Absorption of glucose, sodium, and water by the human jejunum studied by intestinal perfusion with a proximal occluding balloon and at variable flow rates

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SUMMARY A perfusion technique with a proximal occluding balloon has been used to study the absorption of glucose, sodium, and water from the human jejunum at different rates of flow. The absorption of glucose and water was significantly higher with the balloon deflated than inflated, probably because of reflux of infused solution above the point of infusion. Above the inflated balloon 0 to 4.2 ml/min of endogenous secretions could be recovered. Increasing flow rates increase the glucose absorption rate; a single relationship could be found between the glucose load and glucose absorption rate, and single values for the maximum velocity and for the half saturating load were calculated whatever the infusing rate and the initial glucose concentration. The stimulating effect of glucose on water and sodium movement increases gradually when the initial sugar concentration varies from 14 to 133 mM/l. Above this concentration a drop in water and sodium movement is observed, although the initial sodium concentration is kept constant. High flow rates result in a decrease of water absorption and an increase in sodium and potassium secretion rates.

Intestinal perfusion techniques have been widely used to study the absorption of sugars, water, and electrolytes in man (Holdsworth and Dawson, 1964; Gray and Ingelfinger, 1966; Whalen, Harris, Geenen, and Soergel, 1966; McMichael, Webb, and Dawson, 1967; Fordtran, Rector, and Carter, 1968). Nevertheless a controversy still persists about the use of a double or a triple lumen tube (Sladen and Dawson, 1968; Fordtran, 1969). Moreover, although a recent paper deals with the effects of flow rates on the absorption of glucose (Sladen and Dawson, 1969b), the exact consequences of varying flow rates have not yet been reported, especially with regard to the kinetics of glucose absorption and the movement of water and electrolytes. The present study was undertaken to investigate these two problems further.

Methods

Twenty-nine subjects, aged between 25 and 65 years, were studied. They were either patients with minor

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abdominal complaints or entirely normal subjects. None had any evidence of small bowel disease.

A technique utilizing a proximal occluding balloon was used (Phillips and Summerskill, 1966) to prevent contamination by endogenous secretions and reflux of the infused solution above the infusion point. Immediately above the balloon a tube allowed continuous aspiration of the gut. The infusion tube was immediately below the balloon. The test segment was 25 cm long and perfusate was aspirated via a third tube. There was a fourth tube to inflate the balloon (Fig. 1).

The tube was swallowed by the subject and perfusion started when the infusing point was at the duodenojejunal junction; this took between 12 and 36 hours and the position of the tube was checked fluoroscopically. The subjects fasted (no food or fluid allowed) for 12 hours before the test.

At the beginning of the infusion, the balloon was inflated with 40 ml of air and its occluding effect was checked when bromsulphthalein introduced above the balloon was not recovered at the sampling site

Fig. 1 Diagram of the tube with a proximal occluding balloon.

and also the perfused glucose-containing solution above the balloon. This occluding effect was tested several times during the infusion periods. These criteria were fulfilled in all experiments.

Four rates of infusion were used in three different groups of subjects: six subjects were infused at 8 ml/min, 13 subjects at 10 ml/min, and 10 subjects were infused on two successive days at 15 and 20 ml/min. At the rates of 10, 15, and 20 ml/min four solutions were used and contained increasing concentrations of glucose, namely, 66, 133, 200, and 260 mM/l. These will be referred to respectively as solutions I, II, III, and IV. The concentration of sodium chloride concentration was 50 mM/l (except for solution IV where the concentration had to be reduced to 18 mM/l to maintain isoosmolarity). Mannitol was added to solutions I and II in concentrations of 125 and 60 mM/l respectively to produce isotonicity. However, the composition of solutions infused at 8 ml/min was slightly different: the glucose concentrations were 14, 33, 66, 133, and 260 mM/l. The sodium chloride concentration was 60 mM/l (except in the last solution where it was 18 mM/l). Mannitol was also added to reach isotonicity. The solutions all contained polyethylene glycol (PEG) in a concentration of 1 g/l, and they were adjusted to pH 7. The exact number of subjects infused at each rate and at each concentration is indicated in Table III.

Perfusing solutions were warmed to 38°C and introduced by a constant rate infusion pump (Technicon). They were infused in each subject in a random order, and the effect of the balloon was studied by perfusing the same solution with the balloon inflated and then deflated or vice versa. Each solution was infused during an equilibration period of 45 minutes in order to obtain a steady state; jejunal samples were then collected by siphonage or gentle suction for three successive periods of 10 minutes. The mean percentage discrepancy between triplicate PEG concentrations (ie, range expressed as percentage of the mean) did not exceed 10%.

Glucose was estimated by reduction of potassium ferricyanide on a Technicon AutoAnalyzer. Sodium and potassium were measured by flame photometry and chloride by colorimetry on a Technicon AutoAnalyzer. Polyethylene glycol was determined in duplicate by the method of Hyden (1956) and osmolarity by freezing point depression on a Knauer osmometer.

The absorption rates were calculated as follows:

$$\text{Water absorption} = V \times \left(1 - \frac{\text{PEG}_I}{\text{PEG}_D} \right)$$

$$\text{Solute absorption} = V \times \left(S_i - S_D \frac{\text{PEG}_I}{\text{PEG}_D} \right)$$

where V is the infusion rate, S_i and PEG_I are the infused concentrations of solute and PEG, and S_D and PEG_D are the solute and PEG concentrations in the distal samples.

Results

ESTIMATION OF REFLUX AND ENDOGENOUS SECRETIONS

Above the inflated balloon a volume of 0 to 300 ml could be recovered within 70 minutes of infusion. The rate of recovery of these endogenous secretions revealed very large individual variations. In the same subject considerable changes within time were observed. Sometime the flow rate sampled above the balloon was spasmodic with sharp increases followed by total interruptions.

Above the deflated balloon fluid containing glucose and PEG was recovered in 14 out of 22 studies in which these substances were estimated. These concentrations of sugar and PEG were also highly variable; they were sometimes very low, compared to the infused solution, and sometimes comparable to the concentrations measured at the sampling site.

The comparison of absorption of glucose with inflated or deflated balloon disclosed highly signi-

Infusion Rates (ml/min)	Solution Infused with Balloon Inflated and Deflated	No. of Perfusions	Percentage Increase in Glucose Absorption with the Balloon Deflated (mean and range)	Significance of the Observed Difference (paired t test)
10	II	9	+ 20 (- 22 → + 65)	P < 0.05
	III	8	+ 25 (+ 2 → + 100)	P < 0.01
	IV	9	+ 25 (+ 9 → + 62)	P < 0.002
15	II	9	+ 24 (0 → + 110)	P < 0.01
	III	10	+ 34 (+ 2 → + 150)	P < 0.01
20	II	8	+ 31 (- 10 → + 108)	P < 0.01
	III	8	+ 28 (+ 13 → + 56)	P < 0.005

Table I Comparison of glucose absorption with the balloon deflated and inflated¹¹Negative values indicate that glucose absorption was, in a few experiments, higher with the balloon inflated.

Solution	Water Absorption (ml/min/25 cm; mean ± SE)			
	Infusion Rate (ml/min)			
	10	15	20	
II Balloon inflated	1.43 ± 0.45	0.84 ± 0.93	0.96 ± 0.76	
III Balloon deflated Balloon inflated	1.13 ± 1.5 0.73 ± 0.54	1.54 ± 0.62 0.33 ± 0.86	1.76 ± 0.40 0.33 ± 1.20	P < 0.05 P < 0.025
IV Balloon deflated Balloon inflated	1.57 ± 1.57 0.31 ± 0.56	1.92 ± 2.15	1.40 ± 0.97	
Balloon deflated	0.74 ± 0.34	Not done	Not done	

Table II Comparison of water absorption from solutions II, III, and IV at three different infusion rates with the balloon inflated and deflated¹¹At 15 and 20 minutes solution IV was only perfused with the balloon inflated.

ficant differences (Table I). The mean absorption rate was always higher when the balloon was deflated with a mean increase ranging from 20 to 34% of the absorption rate when the balloon was inflated; the difference was significant whatever the absorption rate and the solution infused. But, here again, individual variations were large (Table I). In some subjects perfusion with the balloon deflated resulted in an increase of more than double the glucose absorption rate, whereas in four out of 61 perfusions the absorption rate was slightly lower.

Absorption rate of water was significantly higher when the balloon was deflated for infusion rates of 15 and 20 ml/min, but it was not significantly higher at 10 ml/min (Table II). No significant difference could be observed for sodium movements whether the balloon was inflated or deflated.

The validity of the steady state reached at the end of the equilibration period with the balloon inflated or deflated was compared. This was done by estimating the dispersion of PEG concentrations in the three consecutive 10-minute samples. For each solution infused the value and standard deviation of PEG concentrations were calculated for the three samples collected. The dispersion was expressed as the ratio of the standard deviation to the mean PEG value. These ratios obtained with the balloon inflated and

deflated were compared by the paired t test. Dispersion was found to be statistically higher with the balloon deflated when 10 ml/min or 15 ml/min were infused (P < 0.05), whereas it was not significantly different at an infusion rate of 20 ml/minute.

All the results reported below concern only those perfusions performed with the balloon inflated.

EFFECTS OF VARYING FLOW RATES ON GLUCOSE ABSORPTION AND ITS KINETICS

This effect was tested at four different rates of infusion (8, 10, 15, and 20 ml/min), with at least four different concentrations at each rate.

The results (Table III) indicate that, as a whole, the absorption rate is higher when the infusion rate of the same solution is increased. The difference observed between glucose absorption (for the same initial concentration) at increasing rates of flow can be seen from the data given in Table III. For an initial glucose concentration of 66 mM/l the difference on glucose absorption rate is significant between 10 and 15 ml/min (P < 0.01), and between 15 and 20 ml/min (P < 0.05); for an initial concentration of 133 mM/l there is a significant difference for infusion rates between 8 and 20 ml/min (P < 0.025) and between 10 and 20 ml/min (P < 0.05); for an initial concentration of 200

Initial Glucose Concentration (mM/l)	Glucose Absorption Rate ($\mu\text{M}/\text{min}/25\text{ cm}$; mean $\pm$ SE)			
	Infusion Rates (ml/min)			
	8	10	15	20
14	109 $\pm$ 5 (6) ¹	—	—	—
36	262 $\pm$ 18 (6)	—	—	—
66	472 $\pm$ 60 (6)	459 $\pm$ 46 (10)	585 $\pm$ 112 (9)	719 $\pm$ 115 (9)
133	728 $\pm$ 186 (6)	763 $\pm$ 128 (13)	885 $\pm$ 262 (9)	913 $\pm$ 162 (8)
200	—	841 $\pm$ 188 (12)	971 $\pm$ 206 (10)	1,091 $\pm$ 310 (8)
260	820 $\pm$ 263 (3)	1,019 $\pm$ 164 (9)	999 $\pm$ 257 (9)	1,042 $\pm$ 261 (9)

Table III Glucose absorption under the inflated balloon at six different concentrations and four different infusion rates

¹The figures in parentheses indicate the number of subjects in each group.

mM/l the difference is significant between 10 and 20 ml/min ($P < 0.05$); and no significant difference was found at a rate of 260 mM/l. It can be noticed that (1) at the lowest concentration of sugar (66 mM/l) it is easy to demonstrate increasing absorption with increasing rates; (2) at the highest concentration (260 mM/l), whatever the rate, no significant difference can be shown; (3) for the intermediary concentrations (133 and 200 mM/l) the difference is significant only between 8 or 10 ml/min and 20 ml/min.

If glucose absorption is not expressed as a rate, but as a percentage of infused load, it becomes evident that, at any concentration, the higher the rate, the lower this percentage.

The saturation phenomenon can be shown at each infusing rate by plotting the absorbed glucose against the arithmetic mean glucose concentration. Apparent maximum velocity (V_{max}), and half saturation concentration (K_m) can be calculated by the method of Lineweaver and Burk, ie, by plotting of the reciprocal of the absorption rate against the reciprocal of the arithmetic mean glucose concentration for the different rates. The values for K_m (expressed in mM/l) and for V_{max} (expressed in mM/min/25 cm.) are respectively 81.3 and 1.36 at an infused rate of 8 ml/min; 99.7 and 1.48 at a rate of 10 ml/min; 43.0 and 1.13 at 15 ml/min; and 26.8 and 1.10 at 20 ml/min. It is evident from these figures that the kinetic parameters are different according to the infusion rate, the most striking difference being for apparent K_m values which dramatically decrease for an infusion rate above 10 ml/min. Therefore one must admit that the values for these parameters are dependent upon the experimental conditions, especially the infusion rate.

However, a different way of expressing the results can account, at least partially, for this unexpected finding. If the glucose absorption rate is plotted against the glucose infusion rate (instead of against the mean glucose concentration), a single curve can

be drawn between the points of all experiments whatever the infusion rate (Fig. 2).

The validity of this assumption has been assessed by comparing for each separate infusion rate the regression lines of the Lineweaver and Burk plotting of the reciprocal of the absorbed glucose against the reciprocal of the glucose load. Covariance analysis was done on an IBM computer and showed that the regression lines were identical for 10, 15, and 20 ml/min at a 5% level. It thus became possible, again using the Lineweaver regression line, to draw a general regression line between all individual results obtained at these three infusion rates. The equation of this line was:

$$\frac{I}{GA} = 0.870 \times \frac{I}{GL} + 0.000757,$$

where correlation coefficient = 0.834, $P < 0.0005$,

number of points = 112, $\frac{I}{GA}$ = reciprocal of glucose absorption rate ($\mu\text{M}/\text{min}/25$), and $\frac{1}{GL}$ = reciprocal of infused glucose load ($\mu\text{M}/\text{min}$).

From this equation a common value for the kinetic parameters can be drawn, such that $V_{\text{max}} = 1,320 \mu\text{M}/\text{min}/25\text{ cm}$ and $K_m = 1,150 \mu\text{M}/\text{minute}$.

This value of V_{max} probably represents the actual maximum absorption rate of the test segment whatever the rate and the concentration of glucose infused. The value of K_m can be interpreted as the half saturating glucose load. This single value for K_m easily accounts for the apparent decrease of K_m quoted above when mean glucose concentrations were used for calculation.

It must be noted that, although results obtained at 8 ml/min fit quite well with the single curve (Fig. 2), covariance analysis showed that, at this rate, the Lineweaver regression line was statistically different from the regression lines for infusion of 10, 15, and 20 ml/min.

Fig. 2 Glucose absorption rates related to the glucose load. Note that the results obtained by Sladen and Dawson (1969b) in the distal 30 cm segment (for which the three-lumen method was used) fit quite well with the single curve proposed.

WATER AND SODIUM MOVEMENTS

The rates of water and sodium net movements are shown in Tables IV and V.

Effect of increasing glucose concentrations on water and sodium movements

It appears from Tables IV, V, and VI that water absorption increases and sodium secretion decreases when the glucose concentration in the infused solution varies from 14 mM/l up to 133 mM/l. This effect is more apparent at an infusion rate of 8 ml/min because four glucose concentrations below 200 mM/l have been tested. The same phenomenon is found when glucose concentrations of 66 and 133 mM/l are infused at 10, 15 or 20 ml/min.

However, for an initially higher concentration of glucose (200 mM/l) even though the concentration of infused sodium remains unchanged, water absorption decreases and sodium secretion increases significantly. This general tendency of less water and sodium to be absorbed (or more to be secreted) is considerably more marked when the initial glucose concentration is 260 mM/l (but in this case initial sodium concentration is only 17 m-equiv/l).

Effect of flow rate on ionic movements

Tables IV, V, and VI show that there is a general

trend toward less water and sodium being absorbed (or more being secreted) when the infusion rate increases. The increase in sodium secretion is significant between 10 and 15 ml/min or 10 and 20 ml/min, or both, at any initial glucose concentration. The differences of water absorption for the same initial solution infused at 10, 15, or 20 ml/min are not significant, except for solution IV where water movements at 10 ml/min are significantly different from those observed at 15 ml/min ($P < 0.01$) and 20 ml/min ($P < 0.001$). It is to be noted that these results cannot be validly compared with those measured at 8 ml/min, since the initial sodium concentration was higher at this rate of infusion.

Table VII shows that the flux of potassium from blood to lumen (the infused solutions were free of potassium) increases significantly with increasing flow rate.

The correlation between sodium and water movements was always good, whatever the rate and the initial glucose concentration ($P < 0.001$). Iso-osmotic absorption has always been maintained: osmolarity of the samples always fell between 275 and 305 mOsm/l, and was not significantly different from the osmolarity of infused solutions.

Initial Glucose Concentration (mM/l)	Water Absorption Rate (ml/min; mean $\pm$ SE)			
	Infusion Rate (ml/min)			
	8	10	15	20
14	-1.34 $\pm$ 0.49			
33	0.02 $\pm$ 0.40			
66	1.02 $\pm$ 0.30	0.22 $\pm$ 0.40	0.28 $\pm$ 0.64	0.28 $\pm$ 0.59
133	1.95 $\pm$ 1.30	1.43 $\pm$ 0.45	0.84 $\pm$ 0.93	0.96 $\pm$ 0.79
200	—	0.73 $\pm$ 0.54	0.33 $\pm$ 0.86	0.33 $\pm$ 1.20
260	-0.30 (-0.02 $\rightarrow$ -0.61)	0.31 $\pm$ 0.56	-1.02 $\pm$ 0.75	-1.57 $\pm$ 0.72

Table IV Comparison of water absorption rates from six different solutions infused at four different rates¹

¹Negative values indicate net movement towards the lumen.

Initial Glucose Concentration (mM/l)	Sodium Movement (μ -equiv/min; mean $\pm$ SE)			
	Infusion Rate (ml/min)			
	8	10	15	20
14	-284 $\pm$ 101			
33	-194 $\pm$ 95			
66	-119 $\pm$ 57	-186 $\pm$ 45	-218 $\pm$ 103	-281 $\pm$ 86
133	-70 $\pm$ 105	-132 $\pm$ 39	-213 $\pm$ 88	-183 $\pm$ 91
200	—	-156 $\pm$ 44	-243 $\pm$ 116	-257 $\pm$ 112
260	-266 (-187 $\rightarrow$ -337)	-346 $\pm$ 108	-460 $\pm$ 146	-543 $\pm$ 145

Table V Comparison of sodium movements from six different solutions and at four different perfusion rates¹

¹Because of the low initial sodium concentrations this ion is always secreted into the lumen.

Infusion Rate (ml/min)	Solution			
	I	II	III	IV
10-15	ns	P < 0.01	P < 0.05	ns
15-20	ns	ns	ns	ns
10-20	P < 0.01	P < 0.05	P < 0.025	P < 0.025

Table VI Significance of the differences observed between sodium secretions for the same initial solution infused at three different rates

Initial Flow Rate (ml/min)	Potassium Secretion (μ -equiv/min/25 cm)
10	36 $\pm$ 5 (39) ¹
15	45 $\pm$ 9 (40)
20	50 $\pm$ 10 (35)

Table VII Potassium secretion measured with three different flow rates

¹Figures in parentheses indicate the number of perfusions for each rate.

Discussion

The use of a double- or a triple-lumen tube for intestinal perfusion has been extensively discussed in recent years. Studies on monosaccharides (Holdsworth and Dawson, 1964), disaccharides (Gray and Ingelfinger, 1966; Gray and Santiago, 1966; McMichael *et al*, 1967), and amino acids (Adibi, 1969) as well as investigation of the interrelationships between the absorption of sugars, water, and electrolytes (Sladen and Dawson, 1969a) have been performed with the double-lumen tube. It has been claimed that double- and triple-lumen methods provided similar results for the measurements of water and sodium absorption (Sladen and Dawson, 1968). However, the two-lumen tube has been severely criticized (Fordtran, 1969) because of two errors inherent in this method: contamination of the test segment by endogenous secretions and reflux of the infused solution proximal to the infusion point. This discussion culminated in an exchange of letters

in *Gut* (Soergel, 1969). The use of a double-lumen tube with a proximal occluding balloon (which could be inflated or deflated) appeared to be a suitable method to investigate this problem. Our results undoubtedly outline the two errors of the double-lumen tube quoted above.

The contamination of the test segment by endogenous secretions was proved and quantitated by recovery above the inflated balloon of a volume varying between 0 and 300 ml within 70 minutes, ie, 0 to 4.2 ml/min. These results are in agreement with those reported by Phillips and Summerskill (1966) and by Whalen *et al* (1966) who, using a different method, showed that the flow rate of fasting intestinal contents into the segment studied was 2.16 ± 1.32 ml/min in the jejunum. Besides the large individual variations of the flow rate of this contaminating fluid, our results reveal its important variability in the same subject, since sampling of this fluid above the inflated balloon was strikingly spasmodic. These variations easily explain why the steady state was not so good with the balloon deflated than inflated for infused rate of 10 and 15 ml/min. It is likely that the contaminating effect is less troublesome when higher rates are infused, and that its variations affect the equilibration much less: this is what we found for an infusing rate of 20 ml/minute.

The reflux of infused solution proximal to the infusion point was demonstrated by recovery 10 to 12 cm above this point of a fluid containing glucose and PEG. Fordtran (1969), by comparing absorption of tritiated water and ^{14}C urea with the double- and triple-lumen tube, found that fluid infused at 10 ml/min spreads in a proximal direction for a distance of 20 cm.

These two artifacts inherent in the double-lumen tube influenced in a different way absorption of glucose, water, and sodium. The reflux above the infusion point resulted in an unknown and presumably variable increase in length of the test segment which resulted in turn in an overestimation of glucose absorption. This overestimation could be as much as 150% of the measured value when the balloon was inflated with the mean ranging between 20 and 34% according to the solution and the rate infused. This probably explains why we found glucose absorption much lower than other workers who used a double-lumen tube. Holdsworth and Dawson (1964) infusing at a rate of 20 ml/min a solution containing 25 g/l of glucose measured an absorption rate of about $53 \mu\text{mol/min/cm}$ and calculate a value of V_{max} of $135 \mu\text{mol/min/cm}$. At the same infused rate and with a solution containing 24 g/l of glucose we measured an absorption rate of $36 \mu\text{mol/min/cm}$ and a V_{max} of $44 \mu\text{mol/min/cm}$. Under identical experimental conditions, but with the

balloon deflated, we found an absorption rate of $48 \mu\text{mol/min/cm}$. On the other hand, it is evident that the pollution phenomenon does not influence glucose absorption, since fasting intestinal fluid contains negligible amounts of glucose.

When the balloon was inflated water absorption was found to be higher at infusion rates of 15 and 20 ml/min. This result is at variance with the opinion generally held that the double-lumen method underestimates the absorption of water (Whalen *et al*, 1966; Fordtran, 1969). Our results probably mean that under our experimental conditions the proximal reflux prevailed over the effect of the pollution phenomenon.

Sodium absorption is also said to be underestimated by the double-lumen tube. However we could not find any statistical difference whether the balloon was inflated or deflated. This result is rather difficult to account for, since, with the low concentration of sodium we infused, both reflux and contamination should have resulted in an increased sodium secretion when the balloon was deflated. The large individual variations observed in sodium movement may account for this result.

It is necessary to discuss a possible inhibitory effect of the balloon itself on the absorption mechanism. Phillips and Summerskill (1966) have demonstrated that, during inflation, the motility of the gut was reduced. Studies on correlation between intestinal absorption and motility did not demonstrate any inhibitory effect of hypomotility on sugar absorption. Cummins and Almy (1953) failed to alter, by using bantnine, the glucose tolerance test when glucose was introduced in the duodenum, and Fordtran, Soergel, and Ingelfinger (1962) found that atropinization increased the intestinal absorption of xylose. Groissier and Farrar (1960) conclude that hypomotility probably does not play a critical role in the absorption of isotopic sodium in the normal small intestine, although Higgins, Code, and Orvis (1956) found rather opposite results. Moreover studies on water and ions absorption done with the proximal occluding balloon (Phillips and Summerskill, 1967) and with the triple-lumen tube (Rambaud, personal communication) provided similar results. Thus an inhibitory effect of the inflated balloon on the absorption of sugars, water, and sodium seems unlikely, although not impossible. Finally, it can be concluded from our results with the balloon inflated and deflated that (1) both pollution and reflux do occur with the balloon deflated, and (2) under our experimental conditions absorption rates were more affected by the reflux than by the pollution.

Our results confirm that glucose absorption is a saturable phenomenon. This fact has already been found *in vitro* and *in vivo* by intestinal perfusions in

man (Schedl and Clifton, 1961; Holdsworth and Dawson, 1964) and has been interpreted as evidence for a carrier transport system for glucose. Increasing the flow rate increases glucose absorption rate. This effect is more important when infused glucose concentration is low, ie, when a load-limited situation is approached. But, this effect is still present for higher glucose concentrations, whereas load limitation can no longer be invoked, as shown in Table III and Figure 2. For the highest glucose concentration, the increase in glucose load does not affect absorption rate of this sugar, presumably because saturation of the segment is approached, as will be discussed below.

There is a decrease in values of apparent K_m for high infusion rates when 'traditional' Lineweaver and Burke plotting is used (ie, when the reciprocal of glucose concentration is plotted on abscissae). This result would theoretically mean that as the infusion rate increases, the apparent affinity of the carrier for glucose would be higher. Such an interpretation is rather difficult to accept. The difference in concentration along the segment does not seem to explain the variation of apparent K_m with the rate of infusion. Sladen and Dawson (1969b) predict that glucose absorption would be better correlated with the initial glucose concentration (G_i) when the fractional fall in concentration ($f = \text{drop in concentration down segment}/G_i$) is low (ie, less than 60-70%). In our experiments f was almost always less than 70%. Therefore the factor

$$F \left(f = \frac{1}{f} \log_e \frac{1}{1-f} \right) \text{ which takes the concentration}$$

profile into account varies little with the rate of infusion and the initial glucose concentration. Consequently, an attempt to introduce this factor F in our calculation (by plotting the reciprocal of the glucose absorption rate against F/G_i) did not give a single value of K_m for the four rates of infusion. However, if glucose absorption rate is plotted against glucose load, as initially proposed by Holdsworth and Dawson (1964), the discrepancy between the infusion rates disappears, a common curve can be drawn, and single values of K_m and V_{max} can be calculated. Thus it can be presumed that this value for V_{max} (1.320 mM/min/25 cm or 53 $\mu\text{M}/\text{min}/\text{cm}$) represents the actual maximum absorption capacity of the segment, and that it is almost reached for a load of 3 mM/min. However, Holdsworth and Dawson (1964) found a much higher theoretical value for V_{max} (135 $\mu\text{M}/\text{min}/\text{cm}$) and could also measure higher absorption rates (about 70 $\mu\text{M}/\text{min}/\text{cm}$) for an infused glucose concentration of 50 g/l. This discrepancy is probably

explained by the reflux of infused solution as discussed above. Figure 2 shows that half the saturating load for a 25 cm long segment of jejunum is of 1.15 mM/min. This value cannot be properly called apparent K_m since it does not represent a concentration but a load. It accounts easily for the effect of the rate on 'traditional' K_m : the higher the rate, the lower the concentration which will give half-saturation.

This method of interpreting glucose absorption kinetics can be applied to the results published by Sladen and Dawson (1969b) concerning the effects of flow rate on glucose transport. These workers found for a theoretical segment 15 cm long an absorption rate of about 1.05 mM/min/15 cm and this figure was not influenced by more than a two-fold increase of the infusion rate. The authors interpret this result as a maximum absorption rate for a given initial concentration, and not as a saturation of the absorption capacity itself. In fact, it seems likely that this absorption rate does indeed represent the actual saturation of the segment 15 cm long, and presumably of a longer segment: if one assumes that V_{max} is proportional to the segment length, the theoretical maximal velocity should be of about 0.8 mM/min/15 cm. The results published are higher presumably because of reflux above the infusion point. However, the reflux artifact cannot be invoked for the distal 30 cm long segment, since the absorption was measured in this case with a triple-lumen method, and under these experimental conditions absorption rates measured at three different infusion rates fit quite well with the single curve we proposed, as shown in Figure 2.

Glucose does stimulate sodium and water absorption as shown on Tables IV and V. Under our experimental conditions, this phenomenon resulted in an increase in water absorption and a decrease in sodium secretion. Although unidirectional fluxes of sodium were not measured in our study, it seems logical to assume a priori that glucose stimulates the lumen-to-blood movement of sodium. Schultz and Zalusky (1964), who employed a short-circuited preparation of rabbit ileum, showed that the increase in the short circuit current induced by the addition of glucose to the solution perfusing the mucosal side of the preparation could be entirely attributed to an increase in the rate of active sodium transport from mucosa to serosa. Summers and Schedl (1968) found also that intraluminal glucose increased net absorption and lumen-to-blood movement of sodium on perfused small intestine of rats *in vivo*. However, Nelson and Beargie (1965) found in the dog jejunum that glucose absorption was correlated with the movement of sodium from blood to lumen and not with the reverse movement. Nevertheless,

their experimental conditions may have modified the function of the intestine.

This stimulatory effect of glucose on water and sodium movement was found to increase gradually with solutions containing 14 to 133 mM/l of sugar. However, Schultz and Zalusky (1964) found on rabbit ileum, a saturation of the stimulating effect on sodium transport for glucose concentration of 20 mM/l. Analogous results were reported by Malawer, Ewton, Fordtran, and Ingelfinger (1965) and recently by Sladen and Dawson (1969a) by intestinal perfusion in man: these authors found that the effect of glucose on water transport was gradual but reached a maximum for an initial glucose concentration of 56 mM/l and that the effect of the sugar on sodium absorption was of the 'all-or-nothing' type, the maximum effect being observed for an initial glucose concentration of 14 mM/l. This discrepancy might well be explained by the experimental conditions. Sladen and Dawson (1969a) used higher and variable initial sodium concentrations (inversely related to initial glucose concentrations) whereas we used lower and constant initial sodium concentrations. In this connexion it can be noticed that Fordtran *et al* (1968), in a series of experiments where sodium concentration was kept constant in the segment studied, found that the stimulating effect of glucose or sodium absorption increased when concentration of the sugar infused varied from 0 to 105 mM/l (while the mean glucose concentration in the test segment ranged between 0 and about 40 mM/l). It remains that the mechanism of the effect of glucose on sodium and water movement cannot be soundly discussed from our data, and it is not possible to determine whether the sugar exerts its primary effect on sodium or on water.

For an initial glucose concentration of 200 mM/l, sodium secretion increases and water absorption decreases although the initial sodium concentration was kept constant. An analogous effect was reported by Holdsworth and Dawson (1964) when they compared absorption of water from solutions containing 140 and 280 mM/l of glucose. In this work, however, sodium concentration was not kept constant, since isoosmolarity was adjusted by NaCl. More convincing evidence of this phenomenon was then reported by McMichael *et al* (1967), who also observed a drop in water absorption with 5% maltose solution, containing 80 m-equiv/l Na, which gave the same absorption of glucose as a 5% glucose solution.

Our results with constant sodium concentrations confirm that above a given level of intraluminal glucose concentration both water and sodium absorption drop. This effect seems actually to be correlated with the glucose concentration and not

with the glucose absorption rate, whereas at a rate of 10 ml/min this phenomenon appears when glucose absorption rate increases from 763 $\mu\text{M}/\text{min}/25\text{ cm}$ (for an initial glucose concentration of 133 mM/l) to 841 $\mu\text{M}/\text{min}/25\text{ cm}$ (for an initial glucose concentration of 200 mM/l); it does not appear when, at 20 ml/min, sugar absorption rates vary from 719 $\mu\text{M}/\text{min}/25\text{ cm}$ (for an initial glucose concentration of 66 mM/l) to 913 $\mu\text{M}/\text{min}/25\text{ cm}$ (for an initial glucose concentration of 133 mM/l). At the latter rate, the drop in water and sodium absorption occurs only when initial glucose concentration reaches 200 mM/l. The mechanism of this phenomenon is by no means clear. Saturation of the stimulatory effect of glucose on water and sodium movements cannot be invoked, since, in this hypothesis, these movements should remain constant and not decrease. This phenomenon is much more important with an initial glucose concentration of 260 mM/l presumably because of the low initial sodium level.

High flow rates result in a decrease in water absorption rate and increase in sodium secretion. This phenomenon may be interpreted as an increase in the movement of water and sodium from blood to lumen. This hypothesis is suggested by the significantly higher secretion of potassium from blood to lumen as shown in Table VII (the infused solution was free of potassium). The mechanism of this supposed increase in hydroionic movement from blood to lumen remains unexplained.

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