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2-Deoxyglucose Transport by Intestinal Epithelial Cells Isolated from the Chick

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Summary. Characteristics of 2-deoxyglucose uptake (2DG) by intestinal epithelial cells isolated from chickens were evaluated as a means of discriminating between the concentrative transport system for monosaccharides, associated with the mucosal brush border, and other possible routes of monosaccharide entry. 2DG was chosen as it is not a substrate for the mucosal transport system. The deoxysugar enters via a saturable pathway which is not Na^+-dependent, is not inhibited by K^+, does not accumulate solute against a concentration gradient; exhibits a high sensitivity to inhibition by phloretin; is relatively insensitive to phlorizin inhibition; and has low affinity [but high capacity relative to Na^+-dependent mucosal transport of 3-O-methylglucose (3-OMG) and other monosaccharides]. These characteristics confirm those established in an earlier report for Na^+-independent uptake of 3-OMG. Complications encountered in the use of 2DG as a test sugar include significant rates of metabolic conversion to an anionic form which presumably is a phosphorylated species. Methods for distinguishing between transport and subsequent metabolism are described. Inhibition of 2DG entry by several other sugars is described and inhibitory constants (K's) given for each.

Recent work from our laboratory has indicated that chick intestinal epithelial cells possess two distinct transport systems for 3-OMG (Kimmich & Randles, 1975). One of these transport systems was characterized as a Na^+-independent event which exhibits a marked sensitivity toward phloretin, in contrast to the well-defined brush border system which exhibits Na^+-dependence, and little or no susceptibility to phloretin. The two systems differed in several other particulars including sensitivity toward phlorizin, interaction with K^+; kinetics; substrate specificity; and ability to establish concentration gradients.

Characterizing the Na^+-independent system using 3-OMG as substrate represents an awkward situation in light of the fact that substrate can also be transferred by the Na^+-dependent system (Csaky & Thale, 1960; Goldner, Schultz & Curran, 1969*b*). One wonders if all or many of the observed differences might be related to a single transport system showing

different behavior in the absence of Na^+ than it exhibits when Na^+ is present. For this reason, it seemed desirable to characterize the newly described system using a substrate which does not interact with the Na^+-dependent carrier, so that experiments could be performed under more physiological conditions (presence of Na^+) comparable to those used to define Na^+-dependent transport.

Our earlier work indicated that 2-deoxyglucose interferes with Na^+-independent uptake of 3-OMG more than any other monosaccharide tested (Kimmich & Randles, 1975). Moreover, 2-deoxyglucose is not a substrate for the Na^+-dependent carrier (Crane, 1960; Goldner, Hojjar & Curran, 1969*a*; Kimmich & Randles, 1975). It therefore seemed likely that characteristics of the Na^+-independent carrier system might be determined rather specifically with 2-deoxyglucose as substrate, regardless of the ionic composition of the incubation medium. The following work was undertaken with this aim in mind, and describes the nature of 2-deoxyglucose uptake by isolated intestinal epithelial cells.

Materials and Methods

Intestinal epithelial cells were prepared from 6 to 8-week-old chicks by methods described in detail previously (Kimmich, 1970*a*, 1975). In each experiment cells were separated from the suspending medium using the centrifugation technique reported in earlier studies (Kimmich, 1975; Kimmich & Randles, 1975). The usual isolation and incubation media consisted of (in mM) 80 NaCl, 100 mannitol, 20 Tris-Cl (pH 7.4), 3 K_2HPO_4, 1 $MgCl_2$, 1 $CaCl_2$ and 1 mg/ml BSA. In those cases where Na^+-free medium was employed, 140 mM mannitol, or 80 mM Tris-Cl replaced the NaCl. Total osmolarity was 300 mosmoles in each case. Except for the cases noted in the text, 2-deoxyglucose was employed at a specivity of 0.2 μC/μmole. ^{14}C-2-deoxyglucose was purchased from Amersham-Searle. Incubation temperature was 37 °C in each case.

In each experiment in which metabolism of 2-deoxyglucose was assessed, a 100-μl sample of a 3% perchloric acid extract derived from cells which had accumulated the labeled deoxysugar was placed on a 4-cm column of BIO · RAD AG 1-X4 resin held in small Pasteur pipet. The column was eluted with 2.0 ml of water and the effluent collected. A second sample of effluent was collected while the column was eluted with 2 ml of 1 N HCl. Both samples were evaporated to dryness by heating to 150° in a drying oven. The dried samples were solubilized in 1 ml of toluene-diluted tissue solubilizer (Beckman Biosolve BBS-3 : toluene in a 1 : 2 ratio), and taken to a final volume of 10 ml with toluene containing the scintillation fluors (POPOP and PPO). A second aliquot of cell extract was solubilized and counted directly in order to allow calculation of recovery of isotope from the ion-exchange columns.

Results

The influx of 1 mM ^{14}C-2-deoxyglucose into isolated chick intestinal cells is shown in Fig. 1, for cells prepared and incubated in the absence of

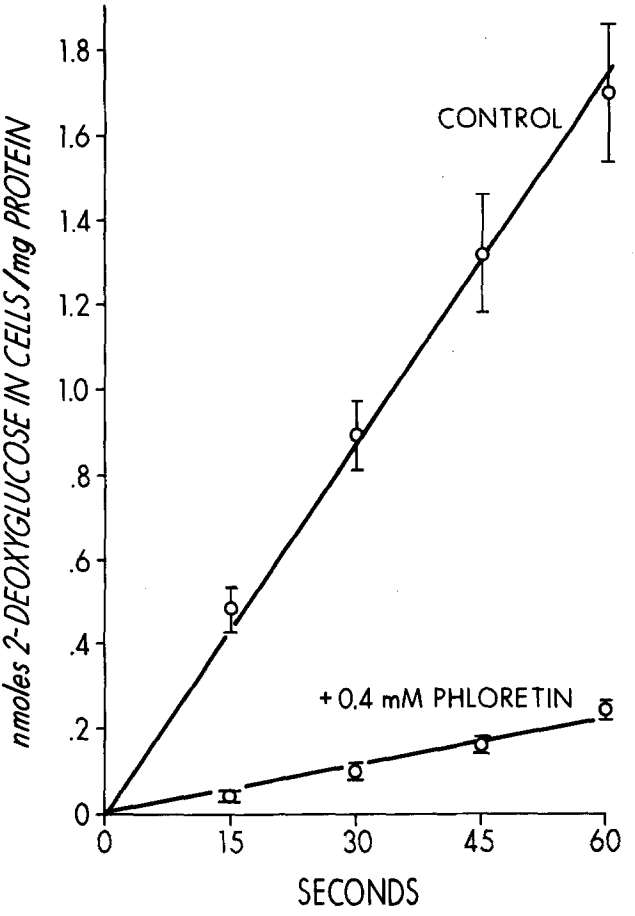

Fig. 1. Unidirectional influx of 1 mM 2DG into isolated chick intestinal epithelial cells, and the effect of 0.4 mM phloretin. Mean and standard error of 5 separate determinations are shown for each time point

Na^+. The observed mean rate is 1.7 nmoles/min × mg protein or about 2.5 times more rapid than the influx of 3-OMG studied under comparable conditions (Kimmich & Randles, 1975). Flux is linear during the one-minute interval of study, indicating that a true unidirectional flux was measured rather than a net flux complicated by significant backflux. In the presence of 0.4 mM phloretin, the influx is 85% inhibited, to a value of 0.245 nmoles/min × mg protein. This value does not differ significantly from that determined for 3-OMG in the presence of maximally effective concentrations of phloretin, and has been attributed to a non-carrier mediated entry route for the sugar (Kimmich & Randles, 1975).

When Na^+ is included in the incubation medium, there is a modest increase (approx. 25%) in the influx rate of 2-deoxyglucose entry, in sharp

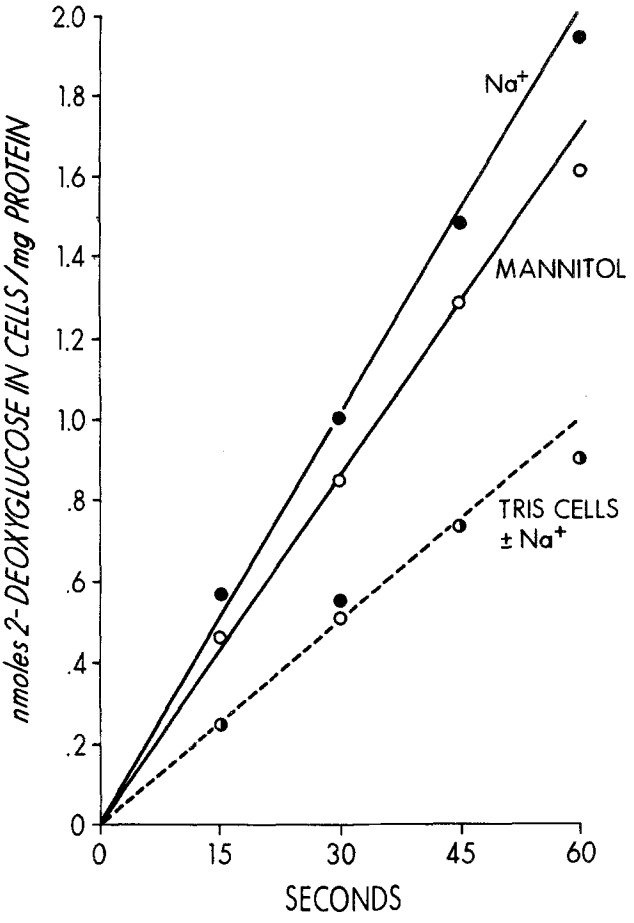

Fig. 2. Effect of Na^+ on unidirectional influx of 1 mM 2DG into isolated chick intestinal epithelial cells. Upper curves show effect of replacement of mannitol by equimolar NaCl. Lower dashed line shows replacement of Tris-Cl by equiosmolar NaCl. Solid data points represent values determined in presence of Na^+, in each case

Table 1. Comparison of rates of 2-deoxyglucose uptake in the presence of Na^+ or K^+ as the primary cation in the incubation medium[a]

Time (sec)	nmoles 2-deoxyglucose taken up/mg protein	
	125 mM Na^+	125 mM K^+
15	0.54	0.55
30	1.06	1.09
45	1.43	1.36
60	1.74	1.72

[a] For these experiments, mannitol was absent from the incubation medium and NaCl or KCl was used at a concentration of 125 mM in order to maintain the same osmolarity as in the usual incubation medium.

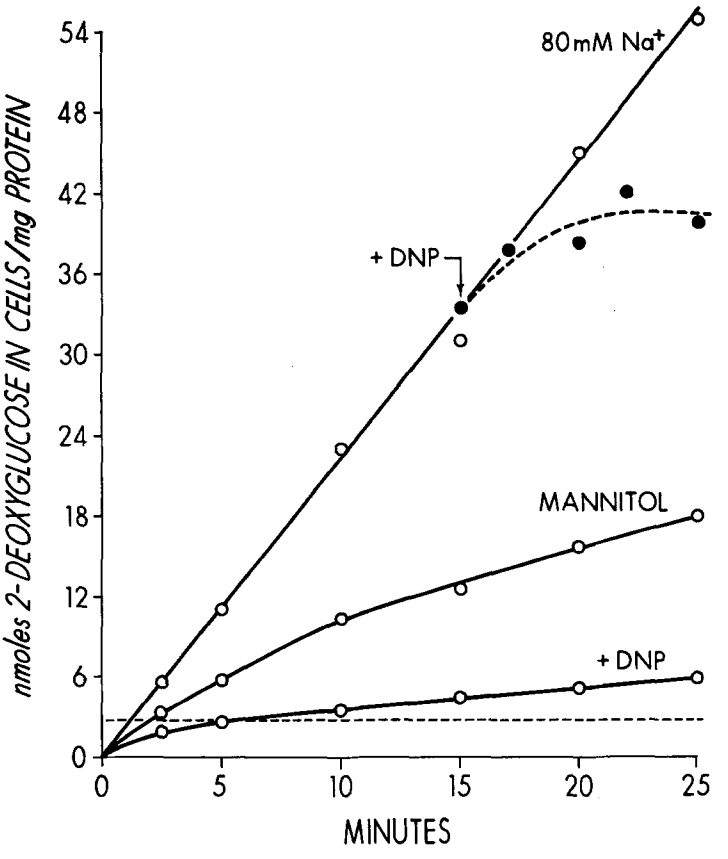

Fig. 3. Effect of Na^+ or DNP on long-term uptake of 1 mM 2DG by isolated intestinal epithelial cells. Data points for the upper 2 curves are the means of 3 separate experiments. Solid data points show effect of DNP (200 μM) added to the control experiment after 15 min uptake had been allowed. The horizontal dashed line represents the degree of uptake expected for simple equilibration of 1 mM 2DG between cellular and extracellular water

contrast to the 10-fold increase in 3-OMG entry induced by Na^+. These data are shown in Fig. 2. If Tris-Cl rather than mannitol is used as a replacement for NaCl, there is no enhancement in the rate of uptake of 2-deoxyglucose when the $Tris^+$ is replaced by Na^+ (Fig. 2), although the rate of uptake is somewhat slower than for cells prepared in the presence of mannitol. It seems likely that the apparent Na^+-dependence shown in Fig. 2, in reality results from different characteristics of the cell membrane when compared in media differing in ionic strength, rather than reflecting a true Na^+-dependence for the 2-deoxyglucose carrier. This idea can be confirmed by comparing the rate of 2-deoxyglucose uptake by cells prepared in media in which equimolar KCl replaces NaCl, to those in which NaCl is used throughout the preparation (Table 1). In this case,

Table 2. Chromatographic separation of unionized and anionic forms of 2-deoxyglucose in extracts of intestinal cells incubated with 1 mM ^{14}C-2-deoxyglucose[a]

nmoles ^{14}C-material extracted/mg protein	nmoles solute eluted/mg protein			percent recovery
	H_2O eluate	HCl eluate	Total eluate	
		Control		
19.6	9.1	7.7	16.8	85.8
19.4	8.4	7.8	16.2	83.5
23.2	11.3	9.2	20.5	88.3
Avg. 20.7	9.6	8.2	17.8	86.0
		+DNP		
4.3	2.8	1.2	4.0	92.0
3.6	2.4	1.0	3.4	94.5
3.6	2.4	1.1	3.5	97.2
Avg. 3.8	2.5	1.1	3.6	94.7

[a] The cells were incubated at 37 °C with ^{14}C-2-deoxyglucose for an interval of 20 min prior to extraction with perchloric acid.

absolutely no dependence on Na^+ is detected for the deoxyglucose entry and the rate matches that observed in the presence of mannitol. Apparently, the low rate of entry when Tris is the primary cation reflects a poorly reversible inhibitory effect of $Tris^+$, which is not mimicked by K^+. In light of the severely inhibitory effect of K^+ on Na^+-dependent entry of 3-OMG (Basackova & Crane, 1965; Kimmich, 1970*b*; Kimmich & Randles, 1973), these data further indicate that the two sugars enter via systems which exhibit differing characteristics.

If 2-deoxyglucose uptake is monitored for more extended time intervals, the data shown in Fig. 3 is obtained. A prolonged interval of net uptake is typically observed which is nearly linear for periods as long as 25 min when the incubation is performed in the presence of Na^+. This should be contrasted with 3-OMG uptake, which comes to a steady state within approximately 10 min (Kimmich, 1970*b*). In the absence of Na^+, 2-deoxyglucose uptake also tends toward a steady state within the period of observation, at a cellular concentration well below that observed when Na^+ is present (Fig. 3). Despite the apparent concentration gradient of 2-deoxyglucose maintained in each case ($\pm Na^+$) the sugar is not present in the cells in a readily diffusible form. This can be demonstrated by adding a metabolic inhibitor after a period of uptake has been allowed. Further uptake of deoxyglucose is then prevented, but in the succeeding 10-min interval the cells do not lose any of the previously accumulated sugar, as

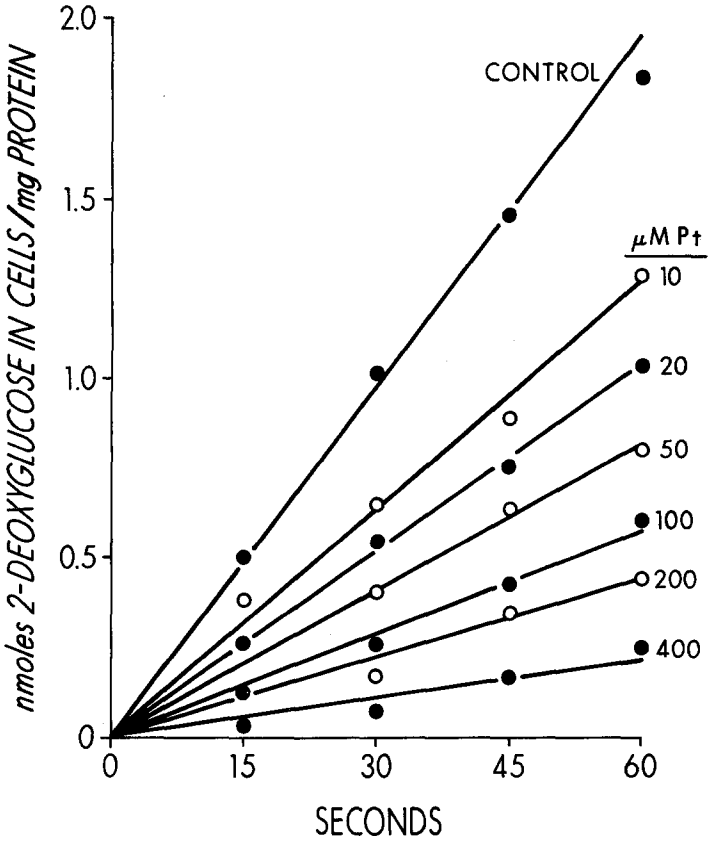

Fig. 4. Effect of various phloretin concentrations on unidirectional influx of 1 mM 2DG into isolated chick intestinal epithelial cells

would occur if the sugar were present in free form at a concentration higher than that provided in the medium. Nonmetabolized 3-OMG is readily lost when DNP is added to the cells after uptake has occurred, until a distribution ratio of unity is reached and maintained (Kimmich, 1970*a*, *b*). When DNP is present from the beginning of the experiment 2-deoxyglucose concentration in the cell also slightly exceeds the extracellular concentration. Apparently, in the absence of a metabolic inhibitor, a larger portion of the 2-deoxyglucose taken up is present in a different form, most likely due to an ATP-dependent phosphorylation as has been demonstrated in other cell types (Letnansky, 1964; Elsas & MacDonnell, 1972; Kleinzeller & McAvoy, 1973). The anionic nature of this new species of deoxyglucose can be readily demonstrated, if the cell extracts are treated with an ion exchange resin in the cationic form. As shown in Table 2, extracts from cells incubated with inhibitor, have only 15% as much 2DG in a form

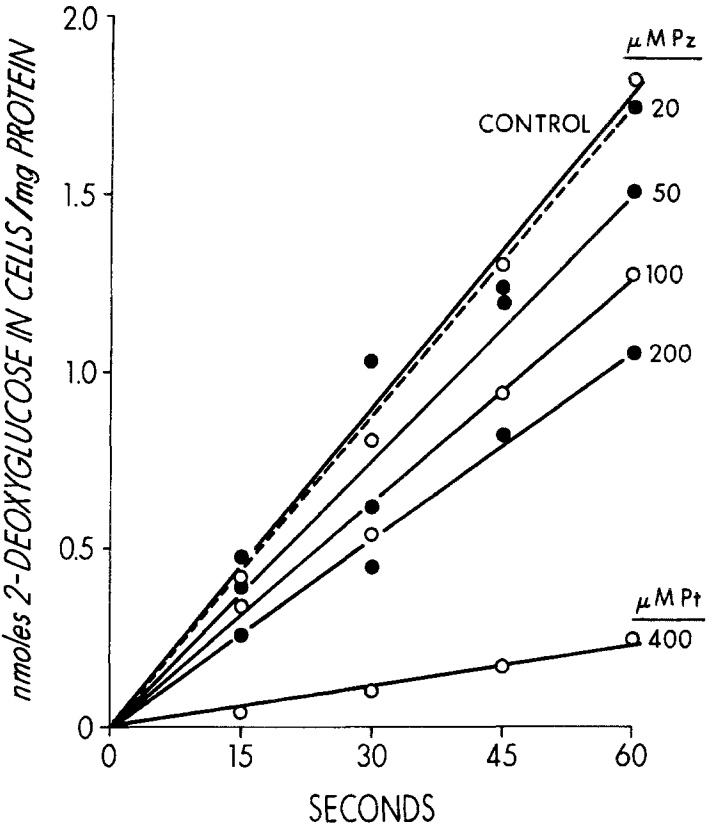

Fig. 5. Effect of various phlorizin concentrations on unidirectional influx of 1 mM 2DG into isolated chick intestinal epithelial cells, as compared to the effect of 400 μM phloretin

which is eluted with HCl as cells incubated without inhibitor. Of the total amount of 2DG associated with the two types of cells, a higher percentage elutes with HCl for the control cells (46%) than for the inhibited (30%). Furthermore the nonionic form of 2DG (H_2O eluate) present in the inhibited cells (2.5 nmoles/min × mg protein) is in good agreement with the amount expected if the free monosaccharide is equilibrating between cellular and extracellular water.

If 2-deoxyglucose entry is mediated via the same carrier responsible for Na^+-independent uptake of 3-OMG, influx of the deoxysugar should exhibit much greater sensitivity toward phloretin than to the corresponding glycoside, phlorizin. Figs. 4 and 5 illustrate that this is exactly what is observed. Twenty μM phloretin exerts a half maximal inhibition of 2-deoxyglucose influx, but 20 μM phlorizin has no significant effect. More than 200 μM phlorizin is required to produce an inhibitory effect equal to

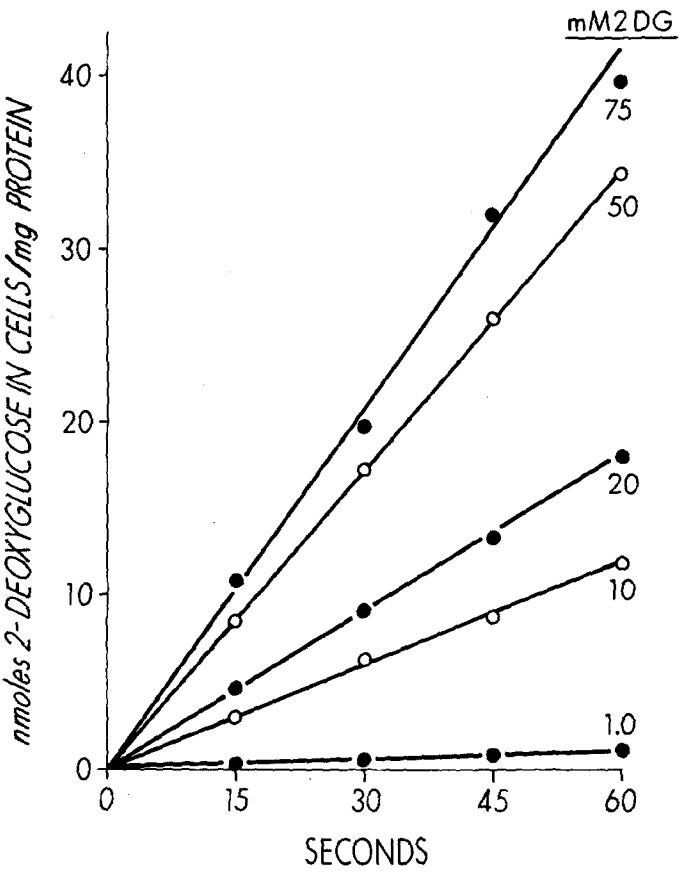

Fig. 6. Unidirectional influx of various concentrations of 2DG into isolated intestinal epithelial cells

that induced by 20 μM phloretin. Concentrations of phloretin greater than 400 μM produce no greater effect than that shown for 400 μM, and the rate obtained under these conditions is the same as the rate observed for 3-OMG with an optimally effective phloretin concentration (200 μM) (Kimmich & Randles, 1975).

Fig. 6 illustrates the results obtained from a kinetic study of 2-deoxyglucose influx. If phloretin-sensitive fluxes for each different substrate concentration are used to construct an Eadie-Hofstee plot, a linear relationship is obtained as shown in Fig. 7. Kinetic values derived graphically indicate a K_T of 27 mM and a V_{max} of 42 nmoles/min × mg protein. The V_{max} is identical to that obtained for 3-OMG in the absence of Na^+, lending credence to the possibility that each sugar utilizes the same carrier under these conditions.

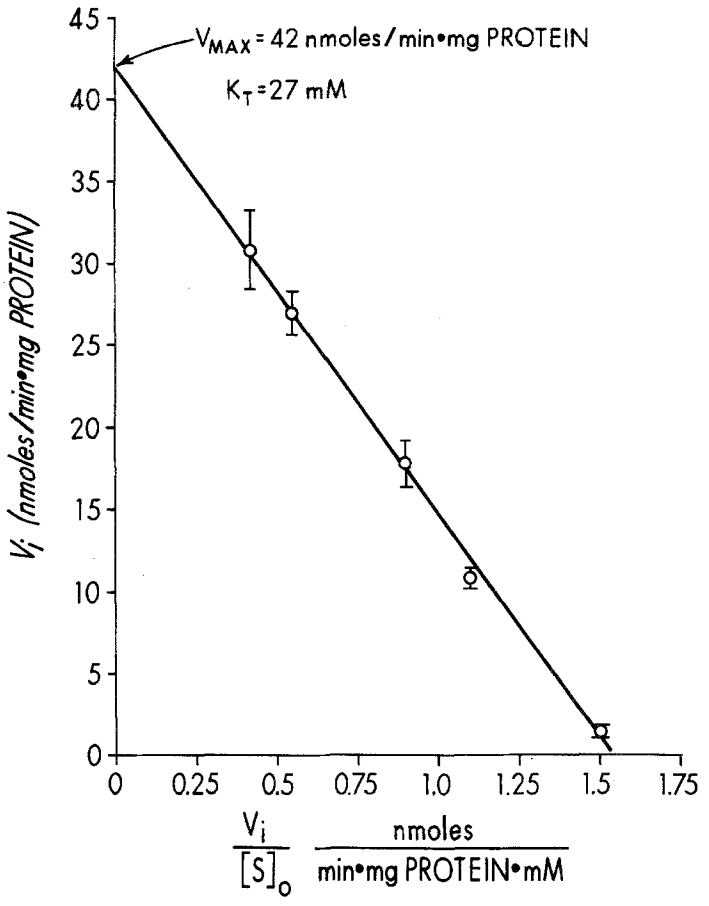

Fig. 7. Eadie-Hofstee plot of uptake of 2DG by isolated intestinal epithelial cells. Rates were corrected for nonphloretin sensitive entry which is believed to represent noncarrier mediated (diffusional) entry. Rates plotted are means ± standard error for 3 separate determinations of influx at various 2DG concentrations as derived from experiments similar to the one shown in Fig. 6

In addition to the differences already mentioned for the Na^+-dependent and Na^+-independent sugar entry systems, they also differ in susceptibility to inhibitors of monovalent ion transport. Ouabain is completely without effect on long-term accumulation of 2-deoxyglucose even after *apparent* concentration gradients of the sugar have been established as shown in Table 3. Active 3-OMG accumulation is nearly completely prevented by this concentration of ouabain as reported earlier (Kimmich, 1970*b*).

Finally, all of the sugars which were shown to inhibit the Na^+-independent uptake of 3-OMG (Kimmich & Randles, 1975), likewise interfere with the uptake of 2-deoxyglucose. Certain sugars inhibit the uptake of

Table 3. Effect of Quabain on Uptake of 2-Deoxyglucose by Isolated Intestinal Epithelial Cells, as Compared to the Effect of Phloretin[a]

Time (min)	nmoles 2-deoxyglucose taken up/mg protein		
	Control	+Ouabain	+Phloretin
2.5	2.3	2.3	0.49
5	3.7	3.5	0.89
10	5.7	5.4	1.68
15	7.7	7.0	2.72
20	8.7	9.1	3.36
25	11.4	10.3	4.19

[a] Ouabain and phloretin were employed at a concentration of 0.25 and 0.20 mM, respectively.

Table 4. Effect of various monosaccharides on initial rate of influx of 2-deoxyglucose into isolated intestinal epithelial cells

Test sugar[a]	n	2 DG influx rate[b] nmoles/min · mg protein	% Control	% inh. P_t sens. rate	Calculated[c] K_I (mM)
None	6	1.70	100	0	
Ribose	3	1.66	98	3	∞[d]
Fucose	3	1.65	97	4	∞[d]
Fructose	3	1.47	86	16	185.0
Xylose	3	1.38	81	23	133.0
Mannose	3	1.14	67	40	64.8
Galactose	3	1.19	70	36	74.4
Glucose	3	0.9	53	56	48.3
3-OMG	2	1.21	71	35	78.7
None +0.4 mM P_i	6	0.28	17	100	–

[a] Each test sugar was employed at a concentration of 50 mM.

[b] Rates given are means for number of determinations indicated. 2-deoxyglucose concentration = 1 mM.

[c] K_I's calculated assuming purely competitive inhibition kinetics between the test sugar and 2-deoxyglucose.

$$K_i = \frac{K_T[I]\, v_i}{V_m[S] - v_i[K_T + S]}.$$

[d] Influx rates of 2-deoxyglucose in the presence of 50 mM ribose or fucose is not statistically different from the control, so these 2 sugars are presumed not to interact with the 2-deoxyglucose carrier.

neither test sugar. The order of effectiveness for seven different sugars is glucose > galactose = mannose > xylose > fructose > fucose = ribose as shown in Table 4. This is the same sequence as established earlier with 3-OMG as the test sugar. Assuming that the inhibition of 2-deoxyglucose uptake by other monosaccharides represents competitive inhibition of a

common carrier, it is possible to calculate the K_I for each sugar making use of the relationship,

$$K_I = \frac{K_T[I]\, v_I}{V_M[S] - v_I(K_T + [S])}$$

which can be readily derived from the equation for initial velocity of substrate uptake in the presence of a competitive inhibitor. The kinetic parameters K_T and V_M refer to the Michaelis constant and maximal velocity for 2-deoxyglucose entry; v_I refers to the initial velocity of 2-DG entry in the presence of a given concentration of an inhibitory sugar $[I]$, and $[S]$ is the 2-deoxyglucose concentration. A K_I for each sugar is given in Table 3. The value calculated for 3-OMG is 79 mM which is in excellent agreement with the K_T determined in earlier work by direct measurement (Kimmich & Randles, 1975). Even glucose apparently does not have as high an affinity for the carrier as 2-deoxyglucose. The K_I for glucose (48 mM) is nearly twice the K_T obtained for 2-deoxyglucose. Fucose and ribose probably do not interact with the Na^+-independent carrier or do so in a very limited fashion ($K_I > 500$ mM).

Discussion

The data presented above show clearly that a transport system for monosaccharides, distinct from the Na^+-dependent system associated with the mucosal brush border can be demonstrated for isolated chick intestinal epithelial cells with the use of 2-deoxyglucose as substrate. Features which distinguish the new system from the well-known Na^+-dependent system can be listed as follows:

1) lack of Na^+-dependence
2) lack of inhibition by elevated K^+ concentrations
3) no active uptake of free sugar
4) relatively high sensitivity to phloretin
5) relatively low sensitivity to phlorizin
6) lack of inhibition by ouabain or other inhibitors of cellular Na^+ extrusion
7) substrate specificity which includes 2-deoxyglucose, mannose, and perhaps fructose, but does not include L-fucose.
8) kinetic characteristics which indicate relatively poor affinity (high K_T), but high capacity (high V_{max}).

The use of deoxyglucose as substrate for characterizing a transport system poses a number of troublesome problems. Most of these difficulties

originate as a result of metabolism of the sugar which the intestinal epithelial cells can accomplish in common with a number of other cell types as reported by other investigators (Letnansky, 1964; Elsas & MacDonnell, 1972; Kleinzeller & McAvoy, 1973). Because of conversion of free 2-deoxyglucose to 2-deoxyglucose-6-phosphate by hexokinase (Sols & Crane, 1954; McComb & Yuskok, 1959) conclusions regarding a lack of energy dependence for the carrier are not entirely straightforward. Obviously, DNP and other metabolic inhibitors cause a pronounced effect on total uptake of 2-deoxyglucose by the cells as shown in Fig. 3. The observed inhibition might simply be due to a decreased degree of phosphorylation induced by a sharp decrease in the cellular ATP pool when the inhibitor is present. This possibility is reinforced by the fact that an anionic form of the labeled sugar can be detected in extracts prepared from cells which had been preincubated with ^{14}C-2-deoxyglucose. In fact, as much as half of the total radioactive sugar present in the control extracts is retained by passage through a column, containing resin in a cationic form. Cells incubated with DNP have less than 15% of much of the anionic sugar species which is consistent with earlier observations that more than 85% of their ATP pool is depleted under these conditions (Kimmich, 1975). Recovery of the total radioactivity applied to the column is greater than 90% in each case.

Lack of accumulation of free sugar against a concentration gradient is indicated by the fact that no efflux of labeled sugar occurs from cells which have been incubated with 2-deoxyglucose for an interval of time, and then suddenly exposed to DNP. Net uptake of sugar ceases subsequent to addition of the inhibitor, but no loss of label occurs as would be expected if the concentration gradient of solute represented free nonphosphorylated sugar. Rapid efflux of a nonmetabolized sugar which has been actively accumulated by the Na^+-dependent carrier has been demonstrated under similar conditions (Kimmich, 1970*b*).

Finally, lack of energy dependence for sugar uptake by the Na^+-independent carrier is indicated by the fact that unidirectional influx of 2-deoxyglucose is unpertubed by various inhibitors of energy transduction such as uncouplers. Apparently the prolonged intervals of linear sugar uptake observed under control conditions represents transport followed by rapid phosphorylation of cellular 2-deoxyglucose, and maintenance of free intracellular sugar at a low concentration. When phosphorylation is minimized or prevented by inclusion of an inhibitor, sugar penetration continues at a similar rate for a short interval, but linearity is lost more quickly (Fig. 3), as backflux of free sugar becomes significant. The sequence

of events for 2-deoxyglucose uptake can be represented as follows:

$$2DG_0 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 2DG_i \xrightarrow[\text{Hexokinase}]{\text{ATP}} 2DG-6P+ADP$$

in which phosphorylation effectively puts the sugar in a form which is unavailable for supporting backflux through the carrier-mediated transport system (*i.e.*, $k_2[DG]_i \ll k_1[DG]_0$). This model represents a modification of one originally proposed by Kleinzeller and McAvoy (1973) in which phosphorylated 2DG was envisioned as being in equilibrium with the intracellular pool of free sugar. Our data more nearly conforms to that described by Elsas and MacDonnell (1972) for hamster jejunum, in which 2DG-6-P does not readily serve as a source of free 2DG. Apparent sensitivity of the transport system to metabolic inhibitors, in reality, simply reflects inhibition of phosphorylation, an increase in $[2DG]_i$ and elevated rates of backflux of free sugar. The total cellular pool of solute in all forms can be limited by excluding metabolic conversion. At present, we have no adequate explanation for the fact that more 2DG is present in the nonionic fraction (H_2O eluate) of cells incubated without metabolic inhibitor (9.6 nmoles/mg protein) than would be expected for an equilibrium distribution of free monosaccharide between extracellular and cellular water ($\sim$2.5 nmoles/mg protein). The discrepancy might be explained if a portion of the phosphorylated sugar is hydrolyzed during preparation of the cellular extracts as has been shown to be true for procedures involving treatment with $ZnSO_4$ and $Ba(OH)_2$ (Kleinzeller & McAvoy, 1973) although this possibility was not pursued. We do not feel the difference represents active accumulation of free sugar or uncoupler would be expected to induce a release of radioactive solute as pointed out earlier.

Another confusing aspect related to the use of 2-deoxyglucose as a test substrate involves the significant difference in degree of uptake of 2DG in the presence *vs.* the absence of Na^+. That the observed difference does not represent an effect of Na^+ directly on the sugar carrier is indicated by the fact that no significant differences in unidirectional influx of 2DG are noted $\pm Na^+$ as shown in Fig. 2 and Table 1. Longer term effects of Na^+ might reflect differences in rate of phosphorylation of free sugar by hexokinase, although again no attempt was made to evaluate this parameter.

The remaining characteristics for 2-deoxyglucose uptake which are listed above can be readily defended from the data presented and require little further comment. Collectively they confirm conclusions derived from studies of 3-OMG transport in the absence of Na^+ and thus reinforce earlier conclusions regarding the existence of two separate transport

systems for monosaccharides in intestinal epithelial cells derived from the chicken. Because 2-deoxyglucose does not interact with the Na^+-dependent carrier, its entry in the presence of Na^+ specifically reflects the activity of the non-Na^+-dependent pathway, unlike 3-OMG which can interact with both systems. Furthermore, 2-deoxyglucose has a higher affinity than 3-OMG for the non-Na^+-dependent pathway, so that the rate of entry at low substrate concentrations is more than twice as fast for the deoxysugar. Maximal capacity for the non-Na^+-dependent pathway is apparently the same no matter which sugar is transferred.

While our data does not identify the cellular locus for the Na^+-independent sugar transport pathway, we continue to favor the liklihood of a serosal surface localization in light of work from Bihler's laboratory (Bihler & Cybulsky, 1973) that a Na^+-independent system with specificity for 2-deoxyglucose can be demonstrated in rat intestinal cells which have had their mucosal transport system inhibited with mercury prior to isolation of the cells. Also, Hopfer and Sigrist-Nelson (1975) have recently reported a sugar carrier associated with vesicles prepared from the serosal surface of intestinal epithelial cells which exhibits relatively high phloretin and low phlorizin sensitivity, similar to the characteristics for the 2DG-Na^+-independent carrier described here. A serosal localization for the phloretin sensitive carrier would allow it to function physiologically to deliver solute concentrated in the cell, by activity of the mucosal Na-dependent carrier, to the circulatory system.

At this stage of development, it appears likely that monosaccharide transport systems localized in the baso-lateral membranes of epithelial cells of small intestine and proximal kidney tubule will prove to be fundamentally similar in character. As early as 1970, Silverman, Aganon and Chinard, using a renal perfusion technique, demonstrated that sugar transfer at the serosal surface of kidney epithelium possessed a different substrate specificity and sensitivity to phlorizin and phloretin than transport at the brush border surface. More recently, Kleinzeller and McAvoy (1973) showed that sugar transport at the serosal surface of flounder kidney tubule is not inhibited by ouabain and exhibited only weak sensitivity to phlorizin. Phloretin and elevated K^+ concentrations were not tested. Finally, the experiments of Kinne, Murer, Kinne-Saffran, Thees and Sachs (1975) using vesicles prepared from either brush border or baso-lateral membranes of kidney epithelium, have indicated a baso-lateral transport system showing preference for D-glucose over L-glucose, and only moderate dependence on Na^+ or inhibition by phlorizin. Similar vesicles prepared from baso-lateral membrane of intestinal tissue indicate

a high sensitivity toward phloretin, as mentioned earlier. While experiments with vesicles may be clouded to a certain extent by varying degrees of contamination with membrane material from the other pole of the cell, it seems likely that monosaccharide transport systems with the characteristics listed at the outset of the discussion will eventually be firmly established for baso-lateral membrane of both kidney and intestinal epithelium. Na^+-dependent concentrative transport, and Na^+-independent passive transport systems are apparently associated with the same cell type in intestine because selective inhibition of the Na^+-independent system with phloretin, allows intact isolated epithelial cells to establish twofold greater concentration gradients than can be achieved in the absence of phloretin (Kimmich & Randles, 1975). Similar experiments performed with isolated kidney tubule cells would be of interest.

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References

Bihler, I., Cybulsky, R. 1973. Sugar transport at the basal and lateral aspect of the small intestinal cell. *Biochim. Biophys. Acta* **298**:429

Bosachova, J., Crane, R. K. 1965. Studies on the mechanism of intestinal absorption of sugars. VIII. Cation inhibition of active sugar transport and $^{22}Na^+$ influx into hamster small intestine, *in vitro*. *Biochim. Biophys. Acta* **102**:423

Crane, R. K. 1960. Intestinal absorption of sugars. *Physiol. Rev.* **40**:789

Csaky, T. Z., Thale, M. 1960. Effect of ionic environment on intestinal sugar transport. *J. Physiol.* **151**:59

Elsas, L. J., McDonnell, R. C. 1972. Hexose transport and phosphorylation by hamster kidney cortex slices and everted jejunal rings. *Biochim. Biophys. Acta* **255**:948

Goldner, A. M., Hajjar, J. J., Curran, P. F. 1969*a*. 2-Deoxyglucose transfer in rabbit intestine. *Biochim. Biophys. Acta* **173**:572

Goldner, A. M., Schultz, S. G., Curran, P. F. 1969*b*. Sodium and sugar fluxes across the mucosal border of rabbit ileum. *J. Gen. Physiol.* **53**:362

Hopfer, U., Sigrist-Nelson, K., Murer, H. 1975. Intestinal sugar transport—studies with isolated plasma membranes. *In:* Carriers and Channels in Biological Systems, A. Shamoo, Editor. *Proc. N. Y. Acad. Sci.* (*In press*)

Kimmich, G. A. 1970*a*. Preparation and properties of mucosal epithelial cells isolated from small intestine of the chicken. *Biochemistry* **9**:3659

Kimmich, G. A. 1970*b*. Active sugar accumulation by isolated intestinal epithelial cells. A new model for sodium-dependent metabolite transport. *Biochemistry* **9**:3669

Kimmich, G. A. 1975. Preparation and characterization of isolated intestinal epithelial cells and their use in studying intestinal transport. *In:* Methods in Membrane Biology; IV, E. D. Korn, editor. Plenum Press, New York (*in press*)

Kimmich, G. A., Randles, J. 1973. Effect of K^+ and K^+ gradients on accumulation of sugars by isolated intestinal epithelial cells. *J. Membrane Biol.* **12**:23

Kimmich, G. A., Randles, J. 1975. A Na^+-independent, phloretin-sensitive monosaccharide transport system in isolated intestinal epithelial cells. *J. Membrane Biol.*, **23**:57

Kinne, R., Murer, H., Kinne-Saffran, E., Thees, M., Sachs, G. 1975. Sugar transport by renal plasma membrane vesicles. Characterization of the systems in the brush-border microvilli and basal-lateral plasma membranes. *J. Membrane Biol.* **21**:375

Kleinzeller, A., McAvoy, E. 1973. Sugar transport across the peritubular face of renal cells of the flounder. *J. Gen. Physiol.* **62**:169

Letnansky, 1964. The influence of 2-deoxyglucose on the nucleotide content of Ehrlich ascites carcinoma cells. *Biochim. Biophys. Acta* **87**:1

McComb, R. B., Yuskok, W. D. 1959. Properties of particulate hexokinase of the Krebs-2 ascites tumor. *Biochim. Biophys. Acta* **34**:515

Silverman, M., Aganon, M., Chinard, J. C. 1970. Specificity of monosaccharide transport in dog kidneys. *Am. J. Physiol.*, **218**:743

Sols, A., Crane, R. K. 1954. Substrate specificity of brain hexokinase. *J. Biol. Chem.* **210**:581