CHAPTER V

METABOLIC EFFECT OF INSULIN ON THE KIDNEY OF BLUE ROCK PIGEON (*COLUMBA LIVIA*)

Metabolic effects of insulin is well documented. In liver and muscle it stimulates glycogen synthetase and inhibits phosphorylase. In adipocytes, insulin stimulates lipogenic enzymes and inhibits lipase. In all these tissues, insulin exerts a profound influence on glucose uptake. In addition, the hormone also lowers the cAMP concentration in liver and fat cells by activating phosphodiesterase and blocks gluconeogenesis in liver (Claus *et al.*, 1979). Insulin, thus regulates metabolic activities by three distinct mechanisms : (1) regulates the activities of enzymes such as glycogen synthetase, phosphorylase, PEPCK etc., (2) reduces the concentration of cAMP, thereby counteracting the action of other hormones that act through this nucleotide, and (3) stimulates cellular glucose transport activity. Recent studies by Yuli *et al.*(1982) have shown that insulin induced stimulation of glucose and amino acid uptake in mouse fibroblast is either by recruitment of new transport carriers or by reduction of the translocation activation energy. Since insulin is secreted from pancreas soon after ingestion of food, this hormone mainly deals with assimilation, deposition and homeostasis of metabolites. The hormone may also counteract the actions of other hormones. It antagonizes the gluconeogenic and glycogenolytic effects of glucagon and other

agents (Cahill et al., 1971; Czech, 1977; Kraus-Friedman, 1984; Hers and Hue, 1983). In fact, the glucagon-to-insulin ratio at which all the glucagon effects were abolished was 0.2 (Parilla, 1974). This counteraction by insulin may be through its ability to decrease the cAMP concentration. Fasting and diabetes are two conditions in which cAMP concentration increases in tissues like liver (Exton et al., 1970; Jefferson, 1968; Hers and Hue, 1983).

Most of the understanding of insulin action is based on studies with tissues such as liver, muscle and adipose tissue. Relatively little attention has been given to the action of insulin in kidney. Karokawa and Lerner (1980) reported that there are specific insulin receptors in renal cortical tubules and they suggested that binding of insulin to its receptors may be important for its degradation as well as for bringing about physiological effects on renal function and metabolism. Kidney, in fact extracts and metabolizes approximately 40 % of the insulin in the arterial blood entering the kidney (Chamberlain and Stimmler, 1967). Earlier reviewers (O'Brien and Sharpe, 1965) had stressed that the influence of kidney on carbohydrate metabolism may be due to the kidney's role in binding and metabolizing insulin, in addition to its function as an organ of glucose conservation and gluconeogenesis. Insulin is also known to promote protein synthesis through accelerating amino acid transport into cells (Inui et al., 1983 a,b). It also functions as a growth factor

whose initial mitogenic effect correlates with decreased gluconeogenic function (Brown et al., 1983).

As a major gluconeogenic tissue, kidney performs vital role in glucose homeostasis in birds. To be effective in such functions, kidney has to receive hormonal signals and act concertingly with other tissues. In the present investigation an attempt was made to understand the effect of insulin on the metabolic activities of kidney of domestic pigeon.

MATERIALS AND METHODS

Adult domestic pigeon (Columba livia) of both sexes weighing 250-300 gms were used in the experiments. The birds were acclimated to laboratory conditions for 2 weeks and fed ad libitum with standard diet. The birds were starved overnight and to one group of birds insulin (Zinc suspension i.p. Lente, The Boots Company, India), was injected intraperitoneally at a dose of 1 unit/ml/bird. The control birds received only 1 ml of 0.9 % saline. Two hrs after injection both experimental and control birds were sacrificed by decapitation. Prior to decapitation the blood was collected from wing vein for the measurement of blood glucose level. The kidney was taken out quickly and after recording the weight was used for glycogen and enzyme estimations. Enzymes estimated were alkaline and acid phosphatases, G-6-Pase, phosphorylase, transaminases

Table I: Effect of insulin administration on the metabolic activities of the kidney of Blue rock pigeon.

Parameters	Normal	Control	Experimental
Protein	13.96 ± 1.038	8.93 ± 0.939	12.77 ± 0.539 **
Alk Pase	1.33 ± 0.078	0.61 ± 0.071	0.296 ± 0.02 **
Acid Pase	0.83 ± 0.045	0.599± 0.069	0.271 ± 0.02 **
GOT	90.4 ± 8.696	84.39 ± 4.94	65.29 ± 3.94 *
GPT	151.62 ± 8.346	127.71 ± 5.24	75.2 ± 2.39 ***
Na^+-K^+-ATPase	133.3 ± 18.089	167.50 ± 11.80	58.81 ± 6.35 ***
Phosphorylase	233.56 ± 21.963	245.45 ± 7.65	240.51 ± 13.3 NS
G-6-Pase	0.113 ± 0.022	0.065± 0.017	0.111 ± 0.02 NS
AChE	3.370 ± 0.077	2.399± 0.231	0.545 ± 0.017 ***
LDH	16.00 ± 1.676	42.74 ± 3.76	17.41 ± 1.13 ***
Glucose	120.00 ± 5.262	127.82 ± 9.84	76.51 ± 4.45 ***
Glycogen	0.033 ± 0.009	0.024 ± 0.004	0.011 ± 0.002 **
Body weight	290 ± 4.47	290 ± 7.07	290 ± 8.94
Total kidney weight	1.59 ± 0.07	1.32 ± 0.12	1.11 ± 0.04

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

EXPLANATIONS TO GRAPHS - CHAPTER V

Fig.1. Graphs showing the effect of insulin administration on the blood sugar level and glycogen content in the kidney of blue rock pigeon.

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

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

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

Fig.5. Graphs showing the effect of insulin on AChE and LDH activities in the kidney of blue rock pigeon.

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

FIG. 1 : EFFECT OF INSULIN

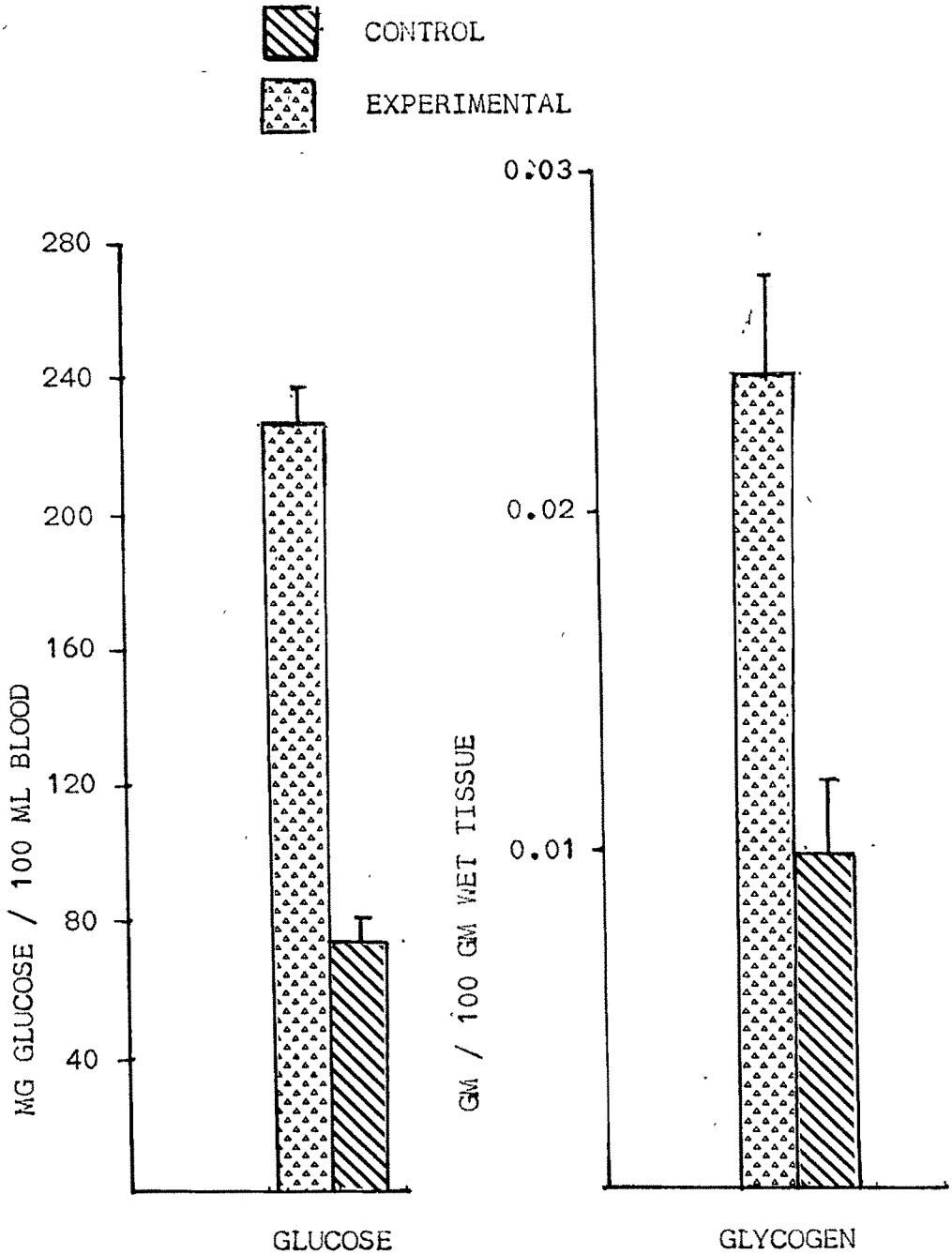


FIG. 3 : EFFECT OF INSULIN

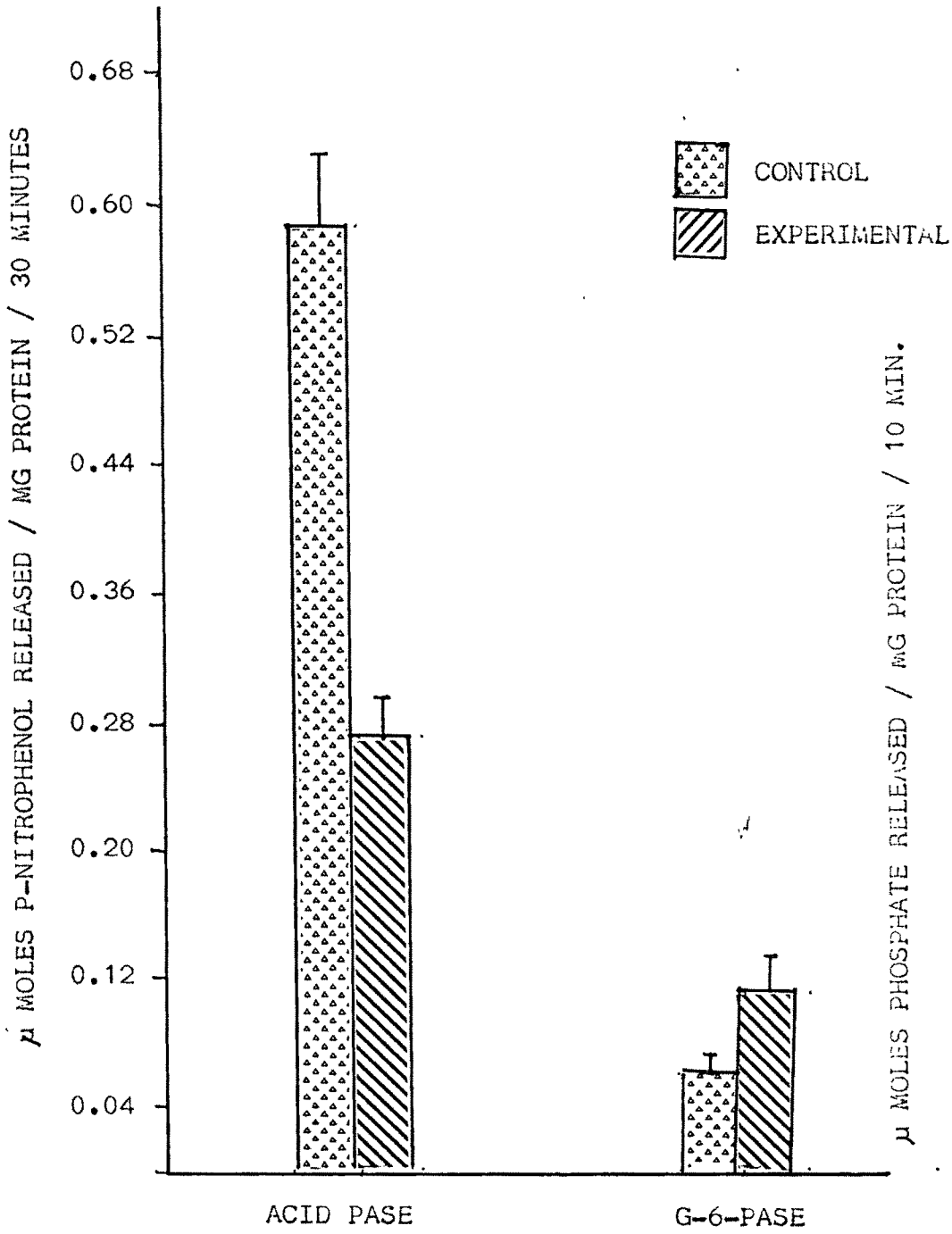


FIG. 4 : EFFECT OF INSULIN

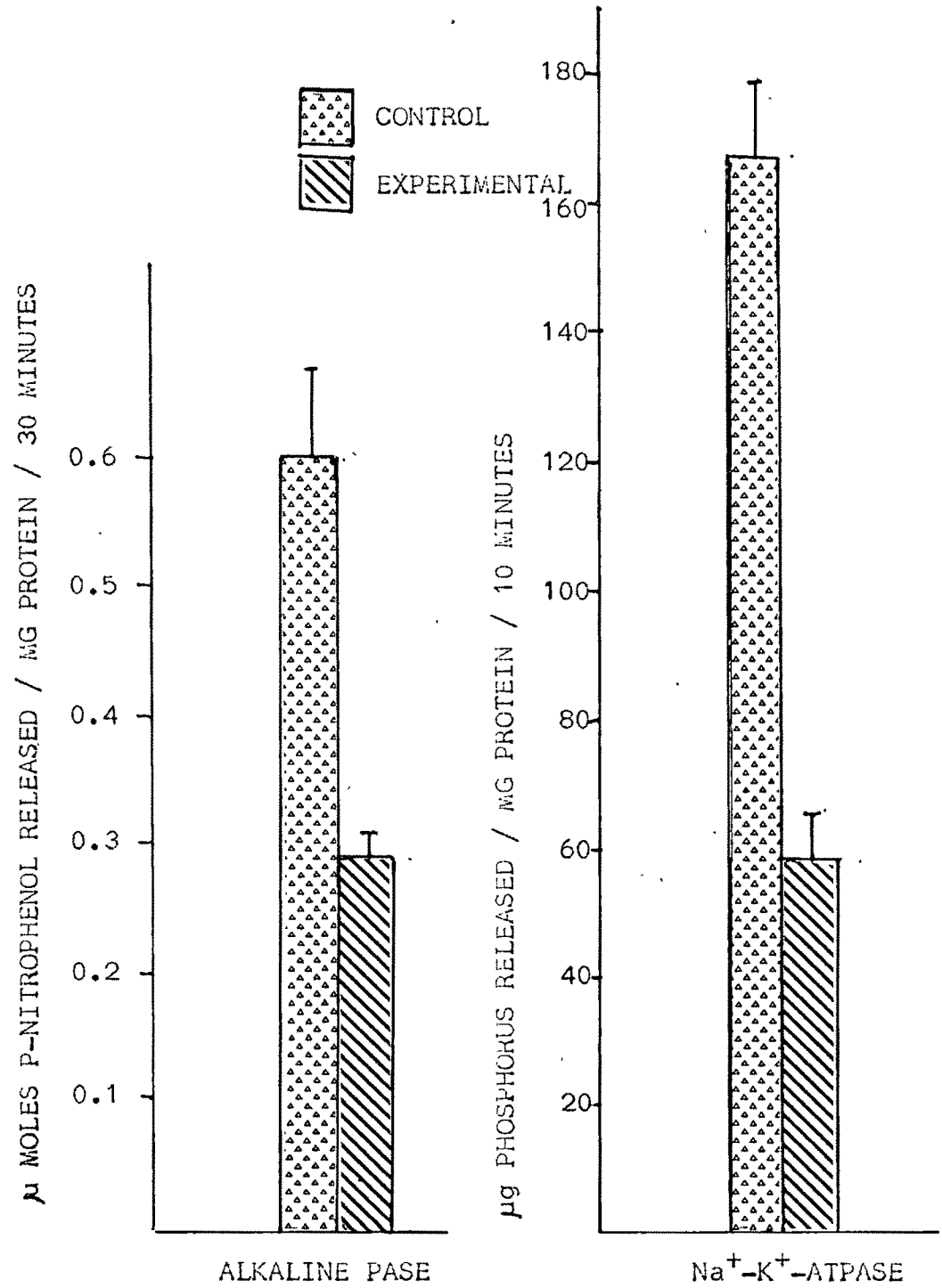


FIG. 5 : EFFECT OF INSULIN

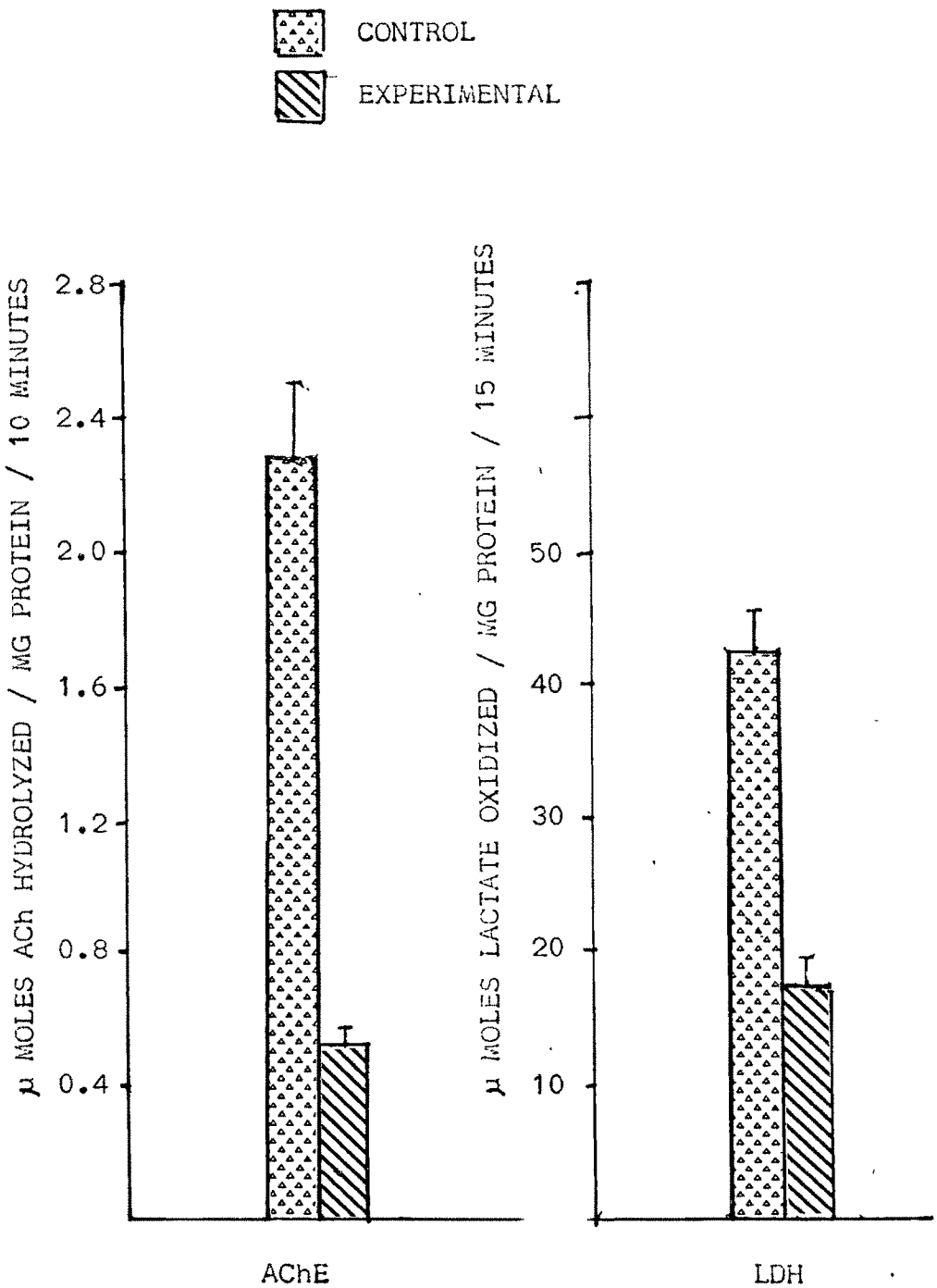
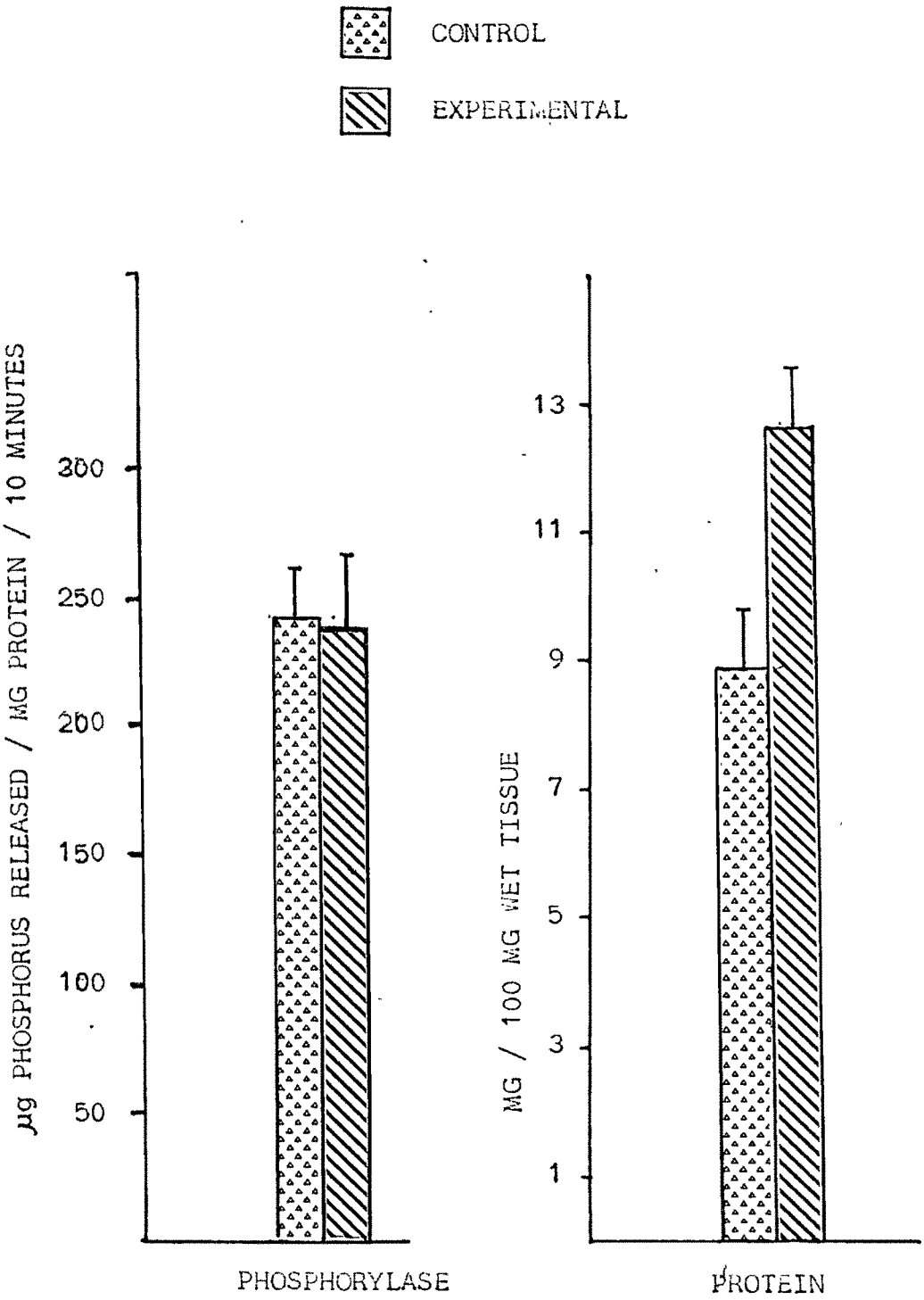


FIG. 6 : EFFECT OF INSULIN



(GOT, GPT), Na^+-K^+-ATPase, AChE and LDH. The methods followed for the estimations are described in Chapter 1. Glycogen and protein content of the kidney were also estimated according to the methods given in Chapter 1.

RESULTS

The data are presented in Table 1 and Figs. 1 to 6.

Administration of insulin produced a hypoglycaemic condition as expected. However, in the kidney glycogen content was found to decrease compared to that in the controls. Protein value, on the other hand showed an increase in experimental birds. The effect of insulin administration on the activities of alkaline and acid phosphatases was a significant reduction compared to control values. Similarly both GOT and GPT showed a significant decrease in the kidney of experimental birds. This trend was also followed by Na^+-K^+-ATPase, Phosphorylase and G-6-Pase, on the other hand showed no significant variation in activities in the kidney of experimental birds from that of control birds. Acetylcholinesterase showed a decrease following insulin administration. So was the case with LDH.

DISCUSSION

One of the characteristic actions of insulin is to counteract the effect of glucagon and catecholamines on the tissues (Kraus-Friedman, 1984). The latter two groups of

hormones are secreted in response to starvation or when under stress of hypoglycaemia. Insulin which is secreted invariably during post prandial conditions, stimulates the uptake of metabolites by tissues as well as conversion of these into storage product. At the same time, insulin also inhibits glucose release, gluconeogenesis and lipolysis. However, administration of insulin into fed rats has no effect on glycogenolysis or gluconeogenesis (Johnson et al., 1972). In fasted rats, insulin blocks the production of glucose induced by hormones such as glucagon and catecholamines by lowering cAMP concentration (Exton et al., 1968).

In the present experiment, insulin did not produce any significant effect on the activities of glycogen phosphorylase or G-6-Pase, indicating that glycogenolysis per se was unaffected in the kidney. However, the activities of both the transaminases (Alanine amino-transferase-GPT and Aspartate aminotransferase-GOT) were found to decrease following insulin administration. Plainly, this indicates that amino acid utilization for gluconeogenesis was suppressed by insulin. The conversion of other gluconeogenic precursors such as lactate was also very much curtailed by insulin as indicated by the reduction in LDH activity. Inhibition of gluconeogenesis by insulin also facilitates more amino acid retention or protein synthesis. In fact, kidney protein values of experimental birds showed significant increase over that of control birds.

Although insulin injection bought about hypoglycaemia in the pigeon,the hormone could not induce enhanced glucose uptake or glycogen deposition in the kidney. The reduction in the activities of both the non-specific phosphomonoesterases also explains the failure of insulin to induce glucose uptake by the kidney. Probably, if administration of insulin was carried out in fed pigeon or along with glucose, kidney might have responded with increased uptake of glucose and glycogen deposition.

The reduction in AChE activity in the kidney indicates reduction in vagal cholinergic activity. It is difficult to state whether this reduction in vagal activity was due to any direct action of insulin or due to hypoglycaemia.

In general, insulin exhibited an inhibitory influence on gluconeogenesis in the kidney of pigeon, eventhough, it failed to induce glucose uptake or glycogen deposition in the tissue.