Biochem. J. (1977) **164**, 1–14
Printed in Great Britain

Steady-State Metabolism and Transport of D-Glucose by Rat Small Intestine *in vitro*

By PENELOPE J. PRITCHARD and JOHN W. PORTEOUS
Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

(*Received* 12 *July* 1976)

1. Conditions of incubation of everted sacs of rat small intestine were selected to ensure that absorption of D-glucose by mucosal tissue from the incubation medium, intracellular metabolism of the absorbed glucose and transport of glucose through the intact intestinal tissue proceeded linearly with respect to time of incubation within stated time intervals. 2. Under these experimental conditions, steady intracellular concentrations of glucose and lactate were demonstrated. 3. The quantitative translocational and metabolic fate of absorbed glucose was determined under these steady-state conditions. About 25% of glucose absorbed from the external mucosal solution was accumulated (temporarily) within mucosal tissue and about 25% transported through the intact tissue into the external serosal solution; the remainder (about 50%) of the absorbed glucose was metabolized, 90% to lactate and 10% to CO_2. Concomitant respiration rates were comparable with those reported for several other preparations of intestine and were stoicheiometrically in excess of the O_2 metabolism required to account for the production of CO_2 from the absorbed glucose. 4. Water transport through the everted sacs proceeded at an optimum rate under the experimental conditions selected. 5. Some other observations are recorded which influenced the design of the experiments and the interpretation of results; these include the initial physiological state of the animal, the anaesthetic used and the ionic composition of the incubation medium.

The metabolism of D-glucose during its transport through preparations of intact intestine *in vitro* has been examined by several authors (Wilson, 1953, 1954, 1956; Wilson & Wiseman, 1954*a*,*b*; Newey *et al.*, 1959; Bronk & Parsons, 1965; Esposito *et al.*, 1967; Leese & Mansford, 1971; Leese & Bronk, 1975; Hanson & Parsons, 1976), but it is not yet possible to state the complete quantitative distribution of glucose after it has been absorbed under steady-state conditions from the external solution bathing the mucosal aspect of intestine *in vitro*, or to define quantitatively the metabolic fate of glucose subsequent to its absorption by the columnar absorptive cells lining the villi of the small intestine.

Evidence has been reported favouring the existence of a (positive) Pasteur effect in intact small intestine perfused via the vascular bed *in vitro* (Lamers & Hülsmann, 1972; Hanson & Parsons, 1976). Tejwani *et al.* (1974) examined the parameters and variables determining the activity of phosphofructokinase in intestinal mucosa and used the results obtained to rationalize earlier claims that the Pasteur effect was absent from a number of preparations of small intestine, including everted-sac preparations. Leese & Bronk (1975) concluded that a negative Pasteur effect operated in the whole mucosal tissue removed from intestine. Evidence for or against the operation of one of the intracellular mechanisms regulating flux of glucose through the glycolytic pathway to pyruvate in small intestine thus appears to depend, at least in part, on the kind of tissue preparation used. No experimental evidence is available on the molecular mechanisms regulating the partitioning of glucose between its two catabolic end products, CO_2 and lactate. There is a similar lack of information on the parameters and variables determining the translocational flux of unchanged glucose through the intestine, or the translocational fluxes of metabolites of glucose from the columnar absorptive cells into the extracellular fluids.

The prime purpose of the present paper is to provide a quantitative statement of the translocational and metabolic fate of D-glucose in rat small intestine under defined conditions *in vitro*; such a statement is fundamental to the elucidation of regulatory mechanisms operating in the intestine during translocation of glucose from the lumen to the circulation. The attainment of this objective demanded the preliminary selection of experimental conditions which ensured that absorption of glucose into epithelial tissue, metabolism of glucose within the tissue and transport of glucose through the whole intestinal tissue (epithelial tissue plus subepithelial and serosal tissue) proceeded linearly with time of incubation

over known periods of time, that glucose absorbed from the external mucosal solution could be quantitatively accounted for chemically and radiochemically, and that glucose metabolized could likewise be accounted for. Such prerequisite conditions do not seem to have been recognized or satisfied heretofore. The first part of this paper deals with the selection of the required experimental conditions; the latter part of the paper provides the quantitative information necessary to attain the objective stated.

Materials and Methods

Materials

All common chemicals were of analytical reagent quality; water was glass distilled. Glucose oxidase (grade II), lactate dehydrogenase, NAD^+ and Tris were supplied by Boehringer Corp. (London) Ltd., London W5 2TZ, U.K., and radiochemicals by The Radiochemical Centre, Amersham HP7 9LL, Bucks., U.K.; peroxidase (type II) was supplied by Sigma (London) Chemical Co., Kingston-upon-Thames KT2 7BH, Surrey, U.K., and Macherey Nagel cellulose 300G by Camlab, Cambridge CB4 1TH, U.K.

Methods

Male Wistar rats (150–200g) were maintained on stock diets and water *ad libitum*; then, to render them docile, they were transferred to a ventilated dark box for 1 h before rapid excision of the small intestine by dissection (Kinter & Wilson, 1965) under diethyl ether anaesthesia. Sacs of small intestine were prepared (Wilson & Wiseman, 1954*a*) by dividing the everted intestine (70–80cm total length) into contiguous 4 or 5 cm segments while immersed in continuously oxygenated Krebs–Ringer phosphate buffer, pH7.2 (De Luca & Cohen, 1964), modified to contain 3mequiv. of Ca^{2+}/litre; sacs were filled with 0.4 or 0.5ml of the same buffer kept continuously oxygenated in a separate vessel. Each sac was suspended in a 50ml stoppered flask containing 40ml of O_2 and 10ml of the same O_2-saturated phosphate-buffered medium, and shaken at 80 oscillations/min during incubation at 37°C. Glucose (10mM) was present initially in both the inner (serosal) and outer (mucosal) solutions unless otherwise stated. In some experiments D-[U-^{14}C]glucose was added at known specific radioactivity to the mucosal solutions only, or alternatively to the serosal solutions only.

After incubation, sacs were removed immediately from flasks, blotted by a standardized procedure, emptied and drained for 20s, then reweighed to determine the final volume of the serosal solution and the final weight of the whole tissue. Mucosal tissue was removed by scraping the sac on a cold plate, weighed, transferred into ice-cold $HClO_4$ (1M; 1ml/g of tissue), centrifuged (1000*g*, 10min), and extracted twice more with half the volume of $HClO_4$. Neutralized tissue extracts and samples of the corresponding serosal and mucosal solutions were analysed at zero time and after stated times of incubation of everted sacs. O_2 consumption and total CO_2 production were followed, under identical conditions of incubation, by Warburg respirometry.

Radiochemical measurements were made in the scintillation fluid of Bray (1960). Counting efficiency was determined against internal standards. Net counts (gross counts−background) were determined on each sample to a standard proportional error of 2% on a single count. CO_2 present initially or produced during incubation of everted sacs was trapped in NaOH after acidification of the complete incubation system; total trapped CO_2 was determined titrimetrically and radiochemically in order to determine its specific radioactivity. Glucose and lactate were separated by t.l.c. on cellulose (Waring & Ziporin, 1964) by using formic acid/ethyl methyl ketone/2-methylpropan-2-ol/water (3:6:8:3, by vol.). The margins of chromatograms, containing reference samples of glucose and lactic acid, were sprayed with aniline phthalate (Vomhof & Tucker, 1965) and with aniline/xylose reagent (cf. Higgins & von Brand, 1966) to locate glucose and lactic acid respectively. Glucose and lactic acid were recovered from the rest of the chromatograms by water extraction, then assayed chemically and radiochemically to determine their specific radioactivities. Model experiments gave 100% recovery of $^{12}CO_2$ and of $^{14}CO_2$ from solutions containing 5–75μmol of Na_2CO_3 and 450–6000 d.p.m.; recoveries of [^{12}C]glucose, [^{14}C]glucose [^{12}C]lactic acid and [^{14}C]lactic acid ranged from 92 to 99% provided that the sample removed from the chromatogram contained not less than 0.1μmol of glucose or lactic acid after chromatographic separation of a mixture of the two solutes. Glucose was determined by a glucose oxidase method (Porteous & Clark, 1965) and lactate as described by Hohorst (1956). Lactate dehydrogenase activity (EC 1.1.1.27) was assayed as described by Kornberg (1955) and pyruvate dehydrogenase (EC 1.2.4.1) as described by Lusty & Singer (1964) after preparation of cell-sap and mitochondrial fractions of intestinal mucosa (Porteous & Clark, 1965). Mucosal tissue homogenates were subjected to starch-gel electrophoresis (Poulik, 1957); sections of the gel were stained for protein (Kohn, 1958) and for lactate dehydrogenase activity (Vessel, 1961). In some experiments, mucosal tissue was analysed for glycogen (Hassid & Abraham, 1957), mono-, di- and triacylglycerols, cholesterol and phospholipids (Lis *et al.*, 1961; Morgan & Kingsbury, 1959; Paul, 1958; Wagner *et al.*, 1961), DNA (Burton, 1956), RNA (Schneider, 1957) and protein (Miller, 1959).

Results and Discussion

Absorption and transport of glucose in contiguous segments of small intestine

Absorption of glucose from the mucosal solution occurred in all segments of the small intestine but to the greatest extent in the length of intestine 10–55cm from the stomach (Table 1); no net transport of glucose into the serosal solution occurred in the proximal 10cm and distal 15cm of a typical 70cm intestine. These results conflict with those previously reported for glucose absorption (Fisher & Parsons, 1950) and are more akin to those of Crane & Mandelstam (1960) showing that tissue accumulation of glucose, galactose or 1,5-anhydroglucitol reached a maximum about midway along the length of golden-hamster intestine. In all subsequent experiments reported in the present paper, sacs were prepared from the length of rat intestine 15–50cm from the stomach to ensure that transport of glucose would occur and to minimize differences between the absorptive (and transport) capacities of sacs prepared from adjacent segments of intestine (Table 1).

Glucose absorption and transport: effects of time of incubation and of initial glucose concentration

In contrast with earlier observations (Wilson & Wiseman, 1954*a*), absorption of glucose from the mucosal solution and transport of glucose into the serosal solution proceeded linearly with time of incubation for at least 30min, then declined progressively (Fig. 1). In all subsequent experiments, incubation of everted sacs was restricted to 20min unless otherwise stated. Maximum glucose absorption by sacs prepared from the proximal and middle thirds of the small intestine occurred with initial extracellular glucose concentrations between 10 and 25mM; apparent saturation of the tissue absorption mechanism in the distal third of the intestine did not occur within the glucose concentration range tested (0–50mM). Transport of glucose across all parts of the small intestine reached a maximum rate with initial extracellular glucose concentrations between 10 and 25mM; 40 and 50mM-glucose markedly inhibited transport of glucose (Porteous, 1977).

Oxygen-dependence of glucose absorption and transport

The ability of everted sacs of intestine to transport glucose in the presence of O_2 and their failure to do so under N_2 has been demonstrated (Wilson & Wiseman, 1954*a*), but the response of such sacs to graded concentrations of O_2 has not been reported. The results of appropriate tests are shown in Fig. 2 and justify the use of 100% O_2 in all other experiments.

Table 1. *Loss of glucose from the mucosal solution (absorption) and gain of glucose in the serosal solution (transport) after incubating everted sacs prepared from different segments of rat small intestine*

Sacs were prepared from 5cm segments of the intestine at the distances from the stomach indicated. Mucosal solutions (10ml) and serosal solutions (initially 0.5ml) contained 10mM-glucose at zero time; incubation lasted 20min at 37°C under 100% O_2. Results shown are mean values (±S.D.) from five experiments using the whole length of small intestine and from a further five experiments using segments of intestine obtained between 15 and 50cm from the stomach.

Origin of the everted sacs (cm from the stomach)	Glucose absorbed from the mucosal solution (μmol)	Glucose transported into the serosal solution (μmol)
0–5	2.1±2.5	−2.0±3.1
5–10	8.1±2.2	−1.1±2.0
10–15	20.0±3.1	2.0±1.1
15–20	24.1±4.0	4.2±0.4
20–25	25.2±3.1	5.1±0.5
25–30	27.6±4.2	5.0±0.4
30–35	27.4±3.8	6.1±0.5
35–40	27.9±4.0	6.5±0.4
40–45	26.4±2.0	7.0±0.5
45–50	25.6±3.0	7.0±0.2
50–55	23.2±2.0	5.0±0.3
55–60	19.3±2.1	−1.0±0.8
60–65	15.2±3.1	−1.5±0.6
65–70	7.8±2.1	−1.2±1.0

O_2 consumption and CO_2 production increased linearly with time of incubation of sacs over the period 0–40min to give a respiration rate of 40μmol of O_2 metabolized/h per g wet wt. of whole intestinal tissue, and a respiratory quotient (CO_2 produced/O_2 metabolized) between 0.9 and 1.0; concomitant lactate formation also increased linearly with time of incubation (Fig. 3).

Glucose and lactate concentration gradients established during glucose transport through everted sacs of intestine

Results shown in Table 2 established the direction of net flux of glucose and lactate through (or out of) intestinal tissue by demonstrating (*a*) that the glucose concentration gradient created across the intestine must have been established as a consequence of two distinct processes, namely concentrative accumulation within mucosal tissue of the glucose absorbed from the external mucosal solution, followed by passage of the accumulated glucose out of the tissue preferentially into the serosal solution; this conclusion is compatible with that reached by using other sugars and different techniques (McDougal *et al.*, 1960; Bihler *et al.*, 1962; Bihler & Crane, 1962), but

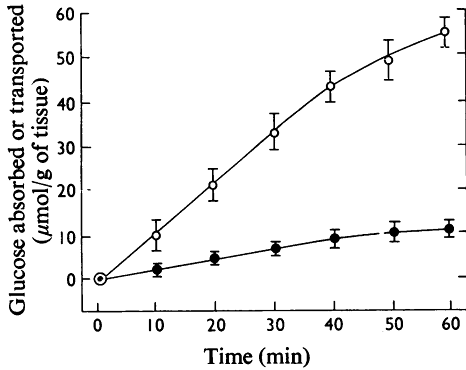


Fig. 1. *Effect of time of incubation on absorption* (○) *and transport* (●) *of* D-*glucose by everted sacs of rat jejunum*

The results shown are the mean values obtained from five sets of six everted sacs, each set of sacs being prepared from the length of intestine between 15 and 55 cm from the stomach, and incubated under standard conditions (37°C; 10mM-glucose; 100% O_2) described in further detail in the text. Bar lines represent the S.D. about the mean values.

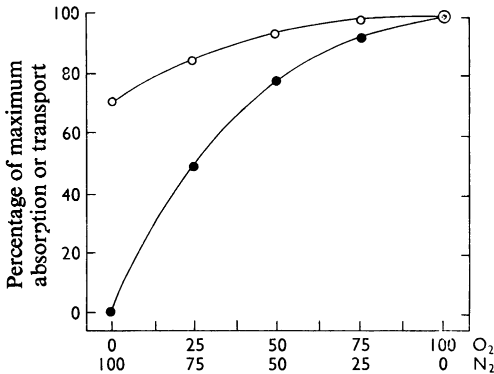


Fig. 2. *Variation in glucose absorption* (○) *and transport* (●) *with O_2 concentration*

Typical results are shown from a set of five sacs (4cm) prepared from the length of intestine 20–40cm from the stomach. Each sac was incubated under the standard conditions (37°C; 10mM-glucose; 20min) detailed in the text except that the O_2 concentration in the gas phase was varied as indicated.

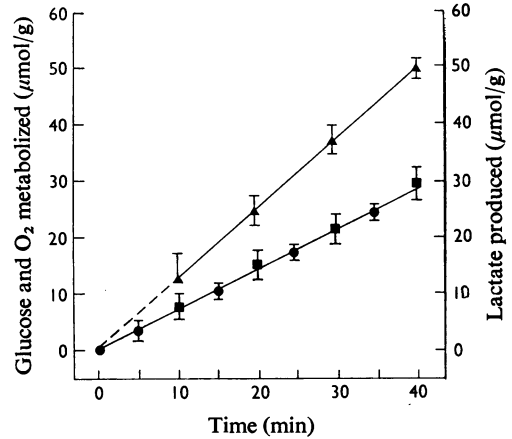


Fig. 3. *Glucose* (■) *and O_2* (●) *metabolized, and lactate produced* (▲), *during incubation of everted sacs of rat jejunum with* 10mM-*glucose at* 37°C *under* 100% O_2 *for various times*

In each experiment, a set of four sacs (4cm) was prepared from the length of intestine 25–45cm from the stomach. Sacs were incubated in 50ml Warburg flasks under conditions detailed in the text; individual sacs were removed after 10, 20, 30 or 40min for determination of total glucose metabolized and total lactate produced. O_2 consumption was monitored at 5min intervals; parallel experiments showed that total CO_2 production also proceeded linearly with time of incubation to give a respiratory quotient between 0.9 and 1.0. The results shown are the mean values from four experiments; bar lines indicate S.D. from the mean.

does not exclude the possibility that passage of glucose out of the columnar absorptive cells across the basal-lateral membrane is facilitated by a stereospecific mechanism located in that membrane (Lauterbach, 1972; Bihler & Cybulsky, 1973; Murer *et al.*, 1974), and (*b*) that the lactate concentration gradient created across the everted sac must be established as a consequence of metabolism of absorbed glucose within columnar absorptive cells followed by passage of intracellular lactate anion plus accompanying cation down the electrochemical potential gradient for lactate anion, towards the serosal solution. It then follows that the brush-border membrane must be effectively less permeable to lactic acid (or lactate anion) than is the basal-lateral membrane of the columnar absorptive cells; no information is available about the structure(s) or mechanism(s) determining this differential permeability of the two membranes to lactic acid or lactate anion.

Tissue concentrations of glucose and lactate (Table 2) were established within the first 5min of incubation and persisted unchanged for at least the next 25min; glucose and lactate did not appear in the serosal solution at concentrations exceeding those found in the mucosal tissue at any time, even when incubations were prolonged to 90min.

Table 2. *Concentrations of glucose and of lactate in the mucosal solutions, mucosal-tissue water and serosal solutions of a series of everted sacs prepared from different positions along the length of rat jejunum*

Nine everted sacs (5cm) were prepared from the length of intestine indicated. Alternate sacs were incubated without glucose in both the mucosal and the serosal solutions, or with 10mM-glucose present initially in both these solutions. Results obtained from the former set of sacs were used to correct gross results obtained from the latter set of sacs. Corrections for the amounts of lactate in the external solutions did not exceed 20% of gross values; in no other instance did corrections exceed 5% of the gross value. Mucosal-tissue water was taken as 75% of mucosal-tissue wet weight (the mean value ± S.D. determined in 15 experiments was 75 ± 3%). The results shown are from a single typical experiment performed to establish the direction of net flux of glucose and lactate in the everted-sac system, and illustrate the approach used in more detailed experiments (Tables 4, 5 and 6).

Origin of the everted sacs (cm from stomach)	Concn. of glucose in the mucosal solution (mM)	Concn. of glucose in the mucosal tissue water (mM)	Concn. of glucose in the serosal solution (mM)	Concn. of lactate in the mucosal solution (mM)	Concn. of lactate in the mucosal tissue water (mM)	Concn. of lactate in the serosal solution (mM)
15–20	8.1	17.0	14.0	0.6	29.0	11.5
25–30	7.5	18.0	15.5	0.4	27.5	10.5
35–40	7.0	16.2	13.4	0.6	26.0	10.0
45–50	7.8	18.0	13.5	0.6	25.0	8.5

Table 3. *Gain or loss of water by the serosal solution of everted sacs of intestine during incubation in the presence or absence of glucose for 20min at 37°C under 100% O_2*

Ten everted sacs (each 4cm long) were prepared in sequence from the length of intestine between 10 and 50cm from the stomach. For convenience of presentation, results from three contiguous sacs have been grouped; Group I sacs were prepared from segments between 10 and 22cm from the stomach, Group II sacs from segments between 22 and 34cm from the stomach and Group III sacs from segments between 38 and 50cm from the stomach. The sac prepared from the segment 34–38cm from the stomach was always incubated with 10mM-glucose present initially in both mucosal and serosal solutions to check that each intestine was absorbing and transporting glucose normally (Tables 1 and 2). Eight intestines were used at each glucose concentration indicated. Each result shown is the mean ± S.D. for 24 determinations. The initial volume of serosal solution in each sac was 0.4ml.

Initial concn. of glucose in the mucosal and serosal solutions (mM)	Changes in the volume of the serosal solution		
	Group I sacs (ml)	Group II sacs (ml)	Group III sacs (ml)
0	+0.04 ± 0.02	+0.02 ± 0.02	+0.01 ± 0.01
1	−0.10 ± 0.02	−0.05 ± 0.03	−0.01 ± 0.01
2	+0.10 ± 0.02	+0.05 ± 0.01	+0.06 ± 0.03
5	+0.15 ± 0.04	+0.12 ± 0.03	+0.10 ± 0.02
10	+0.28 ± 0.06	+0.18 ± 0.03	+0.13 ± 0.02
25	+0.08 ± 0.03	+0.03 ± 0.01	+0.05 ± 0.02
50	+0.03 ± 0.01	+0.02 ± 0.02	+0.02 ± 0.01

Integration of glucose absorption, accumulation, metabolism and transport, lactate secretion and water movement

Lee (1968, 1969) demonstrated the ability of intact rat jejunum to secrete a solution at the serosal surface (mainly via lymph channels), which was iso-osmotic with the solution bathing the mucosal surface of the intestine over a wide range of initial osmolar concentrations. It can now be added (Table 3) that net fluid movement across the rat small intestine into the serosal solution *in vitro* varies with locality along the length of the intestine, but reaches a distinct maximum at all localities when the initial glucose concentration in the iso-osmotic solutions on both sides of the intestine is 10mM. This initial concentration of glucose in the mucosal and serosal solution was adopted for all other experiments.

Since water movement across the intestine *in vitro* is a consequence of net solute transport, the conditions of incubation selected must have been optimum for (i) Na^+ translocation and concomitant glucose translocation from the mucosal solution across the brush border into columnar absorptive cells (Crane *et al.*, 1965), (ii) metabolism of glucose within these cells, (iii) secretion of water and solutes (including glucose and lactic acid or lactate anion) from the mucosal tissue preferentially into the serosal solution, and (iv) operation of transcellular and paracellular routes of solute and solvent movement through intestinal epithelium (Schultz, 1977).

Table 4. *Quantitative metabolic and translocational fate of glucose absorbed from the mucosal solution of everted sacs of rat jejunum*

Four everted sacs (each 4cm, filled initially with 0.4ml of medium) were prepared from the length of a single jejunum between 20 and 50cm from the stomach, incubated and analysed as described under 'Methods' and in Tables 1, 2 and 3, except that D-[U-^{14}C]glucose was added to the mucosal solutions only to give an experimentally determined initial specific radioactivity; the initial concentration of glucose in both mucosal and serosal solution was 10mM. Incubations were terminated after 20min at 37°C under 100% O_2. All radioactivity measurements on glucose and lactic acid were made after chromatographic separation of the two solutes; similar measurements of CO_2 were made after trapping the gas as described under 'Methods'. Values for each of the characteristics (*a*), (*b*), (*c*) and (*d*) of the variables (1)–(7) were determined and calculated for one sac at a time by using the unique values for mucosal-tissue water, initial and final volumes of mucosal and serosal solution for that sac. The mean values shown in the Table were then calculated, along with standard deviations, for the set of four sacs used in this experiment. Values entered against variables (1)–(7) have been corrected to the first decimal place. Values shown against the variables (8)–(11) were calculated in the same way but have been corrected to the second decimal place. Results shown in this Table are analysed further in Table 5, and collated with additional results in Table 6.

		(*a*) Concn. (mM)	(*b*) Amount (μmol)	(*c*) $10^{-4}\times$Radioactivity (d.p.m.)	(*d*) $10^{-4}\times$Specific radioactivity (d.p.m./μmol)
(1) Mucosal solution glucose	Initial	10.0	100.0	1467	14.7
	Final	7.8±0.15	78.2±1.5	1140.9±25.3	14.6±0.4
	Absorbed	—	21.7±1.5	318.6±20.9	14.7±0.9
(2) Mucosal tissue glucose	Initial	0	0	0	0
	Final	16.7±0.9	6.5±0.5	94.5± 6.1	14.5±1.0
(3) Serosal solution glucose	Initial	10.0	4.0	0	0
	Final	13.7±0.3	8.9±0.2	—	—
	Transported	—	4.9±0.2	88.2± 2.5	18.0±0.9
(4) Total CO_2 formed	Initial	—	0	0	0
	Final	—	9.2±0.5	12.1± 1.5	1.3±0.1
(5) Mucosal solution lactate	Initial	0	0	0	0
	Final	0.5 ±0.1	5.0±0.8	35.9± 5.5	7.2±0.6
(6) Mucosal tissue lactate	Initial	0	0	0	0
	Final	25.2±2.4	9.9±0.9	730.7±35.1	7.4±0.5
(7) Serosal solution lactate	Initial	0	0	0	0
	Final	7.8±0.7	5.1±0.4	36.0± 2.3	7.2±0.6

(8) Mean wet wt. of whole intestinal tissue (g): 0.79±0.04
(9) Mean wet wt. of mucosal tissue (g): 0.53±0.03
(10) Mean mucosal-tissue water (ml): 0.39±0.03
(11) Mean increment in serosal solution volume (ml): 0.25±0.01

Quantitative fate of absorbed glucose

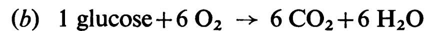
Table 4 records the amounts of glucose absorbed, accumulated and transported at the end of a 20min incubation, the lactate formed, the total CO_2 formed and the observed specific radioactivities of glucose and its metabolites in various compartments of four everted sacs. The distribution and recovery of the absorbed glucose and of the absorbed radioactivity in these sacs is given in Table 5. Since each of these two recoveries in this and similar experiments was close to 100% (Table 6), it is legitimate to conclude from the observed specific radioactivities that all of the glucose accumulated in the mucosal tissue and all of the lactate (in all compartments of the everted-sac system) originated solely in the glucose absorbed from the mucosal solution during the incubation; it follows that pyruvate, the immediate precursor of lactate, must also have originated from the same source. About half of the total CO_2 originated from sources other than the absorbed glucose (Table 6). All calculations of observed specific radioactivities were made on the assumption that glucose transport resulted solely from a unidirectional flux of glucose from the external mucosal solution through the tissue into the serosal solution (Fig. 6). It has been shown (Porteous & Pritchard, 1972) that the calculated specific radioactivity of the glucose entering the serosal solution will then be anomalously high (Table 6) if in fact there is a bidirectional time-dependent flux of glucose across the boundary between the intestinal tissue and the external solution. It is then possible to conclude (Table 6) that the glucose entering the serosal solution originated from the glucose absorbed from the mucosal solution and was not diluted en route by unlabelled glucose derived from other sources.

Stoicheiometry of glucose metabolism during glucose transport through everted sacs

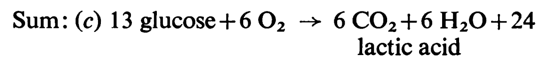
Eleven experiments using unlabelled glucose but otherwise conducted in the same way as those recorded in Tables 4, 5 and 6 gave the following information: glucose metabolized, 13.2 μmol; total lactate formed, 21.5 μmol; total CO_2 produced, 11.6 μmol, all per g wet wt. of intestinal tissue during a 20 min incubation. This last value may be restated as 5.8 μmol of CO_2 produced from the glucose absorbed per g wet wt. of intestinal sac during 20 min if account is taken of the observed and expected radioactivities of CO_2 shown in Table 6.

The stoicheiometry of columnar absorptive-cell metabolism of glucose during translocation of the sugar through the intestine under steady-state conditions *in vitro* therefore approximates closely to:

(*a*) 12 glucose → 24 lactic acid



(*b*) 1 glucose + 6 O_2 → 6 CO_2 + 6 H_2O



Sum: (*c*) 13 glucose + 6 O_2 → 6 CO_2 + 6 H_2O + 24 lactic acid

Results from the independent experiments shown in Table 6 are consistent with these equations.

Table 5. *Distribution of glucose and radioactivity absorbed from the mucosal solution of everted sacs of rat jejunum*

All calculations, except those relating to total lipids, protein and nucleic acids, are based on results shown in Table 4. The amount of glucose absorbed by individual sacs was taken as 100; the amounts of glucose and of metabolites found at the end of the 20 min incubation in various 'compartments' of the everted-sac system were then calculated as a fraction of the glucose absorbed by each sac. The Table records the mean values, together with the standard deviations, for the percentage distribution of the glucose absorbed by a set of four sacs. The distribution of absorbed radioactivity was calculated in the same way. All calculated values have been corrected to the first decimal place.

	Percentage distribution of absorbed glucose	Percentage distribution of absorbed radioactivity
Mucosal-tissue glucose	30.1 ± 2.5	29.8 ± 3.7
Serosal solution glucose	22.5 ± 0.8	27.8 ± 2.3
Total CO_2 formed	7.1 ± 0.7	3.8 ± 0.5
Mucosal solution lactate	11.6 ± 2.6	11.3 ± 1.8
Mucosal-tissue lactate	22.7 ± 0.8	22.9 ± 4.1
Serosal solution lactate	11.6 ± 1.3	11.4 ± 1.3
Total lipids, Total nucleic acids, Total protein	—	0.4 ± 0.1
Recovery of absorbed glucose	105.2 ± 4.9	—
Recovery of absorbed radioactivity	—	107.4 ± 8.6

Lactate dehydrogenase and pyruvate dehydrogenase activities of mucosal tissue

Correlations have been made between the dominant species of L-lactate dehydrogenase isoenzyme present in a tissue and the capacity of the tissue to produce or metabolize L-lactic acid; a unique mode of regulation of the activity of the heart (H_4) isoenzyme has also been proposed (Everse & Kaplan, 1973). Identification of the L-lactate dehydrogenase isoenzyme(s) present in intestinal mucosal tissue was therefore imperative. The electrophoretic migration pattern of mucosal-tissue lactate dehydrogenase (Fig. 4) was like that of skeletal muscle (M_4 isoenzyme) and unlike that of heart muscle (predominantly H_4 isoenzyme). The L-lactate dehydrogenase

Table 6. *Quantitative metabolic and translocational fate of glucose absorbed from the mucosal solution of everted sacs of rat jejunum: proof that all solutes measured had their origin in the glucose absorbed from the mucosal solution*

The results shown are the calculated means (±S.D.) from four sets of four determinations including the set shown in detail in Table 4. The overall recovery of glucose absorbed from all 16 mucosal solutions was 103 ± 9%; the overall recovery of radioactivity absorbed as glucose from the mucosal solutions was 107 ± 12%. The expected specific radioactivities shown in the last column were calculated on the assumption that there was a unidirectional flux of glucose from the mucosal solution to the serosal solution and that neither the absorbed glucose nor its metabolites suffered dilution by unlabelled materials.

	Amount of solute (μmol/g wet wt. of tissue)	10^{-4} × Specific radioactivity of solute (d.p.m./μmol)	
		Observed	Expected
Glucose absorbed from mucosal solution	27.1 ± 2.0	15.0 ± 0.93	14.7 ± 0.10
Glucose accumulated in mucosal solution	8.4 ± 0.65	14.7 ± 0.61	14.7 ± 0.10
Glucose transported into serosal solution	6.0 ± 1.60	20.2 ± 2.07	14.7 ± 0.10
Lactate formed and accumulated in the mucosal tissue	13.4 ± 1.51	7.83 ± 0.82	7.35 ± 0.05
Lactate secreted into the mucosal solution	4.2 ± 0.58	7.34 ± 0.47	7.35 ± 0.05
Lactate secreted into the serosal solution	6.1 ± 0.71	7.42 ± 0.48	7.35 ± 0.05
Total CO_2 formed	12.0 ± 0.54	1.23 ± 0.11	2.45 ± 0.02

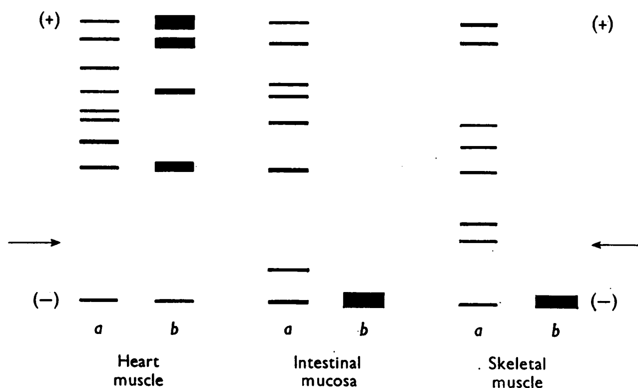


Fig. 4. *Gel-electrophoresis patterns obtained with cell-sap preparations from heart muscle, intestinal mucosa and skeletal muscle of a single rat*

Samples (30–40 μl from the cell-sap fraction of a tissue homogenate prepared at a concentration of 0.5 g of tissue/ml) were applied at positions indicated by the arrows. Half of each gel was subsequently stained (*a*) for protein, the other half (*b*) for lactate dehydrogenase activity as described in the text.

activity of the freshly prepared cell-sap fraction of rat jejunal mucosal tissue was 72 μmol of pyruvate reduced/min per g wet wt. of whole intestinal tissue. The pyruvate dehydrogenase activity of the corresponding freshly isolated mucosal-tissue mitochondria was 0.23 μmol of pyruvate oxidized/min per g wet wt. of whole intestinal tissue.

Additional observations

Mucosal-tissue glycogen. The glycogen content of the mucosal tissue of freshly isolated rat jejunum was 50–75 μg of glycogen/g wet wt. of mucosal tissue, of which about 25% was soluble in cold acid. The total glycogen content of the mucosal tissue decreased by about 30% during incubation of everted sacs for 20 min in the absence of added glucose, and increased, in the presence of 10 mM-glucose, by 30% in sacs prepared from the proximal half of a 70 cm intestine, and by 130% in sacs prepared from distal segments of intestine. Glycogen is thus not a major component of mucosal tissue, does not serve as a significant endogenous substrate during incubation of jejunum *in vitro* and is not a major end product of glucose metabolism in the jejunum *in vitro*.

Glucose incorporation into lipids, proteins and nucleic acids. Less than 1% of the radioactivity of [U-^{14}C]-glucose absorbed by everted sacs was incorporated into mucosal tissue mono-, di- and tri-acylglycerols, phospholipids, cholesterol, proteins and nucleic acids (Table 5) during 20 min incubations with 10 mM-glucose. Glucose is thus transported unchanged from the mucosal solution into the serosal solution, or catabolized en route (Table 6), but not incorporated to any significant extent into larger molecules under the experimental conditions used.

Contribution of submucosal tissue. Intestinal muscle and serosal tissue incubated under the same conditions as those used for everted sacs produced less than 10% of the lactate or CO_2 formed by the whole intestinal tissue. Everted sacs incubated with D-[U-^{14}C]glucose in the serosal solution, rather than in the mucosal solution, confirmed these results.

Effect of metal cation concentration on glucose absorption and transport by everted sacs. Twofold increases or decreases in the K^+, Ca^{2+} or Mg^{2+} concentrations normally used in the mucosal solution had little effect on glucose absorption or transport. Diminution or supplementation of the Na^+ concentration (147 mequiv./litre) normally used in the mucosal solution decreased both glucose absorption from the mucosal solution and glucose transport into the serosal solution. Transport of glucose was always more severely affected than was absorption of glucose; thus halving or doubling the Na^+ concentration in the mucosal solution decreased glucose absorption by 50% but abolished glucose transport completely. Glucose absorption was decreased to half the normal rate when 80% of Na^+ in the mucosal solution was replaced by mannitol, when 40% of the Na^+ was replaced by $Tris^+$ or when 25% of the Na^+ was replaced by K^+. Bihler & Crane (1962) observed a non-linear increase in tissue accumulation of non-metabolizable sugars with increases in Na^+ concentration in the suspending medium up to 217 mequiv. of Na^+/litre; transport of sugars across the tissue into a separate compartment was not possible in these experiments. Our observation of an optimum Na^+ concentration (147 mequiv. of Na^+/litre) for both

glucose absorption and transport does not deny the Na^+-dependence of the glucose absorption step (Bihler & Crane, 1962), but most probably reflects the balance between the Na^+-dependence of this event and the Na^+-dependence of other concomitant events in the whole tissue during glucose absorption, metabolism and transport (Schultz & Zalusky, 1964; Csáky & Hara, 1965; Smyth, 1966; Mitchell, 1967; Robinson, 1967, 1970). Our observations justify the use of mucosal and serosal solutions which were initially iso-osmotic, and the use of a Na^+ concentration of 147 mequiv./litre in the mucosal solution.

Effect of the principal anions in the incubation medium on glucose absorption and transport. Everted sacs prepared from the upper half of rat small intestine exhibited 15–40% higher rates of glucose absorption when incubated in Krebs–Ringer bicarbonate medium (pH 7.2) under O_2/CO_2 (19:1) rather than under the standard conditions used in all other experiments; sacs prepared from the lower half of the small intestine did not show this difference in the rate of absorption of glucose when incubated in the alternative buffer solutions. Transport of glucose into the serosal solution was 20% faster in sacs prepared from all segments of the small intestine when they were incubated under the standard conditions adopted for the present study, rather than in the bicarbonate medium.

Physiological state of experimental animals before anaesthesia. We observed meagre transport of glucose across everted sacs prepared from jejuna of rats which were inexplicably excited before anaesthesia; everted sacs prepared from rats rendered docile as described under 'Methods' invariably exhibited obvious glucose transport, and much more consistent results were obtained between sets of sacs prepared from separate animals. Robinson *et al.* (1966) concluded that the transport function of intestinal epithelial cells *in vivo* was more susceptible to temporary O_2 deprivation than was cell integrity; Folklow *et al.* (1964) and Greenway & Lawson (1966) observed that adrenaline constricted blood vessels in intestinal villi (Fig. 6) and dilated those in deeper intestinal tissue, so diverting blood from the villus vasculature (and adjacent columnar absorptive cells) without affecting total blood flow to and from the intestine as a whole. It is thus possible that the observed poor and variable transport of glucose through everted sacs prepared from excited rats was a consequence of a temporarily restricted O_2 supply to the epithelium *in vivo* resulting from excessive adrenaline secretion immediately before anaesthesia. The Wistar strain of rat is reported (Caygill & Stein, 1967) to be more excitable than other strains.

Choice of anaesthetic. The apparent importance of maintaining the O_2 supply to the intestine during its isolation from the animal suggests that more attention should be given to choice of anaesthetic and to the management of the anaesthetized animal. We avoided phenobarbitone because of its known depression of respiration, and selected a highly volatile anaesthetic in the belief that its rapid dissipation from excised tissue under the conditions described would minimize any possible deleterious effects of the anaesthetic on intestinal functions. Two alternatives to diethyl ether, namely halothane and cyclopropane, were rejected because the former is known to cause hyperthermia (at least in susceptible species) and the latter stimulates release of adrenaline. Levine *et al.* (1970) drew attention to the superior survival of the epithelium of jejunal everted sacs prepared from rats anaesthetized with diethyl ether or sodium pentobarbital rather than with other anaesthetics; the strictures placed by these authors on the everted-sac system apply to conditions of incubation frequently used in the past, but not to the conditions selected for use in the present investigations.

Correlation of translocational and metabolic fluxes in the everted-sac system

The mean values of determinations of variables shown in Tables 6 and Fig. 3 have been used to assemble Figs. 5(*a*) and 5(*b*) in which the structurally and functionally polarized columnar absorptive epithelial cell is represented as a rectangular frame with the striations of the brush border facing the mucosal solution of an everted-sac preparation. The epithelial cell is divided into a cytosolic and mitochondrial compartment, the latter delineated by the inner rectangular frame with a serrated outline. The subepithelial tissue of the core of an intestinal villus, and the musculature of the small intestine (cf. Fig. 6), are together represented by the rectangle with a broken outline in Figs. 5(*a*) and 5(*b*); solutes and water passing from the epithelial cell to the serosal solution, as depicted in these two diagrams, are envisaged as flowing through the vascular and lymph channels of the villus (Fig. 6). In Fig. 5(*b*), the total mean CO_2 produced has been assigned to two origins (glucose and X) in proportions deduced from a comparison of the observed and expected specific radioactivities of the total CO_2 produced (Table 6). The fraction of the total O_2 consumption attributable to oxidation of glucose followed from the stoicheiometry of glucose oxidation; water formed in the same catabolic reaction was similarly deduced. The remainder of the total O_2 metabolized (Fig. 3) has been assigned to the mitochondrial catabolism of the unspecified substrate(s) X; as the composition of the compound(s) X is not known, precise deduction of the metabolic water formed by complete oxidation of X is not possible, but the value shown will not be much in error for compound(s) X which sustained a

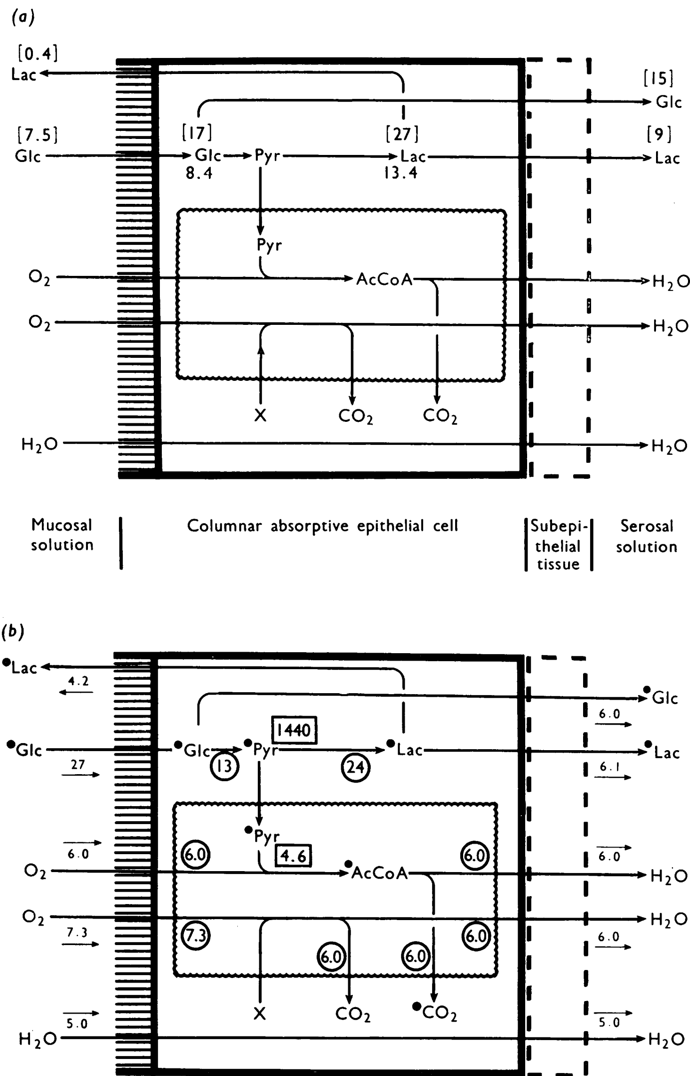


Fig. 5. *Diagrammatic representation of translocational fluxes through and metabolic fluxes within the columnar absorptive cells of everted sacs of rat jejunum*

In (*a*) the abbreviations Glc, Pyr, Lac, AcCoA represent glucose, pyruvate, lactate, acetyl-CoA respectively; X represents unspecified endogenous substrate(s). Values shown thus [15] indicate the observed concentrations of solutes (mM) after a 20min incubation; any accompanying values (e.g. 8.4) indicate the amount of that solute present at the concentration shown. Concentrations and amounts are related by calculations of the kind shown in Table 4. The initial concentration of glucose in the mucosal and serosal solutions was 10mM; the initial concentration of lactate in these two solutions was zero. In (*b*), metabolic and translocational fluxes determined in the same set of experiments are shown. All values in this diagram are stated to two significant digits as μmol/20min per g wet wt. of whole intestinal tissue. The same abbreviations are used for substrates and products, but radioactively labelled solutes are distinguished by a preceding dot (e.g. •Glc); results are taken from experiments (Table 6) in which radioactively labelled glucose was present initially only in the mucosal solution, and are supplemented by results from Fig. 3 (O_2 metabolized). Values shown thus $\overrightarrow{27}$, $\overrightarrow{5}$ indicate the observed amount of solute or water translocated across one or more membranes (translocational fluxes); values shown thus ⑬ indicate intracellular metabolic fluxes. In the same diagram, the lactate dehydrogenase and pyruvate dehydrogenase activities determined on isolated subcellular fractions (see the text) are set within small rectangular frames (thus [4.6]) against the reaction catalysed.

respiratory quotient of 0.82. It has been assumed that the total metabolic water contributed to the observed increment of fluid in the serosal solution. This increment was of the order of 300 μl (17 μmol)/g wet wt. of intestinal tissue during a 20 min incubation (Table 4); the increment in serosal solution volume not accounted for by metabolic water has then been shown (Fig. 5*b*) as water translocated from the mucosal solution through the tissue into the serosal solution. Only one other assumption has been made, namely that steady metabolic and translocational fluxes were established immediately at the start of the incubation period. As steady tissue concentrations of glucose and lactic acid were not shown to be established until the first 5 min of incubation had elapsed, this second assumption may not be strictly valid, but it is unlikely that any significant error has been introduced by making this assumption; measurements of the transformations and translocations collated in Fig. 5 were made after the lapse of 20 min to minimize any such possible error.

So far as we are aware, the results collated in Fig. 5 represent the first attempt to record quantitative steady-state translocational fluxes of glucose and its metabolites through the small intestine *in vitro*, and to correlate such fluxes with concomitant steady-state metabolic fluxes under conditions in which all related components of the system were quantitatively accounted for chemically and radiochemically, and unambiguously traced to the glucose absorbed from one of the two solutions bathing the exterior of the tissue. Of the glucose absorbed from the mucosal solution under conditions sustaining steady translocational and metabolic fluxes, about 50% was found as free glucose (25% transported into the serosal solution plus 25% temporarily accumulated in the mucosal tissue en route for the serosal solution); only 50% of the glucose absorbed from the mucosal solution was metabolized, and of this fraction, about 90% was converted into lactate and 10% into CO_2. The total O_2 metabolized exceeded that required to account for the observed conversion of glucose, via pyruvate, into CO_2.

The flux diagram of Fig. 5 thus poses four separate but related questions. (i) What intracellular parameters and variables determine the partitioning of pyruvate between the two end products of its catabolism, CO_2 and lactate? (ii) In particular, what rate of respiration would be required to shift the proportion of glucose metabolized to CO_2 from the observed value of 10% to, say, 50%? (iii) What increase in the rate of respiration would be required before the Pasteur effect could be demonstrated (that is, before a significant diminution of the total flux of glucose to all end products of glucose catabolism is observed as the rate of O_2 metabolism is increased)? (iv) Are translocational and metabolic fluxes quantitatively interdependent and, if so, what molecular mechanisms determine the interdependence? Does the rate of respiration determine the proportion of the absorbed glucose which is transported unchanged into the serosal solution (that is, into the circulation *in vivo*) or is some other variable or characteristic of the experimental system the determining factor?

With respect to question (i), we have determined the activities of the two enzymes catalysing the first metabolic transformations of pyruvate beyond the branch point in the catabolism of glucose via pyruvate (Fig. 5). It may be significant that the lactate dehydrogenase activity of mucosal tissue was 60-fold greater than that required to accommodate the observed flux of glucose, via pyruvate, to lactate, whereas the pyruvate dehydrogenase activity was little more than twice that required to accommodate the observed flux of glucose, via pyruvate, to CO_2 (Fig. 5). It is tempting to suppose that inhibition of pyruvate dehydrogenase activity in the intact tissue, to one-half of the activity observed in mitochondria isolated from that tissue, might have provided the rate-limiting parameter which could account for the low proportion of glucose converted into CO_2 (Fig. 5). This and related possibilities have been examined in experiments with columnar absorptive cells isolated from rat jejunum (Porteous & Towler, 1975; Porteous, 1977).

Discussion of questions (ii), (iii) and (iv) cannot be separated from an examination of two possible limitations of the everted-sac system, namely the absence of a sustained induced flow of fluid through the capillaries of the villi and underlying submucosal tissue, and the possible limited rate of access of O_2 to the whole intestinal tissue. It is reasonable to assume (Porteous & Pritchard, 1972) that water and solutes leaving the epithelial lining of the everted sac (Fig. 6) reach the serosal solution via venules and lymph ductules in the core of each villus, that is via channels used *in vivo*. But even if none of these channels collapses in an everted sac, the mere absence of a sustained induced flow of fluid through the capillaries of the villi would be expected to diminish markedly the rate of exit of material from the epithelial cells into the serosal solution of an everted sac. The absence of a continuous flow of fluid of physiological osmolarity and at physiological hydrostatic pressure through the capillaries would minimize the normal production of lymph and its unidirectional flow out of the villi of the intestine; the efflux of material from the epithelial lining of the villi into the serosal solution of an everted sac would thus again be impeded. The high concentrations of glucose and lactate in the mucosal tissue (Table 4, Fig. 5*a*) probably reflect a rapid absorption of glucose from the mucosal solution across the brush border of the columnar absorptive cells and rapid metabolism of the intracellular glucose (Fig. 5*a*), but also a relatively impeded efflux of glucose and lactate from the

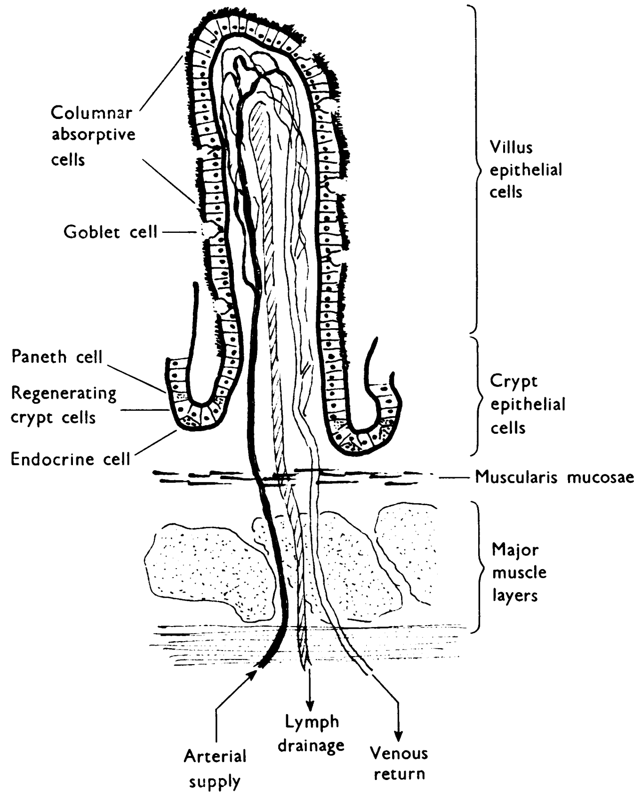


Fig. 6. *Diagrammatic representation of the structure of a villus of small intestine with particular reference to the relationship between the columnar absorptive cells, the vascular circulation and the lymph drainage of the villus*

The lumen of the small intestine *in vivo* is lined by the crypt and villus epithelial cells. In the everted-sac preparation, these epithelial cells are in contact with the extracellular mucosal solution; the extracellular serosal solution of this preparation is adjacent to the two major muscle layers shown (cf. Fig. 5). The mucosal tissue referred to in the text comprises the epithelial tissue plus the subepithelial tissue down to and including the muscularis mucosae. Anastomoses between the arterial supply and the venous drainage, which permit controlled shunting of blood past the villi *in vivo*, are not shown in this diagram.

epithelial cells and subepithelial tissue into capillaries and lymph ducts (Fig. 6) for the reasons just given. We regard this absence of induced flow of fluid through the vasculature and lymph ducts of intestinal tissue as the important deficiency of the everted-sac system (to be set against the many advantages of the system which have made the construction of Fig. 5 possible). Given a less impeded efflux of glucose from the columnar absorptive epithelial cells, it is possible that a smaller proportion of the absorbed glucose would be metabolized and a correspondingly larger proportion transported unchanged into the serosal solution (Fig. 5); a change in respiration rate would not necessarily be called for to achieve this increased transport of glucose from mucosal to serosal solution.

The predominant conversion into lactate of half of the glucose absorbed from the mucosal solution (Fig. 5*b*) might be taken to indicate some inadequacy in the rate of supply of O_2 to the everted sacs, but this is not necessarily a logical conclusion. Other explanations for the relatively high rate of lactate production and relatively low rate of CO_2 production appear to be equally tenable and require examination (Porteous & Towler, 1975; Porteous, 1977). These include the possible inhibition of pyruvate dehydrogenase activity alluded to above, and inhibition of the mechanisms mediating pyruvate translocation from the cell sap into the mitochondrial matrix.

Hanson & Parsons (1976) drew attention to the extracellular barrier to O_2 consumption created by an unstirred layer of fluid in contact with the mucosal

Table 7. *Comparison of respiration rates observed with everted sacs and preparations of vascularly and luminally perfused intestine incubated under defined conditions in vitro*

Respiration rates obtained in the present investigation (Figs. 3 and 6) have been recalculated per g dry wt. of tissue (wet wt./dry wt. of whole intestinal tissue = 6.0, determined on six sets of everted sacs) to provide a comparison with published values; all results are expressed to the nearest 5 μmol of O_2.

Reference	Experimental system	Respiration rate (μmol/h per g dry wt.)
Wilson & Wiseman (1954*a,b*)	Everted sacs, rat jejunum. Krebs–Ringer phosphate medium; 17 mM-glucose; 37°C; 100% O_2; Warburg respirometer, 70–90 oscillations/min; duration 1 h.	890
Bronk & Parsons (1965)	Everted sacs, rat jejunum. Krebs–Ringer bicarbonate medium (pH 7.5); 28 mM-glucose; 38°C; air/CO_2 (19:1); O_2 electrode, stirred medium, 100 rev./min; duration 3–4 min.	180
Levin & Syme (1975)	Everted sacs, rat jejunum. Krebs–Henseleit bicarbonate medium; 28 mM-glucose; 37°C; (*a*) O_2/CO_2 (19:1), (*b*) air/CO_2 (19:1); O_2 electrode, stirred medium; duration 3×5 min.	(*a*) 360–440 (*b*) 120–140
Present paper	Everted sacs, rat jejunum. Krebs–Ringer phosphate medium (pH 7.2); 10 mM-glucose; 37°C; 100% O_2; Warburg respirometer, 80 oscillations/min; duration 5–40 min.	240
Windmueller & Spaeth (1972)	Vascularly and luminally perfused rat small intestine plus caecum plus half of colon. Vascular circuit: fresh rat blood+2.8 mM-glucose equilibrated with O_2/CO_2 (19:1). Luminal circuit: Earle's medium+220 mM-glucose. O_2 consumption monitored continuously as the arteriovenous difference in O_2 content of the vascular circuit; duration 5 h.	310
Hanson & Parsons (1976)	Vascularly and luminally perfused rat jejunum. Vascular circuit: Krebs–Ringer bicarbonate+washed and aged bovine erythrocytes (haematocrit 15%)+10 mM-glucose equilibrated with O_2/CO_2 (19:1). O_2 consumption measured by manometric analysis of vascular perfusion medium; duration 5 min (20–25 or 45–50 min).	385 (mean of two values)

tissue during relatively slow, undisturbed, luminal perfusion of small intestine with an O_2-saturated solution; the presence of such an unstirred layer of solution adjacent to the brush borders of columnar absorptive cells in a vigorously agitated everted-sac preparation cannot be discounted, but seems inherently less likely. A comparison of respiration rates reported for everted sacs, and for the vascularly and luminally perfused intestine, does not reveal a consistent failure of everted sacs to respire as rapidly as the perfused intestine (Table 7). Nevertheless, the respiration rate observed in the present investigation was only 77% of that reported by Windmueller & Spaeth (1972) and only 62% of that reported by Hanson & Parsons (1976). But as Windmueller & Spaeth (1972) observed no net conversion of glucose into lactate, whereas Hanson & Parsons (1976) observed a substantial conversion of glucose into lactate despite a higher respiration rate (Table 7), it may reasonably be concluded from the limited information available that respiration rate is not the only variable determining the proportion of absorbed glucose converted into lactate by the small intestine. Conversely, lactate production cannot yet be used as the sole index of the adequacy or inadequacy of O_2 supply to preparations of intestine in which concomitant metabolic and translocational fluxes are being maintained.

Since respiration, in an intact cell or tissue, is obligatorily coupled to other intracellular and transcellular events, the most pressing problem is to understand how O_2 and all other substrates are metabolized, and their metabolism regulated, in order to sustain concomitant translocational and metabolic fluxes in the intact intestine and within its columnar absorptive cells. Results collated in Fig. 5 represent part of an attempt to attain such an understanding.

We thank Unilever Ltd., Colworth House, Sharnbrook, Bedford, U.K. for a Postgraduate Studentship awarded to P. J. P. Some of the results reported here have been reviewed (Porteous, 1977); we thank the editor (Professor Martin Kramer) and the publishers of *Intestinal Permeation* (Excerpta Medica, Amsterdam) for permission to reproduce Table 6.

References

Bihler, I. & Crane, R. K. (1962) *Biochim. Biophys. Acta* **59**, 78–93

Bihler, I. & Cybulsky, R. (1973) *Biochim. Biophys. Acta* **298**, 429–437

Bihler, I., Hawkins, K. A. & Crane, R. K. (1962) *Biochim. Biophys. Acta* **59**, 95–102

Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285

Bronk, J. R. & Parsons, D. S. (1965) *Biochim. Biophys. Acta* **107**, 397–404

Burton, K. (1956) *Biochem. J.* **62**, 315–323

Caygill, C. P. J. & Stein, W. D. (1967) *Biochem. J.* **105**, 17P

Crane, R. K. & Mandelstam, P. (1960) *Biochim. Biophys. Acta* **45**, 477–482

Crane, R. K., Forstner, G. & Eichholz, A. (1965) *Biochim. Biophys. Acta* **109**, 467–477

Csáky, T. Z. & Hara, Y. (1965) *Am. J. Physiol.* **209**, 467–472

De Luca, H. F. & Cohen, P. P. (1964) in *Manometric Techniques* (Umbreit, W. W., Burris, R. H. & Stauffer, J. C., eds.), 4th edn., p. 119, Burgess Publishing Co., Minneapolis

Esposito, G., Faelli, A. & Capraro, V. (1967) *Arch. Int. Physiol. Biochim.* **75**, 601–608

Everse, J. & Kaplan, N. O. (1973) *Adv. Enzymol. Relat. Areas Mol. Biol.* **37**, 61–133

Fisher, R. B. & Parsons, D. S. (1950) *J. Physiol. (London)* **110**, 281–293

Folklow, B., Lewis, D. H., Lundgren, O., Mellander, S. & Wallentin, I. (1964) *Acta Physiol. Scand.* **61**, 458–466

Greenway, C. V. & Lawson, A. E. (1966) *J. Physiol. (London)* **186**, 579–595

Hanson, P. J. & Parsons, D. S. (1976) *J. Physiol. (London)* **255**, 775–795

Hassid, W. Z. & Abraham, S. (1957) *Methods Enzymol.* **3**, 34–54

Higgins, H. & von Brand, T. (1966) *Anal. Biochem.* **15**, 122–126

Hohorst, H. J. (1956) *Biochem. Z.* **328**, 509–521

Kinter, W. B. & Wilson, T. H. (1965) *J. Cell Biol.* **25**, 19–39

Kohn, J. (1958) *Clin. Chim. Acta* **3**, 450–454

Kornberg, A. (1955) *Methods Enzymol.* **1**, 441–443

Lamers, J. M. J. & Hülsmann, W. C. (1972) *Biochim. Biophys. Acta* **275**, 491–495

Lauterbach, F. (1972) *Hoppe-Seyler's Z. Physiol. Chem.* **353**, 731

Lee, J. S. (1968) *Gastroenterology* **54**, 366–374

Lee, J. S. (1969) *Q. J. Exp. Physiol.* **54**, 311–321

Leese, H. J. & Bronk, J. R. (1975) *Biochim. Biophys. Acta* **404**, 40–48

Leese, H. J. & Mansford, K. R. L. (1971) *J. Physiol. (London)* **212**, 819–838

Levin, R. J. & Syme, G. (1975) *J. Physiol. (London)* **245**, 271–287

Levine, R. R., McNary, W. F., Kornguth, P. J. & LeBlanc, R. (1970) *Eur. J. Pharmacol.* **9**, 211–219

Lis, E. W., Tinoco, J. & Okey, R. (1961) *Anal. Chem.* **2**, 100–106

Lusty, C. J. & Singer, T. P. (1964) *J. Biol. Chem.* **239**, 3733–3742

McDougal, D. B., Little, K. D. & Crane, R. K. (1960) *Biochim. Biophys. Acta* **45**, 483–489

Miller, C. L. (1959) *Anal. Chem.* **31**, 964

Mitchell, P. (1967) *Adv. Enzymol. Relat. Areas Mol. Biol.* **29**, 33–87

Morgan, D. M. & Kingsbury, K. J. (1959) *Analyst (London)* **84**, 409–414

Murer, H., Hopfer, U., Kinne-Saffran, E. & Kinne, R. (1974) *Biochim. Biophys. Acta* **345**, 170–179

Newey, H., Parsons, B. J. & Smyth, D. H. (1959) *J. Physiol. (London)* **148**, 83–92

Paul, J. (1958) *Analyst (London)* **83**, 37–42

Porteous, J. W. (1977) in *Intestinal Permeation* (Kramer, M., ed.), Excerpta Medica, Amsterdam, in the press

Porteous, J. W. & Clark, B. (1965) *Biochem. J.* **96**, 159–171

Porteous, J. W. & Pritchard, P. J. (1972) *Biochem. J.* **127**, 80P

Porteous, J. W. & Towler, C. M. (1975) *Abstr. FEBS Meet.* abstr. 1434

Poulik, M. D. (1957) *Nature (London)* **180**, 1477–1479

Robinson, J. W. L. (1967) *Pflügers Arch. Gesamte Physiol. Menschen Tieren* **294**, 182–200

Robinson, J. W. L. (1970) *Comp. Biochem. Physiol.* **34**, 641–655

Robinson, J. W. L., Antonioli, J. A. & Mirkovitch, V. (1966) *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **255**, 178–191

Schneider, W. C. (1957) *Methods Enzymol.* **3**, 680–691

Schultz, S. G. (1977) in *Intestinal Permeation* (Kramer, M., ed.), Excerpta Medica, Amsterdam, in the press

Schultz, S. G. & Zalusky, R. (1964) *J. Gen. Physiol.* **47**, 1043–1059

Smyth, D. H. (1966) *Excerpta Med. Sect. 23* **1**, 195–205

Tejwani, G. A., Kaur, J., Ananthanarayanan, M. & Ramaih, A. (1974) *Biochim. Biophys. Acta* **370**, 120–129

Vessel, E. S. (1961) *Ann. N.Y. Acad. Sci.* **94**, 877–889

Vomhof, D. W. & Tucker, T. C. (1965) *J. Chromatogr.* **17**, 300–306

Wagner, H., Hörhammer, L. & Wolff, P. (1961) *Biochem. Z.* **334**, 175–184

Waring, P. P. & Ziporin, Z. Z. (1964) *J. Chromatogr.* **15**, 168–172

Wilson, T. H. (1953) *Biochim. Biophys. Acta* **11**, 448–449

Wilson, T. H. (1954) *Biochem. J.* **56**, 521–527

Wilson, T. H. (1956) *J. Biol. Chem.* **222**, 751–763

Wilson, T. H. & Wiseman, G. (1954*a*) *J. Physiol. (London)* **123**, 116–125

Wilson, T. H. & Wiseman, G. (1954*b*) *J. Physiol. (London)* **123**, 126–130

Windmueller, H. G. & Spaeth, A. E. (1972) *J. Lipid Res.* **13**, 92–105