CHAPTER VII

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

Gluconeogenesis from lactate in chicken hepatocytes is greatly influenced by glucagon, cAMP and epinehrine (Dickson and Longslow, 1978; Fister *et al.*, 1982). Glucagon plays a major role in the maintenance of glucose level in birds. Production and release of glucose into blood stream has to be regulated by a sensitive mechanism in view of the fact that birds in general maintain a very high (2-3 times than mammals) plasma glucose concentration (Bell, 1971). Reciprocatively, plasma levels of glucagon in birds are about 19 times higher than in mammals (Samols *et al.*, 1969; Unger, 1971; Fajans *et al.*, 1974; Vanlan *et al.*, 1974; Rabinovitch and Dupre, 1974; Lauret and Mialhe, 1978; Sitbon and Mialhe, 1978; Raheja *et al.*, 1980).

Glucagon stimulates gluconeogenesis not only from lactate and pyruvate but also from substrates like glutamate (Ui *et al.*, 1973) glutamine (Joseph *et al.*, 1978), and propionate (Blair *et al.*, 1973) which join the pathway at the level of oxaloacetate (OAA), or like fructose (Vemeziale, 1971), glyceraldehyde and dihydroxyacetone (Veneziale, 1972) which join at triose phosphate level. The above effects of glucagon are mediated through the action of cAMP-dependent protein

kinase and the phosphorylation of regulatory enzymes. Phosphofructokinase, frutose 2,6-biphosphatase and pyruvate kinase are enzymes that are subjected to hormone regulation. The regulation thus appears to be at the level of fructose-6-phosphate/fructose 1,6-diphosphate and of the pyruvate/PEP interconversions. Glucagon through cAMP dependent protein kinase, phosphorylate phosphofructokinase (PFK), fructose 2,6, diphosphatase (F.2-6 DP) and pyruvate kinase (PK) and activates gluconeogenesis and inhibits glycolysis (Hers and Hue, 1983). It is reported that in isolated chicken hepatocytes glucagon inactivates, through cAMP dependent protein kinase, fructose 2,6 diphosphatase which in turn lowers the level of fructose 2,6 diphosphate (Fister et al., 1982). Fructose 2,6, diphosphate is an activator of phosphofructokinase (Pilkis et al., 1982; Hers, 1983) and lowering the concentration of this metabolites in turn inhibits PFK. In chicken hepatocytes pyruvate kinase is inactivated by a cAMP independent protein kinase (Eigenbrodt et al., 1977). Isolated chicken hepatocytes treated with glucagon showed a decrease in the activity of PFK which was readily reversible in the presence of insulin (Fister et al., 1983). The effect of glucagon is then an inhibition of glycolysis. Fructose 1,6 diphosphatase, a key gluconeogenic enzyme is activated when fructose 2,6, biphosphate level gets lowered. Although, glucagon may stimulate PEPCK enzyme synthesis, major action of the hormone in gluconeogenesis is invariably by suppressing glycolysis in the liver (Hers and Hue, 1983).

Even though the role of glucagon in the regulation of gluconeogenesis and the role of avian kidney as the major site of gluconeogenesis were subjects of intensive investigation, studies on hormonal control of gluconeogenesis in general and the effect of glucagon on the kidney metabolism in particular, is scarce in birds. The present investigation is an apparent attempt to bridge the gap in our knowledge regarding effect of glucagon on avian kidney metabolism.

MATERIALS AND METHODS

Adult domesticated variety of blue rock pigeon (Columba livia) weighing around 250-300 gms were used in the experiments. The birds were acclimated to laboratory conditions for two weeks and fed ad-libitum on standard food containing various grains. The birds were divided into 2 groups and one group was injected 40 µg/animal glucagon in 1 ml of redistilled water. Two injections (i.p) were given each 24 hours apart. The control group of animals received only an equal amount of the vehicle at the same interval. The birds of both the groups were kept under starved condition during the period of glucagon administration (48 hrs). On third day both control and experimental 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 methods followed for estimations of alkaline and acid phosphatases, GOT and GPT (transaminases). 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 I and Figs. 1 to 6.

The most conspicuous effect of glucagon treatment was the tremendous increase in glycaemic level, an almost 5 fold increase over control value. This is accompanied by a drastic reduction in the glycogen content in the kidney. Alkaline and acid phosphatases showed significant decrease in the kidney of glucagon treated birds. Like wise both transaminases showed reduction in the activity levels following glucagon administration. The hormone also brought about a decrease in the activity of Na^+-K^+-ATPase, phosphorylase and LDH in the kidney. G-6-Pase did not show much of variation. The only enzyme, amongst those studied, that showed an increase in the activity was acetylcholinesterase. Protein content of the kidney of experimental birds was significantly lower than that was estimated in the kidney of control birds.

Table I: Effect of glucagon administration of enzyme activities and glycogen content in pigeon kidney and blood sugar level. (Mean ± S.E).

Parameters	Normal	Control	Experimental
Protein	13.956 ± 1.038	16.510 ± 1.550	10.750 ± 0.612 ***
Alk Pase	1.379 ± 0.078	0.296 ± 0.028	0.158 ± 0.015 ***
Acid Pase	0.834 ± 0.046	0.271 ± 0.021	0.096 ± 0.007 ***
GOT	90.400 ± 8.696	57.290 ± 5.860	37.970 ± 4.340 NS
GPT	151.620 ± 8.346	75.220 ± 2.600	24.830 ± 1.560 ***
Na^+-K^+-ATPase	133.300 ± 18.009	53.000 ± 8.380	20.090 ± 2.150 **
Phosphorylase	233.566 ± 21.96	262.600 ± 18.980	52.230 ± 0.921 ***
G-6-Pase	0.113 ± 0.022	0.114 ± 0.022	0.071 ± 0.006 NS
AChE	3.370 ± 0.077	0.555 ± 0.018	1.396 ± 0.038 ***
LDH	16.000 ± 1.679	17.410 ± 1.23	10.750 ± 0.612 ***
Glucose	120.000 ± 5.262	76.510 ± 4.870	356.140 ± 10.840 ***
Glycogen	0.033 ± 0.009	0.024 ± 0.001	0.004 ± 0.0003 ***
Body weight	290 ± 4.49	250 ± 14.50	244 ± 12.69
Total kidney weight	1.59 ± 0.07	1.24 ± 0.10	1.27 ± 0.03

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

EXPLANATIONS TO GRAPHS - CHAPTER VII

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

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

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

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

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

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

FIG. 1 : EFFECT OF GLUCAGON

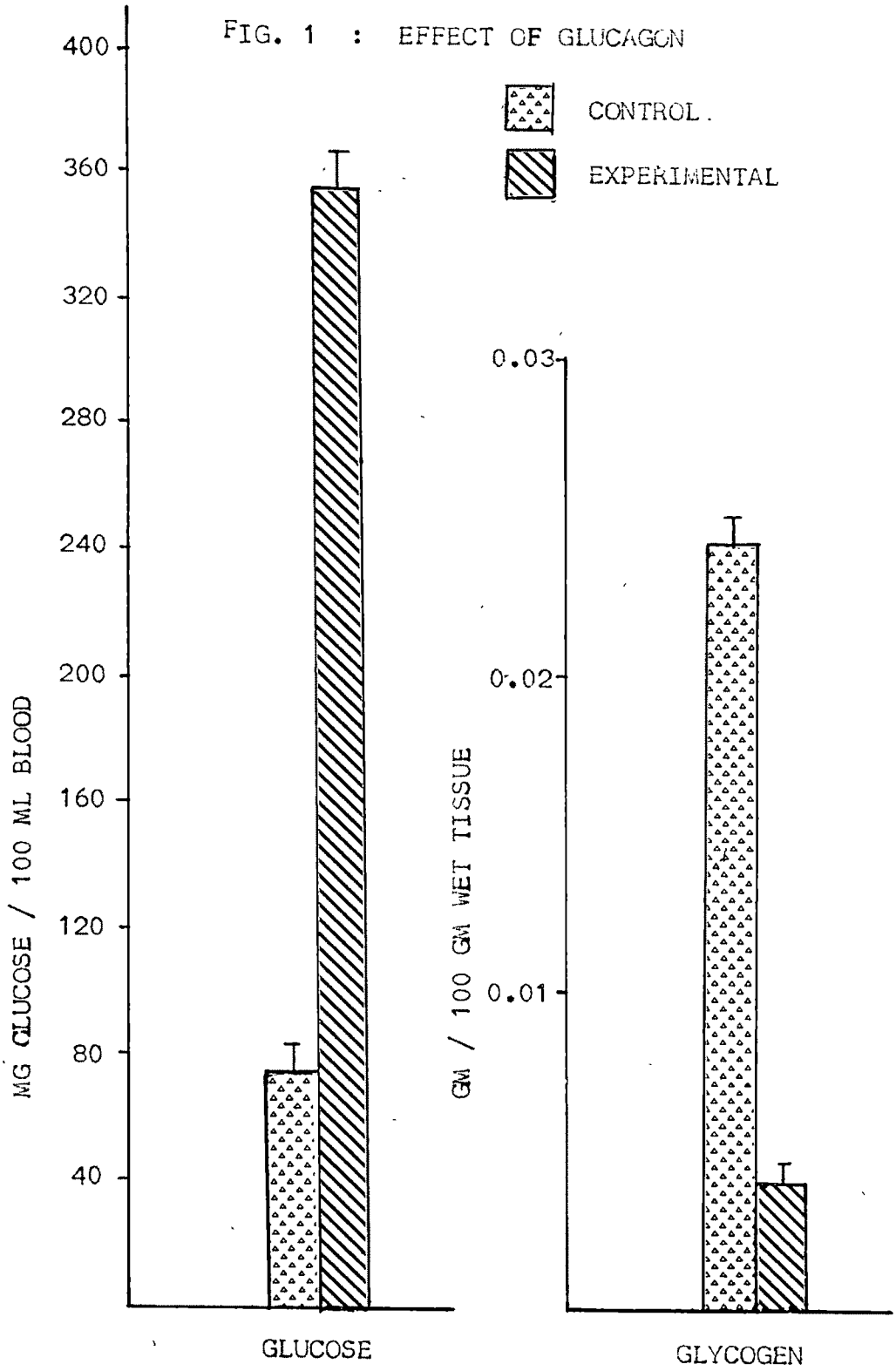


FIG. 2 : EFFECT OF GLUCAGON

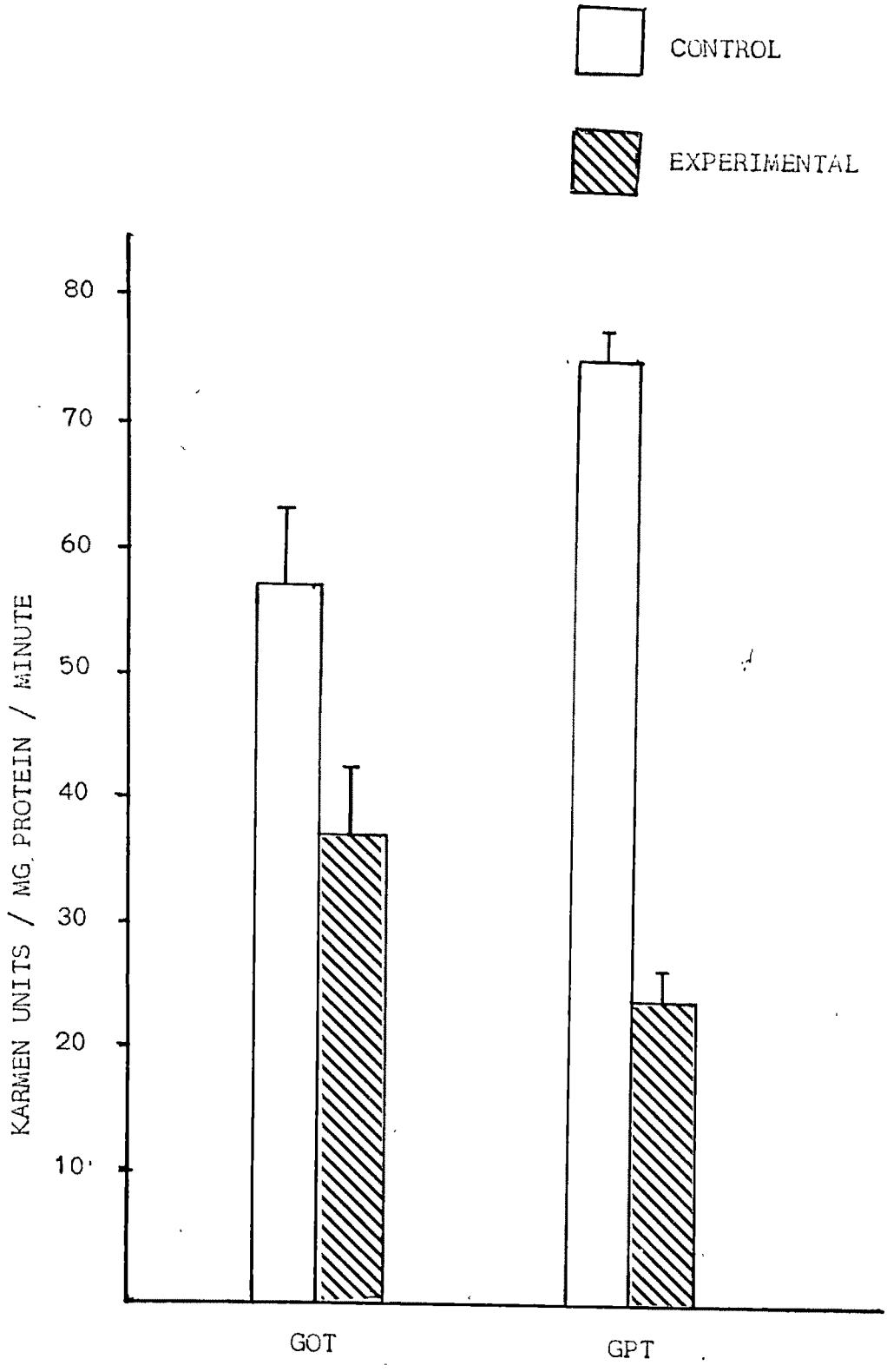


FIG. 3 : EFFECT OF GLUCAGON

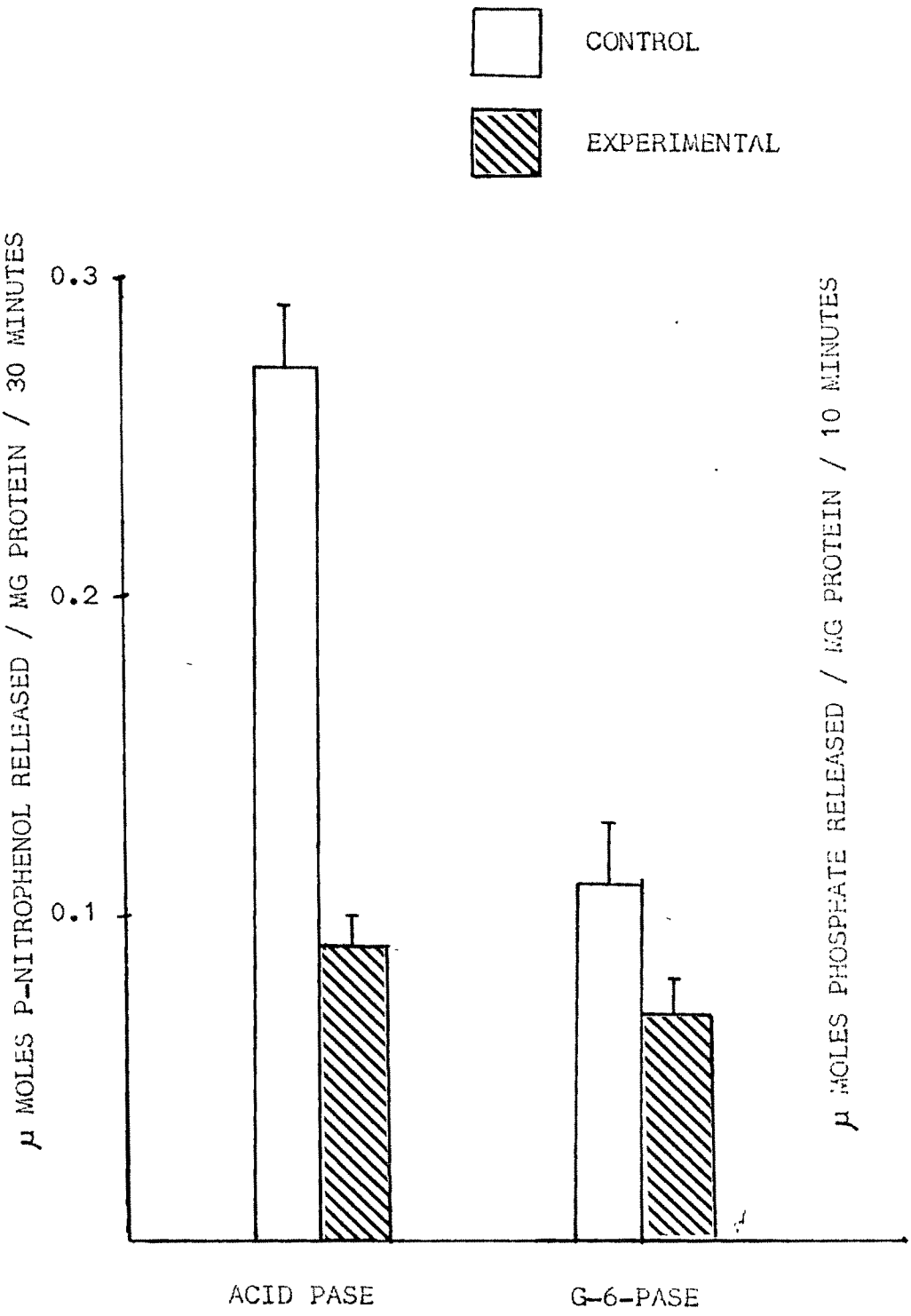


FIG. 4 : EFFECT OF GLUCAGON

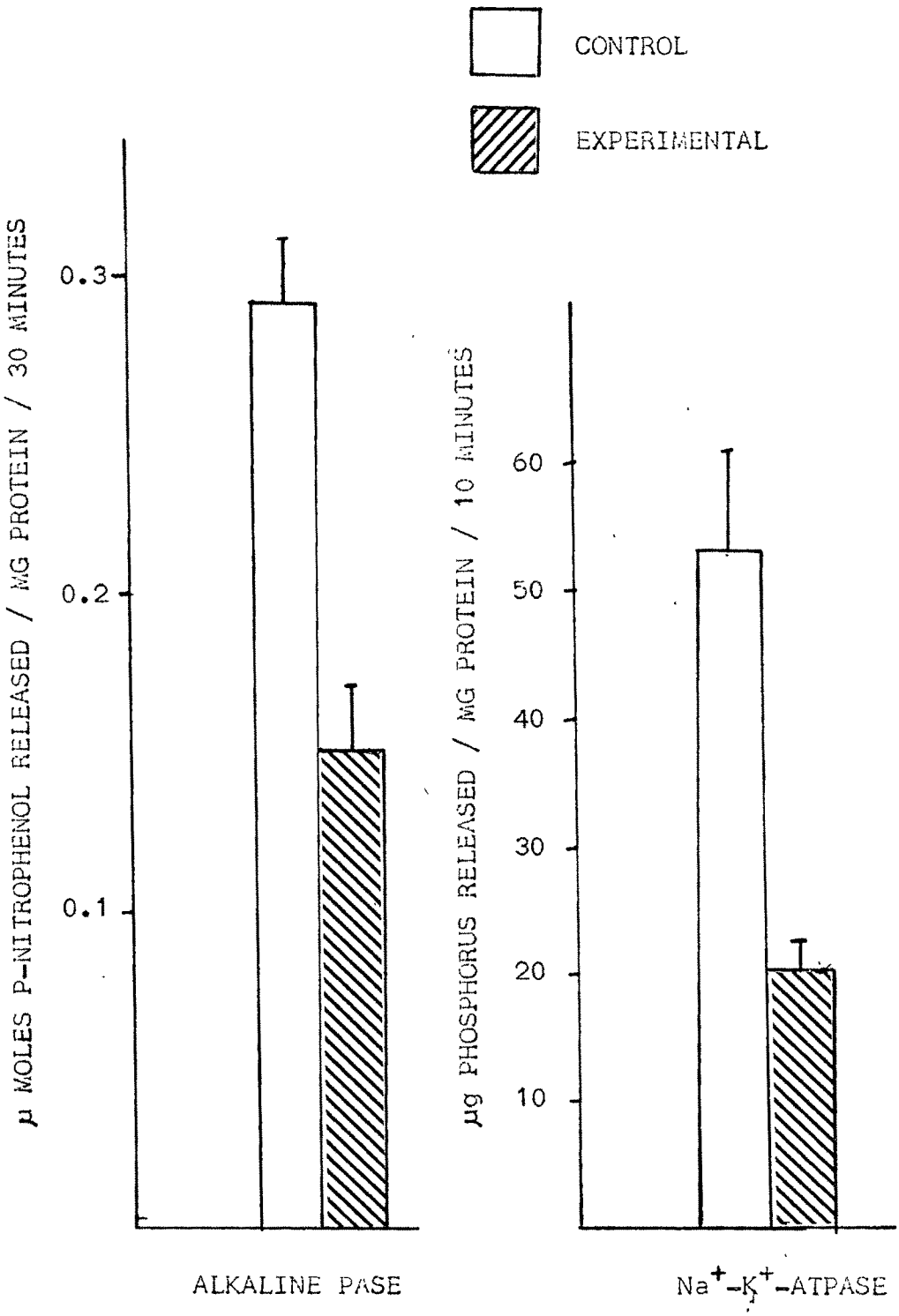


FIG. 5 : EFFECT OF GLUCAGON

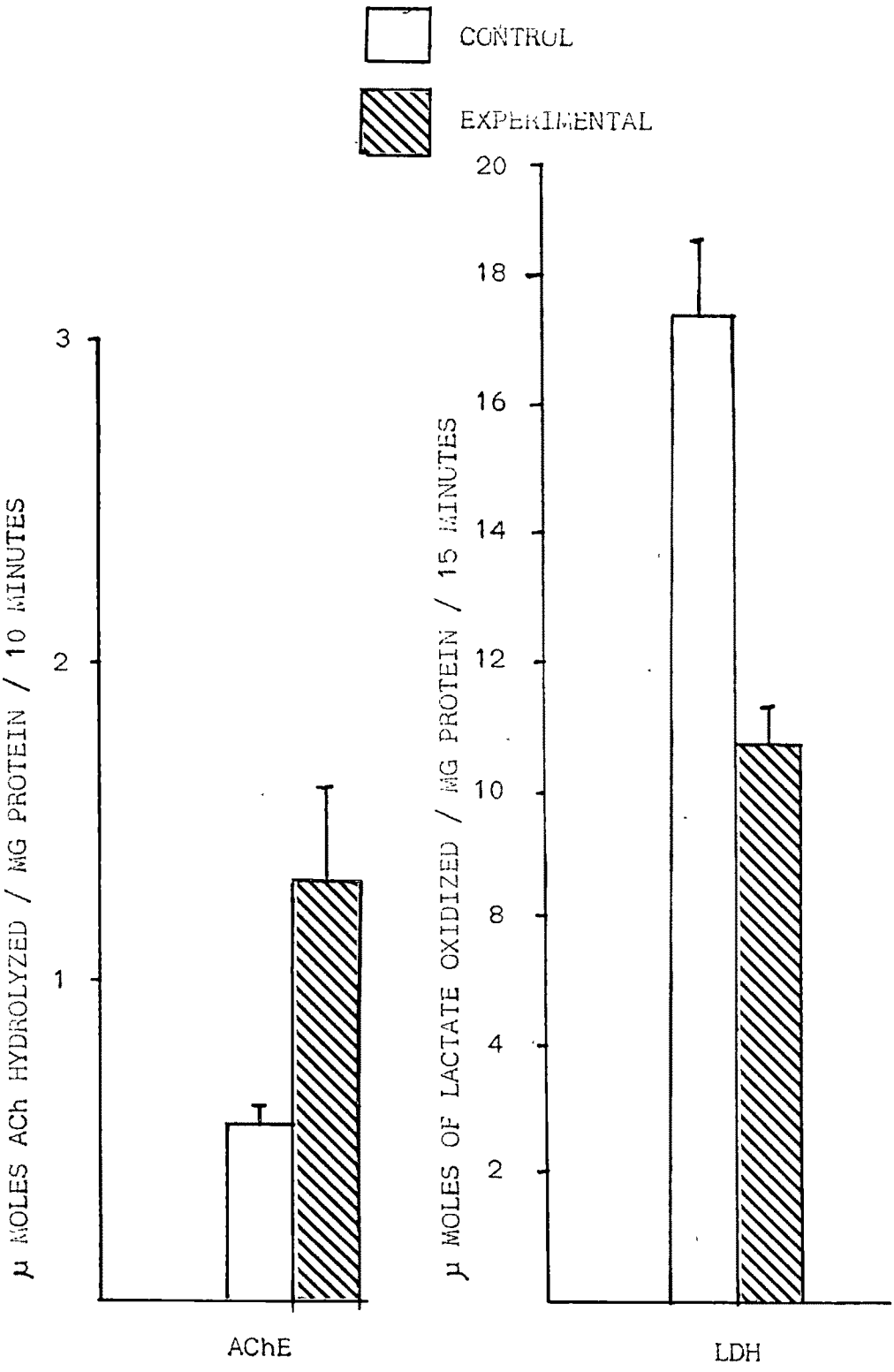
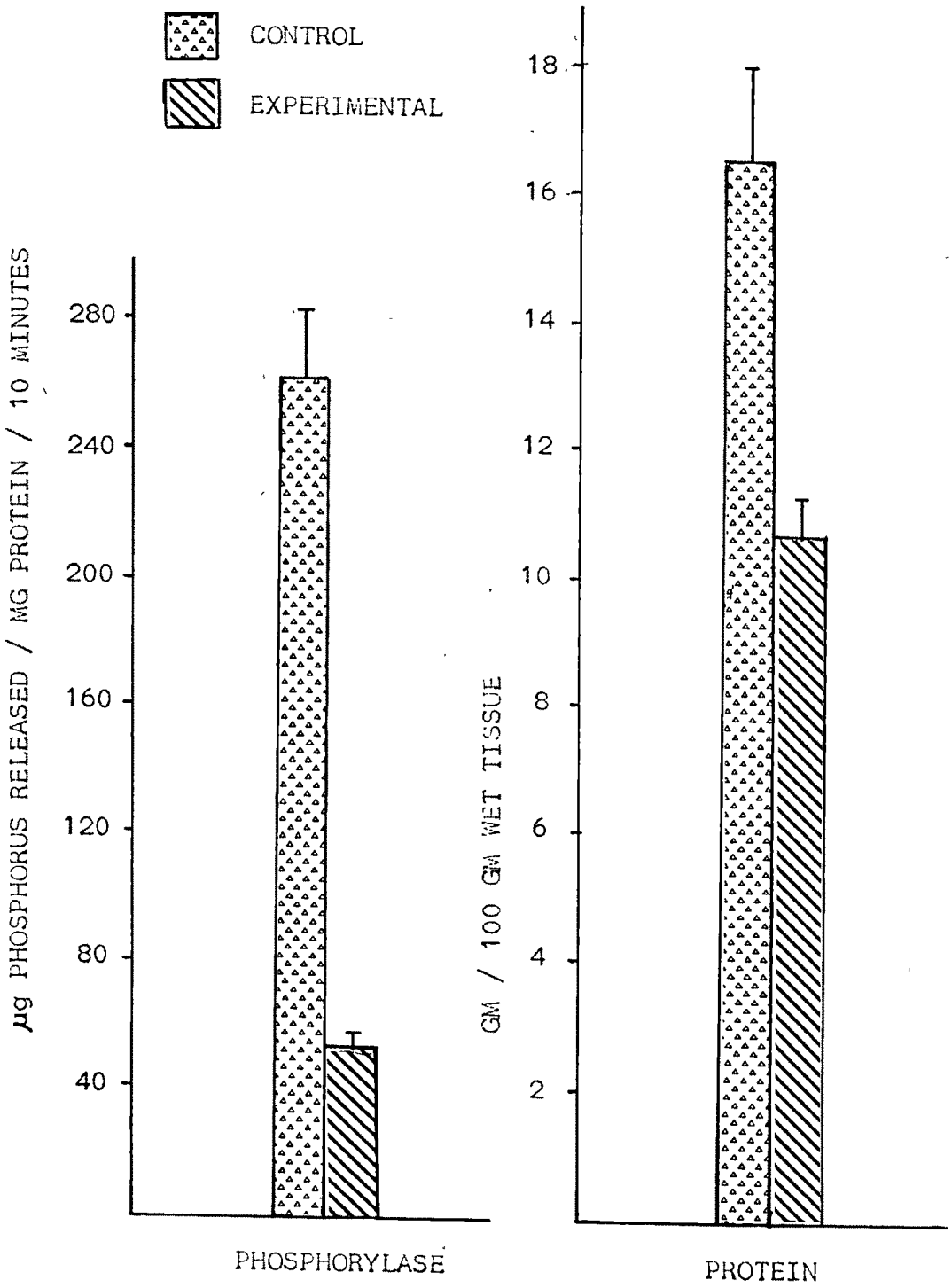


FIG. 6 : EFFECT OF GLUCAGON



DISCUSSION

Glucagon could regulate several key steps in the gluconeogenic pathway. Of these steps, at (a) phosphoenol-pyruvate/pyruvate level and (b) fructose-6-phosphate/fructose 1,6, diphosphate level, futile cycles also exist. Glucagon not only activates the gluconeogenic enzymes but also inhibits the corresponding glycolytic enzymes to prevent futile cycles (Kraus-Friedman, 1983). Phosphoenol pyruvate carboxykinase (PEPCK) is probably activated through a ferroactivator (MacDonald et al., 1978), while pyruvate kinase was inactivated through a cAMP dependent protein kinase (Ljungstrom et al., 1974). F-6-P and F 1-6-P_2 cycle is operated by phosphofructo-kinase (PFK) and fructose 1,6 diphosphatase (F.1-6 DP). PFK is activated by a metabolic activator fructose 2,6,diphosphate. The concentration of this activator increases in isolated liver cells when glucose was added to the medium and decreases when glucagon is added (Hers and Shaftingen, 1982). Thus, glucagon mainly act by inhibiting glycolytic flux by inhibiting key glycolytic enzymes especially those in the 'futile cycles' such as pyruvate kinase and PFK mainly through cAMP dependent protein kinase mechanism. The hormone may also indirectly activate enzymes such as PEPCK and F.1-6 DP.

The inhibition of glycolytic pathway in the avian kidney by glucagon was evident from the fact that both phosphatases were found to be low in the activities. Even the membrane bound Na^+-K^+-ATPase, involved in not only ionic

movements but also glucose and amino acid transport, showed reduction in activity. This indicates a general reduction in metabolite movement into kidney cells. Glucagon is known to alter the amino acid transport in liver and muscle cells *in vitro* in the eel (Inui *et al.*, 1983). Since LDH activity also showed a general reduction, lactate production or utilization could possibly be under inhibition by glucagon.

In the liver, glucagon has a glycogenolytic function by activating glycogen phosphorylase through cAMP. However, long term effect of glucagon coupled to a fasting condition, is an apparent inhibition of glycolysis and stimulation of gluconeogenesis (Hers and Hue, 1983). In the present experiment also, glucagon treatment and starvation produced an inhibitory effect on glycogenolysis as evident from decreased phosphatase activity. In the initial stages of starvation, glycogenolysis must have taken place in the kidney, which was not replenished later even in the event of gluconeogenesis stimulated by glucagon. This was evident from the fact that kidney glycogen content was drastically low in glucagon treated pigeons. G-6-Pase is generally believed to be an enzyme which is not regulated by hormones directly. The control of this enzyme is mainly through allosteric mechanism. This enzyme did not show much variation upon glucagon treatment.

It is difficult to conclude whether glucagon stimulates

gluconeogenesis from substrates such as pyruvate, alanine and aspartate as both GOT and GPT were very low in the kidney of treated birds. But reduction in the protein content points to some amount of amino acid catabolism in the kidney of glucagon treated birds.

In conclusion it could be stated, that major effect of glucagon in gluconeogenesis in the avian kidney was a general reduction in glycolytic activities.