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A RABBIT JEJUNAL ISOLATED ENTEROCYTE PREPARATION SUITABLE FOR TRANSPORT STUDIES

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SUMMARY

1. A method is described for isolating viable enterocytes from rabbit jejunum. Estimates of sucrase and γ-glutamyl transferase activities in cells isolated by this method suggest that they originate from the upper villus only.

2. Isolated cells accumulate both α-methyl-D-glucoside and alanine, maintaining high intracellular concentrations for at least 60 and 40 min respectively. Accumulation of α-methyl-D-glucoside is inhibited by the presence of phloridzin.

3. The cells accumulate ^{42}K and ^{86}Rb in an identical manner. This uptake, which is maintained for at least 60 min, is inhibited in the presence of ouabain. Passive efflux of ^{42}K and ^{86}Rb occurs with rate constants which are virtually identical. The efflux follows a single exponential suggesting that it originates from only one intracellular compartment.

4. It is suggested that the preparation can be used to study the effect of sugars and amino acids on K efflux. The advantages of using such a preparation are discussed.

INTRODUCTION

A major difficulty of studying the transport functions of small intestinal epithelium derives from the anatomical complexity of the tissue. Epithelial cells cover several layers of connective tissue and muscle and this prevents easy access to the basal aspects of the epithelium. Results obtained using whole tissue are, in consequence, difficult to interpret at a cellular level. The characteristics of amino acid transport systems in the brush-border membrane have been explored in short-term uptake studies (Sepúlveda & Smith, 1978), but events occurring within the cell and at the basolateral membrane are more difficult to follow. Techniques involving the use of autoradiography (Paterson, Sepúlveda & Smith, 1982*a*, *b*), vascular perfusion (Cheeseman, 1981; Boyd & Perring, 1982) and membrane vesicles (Mircheff, Van Os & Wright, 1980) have all been developed to overcome part of this difficulty. There is, however, still a need to make direct measurements of ion, sugar and amino acid movements across the basolateral membrane of intact cells. Attempts to prepare such cells in monolayer culture have thus far produced cell lines with the morphology and transport capabilities of crypt cells (Inui, Quaroni, Tillotson & Isselbacher, 1980). The advantages to be gained with the availability of monolayers of fully differentiated enterocytes would be immense, but at the moment the more established use of suspensions of isolated cells provides a good alternative.

The properties of cells isolated from intestinal villi by a number of methods has been reviewed recently by Kimmich (1975). Most of these and other more recent preparations of isolated cells are unable to maintain even modest accumulations of amino acids for more than 10–15 min when used at a temperature of 37 °C (Towler, Pugh-Humphreys & Porteous, 1978; Bradford & McGivan, 1982). Here we develop the hyaluronidase method of Kimmich (1970), shown to be successful with chicken intestine, to isolate jejunal cells from rabbit intestine. These cells are found to retain their functional integrity and transport capabilities long enough to allow K fluxes to be measured during active transport of sugars and amino acids (Brown & Sepúlveda, 1985).

METHODS

Animals

Adult New Zealand White rabbits of both sexes weighing 2·5–3·0 kg were used throughout.

Cell isolation

Cells were isolated from rabbit jejunum using hyaluronidase by a modification of the method described for chicken intestine (Kimmich, 1970). A rabbit was killed by an intravenous injection of sodium pentobarbitone and a section of jejunum about 40 cm long was removed. The tissue was washed in ice-cold phosphate buffered saline (PBS) containing 0·1 mM-DL-dithiothreitol to reduce the mucus content of the preparation. The segment was then everted, tied at both ends and transferred to the isolation medium, containing 1·5 mg hyaluronidase/ml. The incubation at 37 °C in a shaking water bath (75/min) lasted 20 min. The buffer used in this and subsequent incubations was a modified Hanks salt solution and contained (mM): NaCl, 75; $NaHCO_3$, 25; $CaCl_2$, 1·3; $MgCl_2$, 0·5; K_2HPO_4, 0·36; KH_2PO_4, 0·44; KCl, 5; β-hydroxybutyrate, 0·5; D-mannitol, 63; HEPES, 10; pH 7·2; to this was added bovine serum albumin (BSA; 1 mg/ml). β-hydroxybutyrate was present as a non-actively accumulated nutrient for the suspended cells. After the 20 min incubation the intestinal loop was transferred to hyaluronidase-free buffer where cells were released by gentle agitation with a plastic pipette tip. The cells were filtered first through cotton gauze and then through a 200 μm mesh nylon sieve before being washed twice by resuspension and centrifugation.

Preparation of a villus–crypt cell gradient

Cells were isolated from rabbit intestine in sequential populations according to the method of Weiser (1973) with modifications described previously by Rowling & Sepúlveda (1984). The jejunum was removed and washed twice with ice-cold PBS containing 1 mM-DL-dithiothreitol before being everted and filled with PBS. This preparation was then incubated at 37 °C for 15 min in a citrate buffer before being transferred to a PBS buffer containing 1·5 mM-EDTA, 0·5 mM-dithiothreitol and 1 mg BSA/ml. Incubations to detach cells sequentially were carried out for 11, 5, 8, 6, 5, 5, 10, 10, 10, 30 and 30 min respectively at 37 °C with shaking (75/min). Cells from each fraction were collected by centrifugation. For enzyme assays the cells were washed twice in PBS and homogenized using an ultra Turrax homogenizer (20 s, 15000 r.p.m., 4 °C).

Enzyme assays

γ-Glutamyl transferase (GGT) activity was assayed by following *p*-nitroaniline appearance from L-γ-glutamyl-*p*-nitroanilide using glycylglycine as an acceptor (Sepúlveda & Burton, 1982). Sucrase activity was assayed by measuring the liberation of glucose from sucrose as described by Mahmood & Alvarado (1975).

Cytochemical localization of GGT

GGT activity was detected in frozen sections of tissue and in sections cut from frozen cell pellets using a modification of the method of Rutenberg, Kim, Fischbein, Hanker, Wasserkrug & Seligman (1969). The sections were fixed in formal–calcium at 4 °C and then incubated in the presence of

N-γ-glutamyl-4-methoxy-2-naphthylamide, Fast Blue B and glycylglycine at 25 °C. The 4-methoxy-2-naphthylamide released by the action of GGT combines with the diazonium salt Fast Blue B to produce an insoluble red dye, the colour of which is enhanced upon chelation with Cu^{2+}. These sections were viewed after mounting in glycerine–jelly.

Sugar and amino acid uptake measurements

Measurements of sugar, amino acid and ion uptakes were made at 37 °C, using cells at a final concentration of 2–3 mg cell protein/ml. Pre-warmed cell suspensions were mixed with the pre-warmed incubation medium containing ^{14}C-labelled sugar or amino acid (0·2–0·5 μCi/ml) and [^{3}H]inulin as an extracellular space marker (1·5 μCi/ml). Uptakes were terminated by diluting 500 μl cell suspension in 500 μl ice-cold buffer and the cells separated by centrifugation (10000 *g*; 20 s) through a 250 μl layer of the oil mixture di-*n*-butyl phthalate:dinonyl phthalate 3:2 (Sepúlveda, Burton & Brown, 1982). The cell pellets were lysed in 0·5 % (v/v) Triton X-100 and counted by liquid scintillation after protein precipitation with 5 % trichloroacetic acid (TCA). The amount of sugar or amino acid uptake was calculated taking into account the trapped extracellular volume estimated from the amount of [^{3}H]inulin present.

K fluxes

The suitability of ^{86}Rb as a tracer for K was tested by simultaneously measuring ^{42}K and ^{86}Rb uptake by or efflux from these cells. The uptake studies were identical in form to those for sugars and amino acids except that ^{86}Rb was at a concentration of 0·3 μCi/ml and ^{42}K at 2·5 μCi/ml. To measure the release of ^{86}Rb and ^{42}K from the isolated rabbit cells, a concentrated cell suspension (60–120 mg cell protein/ml) was pre-loaded by incubating at 37 °C for 25 min in the presence of ^{86}Rb (3 μCi/ml) and ^{42}K (15 μCi/ml). The rate of ^{42}K and ^{86}Rb loss from these cells was then followed by 100-fold dilution of aliquots of this suspension with radioisotope-free buffer followed by incubation at 37 °C. Timed samples were taken for counting and the incubation terminated as in the uptake measurement experiments. Extracellular ^{42}K and ^{86}Rb associated with the pellet was found to be negligible under these conditions (155 ± 108 nl/mg cell protein; mean ± S.E. of twenty-one determinations). This represents about 6 % of the total water; equivalent to only 0·5 % of the total K present. For both uptake and efflux experiments cell pellets were counted immediately, after cutting the centrifuge tube tips, in an autogamma scintillation spectrometer. A period of 12–14 days was then allowed to elapse for the ^{42}K counts to decay. The samples were then recounted for ^{86}Rb. The decay-corrected ^{86}Rb count was subtracted from the initial counts to estimate pure ^{42}K counts in the pellets. [^{3}H]inulin in the uptake studies was finally counted by liquid scintillation spectrometry.

Intracellular ion concentrations

Cell pellets formed by centrifugation through oil were resuspended in 500 μl water and mixed thoroughly. Protein was precipitated with 5 % (w/v) TCA and the suspensions centrifuged (10000 *g*, 20 s). The clear aqueous supernatants were diluted appropriately with water before K and Na concentrations were determined by emission flame photometry. The protein content of replicate pellets was assayed by the Lowry method and the cellular ion contents expressed as nmol/mg cell protein, after making a correction for the trapped volume, measured using [^{3}H]inulin.

ATP measurements

ATP was extracted from suspensions of cells using perchloric acid (Jawarek, Gruber & Bergmeyer, 1974). The neutralized extract was then assayed for ATP by measuring the hexokinase catalysed production of [^{3}H]glucose-6-phosphate from [^{3}H]glucose (González & García-Sancho, 1981).

Materials

Analar grade chemicals were used throughout. Hyaluronidase (Type III) from ovine testes, bakers' yeast hexokinase (Type VII), DL-dithiothreitol, β-hydroxybutyrate (Na salt), HEPES, bovine serum albumin (Fraction V), L-γ-glutamyl-*p*-nitroanilide and *N*-γ-1-glutamyl-4-methoxy-2-naphthylamide were purchased from Sigma Chemical Co., St. Louis. [^{3}H]inulin, ^{86}rubidium chloride, ^{42}potassium chloride, D-[^{3}H]glucose, methyl(α-D-[U-^{14}C]gluco)pyranoside and L-[^{14}C]-alanine all came from Amersham International PLC, Amersham, Bucks.

RESULTS

Origin of cells isolated using hyaluronidase

Cells isolated by the hyaluronidase method consisted of a mixture of isolated cells and small sheets of epithelial cells connected by tight junctions.

A microscopic examination of jejunum treated with hyaluronidase showed damage to the tissue to be confined, almost exclusively, to the epithelial layer of the villus, with sheets of enterocytes being stripped from the underlying lamina propria (results not shown). This provided initial circumstantial evidence that most of the cells isolated came from near the villus tip.

Further evidence as to the origin of these cells is given in Fig. 1 where the sucrase activity of cells isolated as a villus–crypt gradient by the chelation method is

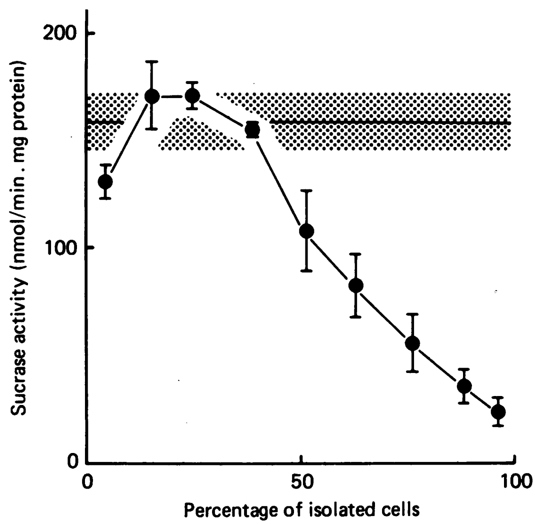

Fig. 1. Sucrase activity of isolated rabbit intestinal cells. Cells were isolated in sequential populations from villus to crypt as described in the text (●) or by the hyaluronidase method (horizontal line). Results are means ± S.E. (given by the shaded area in the case of hyaluronidase cells) of three (●) or eight (horizontal line) experiments. The percentage of isolated cells is based on the cumulative protein content of the sequentially isolated cell fractions.

compared with that found in cells isolated by the hyaluronidase technique. The points show the sucrase activity in fractions of cells isolated sequentially from the villus to the crypt of rabbit jejunum. Activities ranged from 171 ± 16 just below the villus tip to 26 ± 7 nmol/min.mg protein in the crypt region (means ± S.E., $n = 3$). Similar profiles have been described previously for both rabbit (Rowling & Sepúlveda, 1984) and rat intestine (Weiser, 1973) although the specific activities of sucrase in the rat were substantially lower than those found in the rabbit. Sucrase activity in cells prepared by the hyaluronidase method shown by the shaded area in Fig. 1 was 158 ± 13 nmol/min.mg protein ($n = 8$). The chelator isolation method also produced fractions showing a gradient for GGT activity (Fig. 2). Values ranging from 67 ± 5 to 8 ± 3 nmol/min.mg protein ($n = 3$) were obtained for villus and crypt region cell homogenates respectively. Hyaluronidase prepared cells showed a GGT activity of 58 ± 8 nmol/min.mg protein ($n = 10$), again shown by the shaded area on the graph.

Both sucrase and GGT estimates in the hyaluronidase preparation suggest that cells come from the upper third of the villus (less than 30 % of the total protein). This conclusion is also supported by detecting GGT activity by cytochemical methods (not shown). This reveals GGT activity at the brush border of isolated cells, or small aggregates of cells, similar to that seen at the brush-border side of cells in the upper reaches of villi in whole tissue sections.

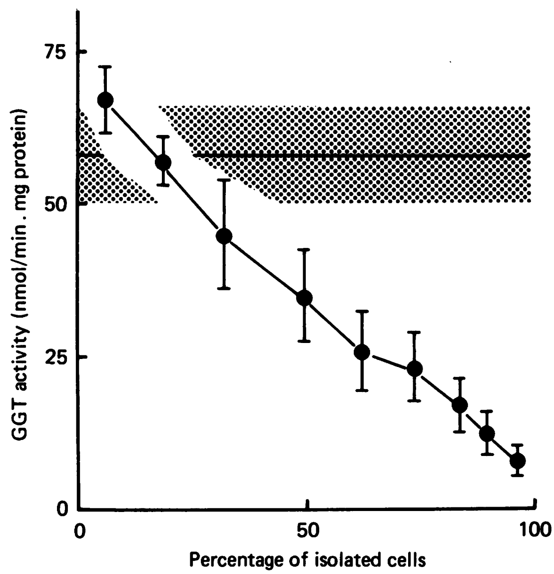

Fig. 2. γ-Glutamyl transferase (GGT) activity of isolated rabbit intestinal cells. Results are means ± S.E. of three (●) or ten (horizontal line) experiments. Other details as in Fig. 1.

Sugar and amino acid accumulation

The sugar α-methyl-D-glucose (α-MG) is a model substrate for the Na-dependent transport system located in the brush border of intestinal epithelial cells (Kimmich & Randles, 1981). The sugar, which is non-metabolizable, was used at a concentration of 0·1 mM to examine the ability of the isolated rabbit enterocytes to establish and maintain intra- to extracellular gradients of actively transported substrates (results not shown). The cells took up α-MG rapidly, reaching a steady-state value of 11·3 nmol/mg cell protein after about 40 min. This value then remained constant during a further 20 min incubation. Virtually all of this uptake was inhibited in the presence of 0·1 mM-phloridzin, a specific inhibitor of Na-dependent hexose transport.

In separate experiments 3-*O*-methyl-D-glucose uptake was measured in the presence of 0·1 mM-phloridzin to estimate the apparent intracellular volume of these cells (Kimmich, 1975). Results from eight experiments gave a mean value of 2.26 ± 0.26 μl /mg cell protein. Similar values were obtained using $^{3}H_2O$ and [^{14}C]inulin to measure total and extracellular water space. Using this number it can be calculated that the steady-state intracellular concentration of α-MG is some 50 times higher than that found in the bathing medium. The concentration of α-MG in the bathing medium was, however, reduced by 26 % over the 60 min time course used to measure uptakes. When this dilution is taken into consideration the intracellular concentration of α-MG approaches 70 times that found in the medium. This value compares very favourably with the 75-fold accumulation described for isolated chicken enterocytes by Kimmich & Randles (1981).

A further characterization of the α-MG uptake by the isolated cells (Fig. 3), shows that the phloridzin-insensitive component of the uptake becomes more prominent as the external concentration of α-MG increases. The phloridzin-sensitive uptake values

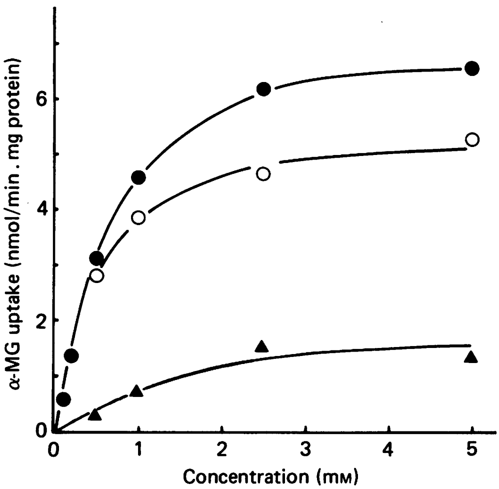

Fig. 3. Concentration dependence of a α-methyl-D-glucoside (α-MG) uptake by rabbit enterocytes. Uptakes were measured during 2 min incubations in the presence (▲) or absence (●) of 0·5 mM-phloridzin. ○, the phloridzin-inhibitable uptake obtained by subtraction.

obtained by subtracting the phloridzin-insensitive values from total uptake were fitted to a rectangular hyperbola (Bliss & James, 1966). Values for K_m and V_{max} were 0·61 ± 0·11 mM and 5·92 ± 0·91 nmol/min.mg protein respectively. The non-linearity of the concentration dependence of α-MG uptake in the presence of phloridzin suggests carrier-mediated transport. A possible pathway for this uptake would be the basolateral membrane and phloridzin-insensitive hexose transport system which has been shown to have low affinity for α-MG in chicken enterocytes (Kimmich & Randles, 1981).

The ability of rabbit cells to accumulate neutral amino acids was also investigated (Fig. 4). Uptake of 0·25 mM-L-alanine at 37 °C is shown to reach a maximum value after 20 min incubation and this level of accumulation was maintained for at least 40 min. The maximum uptake of 6·6 nmol/mg protein is equivalent to a 12-fold concentration above that found in the incubation medium. This value is in good agreement with previous findings for L-valine accumulation in chicken cells (Tucker & Kimmich, 1973) and rat cells with L-leucine (Reiser & Christiansen, 1971), but slightly less than that found for L-alanine accumulation by rat cells (Bradford & McGivan, 1982). In the presence of 10 mM-L-methionine the uptake of 0·25 mM-

L-alanine was greatly reduced (Fig. 4). This is presumably a result of competition between the two amino acids for the same carrier sites. The uptake was also substantially inhibited by the cardiac glycoside, ouabain (Fig. 4), suggesting that a large fraction of the alanine transport is dependent upon the ion gradients which are maintained by the Na pump.

ATP levels in rabbit enterocytes used here were found to be $3{\cdot}98 \pm 0{\cdot}2$ ($n = 7$) nmol/mg cell protein, and remained stable for 40 min. In the presence of dinitrophenol and iodoacetic acid (10^{-4} and 10^{-3} M respectively) ATP levels fell below 0·2 nmol/mg cell protein within 10 min.

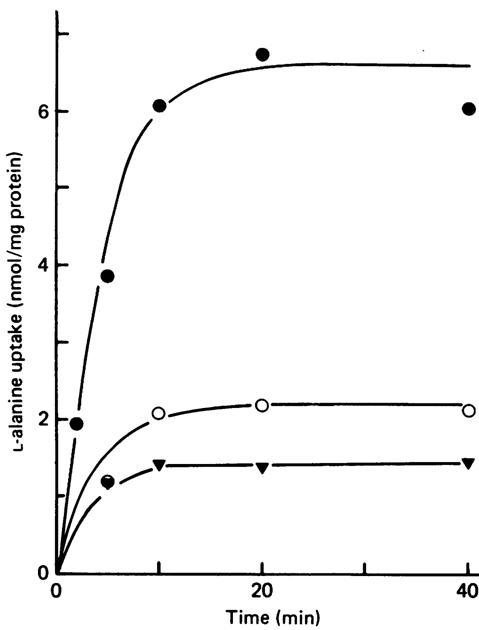

Fig. 4. Time course of L-alanine uptake. The amino acid was used at a concentration of 0·25 mM, in the absence of inhibitors (●), or in the presence of 0·1 mM-ouabain (○) or 10 mM-L-methionine (▼).

Intracellular ion concentrations

The isolated rabbit enterocytes had the ability to maintain their K content at a constant level over a period of 60 min (see Fig. 5). The average K ion content over this period was 156 ± 3 nmol K/mg of cell protein ($n = 4$). The effect of the 0·1 mM-ouabain upon the K content of the cells, also shown in Fig. 5, was to decrease gradually the level to 34 nmol/mg cell protein over 60 min. A more rapid decrease in cell K was seen when the cells were cooled by placing them on ice (Fig. 5). In this case the cells were able to regain their original concentrations when rewarmed to 37 °C.

Na ion levels were found to be about 28 nmol/mg cell protein. In the presence of 0·1 mM-ouabain this value increased to 69 and 86 nmol/mg of cell protein after 15 and 30 min incubation respectively.

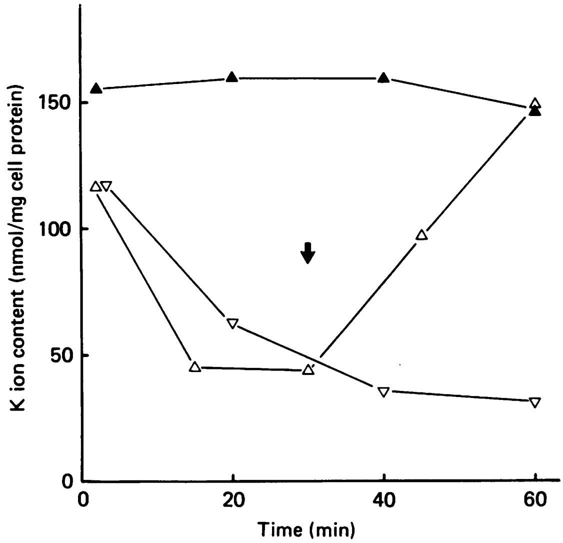

Fig. 5. Intracellular K ion content of isolated rabbit enterocytes. Cells were incubated at 37 °C in the absence of inhibitors (▲) or in the presence of 10^{-4} M-ouabain (△). Another batch of cells was cooled from 37 °C by placing it on ice (▽). At the time indicated by the arrow these cells were transferred back to a water bath at 37 °C.

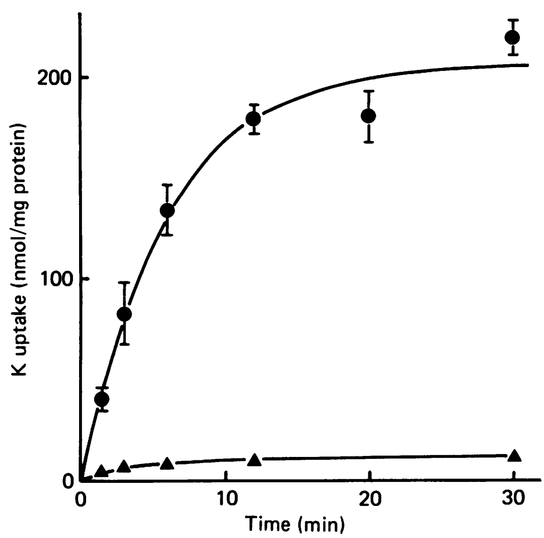

Fig. 6. Time course of K uptake by rabbit enterocytes. Uptake was measured with ^{86}Rb as a tracer for K in the presence (▲) or absence (●) of 10^{-4} M-ouabain. The control points can be described by a single increasing exponential (as shown by the line), of rate constant $0{\cdot}166 \pm 0{\cdot}024$/min and asymptote of 206 ± 10 nmol/mg cell protein. Points are means ± S.E. of experiments with four different cell preparations.

K influx

Fig. 6 shows the time course of K uptake by the isolated enterocytes. The uptake followed an exponential time course with steady-state accumulation of 206 ± 10 nmol/mg of cell protein ($n = 5$) being approached after about 20 min. This uptake was apparently into a single cation pool and seemed to be carried out mainly by the Na pump as only small uptakes are observed in the presence of ouabain (Fig. 6). The results shown were obtained using ^{86}Rb as a convenient tracer for K movements. In a separate series of experiments the suitability of ^{86}Rb as a tracer for K was assessed by comparing its uptake with the simultaneous uptake of ^{42}K. The results of these

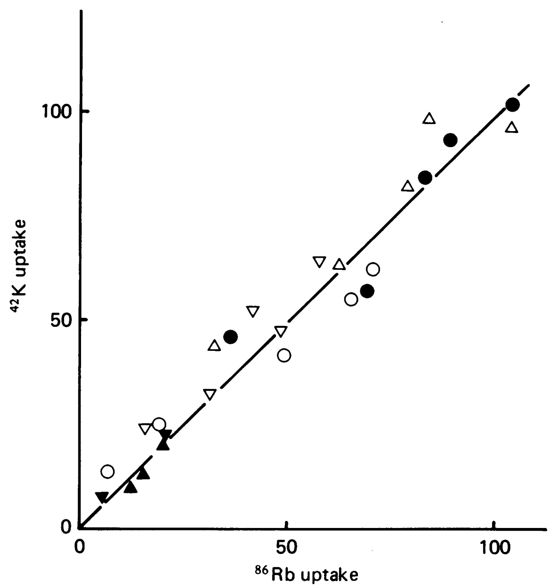

Fig. 7. Comparison of K uptake rates by rabbit enterocytes measured simultaneously using ^{42}K and ^{86}Rb. Points show individual uptake measurements (in nmol K/mg protein) performed with the two isotopes in trace amounts. Experiments were performed in six different cell preparations (different symbols) at various incubation times. The filled triangles correspond to measurements in the presence of 0·1 mM-ouabain. The line shown is the identity line. Linear regression analysis of the points gave a slope of $0{\cdot}95 \pm 0{\cdot}05$ with a correlation coefficient of 0·973.

experiments, obtained in the presence and absence of 10^{-4} M-ouabain are compared in Fig. 7. Uptakes of both isotopes under similar experimental conditions were virtually identical, with all points falling close to the line of identity. This confirms that ^{86}Rb is an appropriate tracer for K uptake measurements in isolated rabbit enterocytes.

K efflux

Cells pre-loaded with ^{86}Rb were used to measure the rate of K efflux. Fig. 8 shows that the loss of K within the time interval studied (about 30 % of the efflux) can be described by a single exponential giving a straight line when plotted semilogarithmically. To test that the efflux measured with ^{86}Rb as a tracer is a true reflexion of K efflux, ^{86}Rb and ^{42}K were used in the same efflux experiments. Both isotopes behaved very similarly, with the points correlating the amount of ^{86}Rb with ^{42}K remaining within the cells at different times all lying close to the identity line (slope $0{\cdot}93 \pm 0{\cdot}07$, $r = 0{\cdot}973$). A semilogarithmic plot of ^{42}K remaining in the cells as a function of time gave a rate constant for its efflux of $0{\cdot}0220 \pm 0{\cdot}0037$/min; the equivalent figure for ^{86}Rb was $0{\cdot}0225 \pm 0{\cdot}0026$/min (means ± S.E. of experiments performed with four different cell preparations).

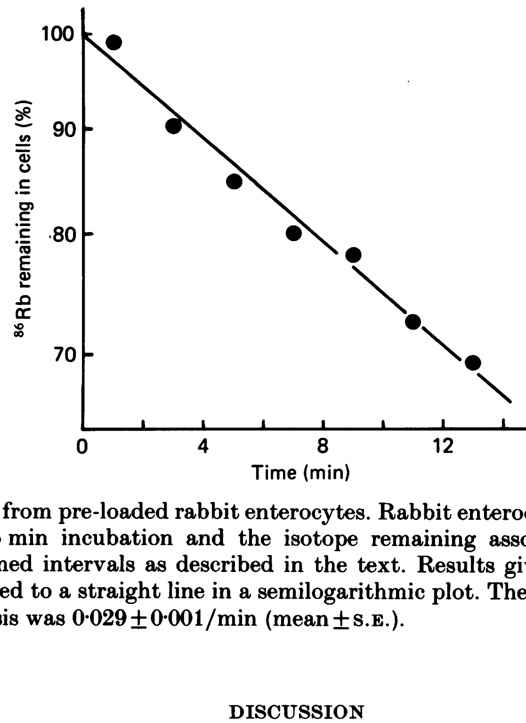

Fig. 8. ^{86}Rb loss from pre-loaded rabbit enterocytes. Rabbit enterocytes were loaded with ^{86}Rb over a 25 min incubation and the isotope remaining associated with the cells measured at timed intervals as described in the text. Results give the means of eight experiments fitted to a straight line in a semilogarithmic plot. The rate constant derived from this analysis was $0{\cdot}029 \pm 0{\cdot}001$/min (mean ± S.E.).

DISCUSSION

Origin of cells

Some of the problems associated with studying transport in whole intestinal preparations *in vitro* arise from the complex anatomy of the tissue. Apart from the difficulties created by the presence of underlying layers of connective tissue and muscle, which are not directly involved with absorption, the epithelium itself is composed of cells at different stages of development. The cells in the crypts which remain largely undifferentiated are responsible for the proliferation of the epithelium. Fully developed enterocytes mature as they migrate from the crypts towards the tips of villi from where they are lost into the intestinal lumen. During this migration the cells acquire their characteristic morphology (Cheng & Leblond, 1974) and enzyme profiles (Nordström, Dahlquist & Josefson, 1968; Webster & Harrison, 1969; Fortin-Magana, Hurwitz, Herbst & Kretchmer, 1970). The transport systems involved in the absorption of sugars and amino acids only occur in the fully differentiated epithelial cells, in this case in the upper region of the villus. This is shown both by the cellular distribution of transported substrate visualized by autoradiography

(Kinter & Wilson, 1965; King, Sepúlveda & Smith, 1981; Smith, 1985) and by observing the changes in membrane potential induced by amino acids in older enterocytes (Smith, Sepúlveda & Paterson, 1983). For these reasons it is important that the population of cells isolated here corresponds to that shown by other methods to transport nutrients. This was established by: (i) measuring sucrase and GGT activities in villus–crypt gradients of sequentially isolated cells and comparing these to those found in hyaluronidase prepared cells, (ii) cytochemically locating GGT activity in the upper villus in sections of whole jejunum and in the brush-border pole of the hyaluronidase prepared cells and (iii) examining hyaluronidase treated tissue histologically to ascertain that damage is restricted to the villus tip. The transport capabilities of the cells in any preparation may vary slightly owing to their site of origin on the villus, and depending on where the villus was located in the intestine. Such differences are, however, largely negated in a well mixed suspension, making the cells ideal for a number of experiments. The use of suspensions of viable enterocytes also overcomes many of the disadvantages of working with whole intestine. Access to the basolateral membranes not only allows direct measurements of ion movements without interference from the underlying tissue, but also ensures that the whole cell is bathed in buffers of known composition free from any localized concentration effects in intercellular spaces. Work of this nature is reported in a subsequent paper (Brown & Sepúlveda, 1985).

Cell viability

The localization of GGT by the cytochemical method suggests that redistribution of this enzyme does not take place as a result of cell isolation. Migration of brush-border membrane enzymes has been reported in individual mouse enterocytes, but not in groups of cells connected by tight junctions (Ziomek, Schuluman & Edidin, 1980). Aggregates of cells are obtained by the use of hyaluronidase and it seems that such preparations are generally better equipped to survive than completely isolated cells. For instance, single guinea-pig enterocytes isolated by incubation in the presence of EDTA failed to maintain an intracellular K concentration higher than that in the bathing medium (Evans, Wrigglesworth, Burdett & Pover, 1971).

The cells used in the present study were able to accumulate sugars and amino acids by mechanisms known to operate in the intact tissue in viable isolated chicken enterocytes (reviews by Munck, 1981 and Kimmich, 1981). The high concentrations of sugar and amino acids achieved upon incubation of the cells in the presence of these substrates indicates that they preserve their energy metabolism and membrane permeability. In agreement with this the rabbit enterocytes are shown to maintain high concentrations of ATP for up to 40 min incubations at 37 °C. This contrasts with observations in another isolated mammalian enterocyte preparation where both amino acid accumulation and ATP levels have been observed to decrease rapidly over a few minutes incubation at 37 °C (Bradford & McGivan, 1982).

It is not possible to say precisely what region of the plasma membrane is involved in this active transport process, but it is reasonable to assume that the phloridzin-sensitive sugar transport, represented by α-MG uptake, occurs through the Na-dependent brush-border transport system. This seems to be the only site of active sugar absorption in the enterocyte (Kimmich, 1981). Active amino acid accumulation may occur through the basolateral membrane, but it is thought that basolateral transport is largely Na-independent (see Munck, 1981). As the active accumulation of alanine observed here is abolished when the Na pump is inhibited by ouabain, it is likely that most of the uptake is Na-dependent and hence occurs through brush-border membrane transport systems.

The intracellular K content of these cells varies from 153 (Fig. 5) to 216 (Fig. 6) nmol/mg cell protein. Estimates of intracellular concentrations of 68–96 mM were made using the value of intracellular water content of 2.26 ± 0.26 μl/mg of cell protein. These concentrations can be compared with intracellular K activities measured in intestinal epithelial cells of different vertebrates which vary from 67 to 100 mM (Lee & Armstrong, 1972; Grasset, Gunter-Smith & Schultz, 1983; Cremaschi, James, Meyer, Rossetti & Smith, 1984). In the context of these comparisons it should be noted that the activity coefficient for K is 0·78 (Robinson & Stokes, 1959). The errors involved in determining intracellular volume and the possibility of different distribution spaces for K and the 3-*O*-methyl-D-glucose make further analysis unwarranted except to say that the concentrations reported here are in rough agreement with previously reported values. Similarly the value of 28 nmol Na/mg of cell protein is equivalent to an intracellular Na concentration of 12 mM, which is in good agreement with previous measurements (Lee & Armstrong, 1972; Cremaschi *et al.* 1984).

Ouabain at a saturating concentration (Rowling & Sepúlveda, 1984) causes a decrease in the cell K concentration (Fig. 5) while the intracellular Na increases presumably because of the Na pump inhibition. Such changes in ion contents could perhaps be predicted from the results showing that ouabain strongly inhibits K uptake by these cells when measured using radioactive tracers (Fig. 6). The Na pump is also inhibited and the cells rapidly lose K when the cells are cooled on ice (Fig. 5). The ability of the cells to recover their intracellular K when rewarmed is further evidence that they are maintaining both energy production and membrane permeability for the 60 min of the incubation.

The data in Fig. 6 are consistent with the notion that extracellular K exchanges with a single pool of intracellular cation, and uptake kinetics are described by a single increasing exponential function. This result is in agreement with other isolated epithelial cells (Zylber, Rotunno & Cereijido, 1975; Sepúlveda *et al.* 1982). Similarly the release of K from pre-loaded rabbit enterocytes is best described by a single decreasing exponential. The rate constant for this efflux was of the same order of magnitude as that reported for other isolated cells (Kristensen, 1980; Valdeolmillos, García-Sancho & Herreros, 1982). Similar kinetic values for uptake and efflux were obtained using ^{42}K and ^{86}Rb as tracers, validating the use of the more convenient ^{86}Rb isotope in future studies.

In summary, it has been shown that epithelial cells isolated from rabbit small intestine and originating from the upper reaches of the villi are capable of actively accumulating sugars and amino acids while maintaining a physiologically normal profile of ions. The preparation is suitable for studies on the relationship between Na-coupled sugar and amino acid transport and K movements *in vitro* as shown in the following paper (Brown & Sepúlveda, 1985).

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