

## A KINETIC APPROACH TO THE STUDY OF ABSORPTION OF SOLUTES BY ISOLATED PERFUSED SMALL INTESTINE

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*(Received 31 December 1973)*

### SUMMARY

1. A new technique has been developed for making serial measurements of water and solute absorption from the lumen of isolated small intestine.
2. The isolated intestine is perfused in a single pass with a segmented flow of slugs of liquid separated by bubbles of oxygen-carbon dioxide mixture. Simultaneous collections are made of effluent from the lumen and of the fluid which is transported across the mucosa. This latter fluid appears to be a fair sample of the tissue fluid.
3. Conditions in the lumen can be changed within less than 5 min. The effects of two or more treatments applied to the same segment of intestine can be determined and the time course of a change in luminal conditions.
4. The rate of appearance of solutes on the serosal side depends on the rate of water absorption, and changes exponentially towards a steady state. The rate constant is a function of tissue fluid volume.
5. In the steady state the concentration of glucose in the tissue fluid is 71 mM when the luminal concentration is 28 mM, and is 45 mM when the luminal concentration is 8.3 mM.
6. For solutes such as glucose for which reflux from tissue fluid to lumen is small relative to flux from lumen to tissue fluid, the time of attainment of a steady state in secretion is usually 50–60 min.
7. For solutes such as sodium for which the reflux is relatively high, the steady state may be reached in 15–20 min.
8. The  $K_m$  for glucose absorption (14–19 mM) is much lower than is found with unsegmented flow perfusion.
9. These findings emphasize problems in interpreting results from other types of intestinal preparation.
10. The rate of glucose absorption from the lumen falls only gradually when the luminal sodium concentration is reduced abruptly. In contrast

the rate of glucose absorption falls suddenly when the luminal glucose concentration is reduced abruptly. This suggests that glucose absorption is not directly dependent on luminal sodium ions.

#### INTRODUCTION

A new technique for study of absorption from isolated intestine has been developed in which serial measurements of rate of absorption from the lumen can be made at short intervals, and also the fluid transported across the mucosa ('secretion') can be collected, as in the method of Smyth & Taylor (1957), though in the present method collections are made at short intervals. The isolated intestine is perfused through its lumen with a segmented flow, i.e. a sequence of slugs of perfusate separated by bubbles of oxygen-carbon dioxide mixture. It is possible to change from one steady state in the lumen to another within less than 5 min; therefore the effects of two or more treatments applied to the same segment of intestine can be determined consecutively, and also the rate of response to a change of luminal conditions.

This technique reveals that the rate of appearance of solutes on the serosal side of the mucosa is a function of the rate of water transport across the intestinal wall. The change in concentration of solutes in the collections of transported fluid following a step change in rate of absorption from the lumen is a simple exponential function of the cumulative volume of water transported. In the simplest instances the rate constant is the reciprocal of tissue fluid volume. Where there are bidirectional fluxes the rate constant is higher, by a factor equal to the ratio of the unidirectional flux rate into the tissue fluid to the net flux rate across the mucosa (Bywater, Fisher & Gardner, 1972).

This technique has been used to make a preliminary examination of the effect of changes in luminal sodium concentration on the rate of glucose absorption, and shows that glucose absorption is not directly dependent on luminal sodium concentration.

#### METHODS

*Animals.* Female albino rats, of a local strain, weighing 200–230 g were used. They had been kept in conditions of controlled temperature and daylight with free access to diet '86' (Oxoid Limited, London) and water for at least 7 days before use.

*Chemicals and reagents.* The salts in the perfusate were AnalaR chemicals (B.D.H. Chemicals, Ltd) except for the choline chloride (B.D.H. Laboratory Reagent) and the choline bicarbonate (supplied as 45% aqueous solution by Sigma). Glucose (AnalaR) and phenol red were also obtained from B.D.H. Gum guaiacum resin was obtained from Brome and Schimmer, London.

*Anaesthesia.* Anaesthesia was maintained by ether inhaled from a mask. Occasionally, urethane (0.7 g/ml.; 5 g/kg body wt.) injected s.c. was used.

*Perfusates.* The normal medium pumped through the intestinal lumen was a Krebs & Henseleit (1932) bicarbonate saline solution modified by halving the concentration of calcium and quartering that of magnesium. To this phenol red (50  $\mu\text{g}/\text{ml}$ .) and glucose (5 mg/ml. or 1.5 mg/ml.) were usually added. The phenol red does not interfere with the absorption of glucose or water (unpublished experiments), and it shows the presence of any leaks. For experiments on sodium depletion, most of the sodium salts were replaced by the corresponding salts of choline or lithium or by mannitol. A bicarbonate saline solution (NaCl 120 mM:  $\text{NaHCO}_3$  25 mM) in equilibrium with 5% carbon dioxide in oxygen at 37° C was used to wash out segments of intestine immediately before their connexion to the perfusion apparatus. All these media were made up in doubly distilled water.

*Analytical methods.* Glucose was estimated on a Technicon AutoAnalyzer by Fisher & O'Brien's (1972) modification of the glucose oxidase method of Hill & Cowart (1966), which uses gum guaiacum extract as chromogen. The s.d. of an estimate of glucose concentration in the range 1–15 mg/ml. is  $\pm 0.016$  mg/ml. (eight observations).

Sodium was estimated on a Unicam SP 90 atomic absorption spectrophotometer.

Total nitrogen was estimated on the AutoAnalyzer as ammonia by the Berthelot phenol-hypochlorite reaction following micro-Kjeldahl ashing (Kaplan, 1965).

Protein was estimated on the AutoAnalyzer by a modification of Failing, Buckley & Zak's (1960) biuret reaction.

*Determination of water content of segments of intestine.* Surface moisture was removed from the lumen by passing a stream of compressed air through the segment for 1 min, and by blotting the outside on absorbent tissue. The segment was then weighed on a tared piece of aluminium foil, and dried at 75° C to constant weight.

*Setting up the intestine.* The removal of the intestinal segment from the animal was as described by Fisher & Parsons (1949). A length of about 40 cm was used in this work, although the technique can be used with segments of other lengths. The oral cannula was inserted into the upper end of the jejunum; this was located as the highest part of intestine which could be withdrawn from the abdominal cavity without traction on the Ligament of Treitz. The blood supply was not interrupted until after the supply of oxygenated perfusate through the lumen had been established.

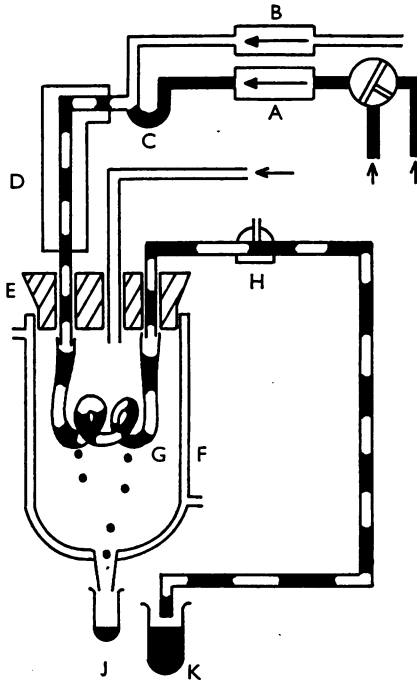
*The perfusion unit.* The apparatus is shown diagrammatically in Text-fig. 1.

Warm moist gas (5% carbon dioxide in oxygen) is pumped by peristaltic pump B into a stream of warm oxygenated perfusate delivered by the other pump (A) to form a sequence of slugs of liquid separated by gas bubbles to which we refer as segmented flow. The pumping rate can be continuously varied over a wide range but is normally 2–4 ml./min for each of perfusate and gas.

This segmented flow is delivered to the inlet cannula which is tied into the intestine. The intestine is housed in an organ chamber (F) which is a water-jacketed glass vessel similar to that used by Fisher & Parsons (1949). The intestine is freely suspended in a series of naturally formed loops on a sling of nylon wool. Gas (5% carbon dioxide in oxygen) saturated with water vapour at 37° C passes through the organ chamber. This serves to oxygenate the serosal surface of the intestine and to blow the transported fluid along the exit tube at the bottom of the chamber to tared collecting tubes at J. We refer to this fluid as 'secretion'.

A modified Starling resistance, H (Fisher & O'Brien, 1972), is connected to the outflow cannula to maintain a distension pressure of 30 cm water so as to expose uniformly the whole mucosal surface to the perfusate and gas. The outflow from the resistance passes to a second set of tared collecting tubes at K. The sets of tubes, J and K, are held in a time operated fraction collector.

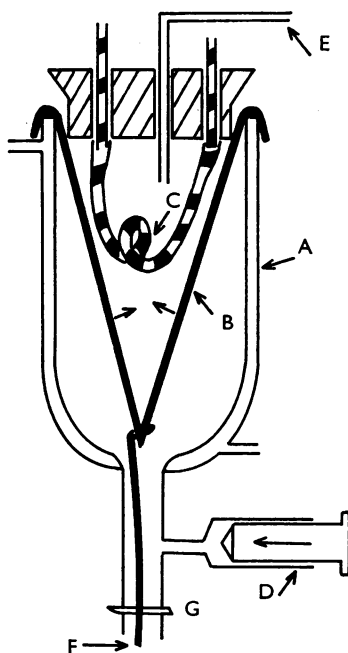
Warm oxygenated saline for washing out the segment of intestine is pumped by an independent peristaltic pump. A by-pass line with a relief valve allows saline to return to the reservoir if the luminal distension pressure exceeds 20 cm water. The perfusate and saline are equilibrated with 5% carbon dioxide in oxygen by means of gas lifts which carry approximately 100 ml. gas per minute into each reservoir. The reservoirs are immersed in a water-bath at 41° C, and this water is circulated through the water jackets by a pump ('Circotherm' supplied by Shandon Southern Ltd, Camberley, Surrey). The temperature of the perfusate at the outflow cannula and of the gas surrounding the intestine is 37–38° C.



Text-fig. 1. *Perfusion unit.* A and B are peristaltic pumps, C is a glass U-tube (internal diameter about 3 mm) with side-arm in which the gas and liquid flows converge to produce the segmented flow. D is a water jacket, made flexible so that when the intestine has been cannulated *in situ* the bung, E, with inlet and outlet tubes can be lifted from the operating table into the organ chamber, F, which is water jacketed. G is the segment of intestine, H is a resistance unit to maintain intraluminal distension, and J and K are collection tubes for the secretion and lumen effluent respectively.

An alternative organ chamber which we use is to insert into the normal organ chamber a thin-walled rubber tube (e.g. a 'Durex' condom) which is tied off and held down at its lower end and fastened at its top end over the opening of the organ chamber (Text-fig. 2). The bung carrying the intestinal cannulae fits into the open top end of this tube which can then be collapsed around the intestine by increasing the pressure in the fluid (either water or air) surrounding it in the organ

chamber. A fine polyethylene tube which passes into the collapsible tube through the bung carries away the transported fluid (secretion) to the fraction collector. The dead space between the intestine and the rubber tube appears to be negligible, and the absorption rates from segments set up in this way are indistinguishable from those for segments set up in the normal fashion. This method has the advantage that collection of secretion is more nearly continuous, instead of in the form of drops of 50  $\mu$ l. or more.



Text-fig. 2. A is the water-jacketed organ chamber.

B is a thin-walled rubber tube which can be collapsed around the segment of intestine C by applying pressure from the syringe at D. The secretion then flows along tube E for collection.

F is a piece of string held under tension by the screw clip, G, so that B is stretched.

*Calculation of absorption rates.* Because the water content of the intestine remains constant throughout the experiment (see later), we assume that the total volume of liquid pumped into the lumen during any collection period is equal to the sum of the volume of effluent issuing from the lumen plus the volume of secretion. This is preferable to assuming an invariant rate of pumping throughout the experiment or to relying on an 'unabsorbable' marker. Thus, the amount of a solute absorbed from the lumen during a collection period is given by

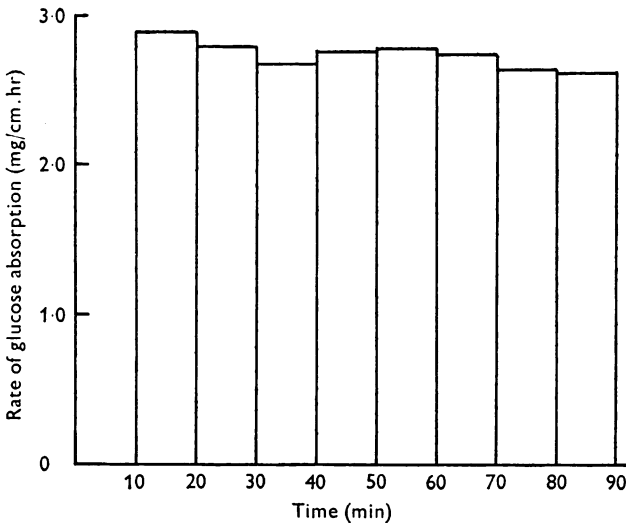
$(W_e + W_s) \cdot C_p - W_e \cdot C_e$  where  $W_e$  is weight of effluent from lumen,  $W_s$  is weight of secretion,  $C_p$  is concentration of solute in perfusate, and  $C_e$  is concentration of solute in effluent from lumen.

The rate of utilization of glucose by the intestine is taken to be the difference between the rate of absorption from the lumen and the rate of secretion at the serosal surface. All rates have been expressed in terms of unit length of intestine, e.g. mg/cm.hr.

## RESULTS

### *Stability of the preparation, and rates of water and glucose absorption*

The rates of water and glucose absorption usually become stable within the first 10 min and remain so over the next 100 min. Some falling off in activity is sometimes seen during the next half-hour but on the few occasions on which the experiment has been further prolonged, it has been



Text-fig. 3. Stability of the preparation. Rates of glucose absorption from the lumen were measured over successive 10 min periods (mean of five experiments).

possible to show continued concentration of glucose for more than 5 hr. Mean rates of glucose absorption from 5 mg glucose/ml. in the lumen measured over eight successive 10 min periods are shown in Text-fig. 3. Table 1 shows a summary of mean rates of absorption from the lumen of glucose and of water, also of mean rates of secretion and utilization of glucose at the steady state corresponding to several luminal concentrations of glucose.

Wilkinson's (1961) method was used to estimate the parameters  $K$  (concentration for half-saturation), and  $V$  (the apparent maximum rate of absorption) in the Michaelis-Menten type of relation for glucose absorp-

TABLE 1. Steady-state rates of absorption, secretion, and utilization by upper 40 cm segments of small intestine\* (means  $\pm$  s.e. and no. of experiments)

Glucose concentration in perfusate (mg/ml.)	Rate of water absorption ( $\mu$ l./cm.hr)	Rate of glucose absorption (mg/cm.hr)	Rate of glucose secretion (mg/cm.hr)	Rate of glucose utilization (mg/cm.hr)
5.0	169 $\pm$ 4 (57)	3.17 $\pm$ 0.10 (57)	2.10 $\pm$ 0.07 (52)	1.19 $\pm$ 0.07 (51)
2.8	174 $\pm$ 12 (9)	2.35 $\pm$ 0.23 (9)	—	—
2.0	179 $\pm$ 14 (9)	1.88 $\pm$ 0.23 (9)	—	—
1.5	130 $\pm$ 4 (17)	1.71 $\pm$ 0.11 (14)	1.04 $\pm$ 0.09 (17)	0.82 $\pm$ 0.12 (13)

\* The 40 cm caudal to the duodeno-jejunal junction.

TABLE 2. Estimates of the Michaelis-Menten parameters relating glucose absorption rate to the luminal glucose concentration:  $v = (V.s/K + s)$  where  $v$  is rate of glucose absorption and  $s$  is glucose concentration in lumen

	$K$		$V$
	mm	mg/ml.	(mg/cm.hr)
Taking $s$ as concentration in fluid entering lumen	19	3.4	5.30
Taking $s$ as concentration in fluid leaving lumen	14	2.5	4.80

TABLE 3. Wet and dry weights and water content of upper 40 cm segments of small intestine. Mean values  $\pm$  s.e. (no. of experiments)

Treatment	Wet weight (mg/cm)	Dry weight (mg/cm)	Water content ( $\mu$ l./cm)
Control	—	—	48.2 $\pm$ 1.5 (7)
1 hr perfusion, normal perfusate	—	—	53.9 $\pm$ 2.6 (4)
2 hr perfusion, normal perfusate	—	—	52.5 $\pm$ 2.9 (2)
1 hr perfusion, normal perfusate followed by 1 hr with low sodium perfusate	64.5 $\pm$ 0.2 (2)	13.8 $\pm$ 0.4 (2)	50.7 $\pm$ 0.6 (2)
Miscellaneous perfused intestines	76.4 $\pm$ 2.9 (16)	12.9 $\pm$ 0.4 (16)	57.7 $\pm$ 2.0 (29)

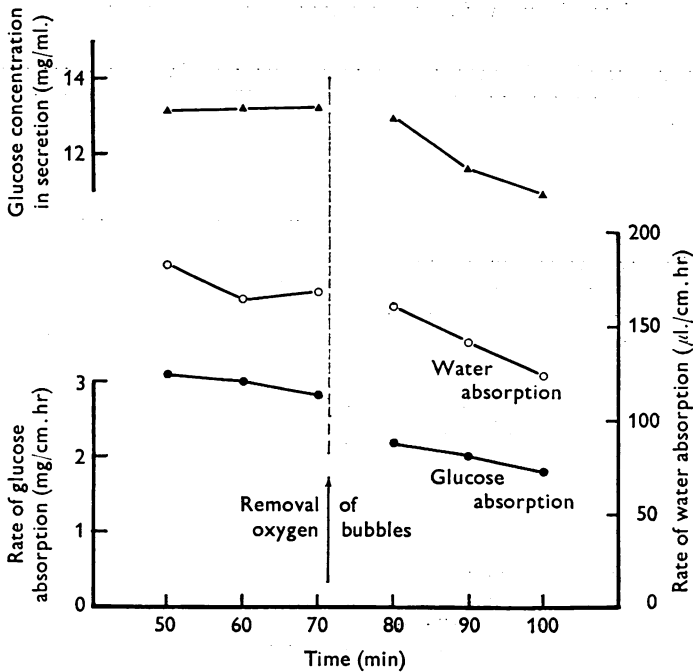
tion  $v = (V.s)/(K + s)$  where  $v$  is the rate of glucose absorption and  $s$  is the luminal glucose concentration. As Sladen (1968) has pointed out, a rigorous treatment is not possible since the glucose concentration ( $s$ ) changes down the length of the intestine as glucose and water are abstracted. Therefore the calculation was made (i) taking  $s$  as the concentration of

glucose in the perfusate pumped into the intestinal lumen and (ii) taking  $s$  as the mean concentration in the luminal effluent. These values are in Table 2.

The water contents of segments of intestine which have been perfused for 1 and 2 hr, and of control segments which have only been washed out with bicarbonate saline are given in Table 3.

*Effect of removal of oxygen segments from the segmented flow through the lumen*

In order to show that the oxygen bubbles segmenting the perfusate stream play a vital role in the survival of this intestinal preparation we have made experiments in which, after a control period, the oxygen bubbles were removed. Pump line B (Text-fig. 1) was removed from the



Text-fig. 4. Effect of replacing a segmented flow through the intestinal lumen by a non-segmented flow. At the arrow, the bubbles of 5% carbon dioxide in oxygen were removed.

oxygen supply and connected in parallel with pump line A to the perfusate reservoir. The basic pumping rate was 2.5 ml./min each of perfusate and oxygen during the control period. The intestine is then supplied with perfusate saturated with oxygen at 37° C at about twice the previous rate,



but with no gas bubbles. The effect of this is to produce rapidly a marked deterioration in the rates of water and glucose absorption (Text-fig. 4).

#### *The stirring effect of a segmented flow*

This stirring effect can be demonstrated as follows.

Blood is sucked up into a vertical glass tube of about 3 mm internal diameter and is allowed to drain out leaving a film of erythrocytes adhering to the walls of the tube. The bottom of the tube is wiped clean and isotonic saline is sucked up gently. When the saline is allowed to drain slowly from the tube, a red spike which moves downwards much faster than does the saline air meniscus, appears in the axis of the saline column (Pl. 1*a*). If the experiment is repeated, but with an air bubble introduced into the saline column, the red spike which develops at the uppermost meniscus is dispersed to the walls of the tube and recirculated when it reaches the lower end of the slug of liquid (Pl. 1*b* and *c*). At all but very low rates of flow the effect is that the upper slug rapidly comes to contain an apparently uniform suspension of erythrocytes whereas the liquid below the bubble remains clear except for the red axial spike (Pl. 1*d*).

The effect of the insertion of gas bubbles into a liquid stream at regular intervals should therefore be twofold: (i) maintenance of near uniformity of concentration across the cross-section of the tube, and (ii) sweeping off the boundary layer more efficiently than in laminar flow.

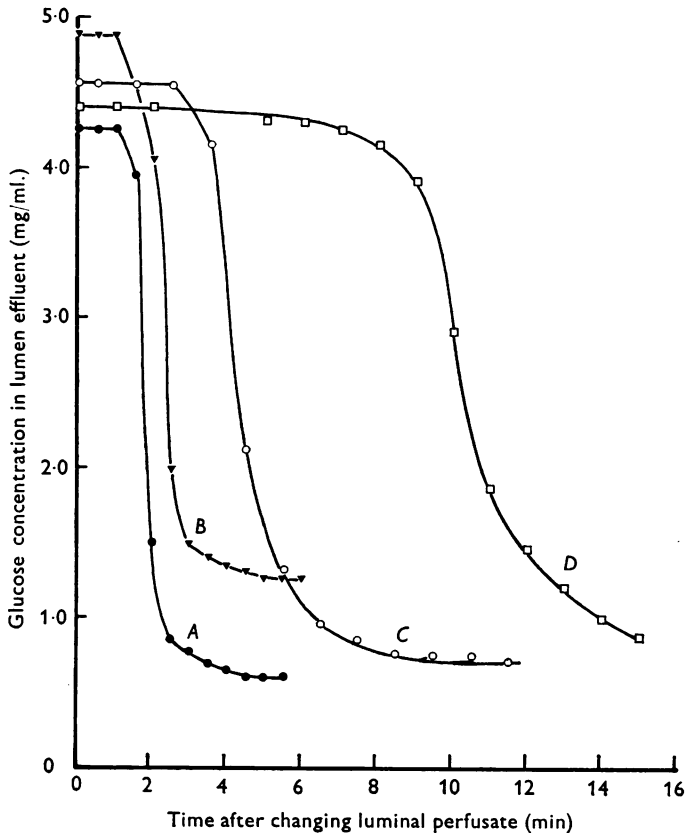
#### *Rate of attainment of a steady state in the lumen*

Text-fig. 5 shows the concentration of glucose in the luminal effluent at intervals after perfusate containing 5 mg/ml. had been replaced by perfusate containing glucose 1.5 mg/ml. Time course (*A*) was obtained with a segmented flow of 4 ml./min of perfusate and 4 ml./min of gas. At the perfusate changeover this rate was doubled for 2 min as is our normal practice. Time courses (*B*) and (*C*) were obtained with unsegmented flows of liquid at 8 and 4 ml./min respectively, and with the rate doubled for 2 min at changeover. Thus, the difference between *A* and *C* is attributable to the bubbles in the segmented flow. Time course *D* represents the effect of non-segmented flow at 4 ml./min with no temporary increase in pumping rate.

These results indicate that not only do the hydrodynamic properties of the system favour a rapid change in the luminal steady state, but also that any change in the rate of absorption of glucose when the luminal concentration is changed must itself be rapid. Text-fig. 6 confirms that a new steady rate of glucose absorption is reached within 5 min of the change of perfusate.

*Rate of attainment of a steady state in the secretion*

It can be shown (see Appendix) that if the secretion from the serosal pole of the mucosal cells mixes uniformly with a constant volume of intestinal extracellular fluid and if the secretion which is collected is a



Text-fig. 5. Time courses of the change in glucose concentration in luminal effluent after the concentration in the perfusate was changed from 5.0 to 1.5 mg/ml.

*A*, normal segmented flow of 4 ml./min of liquid plus 4 ml./min of gas. Flow rate was doubled temporarily for 2 min immediately after the change-over.

*B*, non-segmented flow of 8 ml./min of liquid only. Flow rate was doubled temporarily for 2 min immediately after the changeover.

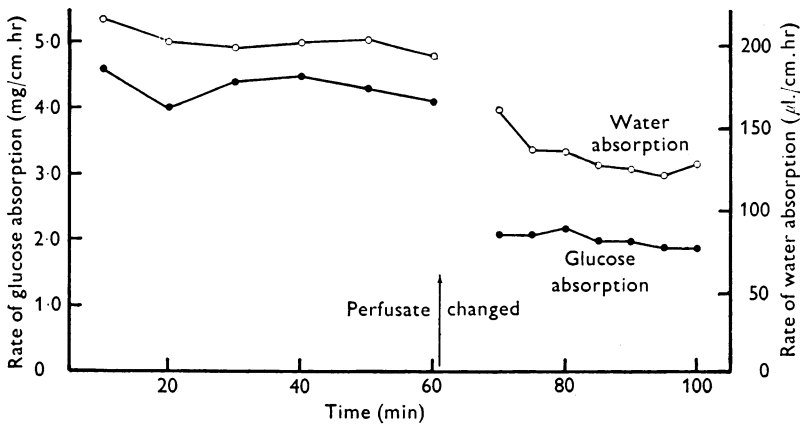
*C*, non-segmented flow of 4 ml./min of liquid only. Flow rate doubled as in *A* and *B*.

*D*, as *C* except that the flow rate was not doubled temporarily.

fair sample of this fluid, then the concentration ( $C$ ) of a solute in the secretion will change exponentially in such a way that

$$C = C_{\infty} + (C_0 - C_{\infty}) e^{-v/V},$$

where  $v$  is the volume of the tissue fluid compartment,  $C_{\infty}$  is the concentration of solute in the fluid entering the extracellular space from the mucosal cells (and the asymptotic concentration in the secretion collected), and  $C_0$  is the concentration of the solute in the secretion at  $v = 0$ .



Text-fig. 6. The effect on absorption of changing the glucose concentration in the luminal perfusate. At the arrow the perfusate which contained 5.0 mg glucose/ml. was replaced by one containing 1.5 mg glucose/ml.

The rate constant for the exponential function is equal to the reciprocal of the volume ( $1/V$ ) of extracellular water with which the secretion mixes. To estimate  $V$  graphically a reliable estimate of the asymptote,  $C_{\infty}$ , is necessary, so that  $\log_e (C - C_{\infty})$  can be plotted against the cumulated volume of secretion,  $v$ . As Wagner & Metzler (1967) have stressed, such a graphical analysis is inefficient, so we have used several digital computer programmes for obtaining best-fit relations to the exponential function. The most satisfactory of these procedures is based on a rapid descent method for minimizing a function (Atkins, 1971). The programme has been run on the IBM 360/50 and ICL 4-75 computers at the Edinburgh Regional Computing Centre.

Intestinal lymph, and therefore intestinal tissue fluid, contains a high concentration of protein (Yoffey & Courtice, 1956) which can be expected to be washed out into the secretion by the water which is transported from the lumen to the serosal surface and it would be predicted that this

washout of protein would have the same rate constant as the attainment of a steady state of glucose concentration in the secretion.

Best-fit constants were computed for a number of experiments in which the glucose and total nitrogen (or protein) concentrations in the secretion were measured, and a typical set of curves for the simultaneous wash-out of endogenous total nitrogen and wash-in of exogenous glucose is shown in Text-fig. 7. Mean values of the calculated parameters are shown in Table 4, and the estimates of  $V$  (29–30  $\mu\text{l./cm}$  length of intestine) clearly are very similar for total nitrogen (or protein) wash-out and glucose wash-in.

The goodness of fit of these kinetics to the single exponential model can be seen from the linear transformations in Text-figs. 8 and 9 for glucose wash-in and protein wash-out respectively.

If the perfusate is suddenly replaced by one containing a different concentration of glucose then, as Text-fig. 10 shows, the new steady state in the secretion is attained in an exponential fashion, although the rate of glucose absorption changes almost instantaneously (Text-fig. 6).

The goodness of fit of the kinetics of glucose wash-out to the single exponential function can be seen from the linear transformation ( $\log_e (C - C_\infty)$  against  $v$ ) in Text-fig. 11.

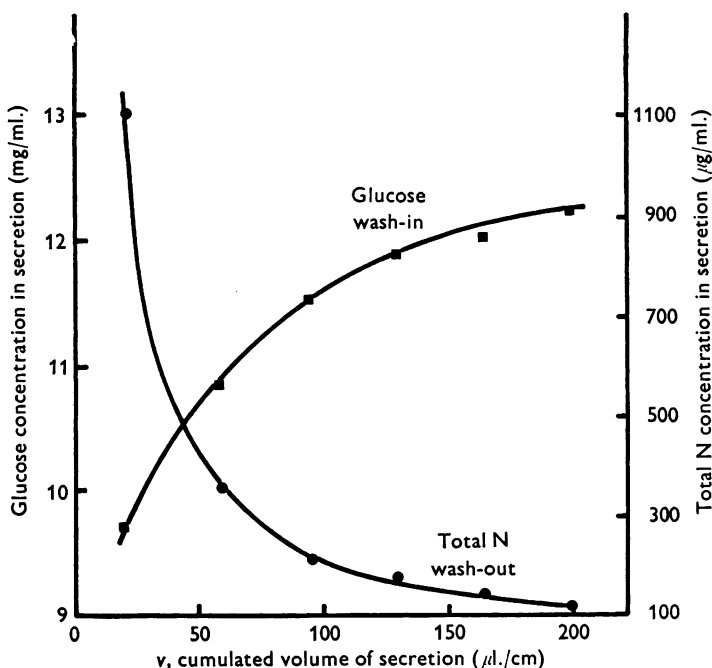
The mean compartment size estimated by the reciprocals of the exponential rate-constants for a number of such glucose wash-out experiments is  $34.9 \pm 2.5$  (19)  $\mu\text{l./cm}$  length of intestine, and this is not significantly different from the values obtained from the glucose wash-in and total nitrogen (or protein) wash-out curves ( $P > 0.1$  by Student's  $t$  test) – see Table 4. The value of  $C_\infty$  for the glucose wash-out experiments shows that the steady-state concentration of glucose in the secretion is 8 mg/ml. when the lumen concentration is 1.5 mg/ml. The sugar is transported actively against a fivefold concentration gradient.

The parameter  $C_0$  gives the concentration of glucose in the secretion when the perfusate with glucose 5 mg/ml. is in the lumen before wash-out has commenced. This value,  $13.6 \pm 0.4$  (19), is not significantly different ( $P > 0.05$ ) from the corresponding estimate made from the glucose wash-in data, for which the mean asymptote,  $C_\infty$ , is  $12.7 \pm 0.3$  (27) mg glucose/ml.

#### *Effect of a change in luminal sodium concentration*

Since the sodium concentration in the cell water and in the submucosal tissue fluid of an isolated preparation must be maintained by continued absorption of sodium from the lumen it is possible that the fall in glucose absorption following sodium deprivation in the lumen (e.g. Riklis & Quastel, 1958) could be at least partially due to a fall in the sodium content of intestinal tissue. If this were so, then the rate of glucose absorption from the lumen would decline gradually when the luminal sodium

was removed, instead of declining abruptly as it does when the concentration of glucose in the lumen is changed. On the other hand, if glucose absorption depends on an electrochemical gradient of sodium across the luminal membrane as Crane (1965) and others have proposed, then the sudden abolition of this gradient should cause an abrupt change in the rate of glucose absorption from the lumen.

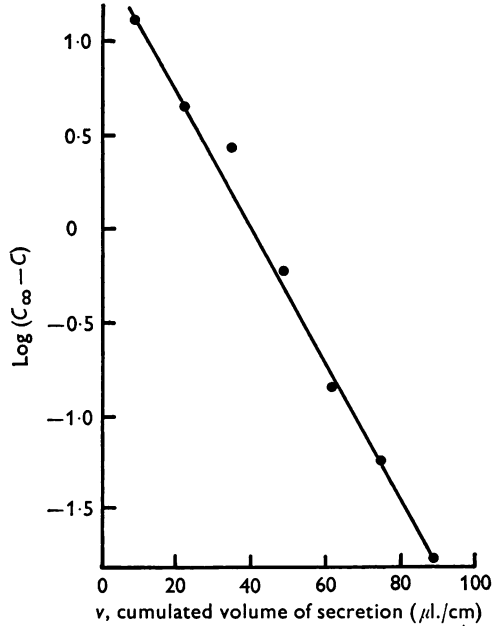


Text-fig. 7. Kinetics of simultaneous wash-out of endogenous total nitrogen and wash-in of glucose in intestinal secretion. The luminal perfusate contained glucose (5.0 mg/ml.).

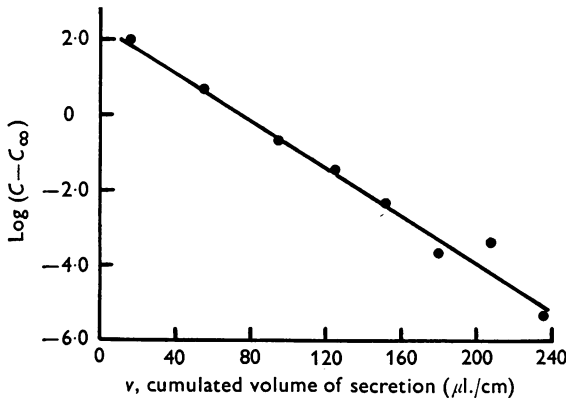
The outcome of three experiments in which the normal perfusate was replaced by one in which the sodium salts, apart from 5 mM-NaCl, had been replaced by the corresponding choline salts is shown in Text-fig. 12, and shows that the fall in glucose absorption rate is gradual (cf. Text-fig. 6).

Text-fig. 13 shows results from similar experiments where the luminal sodium concentration was reduced from normal (144 m-equiv/l.) to 25 m-equiv/l. by replacement of the sodium chloride by choline or lithium chloride or by mannitol. Again the fall in glucose (and water) absorption is gradual, and clearly depends on the nature of the replacing molecule.

The rate of glucose absorption hardly falls any more rapidly than does the sodium concentration in the tissue fluid. Further data for the rate of

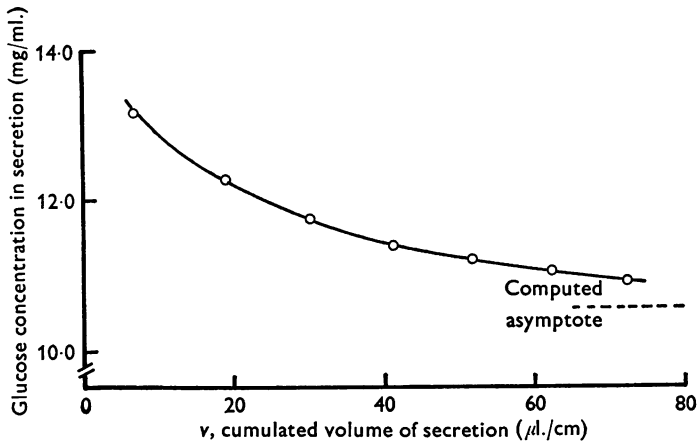


Text-fig. 8. Semi-logarithmic plot of typical glucose wash-in data.

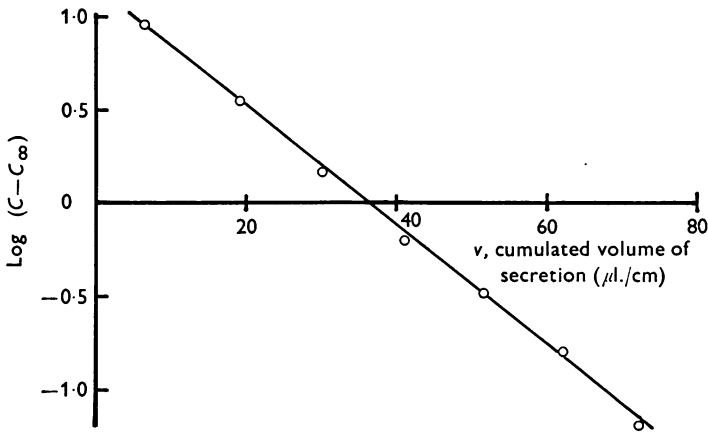


Text-fig. 9. Semi-logarithmic plot of typical protein wash-out data.

glucose absorption and the corresponding sodium concentration in the secretion following depletion of the luminal sodium are shown in Text-fig. 14. There is no simple relationship between them. The course of the wash-out of sodium from the tissue fluid into the secretion appears to fit to a single exponential model (Text-fig. 15). The rate constant for this ( $0.0903 \pm 0.0117$  cm/μl. (4)) is some 3 times that obtained from the wash-out of glucose, total nitrogen or protein. This difference can be explained



Text-fig. 10. Exponential wash-out of glucose in intestinal secretion, after the luminal perfusate concentration of glucose was changed from 5.0 to 1.5 mg/ml.



Text-fig. 11. Semi-logarithmic plot of the glucose wash-out data from Text-fig. 10.

in terms of the bidirectional fluxes of sodium out of the tissue fluid – viz. into the secretion, and back into the lumen (Bywater *et al.* 1972). The ratio of the sodium flux rates appears to be

$$\frac{\text{mucosal to serosal flux}}{\text{serosal to mucosal flux}} = 1.56$$

and this value is in reasonable accord with other values in the literature (e.g. 1.4 for rat ileum, Curran & Solomon (1957)).

TABLE 4. Estimates of parameters in the exponential function.

$$C = C_{\infty} + (C_0 - C_{\infty})e^{-v/V} \text{ (values are means } \pm \text{ s.e.)}$$

	$C_0 - C_{\infty}$ (mg/ml.)	$C_{\infty}$ (mg/ml.)	$V$ ( $\mu$ l./cm)	Number of experiments
Protein wash-out	13.8 $\pm$ 2.0	0.475 $\pm$ 0.067	29.1 $\pm$ 1.7*	9
Total N wash-out	1.46 $\pm$ 0.15	0.101 $\pm$ 0.012	29.6 $\pm$ 2.4	15
Glucose wash-in†	-5.81 $\pm$ 0.47	12.7 $\pm$ 0.3§	29.8 $\pm$ 2.0	29
Glucose wash-out‡	5.50 $\pm$ 0.62	8.08 $\pm$ 0.56	34.9 $\pm$ 2.5*	19
Over-all mean	—	—	31.0 $\pm$ 1.20	72

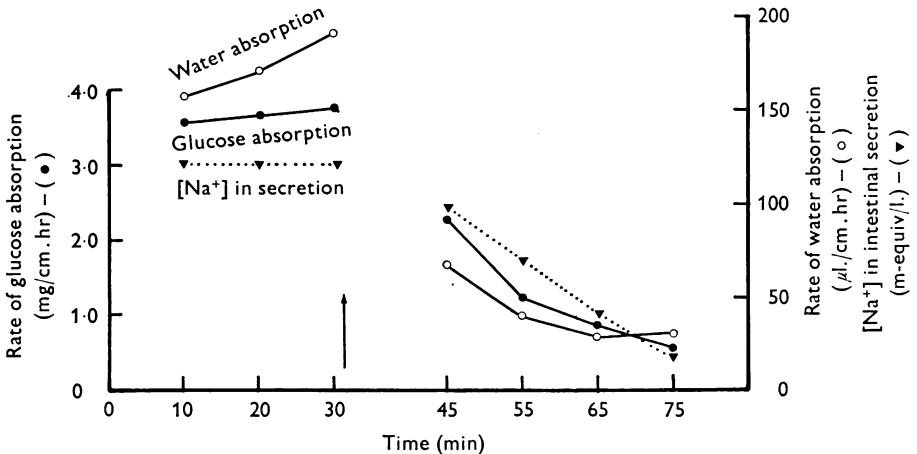
\* Even the extreme mean estimates of  $V$  are not significantly different from one another.

† When perfusion is initiated with 5 mg glucose/ml. in the lumen.

‡ When luminal perfusate containing 5 mg glucose/ml. is replaced by one containing 1.5 mg/ml.

§ Corresponding to a steady-state concentration ratio across the mucosa of 2.55:1.

|| Corresponding to a steady-state concentration ratio across the mucosa of 5.4:1.

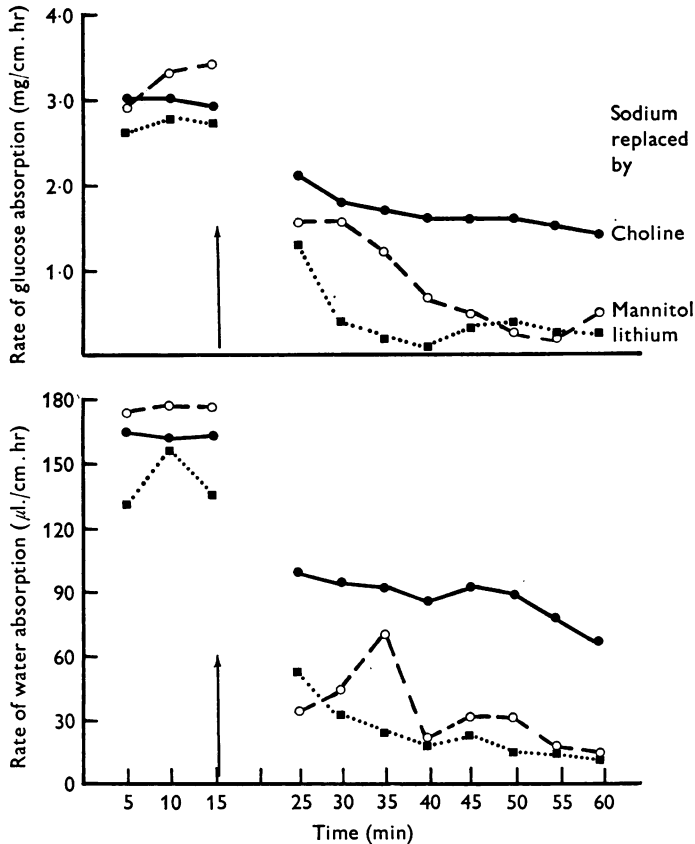


Text-fig. 12. The effect of partial replacement of luminal sodium by choline. At the arrow, the luminal perfusate which contained 144 m. equiv sodium/l. was replaced by one which contained 5 m-equiv sodium/l.

#### *Effect of oxygen and nutrient deprivation while setting up the intestine*

In three experiments the segment of intestine was prepared for perfusion in the normal manner except that the mesentery was cut off and the segment left in cold saline after cannulation for 15 min before the commencement of perfusion. In three other experiments a stream of moist oxygen was passed through the intestinal lumen whilst the cannulated segment lay in the cold saline.





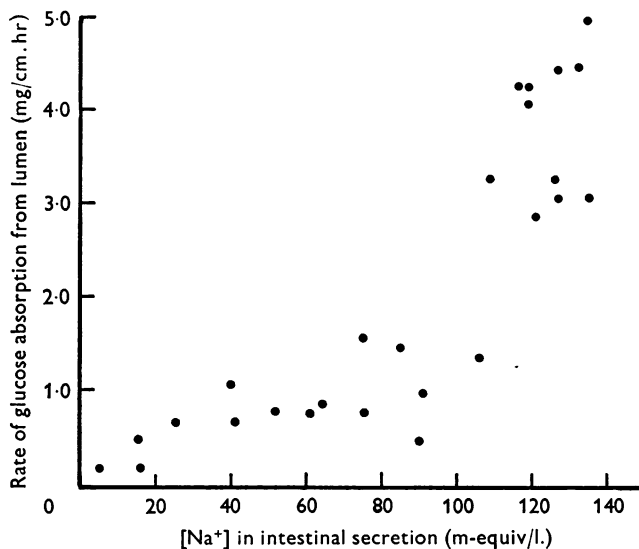
Text-fig. 13. The effect of replacement of luminal sodium chloride by choline chloride (●), lithium chloride (■), or by mannitol (○). At the arrow the luminal perfusate which contained 144 m-equiv sodium/l. was replaced by one which contained 25 m-equiv sodium/l.

The rates of water and glucose absorption in these experiments are shown in Text-figs. 16 and 17 respectively. It will be seen that the rates of absorption increase almost linearly over the first 30–35 min and are stable thereafter at levels corresponding with the normal values shown in Table 1. The stream of oxygen passed through the lumen has no effect on the outcome.

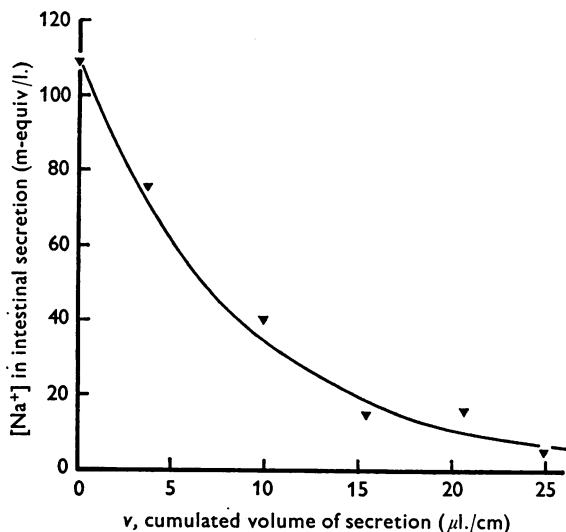
#### DISCUSSION

*The importance of 'segmentation' of perfusate.* Perfusion of 40–50 cm of small intestine at 5 ml./min with perfusate equilibrated with 95% oxygen does not maintain the absorptive activity of the intestine (Text-fig. 4), although the dissolved oxygen supplied to the lumen would be

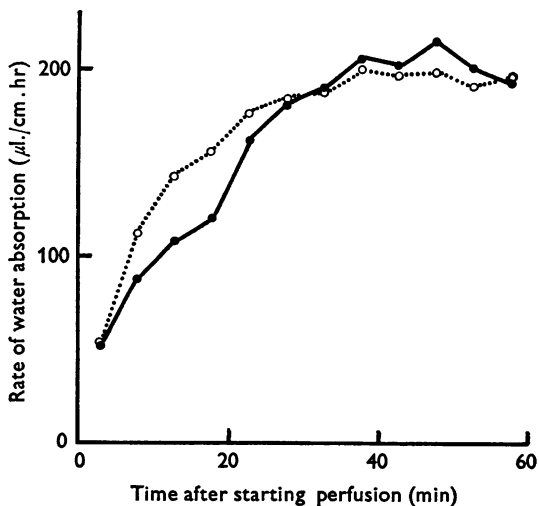
sufficient to support a  $Q_{O_2}$  of  $15 \mu\text{l./mg}$  dry wt. hr. and although the intestinal segment is immersed in moist 95% oxygen. The explanation of this effect is probably that slowness of diffusion of oxygen within the luminal perfusate limits the supply to the mucosa.



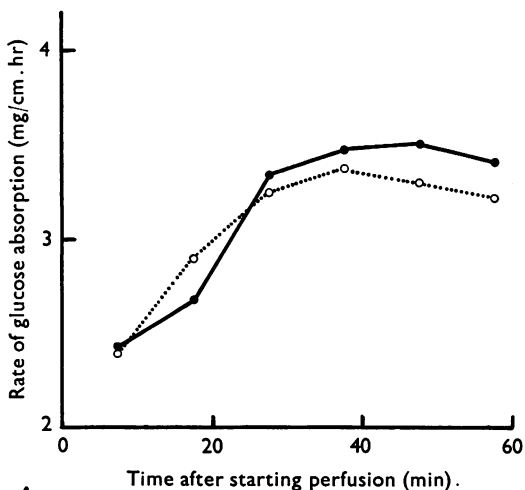
Text-fig. 14. The relation between the rate of glucose absorption and the sodium concentration in intestinal secretion, subsequent to replacement of luminal sodium by choline.



Text-fig. 15. Wash-out of sodium in intestinal secretion subsequent to replacement of luminal sodium by choline.



Text-fig. 16. Rates of absorption of water measured over 5 min periods during the first hour of recovery from interruption of the blood flow for 15 min before perfusion. The interrupted line refers to experiments in which the intestine had been perfused with moist oxygen during the period of interruption (mean of three experiments each).



Text-fig. 17. Rates of absorption of glucose measured over 10 min periods during the first hour of recovery from interruption of the blood flow for 15 min before perfusion. The interrupted line refers to experiments in which the intestine had been perfused with moist oxygen during the period of interruption.

If this is true, then slow perfusion with an unsegmented flow will always yield diffusion-limited rates of absorption. It has been shown previously (Fisher, 1964) that when transport of a solute occurs in two steps of which the first is diffusion across a barrier with a permeability constant  $P$ , and the second one is a saturable process with a maximum velocity  $V$  and a half-saturation concentration  $K$ , then the composite process is a saturable one with the same maximum velocity  $V$ , but with a larger half-saturation concentration, equal to  $K + V/P$ .

When  $P$  is small, the kinetics of such transport are indistinguishable from diffusion kinetics. This may be the explanation of the findings of Schanker, Tocco, Brodie & Hogben (1958) which led them to propose from experiments with slow perfusion rates that absorption of the organic solutes which they used as 'models' of drugs was due to simple diffusion, and could also explain the very high  $K$  values for glucose absorption which were reported in slow perfusion experiments by Dawson & McMichael (1968). Their value of 271 mM can be contrasted with ours of 14–19 mM (Table 2).

As we have shown, 'segmentation' of luminal perfusate flow causes efficient mixing so that limitation of absorption by luminal diffusion is practically eliminated in our preparation. We can also change from one steady state in the lumen to another within less than five minutes and the rapidity with which the rate of glucose absorption responds to a change in glucose concentration suggests that there is no significant unstirred layer between the brush border and the luminal perfusate (Text-fig. 6). The segmentation makes it possible to use sufficiently low rates of perfusion to give large enough concentration differences between perfusate and luminal effluent to allow the precise measurement of absorption.

*Kinetics of absorption in relation to tissue accumulation.* Although the rate of glucose uptake from the lumen becomes stable to within a few per cent within 20 min of setting up the preparation (Text-fig. 3), the rate of its appearance in the secretion is slower, and changes in a fashion corresponding closely with the hypothesis that the fluid secreted by the mucosa mixes intimately with the tissue fluid, so that the fluid expressed on to the serosal surface and then collected is a fair sample of a tissue fluid of uniform composition. The anatomy of the mucosal folds lends support to the notion of uniform mixing: they contain a network of connective tissue fibrils which can be expected to disperse randomly streams of fluid flowing from the mucosa to the centrally placed lymphatics.

As we have shown, the time course of change in concentration of a solute in the secretion is variable, but it corresponds to the variation in rate of water secretion by the intestine. Tissue accumulation of a solute will, in many instances, be directly dependent on accumulation in the

tissue fluid, and therefore dependent on the rate of water absorption. Where this is very low, tissue accumulation is a direct measure of absorption, but when water absorption occurs at the mean rate of over  $150 \mu\text{l./cm. hr}$  which is observed in our experiments (Table 1) accumulation will have reached half its ultimate extent within 10 min and will have virtually ceased within 30 min.

Accumulation of solute in the tissue fluid is therefore crucially dependent on the rate of water absorption and is not a reliable independent measure of rate of solute absorption.

*Kinetics of solute accumulation in tissue fluid.* The same considerations lead to the conclusion that only when water absorption is very lively will the cumulative appearance of a solute in the serosal fluid be a reliable measure of absorptive activity, especially in experiments taking only 30 min, since it is only well after this time, even in a fully active preparation, that the rate of solute transfer from tissue fluid to serosal fluid will approximate to the net rate of transfer from mucosa to tissue fluid.

This explains why the concentrations of glucose in the serosal fluid which Smyth & Taylor (1957) collected are substantially lower than the steady-state concentrations reached in our corresponding experiments with this preparation. Smyth & Taylor pooled the fluid transported over the first hour of perfusion. During this period the composition will only be approaching the steady state (see our Text-fig. 7) and so the mean concentration will be only some 60–70 % of the asymptotic (steady-state) value.

*The effect of interruption of circulation on absorption.* The experiments illustrated in Text-figs. 16 and 17 show that there is a profound but reversible depressive effect of interrupting the blood circulation to the intestine for a period between removal from the animal and institution of perfusion. In our conditions recovery after 15 min of isolation occupied the first 30 min after starting perfusion, which is the period often used in studies of everted sacs. Factors which shorten or lengthen this period could have profound and misleading effects on apparent absorptive activity. Therefore we routinely adopt the precautions of Fisher & Parsons (1949) in commencing perfusion before killing the animal.

One other thing which is clear from these experiments is that the deleterious effects of interrupting the circulation are not due to oxygen lack as had been assumed by Fisher & Parsons (1949) since they are not affected by perfusing the isolated unperfused intestine with moist oxygen during the period of interruption.

The considerations discussed in the last four sections emphasize the caution which must be exercised in interpreting the results of experiments

made on everted sacs, tissue slices or rings, or on segments perfused at low flow rates. We believe that this new preparation obviates many of these difficulties.

*The interpretation of the rate constant of concentration change of solutes in intestinal secretion.* The simple exponential dependence of solute concentration in secretion on the cumulative volume of secretion which is seen in the glucose wash-in, glucose wash-out and nitrogen wash-out experiments (Table 4), yielding the same rate constant in all instances, is entirely consistent with the complete mixing of a constant product of mucosal absorptive activity with the content of a tissue fluid compartment of constant size. The estimate of volume of this compartment is  $30 \mu\text{l./cm}$  of intestine. This is plausible in relation to the known value of total water content ( $50 \mu\text{l./cm}$ ) and dry weight ( $13 \text{ mg/cm}$ ) (Table 3).

The much more rapid equilibration of sodium with the tissue fluid following a change in sodium concentration in the luminal perfusate indicates that the unidirectional flux of sodium into the tissue fluid must be much higher than the net flux. Since the rate constant is 2.8 times the reciprocal of the tissue-fluid volume, it follows that flux from the lumen is 2.8 times the net flux, and so reflux across the mucosa into the lumen is  $(2.8 - 1) = 1.8$  times the net flux. We have already used this type of analysis to establish unidirectional flux rates of deuterium oxide across mucosa (Bywater *et al.* 1972). The fact that equilibration occurs exponentially suggests that reflux of sodium from the tissue fluid to the lumen occurs at a rate proportional to tissue fluid sodium concentration.

*The effect of changes in lumen perfusate composition on glucose absorption.* We have shown that the rate of glucose absorption from the lumen changes promptly when the glucose concentration changes: a new steady state is set up within 5 min of changing to a new perfusate (Text-fig. 6), so that it can safely be assumed that there is no unstirred layer at the mucosal surface, so far as glucose is concerned.

The findings that glucose absorption from the lumen falls only slowly when the lumen sodium concentration is reduced abruptly (Text-figs. 12 and 13) therefore suggests that glucose absorption is not directly dependent on the lumen sodium concentration. It does not seem likely that it is directly dependent on tissue fluid concentration.

Thus the widely recognized interaction(s) between sodium ions and the mechanism(s) for glucose absorption cannot be solely at the luminal membrane. Crane, Miller & Bihler (1961) proposed an allosteric carrier with binding sites for glucose and sodium at the luminal membrane. Crane further developed his hypothesis to suggest (Crane, 1965) that the driving force for glucose absorption comes from the facilitated diffusion of sodium ions down their electrochemical gradient into the mucosal cells on the

sodium-glucose carrier. In this model the intracellular concentration of sodium is kept low, and hence this gradient is maintained, by a ouabain-sensitive sodium pump at the serosal pole of the cells. Thus a reduction in luminal sodium concentration down to 5 m-equiv/l. would immediately abolish (and reverse) the downhill gradient, and therefore immediate cessation (or reversal) of glucose absorption would be predicted. But we find that this does not happen. Newey, Rampone & Smyth (1970) drew similar conclusions on the sodium dependence of methionine absorption, but they were unable to exclude the possibility that an unstirred layer had prevented the sodium concentration at the brush border from being reduced as rapidly as desired.

Our evidence at present therefore is more consistent with the view that glucose absorption is dependent on the sodium content of the glucose absorbing cells, as was originally postulated by Csáky (1963).

#### APPENDIX

##### *Kinetics for solute wash-in or wash-out of the extracellular space*

Consider the fluid secreted from the mucosal cell and entering the extracellular fluid (ECF). If the extracellular volume,  $V$ , remains constant, then for every increment of volume,  $dv$ , which enters the compartment an equal volume,  $dv$ , must be displaced and collected as secretion.

Let the concentration of a solute in the ECF be  $C$  and the concentration of that solute in the fluid entering the ECF be  $C_\infty$ . Assume that the fluid from the mucosal cell mixes uniformly with the ECF, and that at any time the secretion collected is a fair sample of ECF.

Then, when a volume,  $dv$  enters the ECF the net change in the amount of the solute in the compartment is  $(C_\infty \cdot dv - C \cdot dv)$ . Hence, the change in concentration of the ECF,  $dC$  is given by

$$dC = (C_\infty \cdot dv - C \cdot dv) \cdot \frac{1}{V}.$$

Integration of this equation, with the condition that  $C = C_0$  when  $v = 0$  yields

$$C = C_\infty + (C_0 - C_\infty) \cdot e^{-v/V}.$$

Hence by measuring values of  $C$  at successive values of  $v$ , estimates of the constants  $V$ ,  $C_0$ , and  $C_\infty$  are possible.

We are grateful to the Medical Research Council for support. We also wish to thank Elizabeth Middleton and Fiona O'Brien for technical assistance, and Dr G. L. Atkins for help with the curve-fitting.

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## EXPLANATION OF PLATE

The stirring effect of segmented flow.



