CHAPTER 7

EFFECT OF ACTH ON GLUCOSE TRANSPORT ACROSS PLASMA-MEMBRANE OF HEPATIC CELLS AND ITS EFFECT ON HEPATIC ENZYMES IN PRESENCE OF INSULIN AND ACTH

Hypoglycaemic activity of pituitary extracts has been noted for over 30 years. The independent demonstrations by Westermeyer and Raben (1954) and Engel and Engel (1945) that Oxy-cell ACTH would lower the blood sugar in intact and adrenalectomized mice and rats suggested that corticotrophin itself had intrinsic hypoglycaemic activity; and that the hormone is hypoglycaemic in action was strengthened further by the observation that when ACTH was taken along with insulin the glucose uptake was still enhanced. Certain characteristics of the ACTH induced hypoglycaemia suggested that it is mediated through an increase in insulin secretion. Engel et al. (1958) in a series of studies with adrenalectomized, intact and eviscerated rats were able to show that corticotrophin caused an increase in brown adipose tissue glycogen of glucose treated rats, and opined that this glycogen deposition was probably attributable to insulin action and that corticotrophin did not potentiate the glycogen depositing effect of a small dose of insulin. Miller and Krake (1963) have reported that oxy-cell ACTH causes no hypoglycaemia in alloxan diabetic mice. Oxy-cell ACTH has been shown to protect adrenalectomized mice from insulin convulsions and a hyperglycaemic diabetogenic effect of corticotrophin has been observed in adrenalectomized rats in which insulin secretion

has been maximally stressed by force feeding a high carbohydrate diet and administering a sub-diabetogenic dose of cortisone. The hypoglycaemic action of corticotrophin under *in vivo* conditions is the result of an increase in insulin secretion which in turn results from a direct effect of corticotrophin on the pancreas. Apart from the influence of ACTH on insulin secretion, ACTH also causes rapid increase in phosphorylase activity in adrenals (Haynes Jr., 1958) and this situation is mediated through 3' - 5' AMP as in the stimulation of hepatic phosphorylase by glucagon. To understand whether ACTH has any direct influence on the hepatocytes of birds in *in vitro* condition, the present study was undertaken.

MATERIALS AND METHODS

The liver slices from healthy adult pigeons were removed and incubated in KRB media containing ACTH alone or ACTH with insulin or ACh in the following combinations.

(1) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + ACTH (0.1 I.U./ml)

(2) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + ACTH (0.1 I.U./ml) + Insulin (1 Unit/ml).

(3) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + ACTH (0.1 I.U./ml) + ACh (15 mg/ml).

The glucose was estimated (Chapter 1) in the medium before or after incubation and the difference was calculated to determine the uptake or release from the liver cells at the end of incubation period.

At the end of the incubation, the liver slices were processed for the estimation of glycogen and enzymes such as ATPase, SDH, LDH, and acid and alkaline phosphatases (Chapter 1).

RESULTS

The data on the effect of ACTH on glucose uptake, glycogen content in the liver, and enzymes are presented in Tables 7-1 and 7-2 and Figs. 7-1 to 7-4; (Glucose and glycogen, Fig. 7-1; $Na^+ - K^+$ ATPase and AChE, Fig. 7-2; Acid and Alkaline phosphatases, Fig. 7-3; LDH and SDH, Fig. 7-4).

Corticotrophin (ACTH) when present alone in the incubation medium induced a moderate uptake of glucose (Table 7-1); (Fig.7-1). In the presence of insulin, ACTH doubled the glucose uptake by the liver cells. In combination with ACh, ACTH did not induce any further increase in the uptake of glucose than that was affected when ACTH was present alone in the medium.

ATPase activity showed a significant reduction in the presence of ACTH, which was so even in the media that contained insulin or ACh. Of the two dehydrogenase studied, SDH showed a significant decrease whether or not ACTH was present in the medium alone or in combination with insulin or ACh. LDH on the other hand showed a slight increase in all three combinations of additives (ACTH, ACTH + insulin, ACTH + ACh). The activities of AChE, acid phosphatase and alkaline phosphatase showed no variations from what was observed in the liver slices prior to incubation.

Fig. 7-1. Effect of ACTH alone or in combination with insulin or acetylcholine (ACh) on glucose uptake and glycogen content in pigeon liver slices under in vitro conditions.

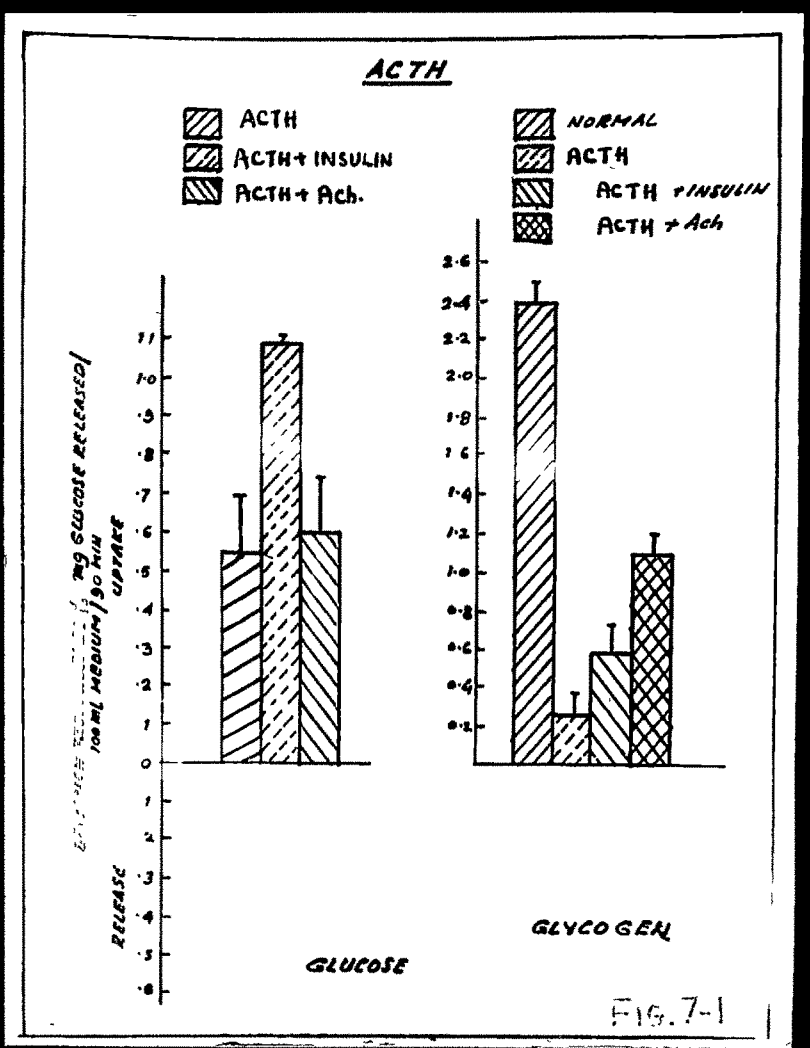
ACTH
ACTH
ACTH + INSULIN
ACTH + Ach.
NORMAL
ACTH
ACTH + INSULIN
ACTH + Ach
RELEASE
UPTAKE
mg GLUCOSE RELEASED/ HUNDRED Tm OF / 90 MIN
GLUCOSE
GLYCOGEN

FIG. 7-1

Fig. 7-2. Effect of ACTH alone or in combination with insulin or acetylcholine (ACh) on acetylcholinesterase (AChE) and Na^{+}-K^{+} ATPase activities in pigeon liver slices under <u>in vitro</u> conditions.

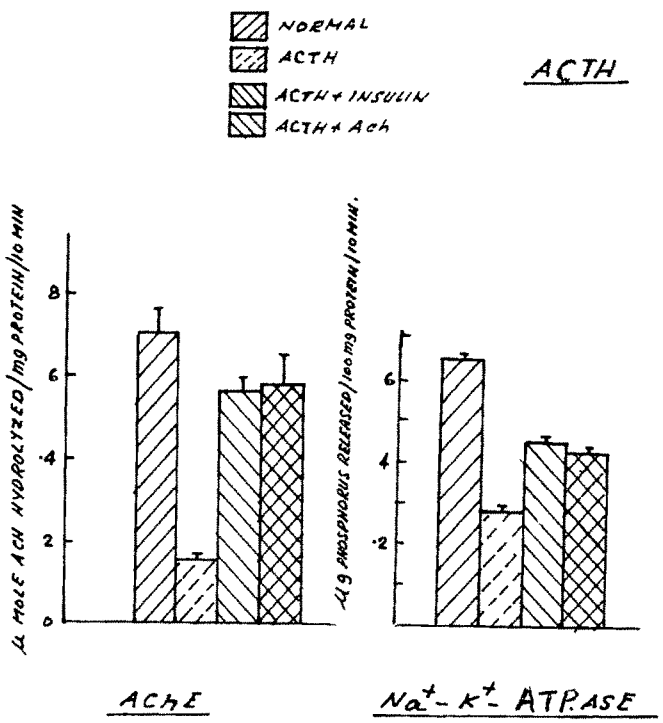
NORMAL
ACTH
ACTH + INSULIN
ACTH + Ach
ACTH
μ MOLE ACh HYDROLYZED / mg PROTEIN / 10 MIN
0
2
4
6
8
μg PHOSPHORUS RELEASED / 100 mg PROTEIN / 10 MIN.
2
4
6
AChE
Na+ - K+ ATPASE

FIG. 7-2

Fig. 7-3. Effect of ACTH alone or in combination with insulin or acetylcholine (ACh) on acid phosphatase (Ac Pase) and alkaline phosphatase (Alk Pase) activities in pigeon liver slices under *in vitro* conditions.

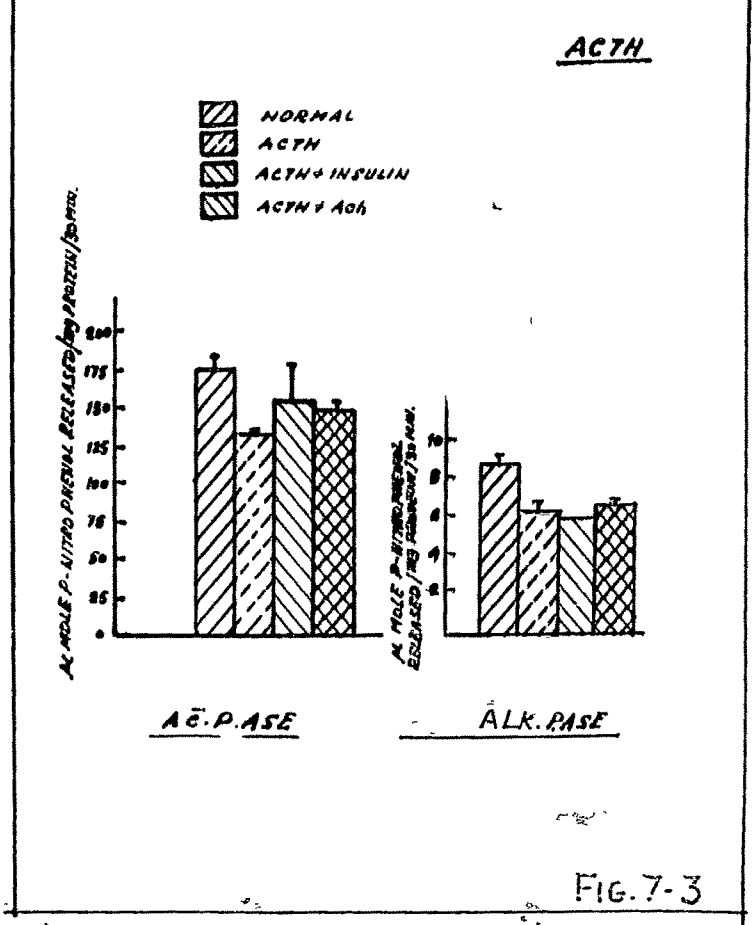
ACTH
NORMAL
ACTH
ACTH + INSULIN
ACTH + Ach
AC. PHOSPHATASE
ALK. PHOSPHATASE

FIG. 7-3

Fig. 7-4. Effect of ACTH alone or in combination with insulin or acetylcholine (ACh) on lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) activities in pigeon liver slices under *in vitro* conditions.

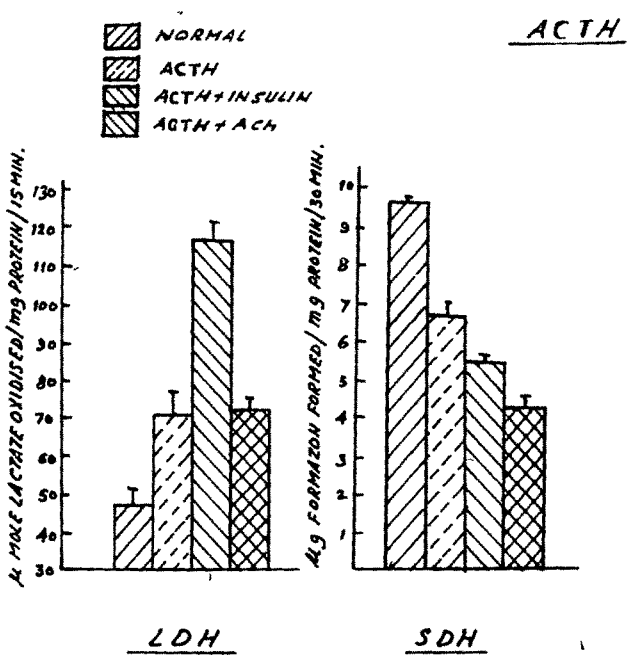
NORMAL
ACTH
ACTH + INSULIN
ACTH + ACh
ACTH
μ MOLE LACTATE OXIDISED/mg PROTEIN/15 MIN.
130
120
110
100
90
80
70
60
50
40
30
μg FORMAZON FORMED/mg PROTEIN/30 MIN.
10
9
8
7
6
5
4
3
2
1
LDH
SDH

FIG. 7-4

Table 7-1

Effect of adrenocorticotrophic hormone (ACTH), alone or in combination with insulin or acetylcholine, on the uptake or release of glucose by pigeon liver slices under *in vitro* conditions. (Mean ± S.E.M)

Additives	Glucose		Glycogen Depletion (2)
	Uptake (1)	Release (1)	
ACTH	0.5424 ±0.1305	-	2.1873 ±0.0198
ACTH + Insulin	1.0995 ** ±0.0828	-	1.8587 ±0.0128
ACTH + ACh	0.6003 NS ±0.1505	-	1.2782 ±0.0813

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

(2) Mg glycogen depletion/100 mg liver.

** $P < 0.02$ NS - Not significant

Table 7.2

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

Enzymes	Control(1) (Tissue)	ACTH	ACTH+ Insulin	ACTH + ACh
Na^+-K^+-ATPase µg phosphorus released/mg protein/ 10 minutes	26.2644 ± 0.5685	11.1848**** ± 0.5300	18.1384*** ± 0.2319	17.6862*** ± 0.1569
AChE µm ACh hydrolysed/mg protein/ 10 minutes	0.7027 ±0.0624	0.1575** ±0.0130	0.5718 NS ±0.1063	0.5891 NS ±0.0734
Acid-Phosphatase. µm P-nitrophenol released/100 mg protein/ 30 minutes	176.29 ± 12.57	133.36 NS ± 5.02	163.65 NS ± 14.79	144.46 NS ± 14.38
Alkaline-Phosphatase µm P-mitrophenol released/100 mg protein/30 minutes	8.9190 ±0.2567	6.4381 NS ±0.04493	6.0820 NS ±0.2953	6.7224 NS ±0.1355
LDH µm lactate oxidised/mg protein/ 15 minutes	47.8737 ± 3.7868	71.0536 NS ± 6.7559	116.2609* ± 5.4060	72.4653 NS ± 2.9369
SDH µg formazan released/mg protein/10 minutes	9.6846 ±0.1742	6.6046** ±0.4364	5.4115*** ±0.1903	4.2017*** ±0.3171

NS - Not significant * $P < 0.05$ ** $P < 0.02$

*** $P < 0.01$ **** $P < 0.001$

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

Glycogen content of liver slices showed a decrease as in other experiments which could at best be considered a non-enzymatic reduction.

DISCUSSION

All the trophic hormones exert their effect on basic cellular processes which in turn subserve the specific function of the cell in which they are acting. Actually the ACTH receptors are present on the adrenals which are the natural target organ. In the present study liver is the experimental tissue which is not the target site of ACTH action. But extra target-organ actions can be expected to occur when sufficient concentration of trophic hormones is present which exert the same or similar effects on basic cellular processes there. Many reports have indicated that ACTH has direct action on several tissues independent of its actions via adrenal cortical hormones. Hypoglycaemic action is one of them. Corticotrophin administration under *in vivo* conditions increased plasma level of insulin (Genuth *et al.*, 1965) and it could be this reason that was mainly responsible for the hypoglycaemic action of ACTH in *in vivo* experiments. However, ACTH also increased the uptake of glucose by liver slices in *in vitro* condition. Hence, it is reasonable to surmise that ACTH has some direct action on the liver slices. The uptake of glucose doubled when both ACTH and insulin were present in the medium indicating that either ACTH had acted synergistically or had facilitated the action of insulin on the cells. This increase on

glucose uptake was seen in spite of the fact that activity of acid phosphatase did not register any change from that which was found in the liver slices before incubation. This action of ACTH on glucose uptake could not be through any effect on membrane permeability, because corticotrophin failed to increase the glucose uptake in the presence of ACh any further than what was observed when it was present alone in the medium. Since the action of ACTH on glucose uptake was additive in the presence of insulin it is possible to believe that the action of ACTH was very similar to what was induced by insulin. Probably the action of ACTH may be through its action on calcium movement into the cell or its release from the bound state.

The decreased SDH activity and the increased LDH activity in the liver slices in presence of ACTH either alone or in combination with insulin or ACh indicate a general reduction in aerobic metabolism while anaerobic reactions are activated. Increased anaerobic glycolysis could also reduce the G-6-P concentration in liver which could lead to a chemiosmotic pull of glucose into the cells.

In other words, the hypoglycaemic action of ACTH is either due to its ability to mimic to a certain extent the action of insulin or due to the increased anaerobic glycolysis and the resulting 'chemiosmotic pull' of glucose into the cells.