CHAPTER IX

EFFECT OF 6-HYDROXYDOPAMINE ON THE METABOLIC ACTIVITIES OF THE KIDNEY OF BLUE ROCK PIGEON (*COLUMBA LIVIA*)

In 1967 Trutnov and Thoenen made the remarkable discovery that an isomer of norepinephrine namely, 6-hydroxydopamine (6-OHDA), produced a destruction of the terminal ground plexus of peripheral sympathetic nor-adrenergic neurons. This phenomenon has since been termed as 'chemical sympathectomy'. It is a relatively selective effect with cholinergic neurons; Schwann cells, glial cells, endothelial and other cell types remaining virtually unaffected at the ultrastructural level. When the compound was injected into the brain, dopaminergic neurons were destroyed along with nor-adrenergic neurons. Since monoaminergic neurons are implicited in numerous different types of behavioural, learning and metabolic processes, such finding of relatively selective morphological and biochemical alterations are of immense importance (Kostrezewa and Jocobowitz, 1974).

Studies using 6-OHDA indicate that the vessels and tubules receive solely adrenergic nerves, which contain acetylcholinesterase (Dibona, 1978). Discrete neurovascular and neurotubular junctions, morphologically consistent with sites of synaptic transmission, are seen with electron microscope. The ultrastructural studies by Dibona (1978) provide a firm support

for a direct effect of adrenergic nerve terminals on renal tubular epithelial cells. In the rat, studies using 6-OHDA suggest that the glomerular arterioles and surrounding tubules receive exclusively adrenergic nerves containing acetylcholinesterase. The physiologic significance of vascular innervation is well established and the renal nerves are considered to exert an influence on renin secretion (Davis and Freeman, 1976). Recent studies have pointed out the possible significance of tubular innervation, by showing an effect of the renal nerves on proximal tubular reabsorbtion of sodium apparently independent of any renal vascular changes (Barajas, 1978). Renal sympathetic nerve activity exerts a number of effects that lead to development of hypertension. (Fink and Brody, 1978). Administration of 6-OHDA into the portal vein selectively destroys the hepatic sympathetic nerves (Lautt and Cote, 1976; 1977) and also prevents the neural stimulation of glucose release (Lautt and Wong, 1978a). Reciprocal changes in gluconeogenic enzyme activity in kidney and liver by VMH lesions is also observed by Nagai et al. (1983). Studies by Dibona and Sawin (1983) suggest that intact renal innervation is required for normal renal sodium conservation and maintenance of body sodium balance during dietary sodium restriction. Chemical symphatectomy resulted in persistent vasoconstriction and increased ptiloerection which minimize heat loss in pigeons (Rintamaki and Nikula, 1982). Some authors have also reported the specific contributions of the kidney and spleen to

cardiovascular homeostasis by differential sympathetic influence on each organ (Weaver et al., 1984). The existence of dopaminergic vasodilator nerves in the renal cortex of the rat is observed by Chapman et al. (1982). Neonatal treatment with the catecholamine neurotoxin 6-OHDA leads to permanent norepinephrinergic denervation in neocortex (Johnson and Hallman, 1982). Intraperitoneal injection of 6-OHDA results in a **nearly** complete functional symphatectomy in the cat liver (Lautt and Cote, 1976). In the intact cat pretreated with 6-OHDA, stimulation of remaining mixed hepatic nerve resulted in a rapid decrease in net hepatic glucose output reaching 1/4th of the control output by 2 minutes (Lautt and Wong,1978b).

Since autonomic nerves are found to have significant direct influence on metabolic activities of liver (Lautt, 1983; Shimazu, 1983), kidney metabolic activities could also be regulated by autonomic nerves. In fact administration of catecholamines produced several changes in the metabolic activities of the kidney of pigeon (Chapter VIII). In the light of this observations, it was deemed worthwhile to investigate the metabolic activities in kidney following chemical sympathectomy.

MATERIAL AND METHODS

Adult domesticated variety of blue rock pigeons (Columba livia) of both sexes weighing 250-300 gms were used in the experiment. The birds were acclimated to laboratory conditions for

two weeks and fed ad-libitum. The birds were divided into two groups. Experimental birds were intraperitoneally injected with 6-OHDA hydrochloride (Sigma Chemicals) dissolved in 0.85 % normal saline containing ascorbate (1 mg/ml), to a concentration of 5 mg/0.5 ml for injection. Two injections were made, 24 hours apart. Only vehicle was injected in control birds. Everytime fresh solutions of 6-OHDA were prepared and used within 2 hours. Both experimental and control birds were kept under starved conditions. 24 hours after last injection, pigeons were decapitated. Prior to decapitation, for glucose estimation, the blood was drawn from the wing vein. Kidney was quickly excised and used for glycogen and enzyme estimations. The enzymes estimated were acid and alkaline phosphatases, G-6-Pase, phosphorylase, Na^+-K^+-ATPase, Transaminases (GOT and GPT), and LDH and AChE. Glycogen and protein were also estimated. The methods followed for these estimations are given in Chapter 1.

RESULTS

The results are presented in Table I and Figs. 1 to 6.

The data show that 6-OHDA produced a significant hyperglycaemic response in the pigeon. At the same time kidney glycogen content showed an increase. Both the non-specific phosphatases, alkaline and acid phosphatases showed a decrease in response to 6-OHDA administration. Among the transaminases GOT activity was increased while GPT level was significantly

Table I : Effect of 6-OHDA administration on the metabolic activities of kidney of pigeon.

Parameters	Normal	Control	Experimental
Protein	13.956 ± 1.038	16.510 ± 1.730	16.570 ± 0.640 NS
Alk Pase	1.378 ± 0.192	0.296 ± 0.025	0.140 ± 0.021 **
Acid Pase	0.834 ± 0.113	0.271 ± 0.024	0.151 ± 0.001 **
GOT	90.4 ± 21.3	57.290 ± 6.560	92.100 ± 1.690 ***
GPT	151.620 ± 26.391	75.220 ± 2.910	48.710 ± 1.880 ***
Na^+-K^+-ATPase	133.30 ± 57.20	53.000 ± 9.87	115.890 ± 7.500 **
Phosphorylase	233.566 ± 21.963	262.600 ± 21.220	125.190 ± 6.750 ***
G-6-Pase	0.113 ± 0.022	0.114 ± 0.005	0.131 ± 0.005 *
AChE	3.370 ± 0.077	0.555 ± 0.021	1.053 ± 0.022 ***
LDH	16.00 ± 5.30	17.410 ± 1.380	9.230 ± 0.106 ***
Glucose	120.00 ± 5.262	76.510 ± 5.450	200.210 ± 5.070 ***
Glycogen	0.033 ± .009	0.024 ± 0.001	0.073 ± 0.002 ***
Body weight	290 ± 4.47	280 ± 4.08	225 ± 6.45
Kidney weight	1.59 ± 0.07	1.27 ± 0.07	1.17 ± 0.08

* $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$, NS - Not significant.

EXPLANATIONS TO GRAPHS - CHAPTER IX

Fig.1. Graphs showing the effect of 6-OHDA administration on blood sugar level in the kidney of blue rock pigeon.

Fig.2. Graphs showing the effect of 6-OHDA administration on GOT and GPT activities in the kidney of blue rock pigeon.

Fig.3. Graphs showing the effect of 6-OHDA administration on acid Pase and G-6-Pase activities in the kidney of blue rock pigeon.

Fig.4. Graphs showing the effect of 6-OHDA administration on Alk Pase and Na^{+}-K^{+}-ATPase activities in the kidney of blue rock pigeon.

Fig.5. Graphs showing the effect of 6-OHDA administration on AChE and LDH activities in the kidney of blue rock pigeon.

Fig.6. Graphs showing the effect of 6-OHDA administration on phosphorylase activity and protein content in the kidney of blue rock pigeon.

FIG. 1 : EFFECT OF 6-OHDA TREATMENT

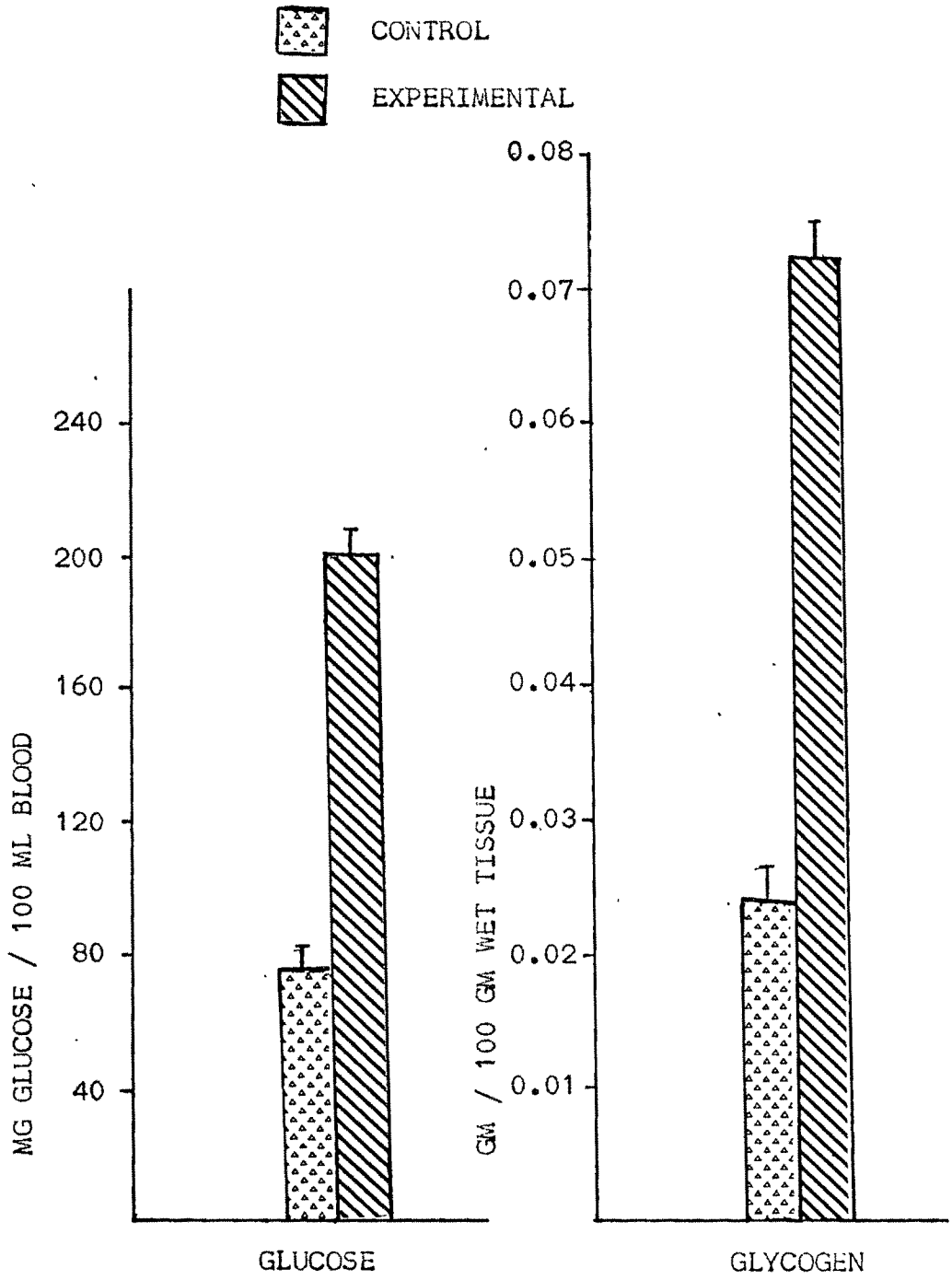


FIG. 2 : EFFECT OF 6-OHDA TREATMENT

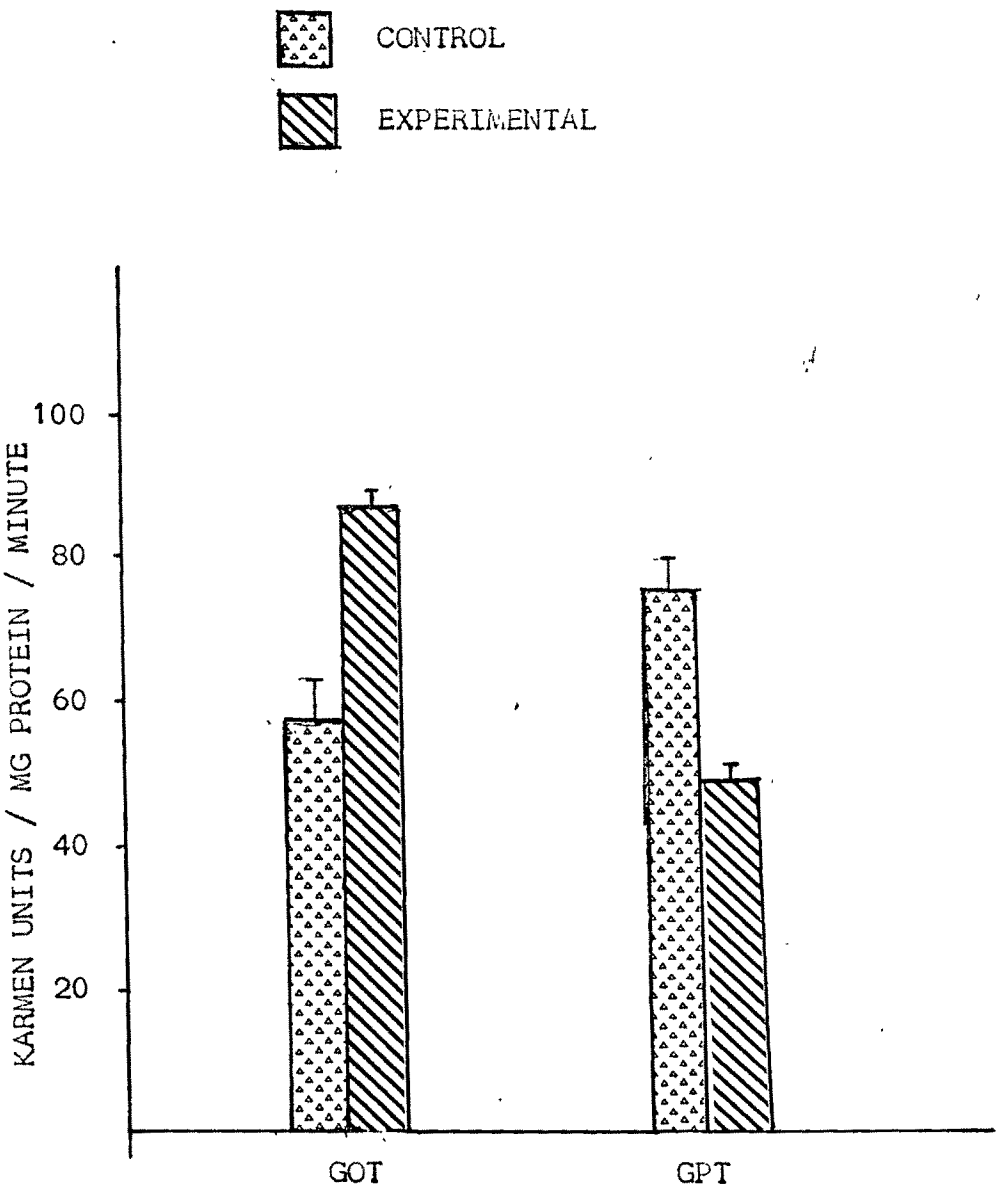


FIG. 3 : EFFECT OF 6-OHDA TREATMENT

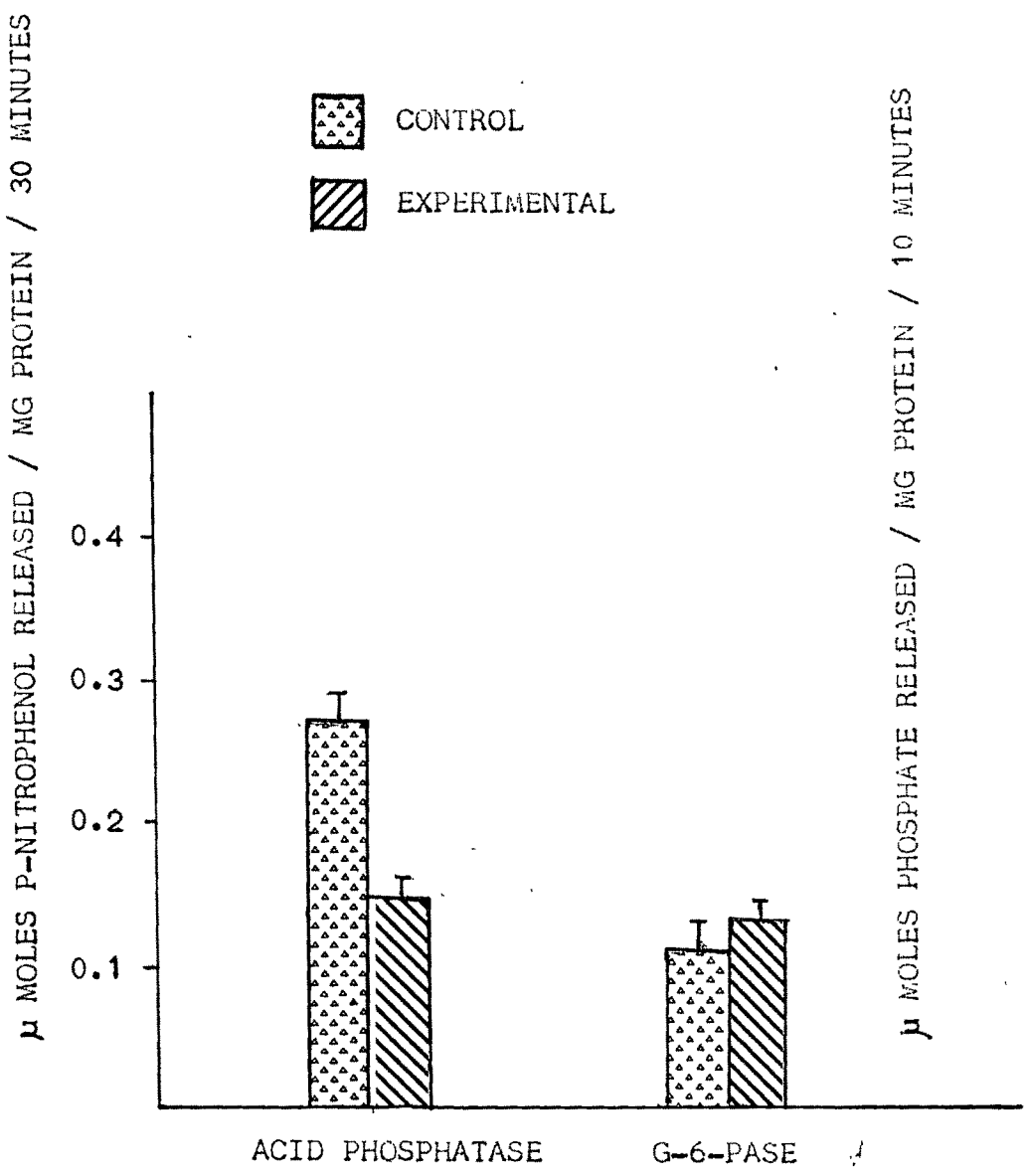


FIG. 4 : EFFECT OF 6-OHDA TREATMENT

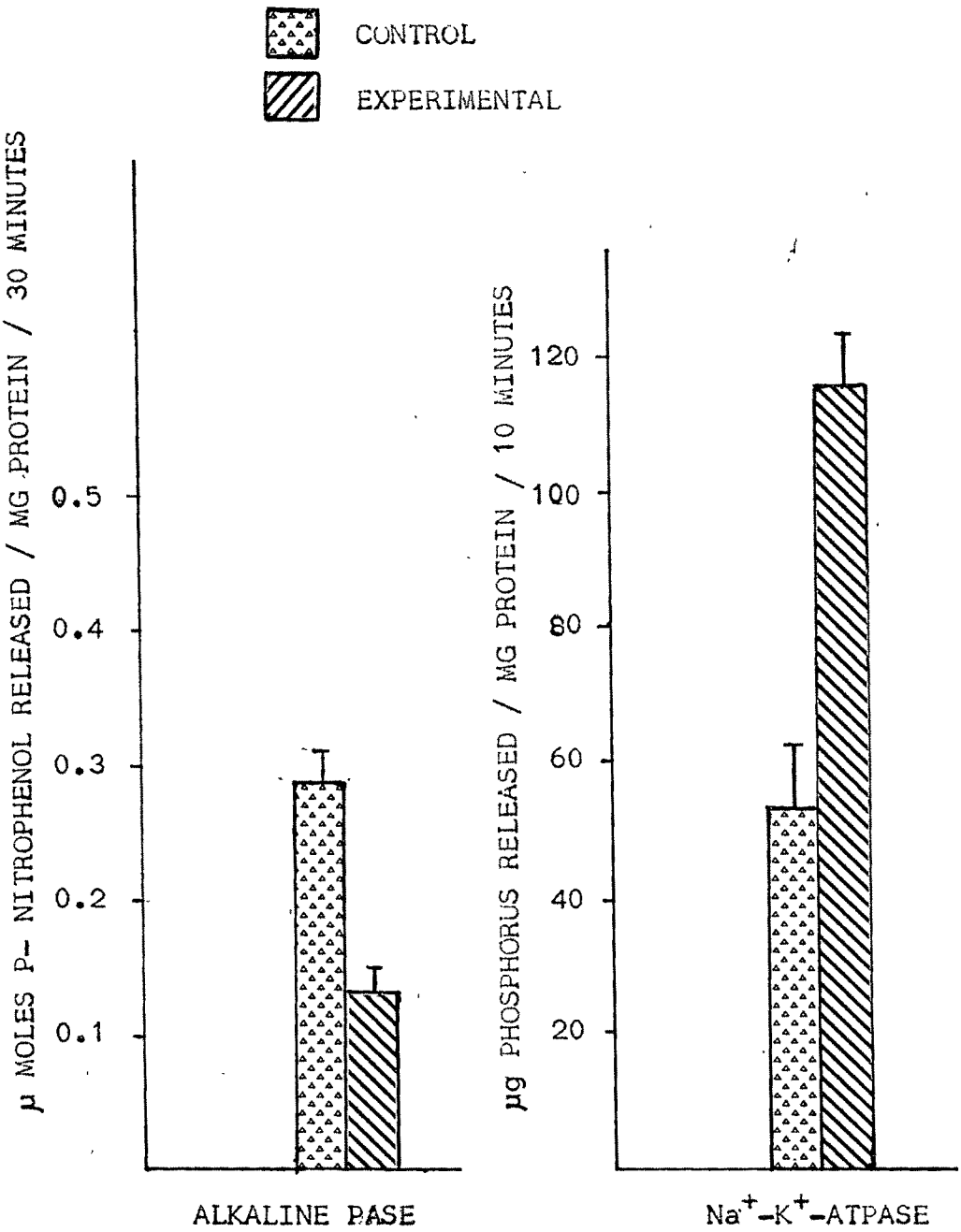


FIG. 5 : EFFECT OF 6-OHDA TREATMENT

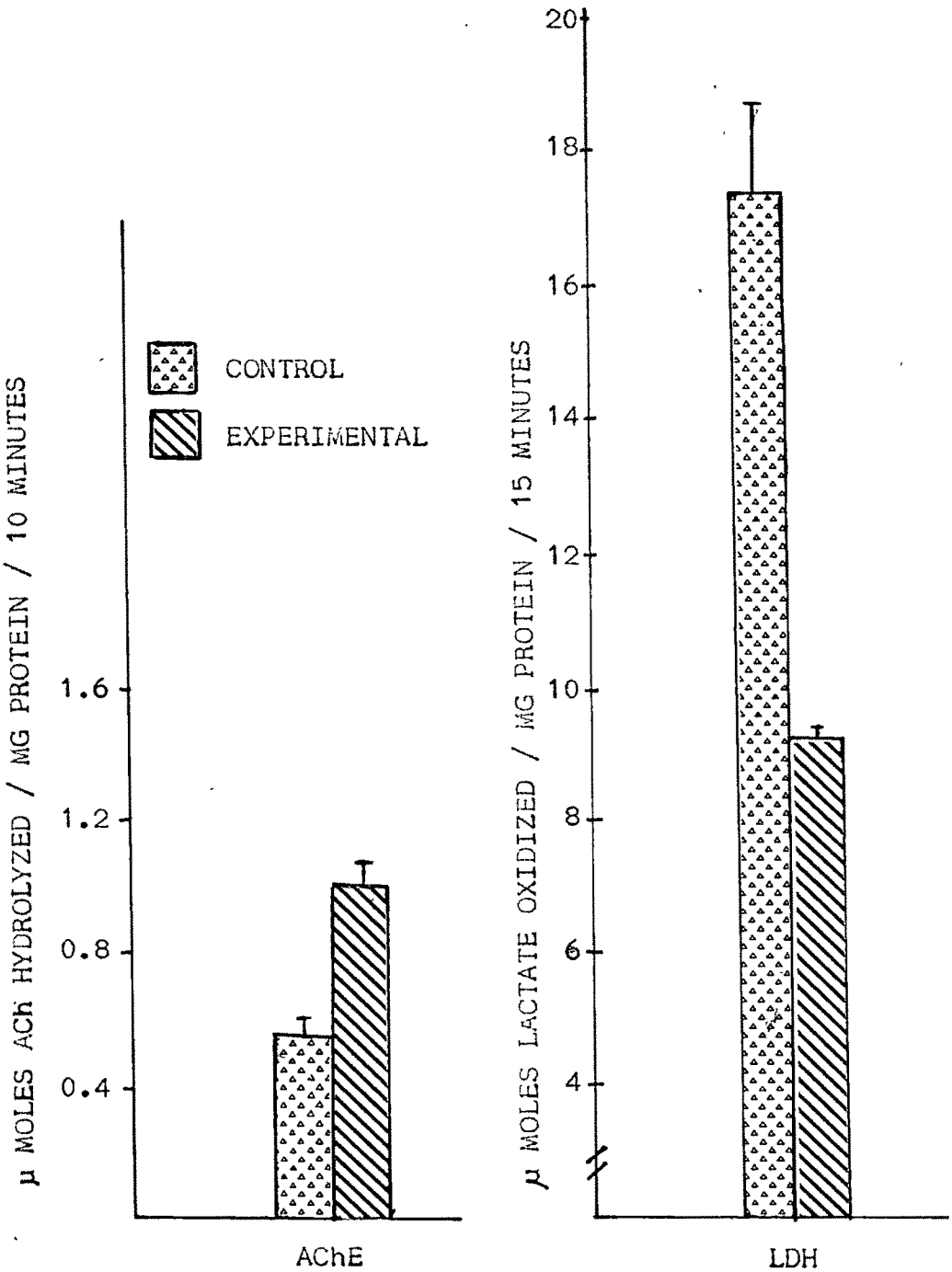
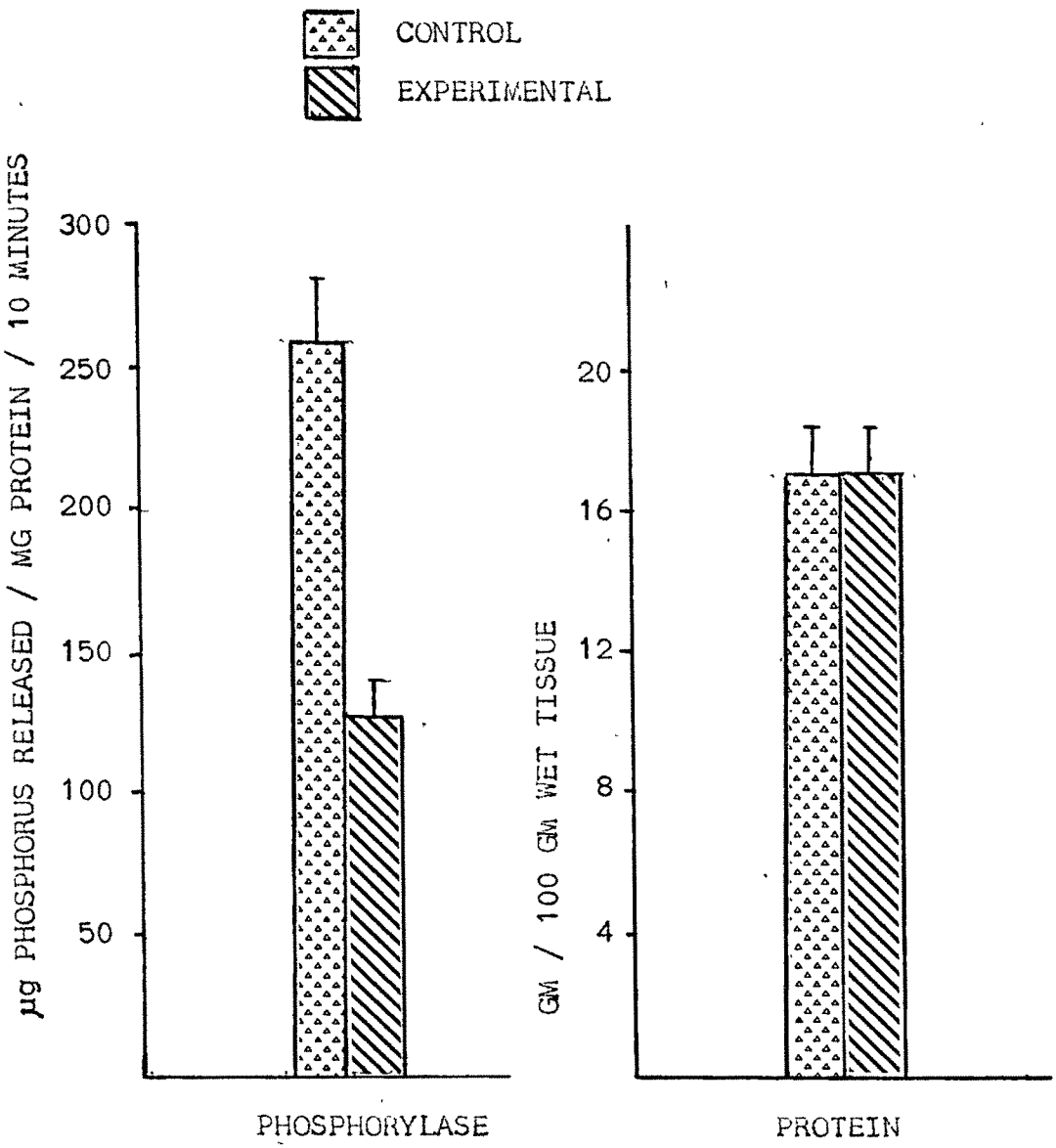


FIG. 6 : EFFECT OF 6-OHDA TREATMENT



decreased. Glycogen phosphorylase showed a decrease in activity with 6-OHDA administration. Na^+-K^+-ATPase and G-6-Pase activities increased significantly in response to 6-OHDA administration in pigeon kidney. AChE activity was increased in the kidney after 6-OHDA treatment. LDH activity too showed an increase in the kidney of 6-OHDA treated pigeons. Protein content did not show any variation in response to 6-OHDA administration. A reduction in general body weight and total kidney weight were observed in the pigeons treated with 6-OHDA.

DISCUSSION

Chemical sympathectomy, by treating pigeons with 6-OHDA for two days, produced hyperglycaemia. Administration of catecholamines (Chapter VIII) as well as glucagon (Chapter VII) also produced the same hyperglycaemic effect. Probably, the starved condition had caused a very high rate of release of adrenaline from adrenal medulla and this in turn produced hyperglycaemia inspite of 6-OHDA administration. The prevailing adrenergic influence was also evident from the metabolic activities in the kidney of 6-OHDA treated pigeons. Non-specific phosphatases, GPT, phosphorylase, LDH all showed decreased activity in the kidney of 6-OHDA treated pigeons, just as in the case of adrenaline injected pigeon kidney (Chapter VIII). Similarly in the kidneys of 6-OHDA treated and adrenaline treated pigeons, AChE and GOT showed increased activities along with an enhanced glycogen content (Chapter VIII). These results indicate that chemical

symphatectomy produced no apparent effect on the kidney metabolic activities in 48 hours starved pigeons because of probable elevated level of adrenaline in blood circulation. In the case of mammalian kidney, sympathetic innervation is mainly concerned with vascular flow and associated glomerular filtration, sodium excretion and even renin release (Davis and Freeman, 1976; Dibona, 1978). In the case of avian kidney also, sympathetic innervation may be concerned with blood flow rate. However, catecholamines present in the blood could influence the kidney cells directly and thus could influence the metabolic activities.