CHAPTER IV

EFFECT OF THYROID HORMONES ON THE METABOLIC ACTIVITIES OF KIDNEY OF BLUE ROCK PIGEON (*COLUMBA LIVIA*).

Thyroid hormones are known to cause hyperglycaemia (Muller *et al.*, 1983). These hormones are also known to regulate metabolic rates of the body in general, and oxidative reactions in particular. Thyroid hormones also have morphogenic actions in most vertebrates. Due to multiplicity of actions, the exact biochemical mechanisms initiated by thyroid hormones are yet to be accounted for. In certain cases the influence of thyroid hormone on a given tissue will be different when other hormones are also present. Hence, while functions of hormone of thyroid gland are discussed, agonistic, synergistic, or antagonistic actions of these hormones to other hormones have to be taken into consideration.

Conventionally, studies on biochemical actions of thyroid hormones are conducted either by extraneous administration of hormones or by thyroid gland ablation. Many tissues are responsive to thyroid hormones. Oppenheimer and coworkers (1974) have reported the existence of binding sites of thyroid hormones in nuclei of rat liver and kidney. The nuclear binding sites are widely dispersed among tissues although the concentration of sites may differ from tissues to tissues. The effect

of binding thyroid hormones such as T_3 (triiodothyronine) is mediated by the formation of new RNA (Tata et al., 1962). Several metabolic effects of thyroid hormones, which may also include thermogenesis, are mediated through their ability to influence membrane bound ATPase enzymes. Some studies have indicated that thyroid hormone regulates the synthesis of Na^+-K^+-ATPase in the kidney and other target organs at nuclear level (Lo and Edelman, 1976 and Lo and Lo, 1981). It has also been observed that thyroidectomy produced proportionate decrease in renal Na^+-K^+-ATPase activity and net Na^+ reabsorption and that administration of T_3 resulted in an increase in both these functions (Katz and Linheimer, 1973). In rat, surgical thyroidectomy decreased renal plasma flow and glomerular filtration rate (Holmes and Discala, 1970; Michael et al., 1972; Katz and Linheimer, 1973; Katz et al., 1975). In kidney, liver and skeletal muscles, ouabain sensitive or Na^+ dependent respiration increased significantly upon T_3 administration to hypothyroid or euthyroid rats (Ismail-Beigi and Edelman, 1970; 1971; Asano et al., 1976).

Thyroid hormones are also known to influence metabolic reactions concerned with glucose uptake, glycogenolysis and gluconeogenesis. In hyperthyroid rat, renal phosphoenol pyruvate carboxykinase (PEPCK) became significantly reduced and L-throxine substitution in hypothyroid rat also produced the same effect (Sibrowski, 1982). T_4 and T_3 were found to

decrease basal phosphorylase 'a' (active form) by increasing phosphorylase phosphatase activity (Malbon and Campbell,1982). Stimulation of glucose uptake was also seen in rat thymocytes *in vitro* by the physiological concentrations of T_3 (Segal and Ingber, 1980).

Avian kidney is metabolically very active especially in gluconeogenic activity. The controls of these metabolic activities in kidney should also rest with hormones as in any other metabolically active tissue. However, no comprehensive studies have been reported so far about the action of various hormones on metabolic activities of avian kidney. In the present study, the action of thyroid hormones on some of the metabolic activities are elucidated.

MATERIALS AND METHODS

Adult domesticated blue rock pigeons (*Columba livia*) weighing around 250-300 gms, acclimated to laboratory conditions for two weeks and fed *ad-libitum* on a standard diet consisting of grains were selected for the experiments. One group of experimental birds received 0.5 mg L-thyroxine (Sigma Chemical Co. U.S.A.) in 0.5 ml 0.9 % saline (pH 8.4). Another group received 0.2 mg triiodothyronine (Sigma Chemical Co. U.S.A.) in 0.2 ml 0.9 % saline (pH 8.4). Control birds received 0.5 ml 0.9 % saline only. Both experimental and control birds received a total of 5 injections (i.p) administered

every alternate day. The birds were sacrificed by decapitation under mild anaesthesia on 11th day. Blood was drawn from wing veins prior to decapitation. The kidney was quickly excised and processed for various estimations. The activities of enzymes such as alkaline and acid phosphatases, transaminases (GOT,GPT), LDH, Na^+-K^+-ATPase, G-6-Pase, phosphorylase and AChE were measured following methods described in Chapter 1. Protein and glycogen content of kidney as well as blood sugar level were also determined as per the methods stated in Chapter 1.

RESULTS

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

Both the non-specific phosphatases in the kidney, did not show any significant variation following T_4 administration. A slight but perceptible increase of alkaline phosphatase was noticed in the kidney following T_3 administration. However, T_3 failed to evoke any change in the acid phosphatase activity from what was noticed in the kidney of control pigeons. Of the two transaminases, GOT did not respond to T_4 or T_3 administration. GPT on the other hand showed significant increase in activity compared to that in control birds. Na^+-K^+-ATPase activity showed no significant change, from what was observed in control birds, following T_4 or T_3 injections. G-6-Pase and AChE were the other enzymes that did not respond to thyroid hormones. Phosphorylase showed significant increase in activity in the kidney of both T_4 and T_3 administered pigeons. The

Table I: Effect of administration of thyroid hormones on the metabolic activities of pigeon kidney (Mean ± S.E)

Parameters	Normal	Control	Thyroxine	Triiodothyronine
Protein	13.96 ± 1.038	12.274 ± 0.507	17.61 ± 1.06	17.16 ± 1.42
Alk Pase	1.379 ± 0.078	0.321 ± 0.041	0.351 ± 0.039 NS	0.414 ± 0.31 *
Acid Pase	0.834 ± 0.046	0.244 ± 0.020	0.216 ± 0.013 NS	0.247 ± 0.041 NS
GOT	90.40 ± 8.696	122.89 ± 9.24	106.56 ± 8.92 *	127.33 ± 7.49 NS
GPT	151.62 ± 8.346	32.93 ± 2.67	118.47 ± 3.87 ***	71.40 ± 6.06 ***
Na^+-K^+-ATPase	133.30 ± 18.08	36.35 ± 1.78	28.97 ± 2.59 *	39.395 ± 8.15 NS
Phosphorylase	233.566 ± 21.96	20.57 ± 2.47	54.13 ± 7.33 **	62.38 ± 2.45 ***
G-6-Pase	0.113 ± 0.022	0.145 ± 0.048	0.082 ± 0.010 NS	0.122 ± 0.012 NS
AChE	3.37 ± 0.077	2.42 ± 0.196	2.43 ± 0.24 NS	1.559 ± 0.214 NS
LDH	16.00 ± 1.676	7.049 ± 0.538	14.276 ± 2.04 ***	12.66 ± 0.89 ***
Glucose	120.00 ± 5.26	92.57 ± 4.83	178.98 ± 10.86 ***	236.28 ± 3.90 ***
Glycogen	0.033 ± 0.009	0.034 ± 0.002	0.002 ± 0.0001***	0.0015 ± 0.0001***
Body weight	290 ± 4.47	281 ± 7.4	225 ± 7.0	270 ± 5.4
Total Kidney weight	1.59 ± 0.07	1.54 ± 0.06	1.38 ± 0.06	1.36 ± 0.04

* $P < .02$, ** $P < .01$ *** $P < .001$ NS - Not significant.

EXPLANATIONS TO GRAPHS - CHAPTER IV

Fig.1. Graphs showing the effect of T_3 and T_4 administration on blood sugar level and glycogen content in the kidney of blue rock pigeon.

Fig.2. Graphs showing the effect of T_3 and T_4 on GOT and GPT activities in the kidney of blue rock pigeon.

Fig.3. Graphs showing the effect of T_3 and T_4 administration on acid phosphatase activities in the kidney of blue rock pigeon.

Fig.4. Graphs showing the effect of T_3 and T_4 administration on Alk Pase and Na^+-K^+-ATPase activities in the kidney of blue rock pigeon.

Fig.5. Graphs showing the effect of T_3 and T_4 administration on AChE and LDH activities in the kidney of blue rock pigeon.

Fig.6. Graphs showing the effect of T_3 and T_4 administration on phosphorylase activity and protein content in the kidney of blue rock pigeon.

FIG. 1 : EFFECT OF THYROID HORMONES

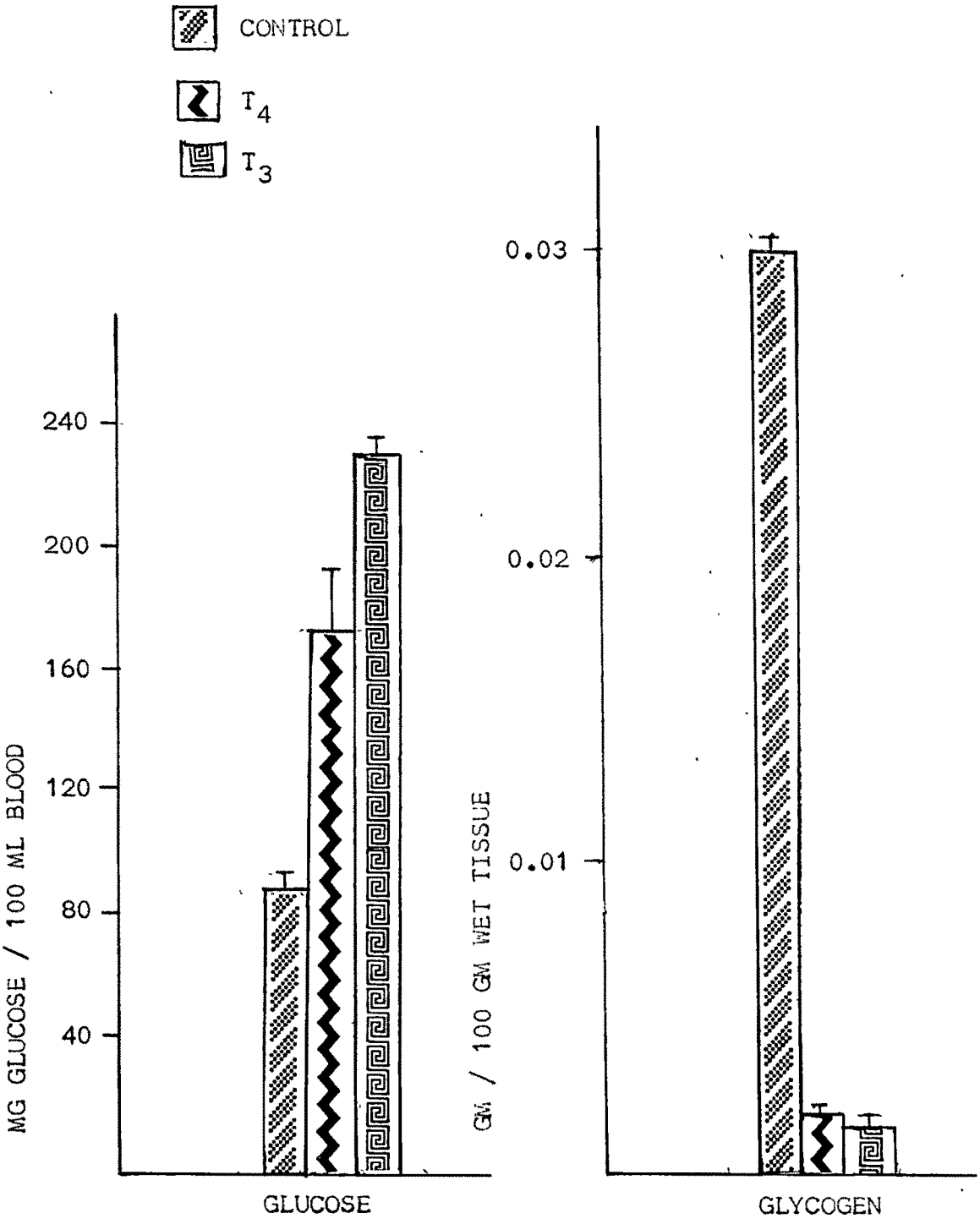


FIG. 2 : EFFECT OF THYROID HORMONES

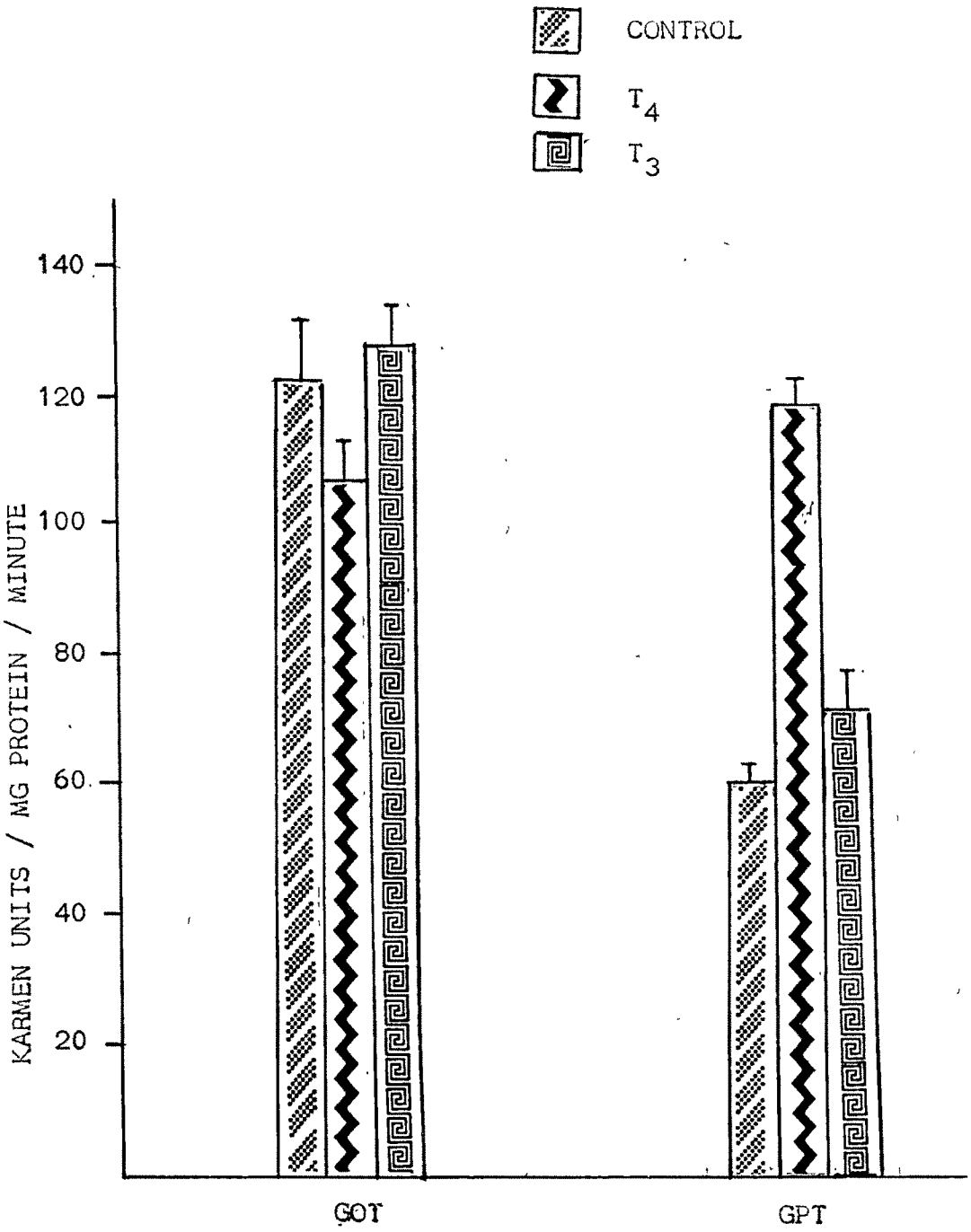


FIG. 3 : EFFECT OF THYROID HORMONES

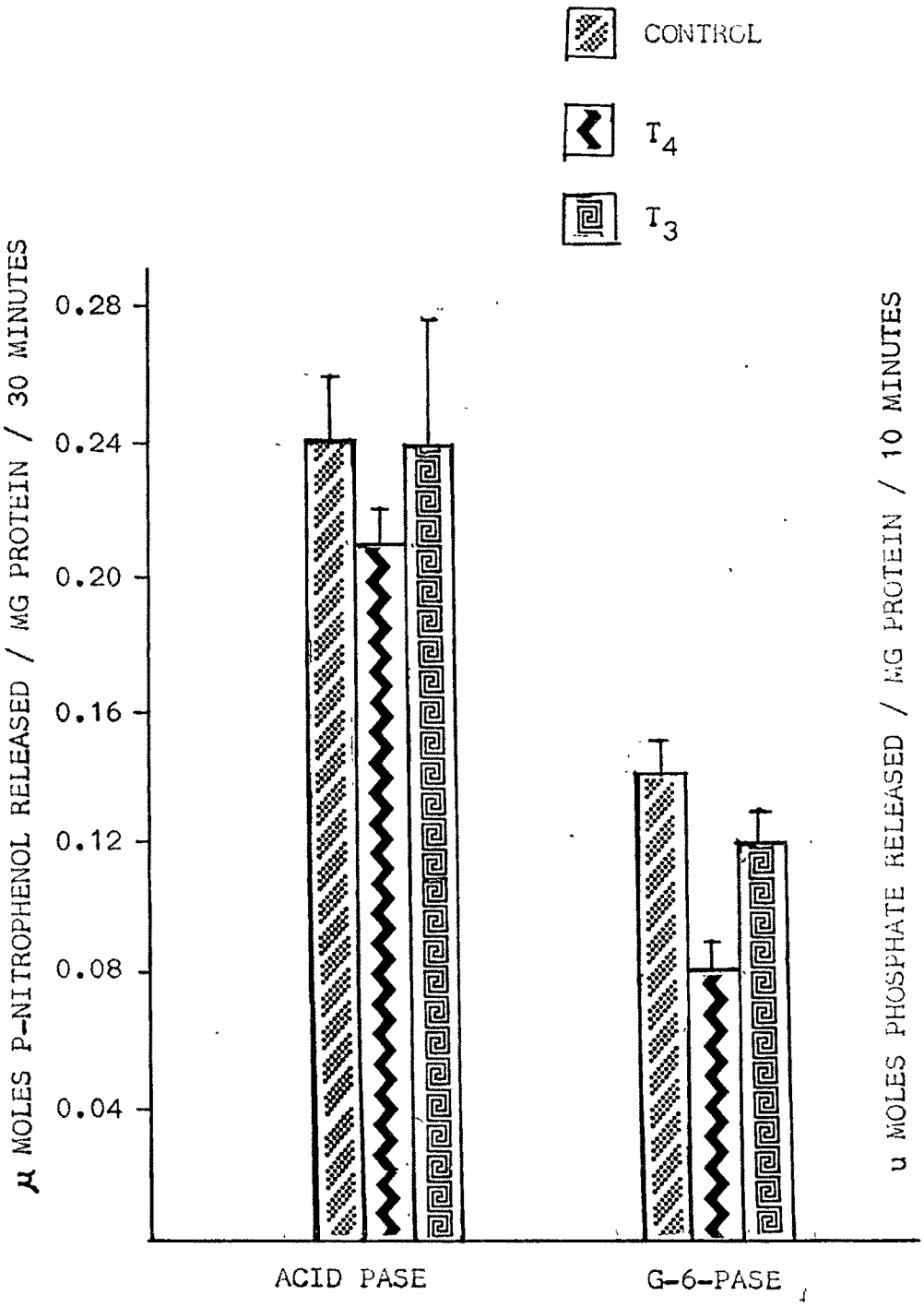


FIG. 4 : EFFECT OF THYROID HORMONES

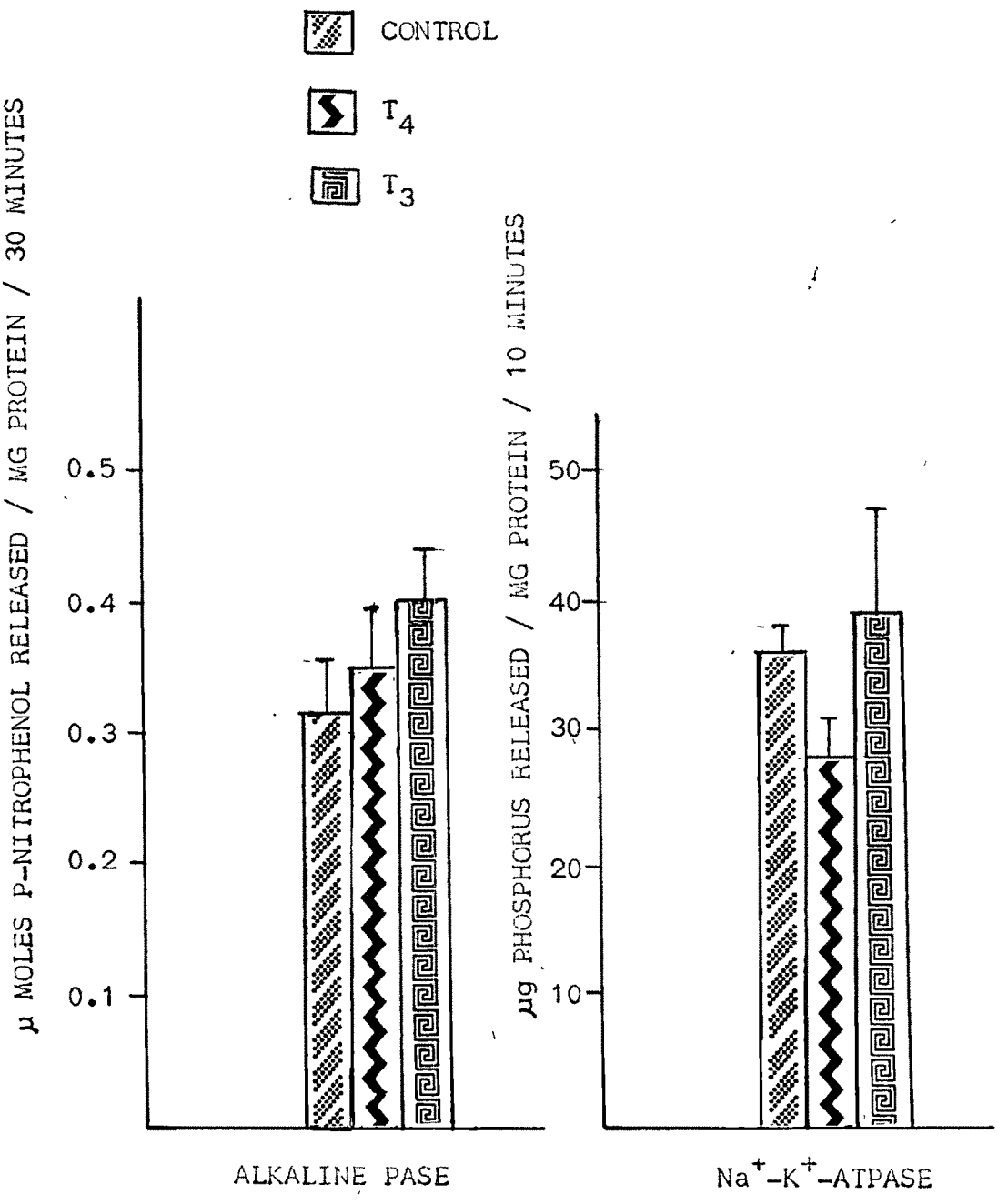


FIG. 5 : EFFECT OF THYROID HORMONES

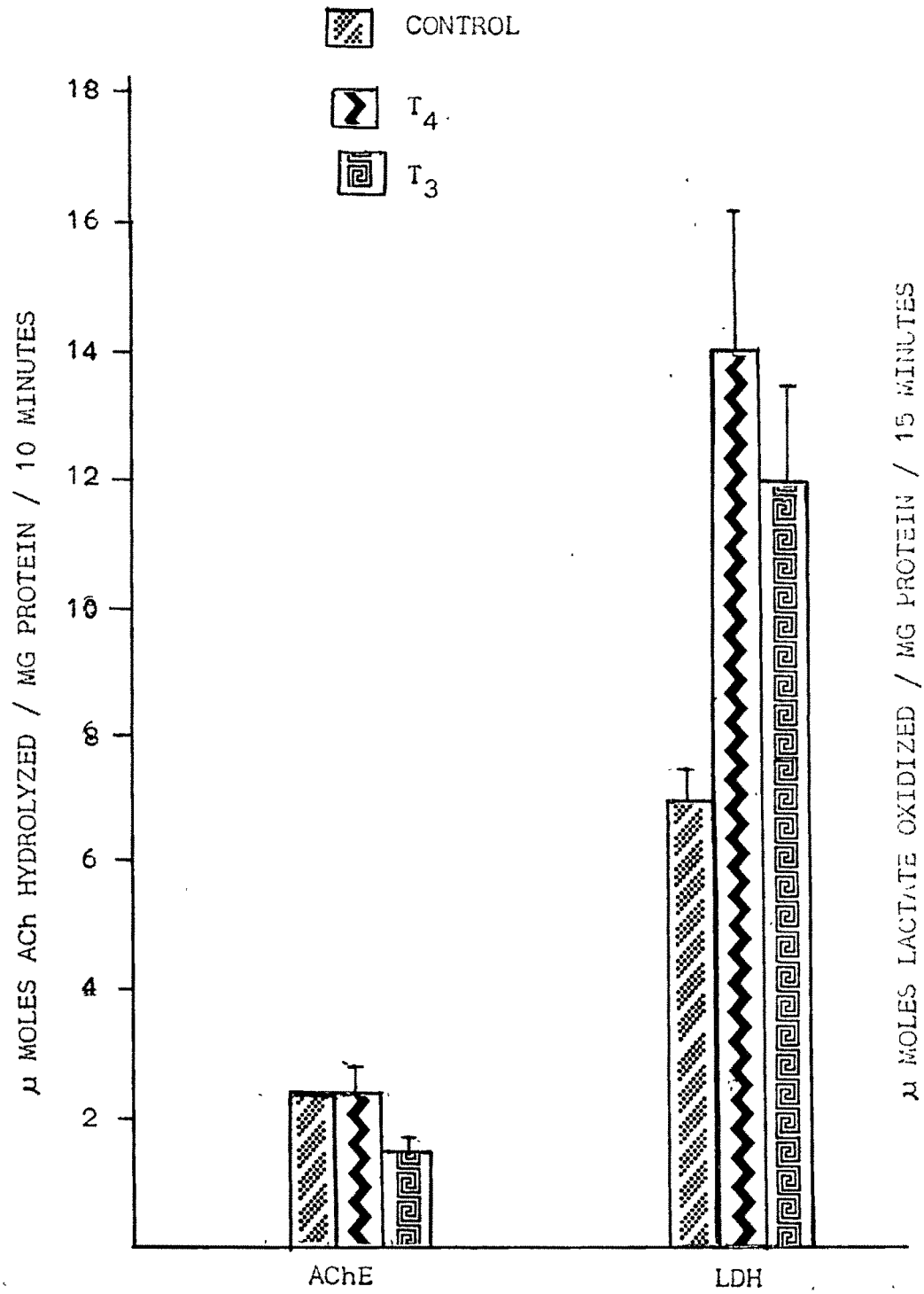
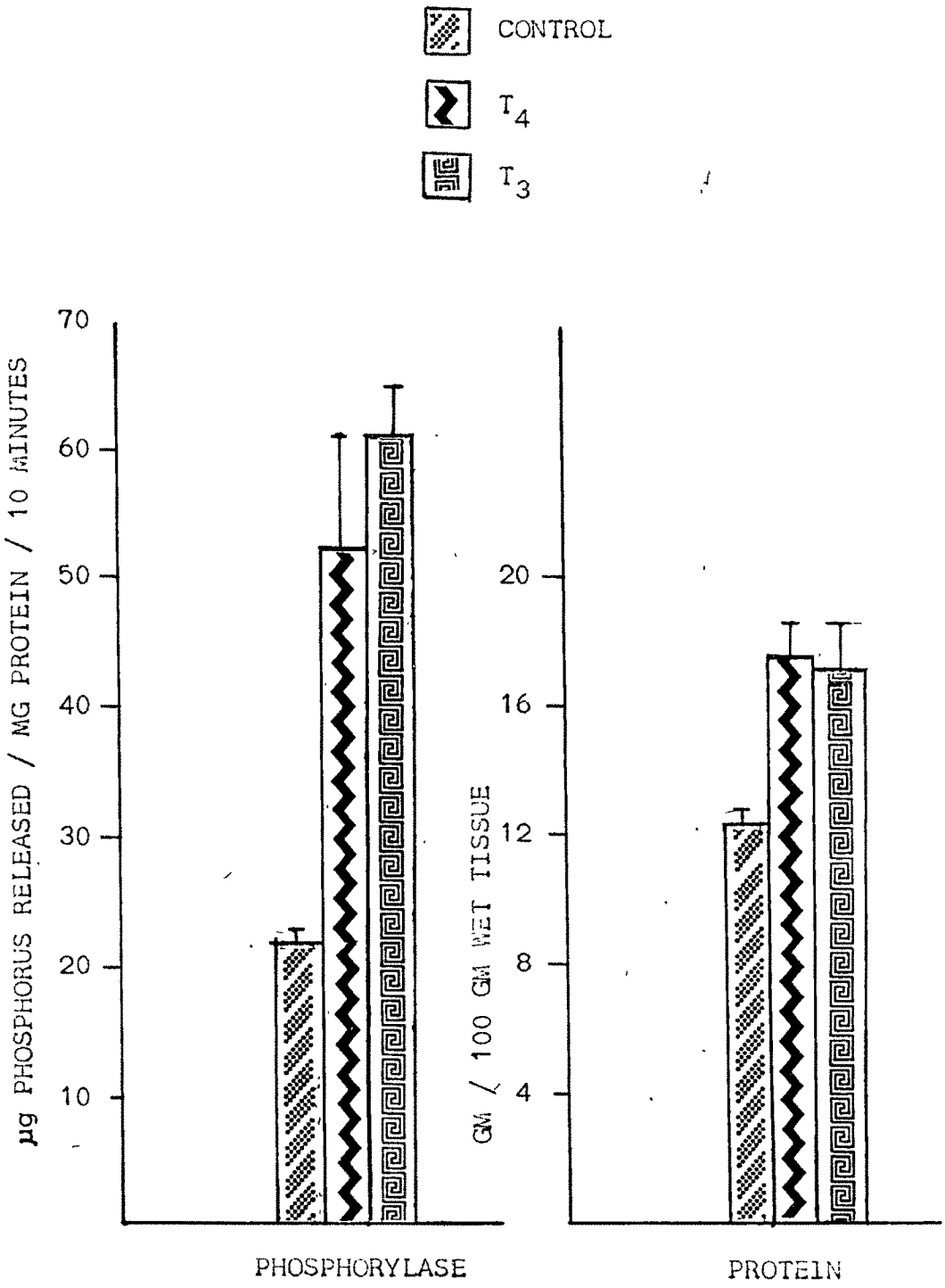


FIG. 6 : EFFECT OF THYROID HORMONES



increase observed in the activity of LDH following T_4 and T_3 treatment, was highly significant. Following administration of T_4 and T_3, glycaemic level increased significantly, while glycogen content in the kidney showed significant depletion.

DISCUSSION

Both T_4 and T_3 elicited a hyperglycaemic response in the pigeon. This was accompanied by sigmificant depletion of glycogen from the kidney, indicating glycogenolysis and subsequent release of glucose. Increased glycogen phosphorylase activity in the kidney lent credence to the contention that both T_4 and T_3 administration brought about glycogenolysis and glucose release from the tissue. In rat hepatocytes, however, T_4 and T_3 brought about a decrease in phosphorylase 'a' activity (Malbon and Campbell, 1982). In this respect, response of avian kidney to T_4 and T_3 differed from that of rat tissues. Similarly, thyroid hormones which were found to bring about both stimulation and induction of Na^+-K^+-ATPase in the rat kidney (Katz and Linheimer, 1973; Lo and Edelman, 1976; Lo and Lo, 1981), elicited no response in the avian kidney.

Since the glycogen content in kidney is so meagre compared to that in liver, major amount of glucose released from kidney following T_4 and T_3 treatment could be formed by gluconeogenesis. Increased gluconeogenic activity in the kidney under the influence of thyroid hormones is indicated by increased

GPT and LDH activities. Lactate and aspartate, the substrates for LDH and GPT, are prominent precursors of gluconeogenic pathway. Since, phosphoenol pyruvate carboxykinase (PEPCK) was not estimated in the present experiments, it is difficult to envisage the action of thyroid hormone on this key enzyme of gluconeogenesis. In hyperthyroid rat kidney PEPCK activity was found to decrease (Sibrowski, 1982). However, in avian kidney such inhibition cannot be expected as both aspartate and lactate have to be converted into oxaloacetate and then into PEP, if these have to serve as gluconeogenic precursors.

In this context it is interesting to note that plasma concentration of gluconeogenic precursor molecules (lactate, pyruvate, alanine and glycerol) were elevated in the hyperthyroid miniature pig but nearly unaffected in hypothyroidism (Muller et al., 1983). This increase was paralleled by a rise in the rate of extraction of gluconeogenic precursors by splanchnic tissues in hyperthyroidism (Wahren et al., 1981). Turnover rates for lactate (Svedmyr, 1966) and glycerol (Tibbling et al., 1969) were reported to be enhanced in the hyperthyroid state. In other words, hyperthyroidic state induces release of gluconeogenic precursors mainly from extrahepatic tissues. These precursors are extracted and converted into glucose by liver. Direct stimulation of gluconeogenesis from alanine in the isolated perfused rat liver of hypothyroid rats by T_3 has been reported (Muller and Seitz, 1980; 1981). Probably, pigeon kidney also may be releasing gluconeogenic

precursors into blood stream under the influence of thyroid hormones.

Another effect of thyroid hormone is on the release of other hormones. Hyperthyroidism induced elevated release of glucagon and insulin (Muller et al., 1983). However, hyperthyroid condition also brings about insulin resistance (Sestoft and Heding, 1981). So, in effect, hyperthyroidic condition mainly enhances the release and action of glucagon, eventhough insulin secretion may also get simultaneously elevated. Thyroid hormones are known to cause partial suppression of acetylcholine receptor synthesis in cultured muscle cells, without affecting the activity of acetylcholinesterase (AChE) (Shainberg et al., 1984). In the present investigation also AChE activity in the kidney was not found to be affected by thyroid hormones. Probably, thyroid hormone treatment may cause ineffectiveness of vagal activity in the kidney without showing any effect on AChE activity.