CHAPTER VI

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

Hyperactivity of parasympathetic system leads to 'reactive hypoglycaemia syndrome' that results in lowered glucose levels and hyperinsulinemia (Nielson, 1926). Vagotomy was found to cure this syndrome (Boulet *et al.*, 1954). Anti-cholinergic drugs were also found to be useful in reducing the effect of this syndrome (Portis and Zitman, 1943; Veverbrants *et al.*, 1969). The reactive hypoglycaemia is reflexely mediated whenever a carbohydrate meal is ingested. The earlier works on this syndrome are suggestive of vagal mediation in bringing about hypersecretion of insulin and thereby the hypoglycaemia and do not consider the direct action of cholinergic system in the uptake of glcuose by tissue cells. Mondon and Burton (1971) showed clearly that acetylcholine (ACh) together with insulin increases the uptake of glucose by liver. ACh is secreted at sinusoidal linings of the hepatic cords where the parasympathetic-cholinergic nerve plexus are demonstrated (Sutherland, 1964). Similarly vagal cholinergic and sympathetic adrenergic fibers are known to innervate the kidney (Thurau, 1967; McKenna and Angelakos, 1968; Nashat, 1974; Moffat, 1975) and are known to regulate the activity of the kidney by counter regulation. In the avian liver the sinusoidal linings show the localization of acetylcholinesterase (Pilo, 1969; Shah *et al.*, 1972b). These authors suggested that the acetylcholine-acetylcholinesterase (ACh-AChE) system is helpful in the assimilation of glucose by the hepatocytes. ACh is known to facilitate glucose transport through a coupling with flow of ions (Willbrandt, 1975). Few studies by Patel (1978) suggest that ACh plays an important role in the regulation of blood sugar level by initiating the glucose uptake by the liver, by stimulating insulin secretion by B-cells of islets of Langerhans as well as by vasodilatory effect on dermal blood vessels.

Vagal parasympathetic nerve, which contains mainly cholinergic fibres innervates kidney, liver, pancreas, GI tract, adrenal, thyroid and skin. The cholinergic fibres secrete ACh at nerve endings. *In vivo* studies carried out by Pilo and Patel (1978) have shown the influence of ACh and insulin on the uptake of glucose by avian and mammalian liver. They showed that mammalian liver in presence of insulin accumulated maximum glucose and addition of ACh only slightly enhanced its uptake, whereas in the avian liver maximum response was for ACh rather than for insulin which lead to the conclusion that the avian liver mostly relies for glucose uptake on the vagal cholinergic influence rather than insuline alone. Studies carried out by Verma *et al.* (1984) have shown that vagus nerve has an inhibitory effect on the gluconeogenic activities on the kidney. Pilo *et al.* (1983) in their studies with pigeon have found that vagotomy results in an altered functional status of kidney and indicated that autonomic nervous system which originates from hypothalamus could control the metabolic activities of kidney in such a way that blood sugar level could, to a certain extent, be maintained.

The above various findings do suggest that the vagal cholinergic fibres have some effect on kidney metabolism. To study, further, the influence of ACh on the metabolic activities and biochemical profile of the kidney of pigeon, the present investigation on the effect of ACh on enzymes concerned with gluconeogenesis was undertaken.

MATERIAL AND METHODS

Adult domestic pigeons (Columba livia) of both sexes, weighing around 250-300 gms were used in the experiment. The birds were divided into two groups and were acclimated to laboratory conditions for two weeks, caged in groups and fed ad-libitum. Experimental group was injected (i.p) with ACh in the dose of 1.5 mg/ml. The control birds were given the same amount of the vehicle. Both the groups of birds were starved overnight. After two hours of injection, both the experimental and control birds were sacrificed by decapitation. Blood samples for glucose estimation was collected from wing veins just prior to decapitation. Kidney was quickly excised from sacrificed birds and processed for enzyme estimations and protein and glycogen contents. The method followed for estimations of alkaline and acid phosphatases, GOT and GPT, G-6-Pase, phosphorylase, Na^{+}-K^{+}-ATPase, LDH and AChE are described in Chapter 1. The methods employed for the determinations of protein and glycogen are also given in Chapter 1.

RESULTS

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

Administration of ACh did not alter the glycaemic level significantly. Similarly, both glycogen and protein contents of the kidney showed no significant changes compared to that of control birds. Of the two non-specific phosphatases alkaline phosphatase showed increased activity whereas acid phosphatase showed a decrease in response to ACh administration. Transaminases too showed altered activities in response to administration of ACh; GOT showing a non-significant increase while GPT showing a highly significant decrease. The same results were obtained for Na^{+}-K^{+}-ATPase and glycogen phosphorylase activities. G-6-Pase activity showed a significant increase in the experimental birds compared to that of control birds. Acetylcholinesterase activity showed decrease following ACh administration. So was the case with LDH.

Table I: Effect of acetylcholine administration on the metabolic activities of the kidney of blue rock pigeon (*Columba livia*) (Mean ± S.E)

Parameters	Normal	Control	Experimental
Protein	13.956 ± 1.038	8.93 ± 0.939	7.47 ± 0.429 NS
Alk Pase	1.378 ± 0.078	0.607 ± 0.070	0.677 ± 0.063 NS
Acid Pase	0.834 ± 0.046	0.598 ± 0.069	0.248 ± 0.017 **
GOT	90.4 ± 8.696	84.39 ± 4.94	93.31 ± 0.261 NS
GPT	151.62 ± 8.346	127.71 ± 5.234	95.14 ± 6.90 **
Na^{+}-K^{+}-ATPase	133.30 ± 18.009	167.50 ± 11.80	61.90 ± 3.06 ***
Phosphorylase	233.56 ± 21.96	245.45 ± 7.65	62.69 ± 5.25 ***
G-6-Pase	0.113 ± 0.022	0.065 ± 0.017	0.155 ± 0.005 **
AChE	3.37 ± 0.077	2.399 ± 0.231	1.87 ± 0.218 NS
LDH	16.00 ± 1.679	42.77 ± 3.76	25.30 ± 1.15 **
Glucose	120.00 ± 5.262	127.82 ± 9.84	141.38 ± 3.61 NS
Glycogen	0.033 ± 0.009	0.024 ± 0.004	0.036 ± 0.006 NS
Body weight	290 ± 4.47	290 ± 8.59	282 ± 8.59
Total kidney weight	1.59 ± 0.07	1.12 ± 0.03	1.30 ± 0.08

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

EXPLANATIONS TO GRAPHS - CHAPTER VI

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

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

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

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

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

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

FIG. 1 : EFFECT OF ACETYLCHOLINE

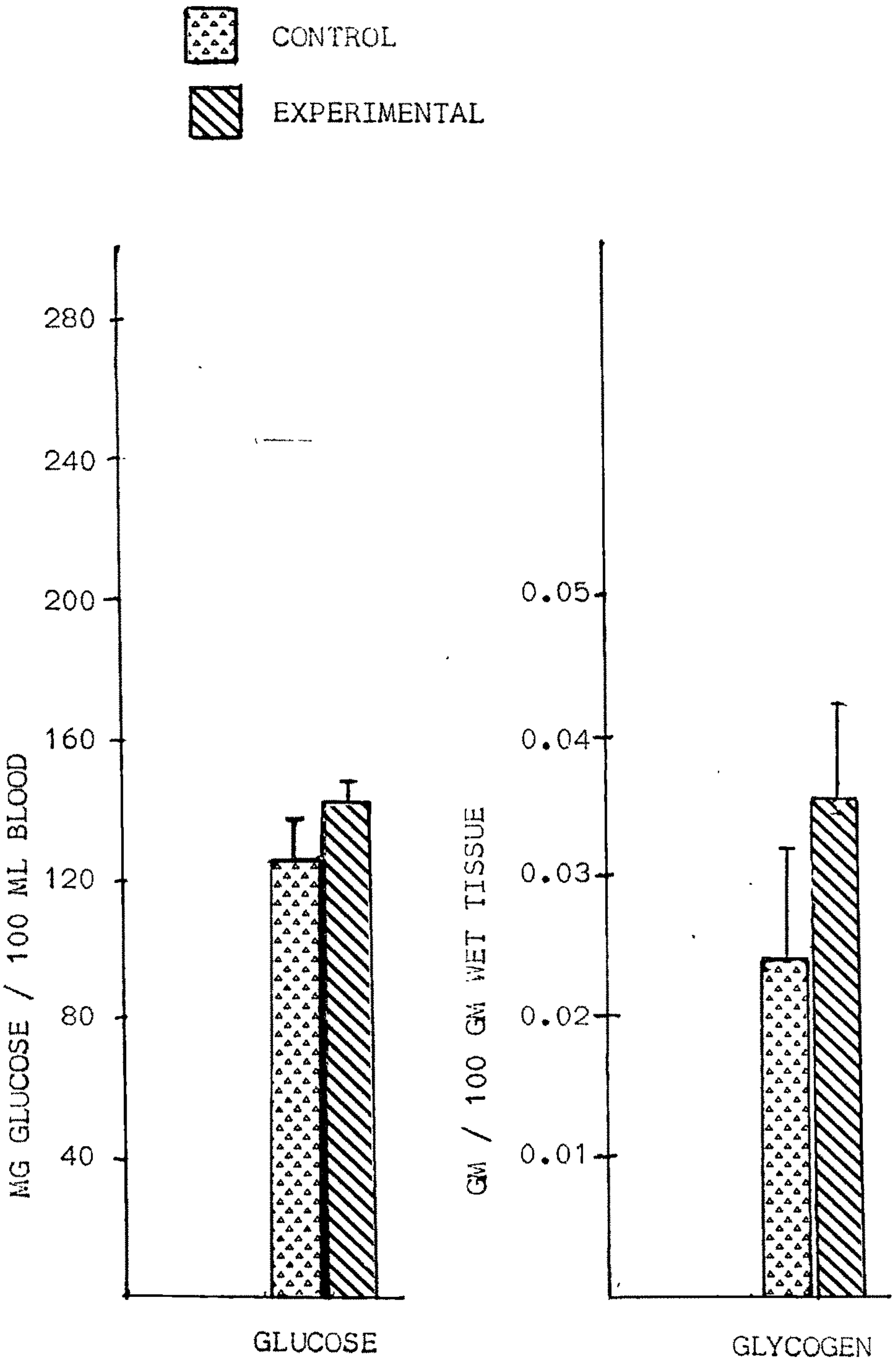


FIG. 2 : EFFECT OF ACETYLCHOLINE

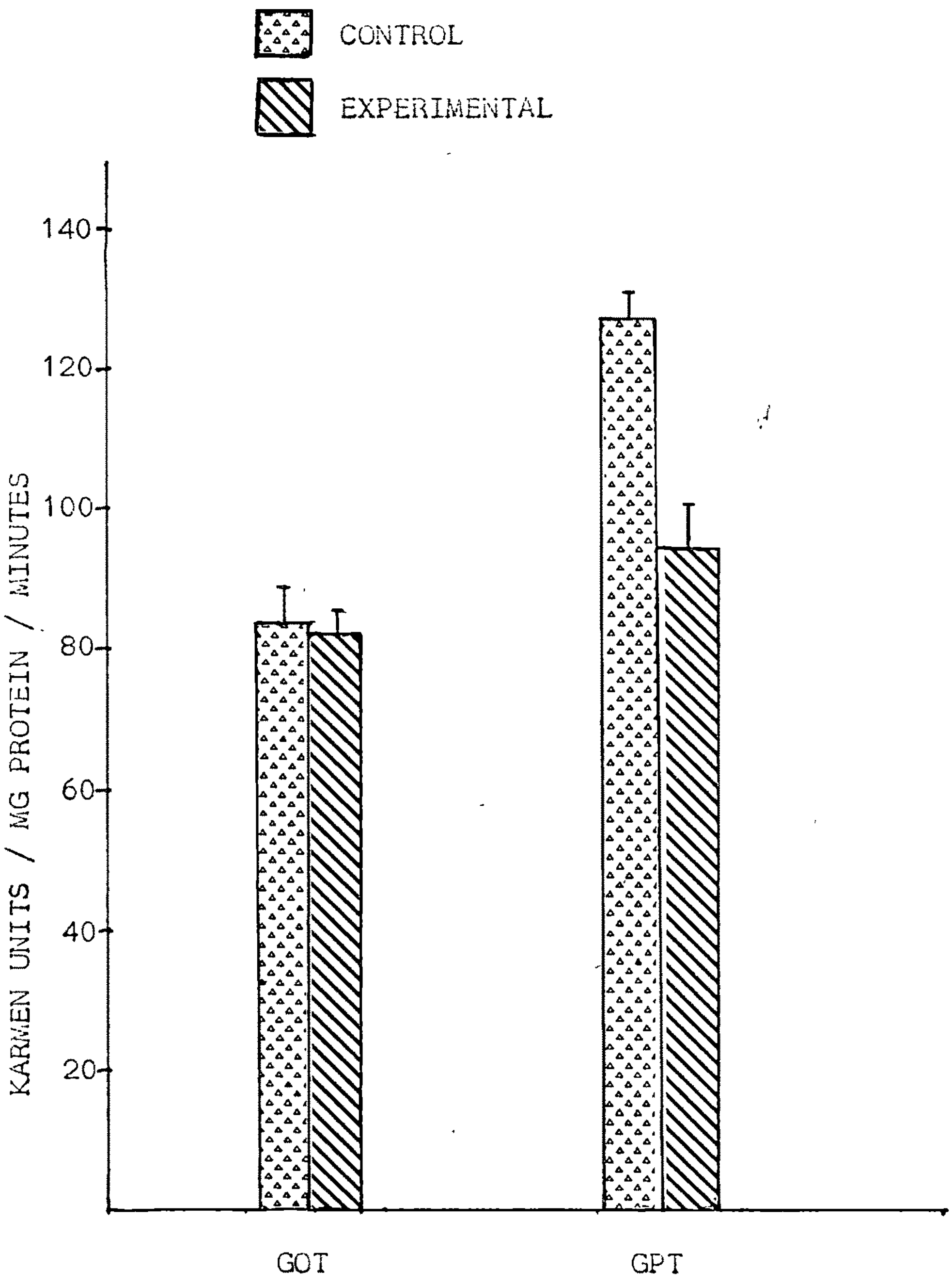


FIG. 3 : EFFECT OF ACETYLCHOLINE

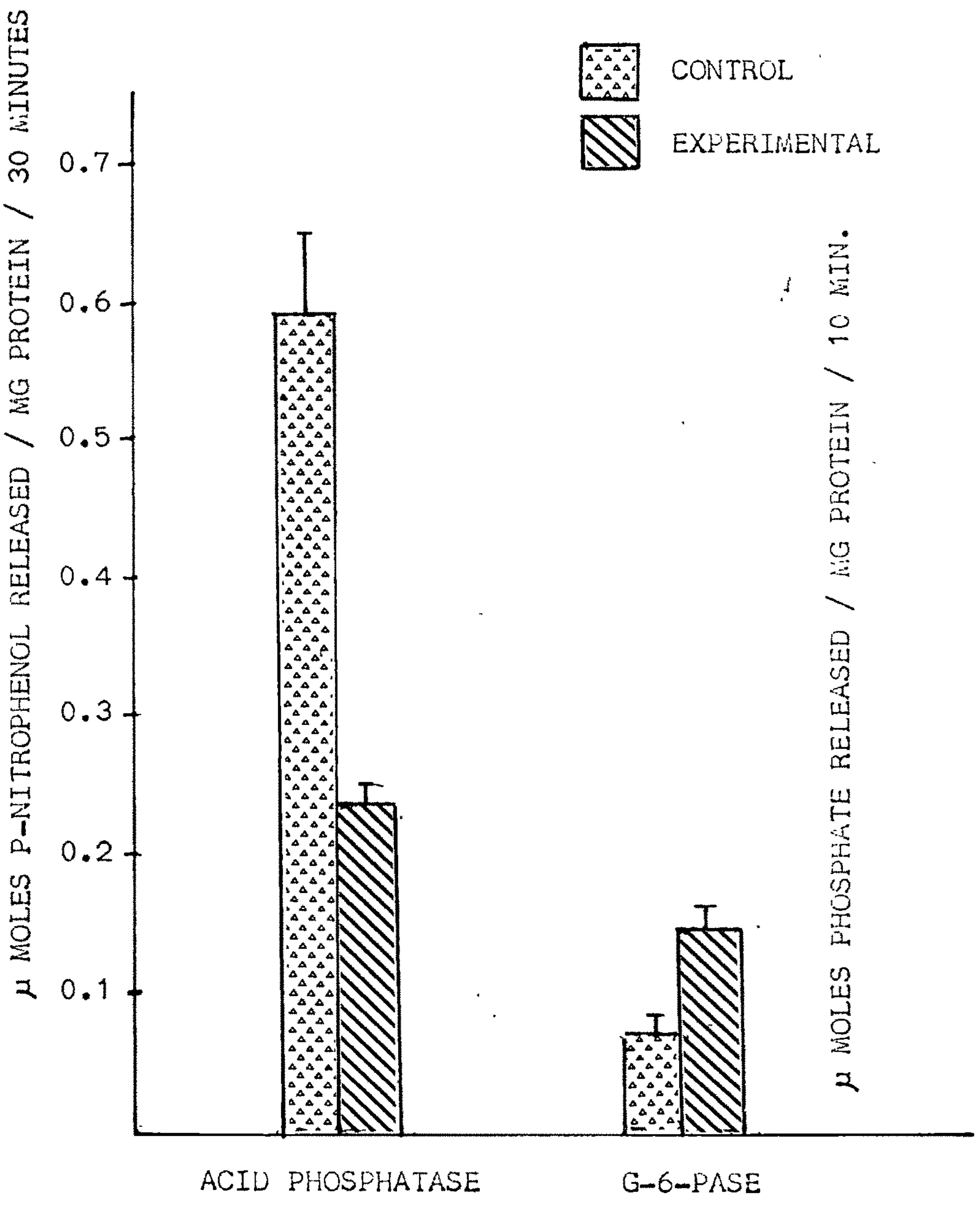


FIG. 4 : EFFECT OF ACETYLCHOLINE

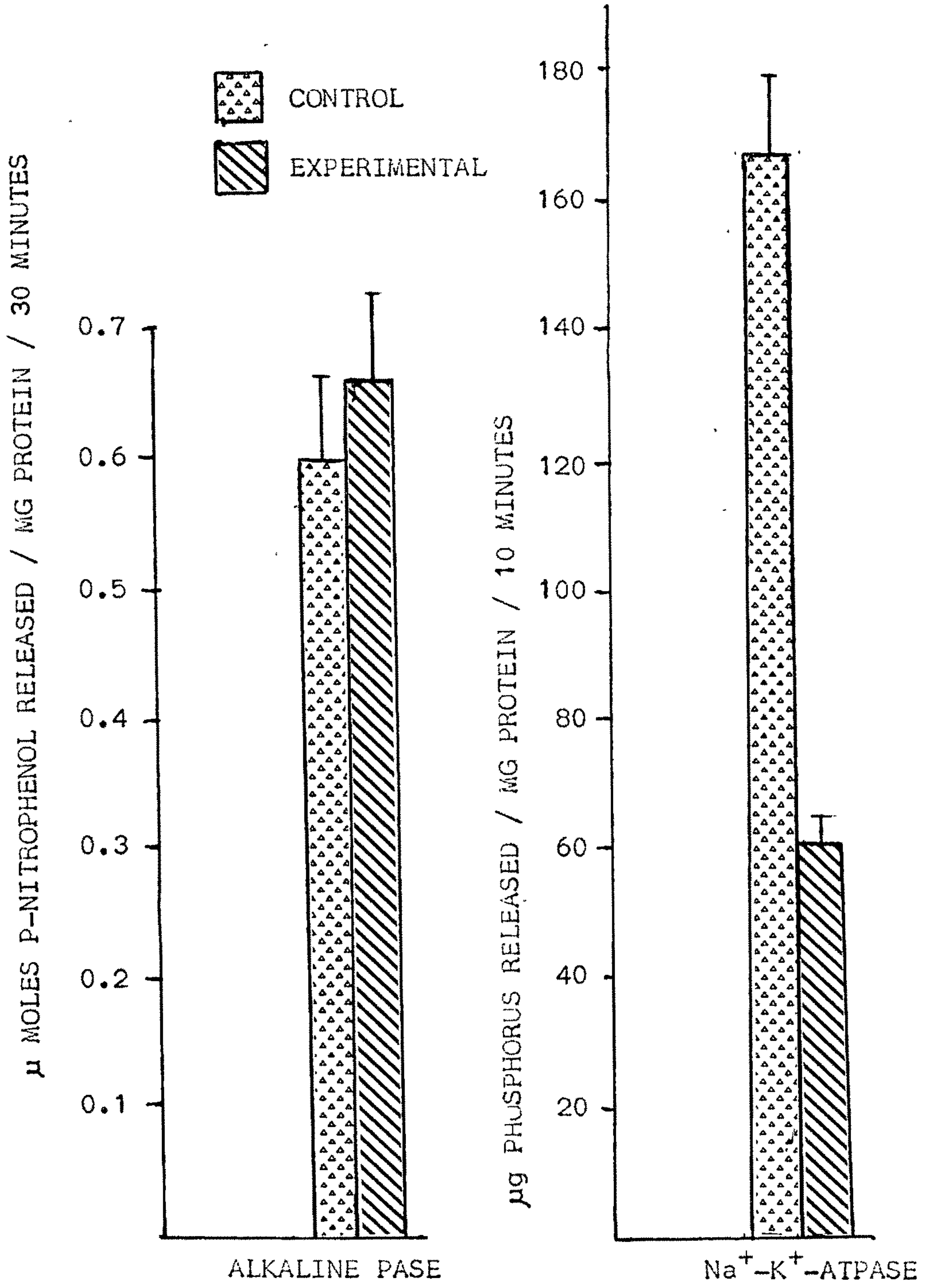


FIG. 5 : EFFECT OF ACETYLCHOLINE

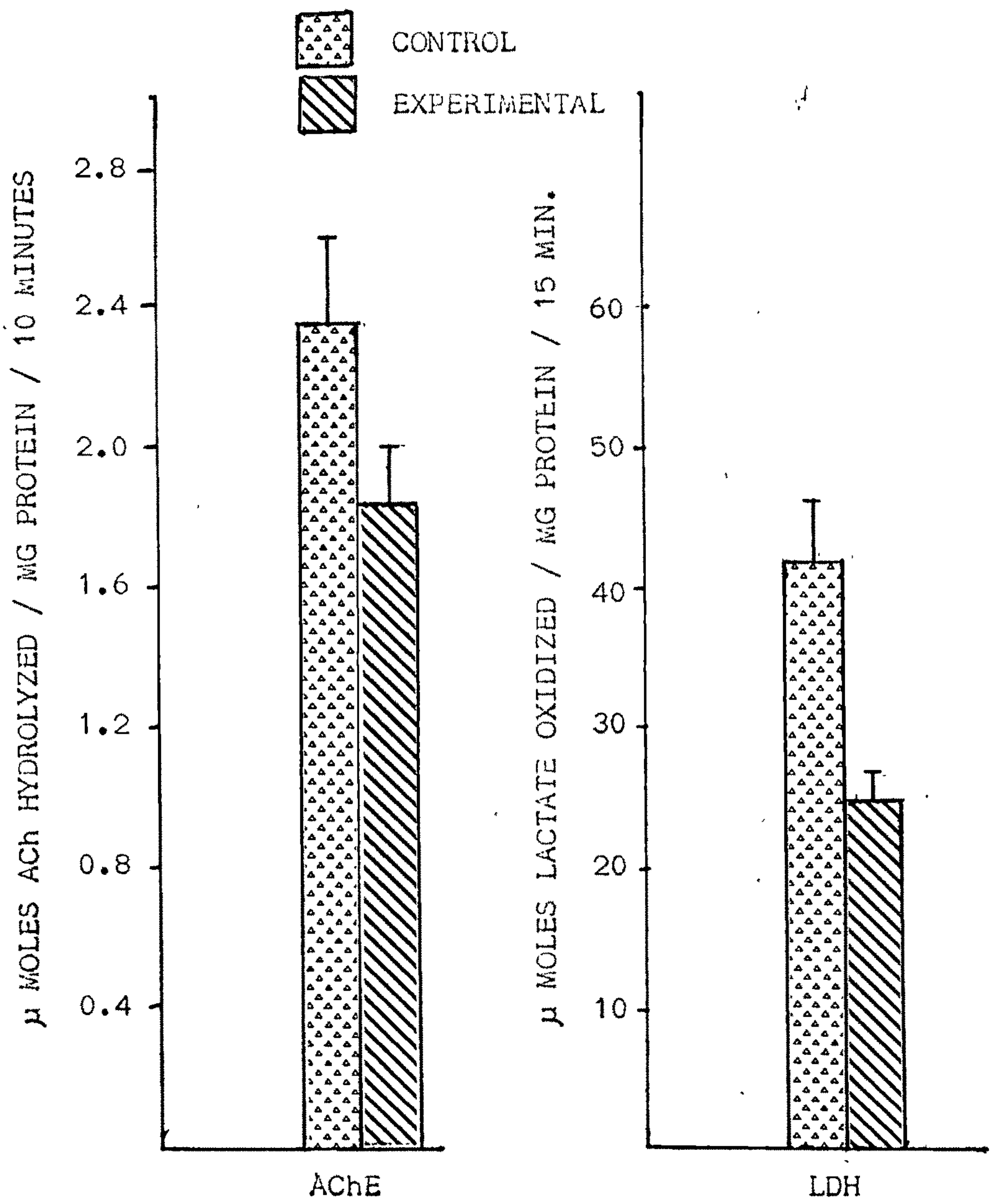
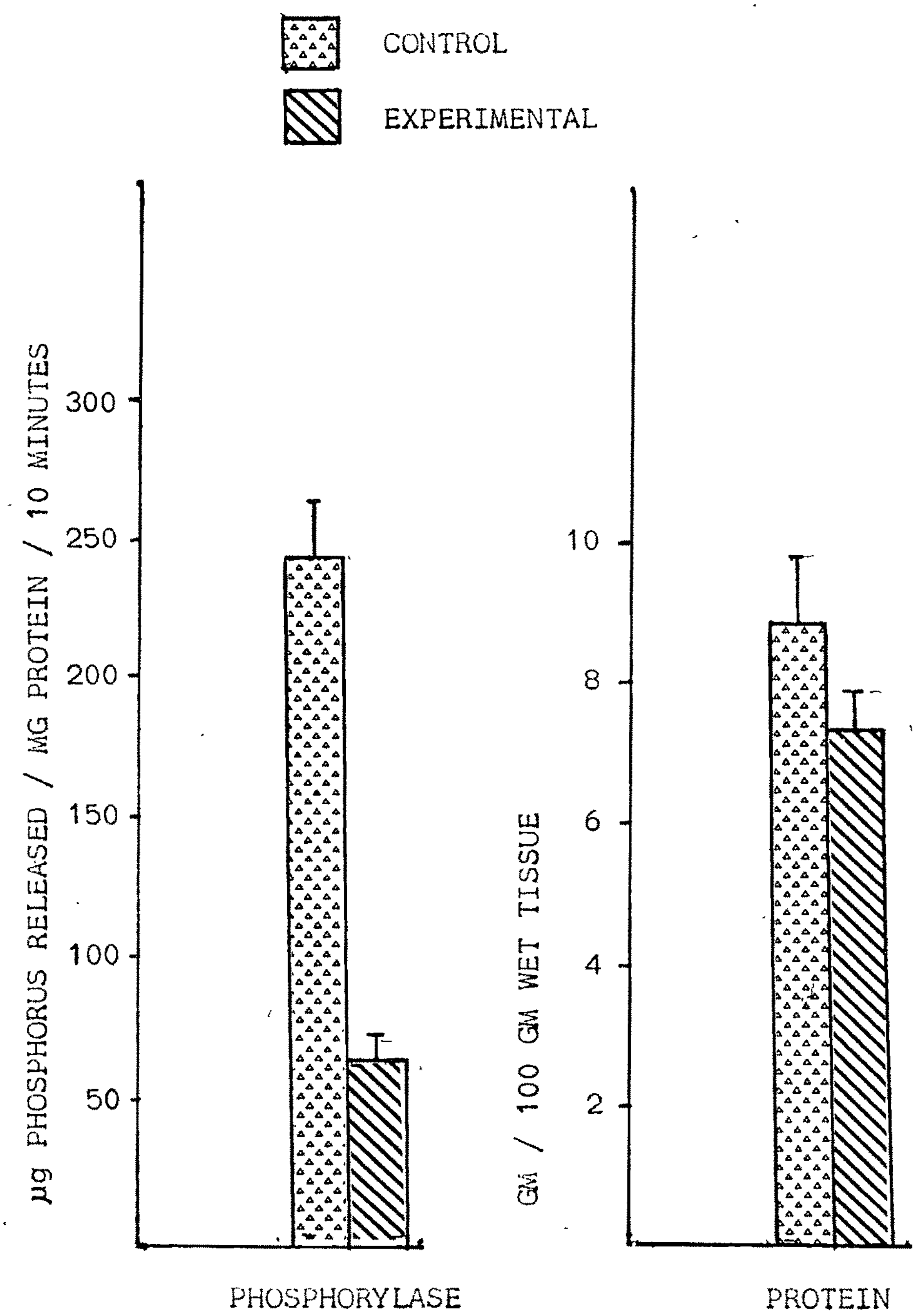


FIG. 6 : EFFECT OF ACETYLCHOLINE



DISCUSSION

It is clear from the data that acetylcholine administration brought about a general reduction in gluconeogenic activity in the kidney of pigeon as evident from decreased activity of transaminase , and LDH. Even the enzymes concerned with glycogenolysis, phosphorylase, showed a decrease. Vagotomy produced an opposite reaction, wherein enzymes such as GOT and GPT showed significant increase in the kidney (Verma et al., 1984) while alkaline phosphatase showed a reduction in the activity (Pilo et al., 1983). ACh administration produced a more or less opposite reaction. In the kidney, acid phosphatase showed a reduction in ACh treated pigeons while alkaline phosphatase activity remained unchanged. Similarly, both GOT and GPT (more so GPT) showed a reduction in the level of activities in the kidney of ACh administered pigeons. Since the effect of ACh, by and large was the opposite of what was seen in the kidney of catecholamones administered pigeons (Chapter VIII), it could be concluded that, like in the case of liver, the kidney metabolic activities are reciprocatively controlled by parasympathetic and sympathetic nerve fibres. However, as the innervation of these fibres are not as profuse as in the case of liver, the degree of control of metabolic actions in the kidney may be far less than that was observed in the case of liver (Mehan, 1985). In the kidney, autonomic nerve fibres could also influence the blood vessels and blood flow, and thereby could indirectly affect the supply of metabolites.

In respect of G-6-Pase, Na^{+}-K^{+}-ATPase and LDH activities in the kidney, the action of ACh resembles that of insulin (Chapter V). However, insulin did not affect the phosphorylase activity in the kidney of pigeon (Chapter V) while ACh produced a significant reduction. As mentioned earlier, hyperactivity of vagal fibres which brings about reactive hypoglycaemia (Nielson, 1926) is coupled with increased insulin secretion. Probably, ACh administration could bring about an increased release of insulin. Hence, the changes in the kidney metabolic activities in response to ACh administration could be partly by direct action and partly through releasing more insulin.