CHAPTER 6

EFFECT OF GLUCAGON ON TRANSPORT OF GLUCOSE ACROSS HEPATIC CELL MEMBRANE AND ON HEPATIC ENZYMES IN THE PRESENCE OF INSULIN OR ACETYLCHOLINE

Glucagon is the major carbohydrate regulator in aves while insulin plays only a supportive role (Sitbon and Mialhe, 1980). In birds the number of glucagon producing cells in the islets is much more than those producing insulin. The plasma concentration of glucagon like wise is more than that of insulin. Moreover, the insulin sensitive membrane components involved in glucose transport are very sparsely distributed in the avian liver. The glucose stimulated insulin release is also very sluggish in birds (Hazelwood, 1977). Due to these reasons glucagon assumes an overall importance in the regulation of carbohydrate metabolism in birds. The release or uptake of glucose by liver cells is a function of glucagon/insulin ratio in the plasma. In birds ACh released by Vagal cholinergic nerves also stimulates glucose uptake by liver cells (Pilo and Patel, 1978). In *in vivo* conditions, no neural or hormonal factors work in isolation; each works synergistically or antagonistically to one another. To understand the action of glucagon in the glucose movement in and out of avian liver cells in the presence of insulin or ACh, the present investigation was planned.

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. The liver was perfused with cold Krebs Ringer Medium (KRB) and then quickly excised. The liver was placed on ice and cut into slices of 100-120 mg weight and were placed in 10 ml flask with 5 ml of KRB medium. The liver slices were incubated for 30 min. at 37°C in a water bath shaker. The slices were incubated in media of following different categories.

(1) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Glucagon (1 ngm/ml).

(2) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Glucagon (1 ngm/ml) + Insulin (1 unit/ml).

(3) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Glucagon (1 ngm/ml) + ACh (15 mg/ml).

The slices, before and after incubation were quickly washed with chilled KRB buffer and homogenised in redistilled water and homogenate was used for assaying enzymes, protein and glycogen as per the methods described in Chapter 1. In order to assess the transport of glucose across the hepatic cell membrane, glucose content of the medium prior to and after incubation was estimated as per the method described in Chapter 1.

RESULTS

The data on the effect of glucagon alone or in combination with insulin or acetylcholine on glucose uptake by liver slices, glycogen content, and enzyme activities in the liver slices are presented in Tables 6-1 & 6-2 and Figs. 6-1 to 6-4 (Fig. 6-1, glucose uptake and glycogen content; Fig. 6-2, Na^+ K^+ ATPase and AChE; Fig. 6-3, Acid pase and alkaline pase; Fig. 6-4, LDH and SDH).

When the medium contained only glucagon, the liver slices released glucose into the medium. However, when insulin or ACh was present along with glucagon, the liver slices took up glucose from the medium. Phosphorylase activity in the slices did not show any significant increase irrespective of the fact that glucagon was present in the medium alone or in combination with insulin or ACh. On the other hand SDH activity was significantly increased in the media containing glucagon only and glucagon and insulin with no change in the medium containing glucagon and ACh. LDH activity also did not show any significant change in any of the media. AChE exhibited a significant decrease in the media containing glucagon and glucagon and insulin, but the activity showed an increase in the medium containing glucagon and ACh. Acid phosphatase activity in the slices did not show much variation while alkaline phosphatase activity showed significant increase in all the three media. ATPase activity showed significant increase in slices incubated with glucagon or glucagon + ACh, but when the medium contained glucagon + insulin, the enzyme activity did not show any change.

Fig. 6-1. Effect of glucagon, alone or in combination with insulin or acetylcholine (ACh) on glucose uptake, and glycogen content in pigeon liver slices under *in vitro* conditions.

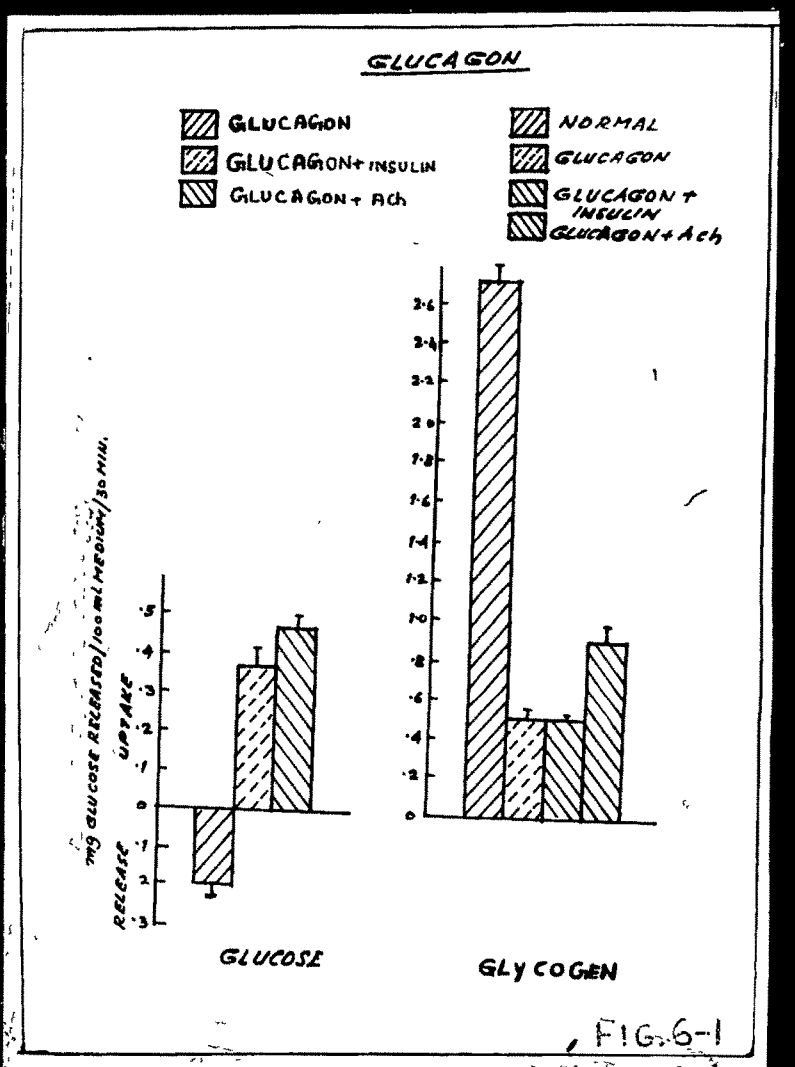
GLUCAGON
GLUCAGON
GLUCAGON + INSULIN
GLUCAGON + Ach
NORMAL
GLUCAGON
GLUCAGON + INSULIN
GLUCAGON + Ach
mg GLUCOSE RELEASED/100 ml MEDIUM/30 MIN.
UPTAKE
RELEASE
GLUCOSE
GLYCOGEN

FIG. 6-1

Fig. 6-2. Effect of glucagon, 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.

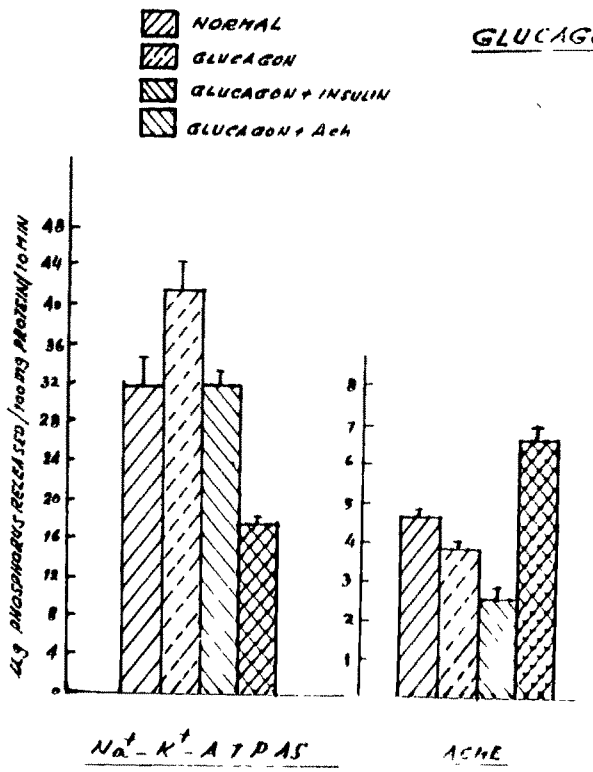
NORMAL
GLUCAGON
GLUCAGON + INSULIN
GLUCAGON + Ach
GLUCAGON
µg PHOSPHORUS RELEASED/100 mg PROTEIN/10 MIN
48
44
40
36
32
28
24
20
16
12
8
4
0
8
7
6
5
4
3
2
1
Na⁺-K⁺-ATPAS
ACHE

FIG. 6-2

Fig. 6-3. Effect of glucagon, 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 *in vitro* conditions.

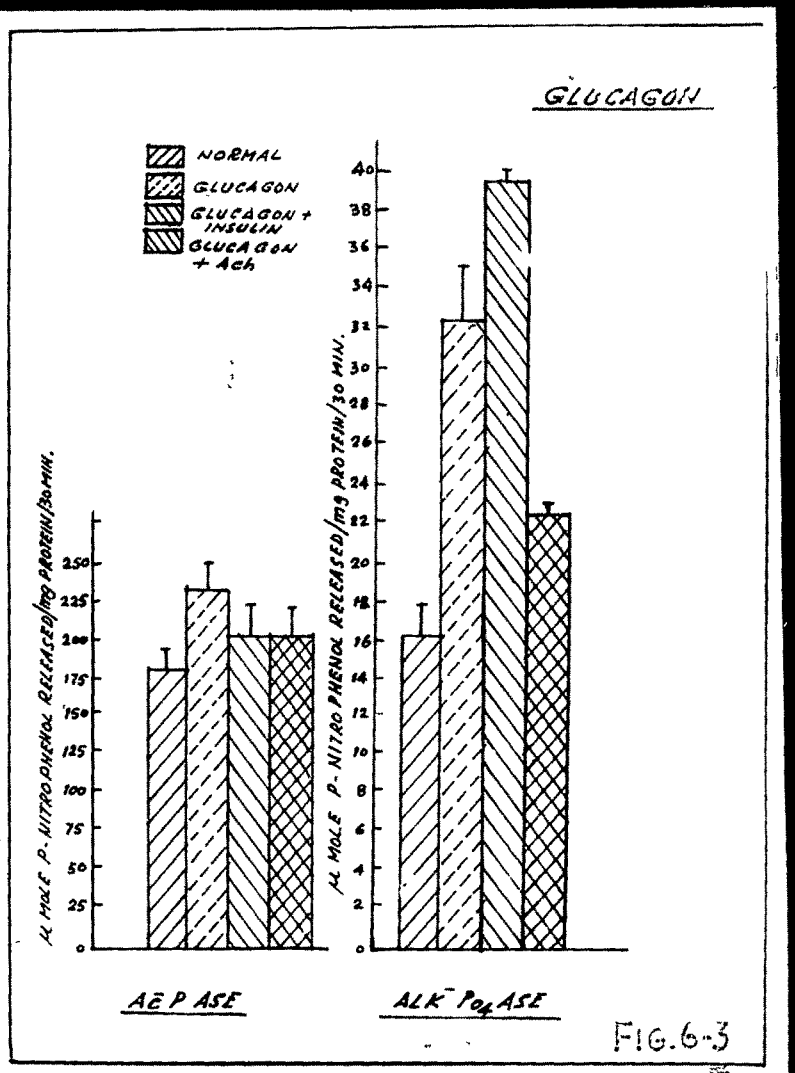
GLUCAGON
NORMAL
GLUCAGON
GLUCAGON + INSULIN
GLUCAGON + Ach
μ MOLE P-NITRO PHENOL RELEASED/mg PROTEIN/30 MIN.
0
25
50
75
100
125
150
175
200
225
250
μ MOLE P-NITRO PHENOL RELEASED/mg PROTEIN/30 MIN.
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
ACPASE
ALK Po4ASE

FIG. 6-3

Fig. 6-4. Effect of glucagon alone or in combination with insulin or acetylcholine (ACh) on lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) in pigeon liver slices under *in vitro* conditions.

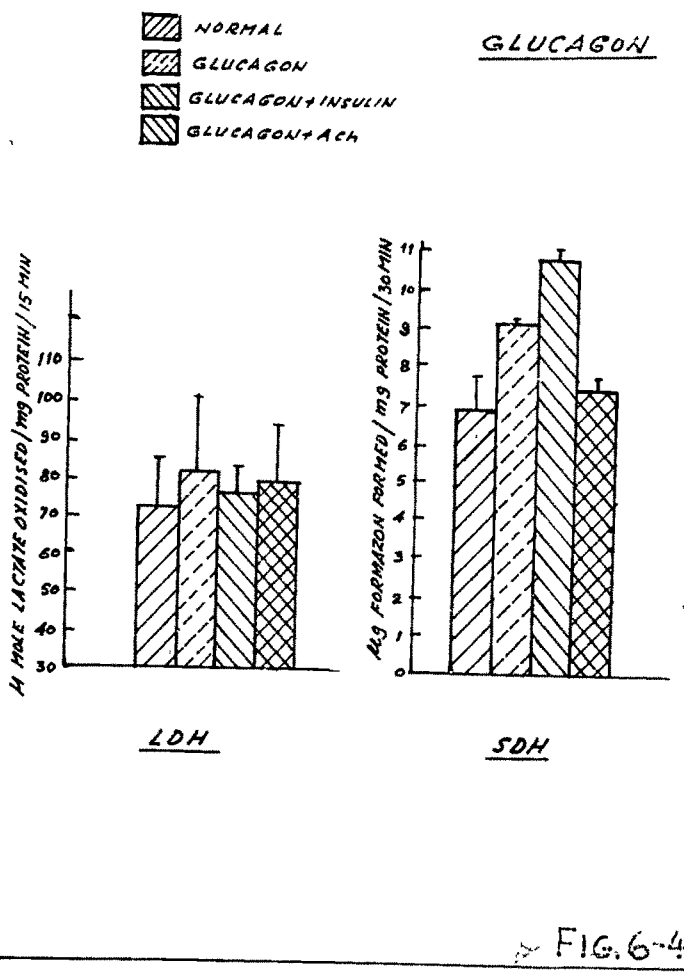
NORMAL
GLUCAGON
GLUCAGON+INSULIN
GLUCAGON+ACh
GLUCAGON
μ MOLE LACTATE OXIDISED/mg PROTEIN/15 MIN
110
100
90
80
70
60
50
40
30
LDH
μg FORMAZON FORMED/mg PROTEIN/30 MIN
11
10
9
8
7
6
5
4
3
2
1
0
SDH

FIG. 6-4

Table 6-1

Effect of glucagon, alone or in combination with insulin or acetycholine 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)	
Glucagon		0.2071 ±0.01802	2.1641 ***
Glucagon + Insulin	0.3703 ±0.0561		2.1858 ***
Glucagon + ACh	0.4746 ±0.0372		1.7719 ***

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

(2) Mg glycogen depletion/100 mg liver.

*** $P < 0.01$

Table 6-2

Effect of glucagon, 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)	Glucagon	Glucagon +Insulin	Glucagon + ACh
Na^+-K^+-ATPase µg phosphorus released/mg protein/ 10 minutes	30.3653 ± 3.1128	41.2343* ± 2.7259	30.7626 NS ± 2.8166	17.4780*** ± 1.2234
AChE µm ACh hydrolysed/mg protein/ 10 minutes	0.4720 ±0.0137	0.3914*** ±0.0163	0.2691**** ±0.0253	0.6681*** ±0.0380
Acid Phosphatase µm P-nitrophenol released/100 mg protein/30 minutes	187.0016 ± 9.6715	240.3283 NS ± 16.8026	205.3766 NS ± 22.4973	205.0250 NS ± 19.7662
Alkaline Phosphatase µm P-nitrophenol released/100 mg protein/30 minutes	16.6000 ± 1.2303	32.6304*** ± 3.8781	41.9748**** ± 4.0559	22.3648* ± 1.6121
LDH µm lactate oxidised/mg protein/ 15 minutes	73.7229 ±12.9798	82.7687 NS ±17.3740	76.9828 NS ± 7.2668	79.4886 NS ±14.6079
SDH µg formozon formed/ mg protein/30 minutes	6.8323 ±0.9922	9.0875*** ±0.1877	10.7954**** ± 0.3269	7.3400 NS ±0.4724
Phosphorylase µg phosphorus released/mg protein/10 minutes	223.5669 ± 21.9631	267.9228* ± 21.2441	229.3578 NS ± 16.2859	247.3627 NS ± 20.4793

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

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

**** $P < 0.001$.

The incubation period was restricted to 30 minutes as glucagon is known to be inactivated beyond that time.

DISCUSSION

Glucagon has specific binding sites in many cells and the glucagon-receptor complex activates the adenylate cyclase enzyme present in the plasma membrane (Sutherland et al., 1962; Davoren et al., 1963; Sutherland, 1972) with consequent rise in intracellular cAMP concentration. cAMP in turn can trigger a series of reactions leading to an increase in phosphorylase activity which ultimately brings about glycogenolysis and release of glucose from liver cells. This characteristic action of glucagon was readily seen in the in vitro studies with liver slices. Glucagon alone in the medium effected a glucose release from the slices. This action of glucagon was countered by both insulin and ACh. Since phosphorylase showed a significant but slight increase in the medium containing only glucagon it could be reasoned that the action of glucagon was mediated through cAMP and insulin or ACh when present along with glucagon had decreased the cAMP concentration. At the same time the alkaline phosphatase activity was found to be high irrespective of the fact that insulin or ACh was present in the medium along with glucagon. This and the fact that acid phosphtase did not show any variation, were mainly responsible for preventing insulin from inducing a glucose uptake as much as it usually did (See Chapter 4) when insulin was present alone in the medium. However, one fact that becomes clear is that insulin

and ACh both could counteract to a certain extent the action of glucagon on glucose release. This effect of insulin on liver to counteract the action of glucagon (Exton et al.,1870) was especially on the formation of cAMP (Jefferson et al.,1968), inactivation of pyruvate kinase (Blair et al., 1976; Feliu et al., 1976) and, on phosphofructokinase (Castano et al.,1979). It is difficult to deduce whether ACh counteracts the action of glucagon in the same manner as insulin does. The fact that both insulin and ACh prevented glucagon from increasing the activity of phosphorylase, points to a similarity of their action against glucagon, especially the action on cAMP. However, the increased AChE activity and decreased ATPase activity observed in liver slices incubated in medium containing both glucagon and ACh may point to a dissimiliarity of action of insulin and ACh in certain other respects.