CHAPTER 2

IONIC INDUCTION OF GLUCOSE TRANSPORT ACROSS MEMBRANE OF LIVER CELLS OF PIGEON UNDER IN VITRO CONDITIONS

The importance of active transport processes in biological systems is now well established. Since past few years number of review articles dealing in whole or in part with transport system have been published (Heinz et al.,1972; Lanyi, 1979; West, 1980). One of the most prominent active transports is the one that is concerned with glucose.

The movement of glucose across cell membrane in general takes place through (1) facilitated diffusion (metabolic removal) (2) chemiosmotic transport (Phosphorylation) and (3) Carrier-mediated transport (Tower, 1962).

The type of glucose transport may vary according to the tissue as well as the physiological state of the cells and concentration gradient differences between extra and intra cellular fluid. Facilitated transport which takes place along a concentration gradient is carrier mediated and insulin insensitive and is found to take place mostly in brain. In liver and muscle there is also a chemiosmotic transport of glucose, induced by insulin against a concentration gradient involving phosphorylation and metabolic removal. The carrier mediated transport which is also against a concentration gradient is

found to take place in erythrocytes, intestinal mucosa, kidney and liver. Erythrocytes and intestinal mucosa require the presence of sodium ions for glucose transport. In ghost cells, the maximum number of receptors for glucose is of the order of 50,000/ghost, a figure of the same general order as has been suggested for ion transport sites (Le Fevre, 1961).

The presence of sodium pump in almost all animal cells has been demonstrated by the observation of either ouabain inhibitable sodium or potassium fluxes between cells and extra cellular media or by the measurement of ouabain inhibitable ATPase activity associated with membrane preparations from the cells (Kaplan, 1983). Under physiological conditions, the predominant but not exclusive transport activity performed by the sodium pump, is the exchange of intra-cellular sodium for extra-cellular potassium with the concomitant hydrolysis of ATP. Both of these movements are against their electro-chemical potential gradients and thus a large portion of the cell energetic output is utilized in maintaining the dis-equilibrium between intra-cellular and extra-cellular Na^+ and K^+ ions. In most systems that have been studied the stoichiometry of the transport is that 3 sodium ions get expelled per 2 K^+ ions taken up for each mole of ATP hydrolyzed, and in the few cases where it has been examined the sodium pump has been shown to be electrogenic (Thomas, 1972; Hoffman et al., 1979). In cultured fibroblasts, the number of glucose transporters increases

when the concentration of glucose in the growth medium is decreased (Martineau et al., 1972). When cultured fibroblasts are transformed, there is a marked increase in the number of glucose transporters in the plasma membrane (Salter and Weber, 1979). It has been shown that number of sodium dependent glucose transporters increases when the cells are grown in medium with a low concentration of glucose and decreases when the glucose concentration in the medium is elevated.

The transepithelial movement of glucose in the small intestine and renal proximal tubule occurs against a concentration gradient. The active step occurs at the luminal side of the cells where glucose entry is coupled to the movement of sodium down its elecrochemical gradient via a specialized carrier localized to the apical plasma membrane. Glucose leaves the epithelial cell by moving down its own chemical gradient on a carrier of the phloretin and cytochalasin B-sensitive type that is localized to the basal plasma membrane. The sodium coupled glucose carrier in the apical plasma membrane has specificity properties that are markedly different from the glucose carrier in the basal membrane (Silverman, 1976). The liver being an endodermal derivative, presence of a sodium dependent carrier mediated glucose transport system as is observed in the apical membrane of the intestine, is easy to understand. In the liver, as in many other tissues the mechanism of sodium dependent transport of glucose involves membrane permeability changes, ionic movements, activation of ionic pump and Na^+-K^+-

ATPase, and ATP utilization. Essentially this glucose transport mechanism is coupled to the transport of ions. Hence factors that alter the permeability of plasma membrane where by Na^+ ions move into the cells should also activate the sodium pump and the carrier molecule involved in the extrusion of Na^+ from cell interior in the exchange of K^+ from extracellular compartment will also take up glucose from extracellular fluid. The glucose transport being coupled with ionic transport, it is possible that other factors which bring about ionic movement should also induce glucose uptake by liver.

To understand the effect of ions on glucose transport by liver cells, liver slices were incubated in Krebs Riger Bicarbonate (KRB) buffer with hypertonic cations.

MATERIALS AND METHODS

Adult pigeons (Columba livia), weighing 180-250 grams, maintained in laboratory conditions on balanced diet, were used for the present experiment. Animals were sacrificed after an overnight starvation. The liver was perfused with cold KRB buffer and then quickly excised. The liver was placed on ice and cut into thick slices weighing 40-50 mg and weighed liver slices were incubated in 10 ml flask with 5 ml of KRB buffer, containing glucose and albumin. The liver slices were incubated for 90 min. at 37°C in a water batn shaker with 120 oscillations/min. The slices were incubated in media of following different

categories.

(1) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + No Additives.

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

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

(4) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + $CaCl_2$ (5 mg/ml).

(5) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + NaCl (5 mg/ml) + KCl (5 mg/ml).

(6) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + KCl (5 mg/ml) + $CaCl_2$ (5 mg/ml).

(7) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + NaCl (5 mg/ml) + $CaCl_2$ (5 mg/ml).

After the incubation the slices were quickly washed with chilled KRB Medium and were digested in KOH for glycogen estimation. The glycogen was estimated in slices removed from liver as well as in slices after incubation. Glycogen was estimated by Anthrone method of Seifter et al., (1950). The glucose concentration was determined in the medium before and after incubation and the differences were calculated. Glucose was estimated by Folin and Malmros (1929) micro method.

RESULTS

The data on glucose uptake or release by liver slices in the presence of ions are presented in Table 2.1. and (Figs. 2-1 & 2-2.).

When liver slices were incubated with only glucose as additive in the medium, no uptake of glucose by the slices was observed, instead a release took place. When the medium contained Na^+ (hypertonic) glucose uptake was found to take place. With hypertonic concentration of K^+ in the medium, the liver slices did not show any uptake of glucose, instead a release of glucose from the slices into the medium was observed. Similarly with Ca^{++} also, the glucose was released into the medium. When the incubation medium contained both Na^+ and K^+ no glucose uptake was observed, but when the medium contained Na^+ and Ca^{++} or K^+ and Ca^{++}, the liver slices showed significant glucose uptake. Glycogen content of liver slices after incubation was seen decreasing in all sets of media and the uniform reduction indicated that a non-enzymic breakdown of glycogen was obviously the cause.

DISCUSSION

Several studies have established the importance of the cationic composition of the incubation medium for glycogen and total carbohydrate synthesis by liver slices *in vitro*. (Buchanan *et al.*, 1949). In the present investigation it has been noticed that glucose transport that took place depended on the movement of sodium into the cells. Sodium ions can move into the cell if

Fig. 2-1. Effect of cations on glucose uptake by pigeon liver slices under *in vitro* conditions.

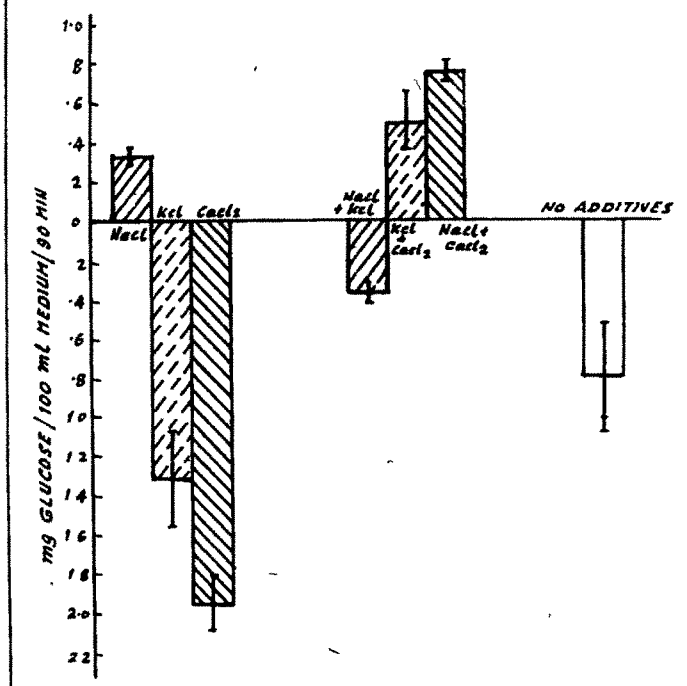
mg GLUCOSE/100 ml MEDIUM/90 MIN
1·0
·8
·6
·4
·2
0
·2
·4
·6
·8
1·0
1·2
1·4
1·6
1·8
2·0
2·2
NaCl
KCl
CaCl2
NaCl + KCl
KCl + CaCl2
NaCl + CaCl2
NO ADDITIVES

FIG. 2-1

Fig. 2-2. Effect of cations on glycogen content in pigeon liver slices under *in vitro* conditions.

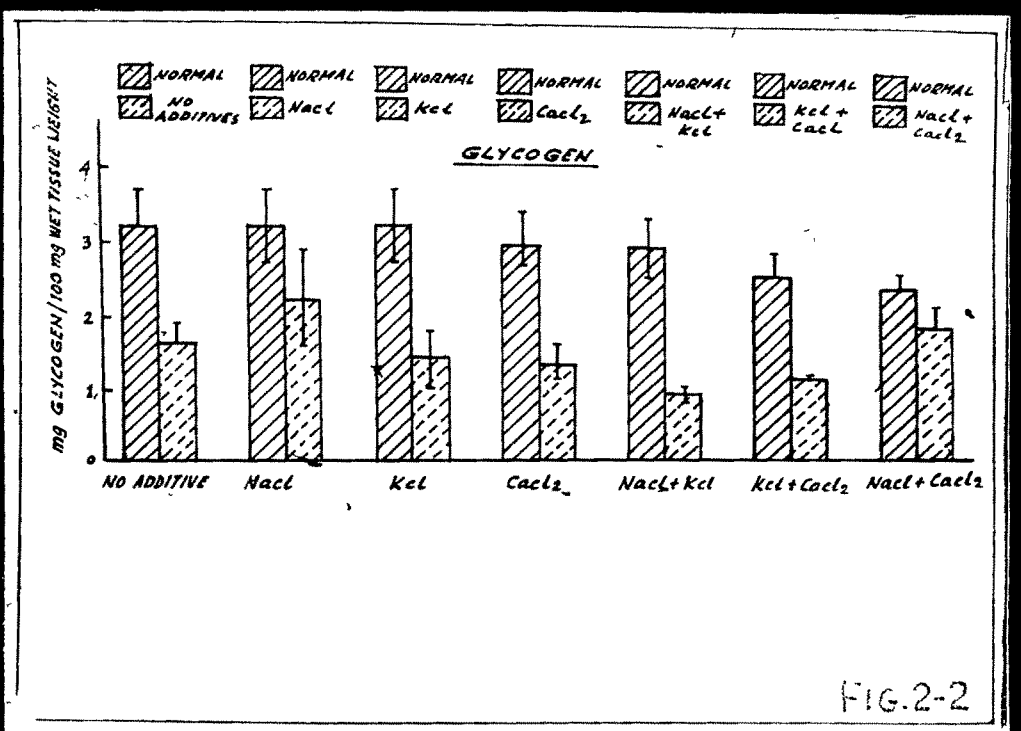
NORMAL
NO ADDITIVES
NORMAL
Nacl
NORMAL
Kcl
NORMAL
Cacl2
NORMAL
Nacl+ Kcl
NORMAL
Kcl + Cacl
NORMAL
Nacl + Cacl2
GLYCOGEN
mg GLYCOGEN/100 mg WET TISSUE WEIGHT
0
1
2
3
4
NO ADDITIVE
Nacl
Kcl
Cacl2
Nacl+Kcl
Kcl+Cacl2
Nacl+Cacl2

FIG.2-2

Table 2-1

In vitro effect of ions on the uptake or release of glucose by pigeon liver slices (Mean ± SEM).

Additives	Glucose		Glycogen Depletion (2)
	Uptake *(1)	Release *(1)	
None	-	0.8045 ±0.2840	1.5560 ±0.2601
NaCl	0.3411 ±0.0404		1.0080 ±0.0665
KCl		1.3175 ±0.2395	1.8120 ±0.1752
$CaCl_2$		1.9550 ±0.1405	1.5940 ±0.3019
NaCl + KCl		0.3646 ±0.0520	1.9560 ±0.3384
KCl + $CaCl_2$	0.5179 ±0.1536		1.4464 ±0.2634
NaCl + $CaCl_2$	0.7628 ±0.0533		0.7634 ±0.1060

*(1) Difference between the glucose concentration in the medium before and after the incubation. Mg glucose/100 mg liver.

(2) Difference between glycogen values of fresh liver slices and liver slices after incubation. Mg glycogen/100 mg liver.

the extra cellular concentration of Na^+ is in hypertonic range or when membrane permeability is altered. In either way, when sodium entered the cells, it activated sodium pump. This fact is experimentally well established in the small intestine which possesses an energy dependent transport process by means of which glucose and certain other sugars can be absorbed against an apparent concentration gradient. This phenomenon has been demonstrated in vivo (Campbell and Davson, 1948) during absorption of sugar from the lumen of intestine segments into the blood stream and in vitro during transfer of sugar from the mucosal to the serosal surface of preparations of intestinal tissues (Darlington and Quastel, 1953). The in vitro techniques which have been used have made it possible to study some properties of the process of active transport such as its response to cationic environment of the medium (Riklis and Quastel, 1958) and its specificity with respect to the configuration of sugars presented for absorption (Crane and Crane, 1956; Wilson and Crane, 1958; Crane and Crane, 1959) and its rate with respect to their concentration (Riklis and Quastel, 1958). Active transport of hexoses by segments of rat deodenum in vitro is accompanied by increase in the electrical potential gradient across the gut uall. The active transport rate, steady state tissue levels of sugar and the exchange rate at the steady state all depend on Na^+, and the rate increases almost linearly with Na^+ concentration. Anaerobic entrance of ordinarily actively transported sugar is also Na^+ dependent and the anaerobic

exchange rate at equilibrium increases with the medium Na^+ level. In the absence of Na^+, the entrance rate of these sugars drops aerobically and anaerobically, to a slow Na^+-independent entrance rate of sugars which are not actively transported. In the present investigation it is noted that Na^+ in the medium did stimulate glucose uptake whereas when Na^+ was replaced by K^+ or Ca^{++} the glucose uptake was inhibited. Na^+ in the medium activates Na^+ pump, as a result, Na^+ and K^+ ions move in and out of the cell respectively. When extrusion of Na^+ and reuptake of K^+ were taking place, glucose could move into the cell. The carrier couples with K^+ and glucose and transfers them towards the inner side of the membrane. Since it implies the existence of a carrier that combines with both ions and glucose, the transport is called Carrier Mediated Flow Coupled Transport. When glucose serves as a substrate for active transport, it is also utilized by the cell and energy generated by its utilization may provide the necessary driving force. However, when non-metabolizable compounds are accumulated, energy is probably derived from another source. Some evidences suggest that a membrane bound Na^+-K^+ activated ATPase is involved in active Na^+,K^+ transport in animal cells. There are reports which show that the association of phosphomonoesterase activity with Na^+,K^+ activated ATPase in membrane of cells, [illegible] activates Na^+,K^+ transport (Tosteson et al., 1961). Evidences are also at hand to show that ATPase in broken human erythrocyte membrane is a part of the system for the active transport of Na^+ and K^+ (Post et al.,

1965). The extrusion of Na^+ and accumulation of K^+ proceed by active transport across the cell membrane. The energy comes from glycolysis, almost certainly by way of high energy phosphate bonds of ATP. In the present experiment it was noted that when K^+ or Ca^{++} was in the medium, glucose uptake was inhibited, instead a release was observed. Early observations too have shown an inhibition of Na^+-dependent ATPase activity by K^+ ions in the presence of low (micromolar) levels of ATP. And it was demonstrated that this was due to the rate limiting release of K^+ from dephosphoenzyme (Post et al., 1965). Na^+ gradient hypothesis is put forward by Crane (1962) to explain the active transport of sugars. According to this scheme it is visualized that both Na^+ ion and sugar molecule bind to a common carrier to form a complex. This complex can be formed at either interface (exterior or cytoplasmic surface), with resulting translocation of cation and sugar in either direction. When sodium is replaced by K^+, uptake is inhibited. When Ca^{++} was added to the medium in combination of Na^+ or K^+, glucose uptake was stimulated. In other words Ca^{++} in the presence of hypertonic cations could induce permeability changes on the plasma membrane and thereby stimulate the glucose uptake. Ca^{++} ions can affect several intracellular mechanisms depending upon the concentration difference between extracellular and intracellular compartments, the manner in which Ca^{++} moves in and out of the cell, whether Ca^{++} binds to receptor (Calmodulin) or not and the cellular characteristic (Rasmussen and Wassman, 1981). Ca^{++} alone did not stimulate glucose uptake but only in the presence of Na^+

and K^+. It can also be said that when Ca^{++} was taken along with Na^+, Na^+ stimulates the Na^+ pump thereby activating membrane bound enzymes like Na^+-K^+ ATPase and alkaline phosphatase, the helping in the transport mechanism or, by elevating cGMP or decreasing cAMP concentrations in the hepatic cells. It is unlikely that Ca-Calmodulin complex is involved in the glucose uptake. Probably, Ca-Calmodulin system may be active in glucagon mediated glucose release.