SOME IN VITRO STUDIES OF CHANGES IN SHORT-CIRCUIT CURRENT OF THE RAT SMALL INTESTINE INDUCED BY SUGARS

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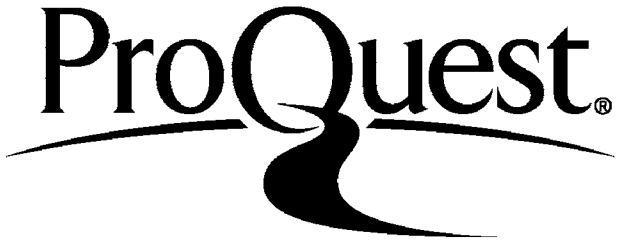

ABSTRACT

The concepts of intestinal sugar transport are almost continuously changing and the detailed mechanisms are not yet fully understood. An attempt has been made to obtain some further information from the study of changes in the short-circuit current measured by a modified technique introduced for the everted intestinal segments of the rat.

Different concentrations of a variety of sugars, some actively transportable, some non-actively transportable and some disaccharides have been tested at 37^{o}C. Glucose and galactose were tested at different temperatures.

The sugar derivatives, ethylidene glucose and benzylidene glucose were found to inhibit competitively the active transport mechanism. Similar inhibition was observed with mannose and lactose. These four compounds form a group of non-transportable sugar inhibitors.

Kinetic analysis was made from the above studies and the Kis and the ΔI maxs were determined for most of the tested sugars including the non-transportable ones.

In the presence of a metabolized sugar (glucose) the intestinal segment maintained its viability over a period of about three hours without a marked effect of the duration. Addition of citrate as a source of metabolic energy improved the level of short-circuit current in the presence and absence of galactose. Anoxic conditions were more effective in depressing the short-circuit current levels in the presence of galactose than in the presence of glucose.

The short-circuit current steady state, once it was established, remained stable throughout the experiment and in the presence of 3-O-methylglucose was approximately equal to the net Na^+ flux from mucosa to serosa.

Approximately al : 1 stoichiometric relationship between Na^+ (ionic-current) and 3-O-methylglucose was observed within a certain range of sugar concentration.

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ABBREVIATIONS

I_{sc}	short-circuit current
ΔI max	maximal change of short-circuit current
Ki	apparent half saturation constant
PD	potential difference
Em	mucosal potential
3MG	3-O-methyl-α-D-glucopyranose
αMG	methyl-α-D-glucopyranoside
βMG	methyl-β-D-glucopyranoside
βPG	phenyl-β-D-glucopyranoside
EG	4,6-O-ethylidene-α-D-glucopyranose
BG	4,6-O-benzylidene-α-D-glucopyranose
IAA	iodo-acetic acid
ATP	adinosinetri-phosphate
ATPase	adinosinetri-phosphatase
ϕNa_{sm}	serosal to mucosal Na^+ flux (efflux)
ϕNa^+_{ms}	mucosal to serosal Na^+ flux (influx)
EA	apparent activation energy
SE	standard error
conc.	concentration
Fig.	figure
min.	minute
cts	counts

CHAPTER 1

INTRODUCTION

I. Hexose Transport Across the Intestinal Membrane

I.a Absorption against the concentration gradient.

The in vivo phenomena demonstrated by Barany and Sperber (1939) showed that the concentration of glucose in a solution placed in an isolated loop of rabbit intestine in situ progressively decreased with time to levels far below the concentration of the blood. Such a result gave an indication that the process under study involved membrane active transport - by definition a net movement of substance across a membranous barrier against the existing electrochemical gradient (Rosenberg, 1954). It seems that in vitro preparations establish similar concentration gradients with certain sugars (Crane, 1968), but fructose among other sugars is absorbed by a different mechanism (Lindquist et al, 1962), which is nonconcentrating (Crane, 1960 b).

Skala et al (1963) have described a large effect of the composition of the bathing medium on in vitro glucose transfer by everted sacs of rat intestine, and they have shown that this effect results from large differences in glucose utilization in the several media. It is known (Wilson, 1956 b) that at least 75% of the glucose entering the mucosal cells of the isolated rat intestine is utilized.

I.b Basis for sugar accumulation against a concentration gradient.

With only few observations (Riklis & Quastel, 1958, Clarkson & Rothstein, 1960, Csaky 1960, Csaky & Thale, 1960) regarding Na^+ dependence of sugar transport at hand, the first suggestion of a mechanistic role for Na^+ in the transport process appeared in 1960

in a review by Crane. In that article, Crane summarised some evidence against previously proposed mechanisms involving phosphorylation (Verzar & McDougall, 1936), mutarotation (Keston, 1954), --- etc., turned to the newly described Na^+ dependence as a possible clue to the mechanistic basis of active sugar transport. However his ideas were both reinforced and modified the following year. At the Prague Symposium on Membrane Transport and Metabolism, Crane, Miller and Bihler (1961) suggested that chemical reactions do not account for the specificity and that "perhaps there is no reaction of any kind beyond the formation of transient complex with membrane carriers". To accommodate the information that they obtained from their experiments with strips of hamster intestine they postulated that a Na^+ - sugar - carrier ternary complex traverses the "diffusion barrier" to the cell interior where dissociation occurs and the Na^+ can then be extruded by an energy-dependent process operating at adjacent membrane sites. Sugar would be effectively trapped by this depletion of cellular Na^+ which would limit efflux via the ternary complex. No provision for anion entry was included in the schematic representation of the ion gradient hypothesis. Furthermore they reported that in order to maintain the asymmetry of the system, metabolic energy seems to be very necessary. The asymmetry, however, need not involve direct interaction of metabolism with the mobile carrier. The primary asymmetry which has been assumed to be present in the epithelial cell is a gradient of Na^+ concentration between tissue and extracellular fluid. The second asymmetry is a direct consequence of the Na^+ gradient and may be assumed to operate synergistically. This is the opposing gradient of K^+ concentration. Finally, a third asymmetry in the system

has been discovered (Crane et al, 1965) that is the affinity. They stated that affinity between sugar and carrier is greatly affected by the Na^+ concentration; the higher the concentration of Na^+ the higher the affinity and conversely. Thus a model for active sugar transport had been suggested (Crane et al, 1961) which accommodated the characteristics of transport as understood at that time, namely: 1) Energy dependence; 2) Michaelis-Menten kinetics; 3) independence from chemical reactions involving the sugar molecule itself; 4) localisation at the brush border membrane; 5) dependence on Na^+; 6) dependence on active Na^+ transport.

Another important development which has changed the thinking about the previously proposed mechanisms (i.e. phosphorylation, mutarotation etc.) of intestinal absorption was the general acceptance of the lipid membrane theory (Davson & Danielli, 1949). From the observation that substances with a high lipid-water distribution ratio can permeate across membranes more readily than those which are more water soluble, it was concluded that the cell is surrounded by a membrane of lipid nature. The first model of cell membrane suggested by Danielli (1949) represented, basically, a layer of protein and a layer of oriented micelles of lipid. This model was fully verified later by electron microscope observations. There is also good indication that the lipoprotein layer on the cell surface is not continuous but contains pores which are, however, too small for visualization by the electron microscope.

However the carrier permits the passage of the sugar molecule across the lipid phase. The chemical nature of the carrier is not known - it has not yet been isolated - but from the kinetics of the

transport, it is assumed that a true combination occurs between the carrier and the substrate, just like the temporary combination of an enzyme with its substrate. The carrier thus is highly specific.

I.c Effects of various concentrations on intestinal sugar absorption.

In a trial to reveal the relations between the rate of absorption and the concentration of the absorbed sugars, Cori (1925) showed that the rate of absorption of glucose and galactose and other sugars are independent of their concentrations. Similar conclusions were reported by many workers, e.g. Barany et al, 1939; Golden and Long, 1942; Small et al, 1959; Faust, 1964. However the experiments by Renevll & Spray, 1956; French et al, 1963; Rider et al, 1967, have failed to show rate limiting kinetics, and the Michaelis-Menten (1913) analysis was not applicable.

Other authors concluded that the rate of glucose absorption appeared to be dependent on the initial concentration - e.g. Omi 1909, MacKay & Bergman, 1933, Abbott et al, 1938, MacKay & Clark, 1941, Vidal-Sivilla, 1950, Larralde & Giraldez, 1958 and Garnier et al, 1960.

Indeed, glucose, galactose and other actively transported sugars can move across the small intestinal mucosa against a concentration gradient and the kinetics of absorption under these circumstances suggest that association with some enzyme system or carrier is involved in their active transport (Fisher & Parsons, 1953a and 1953b, Crane 1960a and 1960b, Schedl & Clifton, 1961, Wilson, 1962, Annegers, 1964a, Holdsworth & Dawson, 1964, Bihler, 1969, Kimmich, 1970 and Modigliani et al, 1971. Furthermore, this concept has been supported by Asano,

1964, Barry et al, 1965, Schultz & Zalusky, 1964a and 1964b, Lyon & Crane, 1966a and 1966b, Lyon, 1967, Debnam et al, 1970, 1971, 1972 and 1973, and Levin & Syme, 1971, on the bases of electric parameters.

I.d Intestinal sugar transport pathway(s).

When two substrates compete with one another in a biochemical process it is presumed that their pathways have at least one step in common. Thus the competition between different sugars, including glucose, galactose ---etc., for the transport mechanism of the small intestine was tested in vivo (Cori, 1926, Annegers, 1964a and Holdsworth & Dawson, 1964) and in vitro (Fisher & Parsons, 1953b, Riklis, Haber & Quastel, 1958, Crane 1960a and Jorgensen et al, 1960 and 1961). They concluded that actively transported sugars do compete for a common pathway in the intestine.

On the other hand, Debnam & Levin (1971 and 1974) and Levin & Syme (1971) concluded that there are multiple mechanisms for the active transfer of hexoses across the small intestine.

I.e Influence of changes in temperature on active absorption of sugars.

In vivo studies showed that at low temperatures absorption of sugars either was very weak or ceased (Verzar & Wirz, 1937, Rafferty & Maclachlan, 1941) with rat intestine, (Vogel, 1953, Cordier & Worbe, 1954, Csaky & Fernald, 1960) with frog intestine and Hussein & Muflih (1969) with mice intestine. Cordier & Piery (1950) reported that raising the chamber temperature from 18° up to 40°C did not modify the rate of rat intestinal absorption of glucose.

In in vitro preparation it seems that the cells at low temperatures become relatively impermeable for sugars (Vogel, 1953 and Csaky & Fernald, 1960) ~~with~~ FOR frog intestine; (Crane et al, 1957) with Ehrlich ascites tumor cells and (Carlisky and Haung, 1962) with mucosal membrane of dogfish intestine.

In the literature, few studies in this area have been reported on the effects of temperature changes on the electrical parameters induced by actively transported sugars. However with in vitro intestinal preparations the increase in temperature of the bathing solution which contained glucose seems to result in a clear rise in the electrical parameters (Sawada & Asano, 1963) with rat; (Schultz & Zalusky 1964a 1964b) with rabbit and (Quay & Armstrong, 1969a) with bullfrog.

I.f Effects of anoxia on intestinal active absorption of sugar.

The publications of in vitro sugar absorption have suggested that energy yielding cell metabolism is essential for tissue/medium concentration gradient to be established. Air or O_2 rather than N_2 must occupy the gas space of the experimental flask (Wilson & Wiseman, 1954). Nitrophenol inhibitors of aerobic phosphorylation are effective in an appropriate concentration range (Crane & Mandelstam, 1960).

However, the rate of glucose absorption under anaerobic conditions was reduced to less than the rate of absorption under aerobic conditions (Darlington & Quastel, 1953, Fisher & Parsons 1953b, Wilson 1954, Wilson & Wiseman 1954 , Baker et al, 1961, Bihler & Crane, 1961, Bihler, Hawkins & Crane, 1962 and Faust, 1962). For a short period of incubation (5 - 7 min.) the rate of glucose absorption from the mucosal fluid containing Na^+ was more rapid under anaerobic than aerobic conditions (Faust, 1962).

Baker et al, 1974 stated that in hamster jejunum, galactose influx was reduced 93% by 10 minutes of mucosal anaerobiosis.

Barry et al (1964), Baker et al (1971) and Munk (1972) showed that under anaerobic conditions the electrical responses measured across the intestinal preparations, fall rapidly in the presence or absence of sugar.

I.g Disaccharide absorption in the intestinal epithelial cells.

Hydrolysis of the disaccharides (through the activity of the small intestinal disaccharidases) takes place within the epithelial cells at the outer coat of the plasma membrane i.e. externally to the site of monosaccharide interaction with the transport process (Miller & Crane, 1961a). This is consistent with the findings obtained by Miller & Crane, 1961b, Crane, 1962, Dahlqvist & Brun, 1962, Newey et al, 1963 and Doell et al, 1965. A more specific localisation was carried out by Eichholz & Crane, 1965 and Overton et al, 1965 who succeeded in recovering a plasma membrane fraction from disrupted brush border with which the disaccharidase and alkaline phosphatase remain quantitatively associated.

Semenza et al (1964) have found that the effect of Na^+ on sucrase activity was closely similar in kinetic parameters to the effect of Na^+ on sugar transport. Then Semenza and Crane (1966) concluded that the transport process and the disaccharidases share the same Na^+ interaction site.

There are at least six different disaccharidases including lactase, maltase, isomaltase, sucrase and trehalase. The activity of these vary

in different species and in different regions of the gut (Dahlqvist, 1961, Dahlqvist et al, 1961, Auricchio et al, 1963, 1965 and Gray et al, 1965).

Kohn, Smyth and Wright (1966, 1968) have reported that maltose and sucrose, but not lactose, stimulate the potential across the rat small intestine in vitro. And they stated that the effect appears to depend on the extent of hydrolysis of disaccharide.

I.h The transport of 3-O-methylglucose (3MG).

The study of unnatural sugars in intestinal absorption was initiated with the aim of examining the significance of the chemical configuration of the sugar in the process (Csaky, 1942). The first such study dealt with glucose molecules with very simple substitutions, a hydroxyl (OH) was replaced with an oxymethyl ($-OCH_3$) group. It was found that if the substitution took place in the third position, the resulting 3-O-methyl-glucose is absorbed in the same way as glucose, whereas substitution in the 2, 5 or 6 positions completely abolished the fast absorption. 3-O-methylglucose was later shown to be a rather valuable tool in the study of intestinal sugar absorption, inasmuch as this sugar does not undergo a chemical change in the process of cellular metabolism but it is actively transported (Crane, 1960b and Wilson 1962).

As a result of such studies a structural requirement has developed which seemed to be essential for a sugar to be actively transported. The sugar had to have a pyranose structure (an oxygen bridge between the first and fifth carbon), it had to have an aldehyde group in the first carbon, and it had to have a free hydroxyl group in the second

position (Crane, 1960b and Wilson, 1962).

However, the metabolically inert behaviour of 3-O-methylglucose allows a great deal of benefit to be derived from following its path step by step in the transport system, e.g. (Csaky & Wilson, 1956 and Csaky & Glenn, 1957). So it gives a good chance for a better understanding of the transport process, namely the Na^+-sugar coupling phenomenon (Csaky, 1961 a & b, 1963b, 1964, Csaky & Fernald, Hartzog, 1961, Csaky & Lassen, 1964, Csaky & Hara, 1965, Schultz & Zalusky, 1964b, Goldner, Schultz & Curran, 1969).

II. Requirement for Na^+.

Observations suggesting that Na^+ influences the transport of other solutes across cell membranes occur through the early literature e.g. (Reid, 1900). However the influence of Na^+ on the transport of a variety of solutes across animal cell membranes was first identified clearly in the 5 year period between 1958 and 1963. In 1958 Riklis & Quastel reported that the absorption of glucose by isolated guinea pig small intestine depended markedly on the presence of Na^+ in the solution bathing the mucosal surface. Thus a new era of sugar transport investigation had been born.

II.a The relationship between entry of sugars and intestinal Na^+ transport.

Dependence of sugar transport on Na^+ appears to be restricted to processes capable of bringing about net movement of sugar against a concentration difference. This concept has been reported in toad intestine in vitro (Csaky, 1960, Csaky & Thale, 1960), in rat intestine in vivo (Clarkson & Rothstein, 1960, Csaky & Zollicoffer, 1960, Barry et al, 1962, 1964, Csaky, 1963a, Levinson et al, 1966) and in vitro (Parsons et al, 1961, Barry et al, 1962, 1964, 1965, 1969, Faust, 1962, Capraro et al, 1963 a & b, Harrison & Harrison, 1963, Sawada & Asano, 1963, Asano, 1964, Lyon & Crane, 1966 a & b, Lauterbach, 1967, Taylor et al, 1968), in hamster small intestine in vitro (Bihler and Crane, 1962, Bihler, Hawkins & Crane, 1962, Bihler, 1969, Crane, 1962, 1965, Crane et al, 1965, Alvarado, 1964, 1965, Caspary & Crane, 1968), in dog intestine in vivo (Annegers, 1964b, Heaton et al, 1969), in chicken small intestine in vitro (Alvarado, 1965, 1967, Alvarado et al, 1967), in bullfrog intestine in vitro (Lassen & Csaky, 1966, Quay & Armstrong,

1969 a & b), in guinea pig small intestine in vitro (Lauterbach, 1967), in human intestine in vivo (Schedl et al, 1963, Holdsworth et al, 1964, Fordtran et al, 1968, Olsen et al, 1968, Sladen, 1969), in rabbit intestine in vitro (Schultz & Zalusky, 1963 a & b, 1964 a & b, 1965, Goldner et al, 1969), and in tortoise small intestine in vitro (Wright, 1966). Also this concept has been supported by Crane (1968), Wasserman (1968), Schultz & Curran (1970), Kimmich (1973) and other authors some of whom will be quoted in the following sections.

II.b Na^+ transfer and pumping sites.

To account for Na^+ transfer in the intestine various Na^+ pumps have been postulated which may be either electrogenic or non-electrogenic. These postulations are as the following:

1. - The endogenous Na^+ pump which is the movement of Na^+ in the invitro intestine when no added transferable or metabolizable substance is present. It is implicit in the work of Barry et al, (1965).

2. - The pump related to hexose transfer, was postulated by Crane, Miller & Bihler (1961), elaborated by Schultz & Zalusky (1964b & 1965) and included in the scheme of Barry et al, (1965). An essential feature of this postulation is that Na^+ transfer is linked to hexose (or amino acid) transfer and not to hexose metabolism. This view has been stressed again by Taylor et al, (1968).

3. - The non-electrogenic Na^+ pump related to hexose metabolism, was postulated by Barry et al (1965). It acts in moving Na^+ towards the serosal side. The concept of this pump is in keeping with the increased fluid transfer caused by metabolized hexose (Barry et al,

1969) e.g. fructose (Barry et al, 1964) and mannose (Duerdoth et al, 1965).

4. - The non-electrogenic Na^+ pump related to galactose transfer was postulated by Taylor et al, (1968), to explain why the Na^+ transfer in the presence of galactose was smaller than in the presence of glucose. It could be a NaCl pump or Na^+ and Cl^- pumps working at the same rate and it does cause fluid movement toward the mucosal side in addition to Na^+ and Cl^- movement.

The energy required for sugar accumulation is introduced into the system at the site of Na^+ translocation (Crane, Miller & Bihler, 1961, and Crane, 1968); Na^+ pumps require ATP (Caldwell et al, 1957 and Dunham, 1957).

The result obtained by Bihler and Crane (1962) and Bloom et al (1962) suggested that if entering Na^+ is not expelled luminally from the brush border it must at least be exchanged for some other cation, e.g. K^+, H^+. This was supported by Taylor (1962) in the intestinal mucosa of the guinea pig. However Crane (1968) reported that there are substantial reasons to believe that outward translocation of Na^+ occurs at the brush border as well as at the basal pole.

On the other hand, Schultz & Zalusky (1964b) and Schultz & Curran (1968) reported that Na^+ pumping is a property exclusive to the basal membrane of the cell.

II.c Relation of Na^+ transport to cell metabolism.

Early experiments were performed primarily with glucose and considerable controversy developed over the question of whether glucose

metabolism is a requirement for intestinal salt and water transport. Curran's data (1960) clearly showed that glucose produces a pronounced increase in the mucosal to serosal flux of Na^+ ~~flux~~ in rabbit ileum. However, the kinetics of the response in Na^+ flux indicated that the sugar was acting primarily by providing energy to a vectorial Na^+ pump. Similar conclusions have been reported by Clarkson et al, (1961) for rat intestine which they said has very little "stored energy" but must be continuously supplied with glucose, and Schultz & Zalusky (1963a) for the rabbit ileum.

On the other hand, other experimental results indicated that enhanced metabolic activity might be an insufficient explanation for the effects of sugars on potential difference and short-circuit current. Barry et al, (1961), reported that phloridzin in low concentration was able to inhibit most of the glucose-induced potential in the rat intestine. They concluded that their 'findings suggested phloridzin affects Na^+ entry not necessarily directly, but possibly by preventing glucose entry'. This concept was presented in more detail (Crane et al, 1961) one year later.

However, metabolism may be involved at least indirectly. This concept was emphasised by a number of reports which indicated that metabolic inhibitors such as 2,4-dinitrophenol (Barry et al, 1961, Sawada et al, 1963, and Asano, 1964), iodoacetate (Sawada et al, 1963 and Barry et al, 1964), and other metabolic inhibitors were all able to inhibit the increase in potential difference and short-circuit current elicited by actively transported sugars. The same inhibitors also inhibit active sugar transport.

II.d The direct coupling between sugar and Na^+ transport.

Strophanthin which is thought to be a rather specific inhibitor of active Na^+ transport also inhibits active accumulation of several sugars in hamster intestine (Crane et al, 1961). Similar effects were also reported for ouabain by Csaky (1961a, 1963b, 1964) and Csaky et al, (1961, 1964, 1965) on 3-O-methylglucose transport by preparations of frog intestine. Schultz & Zalusky (1963a, 1964a, 1964b, 1965) reported that ouabain significantly inhibits the increase in transmural potential differences caused by actively transported sugars in rabbit ileum. Sawada & Asano (1963), reported similar effects for rat intestine. However, the common requirement for Na^+ by several active transport systems led Csaky (1963a) to consider the possibility that Na^+ might play a rather general role in the mechanism of active transport and that Na^+ dependence might not necessarily reflect a Na^+ requirement for the substrate carrier itself. This idea was supported by his earlier observation (Csaky & Thale, 1960, Csaky & Zollicofer, 1960 and Csaky 1961b). However, Csaky (1963a) suggested that cellular Na^+ is required for function of an ATPase which is perhaps "involved in all active transport processes".

A role for Na^+ in coupling energy expenditure to active transport events was perhaps also suggested by the fact that only sugars which are capable of being accumulated against a concentration gradient exhibit a dependence on Na^+ (Bihler & Crane, 1962, and Bihler, Hawkins & Crane, 1962). Other sugars while they may be capable of penetrating the intestinal cell show no such Na^+ dependence, (Bihler & Crane, 1962, Bihler, Hawkins & Crane, 1962 and Bihler, 1965). This fact became obvious largely from the findings that the structural requirements

that determine Na^+ dependence of sugar entry (Bihler & Crane, 1962, and Bihler, Hawkins & Crane, 1962) was exactly the same molecular requirement which a substrate must possess in order to be actively accumulated (Crane & Krane, 1956, Wilson & Crane, 1958, and Crane, 1960b).

II.e The indirect coupling between sugar and Na^+ transport.

Csaky & Hara (1965) reported that ouabain (10^{-5}M) was only effective in inhibiting the transmural flux of 3-O-methylglucose when added to the medium bathing the serosal surface of frog intestine and was completely ineffective when added to the mucosal medium only; and measurement of the degree of penetration of the tissue by (^3H) ouabain indicated that relatively little ouabain crosses the tissue barrier from one surface to the other. This report was one of the first to raise significant doubt with regard to the validity of a direct coupling between sugar transport and active Na^+ transport.

However, Asano (1964) was the first to point out that Na^+ entering the cell on the sugar carrier need not necessarily be returned to the mucosal compartment as was originally stated by Crane et al, (1961), and extrusion entirely via an ouabain-sensitive serosal pump might preclude an effect of mucosal ouabain, but still account for an increase in short-circuit current and potential difference by the tissue in response to active transport sugars. It is this concept that seems to have dominated the interpretation of changes in the electrical activity induced by sugar transport during the early studies of the phenomenon (Schultz & Zalusky, 1963a, 1963b, 1964b, 1965, Asano, 1965, Lyon & Crane 1966 a & b, and Levin 1966). The sequence of events envisioned includes an increased cellular entry of Na^+ at the mucosal

boundary in association with the sugar carrier, a resultant increase in cellular Na^+ concentration, and consequent increased activity of the serosal Na^+ pump leading to an increased short-circuit current and potential difference maintained by the tissue (Schultz & Zalusky, 1963a, 1964b, Barry et al, 1965, Taylor et al, 1968, Quay & Armstrong, 1969a, White & Armstrong, 1971, Field et al, 1971, and Munk, 1972). While several more recent observations indicated some shortcomings in this explanation, these will be considered in a later section. However, Bihler & Crane (1961) and Bihler, Hawkins & Crane (1962) concluded that intestinal active transport of sugar has two components, namely, Na^+ dependent, energy-independent entrance and Na^+ dependent, energy-dependent accumulation against a concentration gradient. Barry et al, (1965, 1967, 1969) stated that their results indicated that there are two Na^+ pumps associated with hexose transport in rat jejunum, both pumps moving Na^+ towards the serosal side. One is an electrogenic pump stimulated by hexose transfer and which results in a rise in short-circuit current and potential and the other is a neutral pump which is stimulated by hexose metabolism and which does not affect the potential. Lauterbach, (1971 and 1972) has reported an active secretion of ouabain from serosal to mucosal surfaces by isolated guinea pig strips.

II.f Site of Na^+ interaction with the sugar transport system.

The site of interaction of sugars with the absorptive process cannot easily, like an enzyme, be isolated and identified unequivocally. The use of glycoside phloridzin was one of several approaches for the deduction of the locus of the sugar accumulation components. Several

reports have pointed out the brush border boundary of the epithelial cells (Ponz & Lluch, 1955, Parsons et al, 1958, Crane & Mandelstam, 1960, Bihler & Crane, 1961, Miller & Crane, 1961a, Kinter & Wilson, 1965, Lyon, 1967, Stirling, 1967, Stirling & Kintner 1967, and Kimmich, 1970). However, Newey et al, 1959, were the first to show that phloridzin inhibits sugar entry into intestinal tissue when added at the mucosal, but not the serosal surface. Alvarado & Crane, 1962 demonstrated that phloridzin exhibits competitive inhibition kinetics on sugar transport by hamster small intestine. They suggested (1964) that phloridzin might serve as a substrate for the sugar carrier and consequently penetrate the cell. Miller & Crane, (1963) stated that phloridzin's presence at high concentration restricted the tissue distribution of glucose to the extracellular space. Stirling (1967) indicated that phloridzin is poorly transported in the small intestine.

III. Some Other Observations on Intestinal Electrical Parameters Induced by Sugar Transport

III.a Influence of sugar transport on undirectional Na^+ fluxes.

As men-tioned above some of the data regarding sugar-induced-changes in intestinal electrical activity is consistent with that obtained in studies of sugar accumulation. But several observations are difficult to accommodate within the limits of the simplified concepts that have thus far been considered. For instance, if the electrical activity of the tissue is due largely or entirely to the Na^+ extrusion system located at the lateral-serosal boundary of the intestinal epithelial cells, it is apparent that events leading to additional mucosal entry of Na^+, such as sugar transport, should produce an enhanced net transfer of Na^+ from mucosal to the serosal surface of the tissue. Indeed this has been observed by Schultz & Zalusky (1963a, 1964a, 1964b), Barry et al, (1965), Taylor et al, (1968), Quay & Armstrong (1969a), Field et al, (1971), White & Armstrong, (1971) and Munk, (1972). Little or no changes in serosal to mucosal fluxes would be expected. Instead, some reports show that the enhanced net flux of Na^+ under certain conditions is entirely due to a decrease in serosal to mucosal Na^+ flux. Some of the clearest examples are those reported by Schultz & Zalusky (1963a, 1964a, 1964b) for rabbit ileum a tissue in which the observed short-circuit current is entirely accounted for by the net flux of Na^+ either in the presence or absence of an actively transported substrate species (Schultz & Zalusky, 1963a, 1964a, 1964b, 1965). In one of these examples it can be observed that over 90% of the observed change in net flux of Na^+ is accounted for by the decrease in serosal to

mucosal flux. Similar data was reported by Quay & Armstrong (1969a), for bullfrog intestine.

Robinson (1970) has concluded that the activity of the $(Na^+ + K^+)$-ATPase, which is isolated from rat, guinea pig and mouse, does not reflect the entire Na^+ transport capability of the intestinal epithelium.

Kimmich (1972) and Kimmich et al, (1972, 1973) observed that intestinal cells depleted of ATP by pre-incubation with 2,4 dinitrophenol, do exhibit sugar entry which is Na^+ dependent and phloridzin sensitive, but insensitive to inhibition by K^+.

III.b Extracellular shunt pathways for monovalent ions and intestinal tissue potential profiles.

Apparently intercellular fluxes of monovalent ions contribute significantly to the total ion fluxes observed in intestinal tissue (Clarkson, 1967, Taylor et al, 1968, Rose & Schultz, 1971, White & Armstrong, 1971, Frizzel & Schultz, 1972, and Schultz, 1972).

However, the concept of Na^+ shunt pathways was not resurrected until 1970 with the advent of the use of microelectrodes for studying transmembrane potentials of intestinal epithelial cells in response to active sugar transport. White & Armstrong (1970) and Rose & Schultz (1970) reported successful attempts with bullfrog and rabbit ileum respectively. In both species the cell interior was shown to be negative with respect to either the mucosal or serosal bathing solutions and the serosal side positive with respect to the mucosal side. Addition of actively transported sugars produced a decrease

in the mucosal to the cell potential (Em became less negative), but no change occurred with non-accumulated solutes were added. A year later, White & Armstrong (1971) and Rose & Schultz (1971) extended their studies with more details. White & Armstrong (1971) concluded that a decrease in mucosal potential associated with Na^+ entry on the sugar carrier might be sufficient in itself to account for the somewhat smaller change observed in transmural potential, i.e. no change in serosal potential need necessarily occur. (Schultz, Fuiz & Curran, 1965, 1966, Csaky & Esposito, 1969, Koopman & Schultz, 1969, Armstrong, Musselman & Reitzug 1970, and Lee & Armstrong, 1972) have reported no change or a decrease in intestinal tissue Na^+ concentration following an interval of sugar accumulation. Furthermore, increases in tissue water have been reported (Schultz et al, 1966, Csaky & Esposito, 1969, and Armstrong et al, 1970). In addition, White & Armstrong (1971) suggested that the rather slow changes in potential which they observed after an initial rapid phase might reflect changes related to cell swelling. Also recall that a significant increase in mucosal to serosal unidirectional flux of Na^+ has, with one exception (Field et al, 1971) not been observed for rabbit ileum (Schultz et al, 1963a, 1964a, 1964b) or bullfrog intestine (Quay & Armstrong, 1969a) in response to sugar accumulation. Frizzel & Schultz (1972) have studied the nature of Na^+ influx into rabbit ileum and concluded that 85% of the total tissue conductance is associated with intercellular shunts. Their data indicate that about 90% of the Na^+ entering the tissue at the mucosal surface is returned to the mucosal side across the same boundary. This brush border Na^+ efflux might be due to either exchange diffusion or active extrusion of Na^+ as has been previously pointed out (Schultz et al, 1967).

Also the possibility of active NaCl extrusion at the brush border was suggested (Taylor et al, 1968) to explain the discrepancy between the I_{sc} and the net Na^+ influx in the rat jejunum during active accumulation of galactose. However, it might be noteworthy that a 5-7 fold decrease in cellular Na^+ concentrations causes only a slight decrease (<20%) in unidirectional Na^+ influx.

Gilles-Bjallien & Schoffeniels (1965) using Greek tortoise intestine and Wright (1966) using tortoise and hamster intestine have noted that actively transporting sugars cause an increase in serosal potential with little or no change in Em, in contrast to the work discussed above for rabbit ileum and bullfrog intestine.

IV. An Alternative to the Ion Gradient Hypothesis.

A number of observations - some of them have been mentioned in the foregoing pages - which are difficult to reconcile with the ion gradient hypothesis have lead Kimmich & Randles (1973) to introduce an alternative schematic model. This model which represents a modification of an earlier version that was submitted by Kimmich (1970) is also discussed in detail in a review by Kimmich (1973). The basic premise of the model as he said considers the possibility that the basic set of energy transduction events may occur in the brush border membrane of the intestinal epithelial cell, which serves to support a variety of energy-dependent transport events. The energised intermediates ($E_1 \sim P$ and $E_2 \sim P$) which may associate with ($Na^+ + K^+$) activated ATPase might serve as a means of coupling the two transport systems i.e. active Na^+ transport and Na^+ dependent solute transport. Furthermore, Kimmich & Randles (1973) suggested that energy associated with the second membrane-bound energised intermediate ($E_2 \sim P$) might be trapped by closely associated membrane components (X,Y, and Z), and used to support a variety of specific active transport events such as sugar, amino acid or K^+ accumulation. These transport components might themselves exhibit ion requirements in order to possess functional activity responsible for sugar or amino acid entry. Functional status of the carrier (e.g. altered mobility or substrate affinity) might be conferred either by direct energization from $E_2 \sim P$ or by Na^+ binding or perhaps by a combination of these two events.

The enzyme is thought to represent an integral part of the so-called Na^+ pump, and has been described as a part of brush border

membrane preparation (Taylor 1962, Berg & Chapman 1965, Quigley & Gotterer 1969, 1972). But the evidence obtained by Fujita et al, (1971) was inconsistent with the latter viewpoint. However, the energized intermediates are known to be generated by a reaction which requires intracellular Na^+ and ATP, and are discharged in the presence of extracellular K^+ (Sen et al, 1969, Fujita et al, 1971). No other monovalent ion can satisfy the role played by Na^+, although other ions can compete for the Na^+ site in a non-functional manner (Post et al, 1965). However, Kimmich (1973) suggested that sugar transport would be closely related to events which establish cellular Na^+ gradients, but not directly dependent on such gradients, and therefore his model allows for those situations where active sugar transport has been observed while a reversed Na^+ gradient was imposed. Then he stated that if monovalent ion and solute transport are driven by a common energy transducing system, sugar transport would interfere with Na^+ extrusion at the brush border, and therefore lead to increases in transmural potential, short-circuit current, ϕ^{Na}_{sm} would decrease and ϕ^{Na}_{ms} might increase, but not of necessity. An increase in the latter parameter would depend on increased rates of Na^+ extrusion at lateral-serosal cell boundaries, which in turn would depend on elevated cellular Na^+ concentrations. The latter response has not been observed even with the use of microelectrodes introduced intracellularly (Lee & Armstrong, 1972). Moreover, a number of questions have been raised in recent years regarding the sufficiency of energy inherent in cellular ion gradient for totally accounting for observed solute concentration gradient (Schafer et al, 1968, 1972); and the fact that active sugar and amino acid transport may occur even when

cellular Na^+ gradients are reversed and outwardly directed (Schafer et al, 1968, 1971, Newey et al, 1970, Kimmich et al, 1970, 1973, Potashner & Johnstone, 1971) have cast further doubt on the concept of energy input provided primarily by cellular ion gradients.

Finally, Gibb & Eddy (1972) have presented data which indicate that the membrane potential may be an important determinate of amino acid transport capability in ascites cells.

V. The Present Work

During the last ten years, the phenomenon of the short-circuit current has held the attention of some authors as a method of studying the sugar accumulation against concentration gradients in the intestinal mucosa in view of the ion gradient hypothesis. Most of the studies in this field have dealt with flat sheets of intestinal preparations (Asano, 1964, Schultz & Zalusky, 1964a, 1964b, Taylor et al, 1968, Quay & Armstrong, 1969a, Baker et al, 1971). Field et al, (1971) used flat stripped and unstripped sheets of rabbit ileum.

Barry et al (1965) used the everted intestinal sac of the rat; in their investigations they hung the intestinal sac of the rat in the bathing solution throughout the experiment which took only 15 or 45 minutes. However, at about the same time that this project was started in 1972, Munk used both the everted rat jejunum and Ussing & Zerahn (1951) technique. He reported that the I_{sc} obtained by Barry et al (1965) in sacs of everted rat jejunum was about 40% of that obtained in his investigation.

The technique of intestinal sac evertion was introduced into modern studies of absorption in 1954 by Wilson & Wiseman (1954). Since that time many modifications and improvements have been made, e.g. (Crane & Wilson, 1958, Clarkson & Rothstein, 1960, Jorgensen, Landau & Wilson, 1961). Barry et al (1964) and Baker et al (1974) have circulated fluid through everted segments of intestine; studies of change in electrical potential during absorption have been made.

In this study a new device was introduced to the everted sac technique for the first time. The full details of this techique are explained in Chapter Three.

It was hoped that this technique in addition to meeting the basic conditions for substrate transport may support and slightly distend the sac, thus may reduce and stabilize the thickness of the wall and separate the villi. It was thought that such a modification might solve at least partially the difficulties underlying the oxygen supply to the deeper layers of the rat intestinal sacs that were observed by Munk (1972). Thus it was hoped that this device might improve the viability conditions of the sac and increase the survival period of the tissue preparation and thus allow enough time for experimental investigations. This new technique also might give rise to a more stable and consistent short-circuit current. Mounting the intestinal segment on the supporting cannula eliminates the wall contractions and thereby helps in controlling the level of the inside solution. Baker et al (1974) reported that the level of the inside solution fluctuated widely with the hamster intestinal contractions. During the experiment the fluctuation of the inside fluid might produce differences in the hydrostatic pressure which in turn will effect the fluid movement in the intestinal preparation (Wilson, 1956a). To control the hydrostatic pressure more efficiently, recirculation of the fluid through the serosal side of the segment was followed in most of the investigations performed in this study. However, the elimination of the intestinal contractions coupled with the re-circulation of the serosal fluid, served to keep the level of the serosal fluid always similar to the level of the mucosal medium.

Having introduced the gut supporting cannula device into the procedure of the rat intestinal eversion, the following studies were made:-

1. The effects of different concentrations of some actively transportable sugars on the short-circuit current. In addition to glucose, galactose, 3-O-methylglucose and α-methylglucose, some of the tested sugars, namely β-phenylglucose and β-methylglucose were employed in this study for the first time to stimulate the short-circuit current. Glucose and galactose were tested not only at 37°C but at other temperatures.

2. The effects of different concentrations of some disaccharides namely, maltose, sucrose, trehalose, lactose and cellobiose on the short-circuit current. The effects of some of these disaccharides on the short-circuit current had not previously been investigated.

3. Special attention was given for the effects of some of the non-transportable sugars namely ethylidene glucose, benzylidene glucose and mannose on the short-circuit current. The effects of different concentrations of these sugars on the short-circuit current recorded in the absence and the presence of the actively transportable sugars (e.g. 3-O-methylglucose, galactose and glucose) were investigated.

Ethylidene glucose has been shown by Baker & Widdas (1973a and 1973b) to be a non-transportable competitive inhibitor of glucose transfer by the human red cell and benzylidene glucose a related compound has been shown by Novak & LeFevre (1974) to have a higher affinity for the hexose transport system in the human erythrocyte. Mannose (28 mM) has been shown (Barry et al, 1969) to have an inhibitory effect on the resting potential difference when added to the mucosal side of the rat intestinal segment. The authors

added that this effect of the mannose was related to the osmotically induced potentials.

4. Kinetic analysis was made from the above studies, the Ki and the ΔI max were calculated for most of the tested sugars including the non-transportable sugars, namely lactose, cellobiose, ethylidene glucose, benzylidene glucose and mannose which gave negative values of ΔI max.

5. On the basis of short-circuit current the effects of some factors on the viability of the intestinal segments (with the new technique followed in this study) were investigated. In this part of the studies, comparisons between the short-circuit current induced by glucose and galactose under aerobic and anaerobic conditions were made. The age of the preparation and the viability of the intestinal segment was investigated in the presence of a metabolized sugar (glucose) and non-metabolized sugars i.e. 3-O-methylglucose and galactose.

As a source of metabolic energy, sodium citrate was used in some experiments. Citrate has been used as a source of energy for the hamster preparations (Atfield et al, 1972 and Browne et al, 1974).

6. In the rat jejunum it seems that there was a conflict regarding the relationship between Na^+ flux and the short-circuit current in the presence of actively but not metabolized sugars (Barry et al, 1965). These authors found that the rate of active Na^+ transport in the presence of galactose or α-methylglucose accounted for only 25-35% of the short-circuit current. Although this was

reviewed by Taylor et al (1968) by using the flat sheets of the intestine, still it seems of some interest to review this relationship using the new technique of the everted rat intestine.

7. Other investigations were carried out in the area of the subject where there were few publications i.e. the stoichiometric relationship between Na^+ and sugar transport; and therefore more investigations might be needed. Goldner et al (1969) investigated this relationship in the rabbit ileum.

CHAPTER 2.

MATERIALS

Animals

White male Albino rats of the Wistar strain usually weighing 350-500 gm were used in this investigation. The rats were always kept for at least one week in the animal's room (at a constant temperature of 25°C) before use. They were maintained on a standard commercial diet (oxoid, SG1 cubes of Lillco & Sons Ltd.). The food and the water were made available ad libitum.

Chemicals

All chemical reagents were analar grade. The saline-buffer medium used in all experiments was the bicarbonate saline (Krebs & Henseleit, 1932) with the following composition expressed in mM.

NaCl	119.0
KCl	4.7
KH_2PO_4	1.2
$NaHCO_3$	25.0
$CaCl_2$	3.4
$MgSO_4$	1.2

The pH was initially 7.4 and usually no appreciable change was observed over a period of 100-160 minutes of incubation. Fresh saline solution was prepared every three days.

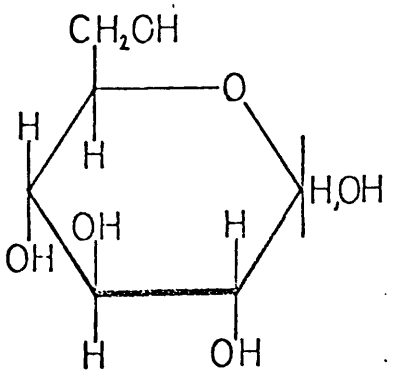
Sugars

Working sugar solutions were prepared from stock solutions. Fresh stock sugar-saline solution was prepared just before the onset of the experiment.

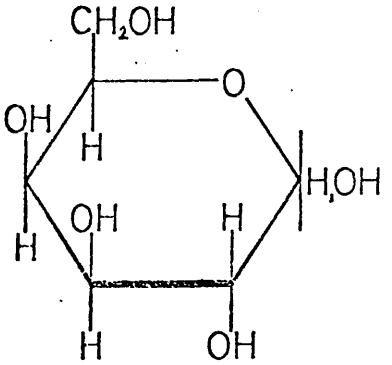
The sugars concerned in this investigation are:

D-Glucose		
D-Galactose		
3-O-Methylglucose	(3-O-Methyl-α-D-glucopyranose)	Koch-Light
α-Methylglucose	(Methyl-α-D-glucopyranoside)	Laboratories
β-Methylglucose	(Methyl-β-D-glucopyranoside)	Ltd. Colnbrook,
β-Phenylglucose	(Phenyl-β-D-glucopyranoside)	Bucks.
D-Mannose		
Insitol		
Ethylidene glucose	(4,6-O-Ethylidene-α-D-glucopyranose)	PREPARED BY D. A. BASKETTER B.Sc.
Benzylidene glucose	(4,6-O-Benzylidene-α-D-glucopyranose)	
Cellobiose		
Maltose		
Lactose		
Sucrose		
Trehalose		

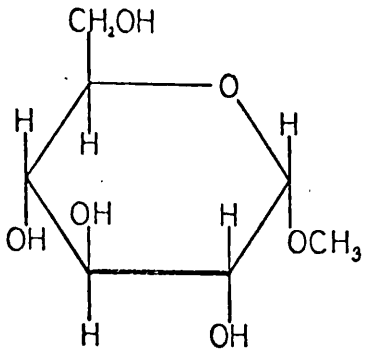
The structural formula for each of these sugars is:



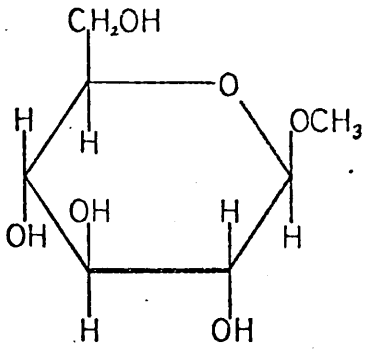
GLUCOSE



GALACTOSE



METHYL-α-GLUCOSIDE



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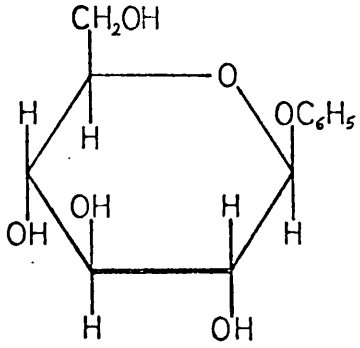
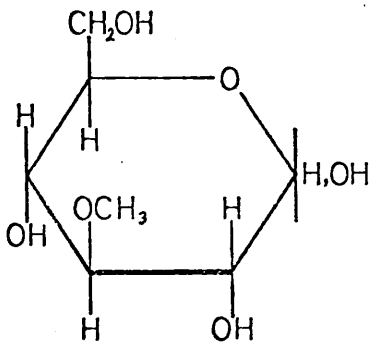
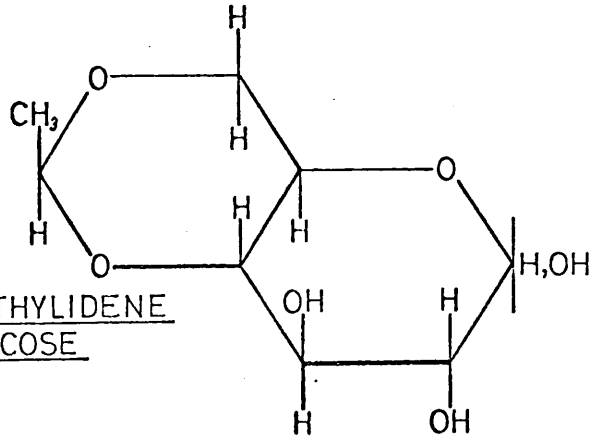


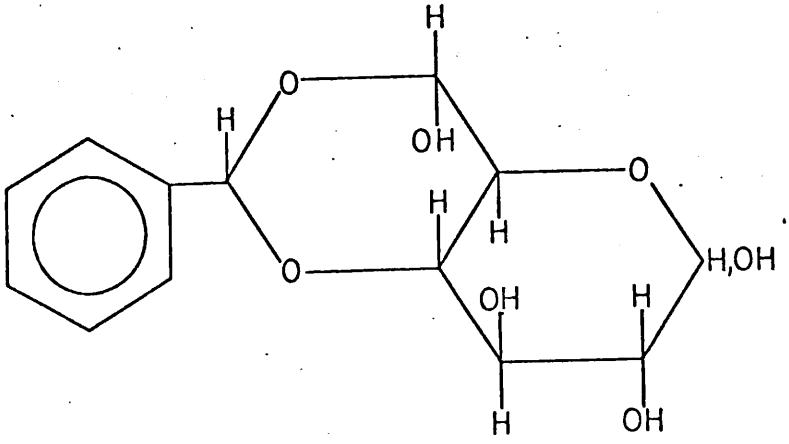
PHENYL-β-GLUCOSIDE



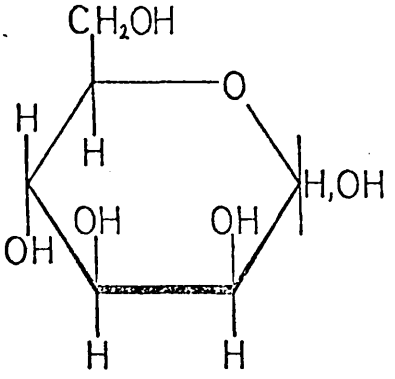
3-O-METHYL GLUCOSE



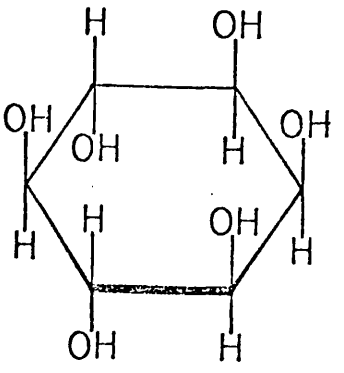
4,6-O-ETHYLIDENE GLUCOSE



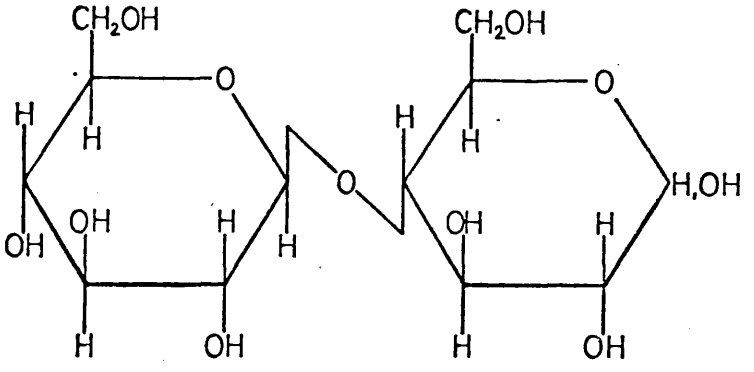
4,6-O-BENZYLIDENE GLUCOSE



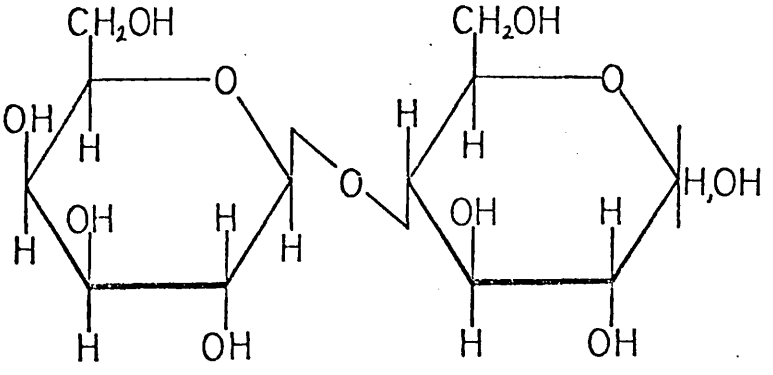
MANNOSE



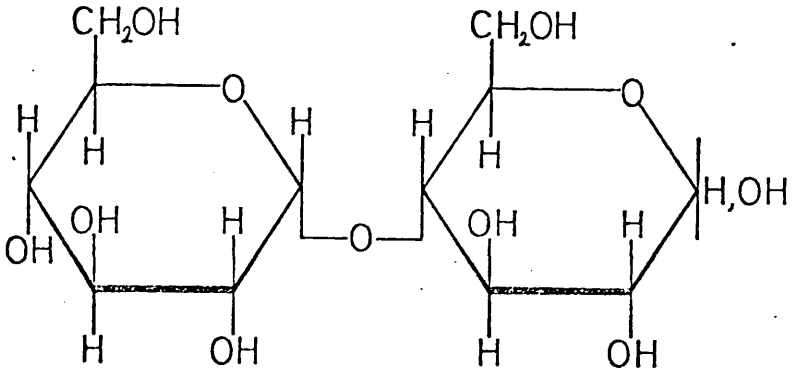
INOSITOL



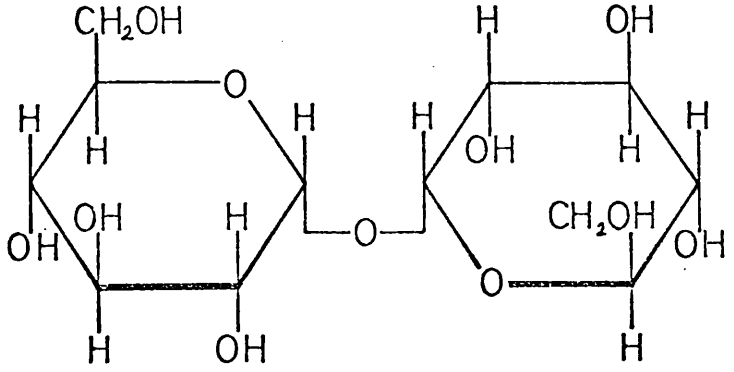
CELLOBIOSE



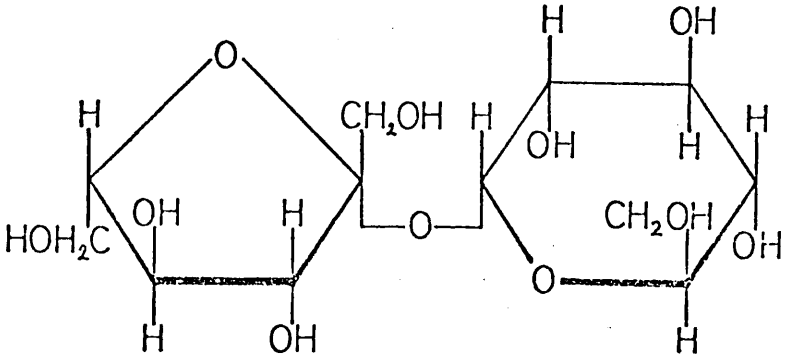
LACTOSE



MALTOSE



TREHALOSE



SUCROSE

Inhibitors

Stock solution of the inhibitor was prepared as required. The serosal stock medium was usually prepared with 50 ml saline solution, but mucosal stock-inhibitor was usually prepared in less than 1 ml saline solution, and it was therefore in a concentrated form.

The inhibitors used in this investigation were:

Iodo-acetic acid

Phloridzin

Ouabain

95% N_2 - 5% Co_2 (to produce anoxia)

Labelled compounds of radioactive isotopes:

3-O-methylglucose-C^{14}-(U)-specific activity 34.3 mCi/mM
(The Radiochemical Centre, Amersham, Bucks.)

Each batch was diluted by the addition of 2.25 ml sterilized isotonic NaCl solution before use.

Na^{22}-sodium chloride injection - specific activity of
25 µCi/mg of sodium.
(The Radiochemical Centre, Amersham, Bucks.)

Na^{24}-sodium chloride injection - specific activity
340 µCi/mg of sodium.
(The Radiochemical Centre, Amersham, Bucks.)

The experimental saline and other experimental solutions were normally continuously gassed with 95% O_2 - 5% Co_2, and kept in the water bath (usually at 37°C) to maintain the temperature.

CHAPTER 3

GENERAL METHODS.

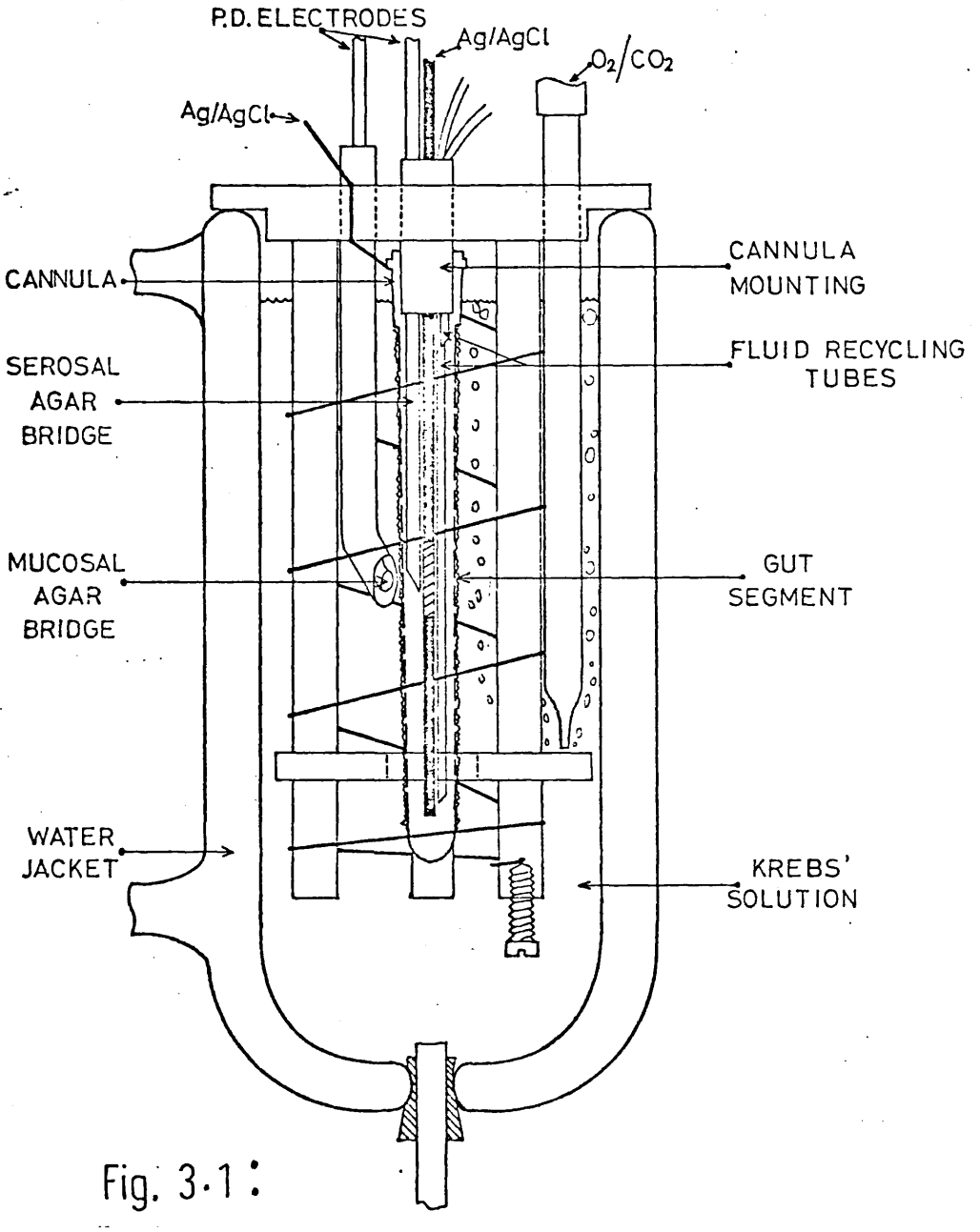


Fig. 3.1:

BATH & ELECTRODE ASSEMBLY

The principle of the method for recording the short-circuit current (I_{sc}) has been described by Ussing & Zerahn (1951). They identified the short-circuit current as the current which reduces the electrical potential across the tissue to zero.

Apparatus:

This is a modification of that described by Barry, Smyth & Wright (1965) for measurement of the short-circuit current across the wall of sacs of the rat's everted jejunum. The latter itself was a modification of another apparatus which has been used by Barry, Dikstein, Matthews, Smyth & Wright (1964) to measure the potential across the wall of sacs of the rat's everted intestine. The apparatus used for short-circuit current, Na^+ fluxes and other studies is diagramatically illustrated in Fig. 3.1. However, agar-KCl bridges were placed on each side of the tissue to monitor the potential, and the external current was introduced by means of two Ag/AgCl electrodes. The inside electrode was a 19-gauge silver wire, and 6cm length of which was plated with AgCl. The outside electrode was a 22-gauge silver wire which was wrapped spirally around three stable columns. The radius of the spiral form of the outside was 1cm; with a total length of 40cm. This electrode was plated with AgCl. All other surfaces of the silver electrodes were covered and insulated by polyethylene tubes. The plating procedure was performed according to Ives & Janz (1961) and the chloride coat was sufficient enough to exceed greatly the current that passed during the experiment. A freshly plated inside electrode was used for each experiment; but the outside electrode could be used many times. So the experimental arrangement of these

electrodes was such that a current of uniform density was applied across the whole length of the piece of intestine used.

Part of the experimental work was carried out by using a current electrode of KCl in 2% agar for the measurements of the short-circuit current across the intestinal wall. The inside current KCl-agar electrode (11cm. long, O.d 1.8mM and I.d 1.0mM) was open in a place about the middle of the segment. This opening was in addition to the opening of the distal end which was held in position about the area of the lower ligature around the intestinal segment. The outside current KCl-agar electrode (8cm. long, O.d 1.8mM & I.d 1.0mM) was held in position inside a glass tube which was one cm. from the central axis of the segment. The lower free end opening of the glass tube was about ½cm. shorter than the distal ligature around the intestinal segment. On the inner side of the glass tube another opening was made just about ½cm. distal to the upper ligature. The current KCl-agar electrode itself was inserted inside the glass tube up to half the way between the two openings. Each of the current electrodes was connected to an electrical conductive medium of 3M KCl. The current was conducted via a 22 gauge silver wire which was embedded in the KCl medium inside a polyethylene tube (60cm. long, O.d 5mM & I.d 3mM).

The potential polyethylene tubes (O.d 1.8mM & I.d 1.0mM) were filled with 3M KCl in 2% agar. The open end of the inside potential tube was held in position in the middle of the intestinal segment. This opening was to establish electrical contact between the KCl-agar and the bathing solution, and thereby to monitor potentials at that area of the serosal surface of the intestinal segment. An outside KCl-agar bridge was used to measure the potential on the mucosal side

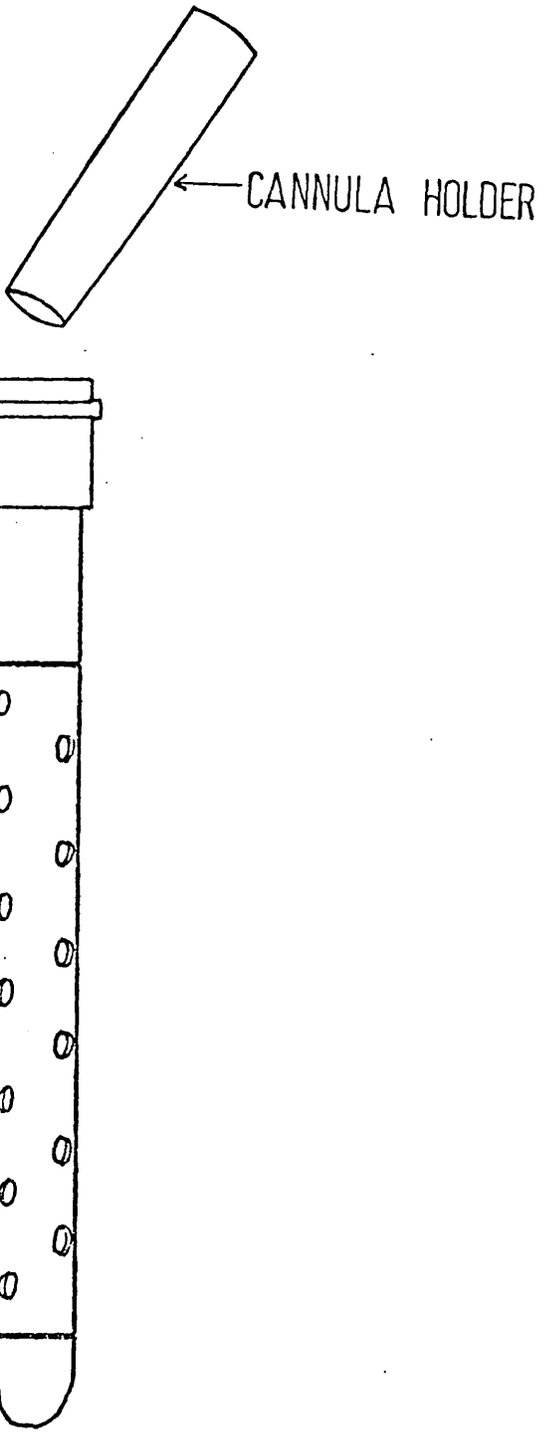


Fig. 3.2: SUPPORTING CANNULA

of the intestinal segment. This consisted of a polyethylene tube held rigidly in position inside a glass tube. The free-end opening of the bridge was adjacent to the open end of the inside bridge and approximately opposite the half-way mark of the intestinal segment.

Gut supporting cannula.

This is a new device introduced in this investigation for the first time. It was thought that this device would serve to hold the intestinal segment in position, rather than have the segment hanging freely in the bathing medium. Moreoever it was hoped that this device will act to minimise the serosal and the ~~muscolosal~~ motility without affecting the mucosa, and thereby a more stable current may be established. Mounting of the everted intestinal segment on the supporting cannula may serve to distend the sac slightly; thus reduce the thickness of the wall and separate the villi. It was thought that such a modification might improve the oxygen supply to the mucosa and to the deeper layers of the intestinal sac and thus improve the viability conditions of the sac. Fig. 3.2 is a diagramatic illustration of the cannula and its holder. It is a cylindrical-shaped tube of toughened polystyrene (O.d 0·57cm and about 7cm long). Its open end is a cone shape that is fitted properly around the tapered lower end of the cannula holder. The latter is a glass tube (5cm. long and O.d 0.5cm.) that passes through the centre of the jacket's cover. The cannula holder is held in position on a clamp with only one cm. of its length passing down through the jacket's cover.

Two shallow circular depressions were made around the cylindrical stem of the cannula. The locations of these depressions are ½cm and

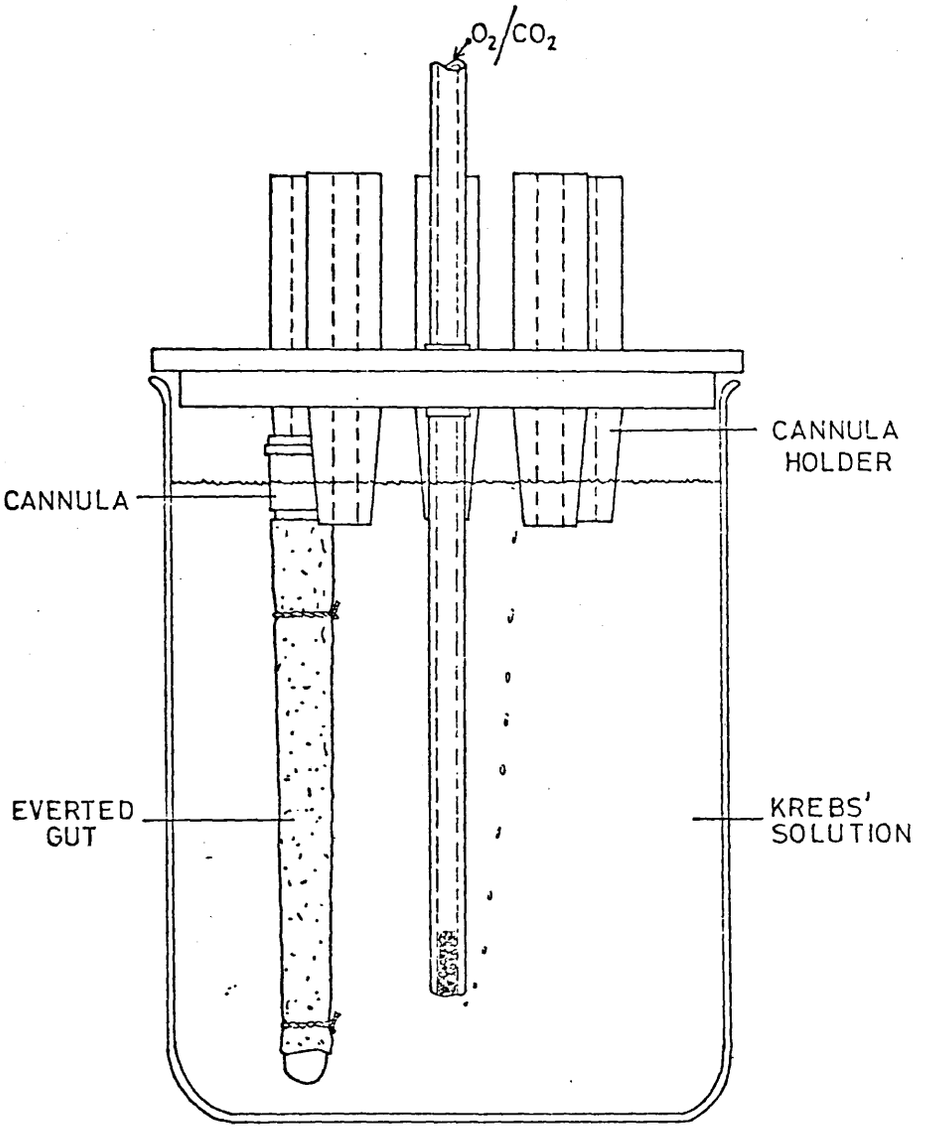


Fig. 3.3: SAMPLE RESERVOIR

5cm far from the distal closed end of the cannula respectively, i.e. the distance between the two depressions is 4.5cm. The area between the two depressions was perforated with 4 rows of small holes. The depressions were coloured, so it is easy to see them even after the intestinal segment is mounted over the cannula, thus the segment can easily be ligated on the cannula at the location of the depressions.

The complete assembly is placed in a water jacket (made of glass) which usually contains 60ml of the mucosal medium. Two similar jackets are used both supplied with water from a water bath (type SB4 of Grant Instrument Ltd.) with a thermostat. All the time during the experiment - except for the case of temperature-variation study - the jacket's medium temperature was kept constant by a continuous recirculation of water at 37°C. The mucosal medium was gassed with 95% O_2 - 5% CO_2. Anoxic conditions were brought about by replacing the O_2 by N_2 gas (i.e., 95% N_2 - 5% CO_2). The serosal medium (5 or 10ml.) was continuously recirculated by the aid of a pump (type MHRE 88 of Watson-Marlow Ltd.) at a flow rate of 2ml/minute. In some experiments the serosal medium was not circulated at all.

Segments reservation.

An arrangement was made (Fig. 3.3) which makes it possible to reserve up to five intestinal segments from the same animal. Five supporting cannulae holders were fixed in a uniform circular manner on the perspex cover of a 150ml beaker. In some experiments, the saline solution included 2-4mM glucose and the beaker was incubated in the water bath at 37°C. The medium was continuously gassed with 95% O_2 5% CO_2. Under these circumstances, the intestinal segments proved to be capable of surviving for more than two hours. Most of the

investigations included here were carried out on one or two preparations that have been taken from the middle part of the small intestine.

The voltage across the intestinal segment was monitored by connecting the agar-KCl bridges through calomel cells to a vibron electrometer (model 33B-2 Electronic Instrument Ltd.). The output to the meter of the electrometer could be fed into an operational amplifier which in turn energised a small D.C. motor which drove a rheostat moderating the potential applied through the current electrodes. This potential was provided from a series of dry cells, 12 volts each, and an appropriate number were applied to the rheostat which acted as a voltage divider. The wiper arm of the rheostat was connected to one of the current electrodes, the other being connected to the low potential end of the rheostat. In the "short circuit current" mode the electric motor drove the rheostat to increase or decrease the current through the tissue as necessary to hold the potential recorded by the electrometer at 0 ± 0.5mv. This negative feedback system was damped by inclusion of extra resistances in series with the Ag/AgCl current electrodes. The current was recorded by measuring the P.D across the 0.5 ohm resistor with a Kipp recording micrograph BD2 (Kipp & Zonen; Delfth. Holland). When using the 0.25m-volt range full scale of the recorder corresponded to 500 microamps. The 0.5 and 1.0mV. settings enabled the range to be increased to 1 and 2 m amps full scale respectively.

A backing off voltage was applied in some cases to limit the amount of short-circuit current which had to be applied. The backing off was not changed once experimental recording had started. The supporting cannulae with a mounted segment of everted gut provided - between the ligatures - an area of mucosa of 9.7 - 10.2 cm^2.

Measurements of short-circuit current, tissue resistance, sugar and Na^+ fluxes thus correspond to this (crude) area of mucosa i.e., without making any allowance for infolding and increases in area due to microscopic topography. This area therefore approximately equals to 10 cm^2.

Experimental Procedures.

The rats were anaesthetized by the intraperitoneal administration of 0.2 - 0.25ml of nembutal-pentobarbitone sodium (No. 8612, Abbott Laboratories Ltd.). To ease the physical process of injecting the nembutal, the rat was usually brought under light ether anesthesia. The ether effect was only temporary and usually lasted for a few minutes. Within about 10 minutes the rat came under complete anaesthesia. The peritoneal cavity was opened by midline incision and the exposed viscera was rinsed continuously with Krebs' saline solution at laboratory temperature. Two openings were made through the intestinal wall, one at each end of the intestine. The proximal opening was connected to a calibrated saline reservoir via a polyethylene tube. Intestinal contents were gently washed out by administrating 25ml Krebs' saline solution (at lab. temperature) from the reservoir. The intestinal contents and the administrated saline solution were allowed to escape spontaneously (without undue pressure) from the distal opening. A cut was made across the intestine at a point about 25cm from the end of the duodenum and another cut at about 8-10cm further down. The mesentery and the blood vessels were then severed. The excised segment was then immersed in the Krebs' saline at laboratory temperature. A length of thin glass rod (2-3mm diameter) was passed along the lumen and

tied to the other end of the segment. The latter was rapidly everted and passed over the supporting cannula and then the rod was cut free. The exposed mucosa was kept wet by rinsing it with Krebs' saline solution and the segment was ligated on the cannula at the site of the two depressions. The serosal medium (0.6ml) was introduced inside the cannula, which was then incubated in the reservation medium. The time that elapsed, between excision of the segment and the onset of incubation was less than three minutes. A spare segment was always prepared from the region which was immediately distal to the first one. The second segment was used in rare cases as an alternative for the first segment when the latter failed, for one reason or another, to give a good response. Infected segments showing patches of thickened mucosa were usually discarded, as they failed to give reasonable response.

Measurements of the short-circuit current (I_{sc}).

The tips of the KCl-agar bridges were positioned as closely as possible to the mucosal and serosal surfaces. The potentials measured in the experimental Krebs' saline medium prior to mounting the intestinal cannula were between 0.1 - 0.2mV. The intestinal cannula was transferred to the apparatus to fit tightly on the holder and the potentials readings were 3-4mV with sugar-free Krebs' solution on both sides. The short-circuit current recording micrograph was zeroed for every experiment and the segment of the intestine was then short-circuited by turning the rheostat manually. The external P.D was applied to the system from the bank of dry cells and the magnitude of the applied P.D. was chosen - with reference to the rheostat position - to be enough for clamping the P.D. at zero

throughout the experiment. The short-circuit current readings in the sugar-free Krebs' solution were higher in beginning, declining rapidly within the first 10-15 minutes, then more gradually during the next 20-30 minutes; both the I_{sc} and the P.D established a stable state from which there was not any more decline. However sugar investigation usually started at this stage. The steady state of the short-circuit current (with or without the use of a small backing off voltage) was considered to be the zero point for any further change that may be stimulated by the sugar added to the experimental medium. Changing the contents of the mucosal solution was accomplished by preparing a new working medium in the second water jacket. The volume (60ml) of the mucosal medium was always constant. Before transferring the whole assembly to this latter jacket, the solution was mixed very well. It was not difficult to transfer the whole assembly which included the intestinal segment from one jacket to the other. The transferring process was usually carried out as quickly as possible and with the utmost care in order not to disturb the efficiency of the system.

Calculations of the short-circuit current (I_{sc}).

Since the chart (on which the I_{sc} was recorded) is divided to equal and uniform units of (μA), it should be easy to calculate the change in the I_{sc} which is induced by the substrate addition at the mucosal or serosal side. As mentioned above, the steady-state-zero point was determined while the intestine was immersed in the sugar-free Krebs' solution. Then the changes in the I_{sc}, related to the sugar, inhibitor, time, temperature, etc. would be measured in relation to the steadystate-zero point. The mean values of the obtained I_{sc} and the standard error (SE) were calculated for each group of readings.

Effects of various concentrations of sugars on the I_{sc}.

Different sugars (monosaccharides and disaccharides) were applied to the mucosal side. The intestinal segment was usually bathed with sugar-free Krebs' solution until the steady state of the I_{sc} was established. Then the whole assembly was transferred to the Krebs' solution that contained sugar at various concentrations for six minutes each, and the sugar-free Krebs' solution was applied again. As mentioned above, two water jacketed baths were used for the purpose and a sugar concentration of 1.1mM was usually used to start with and increased in a step-wise manner up to 22.2mM; higher concentrations were used in some cases. The recorded reading for each concentration was measured from the zero-point at the steady state up to the new recorded reading at the end of six minutes. In this manner the experiments were carried out not only at 37°C, but at temperatures as low as 17° and 27° C.

The Effects of changes in the medium temperature on the I_{sc}.

In this part of investigation, either 4.4mM glucose-saline (Krebs') solution or glucose-free Krebs' solution was used at the mucosal side of the intestinal segment. But 10ml of 22.2mM glucose-Krebs' solution was recirculated at the serosal side of the segment. The temperature of the segment's bathing medium was increased by the aid of the recirculated water in the bath being raised from 22 to 45° C successively.

Effects of the non-transportable sugars on the I_{sc}.

Different concentrations of ethylidene glucose, benzylidene

glucose, mannose, lactose and cellobiose were applied at the mucosal side of the intestinal segment in the presence and absence of the actively transported sugars like 3-O-methyl glucose, galactose and glucose. At the beginning of the experiment the intestinal segment was bathed with sugar-free Krebs' solution until the steady state of the short-circuit current (I_{SC}) was accomplished. Then the whole assembly was transferred to the actively transported sugar medium which stimulated an increment in the I_{SC}. Soon after the steady state of the I_{SC} was established different concentrations of the non-transportable sugar were added to the mucosal side in a step-wise manner. The two water jackets were used alternatively for this purpose. The concentrations of the non-transportable sugars were added simultaneously with the same concentration of the actively transported sugar that previously stimulated the I_{SC}. In other groups of experiments it was chosen to start with a high concentration of 14.7mM ethylidene glucose and work down throughout the experiment until the mucosal medium contained the actively transported sugar only. Ethylidene glucose (20mM) also was added to the serosal side of the segments with the presence of 1.1 or 4.4mM glucose solution at the mucosal side. Other experiments were undertaken by the addition of different concentrations of the non-transportable sugar in the absence of any other actively transportable sugar. Addition of these sugars usually was started soon after the steady state of the I_{SC} was established in mucosal sugar-free Krebs' solution. Krebs' solutions with or without sugar were variously used at the serosal side of the intestinal segment.

Effects of time factor on the I_{sc}.

The effects of elapsed time on the short-circuit current across the intestinal wall was investigated and different sugars were used for this purpose. As in all other investigations included in this work, the intestinal segment was immersed in the sugar-free saline (Krebs) solution until the steady state of the I_{sc} was established. Then the whole assembly was transferred to the second jacket which contained a known concentration of the sugar-saline solution. The recorded increments of I_{sc} were measured at successive equal intervals of time.

Measurements of Na^+ fluxes.

Unidirectional Na^+ fluxes, from mucosa to serosa and serosa to mucosa were determined simultaneously by double labelling with (Na^{24} & Na^{22}), and individually using Na^{22} to assess the effects of ethylidene glucose on the fluxes. About 20 μCi of Na^{24} were added to a known volume (60-64)ml of mucosal Krebs' saline medium. About 5 μCi of Na^{22} were added to 50ml of Krebs' saline solution with or without mannose in a 150-ml-flask and out of which 5ml. was recirculated at the serosal side. The duration of the experiment was usually 140 minutes. The intestinal segment was allowed to stay in the saline-radio-active medium for periods of 20 or 40 minutes. At the end of the latter period a known concentration of 3-O-methyl-glucose was added, and remained in contact with the mucosa for 40 minutes, at the end of which another dose of sugar was added. The serosal medium was withdrawn at the end of each experimental period and simultaneously replaced by fresh aliquots. Withdrawal of the serosal sample took place at 10 or 20 minutes intervals; a 0.5 or 1ml sample from the mucosal side was taken as well. The serosal sample was collected in a small vial (of known weight) and then the weight of the sample was determined accurately by reweighing the vial with the sample. A period of 20 minutes was allowed for the fluxes to be accomplished at a steady state. One ml out of the extracted serosal fluid was taken for counting. Samples were assayed by using an Auto-Gamma Spectrometer (Packard 3375) and all samples were counted for Na^{24} at once. The threshold was set so as to exclude the weaker radiations from the Na^{22}. The mucosal samples were counted for Na^{22} three weeks later. The sugars (3MG or/and E·G) were added to the mucosal side from a concentrated stock solutions. Simultaneously

the I_{sc} was recorded all the way through the experiment.

Examples of calculations of unidirection Na^+ fluxes, net Na^+ flux and the related Isc.

Date of the experiment = 1.8.74.

Na^{24}cts - (control cts + Na^{22}cts in the channel of Na^{24}) = 228, 254 and 241; these cts are related to the periods in between 80-100, 100-120 and 120-140 mins. respectively, i.e. after the addition of the second dose of 3MG, and so the resultant mucosal concentration of the sugar was about 10mM.

The mean cts of 0.1ml. standard mucosal medium (Na^{24}) = 3335

Since each 0.1ml. of 144mM Na^+ solution contains 0.0144mM or 14.4 μmoles of Na^+

$\therefore \frac{3335}{14.4} = 232$

$\therefore$ 228 ÷ 232 = 0.983 μmoles Na^+/1ml/20 mins.

But the extracted serosal fluid weight was 5.61 gm.

So 0.983 x 5.61 = 5.52 μmoles Na^+ transported/20 mins.

$\therefore$ 5.52 ÷ 20 = 0.276 μmoles Na^+ transported/1 min.

In a similar way the other results should be equal to: 0.268 and 0.265 μmoles respectively..

Finally the mean of the three results are≡ 0.2697 ≡ 0.27

Three weeks later 1 ml (for each case) of the mucosal medium was assayed for Na^{22} (in the red channel).

The acquired cts for the same periods above respectively were 7.5, 3.0 and 4.3.

Since the mean cts of 1ml. standard serosal medium (Na^{22}) = 15804

$\therefore$ 144 mM Na^+ x $\frac{1}{1000}$ = 15804

i.e. 144 µmoles Na^+ = 15804

$\therefore$ 15804 ÷ 144 = 110 cts per each µmole of Na^+

$\therefore$ 7.5 ÷ 110 = 0.0682 µmoles Na^+/1ml/20 mins.

Since the mucosal volume at this stage of the experiment = 58ml.

$\therefore$ 0.0682 x 58 = 3.9556 µmoles Na^+ transported/20 mins.

3.9556 ÷ 20 = 0.1978 µmoles Na^+ transported/1 min.

In a similar way the other two results should be equal to 0.0778 and 0.1095 µmoles respectively.

The mean of the three readings = 0.1284 ≡ 0.13 µmoles Na^+ transported/1 min to the mucosal side.

$\therefore$ The net Na^+ flux = 0.27 - 0.13 = 0.14 µmoles/1 min.

Calculation of the net Na^+ flux, from the recorded I_{sc} at the same periods above

Since 1 µA = 0.00062 µmole Na^+/min.

Also since the mean of the I_{sc} recorded during the ~~maintained~~ mentioned periods = 203 µA

$\therefore$ The Na^+ flux = 203 x 0.00062 = 0.1259 ≡ 0.13 µmoles/1 min.

Na^+ net flux µmole/min	I_{sc} µmole/min
0.14	0.13

Measurements of 3-O-methylglucose influx.

A number of experiments were carried out in an attempt to find the relationship between the sugar influx and the I_{sc} and consequently to explore the stoichiometric relationship between 3-O-methylglucose and Na^+. As usual the intestinal segment was first bathed in sugar-free Krebs' solution until the I_{sc} steady state was achieved. Either sugar-free Krebs' or Krebs' mannose (10mM) solution was recirculated at the serosal side through the experiment. 3-O-Methylglucose - C^{14} was added to the mucosal Krebs' solution in two steps. In the first step the concentration of the added sugar was usually about 5mM and it remained in contact with the mucosa for one hour, at the end of which another dose (about 17mM) was added to the medium and remained for another hour. For each step, after allowing a 20 minute equilibration period, its steady state rate of appearance in the opposite solution was determined. Also 3-O-methylglucose influx was assayed using an increasing step-wise mucosal concentration up to 55mM.

The effects of ethylidene glucose on the influx of 3-O-methyl-glucose and the related I_{sc} were also included in this field of study for the first time. Ethylidene glucose was used either on the mucosal or the serosal side. Two different concentrations of ethylidene glucose being attempted at the mucosal side of the intestinal segment. A similar study was carried out using phloridzin (10^{-2} and 2×10^{-2}mM) at the mucosal side.

The sugar and the inhibitor were added to the mucosal side from a concentrated stock solutions. Appropriative volumes of the isotope - C^{14} solution were added to the stock solution of 3-O-methylglucose.

At the end of each experimental period the recirculating serosal medium (5ml) was pumped into a vial (of a known weight) and simultaneously replaced by a new 3MG - C^{14} free medium. The extracted volume of the serosal medium was then determined accurately by weighing it in the vial. Out of this medium, one ml. was added to 10ml. of the scintillation fluid (type NE 260) in a small glass bottle with a tightly fitting stopper. For each step 0.5ml. of the mucosal medium was withdrawn and added to 10ml of the scintillation fluid. All samples were then assayed for C^{14} in the liquid scintillation spectrometer (model 3375).

Examples of calculations.

Experiment date ≡ 1.2.74

Preparation of the stock solution of 3-O-methylglucose - C^{14}.

Since 194 mg (3MG)/litre ≡ 1mM

and since the final volume of the mucosal solution = 61 ml.

and it was desired to work with a concentration of 22mM

produced by adding 1ml of a concentrated solution to 60ml.

of medium, it was calculated that it would be necessary for

260 mg. (3MG) to be dissolved in 0.8 ml. Krebs' saline solution

to which 0.2 ml. C^{14} was added (the radioactive sample contained

only trace amounts of the sugar)

Initially about 0.2 ml. of the 3-O-methylglucose - C^{14} stock medium was added to the mucosal-Krebs' saline (about 60 ml.)

Serosal samples were taken at	Samples No.	cts	Cts/ml.
20 mins.	52		173.6
30 "	53		123.7
40 "	54		132.4
50 "	55		153.1
60 "	56		144.1

At 63 minutes, the rest of the stock sugar - C^{14} was added to the mucosal medium. So the final mucosal medium concentration should be about 22mM.

Further samples were taken at	Samples cts No.	Cts/ml
80 mins.	57	492.7
90 "	58	406.9
100 "	59	403.5
110 "	60	422.1
120 "	61	443.1

Three mucosal samples (0.5 ml each) were taken at	Samples cts No.	Cts/0.5 ml.
03 min.	51	11837
65 min.	62	56490
120 min.	63	56173

From the mucosal counts of the last two samples which were derived from 0.5 ml. of a 22mM(3MG) solution it can be seen by adding:

No. 62 = 56490

No. 63 = 56173

that (No.62 + No.63) = 112663 cts/ml of 22 mM. (3MG)

$\therefore$ 1 ml. of 1 mM $\equiv$ 1 μmole $\equiv$ 5121 cts.

1. Thus the concentration of (3MG) in the initial mucosal medium, i.e. sample No.51 = $\frac{11837 \times 2}{5121} = 4.62 \equiv 4.6$mM
2. And the rate of (3MG) transport into the serosal fluid can be calculated e.g.

   sample No. 52 = $\frac{173.6 \times 4.8}{5121} = 0.162\,\mu$mole/20 min.

   4.8 is the extracted serosal volume (ml) at the end of 20 min.

Similar calculations for the other serosal samples give

53 = 0.116 µmole /10 mins.

54 = 0.127 " " " "

55 = 0.144 " " " "

56 = 0.138 " " " "

57 = 0.433 " " /20 mins.

58 = 0.381 " " /10 mins.

59 = 0.378 " " " "

60 = 0.396 " " " "

61 = 0.415 " " " "

Mean of (53, 54, 55 & 56) = 0.131 µmole/10 mins.

" " " = 0.0131

= 0.013 µmole/min.

Mean of (58, 59, 60 & 61) = 0.393 µmole/10 mins.

= 0.0393

= 0.039 µmole/min.

3. Calculations of Na^+ net flux from the measured I_{sc}, assuming the change in I_{sc} is due only to a change in the net Na^+ movement.

Mean of I_{sc} for (53, 54, 55 & 56) = 45 µA

Mean of I_{sc} for (58, 59, 60 & 61) = 98 µA

Since 1A = 1 coulomb/sec

But 1 mole = 96500 coulombs

So 1A = $\frac{60}{96500}$ = 0.00062 mole/min.

$\therefore$ 1 µA = 0.00062 µmole/min

So

45 x 0.00062 = 0.0279

= 0.028 µmole/min. net Na^+ transferred

98 x 0.00062 = 0.0608

= 0.061 µmole/min. net Na^+ transferred

i.e. 0.028 µmoles Na^+ ≡ 0.013 µmoles (3MG)

or 2.2 ≡ 1.0

and 0.061 µmoles Na^+ ≡ 0.039

or 1.6 ≡ 1.0

RESULTS OF KINETIC STUDIES

CHAPTER 4.

Studies on the effects of different concentrations of sugars on the short-circuit current.

As mentioned in the introduction, it seems that at least there are two different groups of results concerning the relationship between the rate of sugar absorption in the intestine and their concentrations. The first group failed to show rate limiting kinetics and the Michaelis-Menten (1913) analysis was not applicable, but the second group of results showed that the relationship is nonlinear and seems to follow Michaelis-Menten kinetics.

Following a new method i.e., the gut supporting cannula method and on the basis of short-circuit current, the relationship between the rate of sugar absorption and its concentration has been re-examined in this chapter. Glucose, galactose and some glucose derivatives i.e., 3-O-methylglucose, α-methylglucose, β-methylglucose and β-phenylglucose have been used in this study. The method followed mainly was to take an intestinal segment (4.5 cm long) from the region between 25-35 cm from the proximal end of the jejunum (labelled segment-A) or from the region between 35-45 cm from the proximal end of the jejunum (labelled segment-B). The segment then was everted on the supporting cannula and clamped in the apparatus to be immersed in the oxygenated saline (free of sugar) until the steady state of the I_{sc} was established. The serosal fluid usually was also sugar-free saline and remained the same throughout the experiment, except in one case when inositol-Krebs' solution was used (Table 4.4 second set). The mucosal sugar concentration was increased in a step-wise manner at six minute intervals. As shown in Tables (4.1 - 4.4) and Figures (4.1, 4.3, 4.5, 4.7 and 4.9), the applied concentrations of the sugar usually covered the range from 1.1mM up to 22.2mM, but in the case of 3-O-methylglucose a wider range up to 44.4mM was applied.. In most of the experiments the "concentration range" of

one sugar only has been used (Tables 4.1 - 4.3 and Figures 4.1, 4.3 and 4.5) at a time. Also an attempt was made to compare the changes in the short-circuit current induced by two different sugars, one after the other and vice versa, across the same intestinal segment (Table 4.4 and Figures 4.7 and 4.9).

Finally, to investigate the mutual competition of sugars on the basis of short-circuit current, different concentrations of galactose (1.1 - 8.8mM) were mixed with 8.8mM glucose solution in a step-wise manner as shown in Table (4.5).

Results

Figures (4.1 and 4.3) show the I_{sc} obtained for different concentrations of glucose and galactose respectively by using the silver/silver chloride electrodes and the agar electrodes. The discrepancy in the results obtained by using the two different electrodes systems may be related to the fact that the silver/silver chloride electrodes system was built in a concentric fashion with a view of ensuring a more equal distribution of the electrical current. In the case of a nonconcentric system as occurred with the agar electrodes, one may expect that the electrical current instead of being equally distributed tended to be unevenly directed and thus may have introduced an amplification of the measured current before returning the PD to zero. The implication of this discrepancy may be related to some other factors as well e.g., the difference in the resistance of the two systems. Although such amplification would introduce a systematic error in the maximal short-circuit current, it should not affect drastically the determined half-saturation constants.

TABLE 4.1

The changes in the I_{sc} induced by different concentrations of mucosal glucose-Krebs' solution across the intestinal segments (A & B) of the rat.

Agar electrodes and silver/silver chloride electrodes were used for the I_{sc} measurements. Sugar-free Krebs' solution (10 ml) was re-circulated at the serosal side of the segment but for segment (B), 0.6 ml of the same solution was used. The numbers in parenthesis indicate the number of animals used. In all instances, the mean values of the obtained $I_{sc} \pm$ SE are given.

Mucosal glucose conc. (mM)	(5) Segment (A) Ag/AgCl electrodes I_{sc} (μA)	(6) Segment (A) Agar electrodes I_{sc} (μA)	(6) Segment (B) Agar electrodes I_{sc} (μA)
1.1	61 ± 1	124 ± 16	222 ± 23
2.2	116 ± 12	254 ± 26	448 ± 38
4.4	238 ± 22	466 ± 48	711 ± 47
6.6	322 ± 26	593 ± 59	858 ± 60
8.8	380 ± 29	442 ± 64	904 ± 73
13.2	398 ± 32	654 ± 64	905 ± 80
17.6	399 ± 35	638 ± 67	818 ± 97
22.2	388 ± 35	617 ± 65	794 ± 108

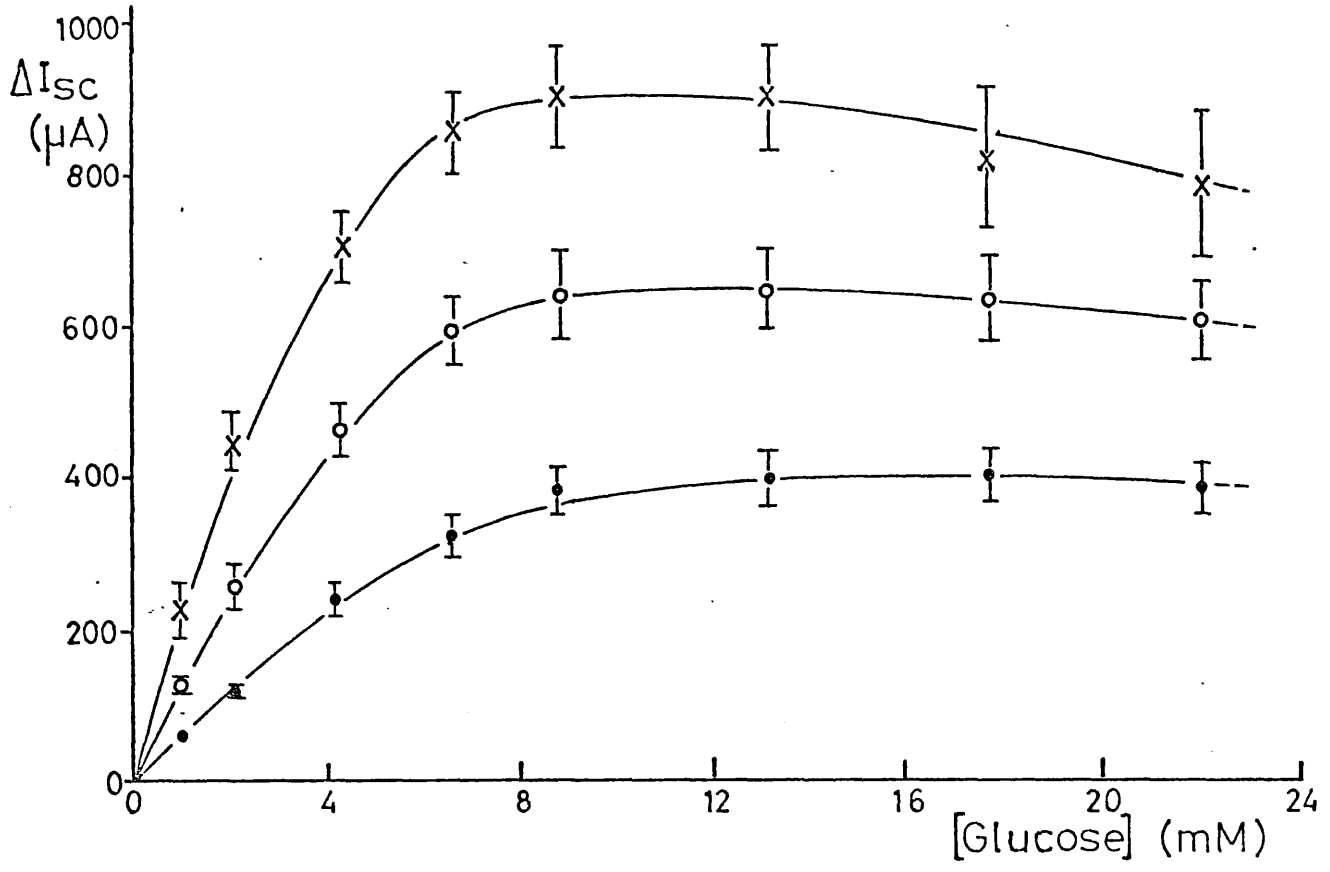


Fig. 4.1 Effect of mucosal glucose on short circuit current (Table 4.1). Segment A: • , Ag/AgCl electrodes; o , Agar electrodes. Segment B: X , Agar electrodes.

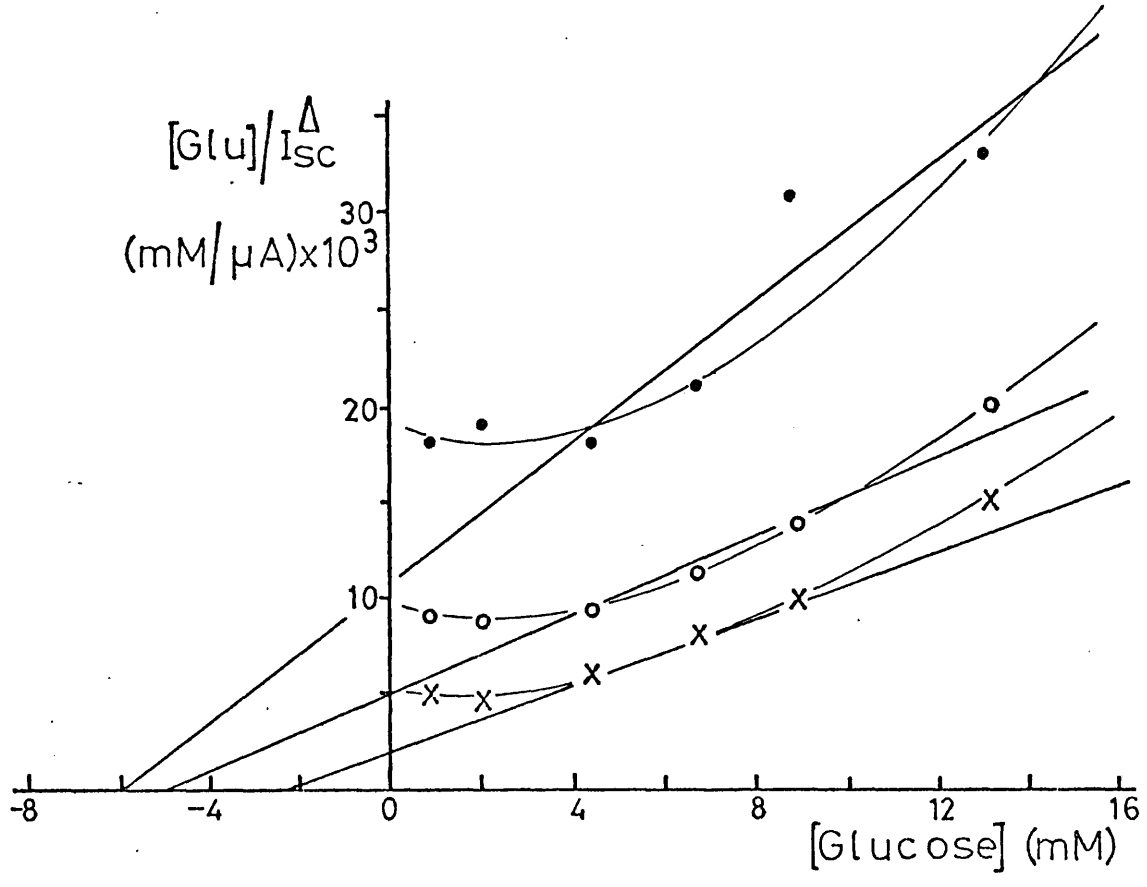


Fig. 4.2 Linear plots of Fig.4.1. Points as above. Regression equations:- • ,y = 1.8x + 11 ; o , y = 1.1x + 5.1; X , y = 0.9x + 2.0.

TABLE 4.2

The changes in the I_{sc} induced by different concentrations of mucosal galactose-Krebs' solution across the intestinal segment (A) of the rat.

Agar electrodes and silver/silver chloride electrodes were used for the I_{sc} measurements. In all instances, sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. The numbers in parenthesis indicate the number of animals used. The mean values of the obtained $I_{sc} \pm$ SE are given.

Mucosal galactose conc. (mM)	(4) Agar electrodes I_{sc} (μA)	(2)* Ag/AgCl electrodes I_{sc} (μA)	
1.1	31.7 ± 3.3	----	----
2.2	65.0 ± 6.2	20.0 ± 2	----
4.4	164 ± 20	47.5 ± 7.4	----
6.6	255 ± 29	64.5 ± 7.4	79.0 ± 4.2
8.8	281 ± 34	75.0 ± 0.0	86.5 ± 4.6
13.2	326 ± 38	83.5 ± 3.5	95.0 ± 3.5
17.6	338 ± 46	72.5 ± 8.8	----------
22.2	324 ± 47	54.0 ±11.3	74.0 ± 4.2
26.6	306 ± 45		
26.6 + 13.2mM glucose	271 ± 40		

* Each column represents the means ± SE of two experiments.

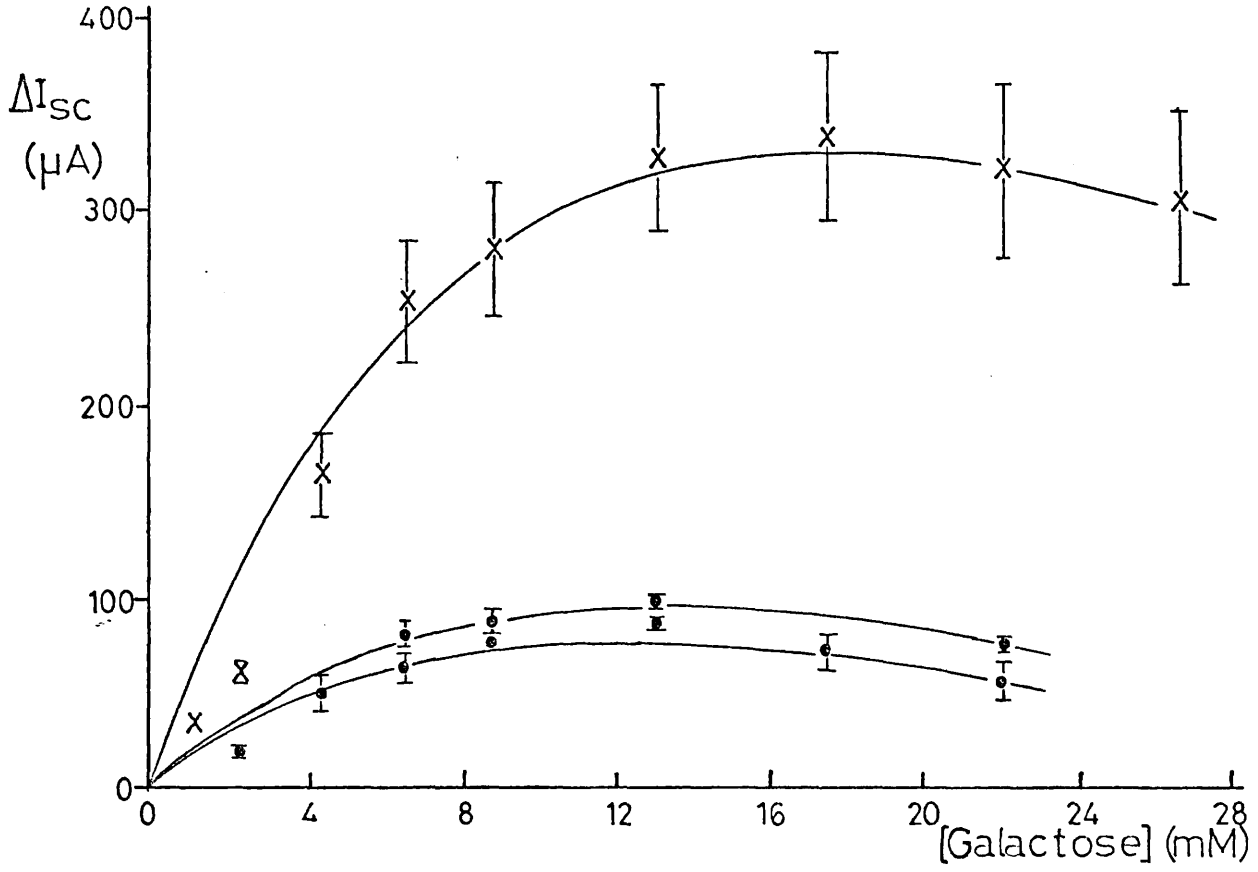


Fig. 4.3 Short circuit current induced by mucosal galactose. (Table 4.2)
• , Ag/Ag Cl electrodes; x , Agar electrodes.

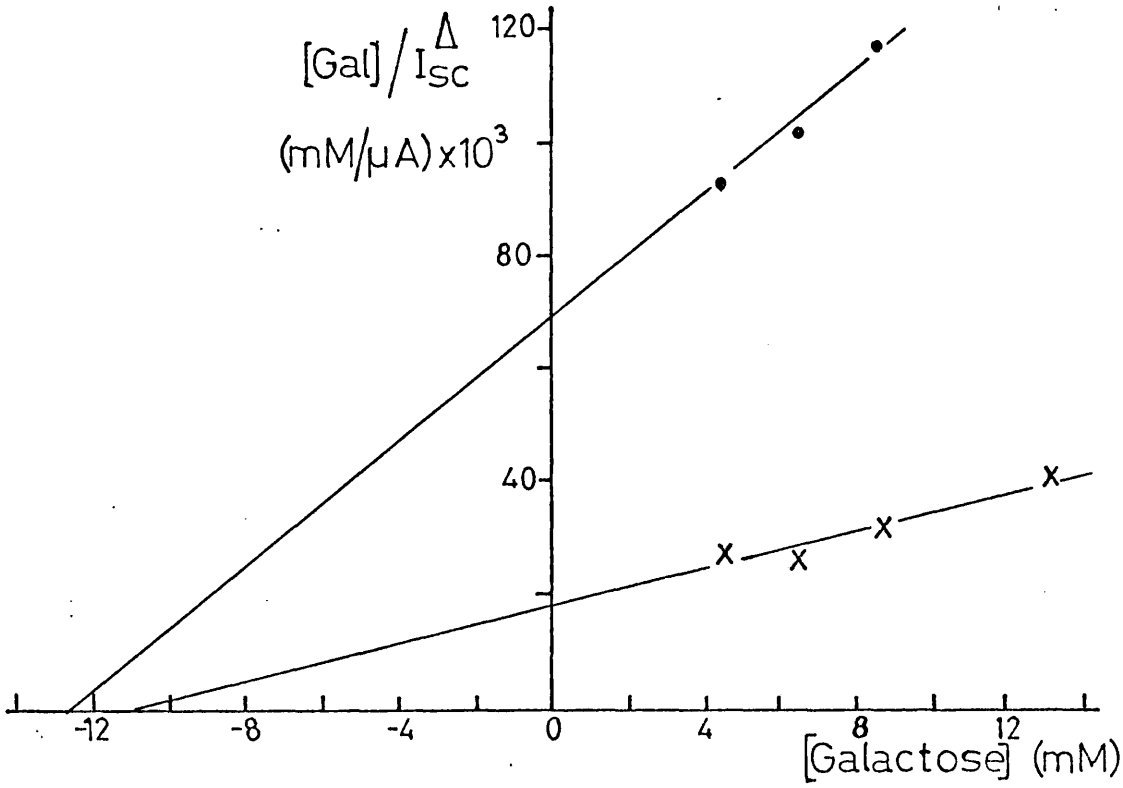


Fig. 4.4 Linear plots of Fig 4.3. Regression equations:- Ag/AgCl electrodes.
• , y = 5.5x + 68 ; Agar electrodes, x , y = 1.6x + 17.7.

TABLE 4.3

The changes in the I_{sc} induced by different concentrations of mucosal 3MG, αMG, βMG and βPG dissolved in Krebs' saline solution.

Silver/silver chloride electrodes were used for the measurements of the I_{sc} across the intestinal segments of the rat. Sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. The numbers in parenthesis indicate the numbers of animals used. The mean values of the obtained $I_{sc} \pm$ SE are given.

Mucosal sugar conc. (mM)	I_{sc} (6) induced by (3MG) (μA)	I_{sc} (6) induced by (αMG) (μA)	I_{sc} (μA) (6) induced by (βMG)	I_{sc} (μA) (3) induced by (βPG)
1.1	-----	31.2 ± 4.0	17.0 ± 2.1	-----
2.2	-----	71.5 ± 9.1	30.2 ± 3.2	-----
4.4	-----	118 ± 12	44.0 ± 4.7	18.0 ± 0.8
6.6	24.0 ± 4.2	137 ± 13	41.5 ± 5.1	23.7 ± 1.4
8.8	-----	135 ± 14	34.3 ± 6.2	25.7 ± 1.9
13.2	50.0 ± 7.1	125 ± 14	30.4 ± 5.2	18.5 ± 1.1
17.6	70.0 ± 10.6	121 ± 15	24.4 ± 5.3	15.7 ± 2.4
22.2	-----	113 ± 15	19.2 ± 4.4	11.7 ± 4.9
24.4	82.5 ± 8.8			
33.3	82.5 ± 8.8			
44.4	75.0 ± 3.5			

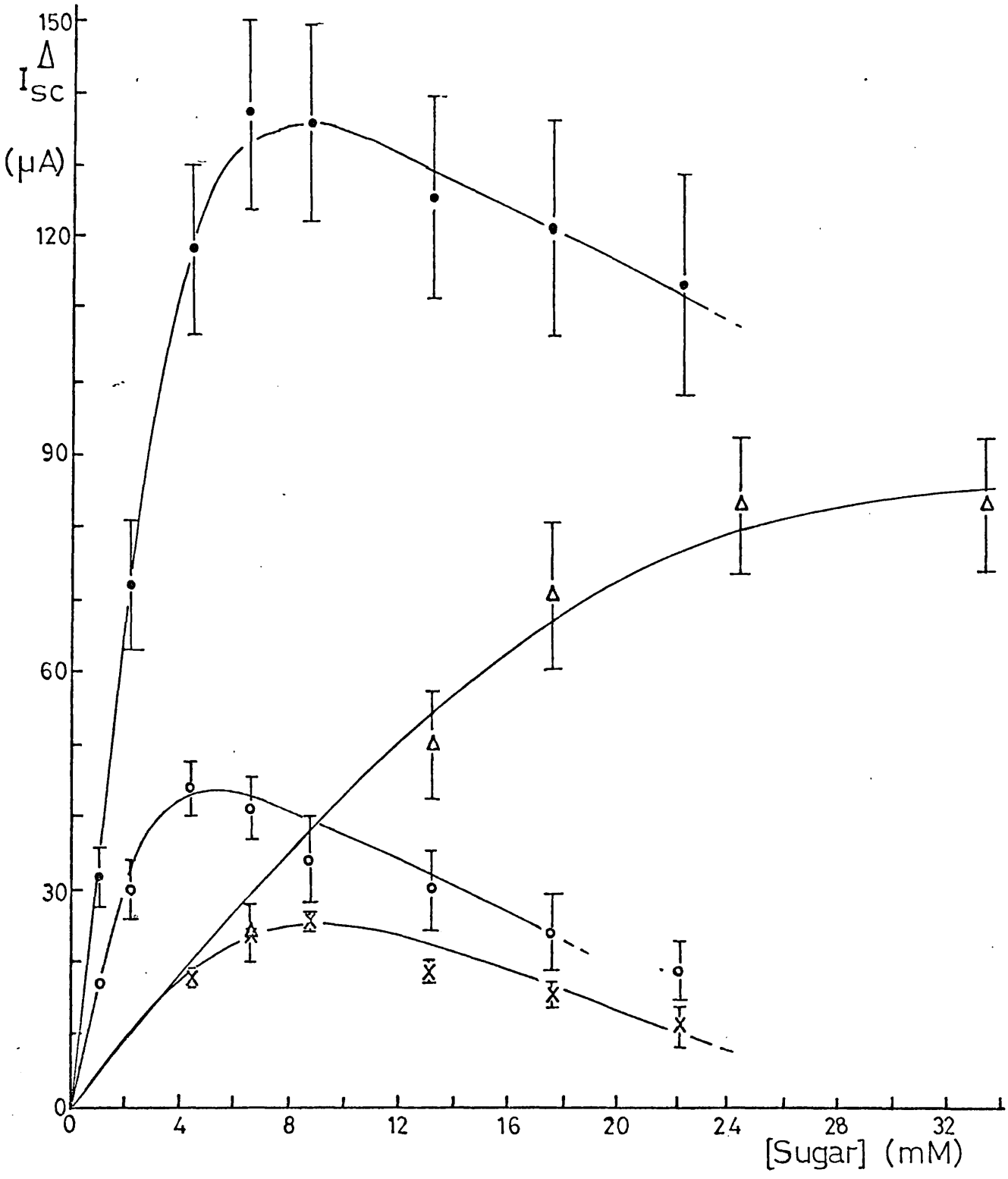


Fig. 4.5 Short circuit current induced by various sugars using Ag/AgCl electrodes. (Table 4.3). • , α-methylglucoside; o , β-methylglucoside; X , β-phenylglucoside; Δ , 3-O-methylglucose.

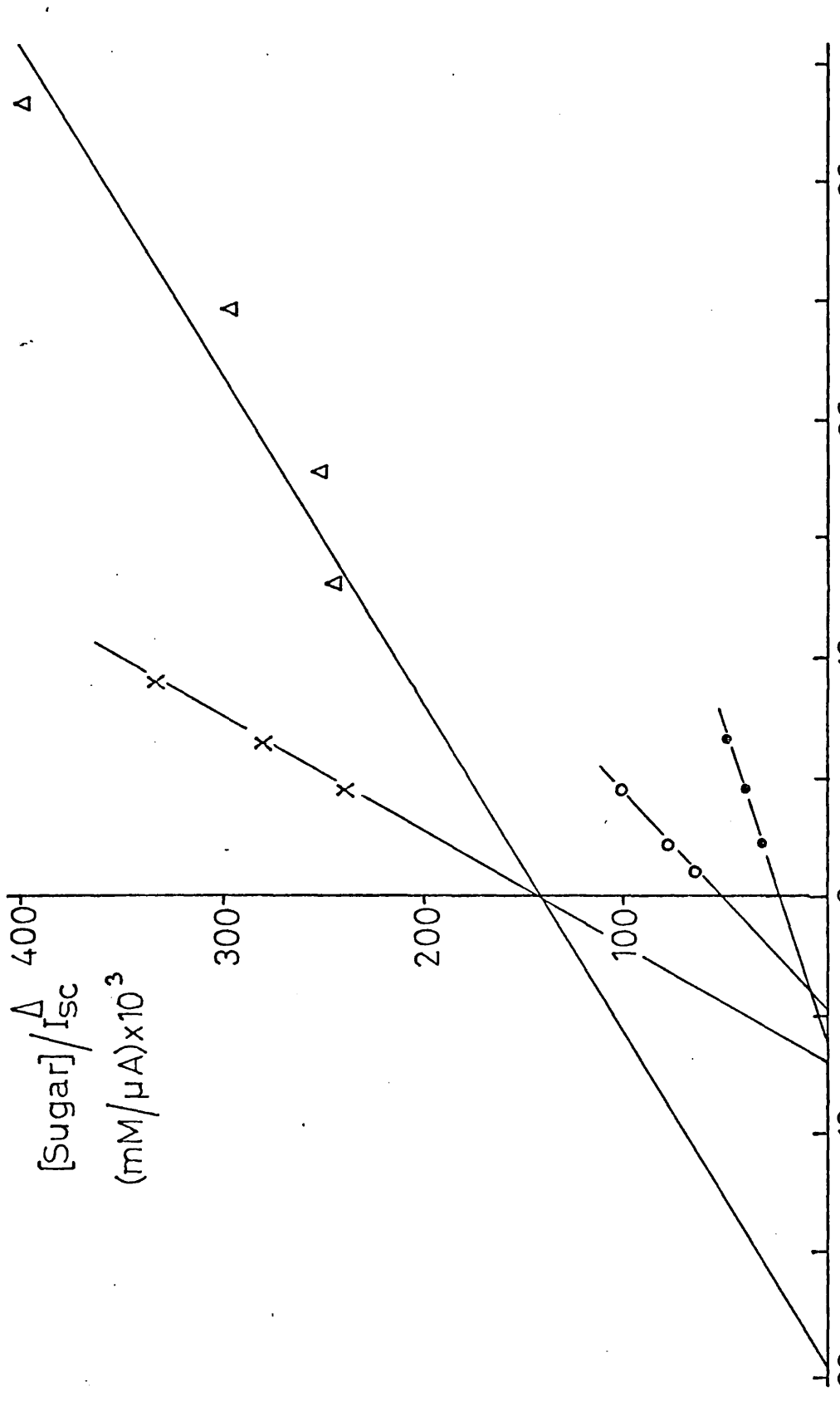


Fig. 4.6 Linear plots of Fig 4.5. Regression equations:- α-methylglucoside • , y = 3.9x + 21.7 ; β-methylglucoside o , y = 10.8x + 51.5 ; β-phenylglucoside X , y = 21.4x + 145 ; 3-O-methylgluoose Δ , y= 7.4x + 141.

Figure (4.1) also shows the I_{sc} obtained (using the agar electrodes) by applying the same range of glucose concentrations at the mucosal side of two adjacent segments. The first segment (A) was taken from the region between 25-35 cm from the proximal end of the jejunum, and the second segment (B) was taken from the 10 cm directly distal to the first one. It can be noticed that although the two segments are adjacent a clear difference in the obtained I_{sc} was found, that in segment (B) being the greater.

The different levels of the sugar concentration gave graded responses in the short-circuit current up to a certain limit whereupon no further appreciable increment was observed. Figures (4.1, 4.3, 4.5, 4.7 and 4.9) clearly show that the relationship between ΔI_{sc} and the added sugar concentration is nonlinear and suggest that the relationship is consistent with Michaelis-Menten kinetics for a saturable enzyme (or carrier) system. If this is so, a straight line may be obtained by plotting the concentration on the abscissa versus the concentration/I_{sc} on the ordinate for each sugar (as recommended by Riggs, 1971). For each case the straight line was obtained statistically by a regression equation (Figures 4.2, 4.4, 4.6, 4.8, 4.10 and 4.12). In these equations, X represents the sugar concentration at the abscissa and Y represents the sugar concentration/short-circuit current at the ordinate. The points that lie on the hyperbola were usually used to draw the straight line. This approach has been followed here specially because the sugar concentrations in excess of a certain level caused the short-circuit current to decline or to flatten out as is clear in most curves. As for as the effects of higher concentrations of sugar on the electrical responses are concerned, Lyon & Crane (1966a) suggested

that osmotic effects which are dependent only on the number of sugar molecules and not on their structural configuration, distort the PD values. Their curve which illustrates the relationship between PD and the glucose concentration is similar to the curves presented here to show the relationship between the I_{sc} and the sugar concentrations.

The lower concentrations were excluded as well when the straight lines were drawn. This was because in most cases the relationship between the I_{sc} and the concentration at the lower levels (about 2.2mM) seemed not to be hyperbolic in shape and practical inspection of the results excluded such points in the linear plots. Asano (1964) used sheets of intestinal wall of rat for the study of the relationship between different glucose concentrations and the electrical parameters. He stated that the relationship is definitely nonlinear and seems to follow Michaelis-Menten (1913) kinetics and he added that the points depicted in his lineweaver - Burk (1934) plot seem to lie on a straight line except for the lower concentration (12.5 mg/100 ml). He also stated that experiments which were carried out to obtain a similar relation between the short-circuit current and the galactose concentrations failed especially at low concentrations of the sugar.

In each case the straight line plot was obtained with at least three points that lie very close to the line. As an example, in (Fig.4.2) most of the obtained readings (Table 4.1) have been plotted; only the points that lie close to the line have been practically used in the regression as illustrated in the Figure. Thus in each case a linear plot was made as recommended by Riggs (1972) and regression method was followed to obtain the Ki and ΔI max (Tables 4.6 and 4.7). Also the half saturation constant of sugar has been calculated

TABLE 4.4

The changes in the I_{sc} induced by different concentrations of mucosal glucose and galactose dissolved in Krebs' solution.

Using the silver/silver chloride electrodes the I_{sc} induced by the two sugars was measured across the same intestinal segment (A) in different orders. In one case glucose was assayed before galactose,in the other galactose was assayed first and glucose second. Sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. Using agar electrodes and 100mM inositol at the serosal side of the segment, similar technique was followed for galactose and 3MG. The numbers in parenthesis indicate the number of animals used.

First Set (4)

Mucosal sugar conc. (mM)	I_{sc} (μA) induced by glucose	I_{sc} (μA) induced by galactose
2.2	50.0 ± 12.4	11.5 ± 3.0
4.4	82.5 ± 20.3	36.5 ± 6.5
6.6	90.5 ± 20.3	56.5 ± 6.5
8.8	92.5 ± 22.1	60.0 ± 7.8
13.2	90.5 ± 22.1	56.5 ± 6.5
22.2	86.5 ± 20.0	42.5 ± 2.7

Second Set (6)

Mucosal sugar conc. (mM)	I_{sc} (μA) induced by galactose	I_{sc} (μA) induced by 3MG
8.3	73.6 ± 16.9	39.7 ± 9.3
12.5	126 ± 20	-----
16.7	125 ± 21	78.9 ± 16.9
20.9	120 ± 19	-----
25.0	107 ± 16	87.6 ± 17.5
33.3		90.1 ± 16.0
41.7		82.4 ± 14.1

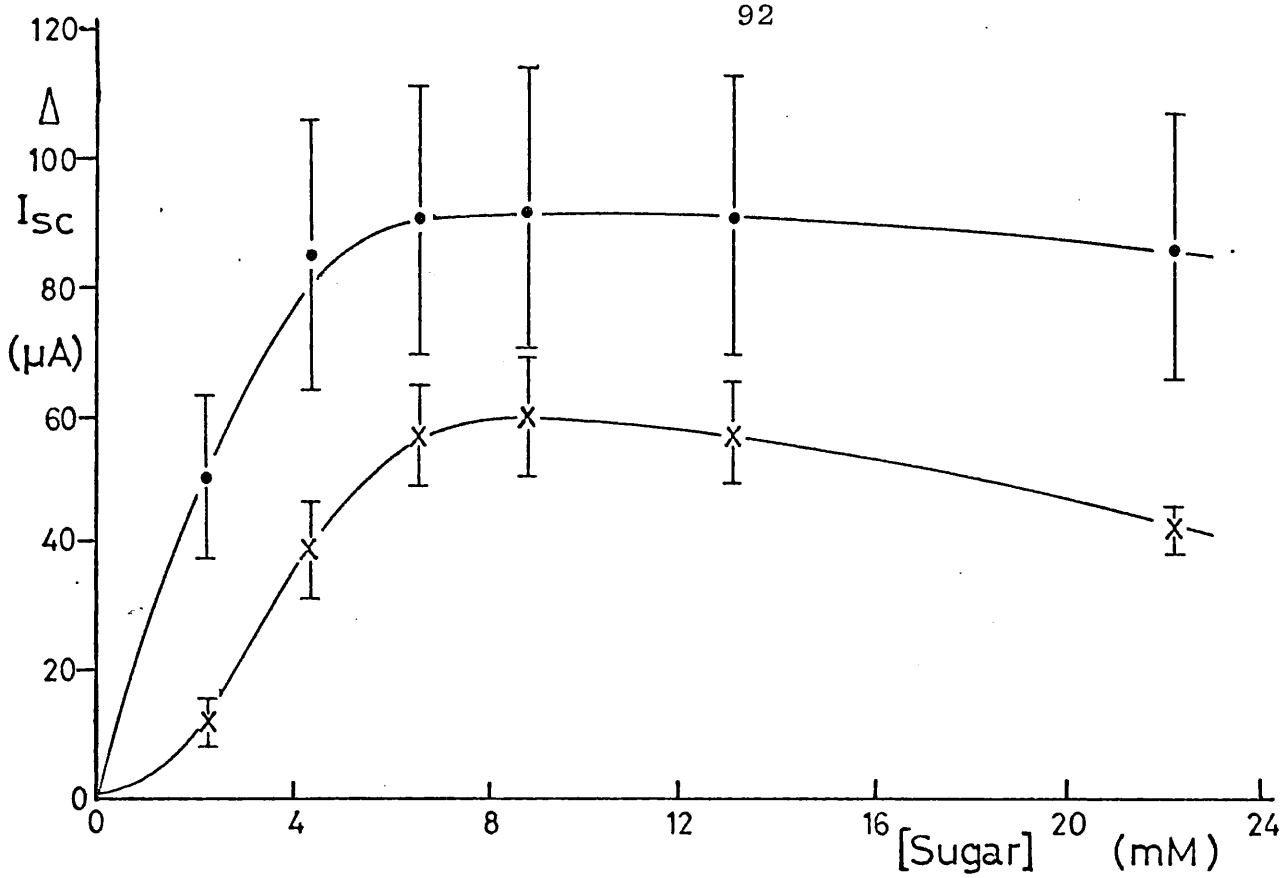


Fig.4.7 Short circuit currents induced by mucosal glucose (•) and galactose (X) in the same segment. (Table 4.4)

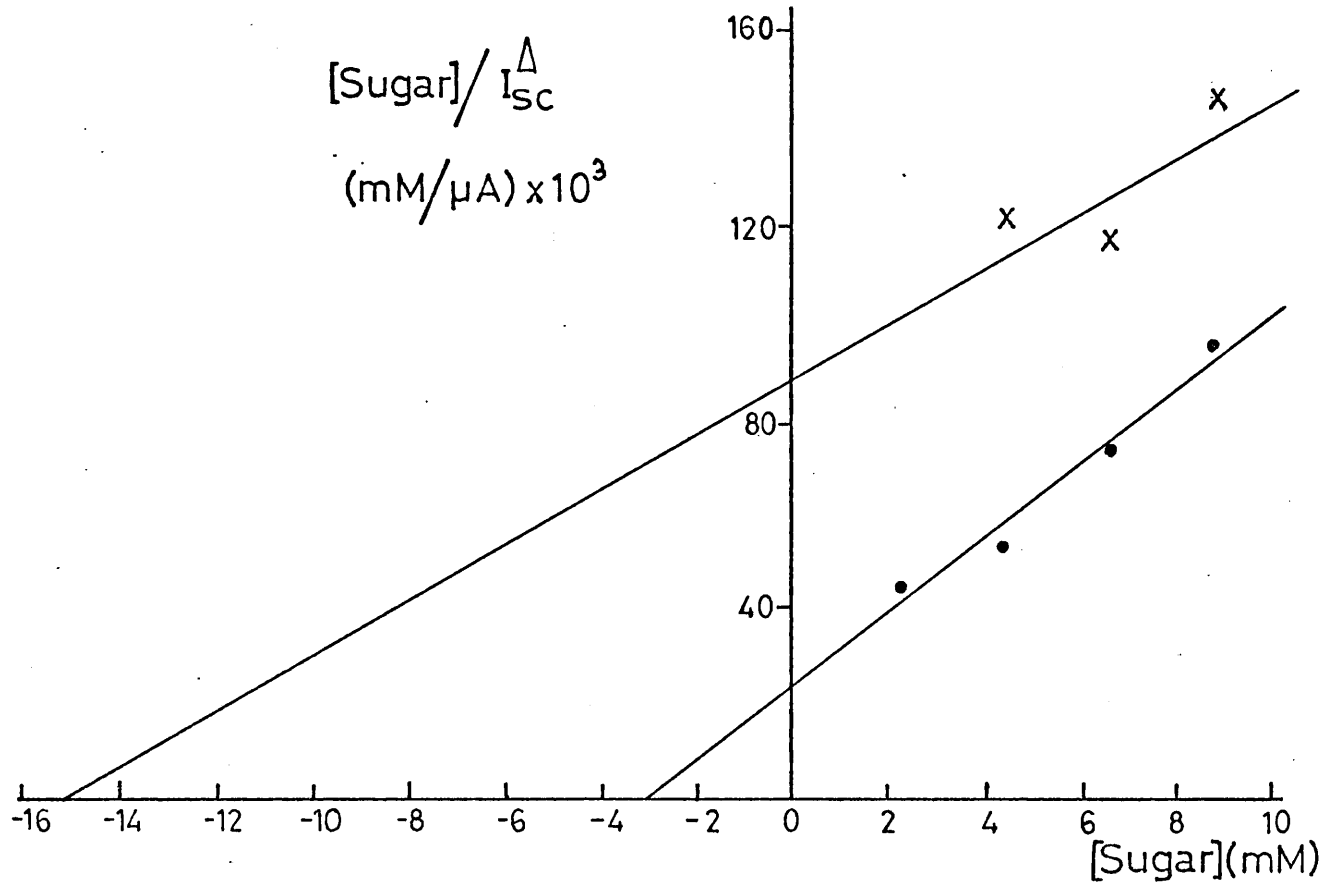


Fig.4.8 Linear plots of Fig 4.7. Regression equations:- glucose, • , y=7.9x + 23.0; galactose, X , y=5.9x + 89.3.

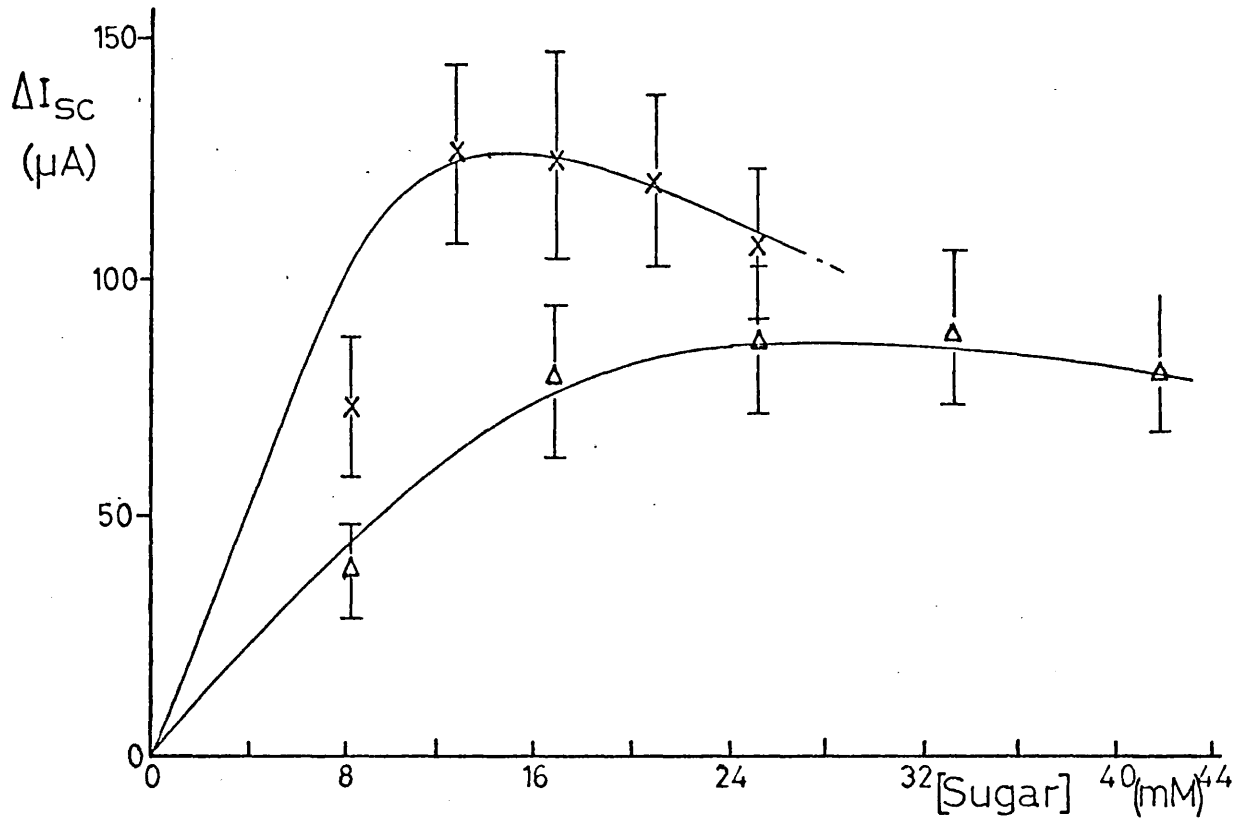


Fig. 4.9 Short circuit current induced by galactose (×) and 3-O-methyl glucose (Δ) across the same segment. Agar electrodes. (Table 4.4).

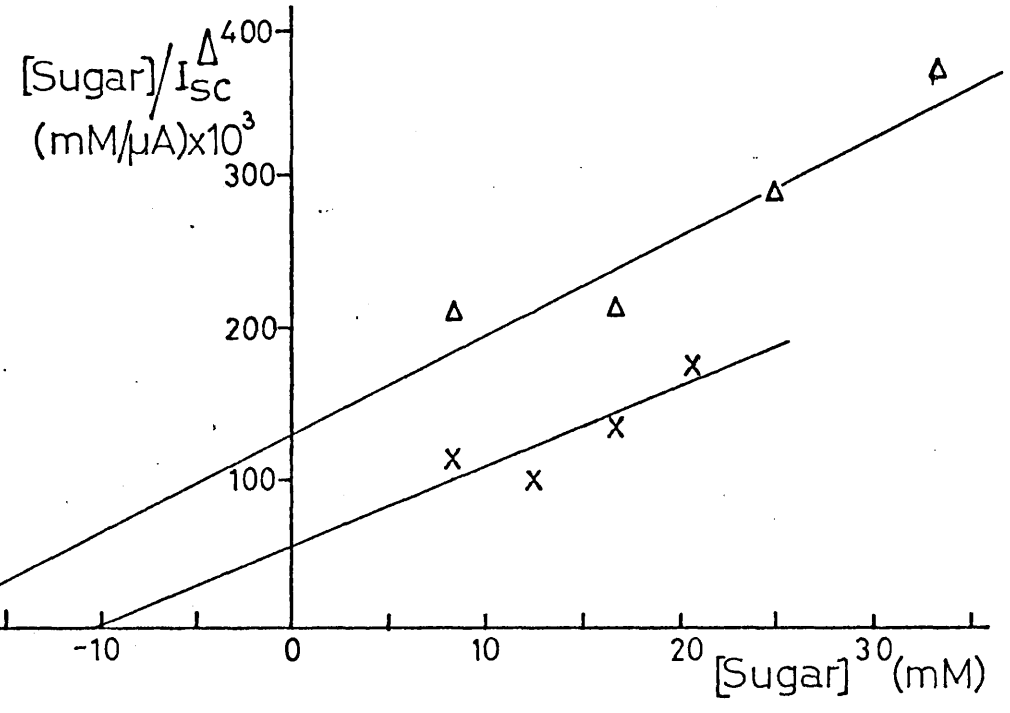


Fig. 4.10 Linear plots of Fig.4.9. Points as above. Regression equations;- X, y = 5.2x + 54.2; Δ , y = 6.7x + 130.

crudely(Kic) by dividing the full saturation concentration of the sugar by two. For each sugar the highest two responses of the I_{sc} were taken to be the full saturation concentrations and Tables (4.6 and 4.7) show the range of the crude estimation of the half saturation concentrations of the sugars and the Ki estimated by the linear-regression method.

Effects of different sugars.

Figures (4.1, 4.3 and 4.5) show the I_{sc} obtained when different concentrations of glucose, galactose, 3-O-methylglucose, α-methylglucose, β-methylglucose and β-phenylglucose were applied. Although from these Figures the comparison of the I_{sc} obtained by different sugars is possible, a closer approach is presented in Figures (4.7 and 4.9) that show the results obtained when different concentrations of two sugars (glucose-galactose and galactose- 3-O-methylglucose) were applied at the mucosal side of the same intestinal segment at a time. The intestinal wall was first bathed with sugar-free saline fluid on both sides (unless otherwise, it will be mentioned) until the steady state was achieved. Then the mucosal side was treated successively with the increasing concentrations of the first sugar for six minutes (e.g. glucose). Before the second sugar (e.g. galactose) was applied, the intestinal segment was bathed with sugar-free saline solution (on both sides) until the steady state was re-established. In half of this investigation the experiments were performed in the order of applying the first then the second sugar and in the other half, the order of application of the sugars was reversed. However, in all investigations a group of experiments was performed for each case; the means $\pm$ SE of the obtained values are shown in the Figures and Tables.

TABLE 4.5

The changes in the I_{sc} induced by combination of different concentrations of mucosal galactose with 8.8mM glucose solution.

Agar electrodes were used for the measurement of the I_{sc} across the intestinal segment (A) of the rat. Sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. Five experiments were performed and the mean values of the obtained $I_{sc} \pm$ SE are presented.

Sugar concentrations glucose + galactose (mM)	(mM)	The obtained I_{sc} (μA)
8.8	8.8	619 ± 49
8.8	4.4	554 ± 51
8.8	2.2	501 ± 56
8.8	1.1	454 ± 58
8.8	0.0	406 ± 60
Increments in the I_{sc} due to galactose solutions (mM)		
1.1		48.0 ± 2.3
2.2		95.0 ± 8.4
4.4		148 ± 12
8.8		213 ± 15

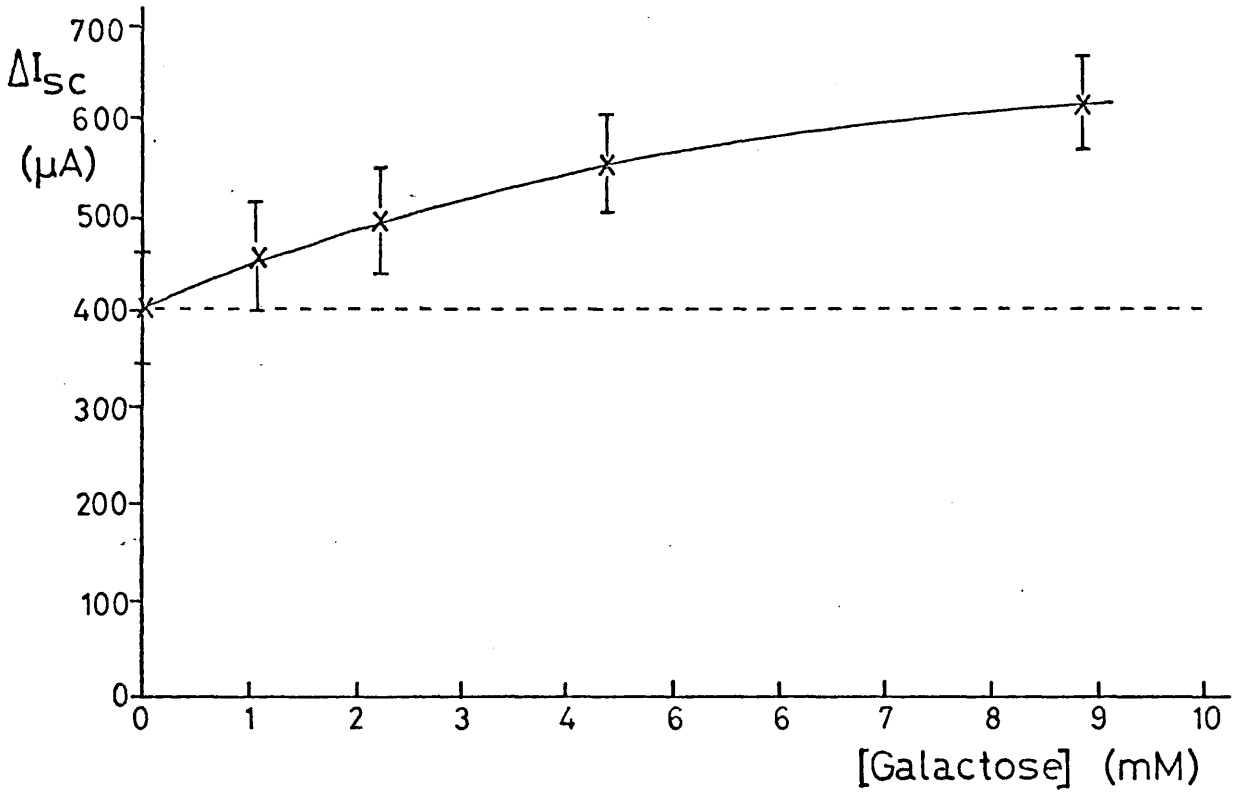


Fig. 4.11 Effect of galactose on the short circuit current induced by 8.8 mM glucose. (Table 4.5)

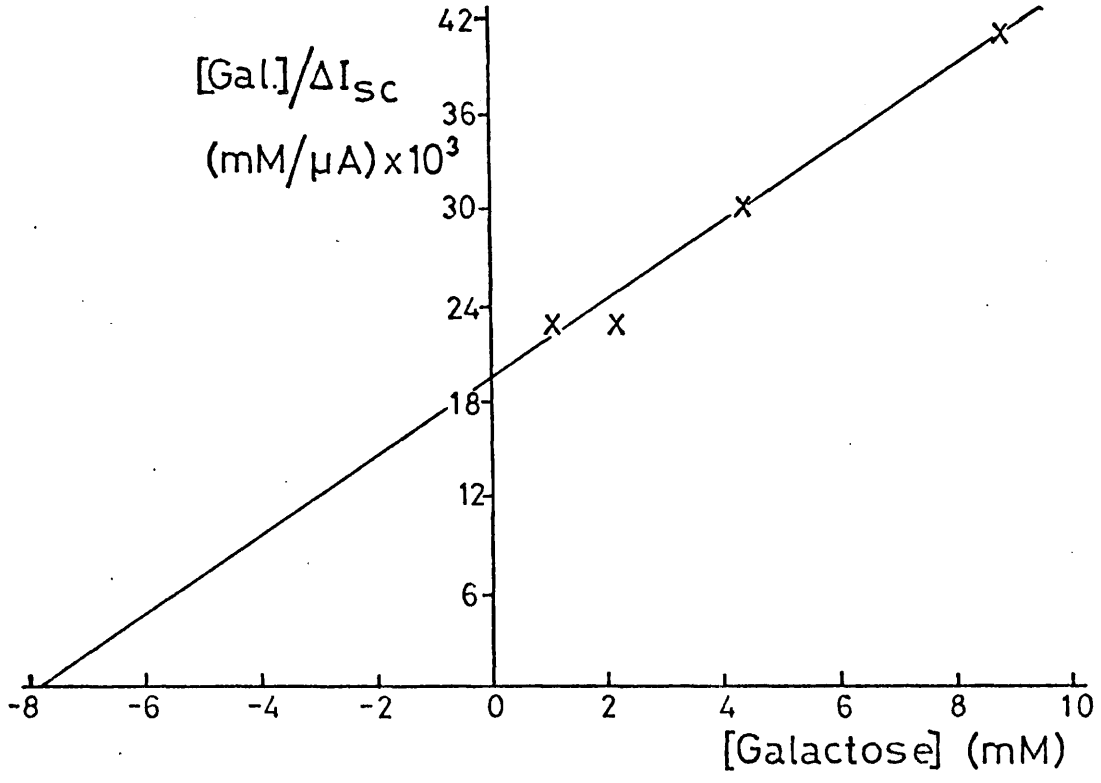


Fig. 4.12 Linear plot of increase in short circuit current due to galactose. Regression equation:- y = 2.5x + 19.0.

It was found that with the sugar-free Krebs' fluid the short-circuit current shows a rapid decrease within the first 10-15 minutes, then a gradual decrease within the next 10-20 minutes when a straight line steady state was clearly achieved and no further decline was noticed. In comparison with other results (e.g. Sawada & Asano, 1963) in which the PD was depressed almost to zero, the depression of the short-circuit current and the PD was never complete. This result will be considered in Chapter 9.

Figure (4.11) and Table (4.5) show the results obtained for the I_{sc} levels induced by mucosal combination of different concentrations of galactose with 8.8mM glucose solution. The short-circuit current first was induced by a combination of 8.8mM galactose + 8.8mM glucose solution; the steady state was achieved. Galactose concentration was successively decreased at six minute intervals until at last only the glucose solution was present at the mucosal side. Decreasing the galactose concentration in a step-wise manner in the range of 8.8-1.1mM gave graded decrease in the I_{sc} level. Analysis of the obtained results (Table 4.7 and Figure 4.12) by the usual mode, gave an apparent Ki of 7.7mM and a derived ΔI max of 405 μA for galactose. These are not very different from the results of galactose when it was used alone; this would support the recent view that glucose and galactose were not competing for a common pathway (Debnam & Levin, 1971, 1974; Levin & Syme, 1971). Also the I_{sc} levels induced by 1.1 and 2.2mM galactose seem to be higher in the presence of glucose than that when the galactose was used alone (Table 4.2). This result is in accordance with Newey, Sandford & Smyth (1965) who reported that galactose transfer in the everted sac of the rat intestine was increased in the presence of glucose. However, in Table 4.2, the result indicates that when the short-circuit

TABLE 4.6

The kinetics of the Na-sugar interaction at 37°C.

The parameters represented in this Table were obtained by using the silver/silver chloride electrodes for the I_{sc} measurements across the intestinal segments of the rat. These parameters were calculated by the regression straight line, as recommended by Riggs (1972). Also the half saturation constants (Kic) of the sugars have been determined directly by dividing the full saturation concentration of the sugar by two. The half saturation constants of some sugars (Ks) which have been obtained from the literature are also represented in this Table.

Sugar	Kic (mM)	Ki (mM)	ΔI max (μA)	Ks (mM)	
Glucose	4.4 - 6.6	6	550	9	(Fisher et al 1953a)
				2.5	(Crane & Wilson 1958)
				7	(Riklis & Quastel 1958)
				4	(Asano 1964)
				4 ± 1	(Schultz et al 1964b)
				13.3	(Lyon & Crane 1966b)
Galactose	8.8 -13.2	12.5	184	35	(Fisher et al 1953b)
				12	(Crane & Wilson 1958)
				10 ± 2	(Schultz et al 1964b)
(3MG)	8.8 -12.2	19.1	135	10	(Crane & Wilson 1958)
				17 ± 3	(Schultz et al 1964b)
(αMG)	2.2 - 3.3	5.6	258	3.9	(Alvarado 1972)
(βMG)	1.1 - 2.2	4.8	93	1.75	(Alvarado 1972)
(βPG)	3.3 - 4.4	6.8	47		
Two sugars assayed using the same segment					
Glucose	2.2 - 4.4	2.9	126		
Galactose	3.3 - 4.4	15.1	169		

TABLE 4.7

The kinetics of the Na-sugar interaction at 37°C.

The constant parameters represented in this Table were obtained by using the agar electrodes for the I_{sc} measurements across the intestinal segments of the rat. The parameters were calculated by the regression straight line, as recommended by Riggs (1972). Also the half saturation constants (Kic) of the sugars have been determined directly by dividing the full saturation of the sugars by two.

Sugar	Kic (mM)	Ki (mM)	ΔI max (μA)	
Glucose	4.4 - 6.6	4.7	925	(Segment A)
Glucose	3.3 - 4.4	2.2	1100	(Segment B)
Galactose	6.6 - 8.8	11.0	621	
Two sugars assayed, one after the other and in different orders, using the same intestinal segment and 100 mM inositol used at the serosal side of the segment.				
Galactose	4.2 - 6.3	10.4	192	
3MG	12.2 -16.6	19.5	150	
Galactose assayed in combination with 8.8 mM glucose solution.				
Galactose		7.7	405	

current had been maximally stimulated by galactose the addition of glucose did not set up a detectable increment in short-circuit current. Schultz & Zalusky (1964b) have reported a similar result in in vitro preparations of the distal rabbit ileum.

Estimation of the parameters (Ki and ΔI max).

Tables (4.6 and 4.7) show a comprehensive comparison of the parameters (Ki and ΔI max) of the sugars used in this study. Also for the sake of comparison, data collected from the literature for the half saturation constants of glucose, galactose, 3-O-methylglucose, α-methylglucose and β-methylglucose in different animals is presented in Table (4.6). In Tables (4.6 and 4.7) it can be noticed that in most cases, there is an obvious discrepancy in the data obtained by the two methods of determining the half saturation constants. This discrepancy may be related to the fact that at higher concentrations of the sugar the I_{sc} responses were clearly reduced and so obscured the real highest responses of the I_{sc}.

Almost similar magnitudes have been found for the Ki of glucose and its derivatives, α-methylglucose (αMG), β-methylglucose (βMG) and β-phenylglucose (βPG) and the highest magnitudes were found for galactose and 3-O-methylglucose (3MG). From the lowest to the highest magnitudes the ΔI max values were as follows: βPG, βMG, 3MG, galactose, αMG and glucose. Also it seems that the ΔI max was depressed when two sugars were assayed on the same segment espeically when inositol was used instead of the sugar-free Krebs' solution at the serosal side. The effects of the order of sugar application and the delay in the application of the second sugar will be considered in detail in

Chapter 8.

Using glucose solution at the mucosal side, both the affinity and the I max were higher for segment (B) than that for segment (A). The Kis obtained for the used sugars seem to be in a good agreement with the results collected from the literature. However, Crane (1960b) has pointed out that it is difficult to compare the values of Km obtained in experiments differing widely in their details.

RESULTS OF KINETIC STUDIES

CHAPTER 5

Studies on the effects of temperature variations on the short-circuit current induced either by glucose or galactose solutions

As mentioned in the introduction, only few reports have been published about the effects of different temperatures on the short-circuit current induced by actively transported sugars across the intestinal wall of mammals. Also using the new device (gut supporting cannula), it was thought that it might be of special interest to reinvestigate the relationship between the temperature variations and the I_{sc} obtained across the intestinal segment in the presence and absence of the sugar.

In this part a trial was made to study the following aspects:

1. - The relationship between the different concentrations of the sugar (glucose and galactose) and the short-circuit current obtained across the intestinal segment of the rat at 27 and 17^{o} C.
2. - The effects of temperature elevation (from 22 to 45^{o} C) on the short-circuit current obtained in the presence and absence of mucosal glucose.

1.- In order to study the first aspect, the following work has been undertaken. The thermostat of the water bath was manually adjusted to 27^{o} C and usually checked with the aid of a thermometer. But to keep the temperature at 17^{o} C ground ice was added now and again throughout the experiment. This was checked by the aid of a thermometer that hung in the water bath. The intestinal segments were always taken from the region in between 25-35 cm. from the proximal end of the jejunum and in this chapter, the agar electrodes were used for the I_{sc} measurements across the segment. As usual the segment was bathed with sugar-free Krebs' solution at both sides (the mucosal and serosal) until the steady state of the short-circuit current was achieved. The mucosal saline solution was then

TABLE 5.1

The changes in the I_{sc} induced by different concentrations of mucosal glucose-Krebs' solution across the intestinal segment of the rat.

Sugar-free Krebs' solution (10ml.) was recirculated at the serosal side of the segment. Agar electrodes were used for the measurements of the I_{sc}, and the incubation temperatures were 27° and 17°C. Six and five experiments were performed for both temperatures respectively; the means ± SE were calculated.

Mucosal sugar conc. (mM)	I_{sc} (μA) induced at 27°C	I_{sc} (μA) induced at 17°C
0.4	----------	8.8 ± 1.0
1.1	58.3 ± 4.2	19.7 ± 1.6
2.2	121 ± 8	30.7 ± 1.7
4.4	206 ± 13	39.8 ± 2.4
6.6	254 ± 15	42.3 ± 2.9
8.8	289 ± 14	44.6 ± 3.1
11.1	315 ± 12	38.0 ± 3.4
13.2	328 ± 12	35.3 ± 3.3
15.4	343 ± 14	----------
17.6	348 ± 14	----------
22.2	353 ± 16	----------

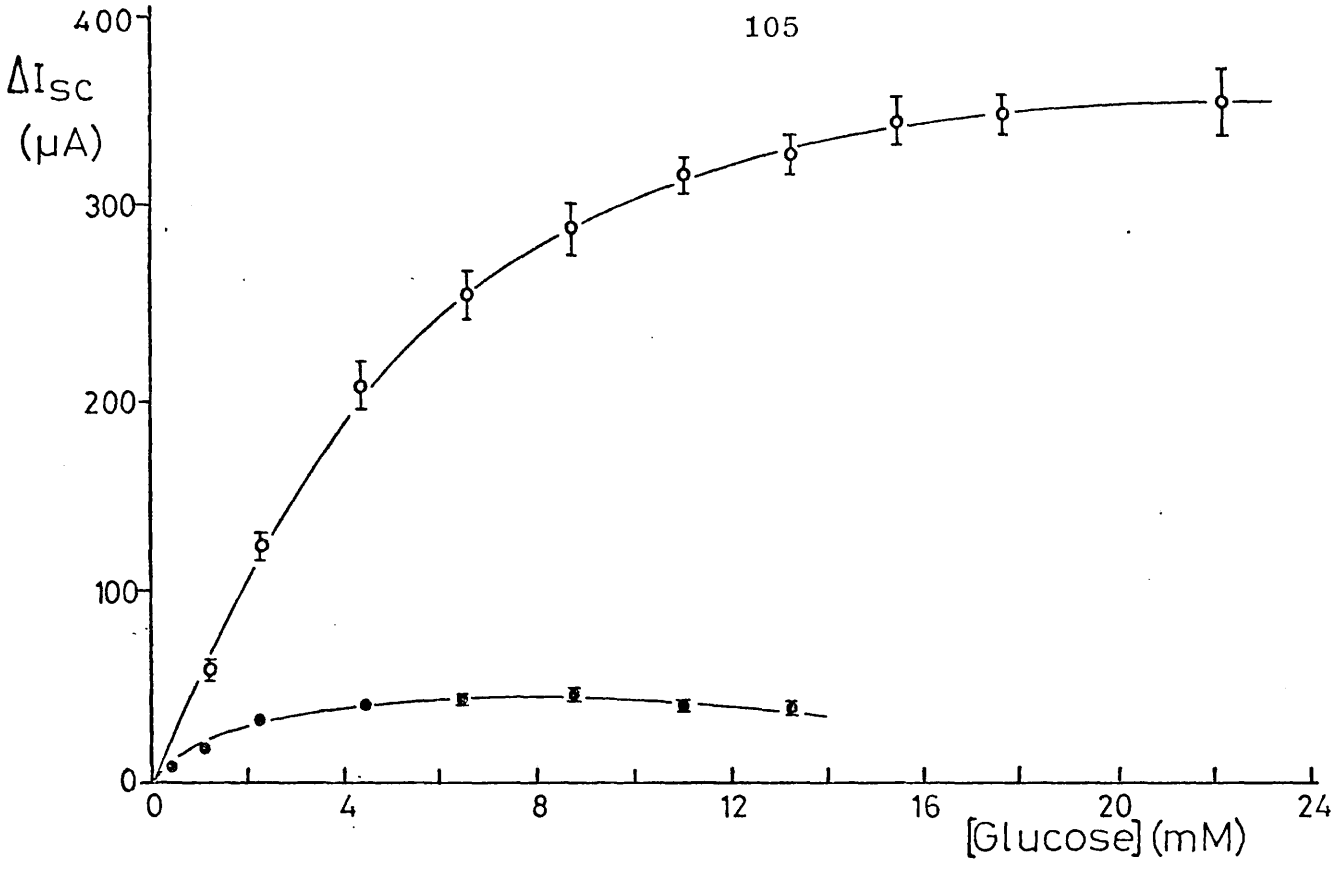


Fig. 5.1 Effect of temperature on the short circuit current induced by glucose. Points :- • , 17°C; o , 27° C. (Table 5.1)

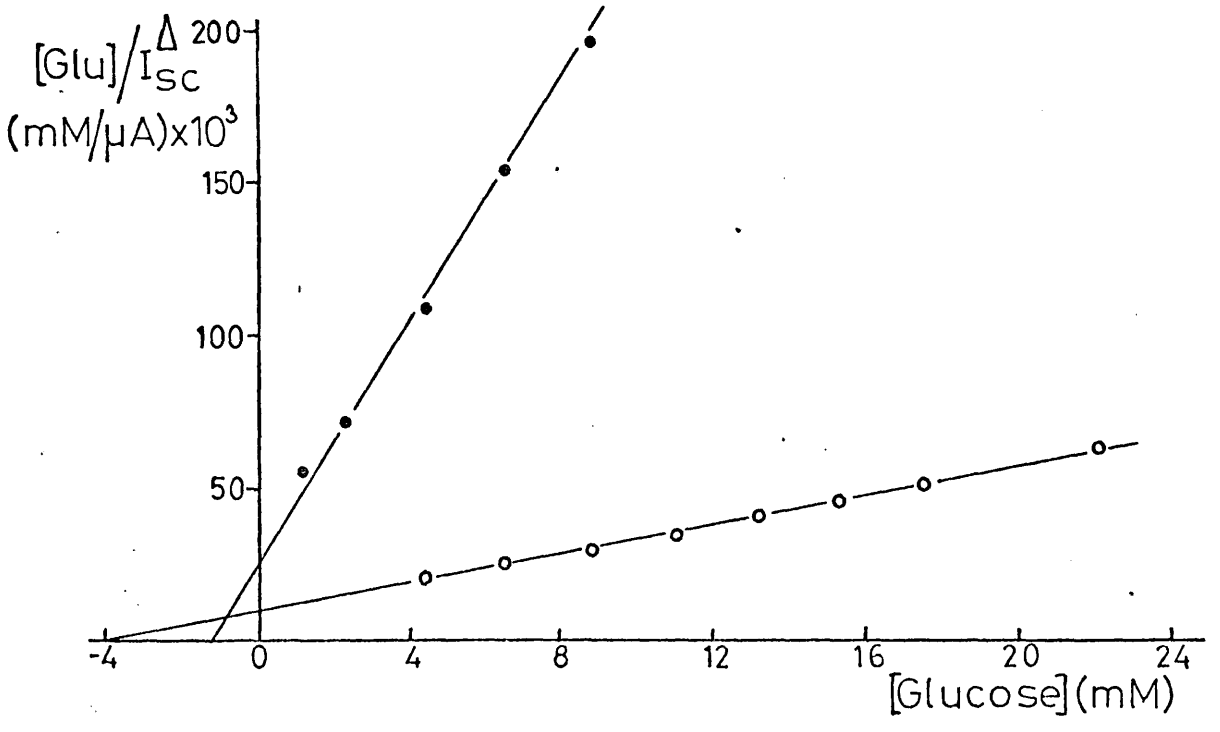


Fig. 5.2 Linear plots of Fig 5.1. Regression equations :- 17°C, • , y = 19.1 x + 29.0; 27°C, o , y = 2.4x + 9.1.

TABLE 5.2

The changes in the I_{sc} induced by different concentrations of mucosal galactose - Krebs' solution across the intestinal segment of the rat.

Sugar-free Krebs' solution (10ml.) was recirculated at the serosal side of the segment. Agar electrodes were used for the measurements of the I_{sc} and the incubation temperatures were 27° and 17°C. Four and six experiments for both temperatures were performed respective, the means ± SE were calculated.

Mucosal sugar conc. (mM)	I_{sc} (μA) induced at 27°C	I_{sc} (μA) induced at 17°C
1.1	17.0 ± 1.7	8.9 ± 0.8
2.2	37.8 ± 3.3	17.5 ± 1.9
4.4	82.5 ± 4.2	30.0 ± 3.8
6.6	131 ± 10	36.7 ± 4.3
8.8	178 ± 18	40.7 ± 4.7
11.1	225 ± 26	43.2 ± 5.3
13.2	262 ± 32	45.5 ± 5.6
15.4	286 ± 31	48.2 ± 6.8
17.6	304 ± 31	----------
22.2	316 ± 31	----------

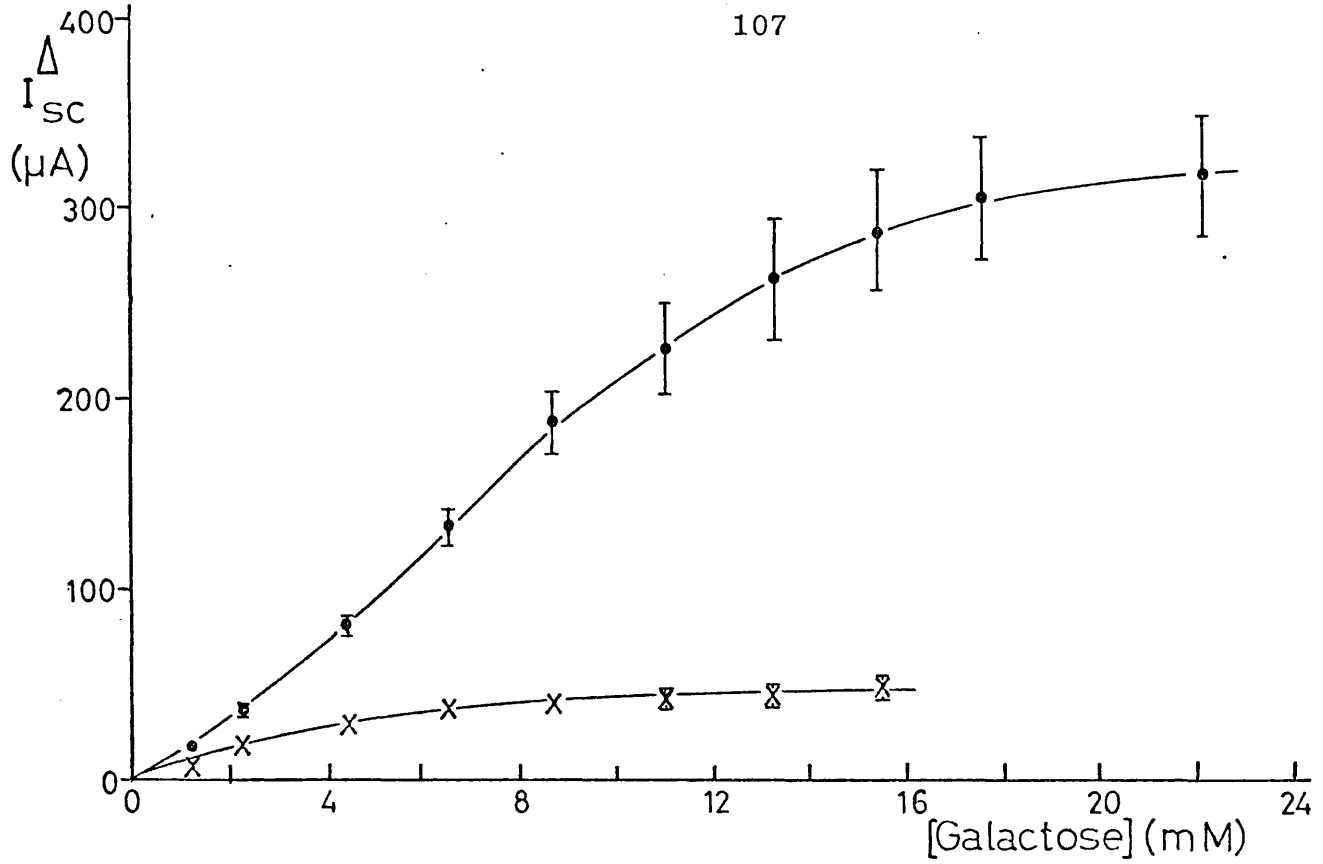


Fig. 5.3 Change in the short circuit current induced by galactose with temperature. Points :- X , 17°C; • , 27°C. (Table 5.2)

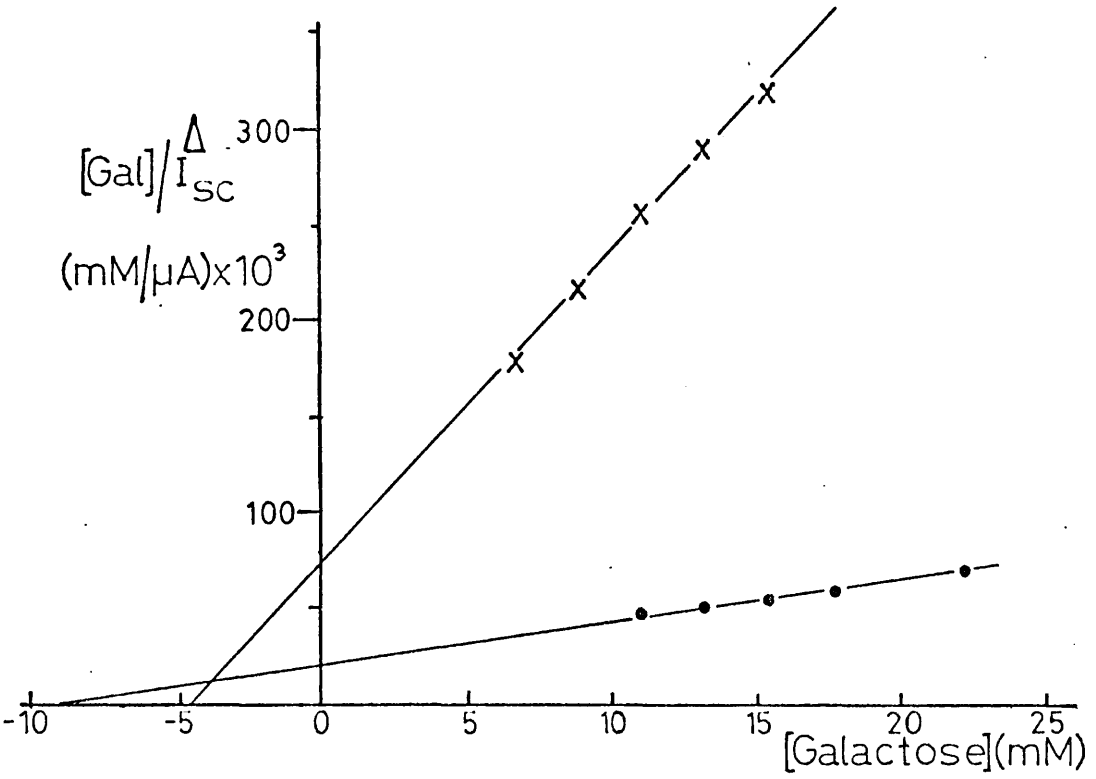


Fig. 5.4 Linear plots of Fig 5.3. Regression equations:- 17°C, X , y = 16.1 x + 75.2 ; 27°C, • , y = 2.2 x + 19.8.

replaced by the sugar-Krebs' saline solution and the concentration of the sugar was increased in a step-wise manner, six minutes for each concentration as indicated in Tables (5.1 and 5.2) and Figures 5.1 and 5.3). The serosal fluid remained unchanged; 10ml of sugar-free saline was recirculated at the serosal side throughout the experiment.

Results.

Figures (5.1 and 5.3) clearly show that at 27^{o} and 17^{o} C; different levels of glucose and galactose concentrations gave graded responses of the short-circuit current. At these temperatures it seems that the effect of osmosis on the short-circuit current at the higher concentrations of both sugars are much less than what have been observed at 37^{o} C (chapter 4.). This can be easily noticed from the curves and Figures (5.1 and 5.3) that show no decline at the higher concentration of glucose and galactose as occurred to the glucose and galactose curves at 37^{o} C (chapter 4.). The results were treated in the same manner described in chapter 4. In each case the linear plot was made (figures 5.2 & 5.4) and regression method was followed to obtain the Ki and ΔI max expressed in electrical terms. In each case a group of 4-6 experiments have been performed; the means and the SE of the results obtained are shown in the Tables and the Figures.

Effects of different temperatures (27^{o} and 17^{o}C) on the I_{sc} induced by step-wise increasing concentration of glucose and galactose.

Figure (5.1) evidently shows that the responses of the I_{sc} in relation to the applied different concentrations of mucosal glucose

TABLE 5.3

The kinetics of the Na-sugar interaction at 27° and 17°C.

The parameters were obtained using the agar electrodes for the I_{sc} measurements across the intestinal segment of the rat. The data was calculated by the regression straight line as recommended by Riggs, 1972. Also for comparison the parameters of glucose and galactose at 37°C which had been obtained in chapter 4 are again represented in this Table.

Sugar	Temperature (°C)	Ki(mM)	ΔI max (μA)
glucose	37	4.7	925.0
	27	3.8	419.0
	17	1.5	51.7
galactose	37	11.0	621.0
	27	8.8	444.0
	17	4.7	62.5

are much higher at 27 than that at 17°C. For example, at 27°C the recorded I_{sc} in the presence of 4.4, 6.6 and 8.8mM mucosal glucose solution are 206 ± 13, 254 ± 15, and 289 ± 14 μA respectively. But at 17°C the obtained I_{sc} in the presence of the same concentrations of glucose respectively are 39.8 ± 2.4, 42.3 ± 2.9 and 44.6 ± 3.1 μA.

For the sake of comparison, it may be of interest to represent again the parameters obtained at 37°C for glucose and galactose (Table 5.3). However, the Ki (mM) of glucose at 37°, 27° and 17°C were 4.7, 3.8, and 1.5 respectively and the Ki (mM) of galactose for the same temperatures respectively were 11, 8.8 and 4.7.

2.- The procedure followed to study the second aspect mentioned above was as follows: the thermostat of the water bath was adjusted to 22°C. The intestinal segments were taken from the same region of the intestine as in the previous section. The segment was immersed in a mucosal sugar-free Krebs' solution. But 10ml. of 22.2mM glucose Krebs' solution was recirculated at the serosal side and remained unchanged throughout the experiment. After the steady state of the short-circuit current was achieved the mucosal saline was replaced by 4.4mM glucose-saline solution for four minutes. By adjusting the thermostat of the water bath the temperature of the fluid bathing the intestinal segment was increased successively up to 45°C as recorded in the first set of Table (5.4). Each temperature was applied for two minutes; at the end of this period the recorded I_{sc} reading was then taken. Following the same technique two experiments were performed with mucosal glucose-free Krebs' solution (second set of Table 5.4).

TABLE 5.4

The changes in the I_{sc} stimulated by temperature elevation of the medium bathing the intestinal segment from 22^{o} to $45^{o}C$.

The agar electrodes were used for the I_{sc} measurements across the intestinal segment of the rat. In the first set the experiments were performed in the presence of 4.4mM mucosal glucose solution and in the second set glucose was omitted from the mucosal medium. In all experiments 10 ml. of 22.2 mM glucose-Krebs' solution was recirculated at the serosal side of the segment. The numbers in parenthesis indicate the number of animals used and the mean values of the obtained I_{sc} ± SE are given.

	The medium temperature (^{o}C)	The time of temperature application (mins)	Obtained I_{sc} (μA)
First set (5)	22	2 - 4	89.0 ± 7.1
	27	2	314 ± 11
	32	2	597 ± 25
	37	2	818 ± 44
	40	2	1044 ± 106
	43	2	1071 ± 133
	44	2	996 ± 134
	45	2	842 ± 110
	45	4	510 ± 45
Second set (2)	22	30 - 45	0.0 (steady state)
	27	2	67.5 ± 5.3
	32	2	55.0 ± 3.5
	37	2	20.0 ± 7.1
	40	2	27.5 ± 5.3
	42	2	10.0 ± 7.1
	44	2	-35.0 ± 17.1
	45	2	-90.0 ± 0.0
	45	4	-248 ± 5.3

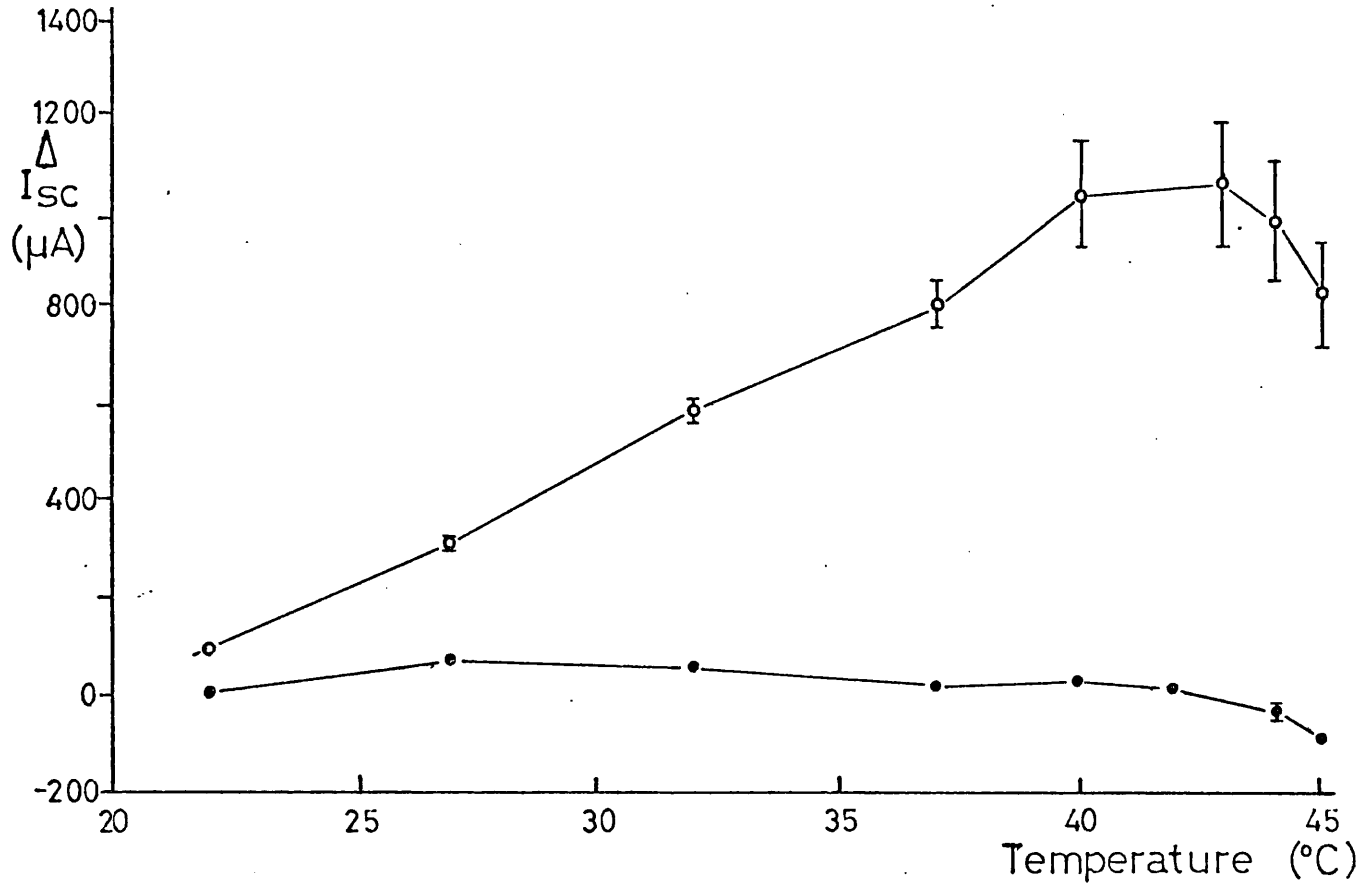


Fig. 5.5 Effect of temperature on the short circuit current in the presence, o ; and abscence, • ; of 4.4 mM mucosal glucose.

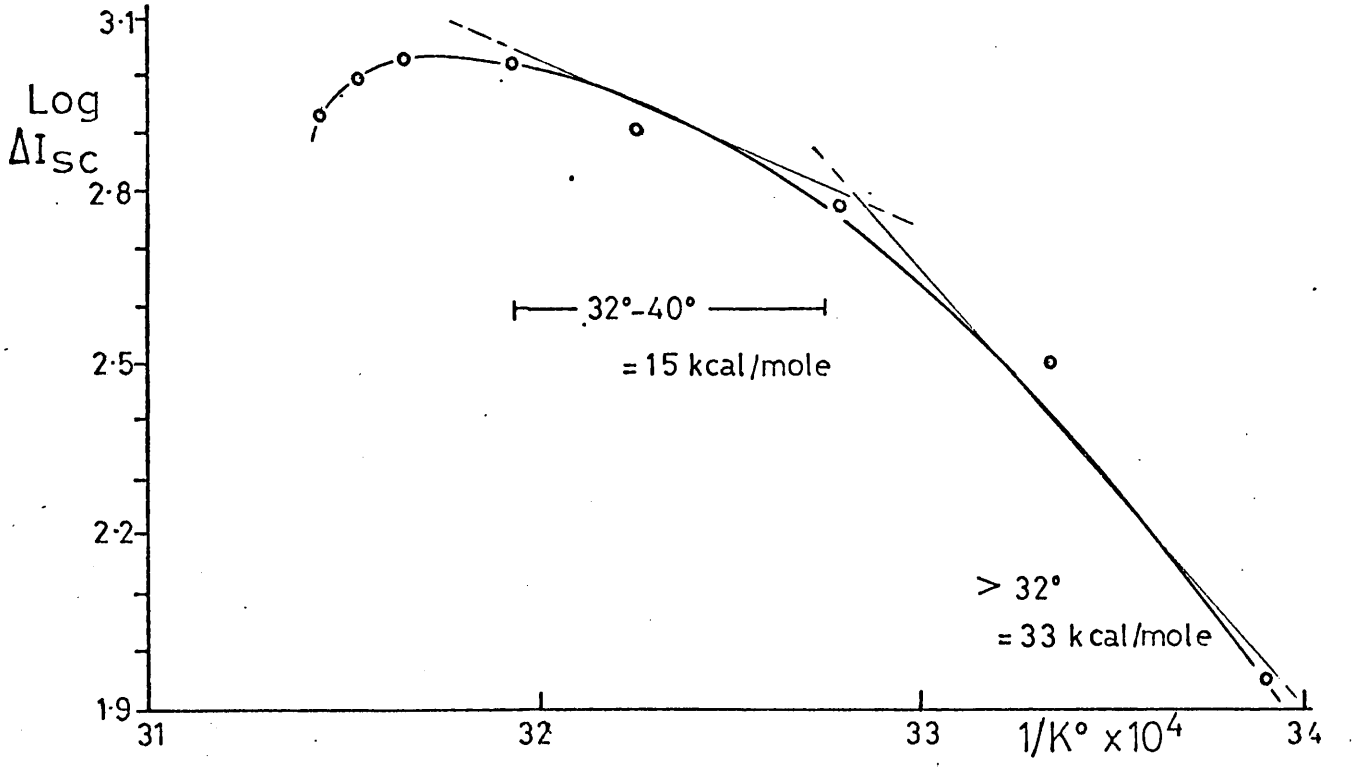


Fig. 5.6 Arrhenius plot of short circuit current in the presence of 4.4 mM muscosal glucose.

Results

Figure (5.5) illustrates the means and SE of the short-circuit current obtained in relation to the applied temperature in the presence and absence of mucosal glucose solution. Evidently the system is highly temperature sensitive, but it is much more sensitive in the presence of mucosal glucose than with the mucosal glucose being omitted. In the presence of mucosal glucose, the relationship between the I_{sc} and the applied temperature seems to be almost linear up to $37^{o}C$. From 37^{o} up to $40^{o}C$ the I_{sc} curve increased faster than the I_{sc} recorded up to $37^{o}C$. However, the I_{sc} response reaches its maximum at $43^{o}C$ and then followed a rapid decline under the effect of further temperature elevation to $45^{o}C$. With the mucosal glucose absent the maximum I_{sc} response was recorded at $27^{o}C$, then followed a gradual decline under the effect of further temperature elevation. At 44 and $45^{o}C$ the recorded I_{sc} depression seems to go further down than the initial point of the I_{sc} steady state at $22^{o}C$. However, Figure (5.5) shows that when glucose was omitted from the bathing mucosal solution, the system becomes relatively temperature insensitive especially at temperatures around $37^{o}C$.

Figure (5.6) shows an Arrhenius plot of the temperature dependence of the short-circuit current in Krebs' solution containing 4.4 mM glucose (Table 5.4). In this Figure, it is clear that, under the condition of the experimental investigation, two straight lines with different slopes were obtained. The first line belongs to the short-circuit current obtained below $32^{o}C$ and the second line belongs to the short-circuit current at temperatures ranging from 32 - $40^{o}C$.

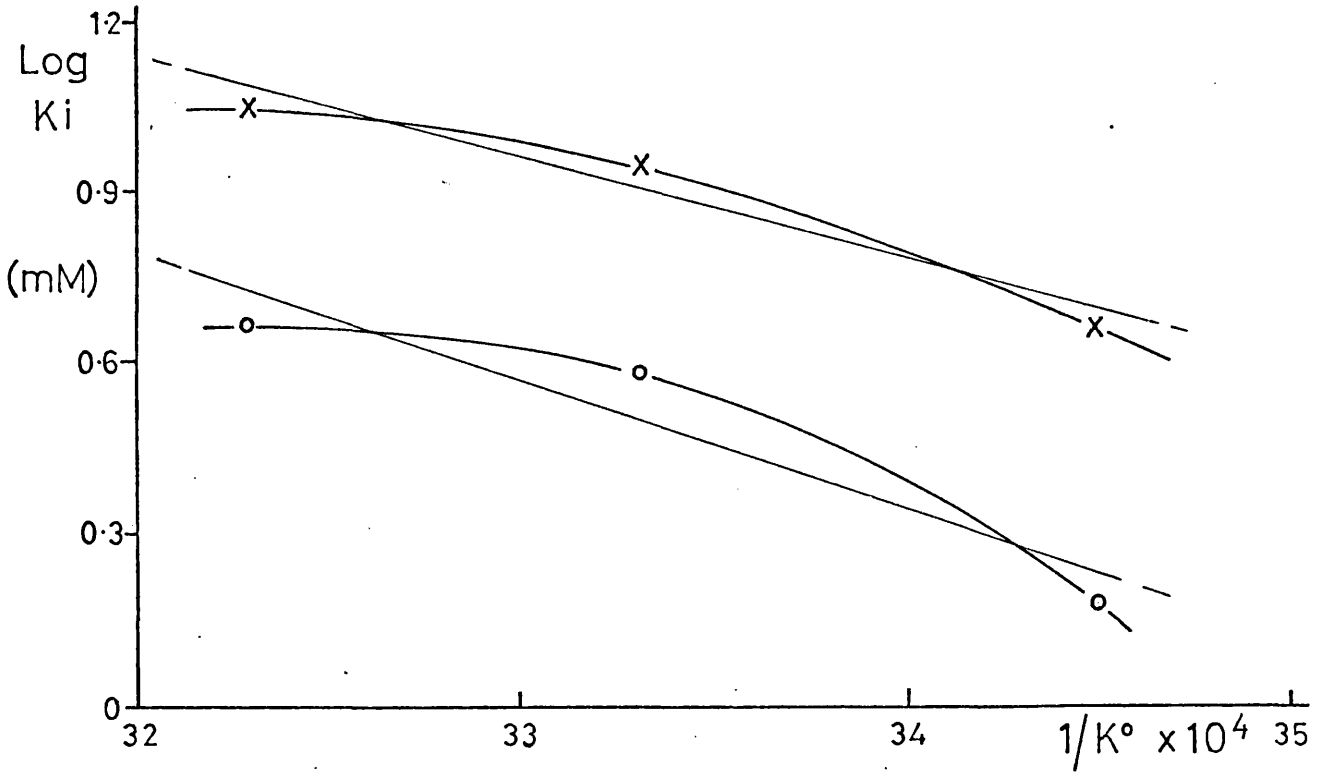


Fig. 5.7 Arrhenius Plots of the apparent half-saturation constants for glucose (o) and galactose (x). (Table 5.3)

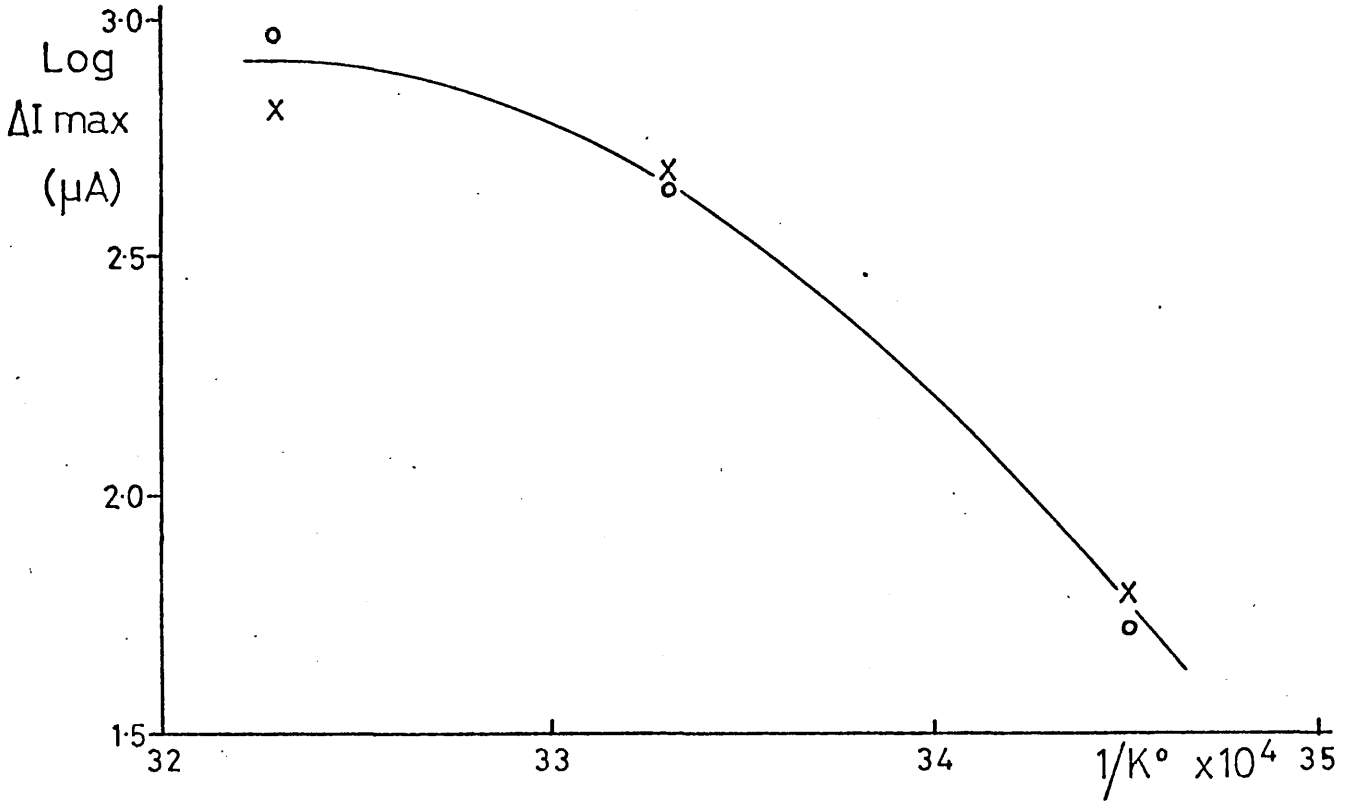


Fig.5.8 Arrhenius Plots of the maximal short circuit current for glucose (o) and galactose (x) (Table 5.3)

After the latter temperature and up to 45°C the line seems to deteriorate in a hyperbolic fashion. The calculated apparent activation energies from the slopes of the two lines respectively were 33.0 and 15.0 kcals./mole.

Figure (5.7) is also an Arrhenius plot of the Ki obtained for glucose and galactose at 17, 27 and 37°C (Table 5.3). The apparent activation energies (EA) calculated from the obtained straight lines of glucose and galactose respectively were 11.0 and 8.3 kcals./mole. Similarly the Arrhenius plot, Figure (5.8) of the ΔI max obtained for glucose and galactose at 17°, and 27° and 37° (Table 5.3) gave different slopes at different ranges of the applied temperatures for each sugar. In this case the calculated apparent activation energies in the presence of glucose were 36.58 and 14.74 kcals./mole respectively at temperatures ranging from 17° - 27° and 27° - 37°C. In the presence of galactose the calculated apparent activation energies for the same ranges of the temperatures respectively were 34.55 and 6.58 kcals./mole. The calculated activation energies obtained in this study are in agreement with that reported (13.2 and 30.0 kcals./mole) by Schultz & Zalusky (1964a and b) on the basis of short-circuit current with rabbit ileum in the presence of actively transported sugar. Nevertheless, the obtained apparent activation energies seems to be greater than those reported for intestinal sugar transport (Cordier & Worbe, 1954 and Csaky & Fernald, 1960). While care must be taken in extracting a conclusion from this information, it is possible - as Schultz & Zalusky (1964b) concluded - that the higher magnitudes of the apparent activation energies obtained,

may be attributed to the fact that the increase in the I_{sc} is the result of the interaction between two carrier-mediated transport systems. Although the apparent activation energies obtained in this study are high, yet little can be said in supporting the idea of the metabolically driven process that can move sodium ions against an electro-chemical gradient. Especially because the activation energy for passive diffusion of sodium ions across cell membranes may be as high as 25 kcal./mole. (Stein, 1967).

RESULTS OF KINETIC STUDIES

CHAPTER 6.

Studies on the effects of different concentrations of disaccharides and glucose on the short-circuit current.

As has been reported in the introduction, extensive studies have been made about the subject of disaccharides absorption in the mucosal cells of the intestine. But as far as the electrical parameters are concerned, only few reports have been found in the literature that have dealt with the subject on the basis of the potential difference across the small intestine of the rat (Kohn, Smyth & Wright, 1966 & 1968). On the basis of the short-circuit current no publication has been found in the literature. However, using the new device "gut supporting cannula", it might be of some interest to investigate the effects of some disaccharides on the short-circuit current across the intestinal segment of the rat. To achieve this goal the following work has been undertaken. Maltose, sucrose, lactose and trehalose were used in this study and glucose was used for comparison. The segment was taken from the region in between 25-35 cm from the proximal end of the jejunum. The segment then was everted on the gut supporting cannula, and clamped in the apparatus; the agar electrodes system was used for the short-circuit current measurements. The intestinal wall was bathed with sugar-free saline solution on both sides until the steady state of the I_{sc} was achieved. Different concentrations of two sugars (maltose-glucose; sucrose-glucose and maltose-lactose) were applied for six minutes each at the mucosal side of the same intestinal segment at a time. The serosal fluid remained unchanged throughout the experiment; 10 ml of sugar-free Krebs' solution was recirculated at the serosal side of the segment. The mucosal side was treated with increasing concentrations of the first sugar. The intestinal segment was then bathed with the sugar-free saline solution again until the steady state of the I_{sc} was established. Different concentrations of the second sugar were then applied. In

TABLE 6.1

The changes in the I_{sc} stimulated by different concentrations of mucosal maltose and glucose solutions.

Using the agar electrodes, the I_{sc} of the two sugars was measured across the same intestinal segment in different orders. In half of the experiments maltose was assayed before the glucose, in the other half the glucose was assayed first and the maltose second. Sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. Six experiments were performed and the mean values of the obtained $I_{sc} \pm$ SE were calculated.

Mucosal sugar conc. (mM)	I_{sc} (μA) induced by maltose	I_{sc} (μA) induced by glucose
1.1	145 ± 12	90.8 ± 9.8
2.2	329 ± 28	204 ± 17
4.4	569 ± 43	376 ± 34
6.6	678 ± 57	508 ± 50
8.8	691 ± 59	554 ± 58
13.2	653 ± 67	542 ± 45
17.6	668 ± 55	540 ± 49
22.2	668 - 52	338 ± 54

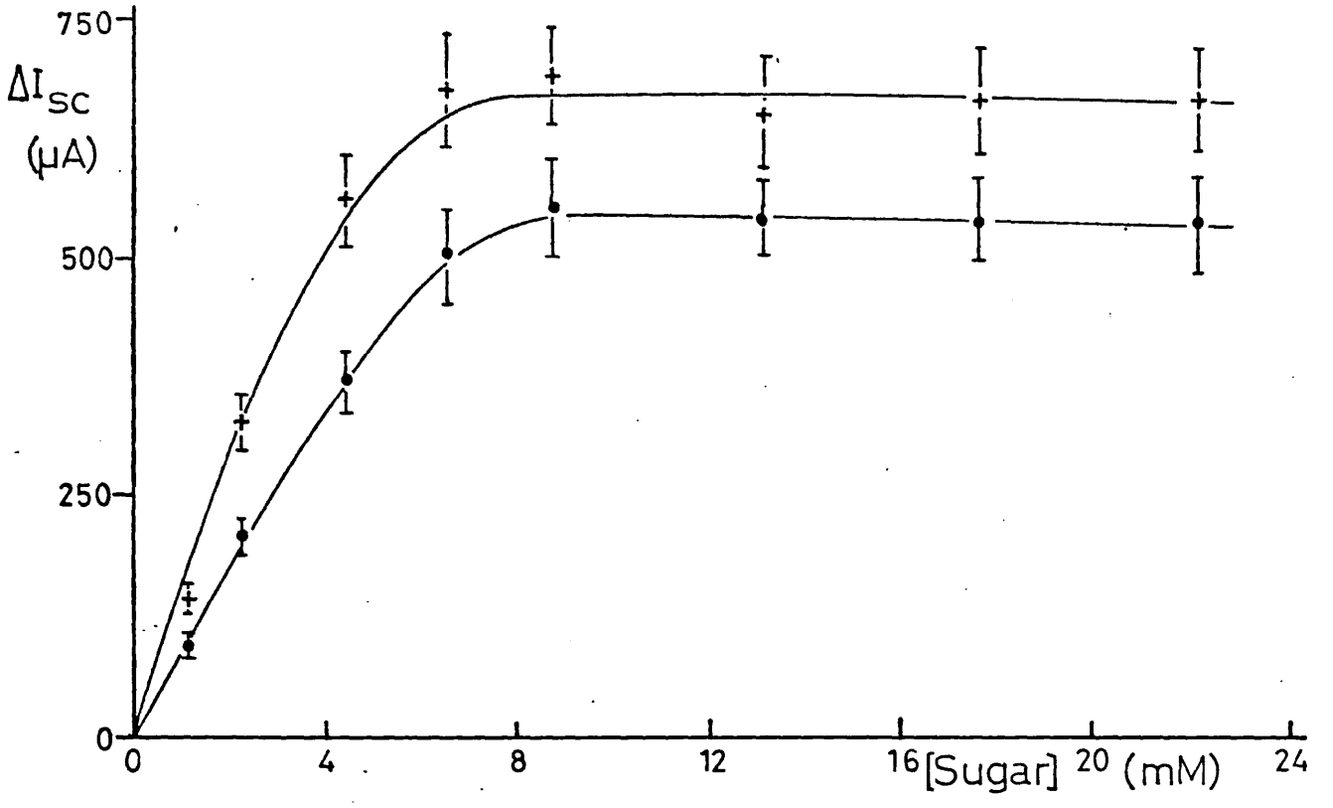


Fig. 6.1 Short circuit currents induced by glucose (•) and maltose (+) in the same segment. (Table 6.1)

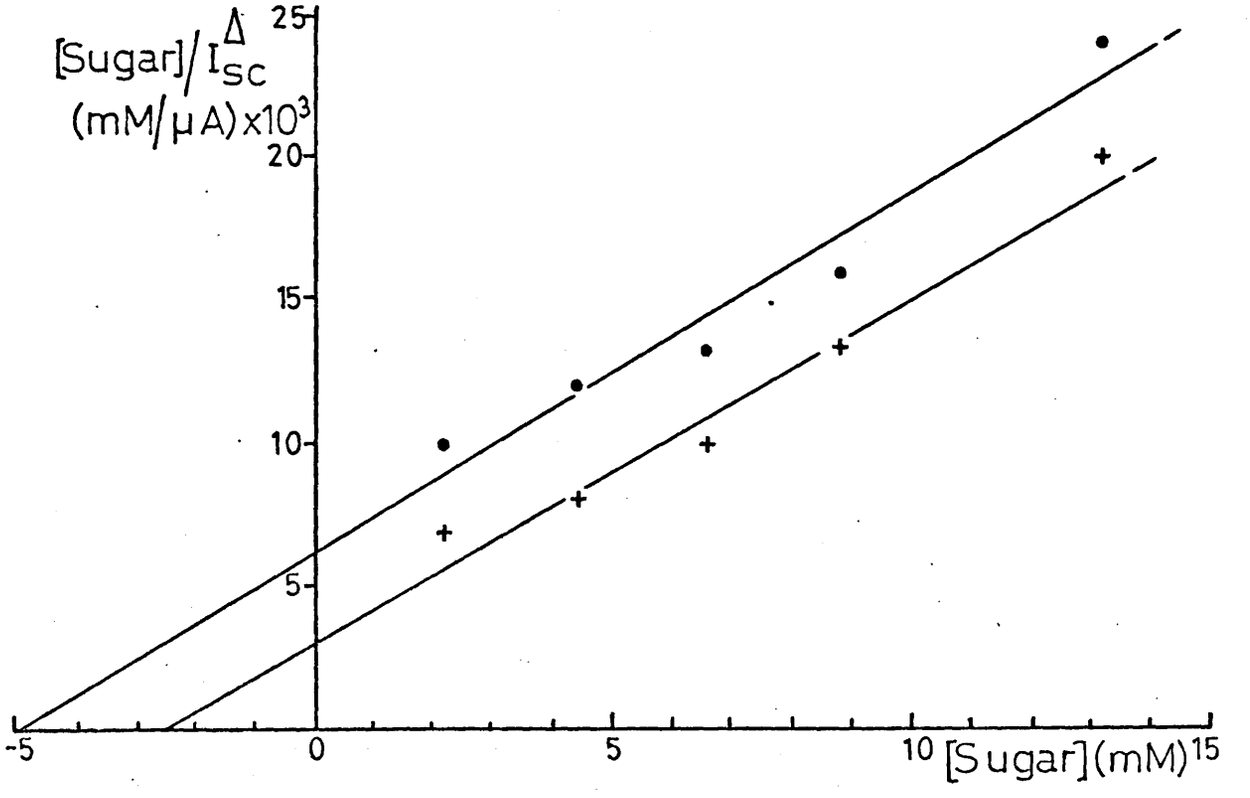


Fig. 6.2 Linear plots of Fig 6.1. Regression Equations :- glucose, • , y = 1.3x + 6.1; maltose, + , y = 1.2x + 3.1.

each case half of the experiments were carried out in the order of applying the first then the second sugar and the other half of the experiments were performed in a reversed order. For each case a group of experiments were performed; the mean and SE of the obtained results are shown in Tables (6.1, 6.2, 6.3 and 6.4) and Figures (6.1, 6.3 and 6.5).

Results

It was clearly found that increasing concentrations of the disaccharides gave graded responses of the I_{sc} across the intestinal segment of the rat. Figures (6.1, 6.3 and 6.5) illustrate that the relationship of the short-circuit current and the added concentrations of the sugars are nonlinear and suggest that the relationship is in agreement with Michaelis-Menten (1913) kinetics. The results (Figures 6.2, 6.4 and 6.6) were treated as described in Chapter 4.

Effects of different sugars

Maltase, sucrase and trehalase, the necessary enzymes for the hydrolysis of maltose, sucrose and trehalose respectively, were reported by many authors (see Semenza, 1968) to be present in the intestinal mucosa of rat.

Figure and Table (6.1) show that maltose and glucose gave rapid responses with the increasing concentrations. The I_{sc} stimulated by added maltose concentrations are higher than those induced by the same concentrations of glucose. However, the I_{sc} obtained with the concentrations of maltose were never as high as twice the I_{sc} induced

TABLE 6.2

The changes in the I_{sc} stimulated by different concentrations of mucosal sucrose and glucose solutions.

Using the agar electrodes, the I_{sc} of the two sugars was measured across the same intestinal segment in different orders. In half of the experiments the sucrose was assayed before the glucose, in the other half the glucose was assayed first and the sucrose second. Sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. Eight experiments were undertaken and the mean values of the obtained $I_{sc} \pm$ SE were calculated.

Mucosal sugar conc. (mM)	I_{sc} (μA) induced by sucrose	I_{sc} (μA) induced glucose
1.1	8.8 ± 1.9	119 ± 15
2.2	24.4 ± 2.7	234 ± 23
4.4	73.1 ± 8.2	423 ± 45
6.6	144 ± 14	441 ± 53
8.8	228 ± 19	594 ± 55
13.2	301 ± 25	611 ± 54
17.6	376 ± 35	601 ± 55
22.2	416 ± 39	585 ± 52

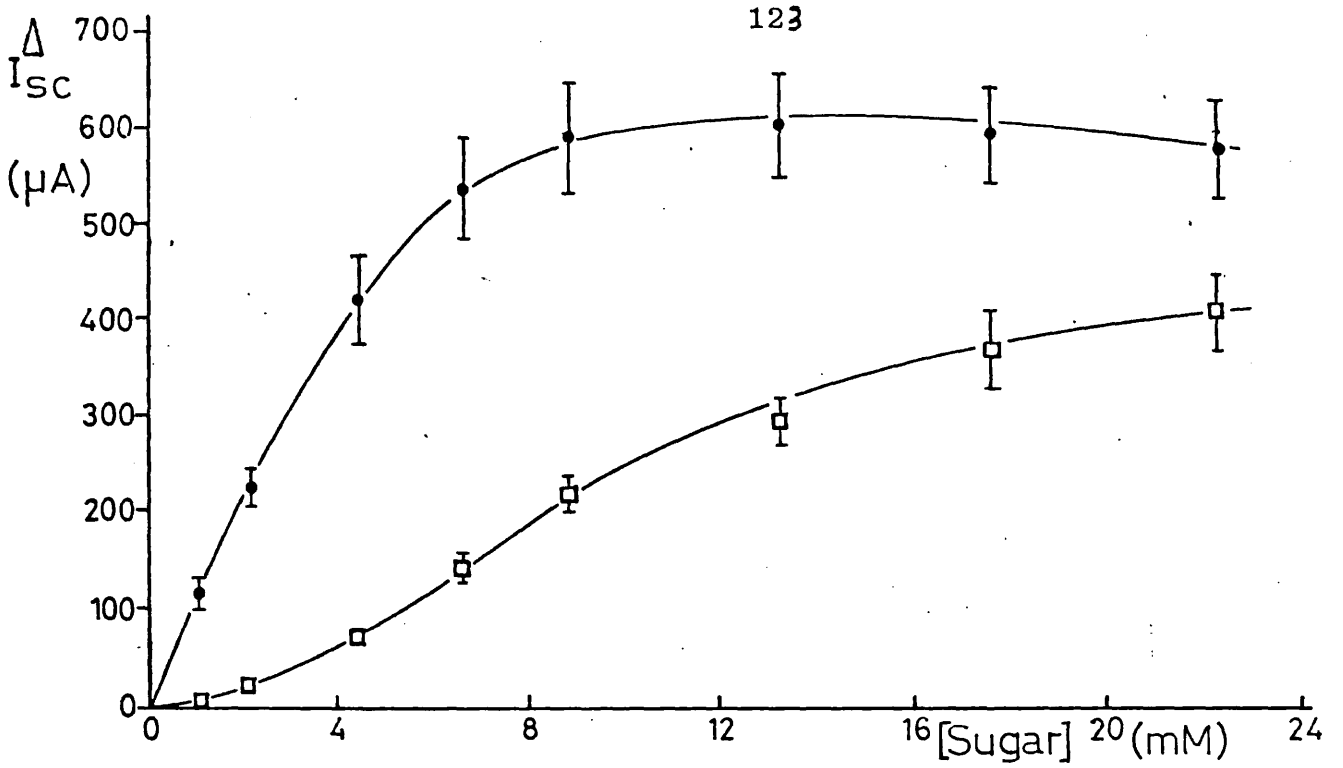


Fig. 6.3 Short circuit currents induced in the same segment by glucose (•) and by sucrose (□). (Table 6.2)

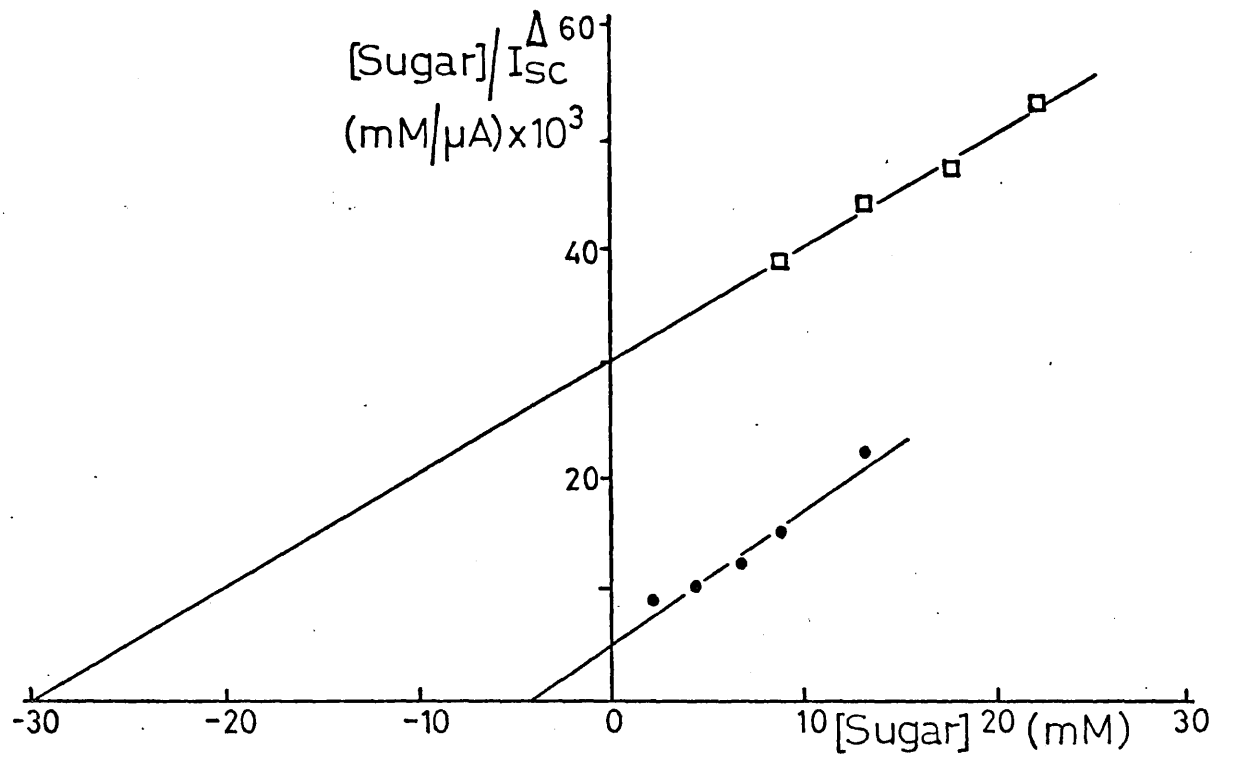


Fig. 6.4 Linear plots of Fig. 6.3. Regression equations :- glucose, • , $y = 1.2x + 5.1$; sucrose, □ , $y = 1.0x + 30.2$.

by similar concentrations of glucose. The I_{sc} stimulated by maltose at low concentrations (1.1 and 2.2 mM) is about 1.6 fold of the I_{sc} induced by glucose at the same concentrations, but with the increase of both sugar concentrations, the I_{sc}-maltose/I_{sc}-glucose decreases. For example, at 8.8 mM the I_{sc}-maltose is 1.25 fold of the I_{sc}-glucose. By contrast, Figure (6.3) and Table (6.2) show that the levels of the I_{sc} induced by different concentrations of glucose are clearly higher than the I_{sc} induced by the same concentrations of sucrose. Also a gradual increase in the I_{sc} was observed with the added sucrose solutions. In the Figure it can be seen that the shape of the sucrose curve is different from the hyperbolic shape of the glucose curve. The shape of the sucrose curve was sigmoid and it may suggest that a competition is involved in the process. It is likely that fructose and glucose yielded from the sucrose hydrolysis may compete for the same pathway regardless of whether fructose will enter the cell on the same pathway as the glucose or not. Lyon & Crane (1966a) found that the graded concentrations of mucosal fructose clearly inhibited the potential difference across the in vitro preparation of the rat's small intestine.

Trehalose: Fungi, yeast, and certain plants contain a disaccharide of two glucose units known as trehalose which also appears in some insects. For the first time an attempt was made to assay the effect of this sugar on the short-circuit current across the intestinal segment of the rat. The usual method was followed; the I_{sc} steady state was obtained with the intestine in sugar-free Krebs' solution. The mucosal Krebs' fluid was then replaced by 4.4 mM glucose-Krebs' solution. At the end of a 15 minute interval a new solution (4.4 mM glucose + 22.2 mM trehalose) was added to remain another 15 minutes when it was replaced by another solution (11.1 mM glucose + 22.2 mM

TABLE 6.3

The changes in the I_{sc} stimulated by combinations of the mucosal glucose and trehalose - Krebs' solution across the intestinal segment of the rat.

Four experiments were performed and the values represent the means ± SE of the I_{sc} measured using the silver/silver chloride electrodes system. Sugar-free Krebs' solution (10 ml.) was recirculated at the serosal side of the segment.

Step No	Mucosal medium glucose (mM)	trehalose (mM)	Time of solutions application (mins.)	I_{sc} (μA) obtained
1	4.4	+ 0.0	15	174 ± 17
2	4.4	+ 22.2	6	209 ± 25
	4.4	+ 22.2	15	226 ± 30
3	11.1	+ 22.2	6	221 ± 27

trehalose). Table (6.3) shows the means $\pm$ SE of the I_{sc} obtained in relation to the applied sugar concentrations within the recorded intervals of time. Addition of 22.2 mM trehalose after a certain magnitude of the I_{sc} (steady state) was stimulated by 4.4 mM glucose, gave rise to another increment of the I_{sc}. Presumably, trehalose undergoes a suitable hydrolysis by the disaccharidase enzyme trehalase. It is also clear that when 11.1 mM glucose-Krebs' solution was added after the I_{sc} had been maximally stimulated by the previous addition of (4.4 mM glucose + 22.2 trehalose) no further increase in the I_{sc} was obtained. A small decline was observed by the addition of the last concentration of glucose, presumably owing to the osmotic effects.

Figure (6.5) and Table (6.4) show the different levels of the I_{sc} obtained by treating the mucosal side of the same segment with increasing concentrations of maltose and lactose in different orders. As can be seen in the Figure, incubation in increasing concentrations of maltose caused increases in I_{sc}; incubation in lactose caused decreases. When the tissue was transferred to the sugar-free Krebs' solution, these effects were partially recovered and after a while a steady state of short-circuit current was achieved. The lactose effects gave rise to a hyperbolic curve, but to the negative side. This effect is evidently different from other mentioned sugars that gave rise to hyperbolae curves to the positive side. The positive hyperbolae are known to be descriptive of a simple association-dissociation process such as may be postulated to occur between a mobile carrier and a sugar molecule. However, the lactose results may suggest that this sugar does not undergo a suitable hydrolysis,

TABLE 6.4

The changes in the I_{sc} stimulated by different concentrations of mucosal lactose and maltose solutions.

Using the agar electrodes, the I_{sc} of the two sugars was measured across the same intestinal segment in different orders. In half of the experiments lactose was assayed before the maltose, in the other half maltose was assayed first and lactose second. Sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. Six experiments were performed and the mean values of the obtained $I_{sc} \pm$ SE were calculated.

Mucosal sugar conc. (mM)	I_{sc} (μA) stimulated by lactose	I_{sc} (μA) stimulated by maltose
1.1	-21.7 ± 6.1	128 ± 8
2.2	-31.7 ± 5.7	273 ± 21
4.4	-39.2 ± 6.4	467 ± 33
6.6	----	524 ± 48
8.8	-46.7 ± 7.4	528 ± 56
13.2	----	509 ± 59
17.7	-62.5 ± 7.1	486 ± 63
22.2	----	469 ± 70
Sugar-free Krebs' fluid	-15.0 ± 4.6	

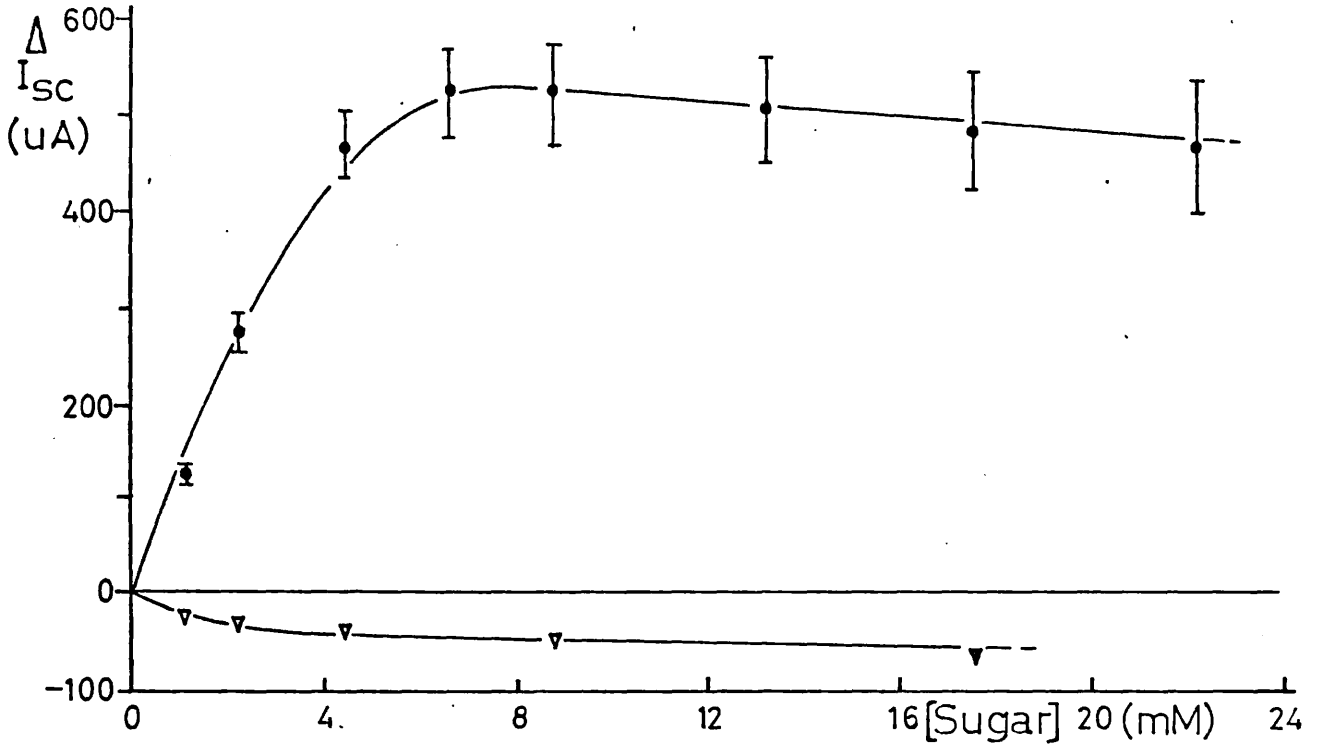


Fig. 6.5 Changes in the short circuit current in the presence of maltose (•) and lactose (▽). (Table 6.4).

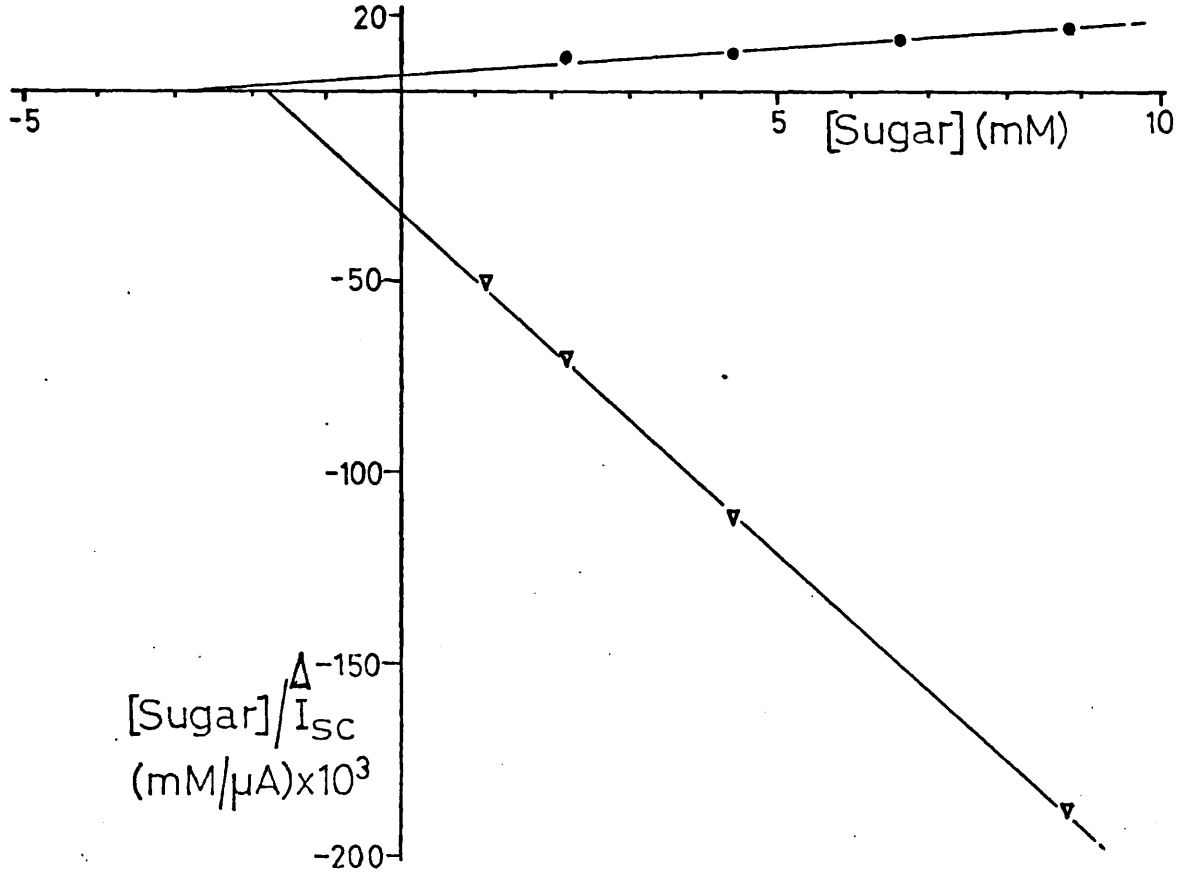


Fig. 6.6 Linear plots of Fig. 6.5. Regression equations ;- maltose, • , y = 1.4x + 4.0; lactose, ▽ , y = -17.8x - 31.7.

that might be because of the absence of the lactase enzyme at the outer coat of the mucosal cells of the small intestine of the adult rat (Alvarez & Sas, 1961, Doell & Kretchmer, 1962 and Koldovesky & Chytil, 1965). Furthermore, the results might suggest an involvement of the sugar-free carrier in Na^+ transport but which may get engaged with the lactose molecule at the outer surface of the mucosal cell and tend to become fixed there and block some of the Na^+ entry to the cell.

Estimation of Ki and ΔI max (Vi max) values

On the basis of the above results of the I_{sc} measurements, the parameters of the used sugar expressed in electrical terms are presented in Table (6.5). The half saturation constant of maltose seems to be just about half of that obtained for glucose, but the ΔI max are almost the same for both sugars; this seems to be consistent with the consideration of the equimolecular concentrations of glucose in both cases.

The sucrose curve (Figure 6.3) shows a different pattern from the hyperbolic shape that has been found to occur for other investigated sugars i.e. glucose or maltose. The sigmoid shape of the sucrose curve suggests a possible interaction of more than one sugar molecule and Na^+ with the mobile carrier. This impression was strengthened from the comparison of the apparent affinities (on the basis of the equimolecular concentrations of glucose) calculated for sucrose, maltose and glucose (Table 6.5). Higher apparent Ki (lower affinity) was obtained for sucrose (29.9 mM), compared to 4.2 - 4.9 mM obtained

TABLE 6.5

The kinetics of the Na-sugar interaction at 37°C.

The parameters were obtained by the regression straight line as recommended by Riggs, 1972. Using the same intestinal segment, the agar electrodes were used for the I_{sc} measurements across the intestinal segment of the rat. The brackets include the sugars which had been applied at the mucosal side of the segment in the same experiment; and different orders of sugar application were followed.

The sugars used	Ki (mM)	ΔI max (μA)
{ maltose }	2.5	812
{ glucose }	4.9	798
{ sucrose }	29.9	990
{ glucose }	4.2	827
{ maltose }	2.8	700
{ lactose }	1.8	-56.8

for glucose and 2.5 - 2.8 mM for maltose. The calculated ΔI max for sucrose seems to be almost within the same order obtained for glucose and maltose.

The values of Ki and ΔI max of maltose in the maltose-lactose experiment are not very much different from the values of the same parameters of the sugar assayed in different experiments with glucose. Lactose showed an inhibiting action of the I_{sc}; however, the inhibition pattern gave rise to a hyperbolic-shape curve at the negative side. An attempt is made to determine the parameters of the sugar by the same method of calculation followed for other sugars (Figure 6.6); these parameters are represented in Table (6.5).

RESULTS OF KINETIC STUDIES

CHAPTER 7

The effects of non-transportable competitive inhibitors of sugar transfer and of some drugs on the short-circuit current.

Bihler (1965) reported that in vitro transport experiments using the hamster small intestine showed that sugars which are not accumulated against a concentration gradient difference belong to two groups. Those in the first group e.g. D-Lyxose enter cellular space slowly and are not affected by inhibitors or Na^+, and kinetics appear to be of diffusion type. The second group e.g. D-Xylose and L-fructose appear to enter by diffusion and by a more specific transport process operating in parallel; this process is Na^+ dependent. Mutual inhibition of entry between these sugars and the actively transported ones is also found. Asano (1964) and Lyon & Crane (1966a) reported that mucosal fructose caused reduction in the recorded short-circuit current and potential difference across the rat intestinal wall. Recently Baker & Widdas (1973a & 1973b) reported that the glucose derivative, ethylidene glucose was found to inhibit glucose fluxes competitively in human red cells, but its penetration into the red cells followed diffusion type kinetics and there was evidence that it could not be translocated on the hexose system. Benzylidene glucose has been shown by Novak & LeFevre (1974) to have a higher affinity for hexose transfer system in the human erythrocyte. Mucosal mannose (28 mM) has been shown by Barry et al (1969) to inhibit the resting potential difference in the rat intestine. It seems that the authors related this inhibition with some other observations to the osmotic effects which were termed as osmotically induced potentials.

In these investigations the mutual inhibition of the sugar entry between ethylidene glucose, benzylidene glucose, mannose and lactose on one hand and the actively transportable sugars i.e. galactose, 3-O-methylglucose, glucose, α-methylglucose and β-methylglucose on the other hand have been examined on the basis of short-circuit

current measurements. Ethylidene glucose was also used at the serosal side of the segment with the presence of 1.1 or 4.4 mM glucose solution at the mucosal side. The effects of different concentrations of the non-transportable sugars on the resting short-circuit current that were recorded with the sugar-free Krebs' solution also have been investigated.

For the sake of comparison some other known inhibitors i.e. phloridzin, ouabain and iodo-acetic acid (IAA) have been examined.

Using the silver/silver chloride or the agar electrodes, the technique followed in this study briefly was to use the segment taken from the regions in between 25 - 35 cm (A) or 35 - 45 cm (B) from the proximal end of the jejunum. Sugar free Krebs' solution was used at the serosal side of the segments throughout the experiment unless otherwise stated. In the presence and absence of the actively transportable sugar, addition of different concentrations of the non-transportable sugar (ethylidene glucose, benzylidene glucose, mannose, lactose and cellobiose) in a step-wise increasing manner for six minutes each was performed when the I_{sc} steady state was achieved. The non-transportable sugar was added simultaneously with the same concentration of the actively transportable sugar that has been used previously to induce the short-circuit current to a higher level. In a similar procedure other inhibitors i.e. phloridzin, ouabain and iodo-acetic acid were applied to the mucosal or serosal side of the segment. Another approach for the study of ethylidene glucose effect was attempted i.e. without having the I_{sc} steady state achieved with tested sugar (glucose). For the latter investigation two concentrations of ethylidene glucose (18.4 and 9.8 mM) were

added in a step-wise decreasing manner for six minutes each, in combination with 1.1 mM glucose solution directly after the I_{sc} has achieved the steady state in the sugar-free Krebs' solution. In all instances, as ilustrated in Tables (7.1 - 7.13) a group of experiments were performed for each case, and the mean values of the obtained I_{sc} $\pm$ SE are presented. Some small differences might be expected in the I_{sc} values obtained in some experiments (Tables 7.2 & 7.3) tested with ethylidene glucose solutions as the sugar was found to contain about 5% glucose. In other experiments a purified ethylidene glucose was used.

Results

Figures (7.1, 7.3, 7.4, 7.6, 7.8, and 7.10) and Tables (7.1 - 7.8) show the results of the inhibitory action of different concentrations of ethylidene glucose, benzylidene glucose, mannose, lactose and cellobiose on the short-circuit current obtained across the intestinal segments of the rat in the absence and presence of the actively transportable sugars. The silver/silver chloride electrodes have been used in most of the experiments for the I_{sc} measurements; in some cases the agar electrodes were used. In these Figures it can be seen that in the absence of the actively transportable sugars, graded reductions in the I_{sc} levels have been obtained with the increasing concentrations of the non-transportable sugar. The relationship between the ΔI_{sc} and the added concentrations of the non-transportable sugars seems to give rise to a negative hyperbolic-shaped curve. It is known that the positive hyperbolic-shaped curve is a descriptive of a simple association-dissociation process such as may be postulated to occur between a mobile carrier and a sugar molecule. However, such a relationship seems to be in accordance with

TABLE 7.1

Inhibition of the I_{sc} by different concentrations of mucosal ethylidene glucose (EG) solution in the absence and presence of sugar (5.5 mM galactose or 17.6 mM 3-O-methylglucose).

Silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segments (A or B). Sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. In the presence of the sugar, the EG was added simultaneously with the same concentration of the sugar which had been used previously to induce the I_{sc}. The numbers in parenthesis indicate the number of animals used in each set. The mean values of the obtained I_{sc} ± SE are given.

(2) First Set		(2) Second Set			(2) Third Set		
Mucosal EG conc. mM	The obtained I_{sc} (μA)	Mucosal galactose mM	Medium EG mM	The obtained I_{sc} (μA)	Mucosal 3MG mM	Medium EG mM	The obtained I_{sc} (μA)
2.2	-22.5 ± 0.4	5.5	+ 0.0	73.5 ± 2.5	17.6	+ 0.0	93.5 ± 13.1
4.4	-40.0 ± 0.0	5.5	+ 4.9	46.5 ± 1.1	17.6	+ 1.6	77.5 ± 12.4
6.6	-49.0 ± 2.1	5.5	+ 9.8	30.0 ± 0.0	17.6	+ 4.9	56.0 ± 11.3
8.8	-58.0 ± 1.4	5.5	+ 14.7	23.5 ± 1.1	17.6	+ 9.8	31.0 ± 4.2
13.2	-65.0 ± 1.4						
17.6	-70.5 ± 0.4						

TABLE 7.2

Inhibition of the I_{sc} by different concentrations of mucosal ethylidene glucose (EG) solution.

Silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (B), and sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. The I_{sc} was first stimulated by sugar alone (2.2 mM of αMG or βMG) until the steady state was achieved. Then the used concentrations of the EG were added simultaneously with the same concentration of the sugar which had been used previously. The numbers in parenthesis indicate the number of animals used for each of the actively transported sugars. The mean values of the obtained I_{sc} ± SE are represented.

First Set (5)	Mucosal Medium (αMG) mM		(EG) mM	The Obtained I_{sc} (μA)
	2.2	+	0.0	116 ± 6
	2.2	+	4.9	112 ± 6
	2.2	+	9.8	105 ± 8
	2.2	+	14.7	98.2 ± 5.1
Second Set (5)	(βMG) mM		(EG) mM	
	2.2	+	0.0	50.0 ± 2.9
	2.2	+	1.6	50.0 ± 4.0
	2.2	+	4.9	49.8 ± 3.5
	2.2	+	9.8	40.6 ± 3.8
	2.2	+	14.7	31.2 ± 3.8

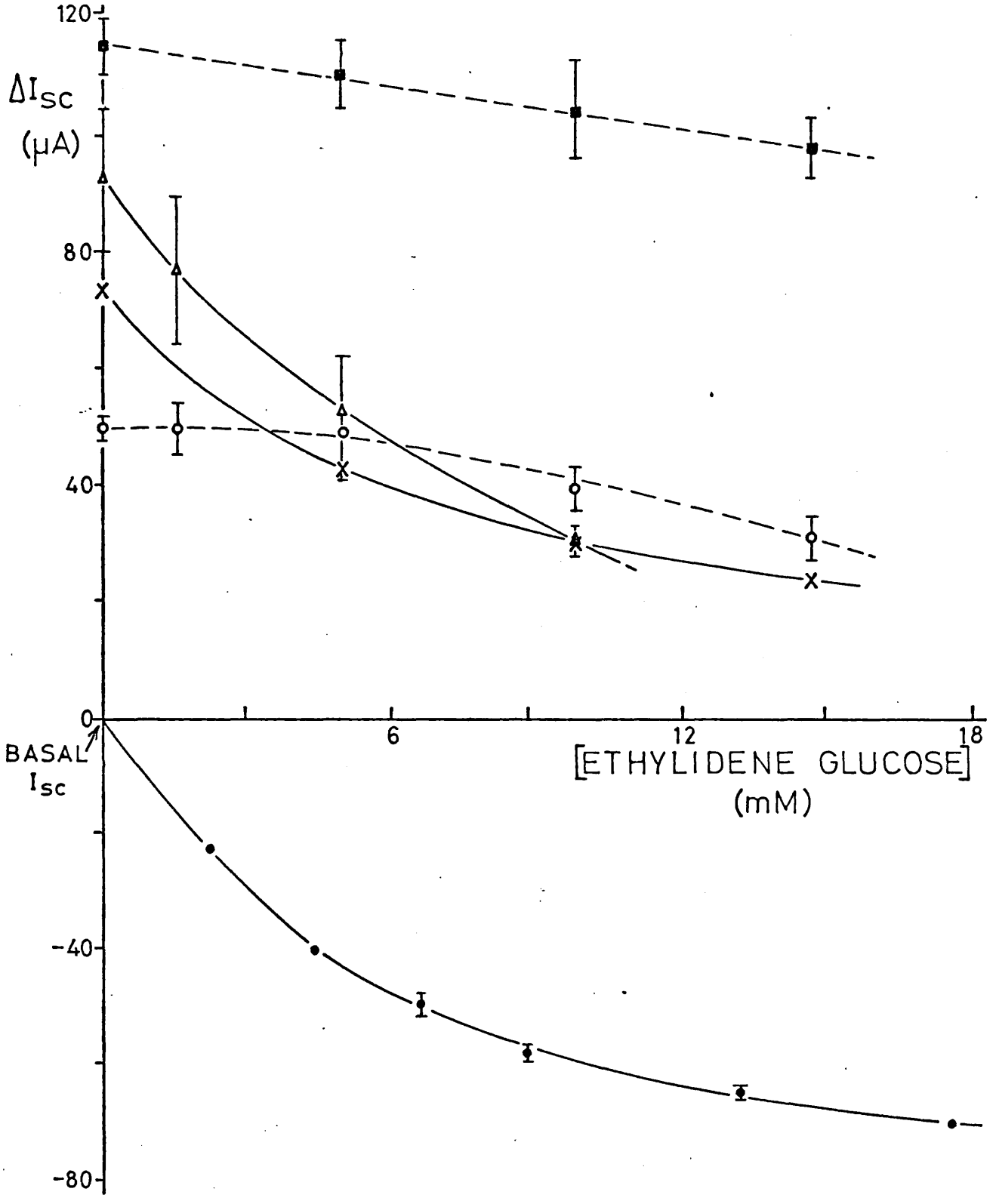


Fig.7.1 The inhibition, by Ethylidene glucose, of the short circuit current induced by various sugars :- ● , basal current; ■ , 2.2 mM α-methyl glucoside; ○ , 2.2 mM β-methyl glucoside; Δ , 17.6 mM 3-O-methylglucose; X , 5.5 mM galactose. (Table 7.1 & 7.2).

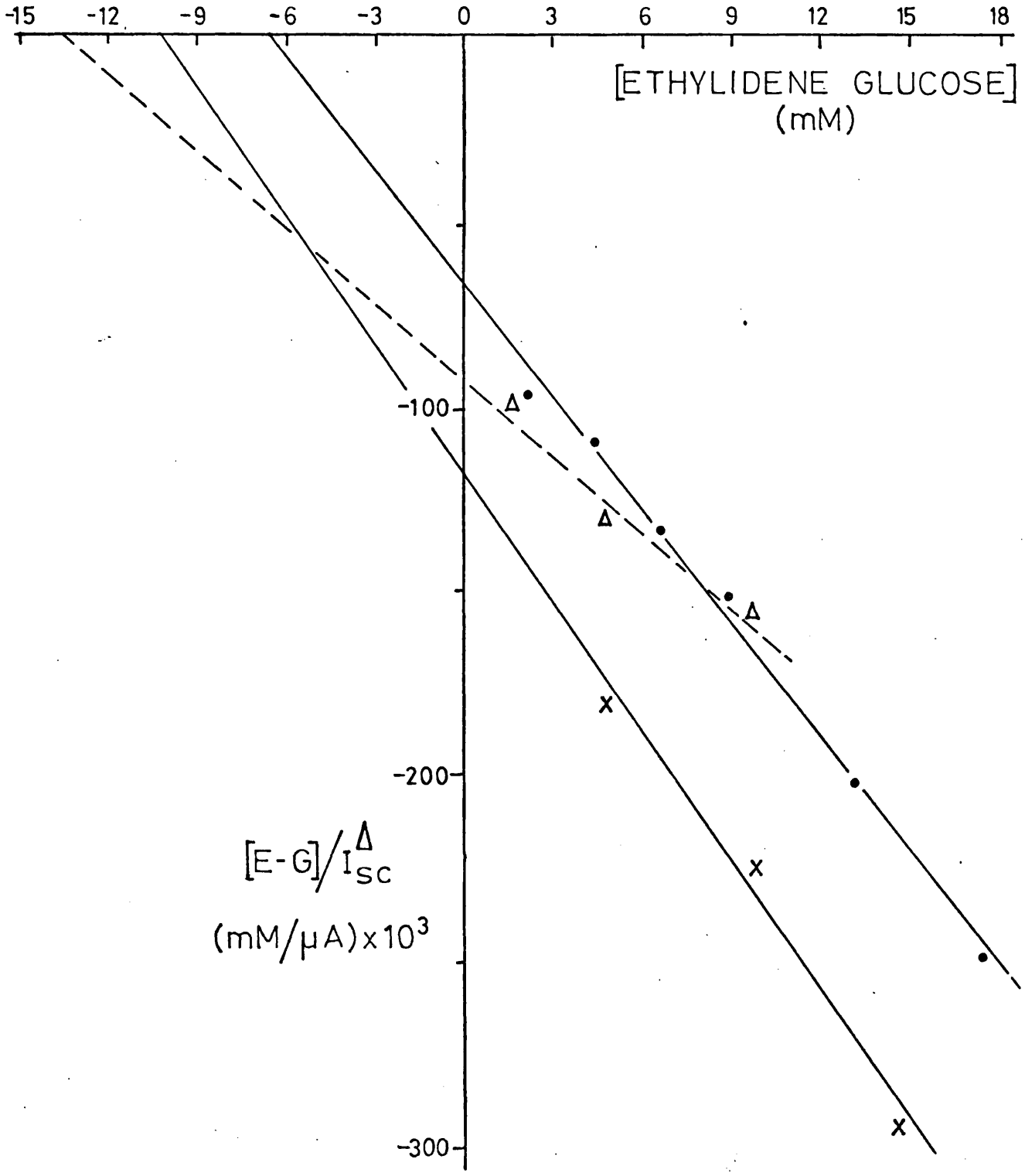


Fig.7.2 Linear plots of inhibition by Ethylidene glucose. Regression equations:- basal current, • , y= -10.3x - 68.5; 17.6mM 3-O-methyl glucose, Δ , y= -6.8x - 92.3; 5.5mM galactose, X , y= -11.5x - 12 0.0

TABLE 7.3

Inhibition of the I_{sc} by different concentrations of mucosal ethylidene glucose (EG) solution.

The agar electrodes were used for the I_{sc} measurements across the intestinal segments (A & B). The used concentrations of glucose and ethylidene glucose were added simultaneously at the mucosal side and 10 ml of sugar-free Krebs' solution was recirculated at the serosal side of the segment. The numbers in parenthesis indicate the number of animals used for each case; the mean values of the obtained I_{sc} ± SE are given.

		(5) Segment (A)		(4) Segment (B)	
Mucosal Glucose mM	Medium EG mM	The Obtained I_{sc} (μA)	The Inhibited I_{sc} %	The Obtained I_{sc} (μA)	The Inhibited I_{sc}%
1.1	18.4	85.0 ± 15.6	45.9	130 ± 28	37.4
1.1	9.8	90.0 ± 24.9	42.7	154 ± 26	26.0
1.1	0.0	157 ± 26		208 ± 33	

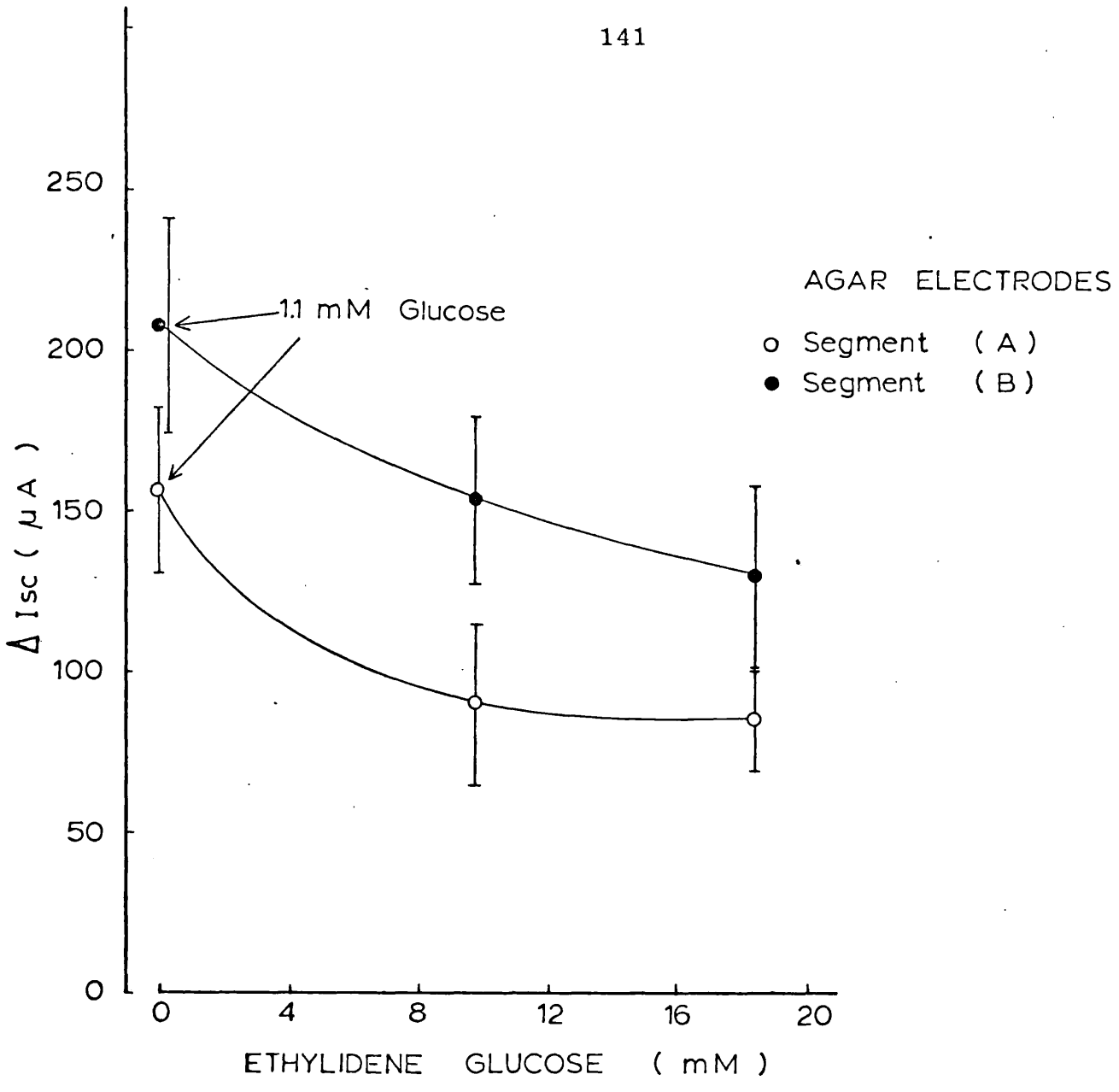


Fig. 7.3 Ethylidene glucose inhibition of the short circuit current induced by glucose (Table 7.3).

TABLE 7.4

Effects of serosal EG on the I_{sc} induced by glucose solutions.

The I_{sc} was induced either by 1.1 mM or 4.4 mM mucosal glucose solution across the intestinal segment (B) of the rat, and in each case the steady state was achieved before applying the EG solution. The serosal saline solution was replaced by 20 mM ethylidene glucose-Krebs' solution to remain for 10 minutes period. A group of experiments were performed for each case of the glucose concentrations; the mean values of the obtained $I_{sc} \pm$ SE are represented. The agar electrodes were used in this measurement.

Number of experiments	Step No.	Mucosal Medium Glucose conc. mM	The Used Serosal Solution	The Obtained I_{sc} (μA)
7	1	1.1	Sugar-free Krebs'	409 ± 23
	2	1.1	20 mM (EG)-Krebs'	370 ± 23
4	1	4.4	Sugar-free Krebs'	733 ± 47
	2	4.4	20 mM (EG)-Krebs'	725 ± 45

TABLE 7.5

Inhibition of the I_{sc} by different concentrations of mucosal benzylidene glucose (BG) solution.

Silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A), and the sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment. In all instances (in the presence and absence of 3MG), the BG concentrations were added after the steady state of the I_{sc} was achieved. The BG concentrations were added simultaneously with the same concentration of the 3MG which had been used previously to stimulate the short-circuit current. The numbers in parenthesis indicate the number of animals used for each case. The mean values of the obtained I_{sc} $\pm$ SE are represented.

The added mucosal BG concentrations mM	(2) First Set The obtained I_{sc} (μA) in presence of 12 mM 3MG	(3) Second Set The obtained I_{sc} (μA) with the saline
0.0	87.0 $\pm$ 3.5	---
0.5	---	-13.3 $\pm$ 2.9
1.0	49.0 $\pm$ 0.7	-22.7 $\pm$ 3.0
1.5	---	-29.3 $\pm$ 4.0
2.0	31.5 $\pm$ 1.8	-32.7 $\pm$ 3.8
3.0	19.0 $\pm$ 0.7	-34.0 $\pm$ 3.7
4.0	13.5 $\pm$ 1.1	-35.7 $\pm$ 3.4
5.0	11.0 $\pm$ 0.7	---

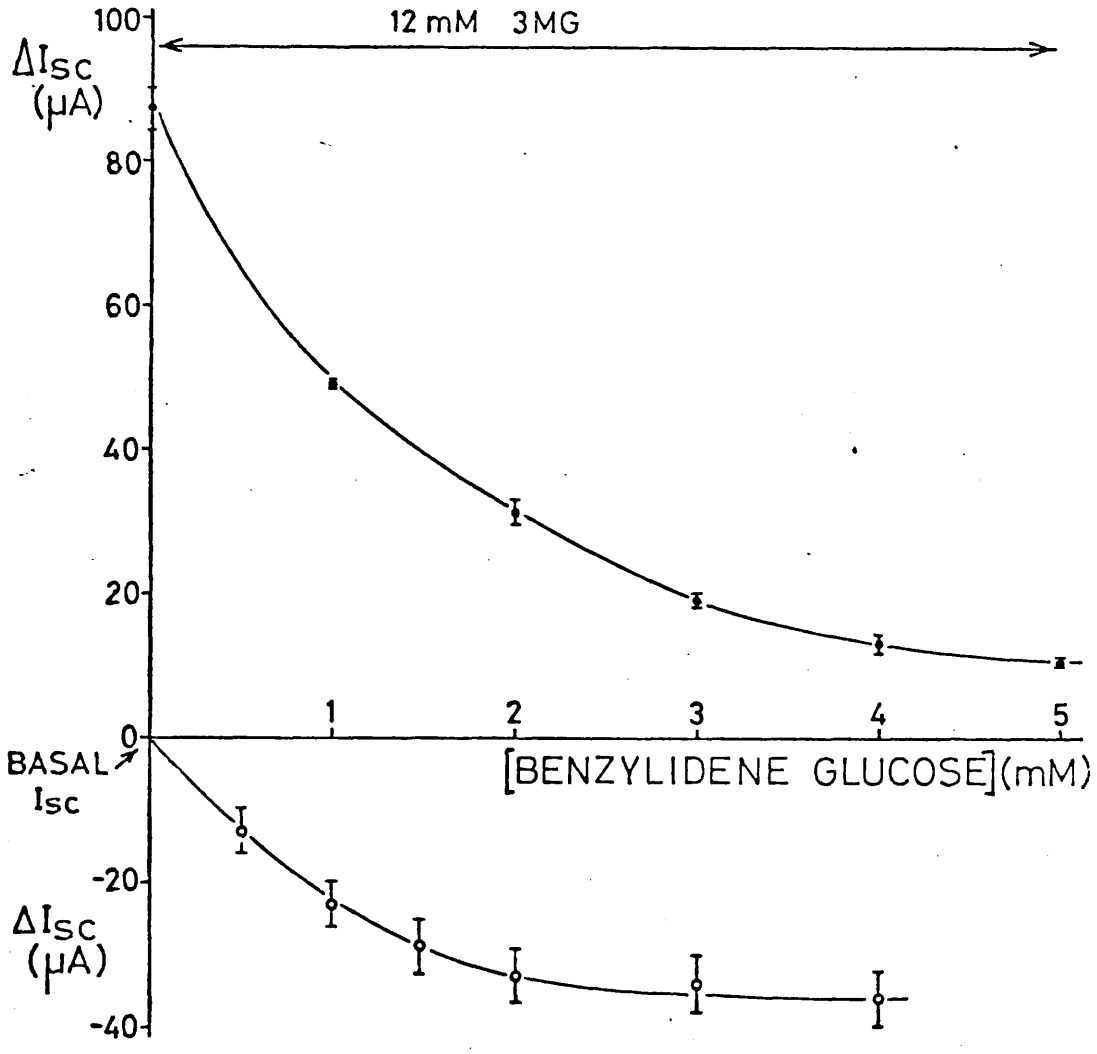


Fig. 7.4 Effects of Benzylidene glucose on the short circuit current in the presence (•) and absence (o) of 12mM 3-O-methyl glucose. (Table 7.5).

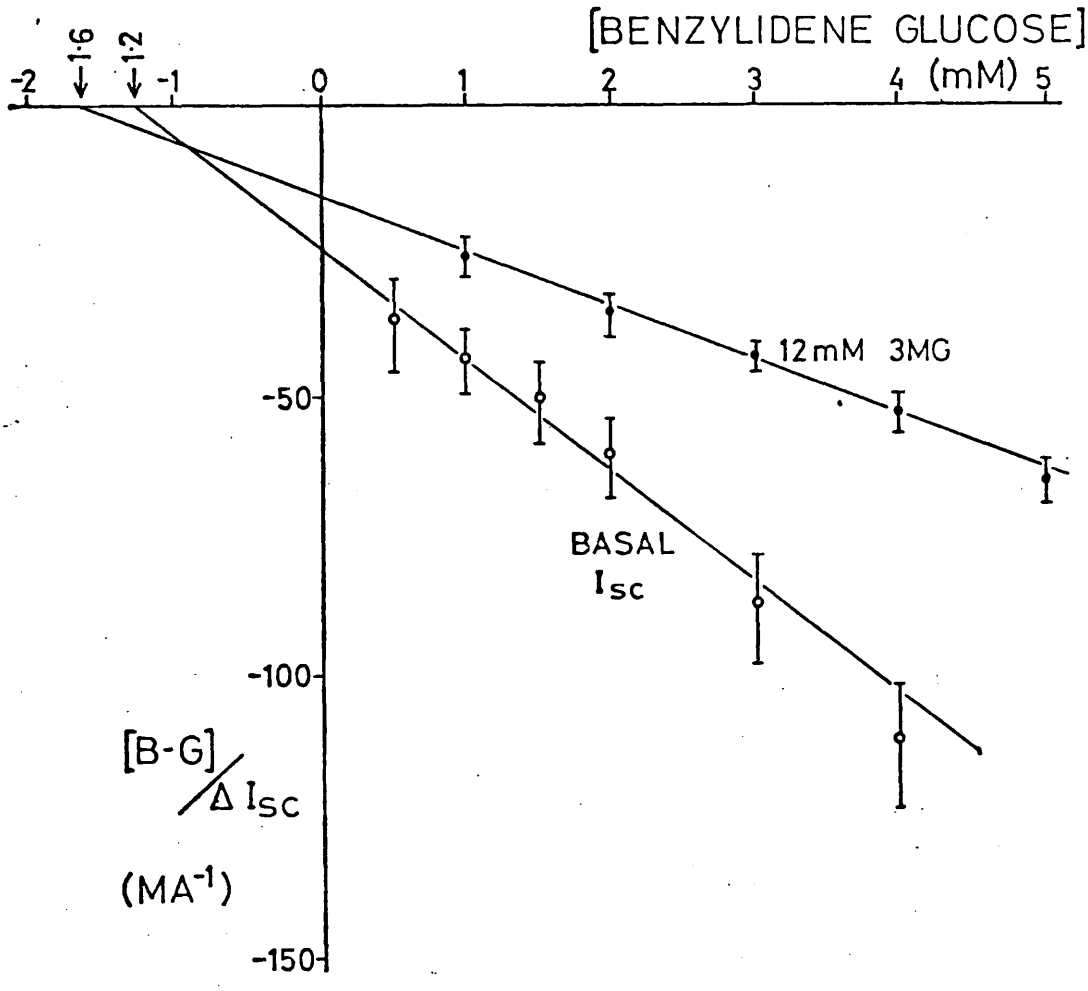


Fig. 7.5 Linear plots of the inhibition due to Benzylidene glucose. Regression equations :- basal current, o , y = -20.1x - 24.3; current induced by 12mM 3-O-methylglucose , • , y = -9.8x -15.8.

TABLE 7.6

Inhibition of the I_{sc} by different concentrations of mucosal mannose solution.

Ag/AgCl electrodes were used for the I_{sc} measurements across the intestinal segment of the rat. Sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment. In all instances (in the presence and absence of galactose) the mannose was added after the steady state of the I_{sc} was achieved. The mannose concentrations were added simultaneously with the same concentration of the galactose (6.6 mM) which had been used previously to stimulate the I_{sc}. The numbers in parenthesis indicate the number of animals used for each case. The mean values of the obtained I_{sc} $\pm$ SE are represented.

The mannose concentrations mM	(2) First Set The obtained I_{sc} (μA) in presence of 6.6 mM galactose	(2) Second Set The obtained I_{sc} (μA) with the saline
0.0	73.5 $\pm$ 3.2	---
2.2	64.5 $\pm$ 3.2	-30.5 $\pm$ 0.4
4.4	55.0 $\pm$ 3.5	-46.5 $\pm$ 1.1
6.6	44.0 $\pm$ 2.8	-59.5 $\pm$ 1.8
8.8	37.5 $\pm$ 3.2	-70.5 $\pm$ 6.0
11.1	31.5 $\pm$ 3.9	---
13.2	29.5 $\pm$ 3.9	-91.5 $\pm$ 4.6
17.6	26.0 $\pm$ 2.8	-102 $\pm$ 6.8
22.2	24.0 $\pm$ 2.8	-110 $\pm$ 9.2
28.8	---	-116 $\pm$ 11.3
33.2	---	-120 $\pm$ 14.1

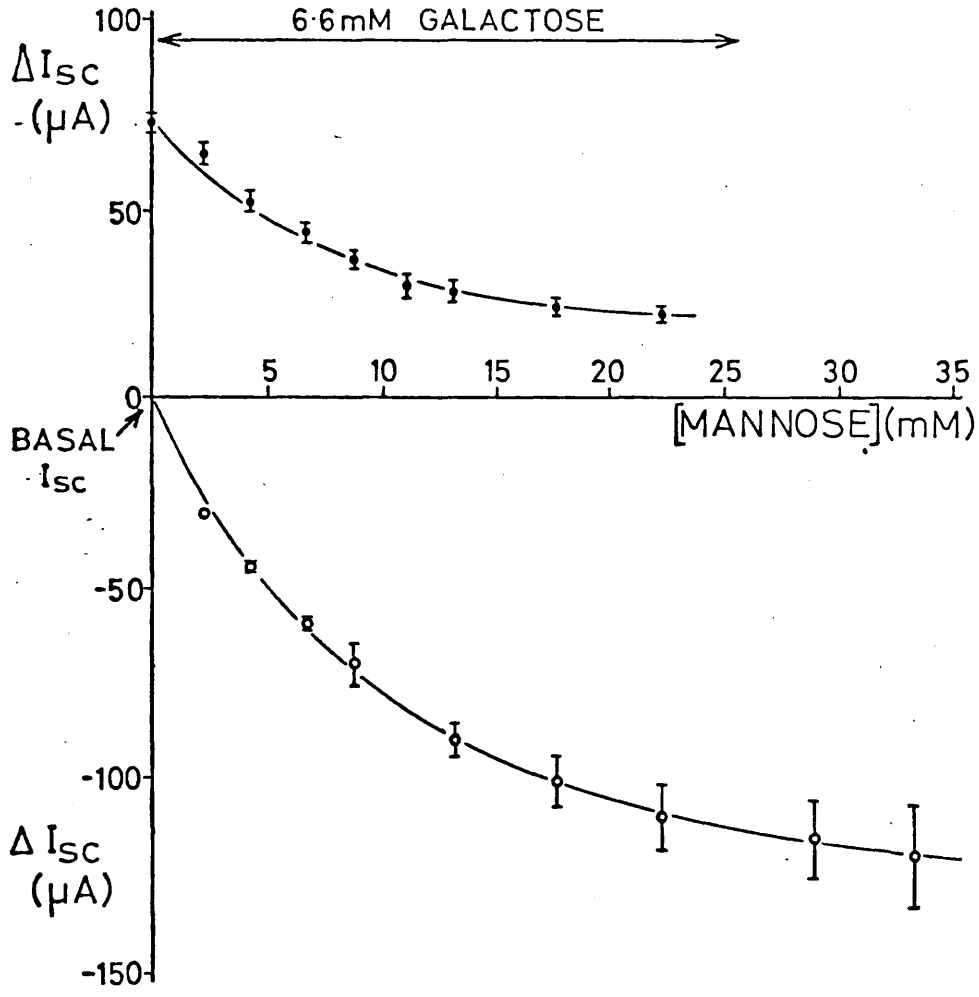


Fig. 7.6 Inhibition of the short circuit current by Mannose. o , basal current; • , current induced by 6.6mM galactose. (Table 7.6).

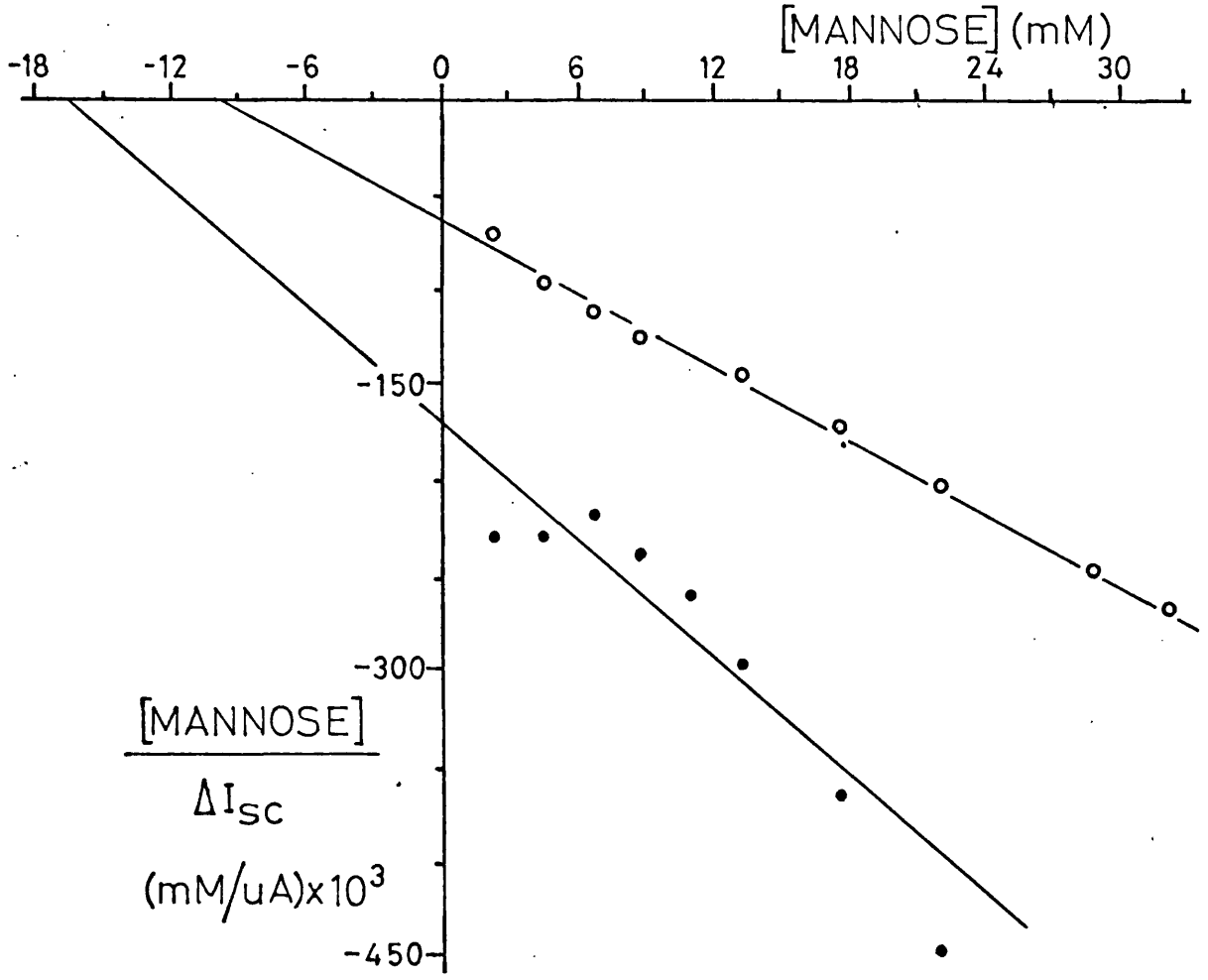


Fig. 7.7 Linear plots of effect of Mannose on, o , basal current and, ● , 6.6mM galactose induced current. Regression equations y = -6.4x -64.1 and y = -10.6x - 178 respectively.

TABLE 7.7

Inhibition of the I_{sc} by different concentrations of mucosal lactose solution.

Ag/AgCl electrodes were used for the I_{sc} measurements across the intestinal segment of the rat. Sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment. In all instances (in the absence and presence of 4.4 mM galactose) the lactose was added after the steady state of the I_{sc} was achieved. The lactose concentrations were added simultaneously with the same concentration of galactose (4.4 mM) which had been used previously to stimulate the I_{sc}. The numbers in parenthesis indicate the number of animals used for each case; the mean values of the obtained I_{sc} $\pm$ SE are represented.

The Lactose concentrations mM	(2) The obtained I_{sc} (μA) in presence of 4.4 mM galactose	(2) The obtained I_{sc} (μA) in presence of sugar-free Krebs' solution
0.0	57.0 $\pm$ 1.4	---
2.2	44.0 $\pm$ 0.7	-15.5 $\pm$ 0.4
4.4	36.5 $\pm$ 1.1	-23.0 $\pm$ 0.7
6.6	31.0 $\pm$ 0.7	---
8.8	29.0 $\pm$ 0.4	-31.0 $\pm$ 0.7
13.2	25.0 $\pm$ 0.0	-35.5 $\pm$ 0.4
17.6	22.5 $\pm$ 0.4	-39.0 $\pm$ 0.7
22.2	---	-40.0 $\pm$ 1.4

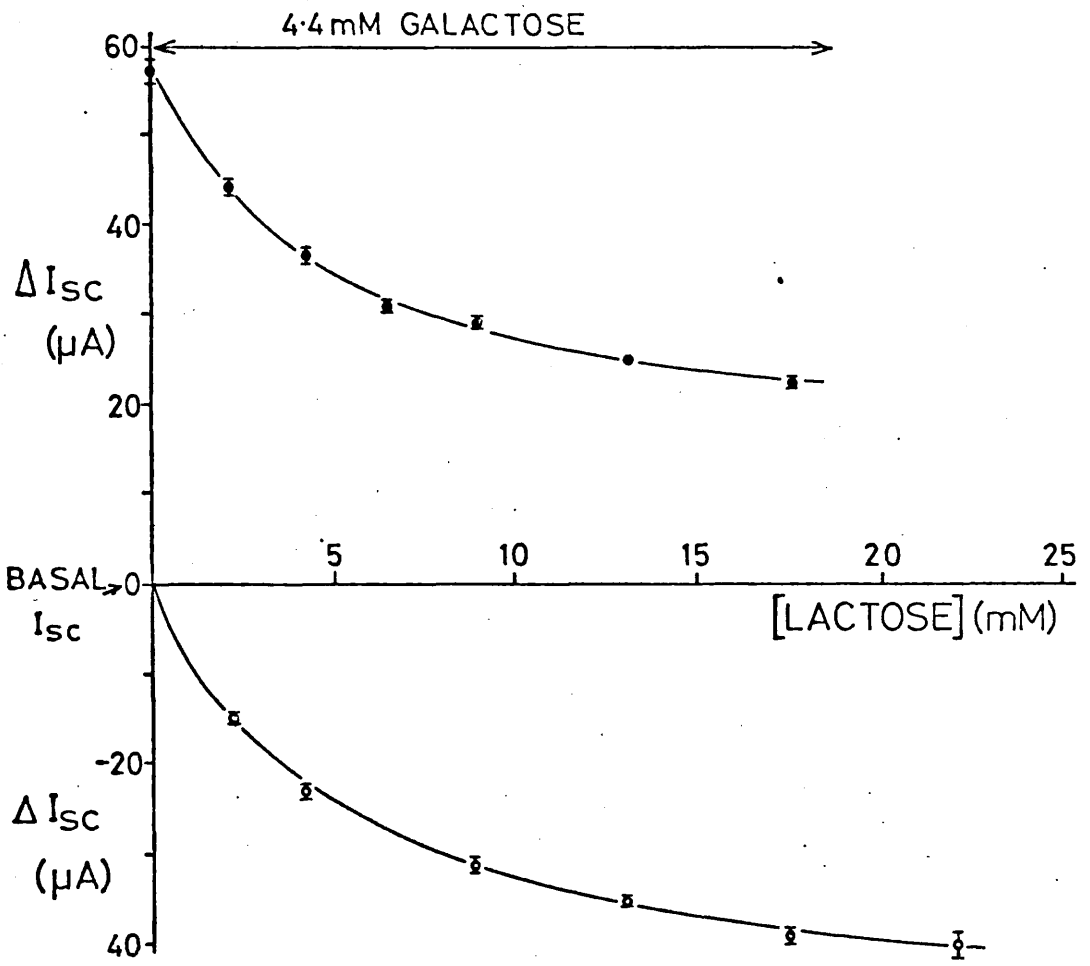


Fig. 7.8 Effect of Lactose on the basal short circuit current (o) and that induced by 4.4mM galactose (•). From Table 7.7.

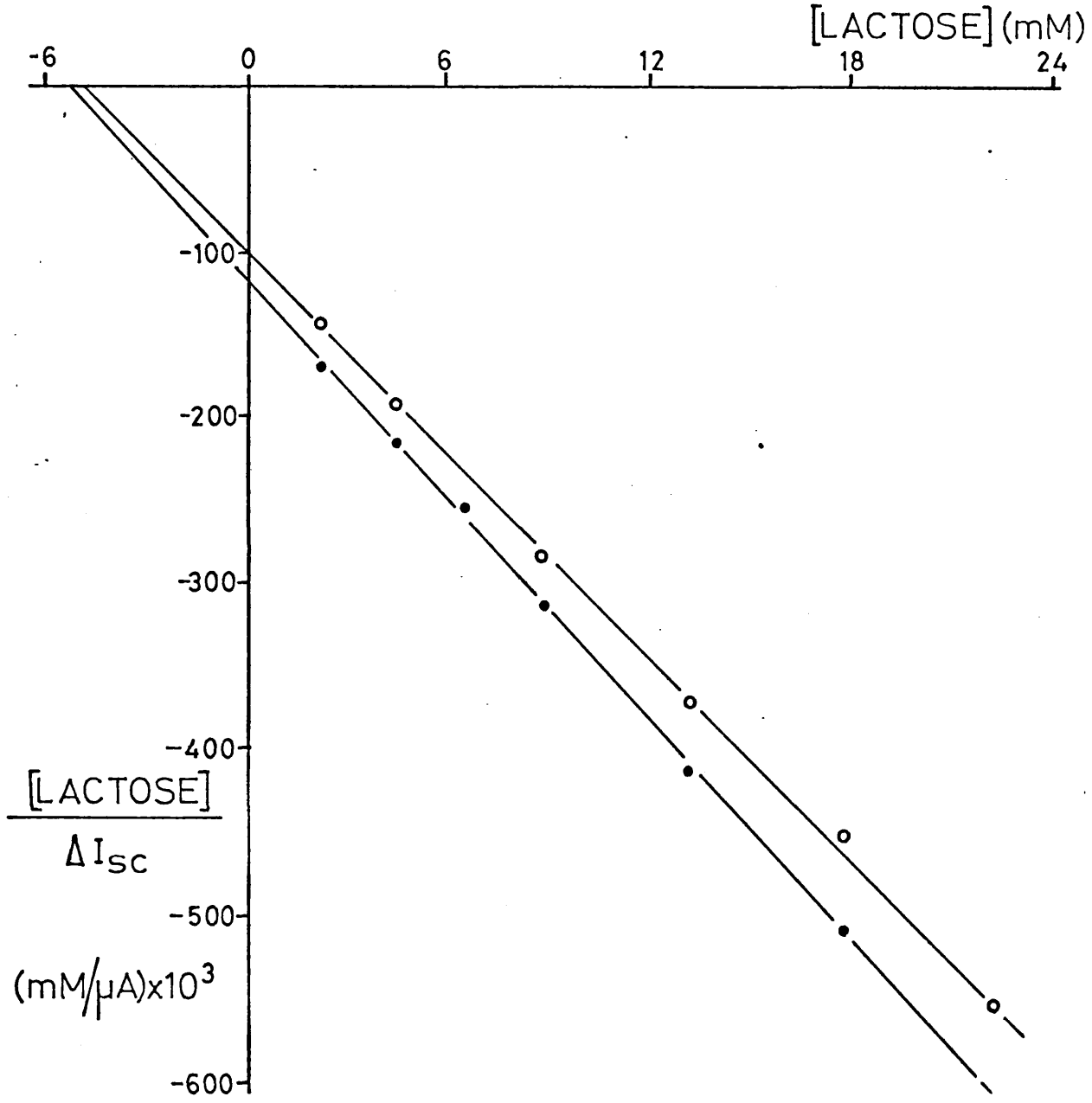


Fig. 7.9 Linear plots of the inhibition due to Lactose. Regression equations:- basal current, ° , y = -20.4x - 101; 4.4mM galactose, • , y = -22.4x - 115.

TABLE 7.8

Inhibition of the basal short-circuit current by different concentrations of mucosal cellobiose solution.

Silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. Sugar-free Krebs' solution (5 ml) was recirculated at the serosal side and 60 ml of the same solution was used at the mucosal side of the segment. Two experiments were performed and the mean values of the obtained $I_{sc} \pm$ SE are given.

Cellobiose concentration (mM)	The inhibited values of the I_{sc} (μA)
2.2	-13.3 ± 2.6
4.4	-19.0 ± 3.4
8.8	-22.7 ± 4.4
13.2	-29.3 ± 3.5
17.6	-33.0 ± 4.5
22.2	-37.0 ± 4.9

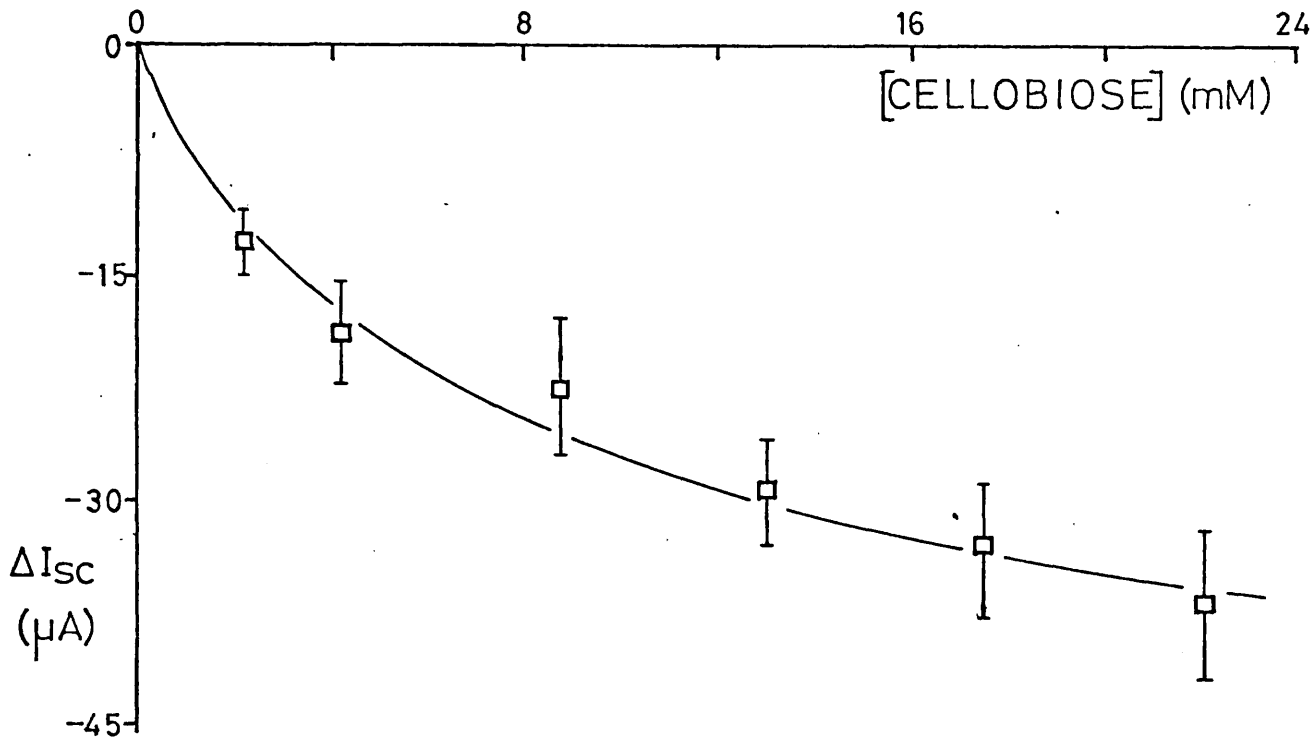


Fig. 7.10 Inhibition of the basal short circuit current by Cellobiose (Table 7.8).

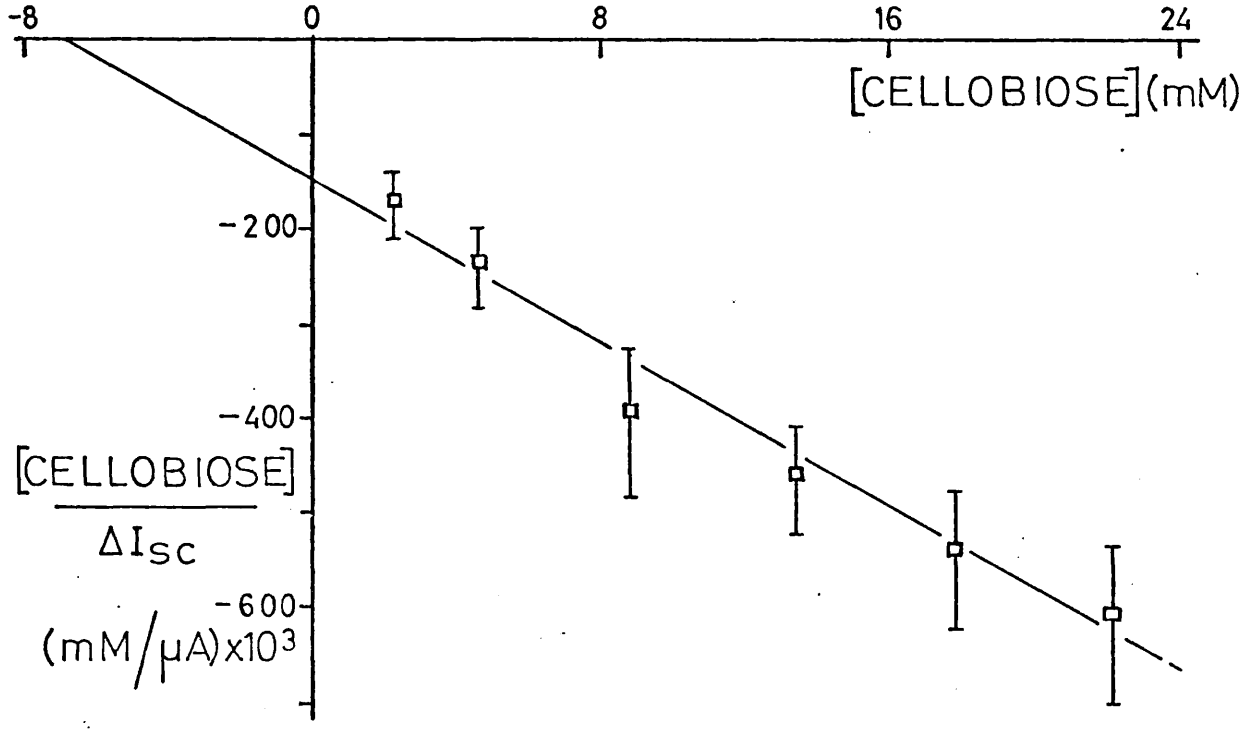


Fig. 7.11 Linear plot of inhibition by Cellobiose. Regression equation $y = -21.6x - 149$.

Michaelis-Menten (1913) kinetics for a saturable enzyme (or carrier) system. Thus the relationship between the concentrations of the non-transportable sugar and the I_{sc} was analysed (Figures 7.2, 7.5, 7.7, 7.9, 7.11) in a similar manner followed for the actively transported sugars in Chapter 4. The Kis and ΔImaxs for the ethylidene glucose, benzylidene glucose, mannose, lactose and cellobiose were determined (Table 7.10). Lactose seems not to be hydrolysed in the adult rat small intestine; it might be because of the absence of the β-glycosidase enzyme necessary for lactose hydrolysis (Alvarez & Sas, 1961, Doell & Kretchmer, 1962 and Koldovesky & Chytil, 1965). Probably for a similar reason, cellobiose seems not to be hydrolysed in the adult rat small intestine. Lactose and cellobiose effects are probably due to the unhydrolysed molecules of these sugars acting extracellularly at the mucosal surface of the cell membrane.

It can be noticed also that there is a good deal of similarity in the inhibitory action of lactose, cellobiose and the other sugars. Thus the results of lactose, cellobiose and the other non-transportable sugars all together may suggest an involvement of the sugar-free carrier system in the Na^+ transport across the mucosa. This carrier system may be inhibited by becoming engaged with the non-transportable sugar molecule at the outer surface of the mucosal cell and tend to become fixed there and so blocking some of the Na^+ entry to the cell. This suggestion might be strengthened by the findings that there was no appreciable inhibition of the short-circuit current when ethylidene glucose (20 mM) was used at the serosal side of the intestinal segment (Table 7.4).

Increasing concentrations of phloridzin in a step-wise manner

TABLE 7.9

Inhibition of the basal short-circuit current by different concentrations of mucosal phloridzin solution.

Silver/silver chloride electrodes were used for the short-circuit current measurements across the intestinal segment (A) of the rat. Sugar-free Krebs' fluid (5 ml) was recirculated at the serosal side and 60 ml of the same solution was used at the mucosal side of the segment. The phloridzin solution was added after the steady state of the I_{sc} was achieved. Two animals were used and the mean values of the obtained $I_{sc} \pm$ SE are represented.

Phloridzin Conc. (M)	The inhibited I_{sc} (μA)
2×10^{-5}	-16.3 ± 0.9
4×10^{-5}	-41.3 ± 6.2
6×10^{-5}	-50.0 ± 7.1
8×10^{-5}	-55.0 ± 8.8
1×10^{-4}	-58.8 ± 9.7

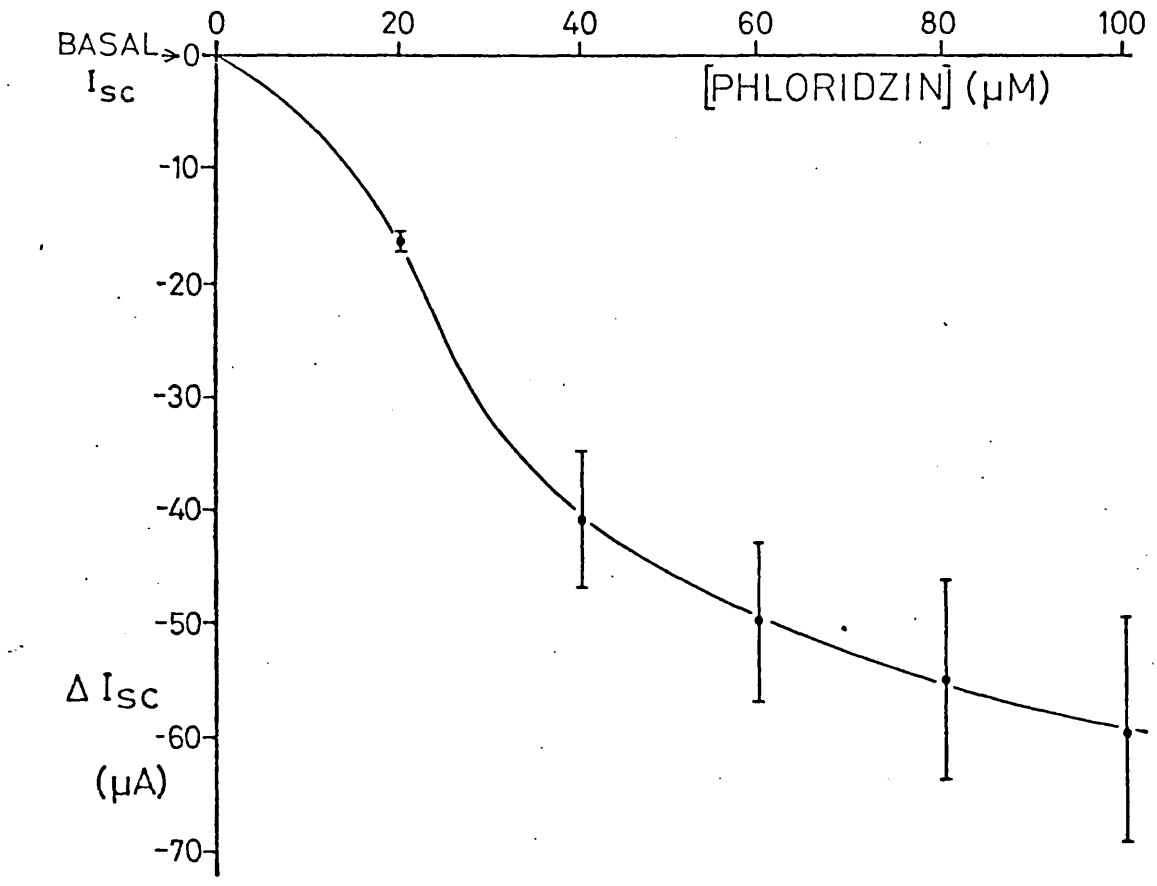


Fig. 7.12 Effect of Phloridzin on the short circuit current in the absence of sugar. (Table 7.9).

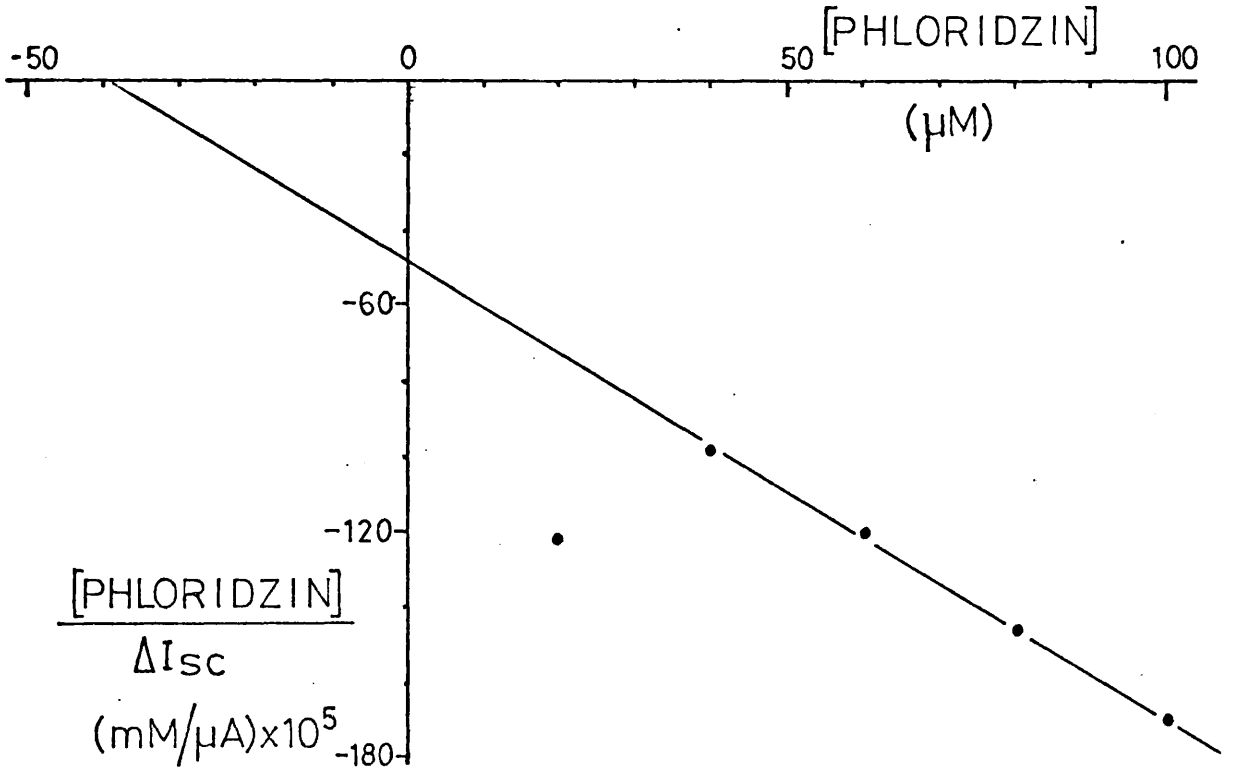


Fig. 7.13 Linear plot of the inhibition due to Phloridzin. Regression equation y = - 1.22x - 48.

(Table 7.9 and Figure 7.12) also gave similar effects to those obtained by ethylidene glucose and other non-transportable sugars in the absence of the actively transportable sugars.

Mannose at a concentration of 33.2 mM inhibited 96% of the basal short-circuit current. This result might suggest that at least a considerable magnitude of the short-circuit current recorded in the sugar free medium at the steady state is related to the Na^+ flux carried by the carrier system.

Similar inhibiting action also was observed for the different concentrations of ethylidene glucose, benzylidene glucose, mannose and lactose in the presence of constant concentrations of the actively transported sugars (galactose, 3-O-methylglucose and glucose) as illustrated in Figures (7.1, 7.4, 7.6, 7.8). A similar approach was also followed for the determination of the Kis and ΔImaxs of the four non-transportable sugars in the presence of the actively transported sugars (Figures 7.2, 7.5, 7.7, 7.9). The estimated parameters are presented in Table (7.10). These results clearly indicate that a mutual inhibition has occurred between the non-transportable sugars (ethylidene glucose, benzylidene glucose, lactose and mannose) and the actively transported sugars (galactose and 3-O-methylglucose).

In the case of using 2.2 mM α-methylglucose (αMG) or β-methylglucose (βMG) Figure (7.1) the inhibitory effects of the used concentrations of ethylidene glucose solution on the I_{sc} induced by either of the two sugars seem to be different from the other used sugars. The inhibitory effects of the ethylidene glucose concentrations on the I_{sc} induced by (αMG) seem to give rise to a declining straight line. The I_{sc} induced

TABLE 7.10

The kinetics of the non-transportable sugars (inhibitors) and phloridzin at 37°C.

The parameters were obtained using the silver/silver chloride electrodes for the I_{sc} measurements across the intestinal segments of the rat, in the absence and presence of actively transported sugar (3-O-methylglucose and galactose). The parameters were estimated by regression line as recommended by Riggs (1972).

The Inhibitor	The added actively transported sugar	Ki mM	ΔI max μA
ethylidene glucose		6.7	97.1
ethylidene glucose	17.6 mM 3MG	13.5	146.3
ethylidene glucose	5.5 mM galactose	10.4	86.9
benzylidene glucose		1.2	49.8
benzylidene glucose	12 mM 3MG	1.6	101.9
mannose		10.1	157.3
mannose	6.6 mM galactose	16.8	94.3
lactose		5.0	49.0
lactose	4.4 mM galactose	5.1	44.6
cellobiose		6.9	46.3
phloridzin		39×10^{-3}	81.9

by (βMG) seems not to be affected by the lower concentrations of ethylidene glucose solution up to 4.9 mM; beyond the latter concentration and up to 14.7 mM the relationship seems to be linear. In this case more work is needed and a wider range of ethylidene glucose might be applied.

Jorgensen, Landau and Wilson (1961) stated that when two substrates compete with one another in a biochemical process it is presumed that their pathways have at least one step in common. They added that transport of sugars across the intestinal epithelial cells is, in all probability, a complex process involving translocation across at least two permeability barriers, adsorption to one or more carriers or enzymes and coupling of a portion of the process to energy yielding reactions of the cell. Theoretically if any one of these steps were common to two transported substances competition might be observed.

However, lactose results might throw a good deal of light on the understanding of the action site of ethylidene glucose, benzylidene glucose and mannose.

Mannose is also a very well known metabolized but non-actively transportable sugar in the animals intestine, and if it penetrates the mucosal cell by diffusion no competition for the energy supply would be expected, thus the current might improve rather than decline. In this case also the quick reduction observed soon after the addition of mannose concentrations might rule out other intracellular inhibition action as the penetration of this sugar into the mucosal cell would be rather slow by the process of diffusion.

Moreover, this suggestion is in accordance with the findings of Baker & Widdas (1973 a & b) in the human red cell for ethylidene glucose

TABLE 7.11

Inhibition of the I_{sc} by (2.3×10^{-1} mM) mucosal phloridzin solution.

The agar electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. The I_{sc} was induced by 8.8 mM mucosal glucose-Krebs' solution, and the phloridzin was added after the I_{sc} steady state was achieved. Glucose-Krebs' solution (10 ml of 22.2 mM) was recirculated at the serosal side of the segment. Six experiments were performed and the mean values of the obtained I_{sc} $\pm$ SE are represented.

Step No.	Mucosal Medium Glucose mM	Phloridzin mM	Time of solutions applications (minutes)	The obtained I_{sc} (μA)
1	8.8 +	0.0	15 - 20	597 $\pm$ 48
2	8.8 +	2.3×10^{-1}	15 - 20	23.3 $\pm$ 6.6
3	8.8 +	0.0	10	60.8 $\pm$ 11.5
	8.8 +	0.0	20	101 $\pm$ 19
	8.8 +	0.0	30	148 $\pm$ 32

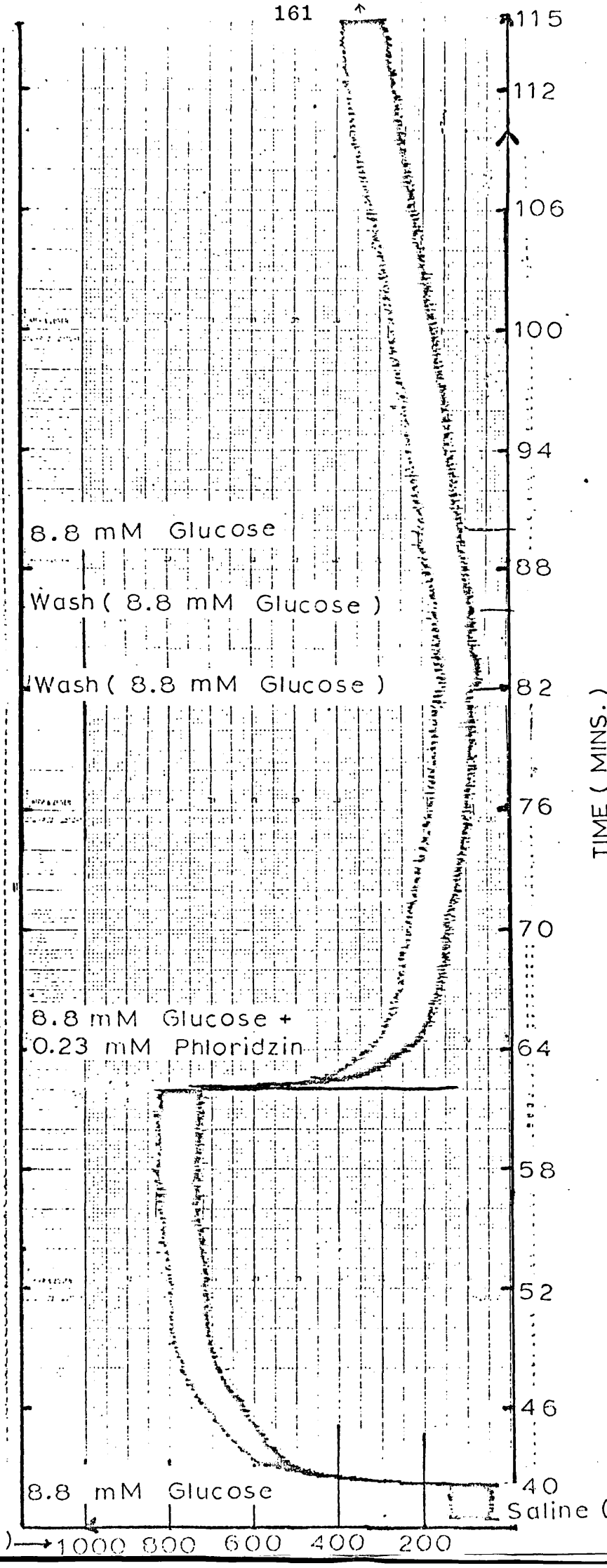


Fig. 7.14 Phloridzin inhibition of the short circuit current induced by glucose.

and Novak & LeFevre (1974) in the human erythrocyte for benzylidene glucose. This conclusion is also consistent with the findings of Bihler (1965) in the hamster small intestine for other non-transportable sugars e.g. D-xylose and L-fructose.

More results will be presented in Chapters 9 and 10 about the effects of ethylidene glucose solution on the fluxes of Na^+ and 3-O-methylglucose across short-circuited intestinal segments of the rat.

The effects of phloridzin, ouabain and iodo-acetic acid (IAA).

The effect of addition of phloridzin (2.3×10^{-1} mM) to the mucosal solution after the I_{sc} had been induced by the addition of 8.8 mM mucosal glucose solution is shown in Table (7.11). Six experiments were performed using agar electrodes for the measurements of the I_{sc} across the rat intestinal segment (A). Glucose-Krebs' solution (10 ml of 22.2 mM) was recirculated at the serosal side of the segment throughout the experiment. Figure (7.14) shows an example of the experiments; in all cases phloridzin was added to the mucosal solution after the I_{sc} steady state had been achieved in the glucose solution. The addition of phloridzin resulted in an immediate fall in the I_{sc} which rapidly reached values approximating those recorded in the absence of mucosal glucose solution. However, in all instances phloridzin remained in the mucosal solution for 15-20 minutes until the I_{sc} levels were just slightly higher than those observed in the absence of mucosal glucose solution. The I_{sc} gradually recovered when the phloridzin was omitted by the addition of a new solution of 8.8 mM glucose. The mucosal and the serosal solutions were changed successively at least twice each for a four minute period. This was done in order to ensure washing out at least most of the phloridzin from the gut. Under these conditions the average rate of the

TABLE 7.12

Inhibition of the I_{sc} by (5×10^{-1} mM) serosal ouabain solution.

The silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. In the first and second set of experiments the I_{sc} was induced respectively by glucose and 3-O-methylglucose (3MG) solutions. These sugars were added to the mucosal side of the segment and the steady state of the obtained I_{sc} usually was achieved before the addition of the ouabain to the recirculated saline solution (10 ml) at the serosal side of the segment. Four experiments were performed for each set, and the mean values of the obtained I_{sc} $\pm$ SE are represented.

First Set

Mucosal Medium glucose conc. mM		Serosal Medium ouabain conc. mM	Time of solutions application (minutes)	The obtained I_{sc} (μA)
4	+	0.0	20 - 30	103 $\pm$ 21
4	+	5×10^{-1}	10	100 $\pm$ 24
4	+	5×10^{-1}	20	93.0 $\pm$ 24.0
4	+	5×10^{-1}	30	86.8 $\pm$ 23.0
4	+	5×10^{-1}	40	81.8 $\pm$ 21.6
Second Set				
(3MG) conc.				
9	+	0.0	20 - 30	75.5 $\pm$ 8.1
9	+	5×10^{-1}	10	67.5 $\pm$ 9.8
9	+	5×10^{-1}	20	59.8 $\pm$ 11.2
9	+	5×10^{-1}	30	56.0 $\pm$ 10.9
9	+	5×10^{-1}	40	54.5 $\pm$ 11.4

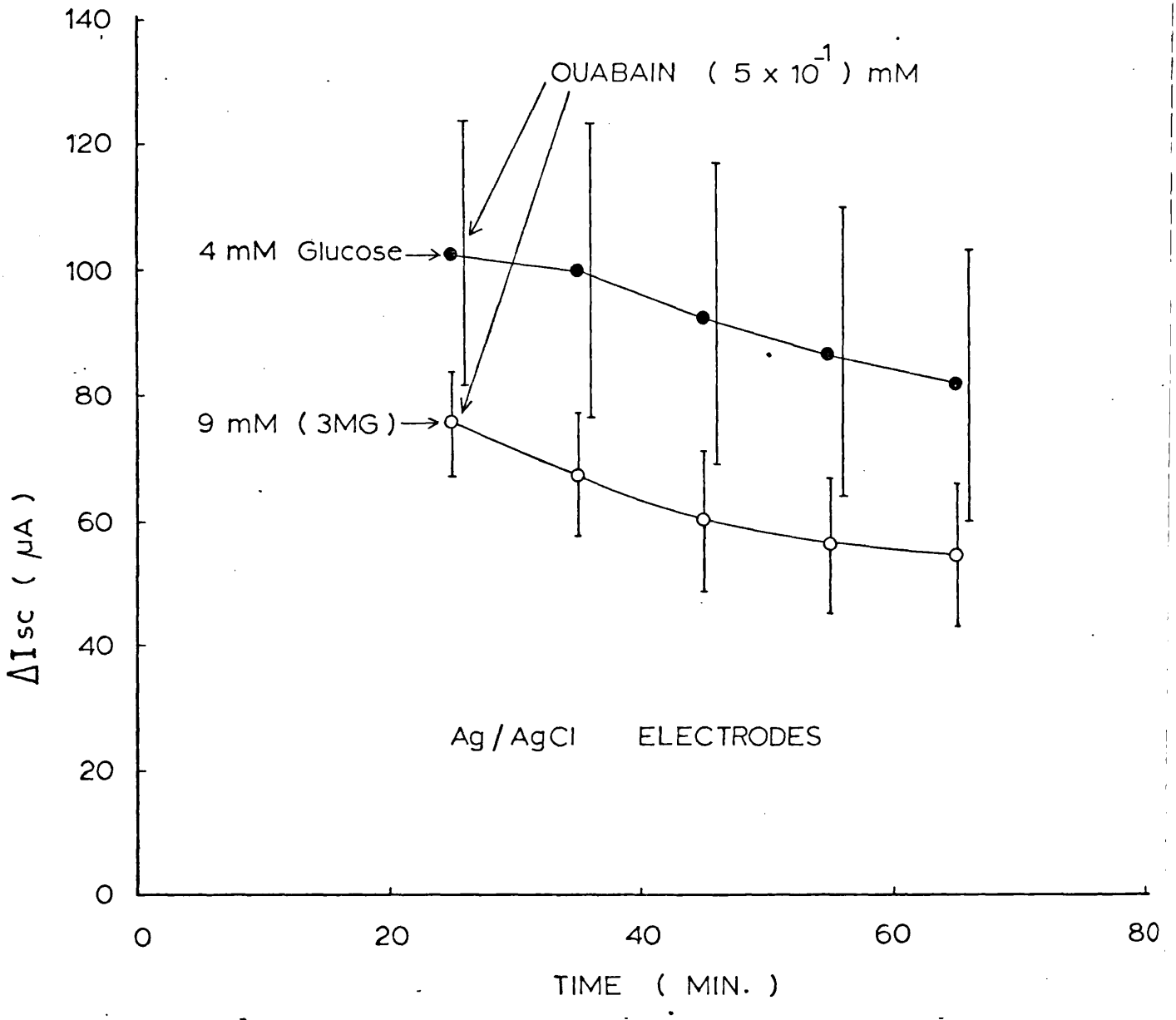


Fig. 7.15 Ouabain inhibition of the short circuit current generated by glucose (•) and 3-O- methyl glucose (o) (Table 7.12).

I_{sc} recovery was about (4 μA/min). More results about the effects of phloridzin on 3-O-methylglucose influx in a short-circuited rat's segment will be presented in Chapter 10.

The inhibitory action of low concentrations of cardiac glucosides on active transport processes in the small intestine and in a wide variety of animal tissues has been well documented. Figure (7.15) shows the effects of ouabain on the I_{sc} induced either by 4 mM glucose or 9 mM 3-O-methylglucose solutions across the intestinal segment (A) of the rat. Using silver/silver chloride electrodes for the I_{sc} measurements, four experiments have been performed for each sugar and the mean values $\pm$ SE of the obtained I_{sc} are presented in Table (7.12). After the I_{sc} steady state was achieved with the sugar solution at the mucosal side, the serosal sugar-free Krebs' solution was replaced by a similar volume (10 ml of 5×10^{-1} mM) ouabain solution. In all cases the addition of ouabain resulted in a gradual decline of the I_{sc} following a brief lag period. In forty minutes time the reductions in the I_{sc} levels due to the ouabain action were only 20.4% and 27.8% in the presence of mucosal glucose and 3-O-methylglucose respectively. Schultz & Zalusky (1964a) using 2×10^{-4} M ouabain at the serosal side of the isolated rabbit ileum reported that in a period of twenty minutes, about 85% inhibition in the I_{sc} stimulated by the sugar. But in the same paper and also in a following publication (1964b), they reported that as time elapsed the I_{sc}, in the presence of sugars, continued to decline from the stable level. However, Lyon & Crane (1966b) reported different species specificity and also differences in jejunal and ileal sensitivity to ouabain. They have performed their experiments with the rat's sacs within the 6 - 7 minutes of the stable levels of the potential

TABLE 7.13

Inhibition of the I_{sc} by (1.3 mM) mucosal iodo-acetic acid (IAA) solution.

The Ag/AgCl electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. The I_{sc} first was induced by 2.2 mM mucosal glucose solution and remained until the steady state was achieved. Then combination of increasing glucose concentrations and the iodo-acetic acid were added in a step-wise manner at the mucosal side of the segment, and finally iodo-acetic acid was omitted from the solution bathing the intestinal wall of the segment. Sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. Six experiments were performed and the mean values of the obtained I_{sc} $\pm$ SE are represented.

Step No.	Mucosal glucose mM	Medium (IAA) mM	Time of solutions applications (minutes)	The obtained I_{sc} (μA)
1	2.2 +	0.0	14 - 20	144 $\pm$ 9
2	2.2 +	1.3	6	108 $\pm$ 5
	2.2 +	1.3	15 - 16	91.2 $\pm$ 5.8
3	4.4 +	1.3	6	64.8 $\pm$ 5.5
4	8.8 +	1.3	15 - 20	25.7 $\pm$ 6.1
5	8.8 +	0.0	15 - 20	-93.0 $\pm$ 10.8

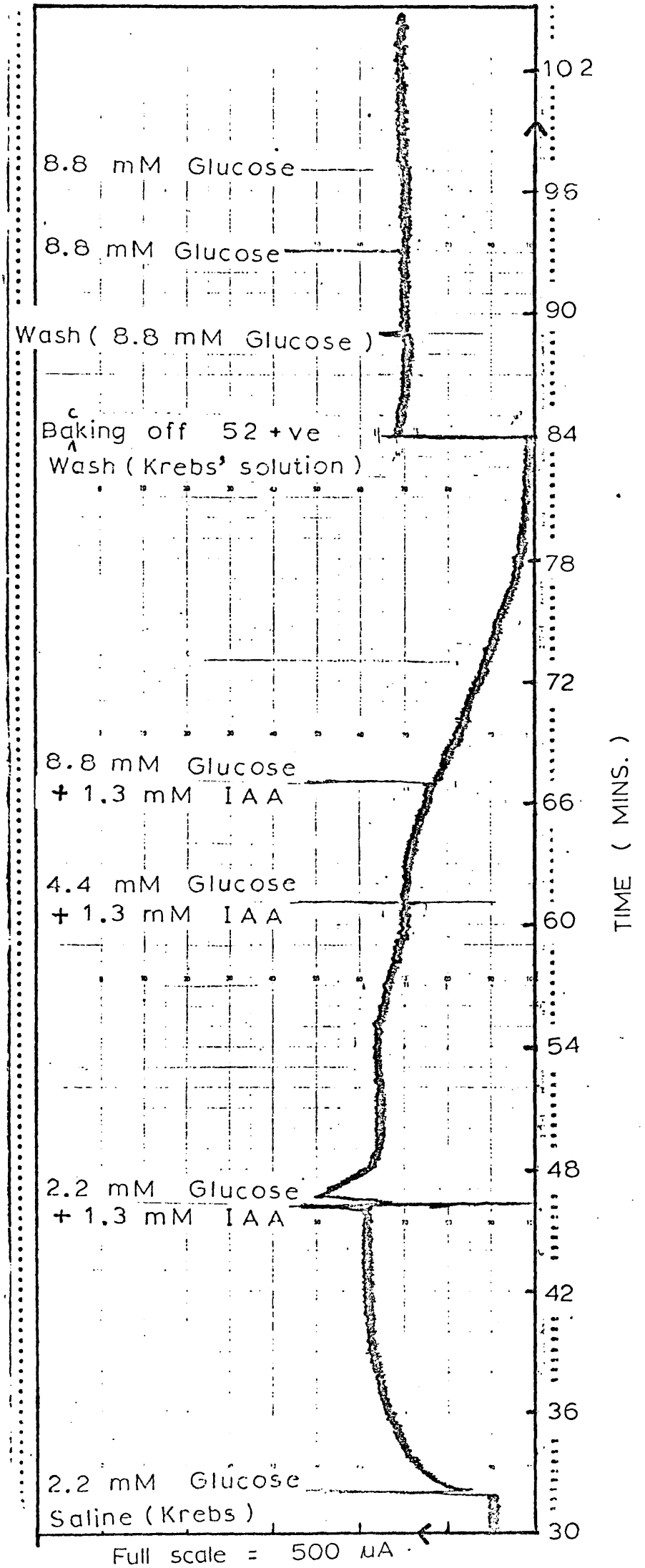


Fig. 7.16 Iodo-acetic acid inhibition of the short circuit current induced by glucose.

difference. As it will be shown later (Chapter 9), using the new device (gut supporting cannula), it was found that the I_{sc}, once a steady state was achieved, was remarkably stable for relatively long periods. However, Figure (7.15) shows that the inhibitory action of the ouabain on the I_{sc} induced by glucose or 3-O-methylglucose solutions gave rise to shallow hyperbolae shaped curves. These results seem to be in agreement with a recent publication by Lauterbach (1972) who found that in the guinea-pig intestinal epithelial cells ouabain permeates the cells both by diffusion and by a saturable transport process.

Table (7.13) shows the inhibitory action of iodo-acetic acid (1.3 mM) on the I_{sc} stimulated by mucosal glucose solutions. Six experiments were undertaken using silver/silver chloride electrodes for the measurements of the I_{sc} obtained across the intestinal segment (A). Figure (7.16) is an example of these experiments; in all instances iodo-acetic acid (IAA) was added to the mucosal solution after the I_{sc} steady state have been achieved with the glucose solution. The addition of IAA resulted in an immediate increment in the I_{sc} which fell rapidly below the previous steady state with the glucose solution (2.2 mM), and seemed to establish a steady state for 6 - 8 minutes but declined continuously afterwards. In all cases no recovery in the I_{sc} was observed by increase of the glucose concentrations or even when the IAA was omitted from the solution after the fall in the I_{sc} have reached the initial steady state with the sugar-free Krebs' solution. However, the inhibitory effect of IAA obtained in this investigation is in agreement with the conclusion obtained by Barry et al, (1964). They reported that iodo-acetate effect is different from that of phloridzin and that iodo-acetate is not a very specific inhibitor of glucose transfer by the intestine but causes general irreversable damage to the gut.

CHAPTER 8

Some studies on the effects of the viability conditions of the intestinal segment on the levels of short-circuit current.

Since a new method of intestinal preparation is followed in this investigation, an attempt is made to study some factors that may effect the viability of the segment and so would affect the levels of the obtained short-circuit current. This study includes the following aspects:

1. Effect of the order of applying the sugar at the mucosal side of the segment on the obtained I_{sc} levels and the difference between the metabolized and the non-metabolized sugars in this respect.
2. Effects of sodium citrate as an energy source on the short-circuit current obtained in the presence and absence of mucosal galactose solution.
3. Effects of anoxia on the short-circuit current levels induced by mucosal glucose and galactose solutions.

1. The first aspect includes some analysis of experiments done in Chapters (4 and 6), i.e. the experiments in which increasing concentrations of two different sugars were applied at the mucosal side of the intestinal segment in different order. These investigations were performed in such a manner that half of the experiments were carried out in the order of applying the first then the second sugar and the other half of the experiments were done in a reversed order. By this means it is clear that the results of each sugar can be classified into two sets according to the order of application of the sugar itself. Thus the first set includes the results of the sugar when it was applied in the first order while the intestinal segment was "fresh" i.e. has not been treated with any other sugar. The second set includes the results of the same sugar as it was applied in the second order, i.e. after the intestinal segment has

TABLE 8.1

The changes in the I_{sc} induced by mucosal galactose and 3-O-methylglucose (3MG) concentrations across the intestinal segment (A) of the rat.

The two sugars were assayed in different orders and the sets (first or second) indicate the order of the sugar application. In three experiments galactose concentrations were used before applying the 3MG concentrations in other three experiments 3MG was used first and the galactose second. The mean values of the obtained $I_{sc} \pm$ SE are given. The agar electrodes were used and inositol-Krebs' solution (10 ml of 100 mM) was recirculated at the serosal side of the segment.

Mucosal Medium Galactose Conc. (mM)	First Set I_{sc} (μA)	Second Set I_{sc} (μA)
8.3	120 ± 6	38.8 ± 6.5
12.5	170 ± 8	67.3 ± 6.4
16.7	184 ± 9	67.0 ± 11.2
20.9	171 ± 7	69.0 ± 12.2
25.0	146 ± 8	68.0 ± 14.7
3-O-methylglucose Conc. (mM)		
8.3	59.3 ± 9.4	20.0 ± 0.9
16.7	111 ± 16	36.0 ± 4.6
25.0	128 ± 20	47.8 ± 4.4
33.3	125 ± 20	55.5 ± 4.3
41.7	109 ± 20	55.5 ± 4.3

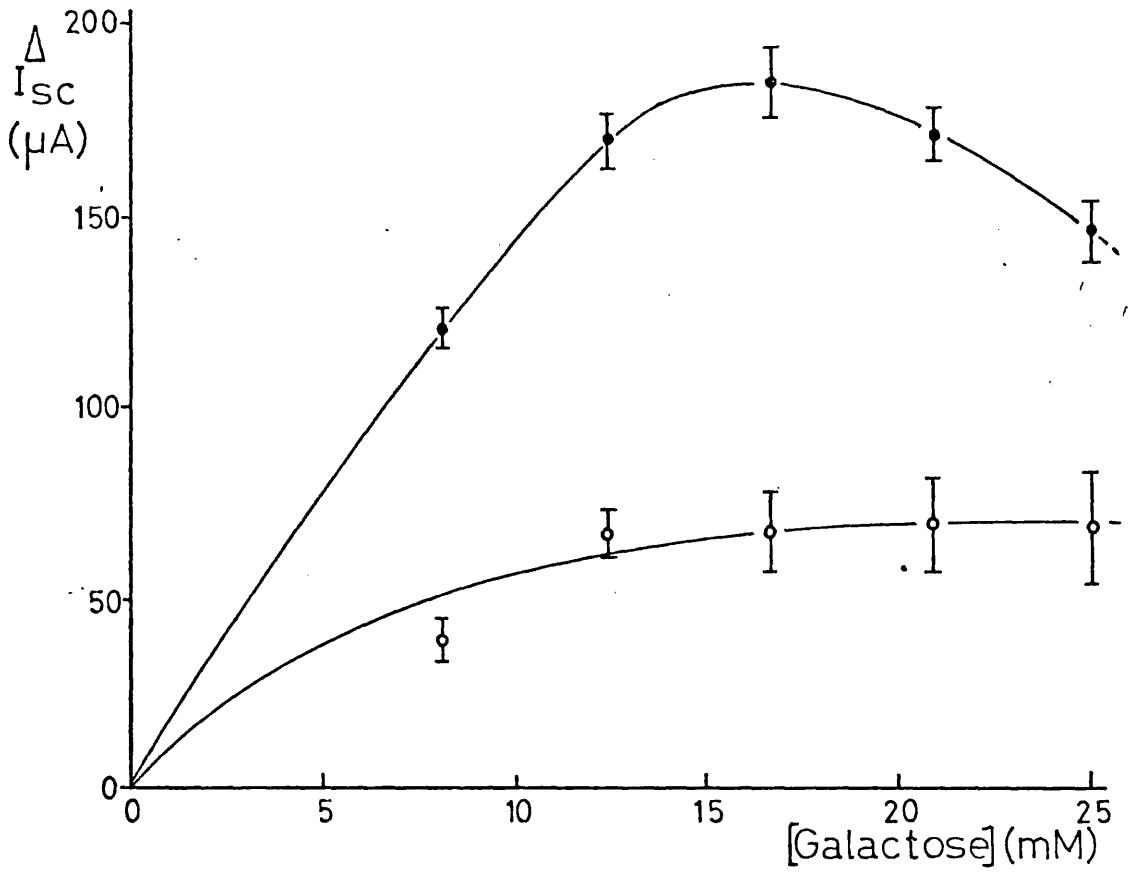


Fig.8.1 The short circuit current induced by galactose before, • ; and after, o ; 3-O-methylglucose. (Table 8.1)

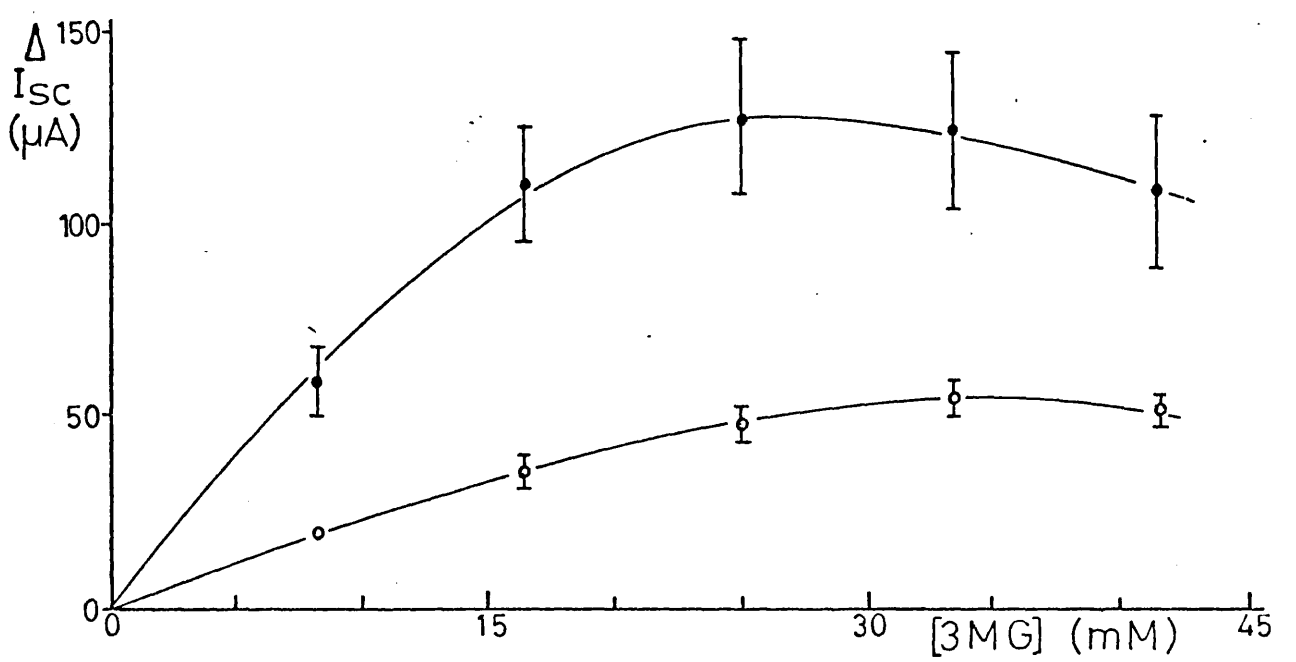


Fig. 8.2 Short circuit current induced by 3-O-methylglucose before, • ; and after, o ; galactose. (Table 8.1)

TABLE 8.2

The changes in the I_{sc} induced by mucosal glucose and galactose concentrations across the intestinal segment (A) of the rat.

The two sugars were assayed in different orders, and the sets (first or second) indicate the order of the sugar application. In three experiments glucose concentrations were used before applying the galactose concentrations and in other three experiments galactose was used first and the glucose second. The mean values of the obtained I_{sc} $\pm$ SE are given. Sugar-free Krebs' solution (0.6 ml) was used at the serosal side of the segment and the agar electrodes were used in this investigation.

Mucosal Medium Glucose Conc. (mM)	First Set I_{sc} (μA)	Second Set I_{sc} (μA)
1.1	75.0 ± 2.4	---
2.2	213 ± 15	38.3 ± 11.2
6.6	515 ± 38	147 ± 23
13.2	665 ± 50	177 ± 24
22.2	672 ± 46	183 ± 27
Galactose Conc. (mM)		
1.1	23.3 ± 2.7	26.7 ± 1.4
2.2	60.0 ± 10.8	70.0 ± 3.5
6.6	172 ± 4	212 ± 7
13.2	252 ± 21	397 ± 10
22.2	298 ± 29	557 ± 2

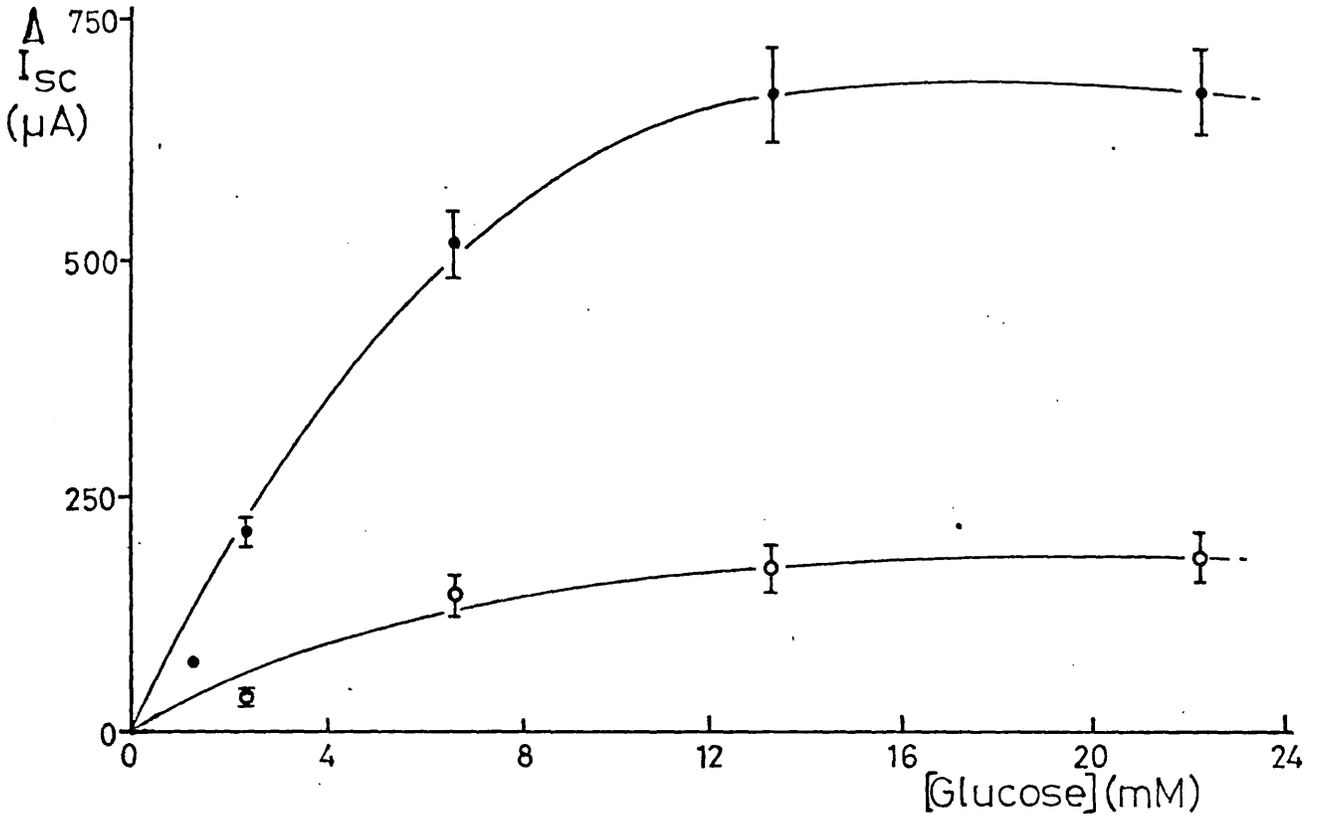


Fig. 8.3 Effect of glucose on the short circuit current before, • ; and after, o ; treatment with galactose. (Table 8.2)

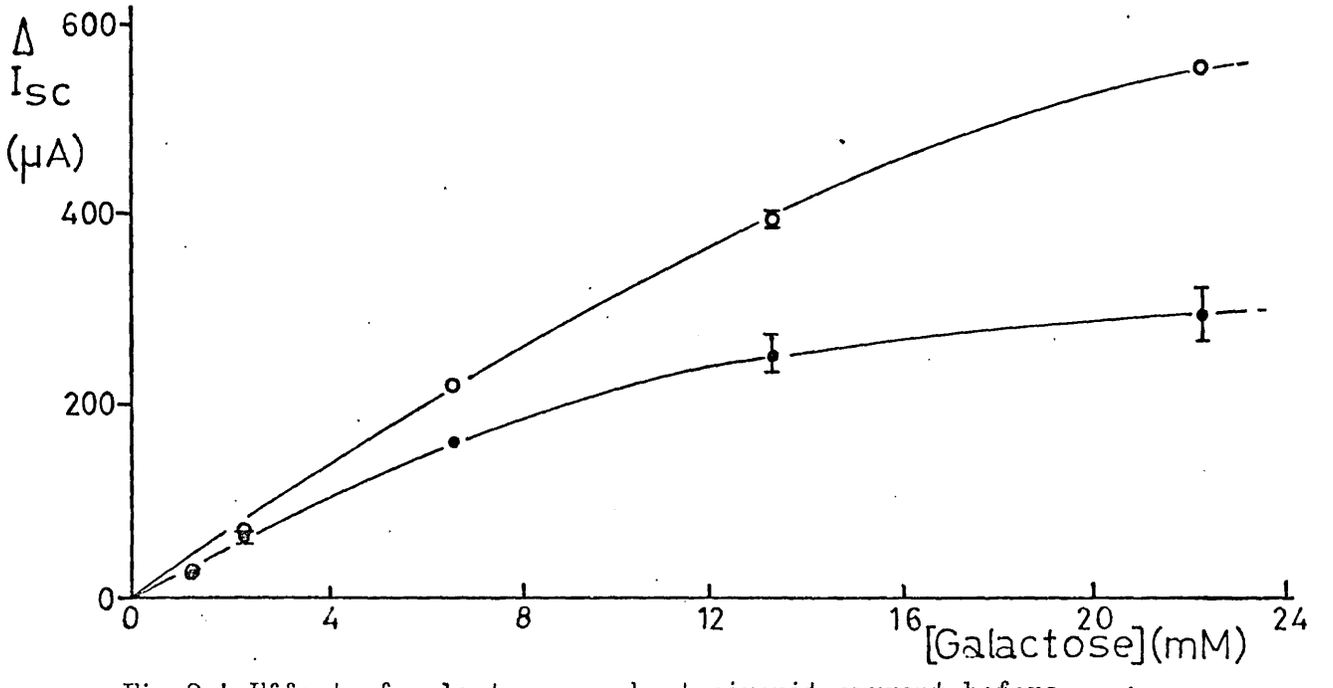


Fig.8.4 Effect of galactose on short circuit current before, • ; and after, o ; treatment with glucose. (Table 8.2)

been treated with different concentrations of another sugar. The time course of such an experiment was 2-3 hours including intervals of application of the different concentrations of the two sugars and the periods needed for the I_{sc} to reach a steady state in a sugar-free medium before the application of each sugar.

Results

Figures (8.1 to 8.3) and Tables 1 & 2 evidently show that the plateau of the hyperbolae for the I_{sc} are much higher when glucose, galactose or 3MG solutions were applied as the first sugar than when the same concentrations of these sugars were applied as the second sugar. The I_{sc} levels of these sugars when applied in the first order state/I_{sc} levels of the same sugars when applied in the second order state respectively are 3.5 - 5.6 folds for glucose, 2.5 - 3.1 folds for galactose and 2.7 - 3.1 folds for 3MG. In these results when these sugars were used in the second order state; either galactose or 3MG had been applied first. But when glucose solutions were applied in the first order state (Figure 8.4) the I_{sc} levels of galactose solutions used in the second order state seems to improve and gave I_{sc} levels even higher than the galactose assayed in the first order state. Almost similar results are obtained for increasing concentrations of maltose (Fig. 8.6 & Table 8.3) when used in the second order state after the glucose concentrations have been assayed in the first order state, and the small differences in the I_{sc} levels stimulated by maltose during the two order states seem to be within the standard error of the experiments. Also Figures (8.5 - 8.9) and Tables (8.3 - 8.5) show that whenever glucose solutions or a disaccharide that could be hydrolysed by the intestinal disaccharidase to produce glucose, were used at the mucosal side of the segment, no striking difference has been noticed

TABLE 8.3

The changes in the I_{sc} stimulated by mucosal glucose and maltose concentrations across the intestinal segment (A) of the rat.

The two sugars were assayed in different orders, and the sets (first or second) indicate the order of the sugar application. In three experiments maltose concentrations were used before applying the glucose concentrations, in other three experiments glucose was used first and the maltose second. The mean values of the obtained I_{sc} $\pm$ SE are given. The agar electrodes were used and 10 ml of sugar-free Krebs' solution was recirculated at the serosal side of the intestinal segment.

Mucosal Medium Glucose Conc. (mM)	First Set I_{sc} (μA)	Second Set I_{sc} (μA)
1.1	102 ± 14	80.0 ± 10.8
2.2	222 ± 23	180 ± 16
4.4	428 ± 47	323 ± 24
6.6	577 ± 72	438 ± 42
8.8	637 ± 81	488 ± 55
Maltose Conc. (mM)		
1.1	155 ± 4	138 ± 20
2.2	343 ± 5	320 ± 46
4.4	583 ± 56	555 ± 64
6.6	653 ± 71	703 ± 86
8.8	650 ± 69	732 ± 90
13.2	632 ± 63	733 ± 87
17.6	603 ± 58	733 ± 77
22.2	597 ± 47	738 ± 73

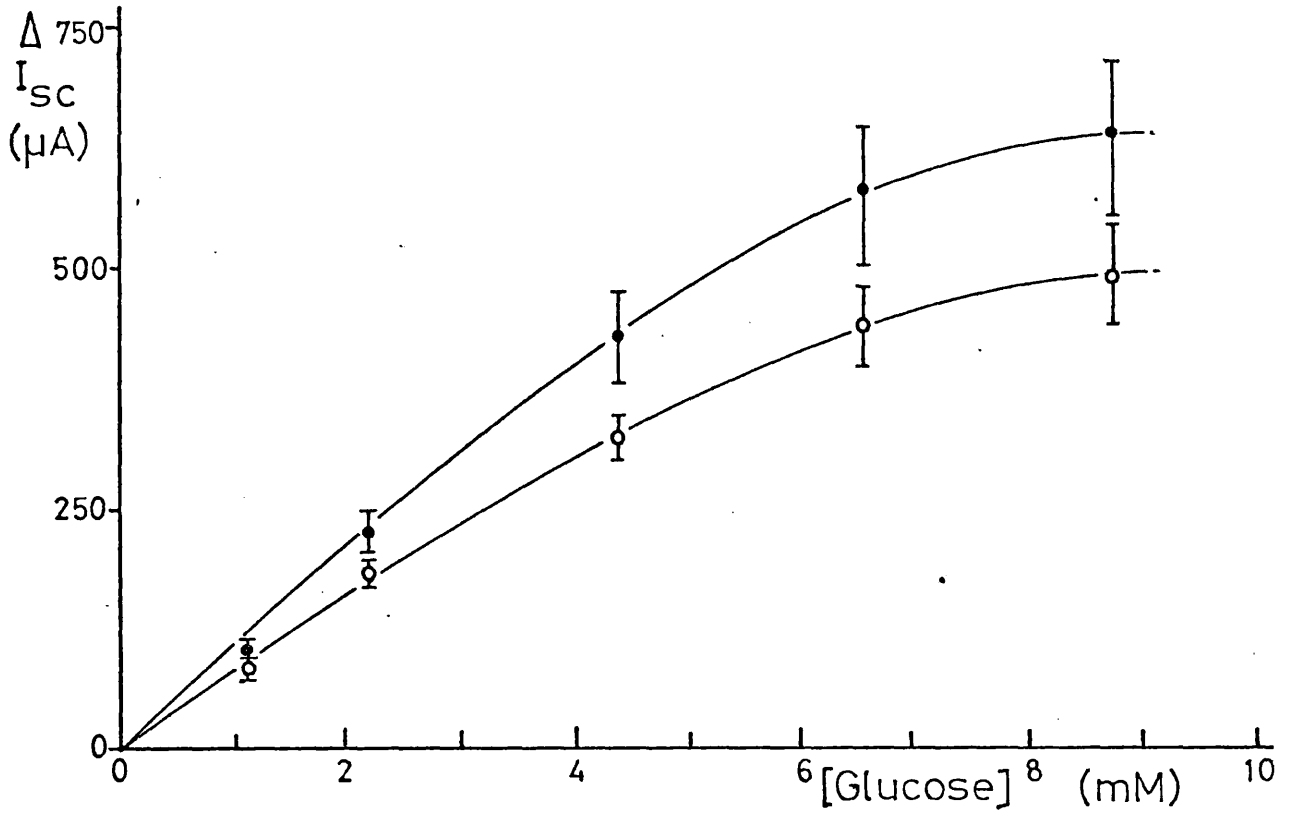


Fig. 8.5 Short circuit current induced by glucose before, • ; and after, o ; treatment with maltose, (Table 8.3).

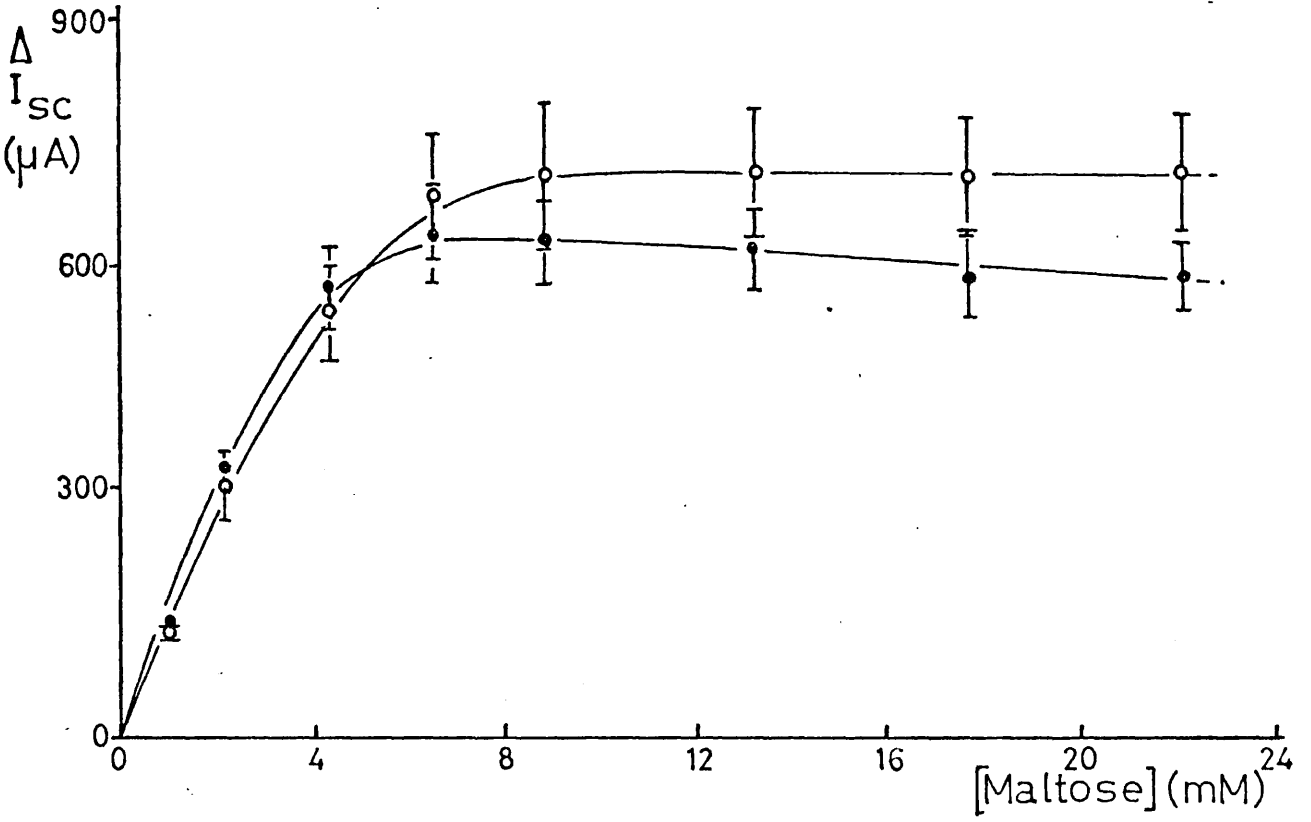


Fig.8.6 Short circuit current induced by maltose before, • ;and after, o ; treatment with glucose. (Table 8.3)

TABLE 8.4

The changes in the I_{sc} stimulated by mucosal glucose and sucrose concentrations across the intestinal segment (A) of the rat.

The two sugars were assayed in different orders, and the sets (first or second) indicate the order of the sugar application. In four experiments sucrose concentrations were used before applying the glucose concentrations, in other four experiments glucose was used first and the sucrose second. The agar electrodes were used and 10 ml of sugar-free Krebs' solution was recirculated at the serosal side of the segment. The mean values of the obtained $I_{sc} \pm$ SE are given.

Mucosal Medium Glucose Conc. (mM)	First Set I_{sc} (μA)	Second Set I_{sc} (μA)
1.1	150 ± 12	88.3 ± 8.9
2.2	280 ± 31	189 ± 13
4.4	511 ± 59	335 ± 25
6.6	643 ± 76	439 ± 20
8.8	693 ± 83	496 ± 23
13.2	695 ± 88	526 ± 19
17.6	675 ± 93	528 ± 22
22.2	645 ± 92	525 ± 25
Sucrose Conc. (mM)		
1.1	11.7 ± 1.4	11.7 ± 1.4
2.2	25.0 ± 4.3	23.8 ± 3.3
4.4	81.3 ± 13.2	65.0 ± 8.1
6.6	165 ± 17	123 ± 16
8.8	246 ± 26	203 ± 22
13.2	333 ± 34	270 ± 29
17.6	425 ± 49	328 ± 35
22.2	468 ± 56	364 ± 41

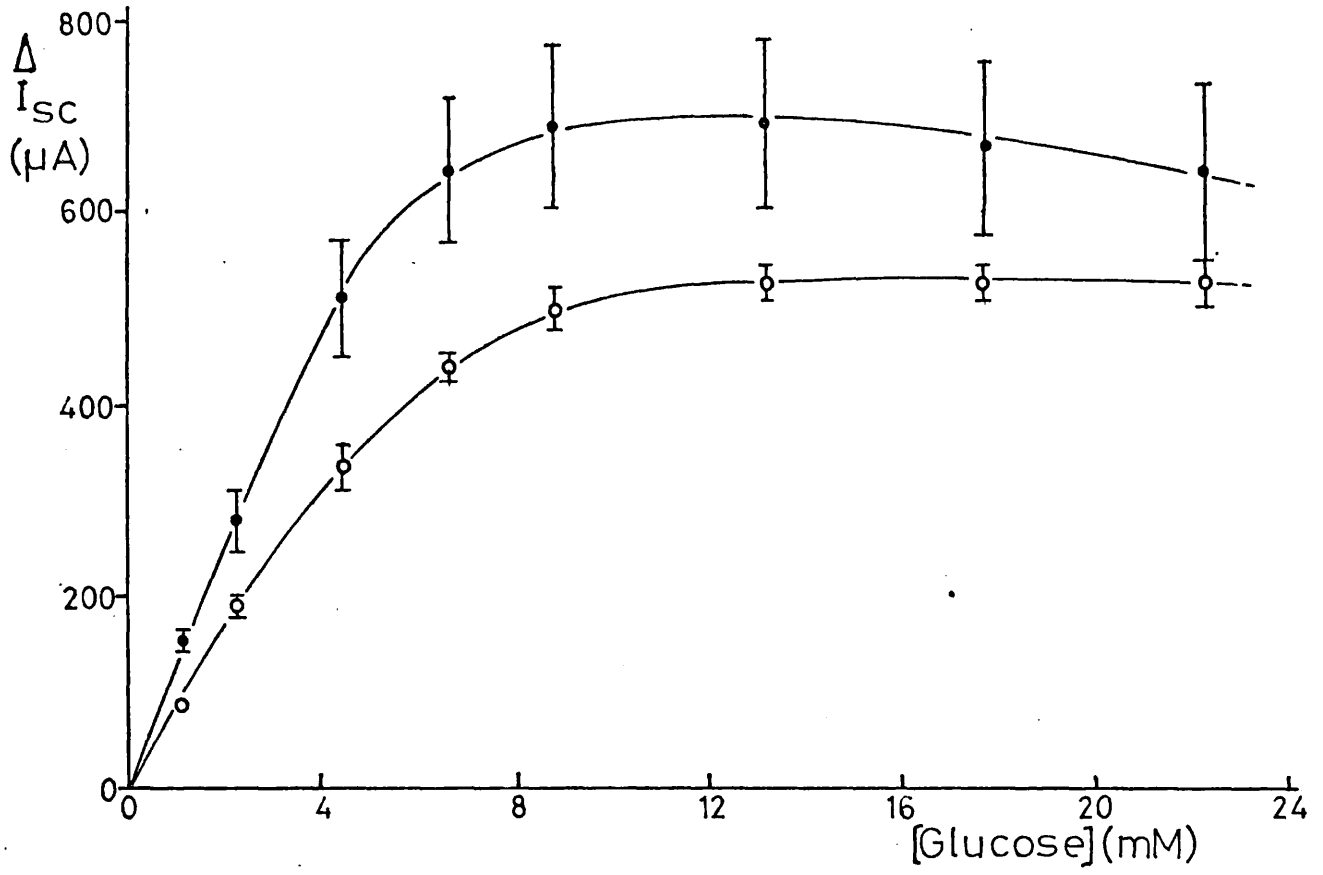


Fig.8.7 Effect of glucose on short circuit current before, • ; and after, o ; treatment with sucrose. (Table 8.4)

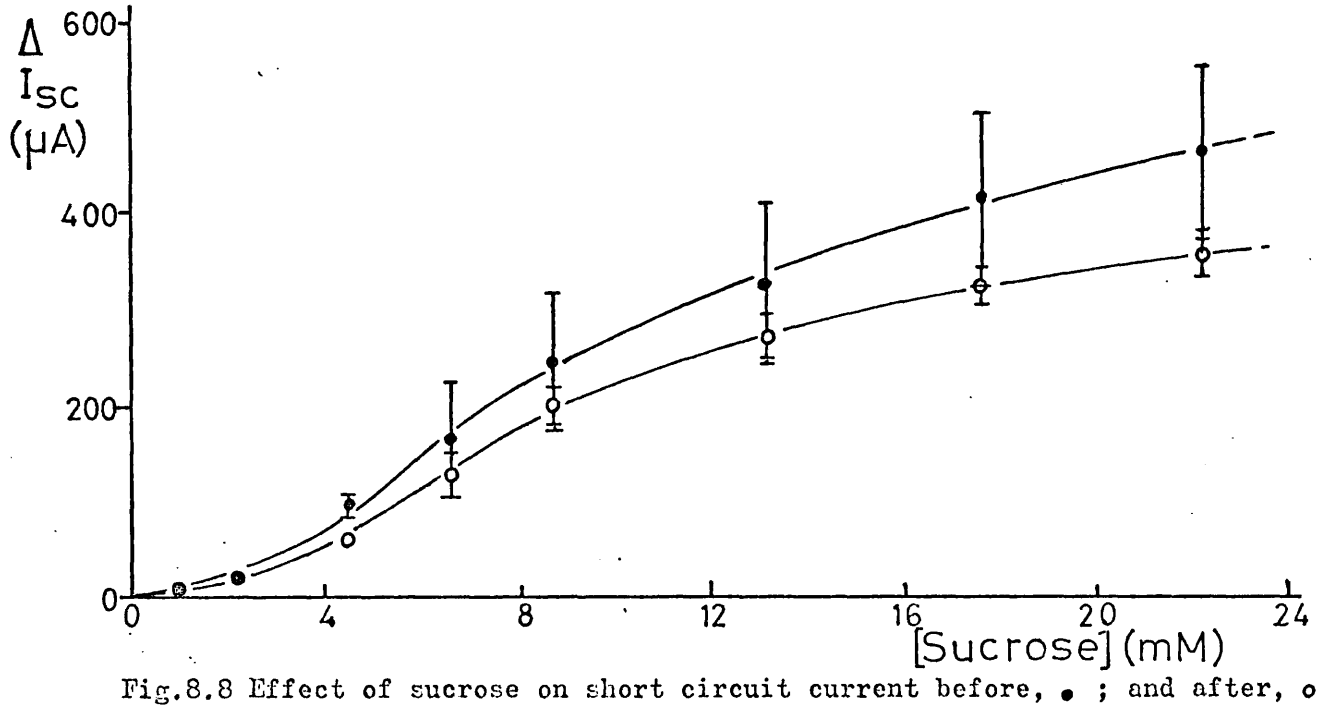


Fig.8.8 Effect of sucrose on short circuit current before, • ; and after, o ; treatment with glucose. (Table 8.4)

TABLE 8.5

The changes in the I_{sc} stimulated by different concentrations of mucosal lactose across the intestinal segment (A) of the rat.

Agar electrodes were used for the I_{sc} measurements, and sugar-free Krebs' solution (10 ml) was recirculated at the serosal side of the segment. While in the first set of the experiments, lactose concentrations were used before applying the maltose concentrations in the second set, the experiments were performed using lactose concentrations after the I_{sc} assessment with the mucosal maltose concentrations; the mean values of the $I_{sc} \pm$ SE were calculated. Three experiments were performed for each set.

Mucosal Medium Lactose Conc. (mM)	First Set I_{sc} (μA)	Second Set I_{sc} (μA)
1.1	-31.7 ± 7.6	-11.7 ± 4.9
2.2	-40.0 ± 7.1	-23.3 ± 5.9
4.4	-48.3 ± 8.3	-30.0 ± 6.2
8.8	-56.7 ± 11.1	-36.7 ± 5.4
17.6	-73.3 ± 9.8	-51.7 ± 4.9

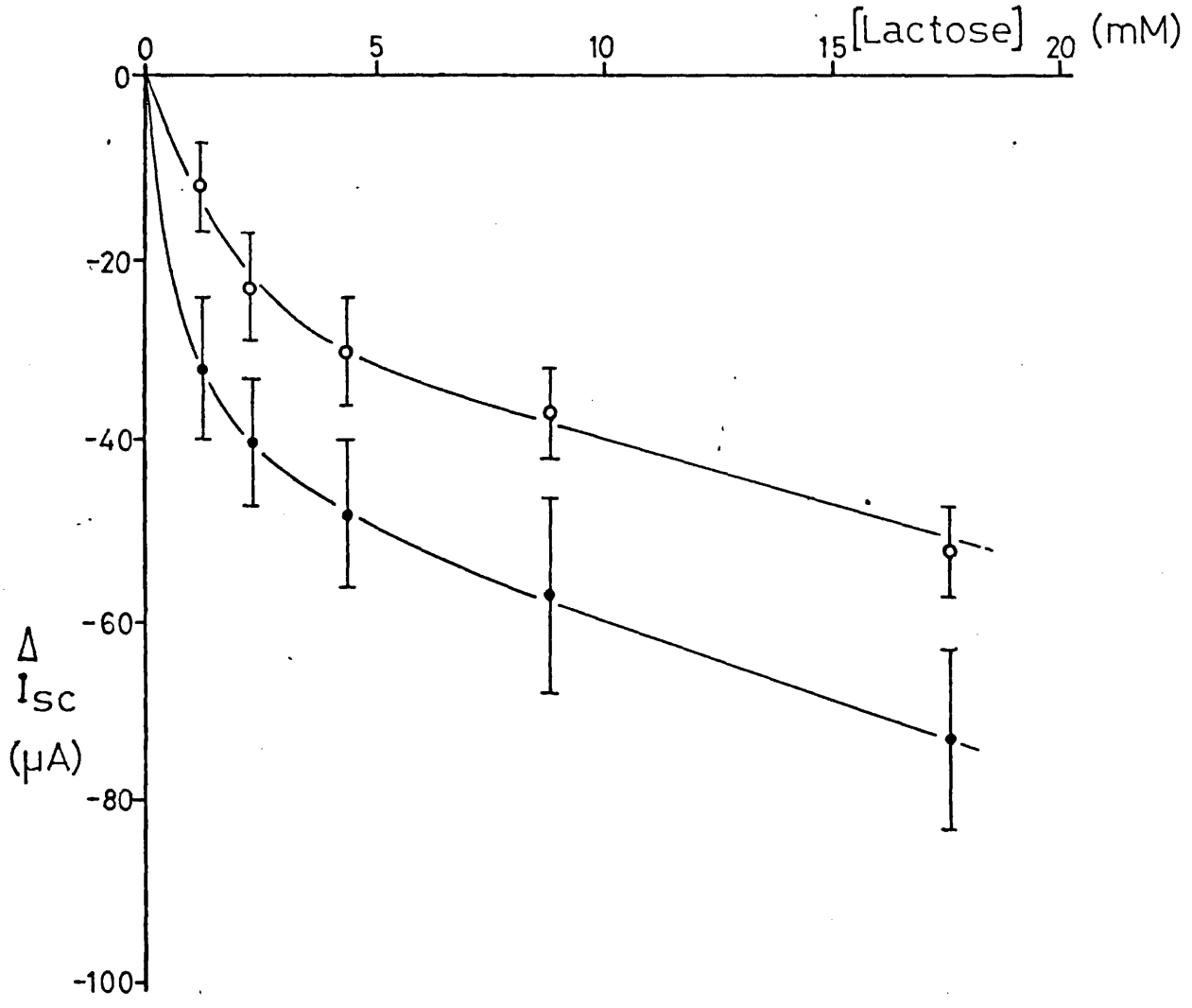


Fig.8.9 Inhibition of short circuit current by lactose before, • ; and after, o ; treatment with maltose. (Table 8.5)

in the I_{sc} levels such as that observed with the 3MG and galactose solutions. The small differences in the I_{sc} levels for the metabolized sugars used in the first and second order states could be simply related to the age of the intestinal segment over the elapsed period of the experiment which took about 150 minutes or more. However, the effect of age on this preparation of the rat intestine to respond to the addition of glucose is very much less than the very early rapid decline in the I_{sc} levels reported by Schultz and Zalusky (1964b) also for glucose, in the rabbit ileum sheets. As far as the age is concerned, Schultz & Zalusky (1964b) reported no difference between 3-O-methylglucose and glucose, but the obtained results (Figure 8.1 and Table 8.1) evidently show that 3-O-methylglucose greatly affected the magnitude of the I_{sc} levels induced by galactose. However, the results obtained in this investigation are in agreement with the conclusion of Clarkson, Cross & Tool (1961) that the rat intestine has very little "stored energy" but must be continuously supplied with glucose.

2. Atfield, Sanford & Smyth (1972) have shown that citrate can provide energy for transfer in the hamster intestine. Also Browne & Smyth, recently (1974) used 7.5 mM sodium citrate as a source of energy at the mucosal side of the everted intestinal sac of the hamster.

In this investigation an attempt was made to use 7.5 mM sodium citrate at the mucosal side of the rat intestinal segment as a source of energy. The intestinal segments were taken from the region in between 25 - 35 cm from the proximal end of the jejunum. The procedure followed was to incubate the intestinal preparation in Krebs' citrate solution at the mucosal side and 5 ml Krebs' fluid

TABLE 8.6

Effects of sodium citrate (7.5 mM) as a source of energy on the I_{sc} levels obtained in the absence and presence of 6.6 mM galactose solution.

Silver/silver chloride electrodes were used for the measurements of the I_{sc} across the intestinal segment (A) of the rat. Sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment. Two experiments were undertaken and the mean values of the I_{sc} $\pm$ SE are represented.

Step No.	Applied Solution	Application time (min)	The obtained I_{sc} (μA)
1	Krebs'-citrate fluid	40	65.0 $\pm$ 3.5
2	Citrate-free Krebs' fluid	4-6	52.5 $\pm$ 5.3
3	Krebs'-citrate fluid	4-6	59.5 $\pm$ 3.9
4	Galactose-Krebs' citrate fluid	15-25	75.0 $\pm$ 5.0
5	Citrate-free galactose Krebs' fluid	13-15	62.0 $\pm$ 9.9
6	Galactose-Krebs' citrate fluid	10	51.0 $\pm$ 14.9

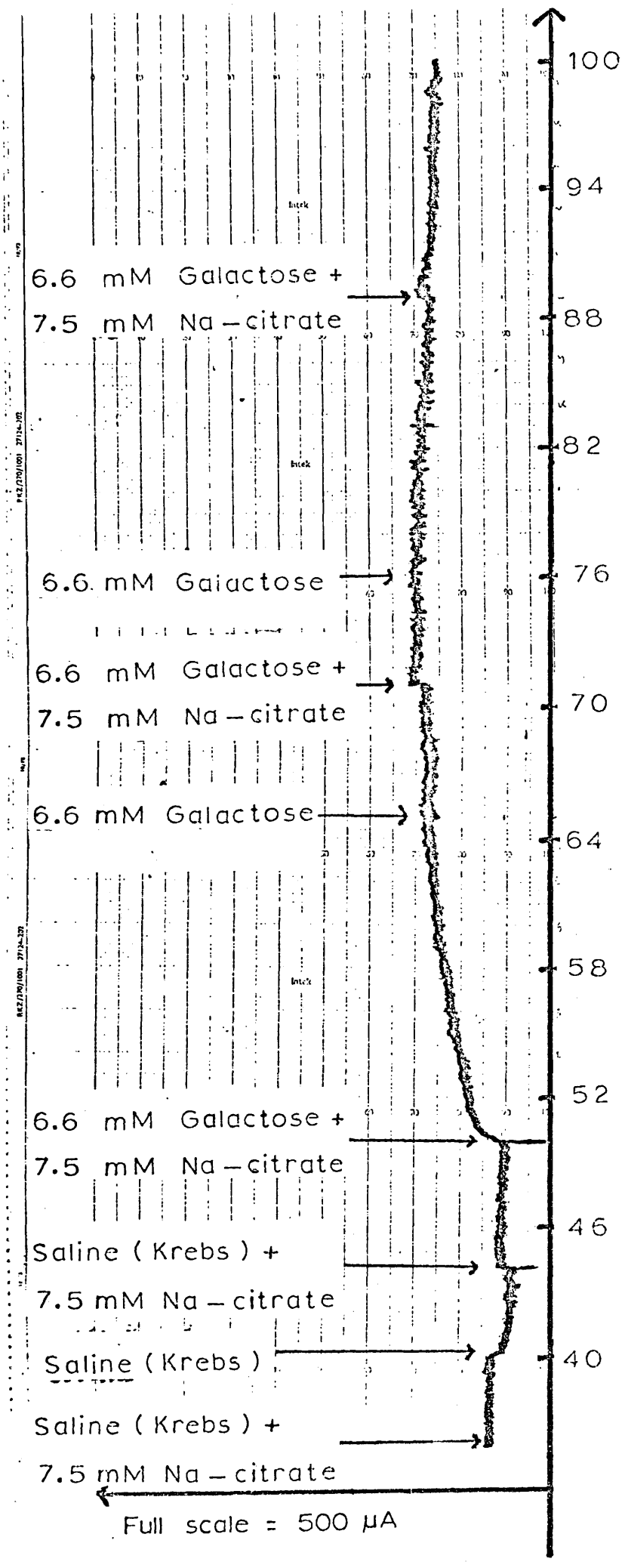


Fig. 8.10 Effects of sodium citrate on the level of the short circuit current in the absence and presence of sugar (galactose).

was recirculated at the serosal side of the segment throughout the experiment. When the I_{sc} steady state was achieved in these solutions, the mucosal Krebs' citrate fluid was replaced by citrate-free Krebs' solution for four minutes and a new solution of the Krebs'-citrate was applied again also for four minutes. Then 6.6 mM galactose-Krebs' citrate solution was added remaining until the I_{sc} steady state was achieved; this solution was then replaced by 6.6 mM galactose-Krebs' solution to remain for 5 - 6 minutes. A new solution of 6.6 mM galactose-Krebs' citrate was applied again for further 5 - 6 minutes. Again galactose-Krebs' solution was applied for 10 minutes and finally replaced by galactose-Krebs' citrate solution. Two experiments were undertaken and the mean of the I_{sc} values obtained $\pm$ SE are calculated.

Results

It has been noticed that in the absence of mucosal sugar the fall of the I_{sc} was gradual and took more time to achieve the steady state when the citrate had been added to the mucosal-Krebs' solution. Figure (8.10) and Table (8.6) illustrate that the I_{sc} level sustained some declination when Krebs'-citrate solution was replaced by normal Krebs' fluid. This fall in the I_{sc} level was at least partially recovered when Krebs'-citrate solution was applied again. In the presence of 6.6 mM galactose solution, no clear difference can be seen in the I_{sc} levels both in the presence or absence of citrate (Table 4.2). However, such a comparison seems not to be easy especially because of the consideration of the individual variation factor which rather tends to promote a wide range of I_{sc} levels. But in this investigation, carried out on the

TABLE 8.7

Inhibition of the I_{sc} by anoxia

The I_{sc} was induced by mucosal galactose-Krebs' solution across the intestinal segment (A) of the rat. Sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment. Using Ag/AgCl electrodes, two kinds of experiments were attempted. In the first set of investigation, three experiments were performed and in the second set two experiments were undertaken. The I_{sc} in these sets were stimulated by 4.4 mM and 6.6 mM galactose respectively. The mean values of the I_{sc} levels $\pm$ SE are presented.

First set

Step No.	Aerobic or anaerobic conditions of the medium	Time allowed for each case (mins)	The Obtained I_{sc} (μA)
1	95% O_2 - 5% CO_2	15	78.3 $\pm$ 6.0
2	95% N_2 - 5% CO_2	8-10	1.0 $\pm$ 0.8
3	95% O_2 - 5% CO_2	10-11	-29.3 $\pm$ 6.6
4	95% O_2 - 5% CO_2 + 4.4 mM glucose	6	-29.3 $\pm$ 6.6

Second Set

1	95% O_2 - 5% CO_2	12-16	96.0 $\pm$ 11.3
2	95% N_2 - 5% CO_2	5-6	43.5 $\pm$ 1.1
3	95% O_2 - 5% CO_2	5-6	70.0 $\pm$ 10.6

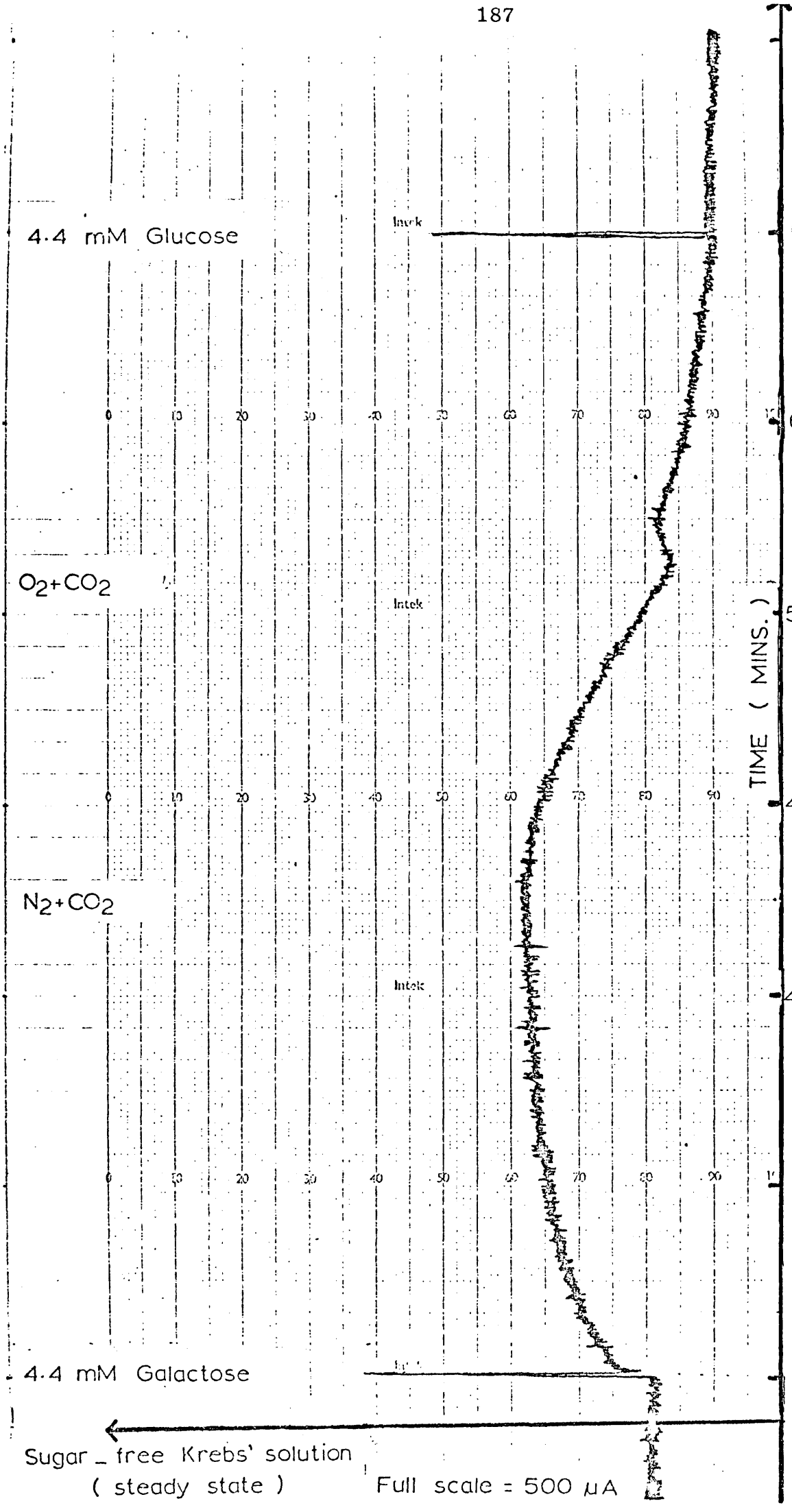


Fig. 8.11 Characteristics of the complete inhibition by anoxia of the short circuit current generated by galactose.

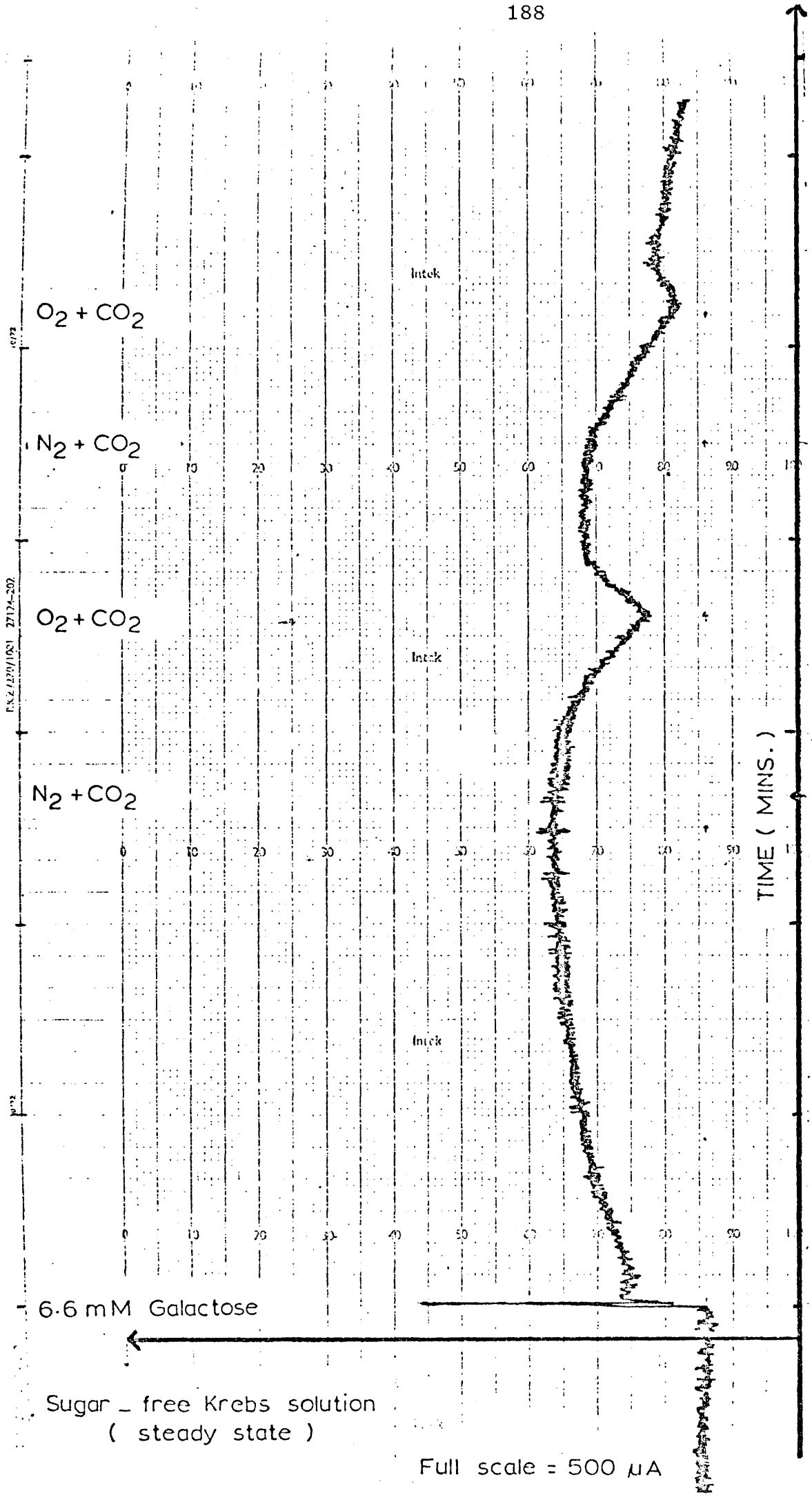


Fig. 8.12 Characteristics of the partial inhibition by anoxia of the short circuit current generated by galactose.

same segment, the results show that omitting the citrate from the galactose solution bathing the mucosal side causes some decline in the I_{sc} level during the period of six minutes solution application. Rapid recovery in the I_{sc} level was observed when the galactose-Krebs' solution was replaced by the galactose-Krebs' citrate solution. When the galactose-Krebs' solution was then applied for 13-15 minutes, a continuous gradual decline in the I_{sc} level was observed which seems not to recover by the addition of citrate to the mucosal galactose-Krebs' solution.

3. For the assessment of the effects of anoxia on the I_{sc} in the presence of mucosal galactose or glucose, the procedure followed mainly was to take two segments from the small intestine of the same animal. For the two sugars respectively, the segments were taken from the region in between 25 - 35 cm and 35 - 45 cm from the proximal end of the jejunum. The segments were bathed in oxygenated sugar-free Krebs' solution at $37^{o}C$ in the reservation beaker. The segments then were clamped in the apparatus and the investigation was carried out on the two segments one after the other and in different order.

Results

Figure (8.11) is an example of the experiments done in the first set of Table (8.7). This Figure clearly shows that the I_{sc} induced by 4.4 mM galactose (93 μA) under aerobic condition, was completely inhibited under the effect of anaerobic condition brought about by replacing O_2 with N_2 bubbles for about 10 minutes. No recovery of the I_{sc} was observed with reoxygenation the mucosal fluid and with replacing the galactose by the glucose solution.

TABLE 8.8

Inhibition of the I_{sc} by anoxia

The I_{sc} was induced by 4.4 mM mucosal glucose-Krebs' solution across the intestinal segment (B) of the rat. Sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment. Using Ag/AgCl electrodes two sets of experiments were attempted. In the first set two experiments were performed, and in the second set three experiments were undertaken. The mean values of the I_{sc} levels $\pm$ SE are presented.

First Set

Step No.	Aerobic or anaerobic conditions of the medium	Time allowed for each case (mins)	The Obtained I_{sc} (μA)
1	95% O_2 - 5% CO_2	10	169 $\pm$ 22
2	95% N_2 - 5% CO_2	26-38	ZERO
3	95% O_2 - 5% CO_2	10	-6.0 $\pm$ 0.7

Second Set

1	95% O_2 - 5% CO_2	15	128 $\pm$ 14
2	95% N_2 - 5% CO_2	14-17	64.3 $\pm$ 11.9
3	95% O_2 - 5% CO_2	10-15	134 $\pm$ 22
4	95% N_2 - 5% CO_2	15	66.7 $\pm$ 11.6
5	95% O_2 - 5% CO_2	10	148 $\pm$ 30

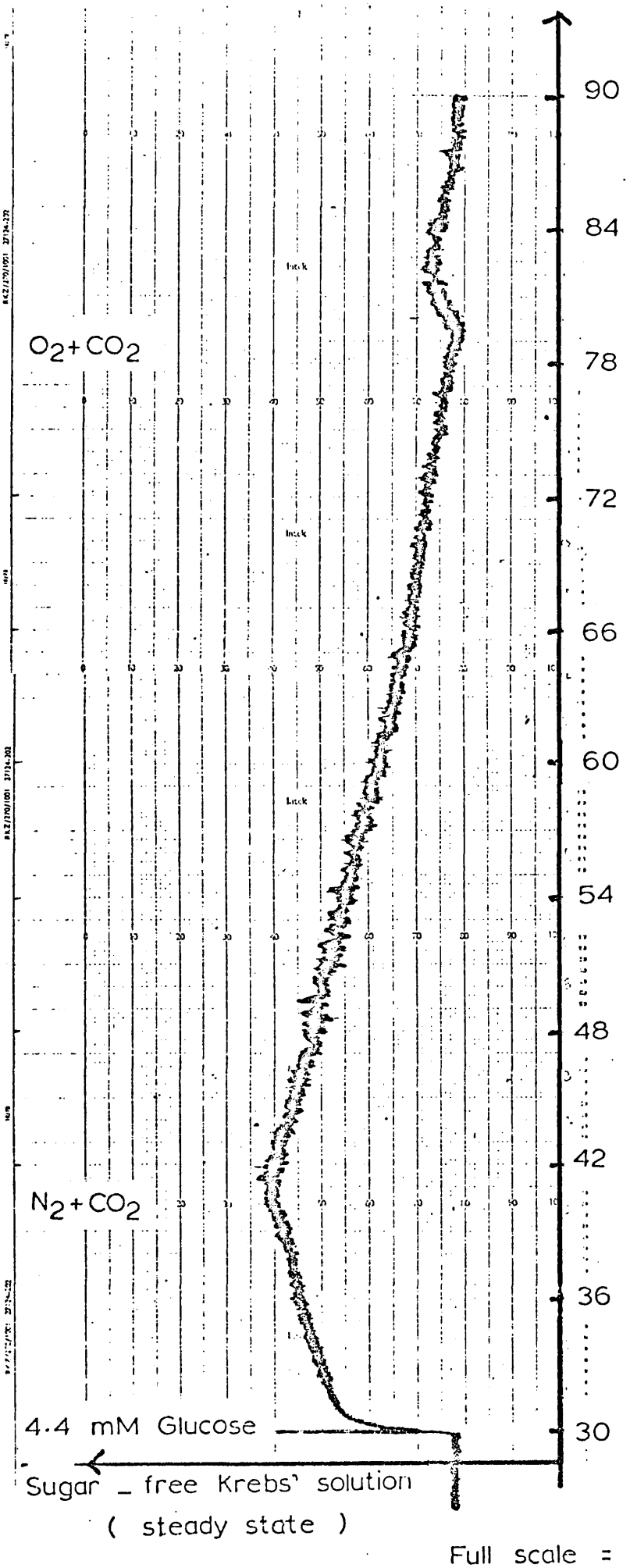


Fig. 8.13 Characteristics of the complete inhibition by anoxia of the short circuit current induced by glucose.

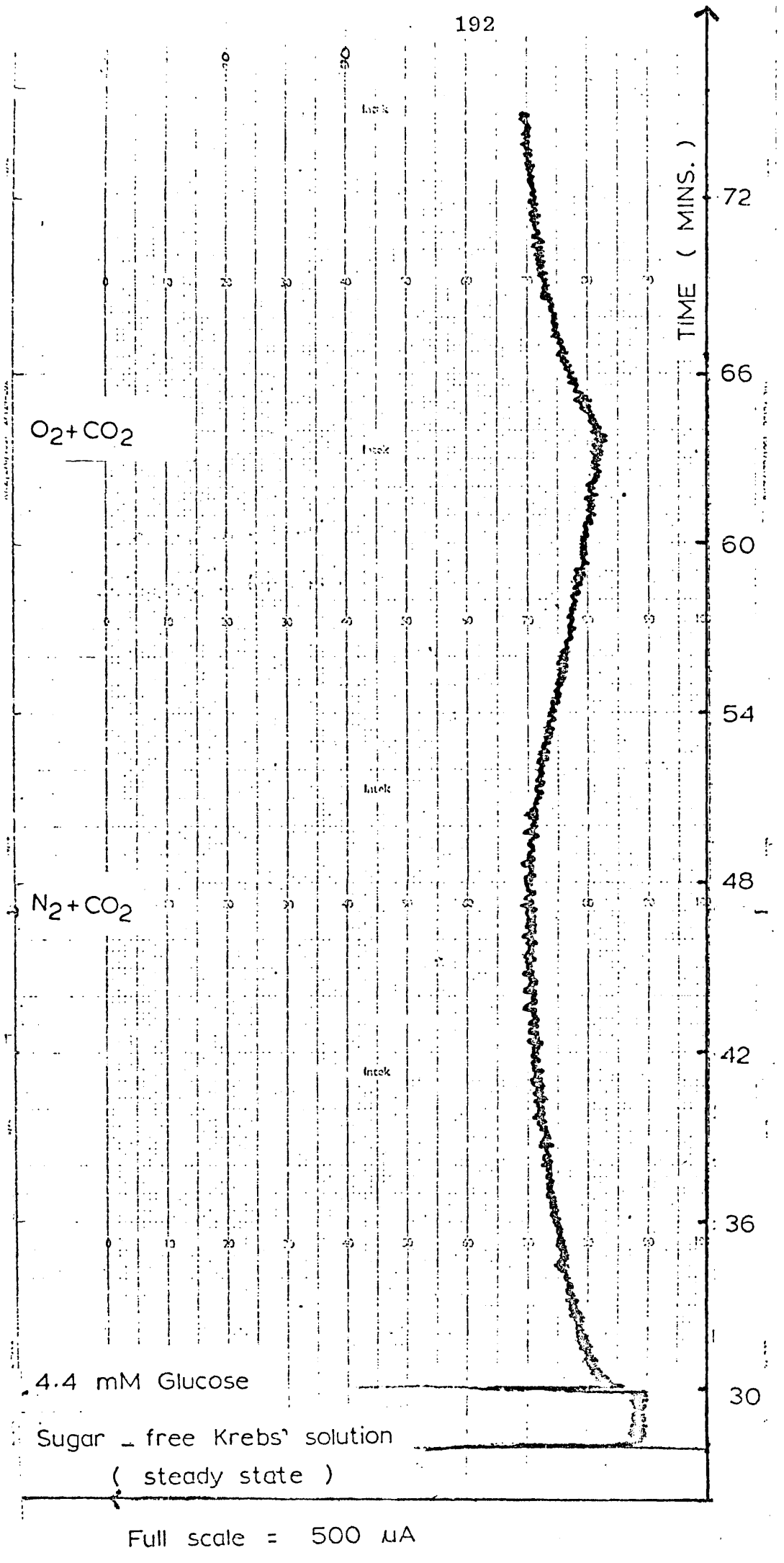


Fig. 8.14 Characteristics of the partial inhibition by anoxia of the short circuit current stimulated by glucose.

Figure (8.12) is the result of one of the two experiments done in the second set (Table 8.7). It shows that the I_{sc} induced by 6.6 mM galactose (112 μA) under aerobic condition fell to 45 μA under the effect of anaerobic condition brought about by replacing O_2 with N_2 bubbles for about 5.5 minutes. In this case, reoxygenation of the mucosal fluid bathing the intestinal segment brought about partial recovery of the I_{sc} within about two minutes and then established a steady state line. Application of anaerobic condition for a further four minutes gave further decline in the I_{sc} to 25 μA. In this case, reoxygenation of the system appeared to induce a small recovery in the I_{sc} (13 μA) within about two minutes which soon after deteriorated again.

Figure (8.13) is the result of one of the two experiments performed in the first set of Table (8.8). It shows the effect on the I_{sc} induced by 4.4 mM glucose solution (200 μA) under anaerobic condition brought about by replacing O_2 with N_2 bubbles for 38 minutes. No substantial recovery in the I_{sc} was observed with reoxygenation of the mucosal fluid, and the small recovery in the I_{sc} obtained within the first three minutes deteriorated soon afterwards.

Figure (8.14) is an example of the experiments done in the second set of Table (8.8). It shows that the I_{sc} stimulated by 4.4 mM glucose solution (93 μA) under aerobic condition fell to 43 μA under the effect of anaerobic condition brought about by replacing the O_2 with N_2 bubbles for about 17 minutes. In this case evidently reoxygenation of the mucosal fluid bathing the intestinal segment brought about full recovery of the I_{sc} within about ten minutes. Also in Table (8.8) it can be noticed that

the I_{sc} obtained under the reoxygenation conditions slightly exceeded the previous I_{sc} induced by the same concentration of glucose under the aerobic condition. In some of these experiments the segment was incubated in sugar-free Krebs' solution for 80 - 90 minutes while the assessment of the galactose solution on the other segment as mentioned above was in progress. Finishing the experiment with galactose, the segment was replaced with a new one (which had been in the oxygenated sugar-free Krebs' solution in the beaker) and which was then clamped in the apparatus for the measurement of the effects of aerobic and anaerobic conditions in the presence of mucosal glucose solution. In this sort of experiment full recovery of the I_{sc} was obtained even after about 150 minutes from the removal of the intestinal segment from the anaesthetized animal. Galactose is different from glucose in the respect that it was not possible to obtain the I_{sc} recovery when anaerobic and aerobic conditions were applied for more than one time successively in the presence of galactose. In the presence of galactose solution reoxygenation of the system brought about only partial recovery of the I_{sc} from the effect of a previous anaerobic condition applied for 5.5 minutes and no substantial recovery of the I_{sc} was observed when the anaerobic condition was extended up to about 10 minutes.

RESULTS OF THE Na^+-SUGAR INTERACTION STUDIES

CHAPTER 9

Some studies on Na^+ fluxes in short-circuited intestinal segments of the rat.

The relation between net Na^+ transport and short-circuit current has been investigated in several in vitro preparations of intestine. The majority of these investigations were performed by using sheets of intestine (Schultz & Zalusky, 1964a, 1964b, Taylor et al, 1968, Quay & Armstrong, 1969a, Field et al, 1971, Munk, 1972). The fewer of these investigations were carried out by using everted sacs of the intestine (Barry et al, 1965 and Munk, 1972). In most instances, the first group of authors reported that the current is approximately equal to the rate of active Na^+ transport from the mucosal side to the serosal side of the intestinal preparation. But Barry et al, (1965) reported that although glucose, galactose and α-methylglucose brought about a significant increase in the short-circuit current across the rat jejunum, agreement between net Na^+ flux and the current is found only with glucose. They concluded that part of the Na^+ moves by a non-electrogenic Na^+ pump which is not related to hexose transfer and the short-circuit current is therefore not necessarily equal to net Na^+ transfer. This conclusion has been supported by Barry et al, (1969) on the basis of the potential difference.

However, in this field of study it seems only few studies have been published using the technique of everted intestine; in addition, as mentioned in the introduction, some results of the first group of authors do not appear to be in accordance with the ion gradient hypothesis. Therefore the relationship between the short-circuit current and the net Na^+ flux has been reviewed in this chapter by following the new technique of the rat intestinal evertion 'gut supporting cannula' which has been introduced and developed in this laboratory. The intestinal segments were taken from the region in

TABLE 9.1

The changes in the I_{sc} induced by mucosal glucose solution across the intestinal segment (B) at successive intervals of time.

In the first set the agar electrodes were used for the measurement of the I_{sc} induced by 1.1 mM glucose-Krebs' solution and in the second set the silver/silver chloride electrodes were used for the measurement of the I_{sc} induced by 2.2 mM mucosal glucose solution. Sugar-free Krebs' solution was used at the serosal side of the segment; in the first set 0.6 ml of the solution was used and in the second 10 ml of the solution was recirculated throughout the experiment. The numbers in parenthesis indicate the number of animals used and the mean values of the obtained $I_{sc} \pm$ SE were calculated.

First set (7)		Second set (6)	
The time minutes	The obtained I_{sc} (μA)	The time minutes	The obtained I_{sc} (μA)
5	280 ± 17	2	87.2 ± 5.5
10	342 ± 21	4	104 ± 8
15	373 ± 23	6	114 ± 9
20	394 ± 23	8	122 ± 9
25	409 ± 23	10	129 ± 9
		12	134 ± 9
		14	138 ± 9
		16	140 ± 9
		18	144 ± 10
		20	146 ± 10

TABLE 9.2

The changes in the I_{sc} induced by mucosal solutions of 9 mM 3-O-methylglucose (3MG) and 2.2 mM α-methylglucose (αMG) across the intestinal segments (A) and (B) respectively.

The silver/silver chloride electrodes were used for the measurements of the I_{sc} throughout the experiment and the readings were taken at 2 minute intervals up to 20 minutes. Sugar-free Krebs' solution, 5 ml and 10 ml respectively was recirculated at the serosal side of the segment. The numbers in parenthesis indicate the number of animals used and the mean values of the obtained $I_{sc} \pm$ SE are represented.

	(4) First set (3MG)	(5) Second set (αMG)
The time minutes	The obtained I_{sc} (μA)	The obtained I_{sc} (μA)
2	47.5 ± 2.1	46.0 ± 2.6
4	56.3 ± 1.9	60.0 ± 4.2
6	63.0 ± 1.7	70.6 ± 5.7
8	68.3 ± 2.0	80.6 ± 7.6
10	71.3 ± 2.1	85.6 ± 8.1
12	74.8 ± 2.0	90.8 ± 8.2
14	78.3 ± 2.3	95.4 ± 9.0
16	82.0 ± 2.7	98.8 ± 9.6
18	83.0 ± 2.5	102 ± 10
20	83.5 ± 2.8	104 ± 10

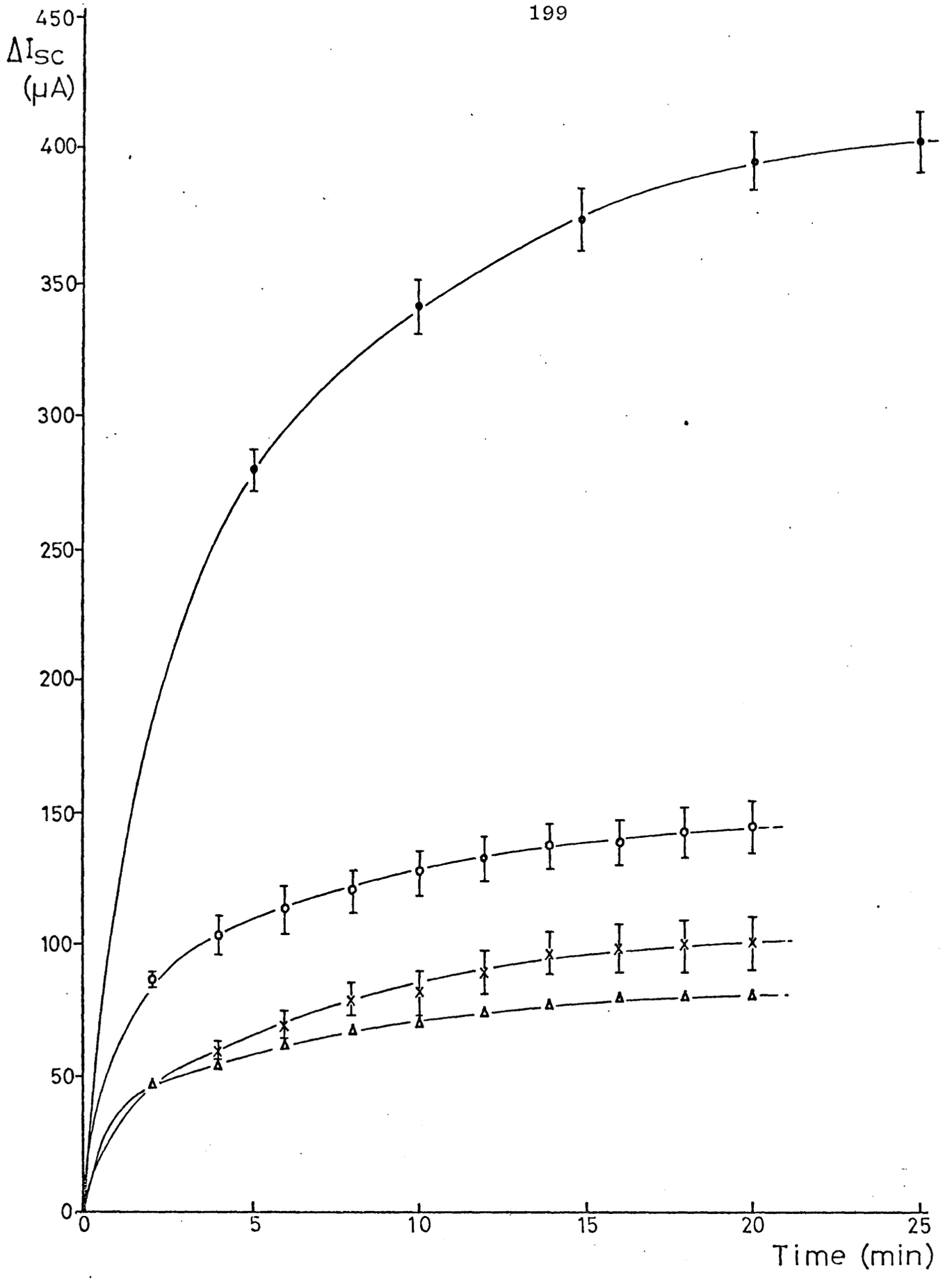


Fig. 9.1 Rate of change of the short circuit current following the application of various sugars to the mucosal surface. • , 1.1 mM glucose (Agar electrodes); o , 2.2 mM glucose; Δ , 9mM 3-O-methyl glucose; X , 2.2mM α-methyl glucoside (all Ag/AgCl electrodes). From Table 9.1 & 9.2.

between 25-35 cm (segement A) or from the region in between 35-45 cm (segment B) from the proximal end of the jejunum. In the case of investigating the electrical resistance of the intestinal wall, in addition to segment (A), segment (C) was taken from the region in between 45-55 cm from the proximal end of the jejunum. Sugar-free Krebs' solution (5 ml) or mannose-Krebs' fluid (5 ml of 170 mM) was recirculated at the serosal side of the segment. Usually silver/silver chloride electrodes were used for the I_{sc} measurements, and the mean values of the obtained data $\pm$ SE were calculated.

Results

Electrical characteristics of the intestinal segments of the rat.

Steady state: Figure (9.1) and Tables (9.1 and 9.2) show the relationship between the I_{sc} induced by the sugars and the time of their application at the mucosal side of the segment. The recorded I_{sc} values were taken successively at the end of 2 or 5 minute intervals up to 20 or 25 minutes period. The increment in the I_{sc} values have been plotted against the time (minutes), and in all instances hyperbolic-shaped curves were obtained. Although the increment rates of the I_{sc} seem to be the fastest within the first few seconds, the steady state of the I_{sc} seems not to be accomplished before 10-20 minutes. This is in agreement with the results obtained by White & Armstrong (1971) who suggested that the rather slow changes in potential which they observed after an initial rapid phase might reflect changes related to cell swelling. In addition, increases in tissue water have been reported (Schultz et al, 1966, Csaky et al, 1969, and Armstrong et al, 1970). It is seen from Figures (9.2 and 9.3) and Table (9.3)

TABLE 9.3

Short-circuit current obtained in the absence and presence of mucosal 3-O-methylglucose (3MG) across the intestinal segment (A) of the rat.

The silver/silver chloride electrodes were used for the I_{sc} measurements and the recorded I_{sc} values were taken at successive intervals of 20 minutes up to 140 minutes. In the first and second sets sugar-free Krebs' fluid (5 ml) and mannose-Krebs' solution (5 ml of 170 mM) respectively were recirculated at the serosal side of the segment. The numbers in parenthesis indicate the number of animals used for each set of experiments and the mean values of the obtained $I_{sc} \pm$ SE were calculated.

(4) First set Sugar-free Krebs' solution at the serosal side.		(3) Second set 170 mM mannose solution at the serosal side.	
Mucosal Medium	I_{sc} (μA) obtained	Mucosal Medium	I_{sc} (μA) obtained
Sugar-free Krebs' solution	79.5 ± 10.1 (Initial steady state)	Sugar-free Krebs' Solution	144 ± 5 (Initial steady state)
Sugar-free Krebs' solution	78.3 ± 10.2 74.5 ± 10.1	Sugar-free Krebs' solution	141 ± 6 137 ± 6 133 ± 6
2.5-3.5 mM 3MG-Krebs' solution	105 ± 10 104 ± 9	20 mM 3MG-Krebs' solution	230 ± 23 208 ± 23 181 ± 22 130 ± 8
10 mM 3MG-Krebs' solution	156 ± 17 158 ± 19 150 ± 17		

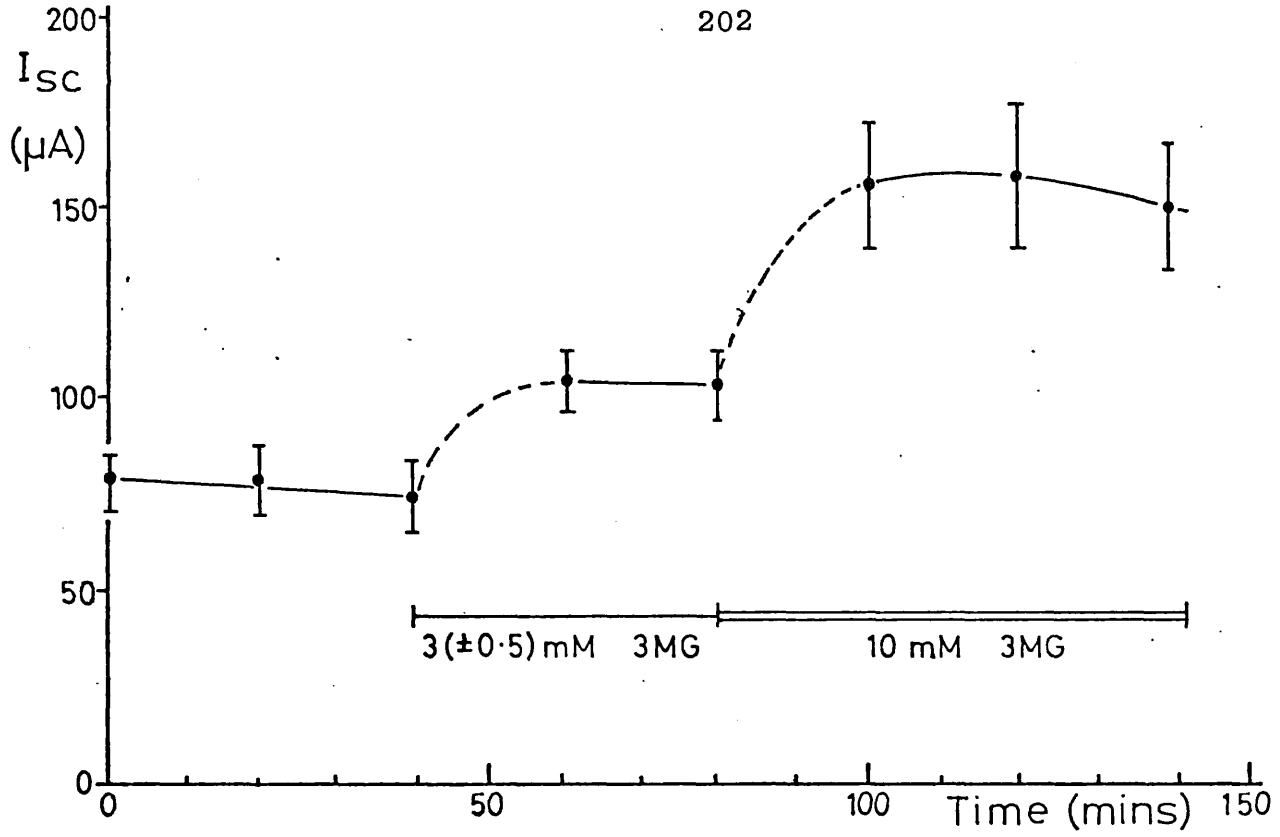


Fig. 9.2 Changes in short circuit current following the application of two aliquots of 3-O-methyl glucose in the absence of serosal sugar. (Table 9.3)

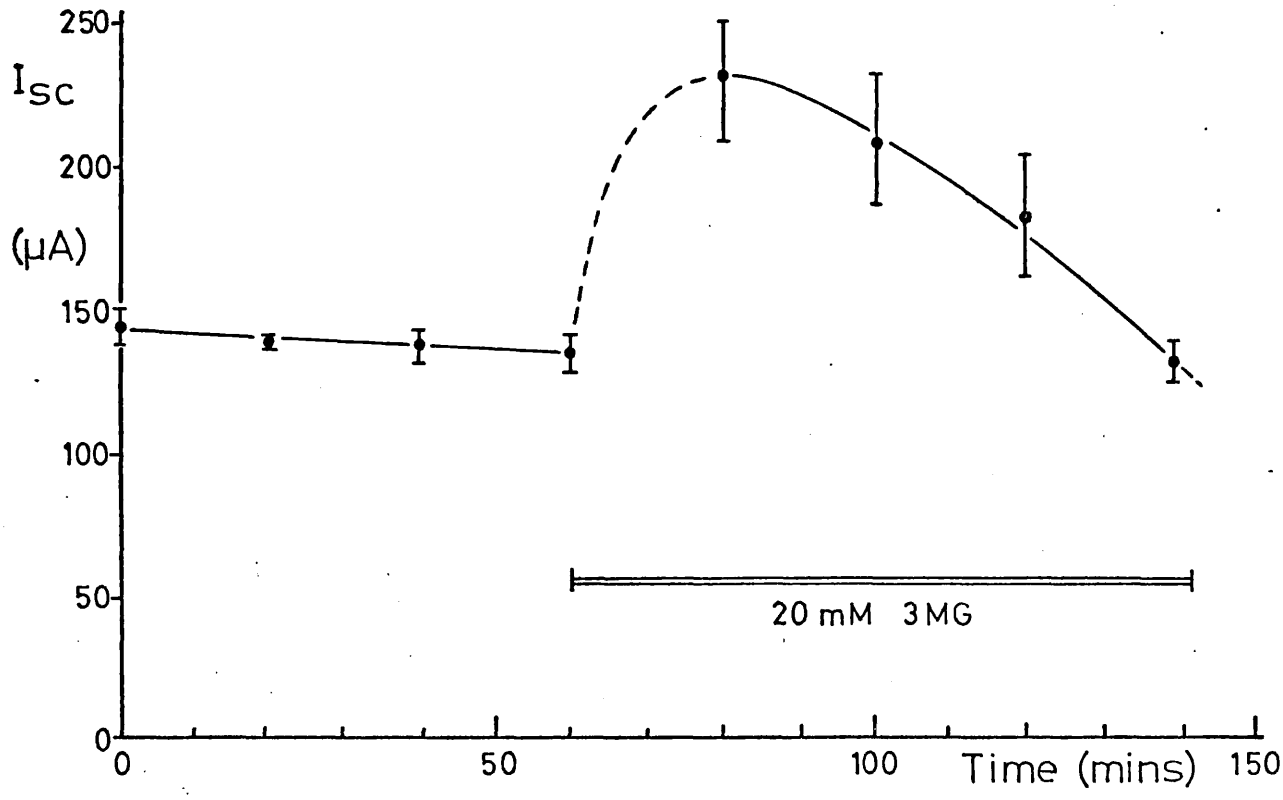


Fig. 9.3 Short-circuit current generated in the absence and presence of 3-O-methyl glucose with 170 mM serosal mannose. (Table 9.3)

that following the initial transient phase, the short-circuit current levels with or without added mucosal 3-O-methylglucose remained virtually constant throughout the experiment. Quay & Armstrong (1969a) in the bullfrog intestine and Field et al, (1971) in the rabbit ileal mucosa, both groups using different techniques, reported a similar steady state of the I_{sc} throughout their experimental time. But Schultz & Zalusky (1964a & 1964b) reported that the I_{sc}, assayed across the isolated rabbit ileum in the presence of mucosal sugar, sustained continuous decline after about 2 minutes of a transient increment phase. The time course of the experiments performed by Barry et al, (1965) including the transient increment phase and the assumed steady state, was only 15 to 45 minutes. However, when 170 mM mannose-Krebs' solution was used (Figure 9.3) at the serosal side of the intestinal segment the I_{sc} values obtained in the absence and presence of mucosal 3-O-methyl-glucose solution seem to be higher than that when sugar-free Krebs' solution was used at the serosal side of the segment. But the I_{sc} induced by the mucosal 3-O-methylglucose in the presence of 170 mM serosal mannose solution sustained continuous decline after a transient increment phase of about 20 minutes. This was probably because of the osmosis effect; it has been reported by Duerdoth, Newey, Sanford & Smyth (1965) that under such conditions mannose would stimulate fluid transfer. Barry et al, (1969) used 168 mM mannose solution at the serosal side for the purpose of initiating fluid transfer across the sac of the rat intestine.

The tissue resistance was also investigated in this study in segments taken from the region in between 25-35 and 45-55 cm from the proximal end of the jejunum. The technique followed in this study was

similar to that followed by Clarkson & Toole, 1964 and other authors e.g. Asano, 1964, Barry et al, 1965, and Quay & Armstrong, 1969a. The use of Ohm's law to calculate the tissue resistance from measurements of short-circuit current and open circuit potential requires that the tissue behaves as a simple d-c resistor. In fact this has been reported to occur for the rat intestine (Clarkson et al, 1964, 1967 and Asano, 1964) and for other animals' intestinal tissues (Schultz & Zalusky, 1964a, and Quay & Armstrong, 1969a).

However, the measurements of the resistance were performed by the following procedure. A calibrated potential control device was introduced in the potential measuring circuit called a 'baking-off device'. By the aid of this device the recorded potential of the short-circuited system (in the presence and absence of the intestinal segment) was manually increased or decreased by one mV at a time. The changes in the short-circuit current occurred due to the one mV potential changes were recorded. This technique was used in the presence and absence of glucose solution at the steady state of the recorded short-circuit current. Similarly the technique was applied with the absence of the intestinal segment while the assembly was immersed in the saline medium (Krebs).

Example of calculations:

Date of experiment - 4 February 1975

The segment was taken from the region in between 25-35 cm from the proximal end of the jejunum.

The mean of six changes in the I_{sc} = 33.4 μA ≡ 1 mV.

Since Ohm's law $\equiv$ $R = \frac{E}{I}$

$= R = \frac{\Delta E}{\Delta I}$

$\therefore$ The resistance of the intestinal segment and the saline solution =

$= \frac{1 \times 10^{-3} \text{ Volts}}{33.4 \times 10^{-6} \text{ Amps.}}$

$= \frac{1}{33.4} \times 1000$

$= 29.94$ ohms.

Similarly the mean of six changes in the I_{sc} in the absence of the intestinal segment = 153.8 μA $\equiv$ 1 mV.

In a similar way of calculation as above the resistance of the saline medium (Krebs) = 6.5 ohms $\therefore$ the resistance of the intestinal segment (10 cm^2) will be:

$29.94 - 6.5 = 23.44$

$= 23.44$ ohms/10 cm^2 of the intestinal mucosa.

The first set of the Table (9.4) shows that the resistance obtained for the intestinal segments in the absence and presence of 8.8 mM and 22.2 mM mucosal glucose solution respectively. Unmarked differences were observed between the presence and absence of the sugar. This unappreciable difference could be due to the time elapsed between the

TABLE 9.4

Resistance of the gut wall of the everted intestinal segments (cannula supporting device) in the absence and presence of the mucosal glucose solution.

In all instances the silver/silver chloride electrodes were used for the current measurements across the intestinal segments and sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment. The segments were taken from the region in between 25-35 (A) and 45-55 cm (C) from the proximal end of the jejunum for the first and second sets of the experiments respectively. For each set three and two animals respectively were used and the mean values obtained are expressed in ohms per 10 cm^2 of the intestinal mucosa $\pm$ SE.

First set:

Substances present at the mucosal side.	Resistance ($_2$ (ohms/10 cm^2)
Sugar-free Krebs' solution	22.8 $\pm$ 0.3
8.8 mM glucose-Krebs' solution	24.6 $\pm$ 0.9
22.2 mM glucose-Krebs' solution	24.6 $\pm$ 0.8

Second set:

Sugar-free Krebs' solution	19.1 $\pm$ 0.3
8.8 mM glucose-Krebs' solution	20.5 $\pm$ 0.7

two measurements as the measured I_{sc} might show a little decline as time elapsed. Quay & Armstrong (1969a) in the bullfrog intestine found that the tissue resistance was independent of the presence or absence of glucose at the mucosal side of the tissue. The resistance of the intestinal wall was independent of the concentration of the glucose present at the mucosal side of the segment. From the Table it can be seen that the segment taken from the middle part of the intestine showed greater values of resistance than the segment taken from the lower part of the intestine. In two experiments (not shown in the Table) the resistance of the intestinal segments taken from the lower part of the intestine was just about 10 ohms/10 cm^2 of the intestinal mucosa, in the presence of 2.2 mM mucosal glucose solution.

Since the resistance measurements depend on the area of membrane under investigation, crude estimation (without allowing for the microvilli and the folds of the mucosal membrane) of the mucosal area was performed. The mucosal area was found to be 9.7 cm^2 and has not appreciably changed in the absence of glucose, but with added glucose solutions the area increased to 10.2 cm^2. For the practical use, approximation of these figures were used and the mucosal area was considered to be 10 cm^2.

Barry et al (1965) reported that the resistance values depend on the region of the intestine chosen for measurement. Field et al (1971) found that the electrical resistance of the isolated rabbit ileal mucosa was about half that of the full-thickness of the ileum. Greater values for the resistance of the everted segments of the rat have been obtained by Barry et al (1965). However, in agreement with

Asano's (1964) conclusion, the low resistance obtained in this study may be attributed to the changes induced by the employed technique or may be explained by the histological findings.

It was noted that in vitro preparation of the small intestine became abnormally permeable to water soluble substances which are passively transported (Hogben, 1960).

Asano (1964) concluded that low resistance obtained in his study could be explained by the large surface area and not by the intrinsic low resistance of the epithelial cell membrane. According to Wilson (1962) the mucosal area is 30 times the serosal area if allowance is made for the villi and 600 times if allowance is made for the microvilli. In agreement with Asano's (1964) conclusion, it may be of some interest to add that the technique of mounting the everted intestinal segment on the supporting cannula employed in this study may induce a good deal of unfolding of the villi and the microvilli, as the cannula introduce a substantial stretching out of the intestine and thus larger surface area may be exposed. However, the technique followed in this study seems to give a low resistance which was associated with a more stable short-circuit current; the latter result has not been successfully observed in many publications as has been mentioned above.

The results of experiments designed to examine the relation between net Na^+ transport and short-circuit current (I_{sc}) across the rat intestinal segment (A) are summarised in Table (9.5) and illustrated graphically in Figures (9.4 and 9.5). The methods of determination and calculation of the unidirectional Na^+ fluxes, net Na^+ flux in relation to the recorded short-circuit current across the intestinal segment

TABLE 9.5

The unidirectional Na^+ fluxes, net Na^+ flux and short-circuit current obtained in the absence and presence of mucosal 3-O-methylglucose (3MG) solution across the intestinal segment (A) of the rat.

The mucosa to serosa and the serosa to mucosa fluxes were determined simultaneously by double labelling with Na^{24} and Na^{22} respectively. At the serosal side of the segment 5 ml of either sugar-free Krebs' solution (first set) or 170 mM mannose-Krebs' solution (second set) was recirculated throughout the experiment. Sampling was carried out at successive intervals of 20 minutes up to 140 minutes. Four and three experiments were performed for the first and the second set respectively. The mean values of the Na^+ fluxes and the $I_{sc} \pm$ SE expressed in μmole/minute/10 cm^2 of the intestinal mucosa are given.

First set

Sugar-free Krebs' solution used at the serosal side of the segment.

Step	Mucosal Medium	Na^+ influx μEq/min/10cm^2	Na^+ efflux μEq/min/10cm^2	Na^+ net flux μEq/min/10cm^2	I_{sc} μEq/min/10cm^2
1	Saline (Krebs)	0.40 ± 0.05	0.13 ± 0.01	0.27 ± 0.04	0.05 ± 0.01
2	2.5-3.5 mM (3MG)	0.29 ± 0.02	0.20 ± 0.03	0.09 ± 0.05	0.07 ± 0.01
3	10 mM (3MG)	0.24 ± 0.01	0.14 ± 0.01	0.10 ± 0.02	0.10 ± 0.01

Second Set

170 mM mannose-Krebs' solution used at the serosal side of the segment.

1	Saline (Krebs)	0.27 ± 0.02	0.21 ± 0.01	0.06 ± 0.03	0.08 ± 0.0
2	20 mM (3MG)	0.26 ± 0.01	0.19 ± 0.0	0.07 ± 0.01	0.12 ± 0.1

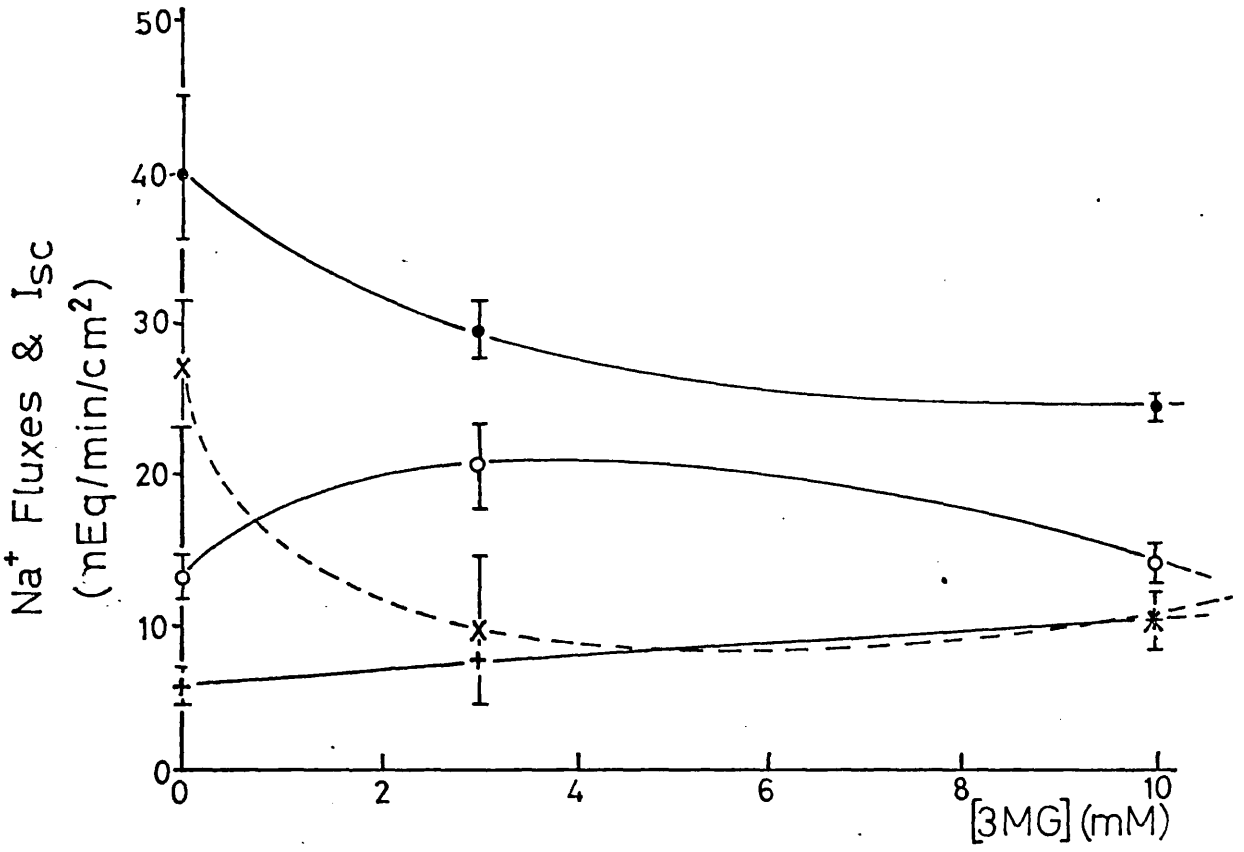


Fig.9.4 Effect of 3-O-methyl glucose on the influx (•), efflux (o) and net flux (X) of sodium ions and on the short circuit current (+) (Table 9.5). No serosal sugar.

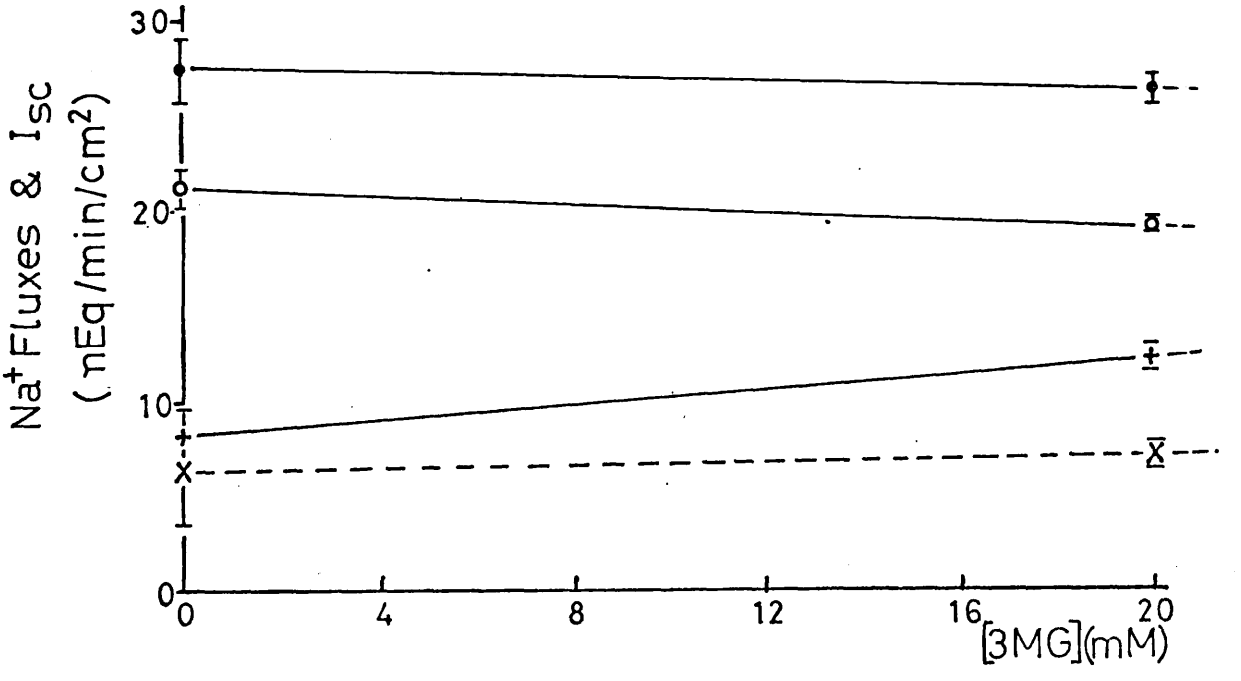


Fig. 9.5 Effect of 3-O-methyl glucose on the influx (•), efflux (o), and net flux (X) of sodium ions and on the short circuit current (+) 170 mM mannose in the serosal medium (Table 9.5).

of the rat are described in the General Method (Chapter Three). Figure (9.4) shows that in the presence of sugar-free Krebs' solution on both sides of the segment, the rate of net Na^+ transport from mucosa to serosa was relatively high and much greater than the I_{sc}. The addition of about 3 and 10 mM mucosal 3-O-methylglucose in two steps for 40 and 60 minutes respectively brought about a marked increase in I_{sc}. The addition of the sugar (about 3 mM) resulted in a clear decrease in the Na^+ influx and either an increase or no significant change in the Na^+ efflux. A decrease in the Na^+ net flux was observed up to a certain level where no further decrease occurred and the net flux became constant and was not affected by the addition of the second concentration of the sugar. Under these circumstances the short-circuit current either was not significantly different or was approximately equal to the net Na^+ flux. These results seem to be consistent with the recent publications (Clarkson, 1967, Taylor et al, 1968, White & Armstrong, 1971, Rose & Schultz, 1971, Schultz, 1972, Frizzel & Schultz, 1972) that the intercellular fluxes of monovalent ions contribute significantly to the total ion fluxes observed in intestinal tissue. These results are also in a good agreement with (Schultz et al, 1965, 1966, Csaky & Esposito, 1969, Koopman & Schultz, 1969, Armstrong, Musselman & Reitzug, 1970 and Lee & Armstrong, 1972) who found that there was either no change or a decrease in intestinal tissue Na^+ concentration following an interval of sugar accumulation. The data obtained by Frizzel & Schultz (1972) indicate that about 90% of the Na^+ entering the tissue at the mucosal surface was returned to the mucosal side across the same boundary. The border Na^+ efflux might be due to either exchange diffusion or active extrusion of Na^+ as has been reported by Schultz et al (1967). Also, as mentioned in the introduction, a significant

increase in mucosal to serosal unidirectional flux of Na^+ has with one exception (Field et al, 1971) not been observed for the intestine of different animals.

The second set of data represented in Table (9.5) and illustrated graphically in Figure (9.5) indicate that, in the presence of sugar-free Krebs' solution and serosal 170 mM mannose-Krebs' solution the short-circuit current(I_{sc})does not significantly differ from the Na^+ net flux. The figure also shows that the addition of 20 mM 3-O-methyl-glucose at the mucosal side brought about a slight decrease in Na^+ efflux and no significant change was observed in the unidirectional Na^+ influx or in the Na^+ net flux. A marked increase in the I_{sc} was noticed and in this case the I_{sc} seems to be greater than the Na^+ net flux.

Comparison of the results obtained in both sets (Table 9.5) show that mannose brought about a decrease in the Na^+ influx and the Na^+ net flux and an increase in the Na^+ efflux and the I_{sc} in the presence of mucosal sugar-free Krebs' solution. When 20 mM 3-O-methylglucose was added to the mucosal side the I_{sc} sustained continuous declination (Figure 9.3) after an initial transient increment phase for about 20 minutes. This result might be due to the osmotic load created by the osmotic gradient between the serosal mannose solution and the mucosal fluid which might oppose the 3-O-methylglucose mucosal to serosal influx and affect the sugar accumulation in the cell and so affect the Na^+ gradient balance or it might be due to competitive inhibition at the serosal border of the mucosal cells. Both possibilities might have operated synergistically.

TABLE 9.6

The effects of mucosal ethylidene glucose (EG) on the Na^+ influx and the I_{sc} stimulated by 12 mM mucosal 3-O-methylglucose solution.

The silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. The I_{sc} and the Na^+ influx were determined simultaneously and the Na^+ influx was assayed by labelling the mucosal sodium with Na^{22} radioactive isotope and sampling at the serosal side was carried out successively at 20 minute intervals. A new 5 ml aliquot of the sugar-free Krebs' solution was recirculated for each interval at the serosal side of the segment. Four animals were used and the mean values of the obtained results expressed in μmole/min ± SE are represented.

Substances present at the mucosal side	Na^+ influx (μmole/min)	Ionic current (μmole/min)
Sugar-free Krebs'	0.31 ± 0.05	0.04 ± 0.01
solution	0.33 ± 0.03	0.04 ± 0.00
12 mM 3MG-	0.33 ± 0.04	0.09 ± 0.01
Krebs'	0.30 ± 0.03	0.10 ± 0.01
solution	0.28 ± 0.02	0.09 ± 0.01
12 mM 3MG-	0.26 ± 0.02	0.06 ± 0.01
Krebs' + 5 mM EG-	0.24 ± 0.01	0.05 ± 0.01
Krebs' solution	0.24 ± 0.02	0.05 ± 0.00

TABLE 9.7

The effects of 14.7 mM serosal ethylidene glucose (EG) solution on the Na^+ influx and the I_{sc} stimulated by 12 mM mucosal 3-O-methylglucose solution.

The silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. The I_{sc} and Na^+ influx were determined simultaneously and the Na^+ was assayed by labelling the mucosal sodium with Na^{22} radioactive isotope and sampling at the serosal side was carried out successively at 20 minute intervals. The (EG) was dissolved in the stock serosal Krebs' solution and a new 5 ml aliquot of the (EG)-Krebs' solution was recirculated for each interval at the serosal side of the segment. Two animals were used and the mean values of the obtained results expressed in μmole/min ± SE are represented.

Substances present at mucosal side	Substances present at serosal side	Na^+ influx (μmole/min)	Ionic-current (μmole/min)
Sugar-free Krebs' solution	Sugar-free Krebs' solution	0.23 ± 0.04 0.25 ± 0.04	0.073 ± 0.01 0.068 ± 0.00
12 mM 3MG-Krebs' solution	Sugar-free Krebs' solution	0.25 ± 0.03 0.24 ± 0.02 0.24 ± 0.02	0.13 ± 0.00 0.12 ± 0.00 0.12 ± 0.00
12 mM 3MG-Krebs' solution	14.7 mM (EG)-Krebs' solution	0.23 ± 0.02 0.23 ± 0.02 0.23 ± 0.02	0.12 ± 0.01 0.12 ± 0.00 0.12 ± 0.00

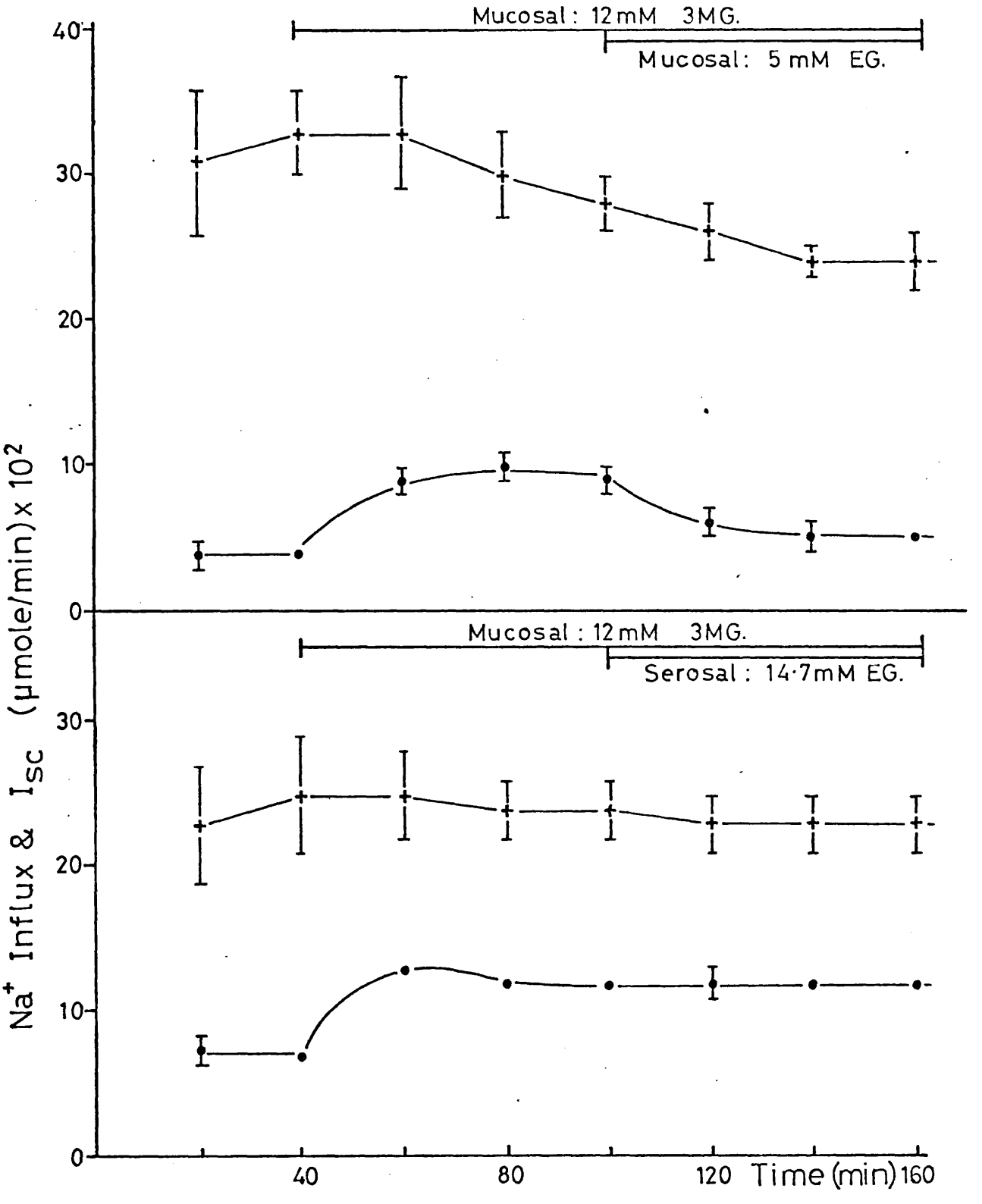


Fig. 9.6 Changes in the sodium flux (+) and the short circuit current (•) following the application of ethylidene glucose to the mucosal medium (above) and the serosal medium (below). From Table 9.6 & 9.7.

Tables (9.6 and 9.7) and Figure (9.6) show the inhibitory action of ethylidene glucose on the Na^+ influx and the I_{sc} stimulated by 12 mM mucosal 3-O-methylglucose solution. The obtained results indicate that the I_{sc} was significantly inhibited by 5 mM mucosal ethylidene glucose solution, but for the Na^+ influx (measured simultaneously with the I_{sc}) the inhibition seems not to be statistically significant. When 14.7 mM ethylidene glucose was used at the serosal side of the segment, unappreciable inhibition in the Na^+ influx and the I_{sc} were obtained.

Also Figure (9.6) shows again that there was not any sign of increment in the Na^+ influx, when the sugar was added to the mucosal side of the segment, but marked increment in the I_{sc} can be observed in the Figure after the addition of the sugar to the mucosal side.

RESULTS OF THE Na^+ - SUGAR INTERACTION STUDIES

CHAPTER 10

Some studies on 3-O-methylglucose influx in short-circuited intestinal segments of the rat.

Since it was found that there was an approximate equivalence between the short-circuit current and the net mucosal to serosal Na^+ flux in the presence of the mucosal 3-O-methylglucose solution (Chapter 9), a basis was provided to study the stoichiometry of the sugar-Na^+ coupling in the light of the increment of the I_{sc} induced by the sugar.

Very few publications have been made about the stoichiometry of the sugar-Na^+ coupling with the carrier system in the intestine of animals. Schultz & Zalusky (1964b) predicted that the stoichiometry of Na^+ and sugar entry on the ternary complex would be 1 : 1. This prediction was successfully confirmed by measuring the increased Na^{22} influx stimulated by the presence of the actively transported sugar 3-O-methylglucose at the mucosal side of strips of the rabbit ileum (Goldner, Schultz & Curran, 1969). Also it was of some interest to study this relationship using the new technique of the rat intestinal eversion on the supporting cannula.

In this chapter as well the effects of mucosal and serosal ethylidene glucose (EG) on the influx of 3-O-methylglucose and the related I_{sc} were also investigated for the first time in the rat intestine. A similar study was performed using phloridzin at the mucosal side of the intestinal segment of the rat.

For this study, the method followed and the calculation of the results are explained in Chapter 3. However, in each case a group of experiments were performed as indicated in the enclosed Tables. The mean values of the obtained results $\pm$ SE were calculated, except for the results included in Table (10.1).

TABLE 10.1

The relationship between the I_{sc} expressed in ionic terms and the influx of the 3-O-methylglucose (3MG).

Silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. The I_{sc} and the sugar influx were determined simultaneously and the sugar influx was assayed by labelling the mucosal sugar with C^{14} radioactive isotope and sampling at the serosal side was carried out at successive ten minute intervals, at the steady state of both the I_{sc} and the sugar influx. Different concentrations of the mucosal (3MG) were tested and a new 5 ml aliquot of the saline - Krebs' solution with or without mannose (10 mM) was recirculated for each interval at the serosal side of the segment. Six animals were used and more than one concentration (2 or 3) of the sugar were usually tested in each experiment.

Mucosal (3MG) conc. (mM)	(3MG) influx (μmole/min.)	The ionic-current (μmole/min.)
3.8	0.011	0.025
4.6	0.013	0.028
5.3*	0.011	0.018
8.0	0.010	0.021
21.2	0.031	0.027
22.0*	0.028	0.035
22.7	0.022	0.028
24.6	0.037	0.040
34.8	0.042	0.042
44.0	0.039	0.022
44.7	0.048	0.037
55.0	0.054	0.036

* Average of two experiments

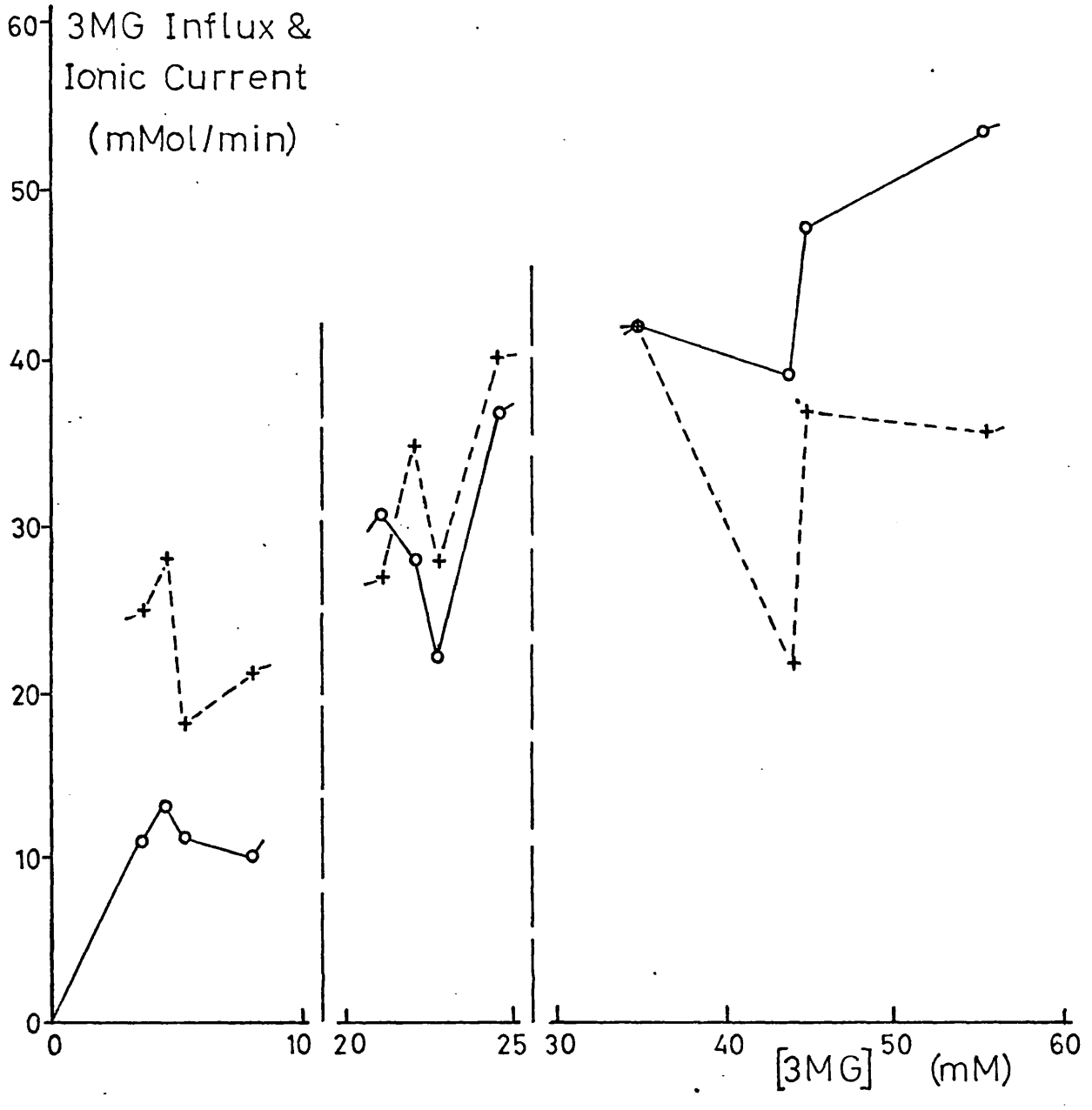


Fig. 10.1 Comparison of the 3-O-methylglucose influx (o)and the ionic-current (+) at different concentrations of 3-O-methylglucose. (Table 10.1)

Results:

Figure (10.1) and Table (10.1) show the stoichiometry of the sugar-Na^+ entry in the rat intestinal segment. 3-O-methylglucose was used which is known to be not metabolized but is actively transported in the Animals intestine (Crane, 1960b and Wilson, 1962). The values obtained for the Na^+ (ionic-current) were calculated from the recorded short-circuit current as it was found that it was approximately equivalent to the net Na^+ flux (Chapter 9). The sugar influx was assayed by labelling the mucosal sugar with C^{14} radioactive isotope and sampling at the serosal side was carried out successively each ten minutes interval at the steady state of both the I_{sc} and the sugar influx. The I_{sc} and the sugar influx were determined simultaneously across the intestinal segment taken from the region in between 25-35 cm from the proximal end of the jejunum. Different concentrations of the sugar were tested at a constant Na^+ concentration (144 mM). From the Figure it can be noted that the ionic-current values were about twice that of the sugar influx at concentrations of the sugar ranging from 3.8 - 8 mM. Lyon & Crane (1966a) stated that the kinetic of the net influx with regard to Na^+ concentration are those of simple association-dissociation phenomenon only when both sugar and Na^+ concentrations are high. However, in this study (Figure 10.1) it is clear that at concentrations of 3-O-methylglucose ranging from 21.2 up to 34.8 mM the stoichiometry of the sugar and the Na^+ (ionic-current) seems to be approximately 1 : 1. However, up beyond the latter concentration i.e. at 44.0, 44.7 and 55.0 mM 3-O-methylglucose concentrations, the sugar entry became greater than the Na^+; presumably this extra sugar entry is related to the diffusion or Na^+ independant facilitated transfer. Debnam & Levin (1972 & 1975) studied the active and passive transfer

TABLE 10.2

The effects of 2.0 and 3.5 mM mucosal ethylidene glucose (EG) on the 3-O-methylglucose (3MG) influx and the ionic-current stimulated by 9 mM mucosal (3MG) solution.

Silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. The I_{sc} and the sugar influx were determined simultaneously and the sugar influx was assayed by labelling the mucosal sugar with C^{14} radioactive isotope. Sampling at the serosal side was carried out at successive ten minute intervals at the steady state of both the I_{sc} and the sugar influx. Sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment and a new 5 ml aliquot of the fluid was used for each interval. Five animals were used and the mean values of the obtained results $\pm$ SE are represented.

Substances used at the mucosal side			The influx of (3MG)	The calculated ionic-current
(3MG) mM		(EG) mM	(µmole/10 min)	(µmole/10 min)
9.0	+	0.0	0.17 ± 0.01	0.44 ± 0.04
9.0	+	2.0	0.16 ± 0.01	0.12 ± 0.05
9.0	+	3.5	0.14 ± 0.01	0.02 ± 0.04

TABLE 10.3

The effects of 10 mM serosal ethylidene glucose (EG) solution on the 3-O-methylglucose (3MG) influx and the ionic-current stimulated by 9 mM mucosal 3MG solution.

Silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. The I_{sc} and the sugar influx were determined simultaneously and the sugar influx was assayed by labelling the mucosal sugar with C^{14} radioactive isotope. Sampling at the serosal side was carried out at successive ten minute intervals of the steady state of both the I_{sc} and the sugar influx. The EG was dissolved in the stock serosal Krebs' solution and a new aliquot of the EG-Krebs' solution was recirculated for each interval at the serosal side of the segment. Two animals were used and the mean values of the obtained results ± SE are represented.

Substances present at the mucosal side	Substances present at the serosal side	3MG influx (μmole/10 min)	The calculated ionic-current (μmole/10 min)
9 mM 3MG-Krebs' solution.	sugar-free Krebs' solution.	0.14 ± 0.0	0.35 ± 0.01
9 mM 3MG-Krebs' solution.	10 mM EG-Krebs' solution.	0.16 ± 0.0	0.29 ± 0.02

TABLE 10.4

The effects of 10^{-2} and 2×10^{-2} mM mucosal phloridzin on the 3-O-methylglucose influx and the ionic-current stimulated by 9 mM mucosal 3MG solution.

Silver/silver chloride electrodes were used for the I_{sc} measurements across the intestinal segment (A) of the rat. The I_{sc} and the sugar influx were determined simultaneously and the sugar influx was assayed by labelling the mucosal sugar with C^{14} radioactive isotope. Sampling at the serosal side was carried out at successive ten minute intervals at the steady state of both the I_{sc} and the sugar influx. Sugar-free Krebs' solution (5 ml) was recirculated at the serosal side of the segment and a new 5 ml aliquot of the fluid was used for each interval. Four animals were used and the mean values of the obtained results $\pm$ SE are represented.

Substances used at the mucosal side			The influx of 3MG	The calculated ionic-current
3MG mM		Phloridzin mM	(µmole/10 min)	(µmole/10 min)
9.0	+	0.0	0.22 ± 0.03	0.33 ± 0.02
9.0	+	1×10^{-2}	0.12 ± 0.02	-0.10 ± 0.01
9.0	+	2×10^{-2}	0.08 ± 0.01	-0.12 ± 0.01

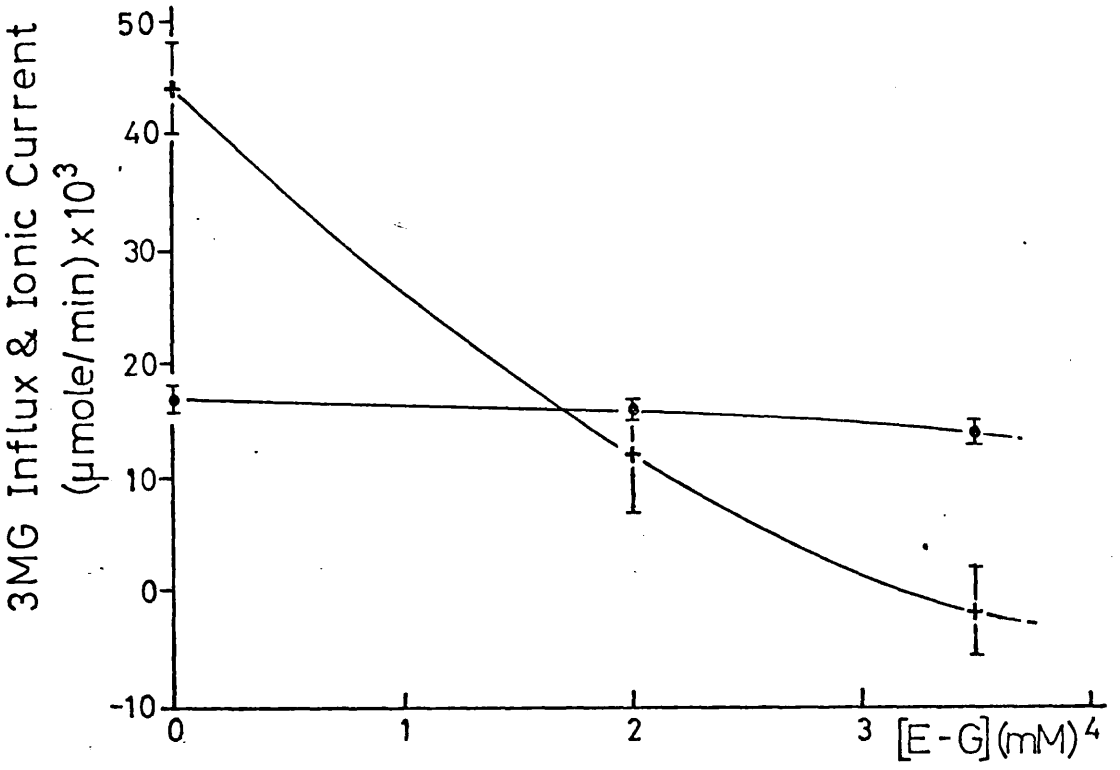


Fig.10.2 Changes in the 3-O-methylglucose influx (•) and ionic-current (+) in the presence of ethylidene glucose. (Table 10.2)

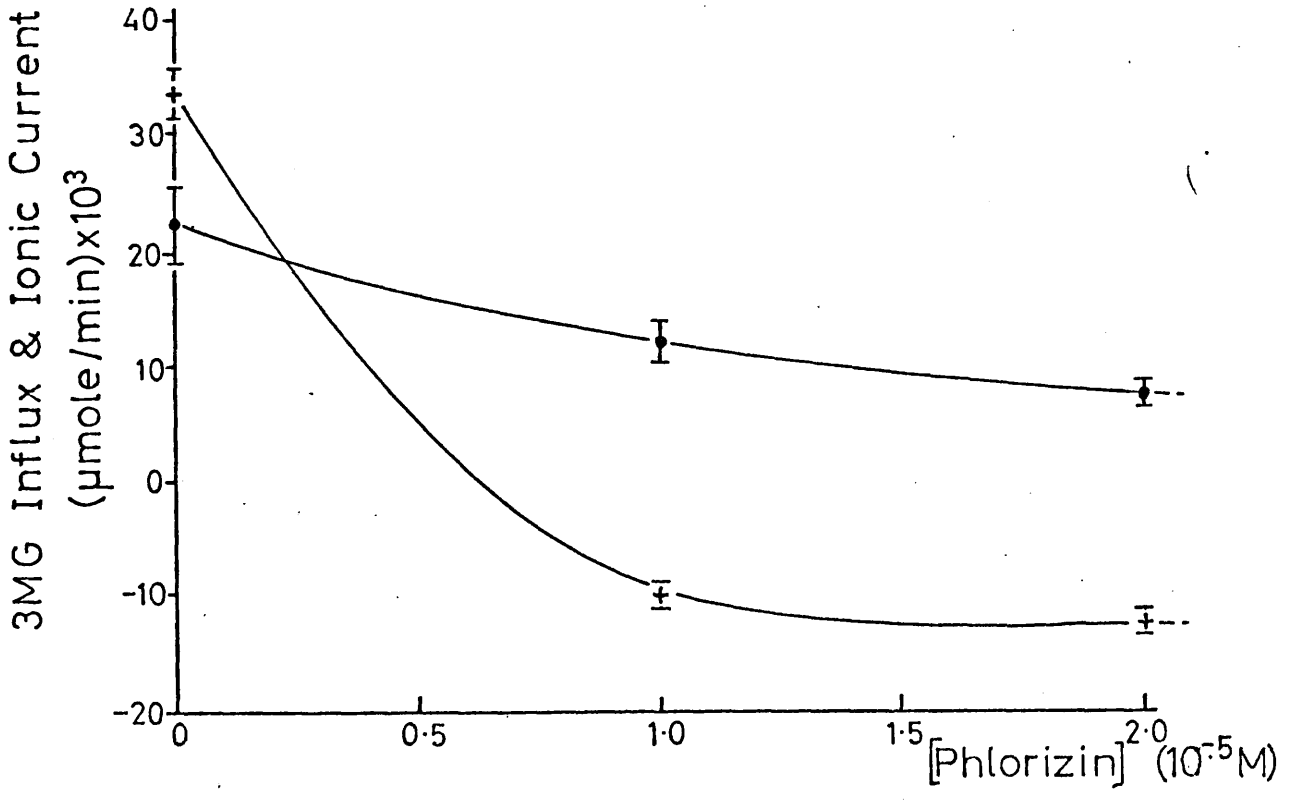


Fig. 10.3 Effect of Phloridzin on the 3-O-methylglucose influx (•) and the ionic-current (+). (Table 10.4).

components of hexoses (glucose, galactose and α-methylglucoside) absorption in rat jejunum in vivo. They stated that the electrical method measured only absorbed hexoses that passed through the electrogenic (sodium linked) active transfer mechanism, while chemical estimations measured this together with hexose absorbed by non-electrogenic processes.

Figure (10.2) and Tables (10.2 and 10.3) show the inhibitory action of ethylidene glucose on the influx of 3-O-methylglucose and on the related ionic-current stimulated by 9 mM mucosal 3-O-methylglucose solution. Figure (10.2) shows the action of 2 and 3.5 mM ethylidene glucose solution (~~pure~~ FREE of glucose) used at the mucosal side of the intestinal segment. The ionic-current was markedly inhibited and the inhibition gave rise to a negative hyperbolic-shaped curve. For the sugar influx unmarked inhibition was observed. When 10 mM ethylidene glucose was used at the serosal side of the segment (Table 10.3) unmarked inhibition of the ionic-current was obtained and a small increase in the influx of the sugar was observed.

Figure (10.3) and Table (10.4) show the inhibitory effects of 10^{-2} and 2×10^{-2} mM mucosal phloridzin on the influx of 3-O-methylglucose and the related ionic-current stimulated by 9 mM mucosal 3-O-methylglucose solution.

From the figure it can be clearly seen that the inhibitory action of the phloridzin gave rise to negative hyperbolae curves for both the ionic-current and the sugar influx. While a drastic action of the phloridzin on the ionic-current was observed, a much smaller effect on the sugar influx was noted. This result might be of some importance and helpful for the understanding of the ethylidene glucose inhibitory action on

the current and the fluxes of the sugar and Na^+. However, it is known that phloridzin is a very potent inhibitor of the sugar entry in the animal's intestine. It is, therefore, to be expected that the inhibition by the sugar-competitive action will be smaller in magnitude unless higher concentrations of the sugar-inhibitor could be used which might be unsafe to do as it might provoke some difficulties e.g. osmosis. It might be remembered that the electrical method measured only absorbed hexoses that passed through the electrogenic (sodium-linked) active transfer mechanism, while chemical estimations measured this together with hexose absorbed by non-electrogenic processes (Debnam & Levin, 1972 & 1975). Furthermore, lactose seemed not to be hydrolysed and translocated across the mucosal cell of the intestine by the carrier (or enzyme) system yet it had inhibited the I_{sc} in a manner which was in agreement with Michaelis-Menten kinetics (Chapters 6 & 7). These results and considerations in addition to the results obtained in Chapters (7 & 9) for the ethylidene glucose inhibitory action might throw a good deal of light on the mode and the site of ethylidene glucose action. A conclusion may be drawn that ethylidene glucose inhibits competitively the sugar active transport system in the rat intestine without necessarily entering the cell.

Ethylidene glucose was found to penetrate human red cells by diffusion (Baker & Widdas, 1973 a & b). Thus in the mucosal cells of the rat intestine intracellular effects would be possible if this sugar inhibited the I_{sc} when used at the serosal side of the intestinal segment, but such an effect was not significantly observed. Thus effects of ethylidene glucose appear more consistent with an action on the mucosal surface and together with benzylidene glucose, mannose, lactose and cellobiose (Chapter 7) may form a family of non-translocatable inhibitors of the sugar transfer system.

CHAPTER 11

DISCUSSION

On the basis of short-circuit current changes induced by sugars applied on the mucosal side of the intestinal segment of the rat, the sugar transfer or uptake into the intestinal epithelial cells - of the new technique introduced into the preparation of the intestinal eversion - obey Michaelis-Menten (1913) kinetics with respect to the sugar concentration. A variety of sugars were tested, namely glucose, galactose, 3-O-methylglucose, α-methylglucose, β-methylglucose and β-phenylglucose. This is in accordance with the current publications especially those done on the basis of electrical methods e.g. (Asano 1964, Schultz & Zalusky 1964b, Lyon & Crane 1966a, Debnam & Levin, 1970, 1971, 1972, 1973, Levin & Syme 1971). In most of the publications it was found that the majority of the experiments were performed by using glucose and to a lesser extent galactose and 3-O-methylglucose were used. The experiments which were carried out by Asano (1964) to obtain a similar relation between the short-circuit current and the galactose concentrations failed especially at low concentration of the sugar. Some other results failed to obey Michaelis-Menten (1913) analysis e.g. (Cori 1925, Small et al 1959, French et al 1963, Faust 1964, Rider et al 1967, Förster 1972). The latter group of the authors have used chemical methods in their investigation of glucose or other sugar transport across the intestine. Chemical methods might measure the absorbed sugar that passed through the electrogenic (sodium linked) active transfer mechanism together with the sugar absorbed by a non-electrogenic processes, while the electrical methods measure only sugars that pass through the first mechanism (Debnam & Levin 1972, 1975). In this respect it would found (Chapter 10) that using 3-O-methylglucose concentrations which exceeded a concentration giving full saturation, the transport measured by the short-circuit current gave lower levels for the sugar

influx than those measured by the use of radioactive isotope C^{14} labelled sugar and measuring that appearing on the serosal side of the intestinal segment. It was concluded that the additional levels of the 3-O-methlyglucose influx measured by the chemical (isotopic) method may be related to simple diffusion or to a more specific mechanism like facilitated diffusion. 3-O-Methylglucose was chosen for such a study because it is not metabolized but is actively transported by the intestinal epithelium (Crane, 1960b, Wilson, 1962). However, it seems likely that the chemical methods might give results that make the kinetic analysis of the active transport mechanism of the sugar more complex.

Robinson (1972) in his comment on experiments done in vivo by Dr Förster stated that studies on the absorption of sugars or amino acids in vivo are nearly always performed by measuring the disappearance of the substrate from the perfusate. He reported that he and others have demonstrated that saturation kinetics of amino acid absorption in vivo are only obtained when the tissue levels of the substrate are measured, a linear relationship between the substrate concentration and velocity of absorption being concommitantly observed when transport is measured as the disappearance from the perfusate. Crane (1960b) reported that the value of the apparent Km ≡ Ki, depends to a large extent on the experimental technique followed. On the bases discussed above it is likely that the values of the Ki obtained (Tables 4.6, 4.7, 5.3, 6.5 and 7.10) by the electrical method are more nearly correct. However, for the sake of comparison, the half saturation constants of some sugars (used in the present study) which have been obtained from the literature for different animals are also presented in Table (4.6).

During the course of study (Chapter 5) it became clear that the system was highly temperature sensitive particularly the increase in short-circuit current in the presence of glucose or galactose solutions at the mucosal side of the intestinal segment. At the lower temperatures i.e. 27^{o} and $17^{o}C$, the system still appeared to be concentration dependent so that Michaelis-Menten kinetics (1913) were applicable. The magnitude of the parameters obtained for both sugars were also temperature dependent. The ΔI max for the two sugars were clearly lower at 27^{o} and $17^{o}C$ than that at $37^{o}C$ (Table 5.3). For the cellular transport mechanisms the higher degree of temperature sensitivity is usually taken as evidence of a chemical reaction which requires energy. Although the apparent activation energies calculated from the Arrhenius plots were high, there is no indication of the rate limiting steps involved.

The kinetic analysis of the effects of the disaccharides on the short-circuit current showed different actions for maltose, sucrose and lactose. Maltose like glucose gave rise to a hyperbolic-shaped curve and the linear plot as recommended by Riggs (1972) gave an apparent Ki of 2.5 mM for maltose and 4.9 mM for glucose and the ΔI max were almost the same for both sugars. This result seems to be consistent with the consideration of the equimolecular concentrations of the glucose in both cases and would indicate that hydrolysis of maltose at the brush border was not rate limiting. The sucrose curve was sigmoid, which may be related to the fact that after the hydrolysis of the sucrose to glucose and fructose, the latter two sugars would have different reactivity for the carrier system. Thus Lyon & Crane (1966a) found that graded concentrations of mucosal fructose clearly inhibited the PD across the in vitro preparation of the rat's small

intestine. This also seems in accordance with the result obtained by Kohn, & WRIGHT Smith, (1968) who found that the PD changes for the sucrose at low concentration was much smaller than that for isomolar glucose but the difference decreased as the concentrations were increased. It is not, therefore, surprising that the short-circuit current changes should be complex. However, the surprising observation of the present investigations was the inhibitory effects observed with lactose. This disaccharide on hydrolysis should yield equimolecular quantities of glucose and galactose both of which are actively absorbed with the stimulation of the short-circuit current. That there should be no stimulation of the short-circuit current might have been explained with intestines from adult rats which lack the β-glycosidase enzyme. In rat small intestine it was reported (Alvarez & Sas, 1961, Doell & Ketchmer 1962, and Koldovsky & Chytil 1965) that lactase is present at birth at high levels and tends to decrease thereafter. It is also well known (see Semenza, 1968) that in small intestine of most animals, the pattern of development of the lactase enzyme is almost the mirror image of that of the other disaccharidases. Thus if lactose was not hydrolysed it would remain extracellular and could only exert an effect at the membrane surface. Such an effect may be competition for a sugar binding site with fixation and prevention of translocation. Similar action also was observed for the cellobiose. These properties of lactose are however shared by a number of sugar derivatives which are non-transportable inhibitors of the red cell hexose-system. Thus ethylidene glucose, benzylidene glucose together with lactose, cellobiose and mannose form a group of non-transportable potential inhibitors of the sugar absorption system in the intestine. Indeed phloridzin may belong to this group since Newey, Sanford, Smyth & Williams (1963) have suggested that the hexosyl unit of the phloridzin plays an important part in the binding

of this inhibitor.

In competitive experiments it was possible to study the increments in short-circuit current produced by varying concentrations of one sugar in the presence of fixed concentration of another sugar. In the case of actively transportable sugars such increments were always positive and with applying different concentrations of galactose in the presence of a fixed concentration of glucose, there was no definite increase in the apparent half-saturation constant for galactose as would be expected from simple competition. Nor was there appreciable reduction in the maximum short-circuit current seen at high galactose concentrations. The presence of glucose thus either increases the maximal transport possible by the mucosa or does not offer simple competition for the galactose transporting system as has been suggested by Debnam & Levin (1971 & 1974) and Levin & Syme (1971). The result seems to be in keeping with the result obtained with the everted sac of the rat intestine by Newey, Sanford & Smyth (1965) who reported that galactose transfer was increased in the presence of glucose. They concluded that glucose may be able to enter the cell without competing with galactose. With sugar derivatives like ethylidene glucose, however, when mixed with a constant concentration of the transportable sugar i.e. glucose, galactose, 3-O-methylglucose, α-methylglucose and β-methylglucose; increasing concentrations of ethylidene glucose have an inhibitory effect that is they produce negative increments in the short-circuit current. As the ethylidene glucose inhibitory action gave rise to a negative hyperbolic curve at least with the glucose, galactose and 3-O-methylglucose it is assumed that the reaction is a membrane one, fixing the sugar transfering mechanism but without allowing translocation on which the short-circuit current depends. On this basis it could be argued that in a two sugar competitive system in

which one sugar is kept constant in respect to the concentration, if increments in the concentration of the other sugar increase the short-circuit current then it must be transportable (actively), whereas if the short-circuit current decreases the second sugar is competitive but is not transportable. The presumption of a mucosal membrane effect is made more likely since ethylidene glucose applied to the serosal side has not been shown to have an appreciable inhibitory effect and when applied on the mucosal side the inhibitory effect begins without delay.

An interesting and important observation of the present study is the inhibitory effect of ethylidene glucose, benzylidene glucose and mannose on the short-circuit current of the intestine in Krebs' solution which is free of transportable sugar. When the everted sac of the intestine (with the supporting cannula technique) was first mounted in a sugar-free medium the short-circuit current declined over about forty minutes to a low steady state. This remained stable to changes in the sugar-free medium. In all instances, addition of different concentrations of ethylidene glucose, benzylidene glucose or mannose brought about further declination in the short-circuit current. In all cases also, the negative increments obtained in the short-circuit current by the addition of any one of these three sugars were concentration dependent in a manner suggesting a saturable effect. This was confirmed by the kinetic analysis to derive the Ki and ΔImax for these sugars. The Kis for inhibiting the basal short-circuit current were almost similar to those obtained in inhibiting the short-circuit current at the steady state obtained with a transportable sugar (such as 3-O-methylglucose or galactose); suggesting that reactions were occurring either at the same sites or

at sites with very similar affinities.

The inhibitory action of the non-transportable sugars described in this work may be a feature of other non-transportable sugars some of which show evidence of competitive inhibition (Bihler, 1965). However, there is a difference between those sugars like mannose which lack the stereo-specificity at carbon atom 2 and the sugar derivatives, ethylidene glucose, benzylidene glucose and lactose which have this stereo-specificity but which have a bulky group at the other end of the glucose molecule.

In summary, competition for a sugar binding site but without translocation is the most probably common feature relating ethylidene glucose, benzylidene glucose, mannose and lactose in their reactions with intestinal mucosa. The corollary to this view would be the hypothesis that at least part of the basal short-circuit current of the everted gut bathed in sugar-free medium was actually being carried (presumably without sugar) on the sugar transporting system. This hypothesis may be made clearer from the finding that mannose (33 mM) inhibited about 96% of the basal short-circuit current across the intestinal segment. Translocation of the Na^+ on the sugar carrier mechanism was implicit in the schematic model suggested for sugar accumulation by rabbit ileum (Goldner, Schultz & Curran, 1969). In this model Na^+ dependent alteration in the carrier mobility was postulated to play an important role in generating the sugar gradient. This was in contrast to the situation reported by Crane, Forstner & Eichholz (1965) and Lyon & Crane (1966a) for hamster and rat small intestines respectively in which the primary effect of Na^+ was to improve carrier affinity. The latter authors reported that measurements of PD indicated that, the increment of potential over the

resting level is dependent upon the Na^+ concentration and at a given Na^+ concentration upon the concentration of glucose. The saturation level for Na^+ appeared to lie between 48 and 72 mequiv. while that for glucose appears to be reached between 10 and 25 mM. Lauterbach (1967) reported that in rats and guinea pigs in vitro preparations there was a strong linear correlation between glucose and sodium absorption if the Na^+ concentration was held constant. The dependence of the res-ting PD on Na^+ concentration observed by some authors e.g. (Lyon & Crane, 1966a) might be in accordance with the above view. Thus in the absence of sugar Na^+ could still react with the transporting system but so far evidence of ionic movement on the system without the presence of sugar has not been clearly demonstrated.

The absence of sugar in the medium does not exclude the possibility of a small leakage of intracellular sugar into the microvillal crypts and reabsorption with Na^+ on the transporting system but after forty minutes incubation any free intracellular sugar would be at a very low concentration and this explanation would appear to be unlikely.

It is interesting to compare the relative magnitudes of the negative increments of the short-circuit current with the positive ones produced by a transportable sugar such as glucose or galactose. Thus lactose (Table 6.5) reduces the sugar-free short-circuit current with agar electrodes by a maximum of about 57 μA against a maximum increment from glucose of about 900 μA. Thus the sugar-free leakage current could be about 6.3% of the sugar stimulated current in the case of glucose. It would be higher for the other sugars with smaller maximal increments of short-circuit current.

In this study, the new technique of the gut supporting cannula introduced in the preparation of the everted rat small intestine proved to have the advantage of longer viability of the intestinal segment up to about three hours. This occurred specially when glucose was used as the testing substrate. The longer viability of the intestinal segment was associated with greater consistency and more stability in the short-circuit current. The results obtained in Chapter 8 showed that no striking effect due to the age of the intestinal segment on the magnitude of the short-circuit current was seen provided that the tested sugar could be metabolized (i.e. glucose) by the intestine. When glucose concentrations were applied in the first order, the I_{sc} levels of galactose, which is known to be poorly metabolized by the rat intestine (Barry et al, 1969) but like glucose actively transferred (Fisher & Parsons, 1953b), used in the second order seemed to improve and gave I_{sc} levels even higher than those obtained ~~with~~ WHEN the same concentrations of galactose ~~was~~ WERE assayed in the first order (Figure 8.4). This seems to be in accordance with the results obtained by Newey et al, (1962 & 1965) who found that glucose metabolism can stimulate glycine and galactose transfer respectively.

It was also observed (Figure 9.2) that following the initial transient phase (15-20 mins) the short-circuit current levels with or without added mucosal sugar remained virtually stable throughout the experiment. Similar cases for the short-circuit current were obtained by Quay & Armstrong (1969a) and by Field et al, (1971). Schultz & Zalusky (1964 a & b) reported continuous decline of the measured short-circuit current just after about two minutes of a transient increment phase. The authors reported that this decline in the short-circuit current was related to the effects of age of

the intestinal preparation. With the addition of glucose or galactose, Taylor et al (1968) obtained similar declines in short-circuit current across the rat jejunum. The time course of the experimental measurements of the short-circuit current in most of the previous work was therefore 15 to 45 minutes (Barry et al, 1965), 50 to 80 minutes (Schultz & Zalusky, 1964 a & b) and 120 minutes (Taylor et al, 1968).

The improved performance in the short-circuit current with glucose in contrast to other transportable sugars strongly suggests that the maximal short-circuit current may not be membrane limited but may depend on a supply of some metabolite or source of energy which glucose can also supply. 3-O-methylglucose which is not metabolized but actively transportable in the intestine had a consistently lower maximal short-circuit current than glucose or galactose. Such an observation seems to be in accordance with the recent schematic model of Kimmich & Randles (1973) as an alternative to the ion gradient hypothesis of intestinal sugar transport. The summary of this model which depends on a direct input of metabolic energy for the sugar transport system, has been explained in the introduction. The present observation also seems to be in agreement with the findings of some other authors e.g. Schafer et al, (1968 & 1972) who raised a number of questions regarding the sufficiency of the energy inherent in cellular ion gradients for totally accounting for observed solute concentration gradient.

The measured short-circuit currents depend on the geometry of the current carrying and voltage measuring electrodes. The simpler agar electrodes gave much higher readings than the concentrically arranged cage of silver/silver chloride current carrying electrodes.

However, provided comparisons are made with experiments done with the same electrode system the qualitative changes are similar. The measurements with the concentric silver/silver chloride cage are probably nearer to the true values and approximately corresponded to the currents calculated from the net fluxes of sodium ions estimated by the double labelling technique of radioactive isotopes for the ion influx and efflux.

The evidence for active sodium transport found in the present study in the jejunal segments of the rat are in accordance with the current reports. Net sodium transfer under short-circuit conditions has been observed in vitro in the small intestine of the rat (Barry et al, 1963, 1965., Clarkson et al, 1964, 1967, and Taylor et al, 1968), rabbit (Schultz & Zalusky, 1963a, 1964a & b, and Taylor et al, 1968) and bullfrog (Quay & Armstrong, 1969a). In most of these studies, however, ileum was used and net sodium flux and short-circuit current were found to be approximately identical, suggesting that sodium transfer was the only active ion transport evident in vitro. This suggestion has been supported by data showing passive behaviour of chloride under the same conditions e.g. (Clarkson et al, 1964 & 1967). An exception is the work of Barry et al (1965) in the rat jejunum. These authors found that net sodium fluxes exceeded short-circuit current in the presence of fructose and the short-circuit current exceeded net sodium flux in the presence of the actively transported but poorly metabolized substrate galactose. Taylor et al (1968) have demonstrated that the discrepancy between these two parameters in rat jejunum incubated with galactose is due to a net secretion of chloride from serosa to mucosa. However, under conditions of the present experiments, net sodium influx takes place down the

electrochemical potential gradient for this ion. By this analogy with commonly accepted ideas on sodium transport in mammalian intestine (Curran, 1968), active ejection of Na^+ from the cell is assumed to occur via a metabolically driven sodium pump in the serosal membrane (Asano, 1964, Schultz & Zalusky, 1964b). It was found e.g. (Schultz & Zalusky, 1964b) and it is confirmed in this investigation that this sodium pump is ouabain sensitive. When 20 mM 3-O-methylglucose was added to the mucosal side in the presence of 170 mM serosal mannose, the short-circuit current sustained continuous declination after an initial transient increment phase of about 20 minutes. This observation was also associated with disturbance of the equality balance between the Na^+ net flux and the short-circuit current, found in the absence of mannose. In addition to the osmotic effects, these observations might be related to competitive inhibition at the serosal side of the mucosal cells. This seems to be a possibility in view of the findings that the addition of mannose to the mucosal side of the segment inhibited the short-circuit current induced by the galactose (Chapter 7). Barry et al, (1969) have reported that 28 mM mucosal mannose inhibited the resting level of the potential difference in the absence of added solute. However, in all instances the detail of the changes in the short-circuit current and Na^+ fluxes in the presence and absence of the sugars have been fully explained in Chapter 9 (Table 9.5 and Figures 9.4 and 9.5). It seems that these results are not different from the recent ideas known about the active sodium transport and Na^+-dependent sugar transport in the intestine of animals (Kimmich, 1973).

The kinetic studies of the present investigation suggest that there must be a stoichiometric relation (at least at one stage of the experiments) between the increments in the (ionic current) or its synonym the net Na^+ flux and the rate of sugar transport. This relationship has been investigated by the addition of different concentrations of 3-O-methylglucose labelled by C^{14} radioactive isotopes at the mucosal side of the short-circuited intestinal segments. The values obtained for the Na^+ (ionic current) were calculated from the recorded short-circuit current, as it was found that it was approximately equivalent to the Na^+ net flux. It was found that at the concentrations of 3-O-methylglucose ranging from 21.2 up to 34.8 mM the stoichiometry of the Na^+ and the sugar entry was 1 : 1. This result is in accordance with the findings of Goldner, Schultz & Curran (1969) who used 3-O-methylglucose at the mucosal side of the strips of the rabbit ileum.

SUMMARY

1. In these investigations changes in the short-circuit current across the intestinal segments of Albino rat produced by a variety of mucosal sugars were studied. The segments were usually taken from the mid part of the intestine.

2. A new device, namely the gut supporting cannula was introduced to the everted sac technique for the first time in this study. The technique was elaborated during the course of the study and the full details of this technique are explained in Chapter Three.

3. Two different geometric arrangements of different current electrodes were used; these were the simpler agar electrodes and the concentrically built silver/silver chloride electrodes. The details of both systems are fully explained in Chapter Three.

4. Different concentrations of glucose, galactose, 3-O-methylglucose, α-methylglucose, β-methylglucose and β-phenylglucose were added to the mucosal side of the intestinal segment at $37^{o}C$. In each case the relationship between the added concentrations of the sugar and the obtained short-circuit current was not linear and a hyperbolic curve was obtained.

5. In the presence of different concentrations of glucose, the two adjacent segments taken from the mid part of the intestine showed significant differences in the levels of the obtained short-circuit current, and it was greater in the distal segment (B) than in the proximal one (A).

6. Within the concentration ranges of galactose and glucose used no mutual competition for the transport system was observed.

7. Different concentrations of glucose and galactose were assayed at 27°C and 17°C. The relationship between the concentrations of the sugar and the short-circuit current produced was similar in slope to that obtained at 37°C for both sugars but the short-circuit current showed lower levels at 27° and 17° respectively. In the presence of mucosal glucose (4.4 mM) the system proved to be highly sensitive to temperature elevation of the bathing medium from 22° to 45°C but not similarly sensitive in the absence of mucosal sugar. Arrhenius plots were made from these studies and the activation energies were determined.

8. The disaccharides, maltose, sucrose, trehalose, lactose and cellobiose concentrations were tested. Different results were observed for these sugars; the short-circuit current seems to depend on the hydrolysis and on the kind of the simple sugars included in the molecular construction of each disaccharide. Some competitive inhibition may have occurred between glucose and fructose yielded on the hydrolysis of sucrose; this differed from the results with maltose which, when hydrolysed, would give two molecules of glucose.

Lactose and cellobiose seemed not to be hydrolysed; they inhibited the short-circuit current. The results suggest an involvement of these sugars with the transport system at the outer side of the mucosal cell without their being transferred into the cell.

9. The inhibition of short-circuit current by different concentrations of ethylidene glucose, benzylidene glucose, mannose and phloridzin gave

rise to hyperbolic shaped curves similar to that obtained with the different concentrations of lactose and cellobiose.

10. Mutual competition for the active sugar transport system was observed between the non-transportable sugars (lactose, ethylidene glucose, benzylidene glucose and mannose) and the actively transported sugars like 3-O-methylglucose, galactose and glucose.

11. Kinetic analysis was made from the above studies and the Kis and ΔI maxs were obtained.

12. On the basis of changes in the short-circuit current induced by the metabolized sugar (glucose) the intestinal segment (with the new modification of the supporting cannula) maintained its viability over a period of about three hours. In this respect a clear effect of the duration on the viability of the system was observed in the presence of non-metabolized sugars like galactose and 3-O-methylglucose but not with the metabolized sugar (glucose).

Using sodium citrate as a source of metabolic energy slightly improved the level of short-circuit current in the presence or absence of galactose.

The anaerobic conditions were more depressant to the short-circuit current induced by galactose than to that induced by glucose.

13. In the presence and absence of sugar, the short-circuit current, once it was established, remained stable throughout the experiment.

14. Using the silver/silver chloride electrode system the short-circuit current induced by 3-O-methylglucose concentrations were found to be approximately equal to the net Na^+ fluxes from the mucosa to the

serosa.

15. Approximately 1 : 1 stoichiometric relationship between Na^+ (ionic-current) and 3-O-methylglucose influx was observed with the sugar concentrations ranging from about 21 to 35 mM.

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