CHAPTER 5

EFFECT OF THYROXINE ON GLUCOSE TRANSPORT ACROSS CELL MEMBRANE OF HEPATIC TISSUE OF DOMESTIC PIGEON, COLUMBA LIVIA

Thyroid hormone is closely related with oxidative reactions and regulation of metabolic rates in the body. Thyroid hormones accelerate metabolic reactions and the mechanism by which the metabolism is accelerated is probably through its action on the membrane bound ATPase enzyme. Thyroxine increases ATP utilization and as a result ATP depletion takes place which is responsible for the increase in oxygen uptake. Many conflicting mechanisms have been proposed to explain the multiple biological actions of thyroid hormones, such as the regulation of basal metabolic rate and effects on protein, lipid and electrolyte metabolism. Although there are no evidences to show that the effect on basal metabolic rate is more fundamental than are the other actions of the hormones, the majority of the attempts are to explain the calorigenic action of thyroid hormones, especially since the discovery that administration of large doses of thyroxine or direct addition *in vitro* leads to uncoupling of oxidative phosphorylation in liver mitochondria (Martius and Hess, 1951). Some studies have also indicated a sort of direct action of thyroid hormone on mitochondria or mitochondrial permeability as seen by swelling or contraction (Tapley *et al.*, 1955) and interaction with dehydrogenases or their prosthetic metal groups (Wolff and Wolff, 1957). There are increasing evidences to

indicate that thyroxine can influence processes not directly dependent on mitochondrial function, such as protein and nitrogen metabolism, glycolysis and lipid synthesis and breakdown (Phillip and Langdon, 1956).

Thyroid thus has direct effect on a large number of metabolic reactions. Recently it has been observed that thyroxine also helps other hormones in exerting their effects on metabolic reactions. Jolin et al., (1983) showed that thyrodectomized diabetic rats showed only a slight increment in the glucose level in blood, and the treatment with insulin reduced the glycemic level but not when injected with thyroxine. Thyroxine in this condition produced its gluconeogenic or glycogenolytic activity. These authors concluded that insulin was needed for thyroxine to manifest its metabolic effects, although they are opposite to that of insulin. Thyroxine, in in vivo conditions thus acts as hyperglycemic agent in presence of insulin. The present experiment was conducted to understand whether thyroxine has the same effect on hepatocytes of birds in the presence of hypoglycemic agents such as insulin and acetylcholine in in vitro conditions.

MATERIALS AND METHODS

Adult pigeons (Columba livia) weighing 180-250 grams maintained in laboratory conditions on balanced diet were used for the present experiments. Animals were sacrificed after 24 hours of starvation period. The liver was perfused with Cold

Krebs Ringer Medium and then quickly excised. The liver was placed on ice and cut into slices. The slices were weighed and were placed in 10 ml flask with 5 ml of Krebs Ringer Bicarbonate buffer with glucose and albumin. The liver slices were incubated for 90 min. at 37°C in a water bath shaker. The slices were incubated in media of following categories.

(1) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Thyroxine (10 µg/ml).

(2) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Thyroxine (10 µg/ml) + Insulin (1 unit/ml)

(3) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Thyroxine (10 µg/ml) + ACh (15 mg/ml).

The slices before and after incubation were quickly washed with chilled KRB buffer and homogenised with distilled water and homogenate was used for enzyme and protein estimations as per methods given in Chapter 1. The incubation medium (before and after incubation) was subjected for glucose estimation and differences were calculated.

RESULTS

The data on the effect of thyroxine on glucose uptake, glycogen content in the liver, and enzymes are presented in Tables 5-1 and 5-2 and Figs. 5-1 to 5-4. (Glucose and glycogen

Fig. 5-1; Na^+-K^+ ATPase and AChE Fig. 5-2; Acid and Alkaline Phosphatases Fig. 5-3; LDH and SDH Fig. 5-4).

Thyroxine when present alone in the medium, stimulated an uptake of glucose (1.0989 mg/100 mg tissue/90 min). The uptake of glucose was even greater (1.2048 mg/100 mg tissue/90 min) when insulin or ACh was also present in the medium. However, thyroxine together with ACh induced only comparatively a small amount (0.4381 mg/100 mg tissue/90 min) of glucose uptake by liver slices than when thyroxine was present with insulin. Na^+-K^+-ATPase, an enzyme immensely affected by thyroxine showed an increase in slices incubated in the medium containing only thyroxine. But when incubation medium contained insulin or ACh together with thyroxine, Na^+-K^+-ATPase showed a significant decrease. SDH showed a slightly significant increase in slices incubated with only T_4 while together with insulin or ACh thyroxine did not produce any change in the activity. LDH on the other hand showed significant increase in all three combinations (Thyroxine, Thyroxine + insulin and Thyroxine + ACh). AChE showed significant reduction only in the medium containing thyroxine with insulin or ACh. Acid phosphatase showed a slightly significant increase in all three combinations while alkaline phosphatase showed a decrease only in those slices incubated in a medium containing thyroxine alone. Glycogen content of the liver slices showed a drastic reduction in all three sets of media.

Fig. 5-1. Effect of thyroxine, alone or in combination with insulin or acetylcholine (ACh) on glucose uptake by and glycogen content in pigeon liver slices under *in vitro* conditions.

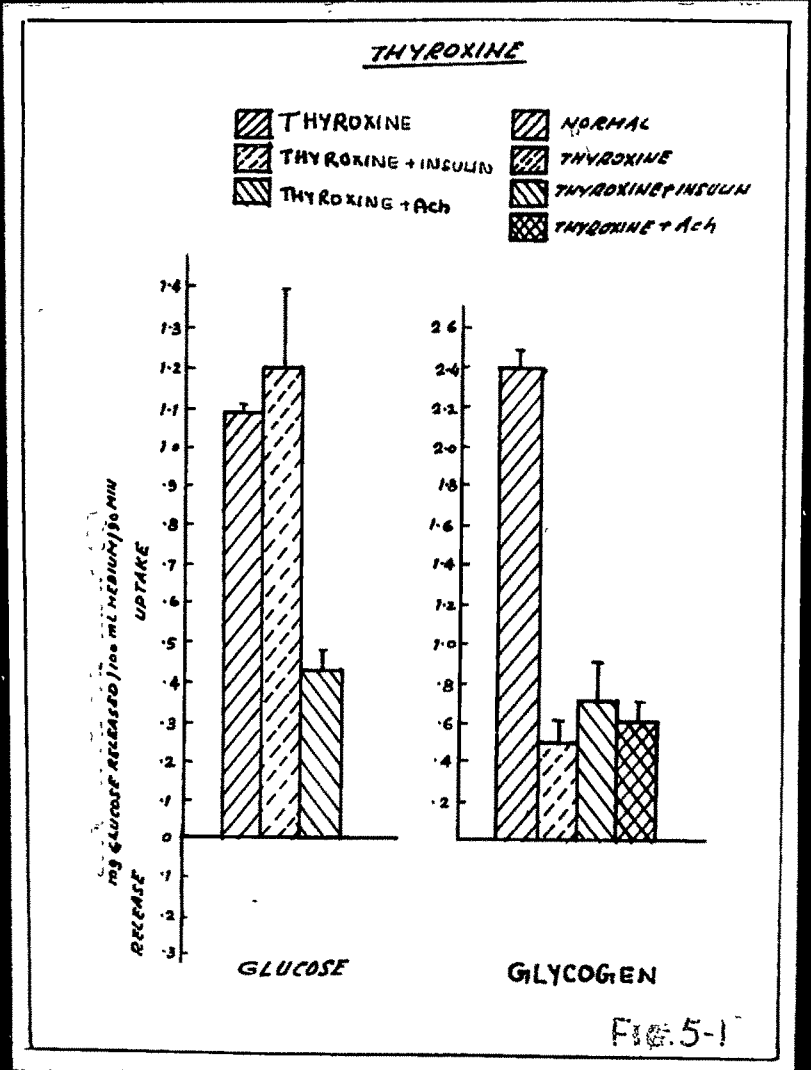
THYROXINE
THYROXINE
THYROXINE + INSULIN
THYROXINE + Ach
NORMAL
THYROXINE
THYROXINE+INSULIN
THYROXINE + Ach
mg GLUCOSE RELEASED/100 ml BLOOD/30 MIN
UPTAKE
RELEASE
GLUCOSE
GLYCOGEN

Fig. 5-1

Fig. 5-2. Effect of thyroxine, alone or in combination with insulin or acetylcholine (ACh) on Na^+-K^+-ATPase and acetylcholinesterase (AChE) activities in pigeon liver slices under *in vitro* conditions.

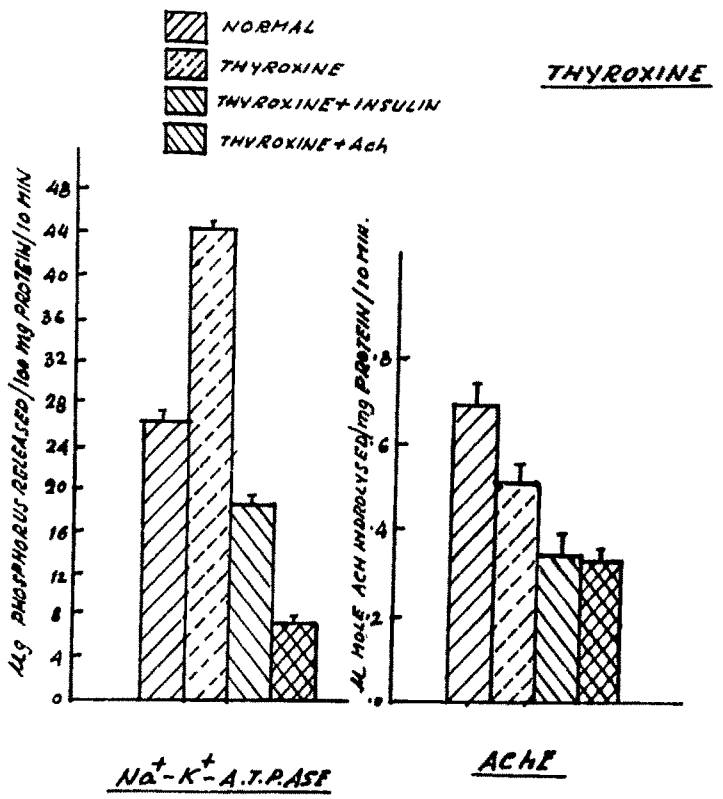
NORMAL
THYROXINE
THYROXINE + INSULIN
THYROXINE + Ach
THYROXINE
µg PHOSPHORUS RELEASED/100 mg PROTEIN/10 MIN
48
44
40
36
32
28
24
20
16
12
8
4
0
µL MOLE ACh HYDROLYSED/mg PROTEIN/10 MIN.
.8
.6
.4
.2
.0
Na+ - K+ - A.T.P.ASE
ACHE

FIG. 5-2

Fig. 5-3. Effect of thyroxine, alone or in combination with insulin or acetylcholine (ACh) on acid phosphatase (Ac Pase) and Alkaline phosphatase (Alk PO_4ase) activities in pigeon liver slices under <u>in vitro</u> conditions.

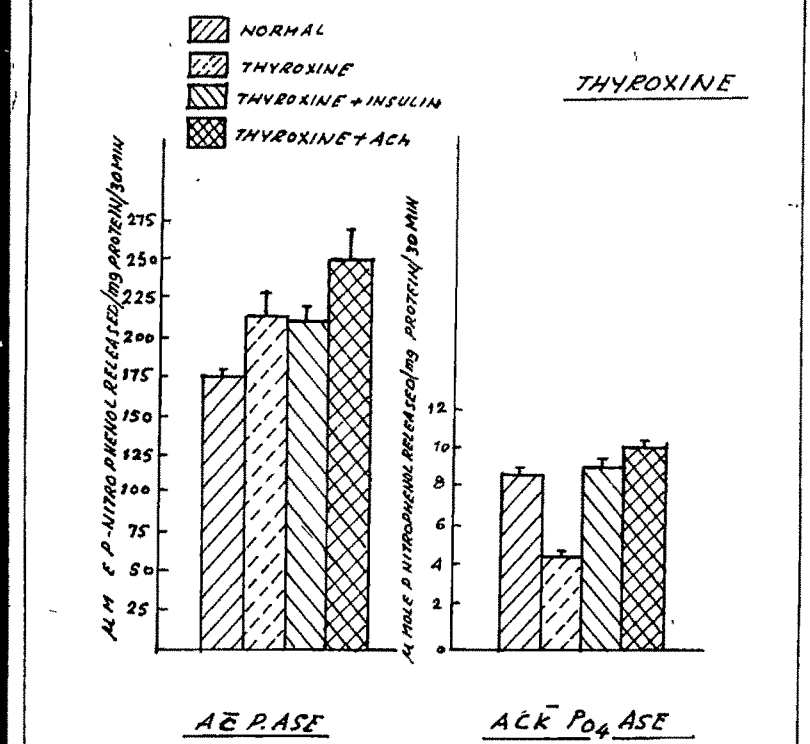
NORMAL
THYROXINE
THYROXINE + INSULIN
THYROXINE + ACh
THYROXINE
μ M ε P-NITRO PHENOL RELEASED/mg PROTEIN/30 MIN
275
250
225
200
175
150
125
100
75
50
25
μ MOLE P NITROPHENOL RELEASED/mg PROTEIN/30 MIN
12
10
8
6
4
2
0
ACETPASE
ACK PO4 ASE

FIG. 5-3

Fig. 5-4. Effect of thyroxine, alone or in combination with insulin or acetylcholine (ACh) on lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) in pigeon liver slices under <u>in vitro</u> conditions.

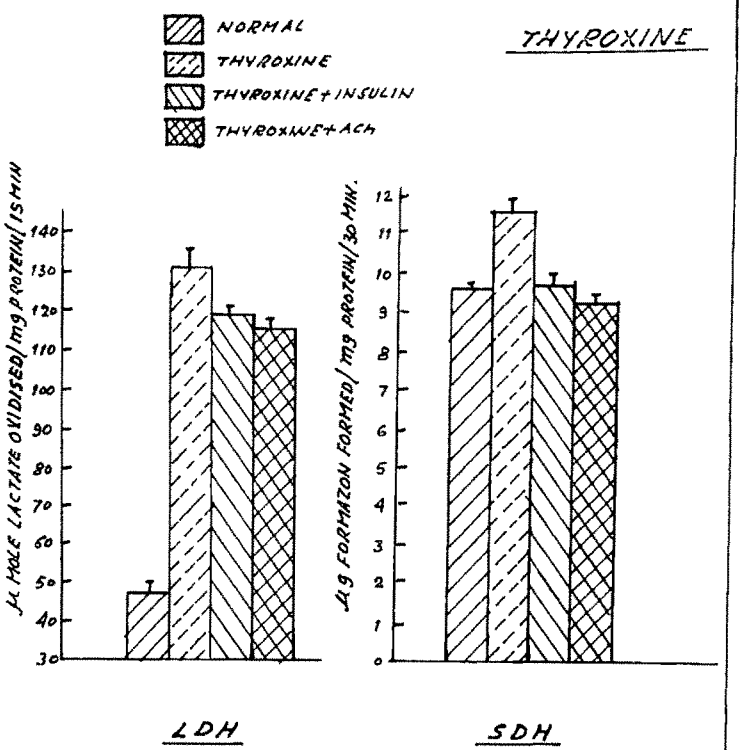
NORMAL
THYROXINE
THYROXINE + INSULIN
THYROXINE + ACh
THYROXINE
μ MOLE LACTATE OXIDISED/mg PROTEIN/15 MIN
140
130
120
110
100
90
80
70
60
50
40
30
μg FORMAZON FORMED/mg PROTEIN/30 MIN.
12
11
10
9
8
7
6
5
4
3
2
1
0
LDH
SDH

FIG. 5-4

Table 5-1

Effect of thyroxine, alone or in combination with insulin or acetylcholine on the uptake or release of glucose by pigeon liver slices under *in vitro* conditions (Mean ± SEM).

Additives	Glucose		Glycogen Depletion(2)
	Uptake (1)	Release (1)	
Thyroxine	1.0983 ±0.07397	-	1.9511 ***
Thyroxine + Insulin	1.2048 ±0.2411	-	1.7143 ***
Thyroxine + ACh	0.4381 ±0.0555	-	1.8047 ***

(1) Mg glucose taken up or released by 100 mg liver.

(2) Mg glycogen depletion/100 mg liver. *** $P < 0.01$

Table 5-2

Effect of thyroxine, alone or in combination with insulin or acetylcholine, on the enzyme activities in the pigeon liver slices under *in vitro* conditions.

Enzymes	Control(+) (Tissue)	Thyroxine	Thyroxine +Insulin	Thyroxine + ACh
Na^+-K^+-ATPase ug phosphorus released/mg protein/ 10 minutes	26.264 ± 0.568	44.782 **** ± 0.767 ****	18.498 **** ± 0.687	6.924 **** ± 0.611
AChE um ACh hydrolysed/mg protein/ 10 minutes	0.702 ± 0.062	0.522 NS ± 0.058	0.363 *** ± 0.057	0.344 *** ± 0.036
Acid Phosphatase um P-nitrophenol released/100 mg protein/30 minutes	176.29 ±12.57	214.28 NS ±17.13	215.29 * ± 8.85	253.51 *** ± 17.04
Alkaline Phosphatase um P-nitrophenol released/100 mg protein/30 minutes	8.91 ± 0.25	4.97 **** ± 0.24 ****	9.33 NS ± 0.24	10.13 ** ± 0.25
LDH um lactate oxidised/mg protein/ 15 minutes	47.87 ± 3.78	131.72 **** ± 5.13 ****	118.65 **** ± 3.16 ****	105.18 **** ± 3.68
SDH ug formozon formed/ mg protein/30 minutes	9.68 ± 0.17	11.57 ** ± 0.56 **	9.69 NS ± 0.40	9.28 NS ± 0.49

(+) Enzyme values of fresh liver slices not subjected to incubation.

NS - Not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.001$.

DISCUSSION

The observation that diabetic rats appeared less diabetic when hypothyroidic, reveals the opposing action of thyroid hormone to that of insulin (Jolin *et al.*, 1983). However, the manifestation of some of the thyroxine actions require the presence of insulin (Jolin and Herrera, 1982; Jolin *et al.*,1983). In rats, thyroxine to a certain extent opposes the glycaemic response effected by insulin. However, in the *in vitro* experiments thyroxine elicited a glucose uptake response by the avian liver slices and this action was additive in the presence of insulin. The action of thyroxine in this respect was very much subdued in the presence of ACh, in spite of the fact that ACh also is capable of inducing glucose uptake by liver slices. Thyroxine thus counteracted the action of ACh more than that of insulin. The difference between the action of thyroxine in *in vivo* condition and in *in vitro* condition may be due to the physiological state of the liver itself. The gluconeogenic and glycogenolytic actions of thyroxine are more prominent in rats when they are subjected to starvation (Llobera and Herrera, 1980; Llobera *et al.*, 1978). The presence of glucagon and catecholamines under conditions of starvation could be expected. Thyroxine under such conditions must be augmenting the action of glucagon or catecholamines. In the absence of these hyperglycemic agents, thyroxine could effect a glucose uptake action by liver cells. In the presence of insulin such effect was amplified. This complementary action of thyroxine in glucose uptake in *in vitro*

conditions does not, however, prove that both thyroxine and insulin affect the liver in the same manner. In fact some of the enzymes studied such as Na^+-K^+-ATPase, SDH and alkaline phosphatase, showed responses to thyroxine alone in the medium and thyroxine in presence of insulin or ACh. In fact enzyme responses were more or less conforming to the gluconeogenic actions of thyroxine. The increased glucose uptake influence of thyroxine in the pigeon liver was not in tune with its action observed on the metabolic machinery of the rat liver cells. The only contention one could extent at this juncture, is that thyroxine must be affecting permeability of the avian hepatocyte membrane and thereby increasing glucose uptake without involving either insulin stimulated or ACh stimulated glucose uptake mechanism.