3-Methyl Glucose Transport in Isolated Epithelial Cells from Rat Small Intestine (37071)

MOHAMMED M. SAYEED AND ARTHUR E. BAUE
(Introduced by H. T. Blumenthal)

Division of Cell Physiology, Department of Surgery, Washington University School of Medicine and The Jewish Hospital of St. Louis, St. Louis, Missouri 63110

A number of studies have been reported recently on non-electrolyte transport functions of the isolated intestinal epithelial cells (1–6). Although this approach as suggested by Reiser and Christiansen (5) might allow examination of the characteristics of the transport processes in a manner similar to that possible in bacteria and red blood cells, possible alterations in the functional capabilities of cells during isolation and the purity of such isolated cells remain critical factors. In addition, the permeability and transport characteristics might not be uniform across the surface of isolated intestinal cells because of the presence of brush border membranes at one end of the isolated cells. Previous reports have indicated clearly the presence of transport mechanisms which can concentrate amino acids (3–6) and sugars (1–4) in isolated intestinal epithelial cells. Reiser and Christiansen (5) found that isolated cells from rat small intestine had the stereospecificity, energy-dependency, and kinetic properties for leucine transport as occurred in the intact intestine. Kimmich (4), who has studied isolated epithelial cells from the small intestine of chickens, reported that both galactose and valine were accumulated by these cells against a concentration gradient by a process which was inhibited by dinitrophenol, ouabain, and oligomycin. Galactose transport by cells of chicken small intestine was dependent on the presence of Na^+ (4), but not on the direction of Na^+ gradient, whether inward as occurs normally or outward as produced by preloading the isolated cell with Na^+. This observation is inconsistent with the requirement of a sodium gradient for concentrative sugar uptake. Although it has been shown that isolated epithelial cells from mammalian small intestine can transport the nonmetabolizable sugar, 3-methyl glucose, against a concentration gradient (3), the Na^+ requirements for this transport system in mammalian cells have not been studied. We have therefore investigated the effects of replacement of Na^+ by K^+ or choline on 3-methyl glucose uptake in isolated epithelial cells from the rat small intestine.

Methods. Fasted albino rats (200–250 g) were sacrificed by a blow to the head. The small intestine was exposed, cannulated at the duodenal and ileal ends, and quickly flushed with chilled oxygenated Ca^{2+} and Mg^{2+} free medium containing 125 m*M* NaCl, 5 m*M* KCl, 10 m*M* glucose, 5 m*M* phosphate buffer, (pH 7.4), 12.5 m*M* $NaHCO_3$ and 1 m*M* EDTA. The intestine was then removed, everted, and filled with the Ca^{2+} and Mg^{2+} free medium. Everted intestine was divided into 6 equal segments and each segment tied off at both ends. The intestinal segments were placed on the paraffin floor of a plastic container with a lid to which 50 ml of the Ca^{2+} and Mg^{2+} free medium with 25 mg % hyaluronidase (Sigma, type III) had been added. The use of the enzyme hyaluronidase and of a medium free of Ca and Mg ions in the isolation of epithelial cells has been reported by other investigators (1, 7). The containers with everted intestinal segments were oxygenated by a continuous stream of 5% CO_2 in O_2 through the container which was shaken in a water bath at room temperature. After 5 min the medium was poured off and examined under a phase microscope. This material was later discarded. Twenty milliliters of fresh Ca^{2+} and Mg^{2+} free medium

with hyaluronidase was then added to the intestinal segments in the plastic containers and shaking was continued for 20–25 min. The medium was then collected into centrifuge tubes and spun at 200 rpm in a refrigerated International Centrifuge (Model PR-2). The resulting pellet was washed twice in a Ca^{2+} and Mg^{2+} free medium without hyaluronidase at 500 rpm. The final pellet (60–100 μl of packed cellular material) was resuspended in 1 ml oxygenated Krebs–Ringer bicarbonate (KRB) (8).

For the measurement of 3-methyl glucose (3MG) uptake, 1 ml of cell pellet suspension was added to an equal volume of 3MG in KRB (final concentration 5 m*M*). The mixture was oxygenated (95% O_2 + 5% CO_2) and incubated at 37° in a Blue M shaking water bath for 20 min. At the end of incubation, the reaction mixture was transferred quantitatively into cytocrit tubes and the cellular material was packed in these tubes by centrifugation at 500 rpm. The volumes of packed cells and supernatant were read directly from the cytocrit tubes. Packed cells were resuspended in distilled water to a volume of 0.5 ml. 3MG was analyzed spectrophotometrically in the supernatant and the suspension prepared from packed cells by the Nelson (9) and Somogyi (10) methods. The "mannitol space" in the packed cell pellet was determined in some experiments by incubating isolated cell suspension with 3 m*M* mannitol in a KRB medium under conditions similar to those employed in the 3MG uptake experiments. The mannitol solution contained trace quantities of [^{14}C]-mannitol (about 10,000 counts/min/ml). At the end of incubation the cellular material was packed and pellet volume determined in the cytocrit tube. Aliquots (0.1 ml) of cell pellet suspensions were placed in vials with 0.4 ml NCS solubilizer (Amersham/Searle Corp.) incubated at 37° overnight and after adding 10 ml Bray's solution (11) radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. One-tenth milliliter aliquots of supernatant and 3 m*M* mannitol solution with the trace amount of radioactive mannitol were added to 10 ml Bray's solution in separate vials and radioactivity measured to determine concentration of mannitol in the medium and the cellular material at the end of incubation. The ratio, μmole mannitol per milliliter pellet/μmole mannitol per milliliter supernatant was % mannitol space in the pellet. The mannitol space in the packed cell pellet was assumed to represent the extracellular space as mannitol is not absorbed by intestinal mucosal cells (12).

In anaerobic experiments, cellular material isolated by the use of Ca^{2+} and Mg^{2+} free medium with hyaluronidase was resuspended in Krebs–Ringer phosphate (KRP) (8) and gassed with 100% nitrogen for 5 min prior to incubation with 3MG (final concentration 5 m*M*) in KRP. This incubation was carried out under similar conditions as in the control experiment except that the reaction mixture was gassed with 100% nitrogen. The inhibitors, iodoacetamide (IA) or 2,4 dinitrophenol (DNP) when used were added to the KRB medium in which isolated cellular material was resuspended and were present in the incubations with 3MG at a final concentration of 10^{-4} *M*. The effect of replacement of Na ion on 3MG uptake was studied in isolated cellular material which was resuspended in modified Krebs–Ringer solution in which NaCl and $NaHCO_3$ were replaced by equivalent amounts of KCl and $KHCO_3$ or choline-Cl and choline-HCO_3. The conditions of incubation of isolated cells with 3MG in the experiments with inhibitors or when Na^+ was replaced were otherwise the same as in the control experiments.

Results. After everted intestinal segments were incubated at room temperature for 5 min in a Ca^{2+} and Mg^{2+} free medium containing hyaluronidase, the medium when examined under the phase microscope was found to contain both cellular and acellular material. The acellular material was probably mucous. The cells were both large columnar cells with prominent brush borders, singly or in aggregates of 2–4, and individual medium or small size round cells without brush borders. The round cells tended to cluster or dissociate on the microscope slide. When a fresh supply of the Ca^{2+} and Mg^{2+} free hyaluronidase solution was added and the segments were shaken at room temperature

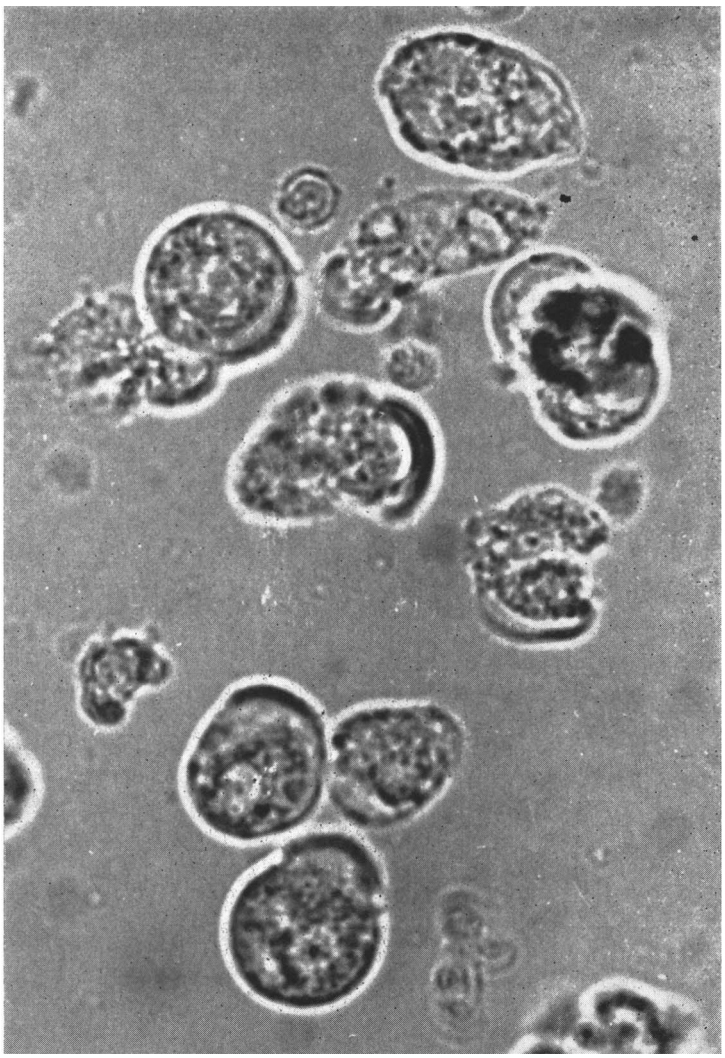

FIG. 1. Isolated epithelial cells from rat small intestine as seen under phase microscope.

for 20–25 min, the round cells without brush borders were present in greatly decreased numbers. The acellular "mucous" was easily washed off by two consecutive centrifugations at 500 rpm in Ca^{2+} and Mg^{2+} free medium without hyaluronidase. This left large epithelial cells which were identified by the presence of brush borders. These cells appeared more spherical in shape than columnar on examination under phase contrast microscope. Aggregates of 2–4 epithelial cells were again seen and represented 10–25% of the population of the cells with brush borders.

The distribution of the nonabsorbable sugar mannitol in the cell pellet after incubation of cells with the sugar is shown in Table I. The cell pellet was consistently found to contain less mannitol than the amount found in an equal volume of supernatant. The difference between total cell pellet volume and the mannitol space was assumed to be the mannitol inaccessible intracellular space and this was found to be approximately 13% of the total cell pellet volume. These determinations yield extra- and intra-cellular spaces of *in vitro* preparations only. The value of 87% extracellular space in our study is roughly comparable to that obtained by

TABLE I. Mannitol Space in Intestinal Epithelial Cell Preparation

micromole mannitol/milliliter			
Animals	Supernatant	Cell pellet	% Mannitol space
1	2.99	2.43	81.27
2	2.99	2.85	95.32
3	3.01	2.81	93.35
4	3.13	3.05	97.44
5	3.07	2.71	88.27
6	3.07	2.51	81.75
7	3.03	2.76	91.09
8	3.07	2.36	76.87
9	2.77	2.65	95.66
10	3.16	2.34	74.05
		Mean	87.51
		±SE	2.67

Stern and Jensen (2) in isolated intestinal epithelial cells and Crane and Mandelstam (13) in the preparation of intestinal villi.

The data on 3MG transport by the isolated epithelial cells is shown in Table II. 3MG was analyzed in the cell pellet at the end of incubation and corrected for amounts found in the extracellular space to give intracellular concentrations. The nonmetabolizable sugar was apparently accumulated by the isolated cells against a concentration gradient. The value for the ratio of cell sugar to medium sugar was approximately 4. This is in agreement with the finding of Huang (3) in isolated epithelial cells from rabbit small intestine. The accumulation of 3MG against a concentration gradient could not be demonstrated in the presence of DNP, IA, with anoxia or with a medium which was free of Na^+.

A net uptake of 3MG by isolated intestinal cells was demonstrable in all experiments and is shown in Fig. 2. The rate of uptake was expressed as μmole of 3MG per ml intracellular volume after 20 min of incubation. Replacement of Na^+ by K^+ or choline reduced 3MG uptake activity to approximately 40% of control. On the other hand under anaerobiosis or in the presence of IA, uptake was decreased to 53% and with DNP it was 65% of control level. The level of inhibition due to Na^+ replacement by K^+ was significantly greater ($p < 0.05$) than inhibition due to anaerobiosis, IA or DNP. Inhibition due to replacement of Na^+ by choline was significantly greater than that due to anaerobiosis and dinitrophenol but not iodoacetamide.

Discussion. Huang (3) has observed a decline in 3MG transport with time at 36° in isolated epithelial cells from rabbit small intestine. He has suggested that this was due to increased efflux of sugar back to the medium as isolated cells unlike intestinal epithelial cells *in situ* had all of the cell surface exposed to the medium and were free of the submucosal and muscular barriers to diffusion. However, the decline in transport from 10 to 20 min incubation was approximately 10% and the concentrative mechanism for sugar was still operative at 40 min though at a reduced rate. In this study an uphill transport of sugar into isolated cells was demonstrated at 20 min at 37°. The ability of isolated epithelial cells to support uphill sugar transport indicates that an increase in efflux of sugar that might occur from isolated epithelial cells would not affect the active sugar absorption. This does not seem unreasonable as surface area of membranes of microvilli in the brush border region, which has been postulated to be the site of active sugar transport (14), in all probability exceeds the area of cell surface without brush border.

The uphill transport of 3MG in isolated intestinal cells was completely inhibited by blocking cellular metabolic energy with anoxia, DNP and IA or by replacement of

TABLE II. 3-Methyl Glucose (3MG) Uptake by Intestinal Epithelial Cells.

Animals	$[3MG]_{Med.}$	$[3MG]_{Cell\ Pellet}$	$[3MG]_{Cell}/[3MG]_{Med.}$
1	5.90[a]	15.81[a]	2.68
2	4.98	11.24	2.26
3	6.00	42.57	7.10
4	6.28	33.78	5.38
5	6.36	34.93	5.49
6	6.20	22.07	3.56
7	5.41	16.82	3.11
8	5.17	8.30	1.61
		Mean	3.89
		±SE	.67

[a] Values have units of millimoles/liter.

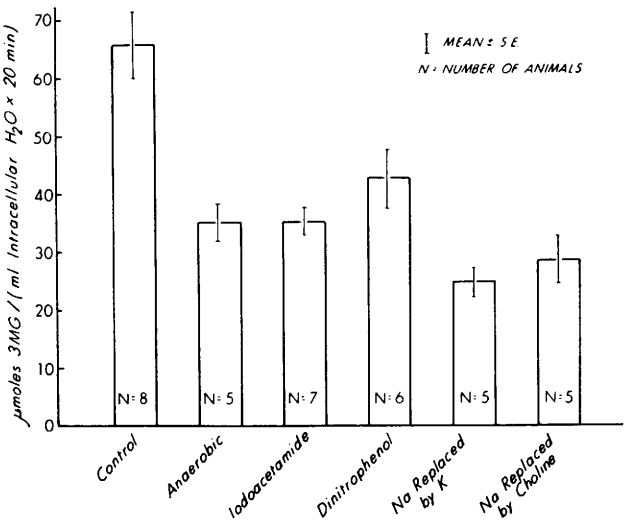

FIG. 2. Uptake of 3-methyl glucose (3MG) by isolated intestinal epithelial cells under various incubation conditions. See text for details of incubation conditions.

Na^+ in the incubation medium. Thus both cellular metabolic energy and Na^+ were required for active sugar transport in isolated epithelial cells. It could not be ascertained whether energy and Na^+ together were required for the formation of an intermediate high energy compound which might be utilized in the active sugar transport, as envisioned in the model of intestinal sugar transport proposed by Kimmich (4), or whether Na^+ was required to establish an inward Na^+ gradient and energy was required for maintenance of the Na^+ gradient by the Na pump as proposed by the "Na gradient" hypothesis (15).

The net influx of 3MG seen at 20 min incubation at 37° (Fig. 2) with the metabolic inhibitors, anoxia or in the absence of Na^+ was probably due to equilibration of sugar between intracellular water and medium by passive diffusion. The passive uptake was slightly lower in experiments where Na^+ was replaced by K^+ or choline than in experiments with DNP or IA. This might suggest that not only active sugar transport but the passive rate of sugar equilibration is also decreased in the absence of sodium ion.

Summary. The transport of nonmetabolizable sugar, 3-methyl glucose (3MG), was studied in the isolated intestinal epithelial cells from rat small intestine. Epithelial cells when incubated in Krebs–Ringer bicarbonate (KRB) medium for 20 min (37°) accumulated 3MG to approximately 4 times its concentration in the medium. Active transport of 3MG into epithelial cells was abolished by: (a) replacement of Na^+ in the KRB with K^+ or choline, (b) addition of 0.1 m*M* dinitrophenol (DNP) or iodoacetamide (IA), or (c) anaerobic conditions. The passive uptake of 3MG into epithelial cells, however, varied under these conditions. The net uptake values as compared to control, approximately, were: 65% with DNP, 53% with IA or anoxia, and 40% in the absence of Na^+. The greater decrease in 3MG uptake in the Na^+ free medium might indicate a facilitation by Na^+ of the passive 3MG transport process.

The technical assistance of Ms. Ann Mitchell is gratefully acknowledged.

This work was supported by grants from U. S. Public Health Service Grant No. HE 12278-04, U. S. Army Contract No. DA DA 17-69-C-9165, and General Research Support Grant No. SO1 RR05491 from NIH.

1. Stern, B. K., Gastroenterology **51**, 855 (1966).

2. Stern, B. K., and Jensen, W. E., Nature (London) **209**, 789 (1966).

3. Huang, K. C., Life Sci. **4**, 1201 (1965).

4. Kimmich, G. A., Biochemistry **9**, 3669 (1970).

5. Reiser, S., and Christiansen, P. A., Biochim. Biophys. Acta **225**, 123 (1971).

6. Reiser, S., and Christiansen, P. A., Proc. Soc. Exp. Biol. Med. **140**, 362 (1972).

7. Perris, A. D., Can. J. Biochem. **44**, 687 (1966).

8. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., "Manometric Techniques," p. 132. Burgess Publishing, Minneapolis (1964).

9. Nelson, N., J. Biol. Chem. **153**, 375 (1944).

10. Somogyi, M., J. Biol. Chem. **160**, 61 (1945).

11. Bray, G. E., Anal. Biochem. **1**, 279 (1960).

12. Hober, R., and Hober, J., J. Cell Comp. Physiol. **10**, 401 (1937).

13. Crane, R. K., and Mandelstam, P., Biochim. Biophys. Acta **45**, 460 (1960).

14. Crane, R. K., Fed. Proc. **21**, 891 (1962).

15. Crane, R. K., Fed. Proc. **24**, 1000 (1965).

Received Sept. 22, 1972. P.S.E.B.M., 1973, Vol. 142.