic state leading to significant glycogen buildup through suppression of phosphorylase and G-6-Pase and lowering of blood glucose level. It can, therefore, be suggested that short term influences of OVX E_2 and E_2 + P treatment does bring forth some interesting immediate metabolic alterations in hepatic tissue, which, if pursued in an extensive and intensive manner, may throw some light on early influence on homeostatic mechanisms. Further, this information may explain as to how the delayed adaptations, as have been reported by several workers (Matute and Kalkhoff, 1973 and John *et al.*, 1973), get settled by prolonged intervals of repeated

administration of gonadal hormones. One of the possible advantages would be to plan a more compatible hormonal schedule in the correction of endocrine disturbances related to physiological adaptability.