J. Membrane Biol. 100, 123–136 (1987)

The Journal of
Membrane Biology

FOREWORD TO THIS SERIES OF PAPERS

The three papers to follow provide evidence for a new theory of intestinal absorption of solutes. It is proposed that the presence of glucose or amino acids in the intestinal lumen activates contraction of the perijunctional actomyosin ring in the apical region of epithelial cells, thereby opening tight junctions to permit mass transport of nutrients by solvent drag through paracellular channels. According to the new theory, the primary role of Na-coupled active transport is to provide the osmotic force for convective (absorptive) flow and to trigger contraction of cytoskeletal proteins. At luminal concentrations of glucose or amino acids normally present after a meal, mass transport by solvent drag becomes the major mechanism for absorption of hydrophilic nutrients.

The evidence to be presented derives from three independent lines of inquiry, each of which is described in a separate paper. Although the approaches and techniques are different in each paper, the results are mutually supportive and provide a unified theory which can explain well-known phenomena that could not be explained by previous theories.

The first paper is based on the clearances (steady-state transepithelial fluxes per unit concentration) of inert solutes of various sizes from the small intestines of anesthetized animals in the presence and absence of glucose. From the results we calculate the fraction of fluid absorption which passes paracellularly and the dimensions of channels in a theoretical porous membrane having the same permeability to inert molecules as the glucose-activated intestinal epithelium. From simultaneous measurements of glucose we also calculate the relative contributions of solvent drag, active transport and back-diffusion to the net flux of glucose at any given rate of fluid absorption and luminal concentration.

The second paper addresses the question of whether Na-coupled transport increases solvent drag solely by virtue of its effects on fluid absorption or whether it also opens epithelial junctions. Transepithelial impedances were measured in isolated intestinal segments perfused with oxygenated fluorocarbon emulsions. Addition of small concentrations of glucose or amino acids to the luminal perfusate induced two- to fourfold decreases of impedance at all frequencies tested. Half-maximal changes of impedance occurred at 2–3 mM, which is close to the K_m for active transport. Impedance as a function of frequency was interpreted in terms of junctional resistance, lateral space resistance and capacitance (surface area) of basolateral membranes. The absolute value of junctional resistance in the glucose-activated segment agrees well with that predicted from the permeabilities to inert solutes measured in the first paper. The absolute capacitance agrees well with that predicted from exposed membrane area as measured in the third paper of the present series. The results support the theory that surface area of lateral membranes and dimensions of epithelial cell junctions are regulated by the concentration of nutrients in the intestinal lumen. It is shown also that this regulation is reversibly dependent on concentration and is sensitive to oxygen tension.

In the third paper we explore the structural correlates of the impedance and permeability changes induced by glucose or amino acids. With the aid of new techniques of perfusion and fixation we show that the functional changes are associated with profound changes in ultrastructure as revealed by light microscopy, transmission electron microscopy and freeze-fracture techniques. Addition of glucose or amino acids induces junctional dilatation and disruption of stand/groove compartments in the zonnulae occludens accompanied by expansion of lateral spaces and condensation of actomyosin in the perijunctional ring. The anatomical results correlate well with the observed effects on permeability and impedance; they provide support for the hypothesis that activation of Na-coupled solute transport triggers the contraction of cytoskeletal elements to open intercellular channels.

Contribution of Solvent Drag through Intercellular Junctions to Absorption of Nutrients by the Small Intestine of the Rat

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Summary. The lumen of the small intestine in anesthetized rats was recirculated with 50 ml perfusion fluid containing normal salts, 25 mM glucose and low concentrations of hydrophilic solutes ranging in size from creatinine (mol wt 113) to Inulin (mol wt 5500). Ferrocyanide, a nontoxic, quadrupally charged anion was not absorbed; it could therefore be used as an osmotically active solute with reflection coefficient of 1.0 to adjust rates of fluid absorption, J_v, and to measure the coefficient of osmotic flow, L_p. The clearances from the perfusion fluid of all other test solutes were approximately proportional to J_v. From L_p and rates of clearances as a function of J_v and molecular size we estimate (a) the fraction of fluid absorption which passes paracellularly (approx. 50%), (b) coefficients of solvent drag of various solutes within intercellular junctions, (c) the equivalent pore radius of intercellular junctions (50 Å) and their cross sectional area per

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unit path length (4.3 cm per cm length of intestine). Glucose absorption also varied as a function of J_v. From this relationship and the clearances of inert markers we calculate the rate of active transport of glucose, the amount of glucose carried paracellularly by solvent drag or back-diffusion at any given J_v and luminal glucose concentration and the concentration of glucose in the absorbate. The results indicate that solvent drag through paracellular channels is the principal route for intestinal transport of glucose or amino acids at physiological rates of fluid absorption and concentration. In the absence of luminal glucose the rate of fluid absorption and the clearances of all inert hydrophilic solutes were greatly reduced. It is proposed that Na-coupled transport of organic solutes from lumen to intercellular spaces provides the principal osmotic force for fluid absorption and triggers widening of intercellular junctions, thus promoting bulk absorption of nutrients by solvent drag. Further evidence for regulation of channel width is provided in accompanying papers on changes in electrical impedance and ultrastructure of junctions during Na-coupled solute transport.

Key Words intestinal absorption · paracellular pathways · solvent drag · epithelial permeability · intercellular junctions · molecular sieving

I. Introduction

Epithelia in the small intestine, renal proximal tubules and gall bladder are classified as "leaky" because of their low electrical resistance and high permeability to monovalent cations [23, 51]. Freeze-fracture techniques [8, 9] indicated that intercellular junctions (zonulae occludens) may provide paracellular shunt pathways that could account for the low electrical resistance and small transmucosal voltage during active sodium transport [23, 50, 52]. The presence of paracellular pathways in various in vitro preparations of intestines containing glucose can also be demonstrated by transepithelial passage of extracellular markers ranging in size from sorbitol to inulin [17, 39, 40, 53]. It is not generally supposed, however, that paracellular pathways provide a major route for intestinal absorption of hydrophilic nutrients such as glucose or amino acids, although this possibility was stated clearly in a review by Crane [11]. In the present paper and in two accompanying papers we provide evidence that absorption of fluid, initiated by Na-coupled active solute transport, can account for a large fraction of normal glucose or amino acid transport by solvent drag through intercellular channels in the epithelia of the small intestine. Our interest in this problem was aroused by the observation that certain muramyl peptides derived from normal intestinal bacteria exert their characteristic biological activity after oral administration. Specifically, synthetic muramyl dipeptide of molecular weight 495 has pyrogenic and somnogenic effects after either intravenous or oral administration [33], and we wished to find out if this highly polar compound is actively transported from the intestinal lumen to blood. To this end we perfused the small intestines of anesthetized rats with a balanced salt solution containing acetylmuramyl-L-alanyl-D-isoglutamine (MDP). Glucose (25 ± 5 mM) was added to stimulate fluid absorption [18] and as a marker known to be actively transported. Phenolsulfonpthalein (PSP), polyethylene glycol (PEG_{4000}) or inulin were added as reference markers to which the intestinal epithelium is allegedly impermeable [7, 29, 48]. We soon found, however, that not only MDP but also the reference markers disappeared from the perfusion fluid at rates that varied greatly with rate of fluid absorption but relatively little with molecular size [46]. Previous investigators have noted that excised intestinal sacs or dissected flat preparations of intestinal wall are slightly permeable to large inert substances such as PEG or inulin, *provided* that glucose or amino acids are present in the incubation medium [39, 53].

In the present paper we show that in the intact glucose-activated intestine lipid insoluble solutes ranging in size from creatinine (mol wt 113) to inulin (mol wt 5500) are carried by solvent drag from lumen to blood through negatively charged paracellular channels which exclude completely the passage of a relatively small quadrupally charged anion (ferrocyanide). From the results we estimate the proportion of absorbed fluid which passes through intercellular junctions, the width of channels which could account for observed coefficients of osmotic drag and osmotic flow and the contribution of passive paracellular convection to normal intestinal absorption of glucose and other biologically important substances.

II. Materials and Methods

A. Operative Procedures and Perfusion Techniques

Rats weighing 250–400 g (Charles River) were anesthetized with pentothal, 30 mg/kg. A *T*-tube was placed in the trachea and the carotid artery cannulated with PE10 tubing leading to a strain gauge manometer. The intestines were exposed and a soft silicone inflow cannula inserted about 5 cm into the duodenum towards the ligament of Treitz. The ileum was ligated near the cecum and incised proximal to the ligature; the intestinal contents were then gently flushed with about 200 ml warm saline. A silicone outflow cannula was then introduced in the ileum about 20 cm from the cecum. The perfusion thus included all of the jejunum plus the proximal half of the ileum; the total length of intestine perfused was usually 50–80 cm out of a total of 90–110 cm of small intestine. The wet wt of the empty rat intestine is about 6 g per 50 cm length.

The ionic composition of control perfusion fluid was NaCl 7.25 g/liter, $NaHCO_3$ 2.1 g/liter, KCl 0.3 g/liter, $MgCl_2 \cdot 6H_2O$ 0.2 g/liter, $CaCl_2$ 0.22 g/liter. The concentration of glucose was usually 25 ± 5 mM, maintained by constant infusion of about 1.5 mmol/hr to compensate for absorption and metabolism. The perfusion fluid was recirculated through the jejeunal-ileal segment by a Sigmamotor Model 120 eccentric disc operating on a loop of rubber tubing. Fluid was pumped at the rate of 3 ± 1 ml/min from a 50-ml graduated cylinder through a 38°C water bath to the duodenal cannula. The reservoir was submerged in ice to minimize bacterial degradation of organic solutes. Fine adjustment of inflow temperature to 38 ± 1°C was made with an electric coil heater at the entrance to the cannula. The outflow was returned to the cold reservoir at a height of 2–4 cm above the inflow in order to maintain a small positive pressure within the intestine at all times. Perfusion pressure at the inflow was recorded continuously by strain gauge; it oscillated sinusoidally from about 2 to 8 cm H_2O with each revolution of the Sigmamotor pump, thus stirring the intestinal contents. Any mechanical impediment to flow through the intestine became manifest as an increase of inflow pressure, and steps were taken to correct the disturbance. This was important because the rate of absorption of fluid was measured by the rate of decrease of volume in the reservoir, it being assumed that the volume of the intestine would be constant if the mean intraluminal pressure remained constant.

B. Intestinal Dimensions

The initial volume of the perfused segment was determined by indicator dilution, usually using PSP as the indicator. For this purpose the initial inflow was adjusted to 4 ml/min and the outflow collected without recirculation in 2-min intervals for 16 min starting from the moment when PSP was first observed at the transparent inflow cannula. The mean transmural pressure was maintained at 4 ± 1 cm H_2O by adjustment of outflow pressure. Sixteen minutes sufficed to reach a steady-state concentration of PSP in the outflow so that dilution volume could be estimated from measured concentrations and volumes of inflow and outflow samples. The initial volume of the intestinal segment was taken as the distribution volume of PSP less the volume of cannulae and connecting tubing. At the end of each experiment (usually 3–4 hr) the volume of each segment was measured by direct drainage; usually the initial and final volumes agreed within 5 ml and the average was used for calculating rates of absorption of fluid per unit intestinal volume. In 21 experiments the mean initial volume calculated from indicator dilution was 0.20 ± 0.055 (SD) ml per cm length; the drainage volume at the ends of the same experiments averaged 0.20 ± 0.054 ml per cm. The internal radius calculated from this volume was 0.25 cm. The area of the *serosal* surface was estimated from the diameter of segments filled with fluid at 4 cm H_2O pressure; the average o.d. measured by micrometer was 0.65 cm, corresponding to 2.0 cm^2 per cm length. This value is not significantly different from that reported in histological studies by Fisher and Parsons [20]. However, it is significantly larger than serosal areas of dissected intestines mounted as flat tissue; when intestinal segments are slit longitudinally and placed on filter paper they may contract to less than 50% of their intact area. The wet wt of the empty rat intestine was 0.128 ± 0.013 g per cm length; this value agrees almost exactly with the tissue volume calculated from the difference between outside and inside diameters. For purposes of the present paper we shall assume that a 50-cm length of rat intestine weighs 6 g, contains a volume of 10 ml and has a serosal area of 100 cm^2. Permeability coefficients of biological membranes are usually expressed per unit surface area, but this has little meaning for intestinal epithelium in which the surface area of the microvilli is many times greater than the histological surface of the mucosa and the permeability of plasma membrane is entirely different from that of intercellular junctions. In order to compare individual experiments with each other and with literature values, we will express permeability coefficients per cm length of intestine or per cm^2 serosal surface.

C. Analytical Methods, Calculation of Net Flux Rates

Glucose was measured by glucose oxidase (Worthington Biochemicals), ferrocyanide by hydrogen peroxide [5], creatinine by the Jaffé reaction and inulin or PEG from labeled compounds (New England Nuclear). A G10 Sephadex column was used to verify homogeneity of inulin or PEG in perfusion fluid or in samples of urine. The amount of solute in the intestinal perfusate at any time was calculated as the product of volume x concentration; net flux rates were calculated as the loss of solute from the perfusate over time after making second-order corrections for loss in the samples.

III. Results

Part A. Impermeability of the Intestinal Mucosa to Ferrocyanide

$Na_4Fe(CN)_6 \cdot 10\ H_2O$ forms the quadrupally charged anion $Fe(CN)_6^{4-}$ (mol wt 212) in solution. The osmotic activity coefficient of a 10 mM solution in 0.15 M NaCl is 0.78 (~39 mOsM) as determined by freezing point. Ferrocyanide has been used intravenously for renal clearance studies in animals [5, 56] and in man [38]. We have found that the perfused rat intestine is impermeable to ferrocyanide in concentrations of 1–20 mM; there is no detectable loss during four hours or more of continuous recirculation of the fluid through the intestinal lumen. In contrast, uncharged solutes such as creatinine or inulin are cleared from the perfusion fluid at rates that depend on the rate of fluid absorption as shown in Part B below. Ferrocyanide therefore has an osmotic reflection coefficient of 1.0 and is an ideal solute for use in measuring L_p, the coefficient of hydrodynamic conductance (otherwise known as the coefficient of osmotic flow).

Figure 1 shows the reversible effects of 10 mM ferrocyanide in reducing rate of fluid absorption in the glucose-activated intestine of an anesthetized rat. Figure 2 summarizes results from several such experiments, showing how the rate of fluid absorption varies as a function of osmolality of ferrocyanide in jejeunal-ileal segments. The slope of the regression line, $\Delta J_v/\Delta$ mOsM, is −0.177 for segments

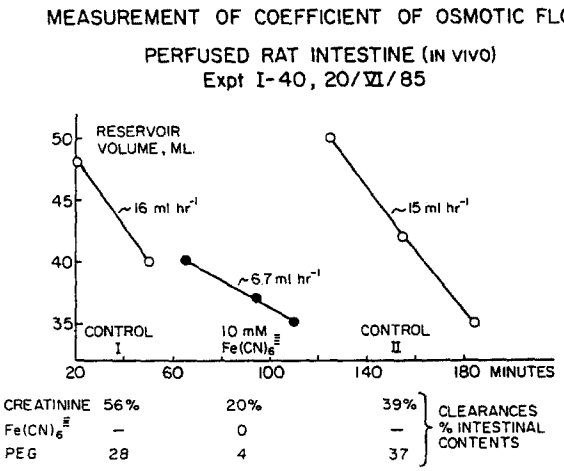

Fig. 1. Effects of 10 mM ferrocyanide on rate of fluid absorption and on clearances of creatinine and PEG from rat small intestine in vivo (segment length ca. 85 cm). This experiment illustrates the reversible effects of ferrocyanide on fluid absorption, the impermeability of the epithelium to ferrocyanide and the method for determination of L_p, the coefficient of osmotic flow, shown in Fig. 2

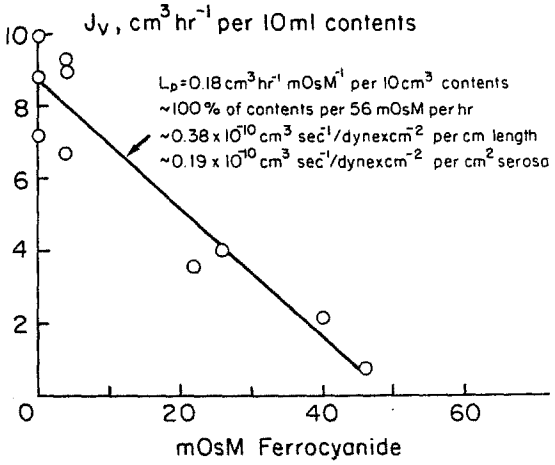

Fig. 2. Coefficient of osmotic flow, L_p, as estimated from effects of ferrocyanide on fluid absorption. Data from four experiments using the protocol illustrated in Fig. 1. Values of J_v are expressed per 10 ml intestinal volume. When converted to cgs units the value for L_p is 0.38×10^{-10} cm² sec^{-1}/dyne cm^{-2} per cm length or 0.19×10^{-10} per cm² serosal surface. This value is not significantly different from that found by Naftalin and Tripathi [40] who used 20 kD PEG instead of ferrocyanide as the osmotically active solute

containing 10 ml fluid, thereby defining the overall coefficient of osmotic flow. When expressed in cgs units this value becomes 0.38×10^{-10} cm^3 sec^{-1}/dyne · cm^{-2} per cm length of segment or 0.19×10^{-10} cm^3 sec^{-1}/dyne · cm^{-2} per cm^2 serosal surface. This value of L_p agrees almost exactly with the value found by Naftalin and Tripathi [40] who used large impermeant polyethylene glycol molecules as the osmotically active solutes in flat sheets of mucosa from rabbit ileum. However, it is several-fold greater than values for L_p estimated by previous investigators using salt [14, 15, 25] or mannitol [22, 54] as the "impermeant" solute. As shown by Naftalin and Tripathi [40] and also in the present paper, the intercellular junctions are highly permeable to uncharged solutes the size of mannitol, and the composite osmotic reflection coefficient of the intestinal epithelium to such molecules is only about 0.2. L_p determined as above is a "lumped" coefficient, which describes hydrodynamic conductance through a very *small* area of large aqueous channels (intercellular junctions) in parallel with hydrodynamic plus diffusional conductance through a very *large* area of plasma membrane (microvilli). In Part B below we show how to estimate the proportion of total fluid absorptive flow that passes through the intercellular channels, thus defining the L_p for these channels separately.

Part B. Paracellular Transport of Inert, Lipid-Insoluble Solutes

Figure 3 summarizes clearances of creatinine, PSP, PEG_{4000} and inulin as a function of rate of fluid absorption, J_v. Clearances are defined as net steady-state transmural flux rates per unit concentration in the intestinal perfusate. Variations of J_v in the range 7–15 ml hr^{-1} were the result of individual differences between different experiments during perfusion with balanced salt solutions containing 20–30 mM glucose; fluid absorption rates in the range 0.5–7 ml hr^{-1} were produced experimentally by addition of osmotically active, inert solute (usually ferrocyanide) to the perfusate. Regression lines and correlation coefficients, r, are shown; some of the correlation coefficients are as low as 0.75, but there is little doubt that clearances of these solutes are approximately proportional to rates of fluid absorption and only slightly dependent on molecular sizes in the range shown. Similar results, expressed in the same units (but not explicitly as clearances) were obtained by Whittembury et al. [58] who used sucrose, inulin and dextrans to demonstrate solvent drag through the gall bladder epithelium of guinea pigs.

Interpretation of Clearance data. Proportion of Fluid Flow through Paracellular Junctions. Dimensions of Aqueous Channels in a Porous Membrane having the Same Coefficients of Solvent Drag, Hydraulic Conductance and Electrical Resistance as the Intestinal Epithelium

Presentation of results in terms of directly measured clearances and rates of fluid absorption per 10 ml intestinal volume as in Fig. 3 provides a simple

estimate of the percentage of intestinal contents absorbed per hour, and this information is useful for estimating the proportions of glucose or amino acids that are carried by the paracellular route after a normal meal as described in Part D below. However, Fig. 3 provides little direct insight into the biophysical properties of the paracellular channels. In the following paragraphs the primary data of Fig. 3 are analyzed in terms of molecular sieving to estimate the dimensions of paracellular channels which could account for observed clearances as a function of flow, for the observed coefficient of osmotic flow described in Part A and for the observed electrical resistance of junctional channels described in the accompanying paper [44].

Contributions of diffusion and solvent drag to the flux of inert solutes through porous membranes can be described in the form proposed by Kedem and Katchalsky [32] and widely used in textbooks and reviews [12, 16, 31, 49].

$$J_s = \omega RT(c_1 - c_2) + (1 - \sigma_f)J_v\bar{c} \qquad (1)$$

where J_s = flux rate of solute, s, J_v = flow of solvent, ωRT = diffusional permeability coefficient, $(1 - \sigma_f)$ = coefficient of solvent drag, c_1, c_2 = concentrations of s at the two ends of the channel, and $\bar{c}$ = mean concentration of s in the channel = $(c_1 + c_2)/2$.

Equation (1) does not take account of unstirred layers which may be present near the surface of absorptive cells or between microvilli near the entrance to tight junctions. Equation (1) is almost identical with the Molecular Sieving Equation first used for studies of capillary permeability [42] and renal glomerular permeability [43]. ωRT is synonymous with $D'A_p/\Delta x$ in the equation of molecular sieving and $(1 - \sigma_f)$ is synonymous with D'/D where D' is the restricted diffusion coefficient and $A_p/\Delta x$ is the cross sectional area per unit path length of the channels. The latter terminology is useful for evaluating channel dimensions as described below. Inspection of Fig. 3 shows that diffusive flux, ωRT, is negligible relative to solvent drag. Thus the clearances of all the solutes shown in Fig. 3 approach zero at low rates of fluid absorption, J_v. If diffusional transport were significant, the lines relating clearance rates to fluid absorption would intercept the clearance axes at positive values; in fact, all the intercepts are close to zero or even slightly negative. For the case of these inert solutes, Eq. (1) becomes

$$J_s = (1 - \sigma_f)J_v(c_1 + c_2)/2. \qquad (2)$$

The flows and concentrations in Eq. (2) refer to channels which allow passage of both solvent and solutes. However, in intestinal epithelia which actively transport ions and organic solutes some of the absorbed fluid must accompany the actively transported solutes intracellularly without passing through the intercellular channels which allow passage of the inert solutes shown in Fig. 3.

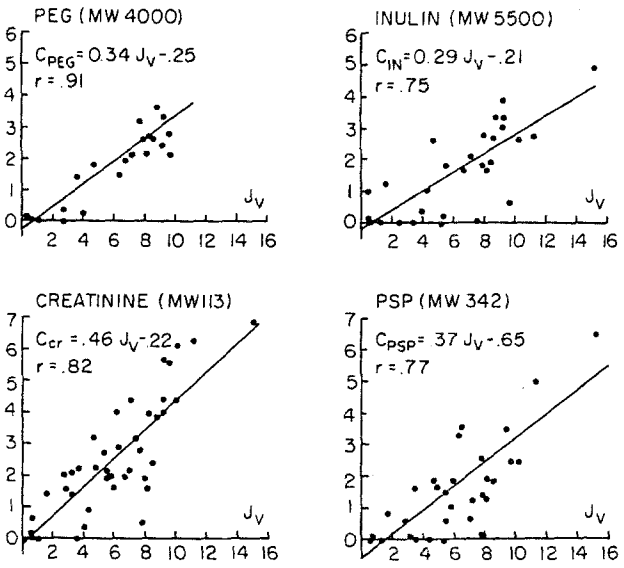

Fig. 3. Clearances of creatinine, PSP, PEG_{4000} and inulin as a function of fluid absorption rates from rat small intestine perfused in vivo. Data from multiple clearance periods in each of 25 rats. Regression lines and correlation coefficients, r, are shown

For inert solutes Eq. (2) becomes

$$J_s = J_v'c_2' = (1 - \sigma_f)J_v'\frac{(c_1 + c_2')}{2} \qquad (3)$$

where c_2' refers to concentration of s at the distal end of the intercellular channel. Solving Eq. (3) for c_2' we have

$$c_2' = \frac{(1 - \sigma_f)}{(1 + \sigma_f)}c_1. \qquad (4)$$

Equations (3) and (4) and the experimental data of Fig. 3 define within narrow limits the fraction, f, of total fluid absorption which takes place through paracellular channels. Thus from Eqs. (3) and (4)

$$J_s = C_sc_1 = (1 - \sigma_f)f \cdot J_v\left[c_1 + \frac{(1 - \sigma_f)}{(1 + \sigma_f)}c_1\right] \div 2. \qquad (5)$$

Differentiating Eq. (5) we have

$$\frac{dC_s}{dJ_v} = f\frac{(1 - \sigma_f)}{(1 + \sigma_f)}. \qquad (6)$$

For the case of creatinine (Fig. 3), $dC_s/dJ_v = 0.46$ whence

$$\sigma_f(\text{creatinine}) = (f - 0.46)/(f + 0.46). \qquad (7)$$

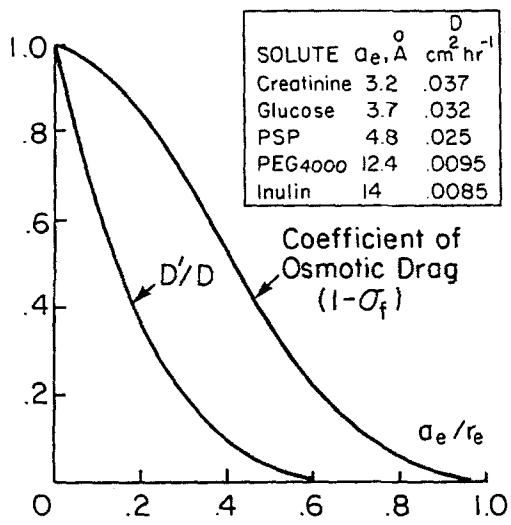

Fig. 4. Coefficients of solvent drag and restricted diffusion as function of equivalent molecular and pore radii, a_e/r_e. This figure, together with the molecular data of the insert, is included for convenience of readers who wish to apply the theory in detail. The curves are drawn from tabular values in reviews by Crone and Christensen [12] and by Curry [16]

It follows from Eq. (7) that the fraction of total absorptive flow that takes place through intercellular channels must exceed 0.46; otherwise σ_f would be negative. This is equivalent to stating that flow through paracellular channels must be at least as large as the creatinine clearance; otherwise it would be necessary to assume that creatinine is transported transcellularly. On the other hand, an upper limit to the fraction of paracellular flow is set by the fact that any channel large enough to allow an inulin clearance of 29% of J_v (Fig. 3) will exert little restriction on the passage of creatinine. For inulin, Eq. (7) becomes

$$dC_{IN}/dJ_v = 0.29 \geqq 0.46(1 - \sigma_f)/(1 + \sigma_f)$$
whence $(\sigma_f)_{IN} = \geqq 0.73$.

Reference to Fig. 4 shows that this requires an equivalent pore radius of at least 50 Å, which would offer negligible resistance to passage of creatinine ($\sigma_f \sim 0$). Therefore f must be very close to 0.46. In the following analysis we take 0.5 as the fraction, f, of total absorptive flow that passes through paracellular channels. Substitution in Eq.(7) of $f = 0.5$ and the observed values of dC_s/dJ_v shown in Fig. 3 leads to values for $(1 - \sigma_f)$ of 0.97, 0.85, 0.81 and 0.79, respectively, for creatinine, PSP, PEG_{4000} and inulin as listed in Table 1. If the coefficients of solvent drag were calculated in the usual way from the Kedem-Katchalsky equation [Eq. (2)] on the assumption that $c_2 = J_s/J_v$ then the composite (whole membrane) coefficients of osmotic drag would be respectively 0.79, 0.66, 0.58 and 0.63 and there would be no simple solution for equivalent channel size as described below. $(1 - \sigma_f)$ is a well-known function of the ratio of molecular radius, a_e, to equivalent pore radius, r_e, or slit width, w_e. This function, together with relevant values for molecular radii and diffusion coefficients, is reproduced in Fig. 4 from Tables published in reviews by Crone and Christensen [12] and by Curry [16]. The corresponding values of equivalent pore radius are 46, 25, 52 and 52 Å for creatinine, PSP, PEG_{4000} and inulin, respectively. The discrepant value of 25 Å for PSP may be ascribed to its negative charge at pH 7.4; for this reason PSP is not suitable for estimation of pore size and the value is placed in parentheses in Table 1. Values of r_e, obtained from creatinine, PEG and inulin are all close to 50 Å and this value is selected for calculation of pore area per unit path length, $A_p/\Delta x$, as follows.

From Poiseulle's Law

$$J_v'/\Delta P = fL_p = A_p/\Delta x \div r_e^2/8\eta$$

or

$$A_p/\Delta x = \frac{8\eta L_p f}{r_e^2}.$$

L_p was evaluated in Part A and found to be 0.38×10^{-10} cm sec^{-3}/dyne cm^{-2} per cm length of intestine, whence $A_p/\Delta x = (8)(0.007)(0.38 \times 10^{-10})(0.5)/(50 \times 10^{-8})^2 = 4.3$ cm per cm of segment. This value for $A_p/\Delta x$ in paracellular junctions agrees well with the value estimated independently from electrical impedance as described in the accompanying paper [44]. Impedance analysis showed that the electrical resistance of intercellular junctions per cm length of glucose-activated perfused rat intestine is 7 Ω, corresponding to $A_p/\Delta x$ of 6.8 cm in junctions filled with perfusion fluid having a specific resistance of 48 Ω-cm at 38°C. Thus it is possible to predict (within 40%) the electrical resistance of intercellular junctions from measured coefficients of hydraulic conductivity and solvent drag of inert, uncharged solutes. Further support for the values of channel dimensions determined as above comes from studies of glucose transport described in Part D below.

Part C. Fluid Absorption and Clearances in the Absence of Luminal Glucose

Table 2 shows that fluid absorption in the absence of luminal glucose is only about 50% of that in the glucose-activated system. Clearances of creatinine, PEG and of inulin were greatly reduced and were

Table 1. Permeability coefficients and estimated dimensions of intercellular junctions in the small intestine (midgut) of the rat

	Creatinine	PSP	PEG_{4000}	Inulin
Clearance, ml hr^{-1} per 10 ml segment	$0.46J_v - 0.22$	$0.37J_v - 0.65$	$0.34J_v - 0.25$	$29J_v - 0.21$
$(1 - \sigma_f)$	0.97	(0.85)	0.81	0.79
r_e, Å	46	(25)	52	52
L_p, cm^3 sec^{-1}/dyne cm^{-2}	0.38×10^{-10} per cm length or 0.19×10^{10} per cm^2 serosal area			
$A_p/\Delta X$, cm	4.3 (from permeability)			
Per cm length	6.0 (from impedance)			

Table 2. Perfusion with or without glucose[a]

	J_v	C_{Cr}	C_{PEG}	C_{IN}
With glucose	8.8 ± 0.6(15)	4.1 ± 0.5(15)	2.6 ± 0.2(8)	2.4 ± 0.4(14)
Without glucose	4.6 ± 0.4(14)	1.4 ± 0.3(14)	1.6 ± 0.3(11)	1.2 ± 0.3(13)

[a] Fluid absorption (J_v) and clearances (C) ml hr^{-1} per 10 ml contents. Means ± SE (N clearance periods).

too small to allow estimates of permeability coefficients and dimensions of intercellular channels by the methods described in Part B. However, in an accompanying paper [44] it will be shown by impedance techniques that the area per unit path length of intercellular junctions in glucose free media is less than half that in glucose or amino acid-activated preparations.

Although no exogenous glucose was added to luminal perfusate in the experiments of Table 2 designated "without glucose," this did not preclude glucose from reaching the epithelial transport system via the blood. The possibility that residual fluid absorption during perfusion with glucose-free perfusion shown in Table 2 is associated with transport of glucose derived from blood is considered in the discussion section.

The effects of glucose on clearance of creatinine from the gastrointestinal tract can be demonstrated, at least qualitatively, in normal unanesthetized rats. For this purpose rats were starved for 24 hr and then given 50–100 mg of exogenous creatinine by stomach tube at 5 p.m. They were then placed in metabolism cages over a fraction collector for urine and given access to either 20% glucose or to water. Absorption of creatinine during the subsequent 15 hr was measured from its urinary excretion after correcting for endogenous creatinine excretion. Ten rats with access to glucose excreted 53 ± 2.4% (SE) of the exogenous load in 15 hr; the same rats given access to water instead of glucose excreted 37 ± 2.3% of the load. The difference is significant at the $P < 0.01$ level and provides some assurance that the results of experiments on perfused intestines of anesthetized rats can be applied to normal animals.

Part D. Intestinal Absorption of Glucose

It was shown by Fullerton and Parsons [24] that absorption of glucose from perfused rat intestine is a function of rate of fluid absorption and of luminal concentration. The concentrations used were from 28 to 246 mM or 10- to 50-fold greater than the half-saturation (K_m) value for active transport. At a concentration of 246 mM the absorption rate of glucose was fourfold greater than at 28 mM, a result clearly incompatible with a glucose carrier having a K_m of 5 mM. The experiments of Fullerton and Parsons depended on individual variations of J_v in different experiments. We have extended the range of variation of J_v experimentally through the use of ferrocyanide as described in Parts A and B above.

Figure 5 shows the total steady-state transmucosal flux, J_T, of glucose as a function of fluid absorption rate, J_v, in perfused jejunal-ileal segments of 16 anesthetized rats. Each point is based on measurements made during 30–90 min of perfusion. Variations of J_v in the range 6–12 ml hr^{-1} are the result of differences between individual rats perfused with balanced salt solutions containing 20–30 mM glucose; fluid absorption rates in the range 1–6 ml hr^{-1} were produced experimentally by addition of osmotically active solute (ferrocyanide) to the perfusate. The concentration of glucose in the recirculating perfusion fluid was maintained approximately constant in the range 20–30 mM by infusion

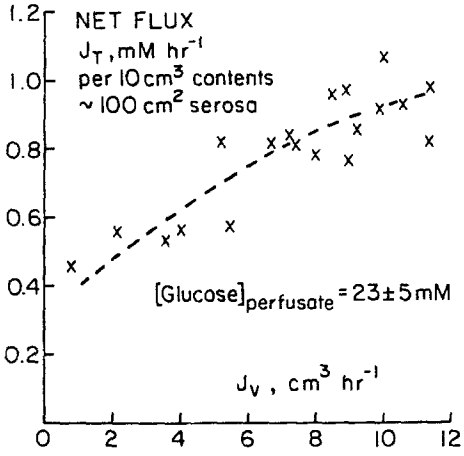

Fig. 5. Net glucose flux *vs.* fluid absorption from rat small intestines perfused in vivo. Data from steady-state measurements in 16 rats. The dashed line drawn through the experimental points conforms to the theoretical analysis of glucose flux described in the text and illustrated semi-diagrammatically in Figs. 6 and 8

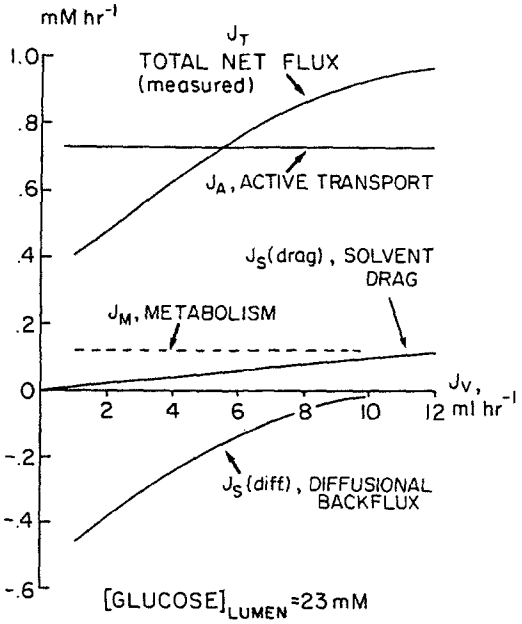

Fig. 6. Components of glucose flux, calculated as described in text from measured total flux (J_T, Fig. 5) and the clearances of inert solutes (Fig. 3). Flux rates are expressed per 10 ml lumen volume (approximately 50 cm length or 6 g wet wt). The contribution of solvent drag is relatively small in this example because the concentrations of glucose in the perfusates were only 23 ± 4 mM; under physiological conditions the luminal concentration of glucose may exceed 300 mM and the contribution of solvent drag exceeds that of active transport as shown in the discussion section (*see* Figs. 7 and 8)

of glucose at a rate estimated to match the rate of loss by intestinal absorption and metabolism. This concentration of glucose is 5- to 10-fold greater than the K_m for carrier-mediated active transport of glucose. Therefore the dependence of glucose transport on rate of fluid absorption shown in Fig. 5 cannot easily be attributed to changes in active transport. The dashed line drawn through the experimental points conforms to the theoretical analysis of glucose flux described below and illustrated semi-diagrammatically in Figs. 6 and 8. It is not significantly different from a straight line drawn through the same experimental points but a linear regression cannot be resolved into the components of glucose flux illustrated in Fig. 6. The following components of total transmucosal flux of glucose, J_T, can be calculated from Fig. 5 and the results obtained in Part B, summarized in Table 1.

1. J_A Rate of Active Transcellular Glucose Transport

2. J_{SD} Rate of Transport of Glucose by Solvent Drag through Intercellular Junctions

3. $J_{Sdiffusion}$–Rate of Back Diffusion of Glucose from Lateral Intercellular Space to Lumen at Abnormally Low Rates of Fluid Absorption

4. c_2–Concentration of Glucose in Mixed Absorbate (Paracellular + Transcellular) and Its Contribution to the Driving Force for Fluid Absorption

1 and 2: Rate of Active Transport J_A and of Solvent Drag J_{SD}

In the in vivo preparation with intact blood supply there is no accumulation of transported glucose in the tissues as there is in in vitro isolated segments [4, 18, 19]. Thus in the steady state

$$J_A = J_T - J_M - J_{SD} + J_{Sdiffusion} \tag{8}$$

where J_M is that part of apical flux used in intracellular metabolism and all other subscripts are defined above. We assume that metabolic rate is about 15% of J_T at normal rates of fluid absorption as found by Atkinson et al. [2] in vivo and by Leese and Mansford [34] in everted sacs. Reference to Fig. 5 shows that this is equivalent to 0.12 mM hr^{-1} per 6 g wet wt (or per 10 ml intestinal volume). The active component of transport is then

$$J_A = J_T - 0.12 - J_{SD} + J_{Sdiffusion} \text{ mM hr}^{-1} \text{ per 10 ml intestinal volume} \tag{9}$$

At normal or high rates of J_V back diffusion is negligible and J_{SD} can be estimated, at least approximately, from the clearances of inert solutes of comparable size. Thus the experimentally determined relations between clearances and J_V for creatinine (mol wt 113) and for PSP (mol wt 342) were respectively (Fig. 3)

$C_{\text{creatinine}} = 0.46\, J_V - 0.22$

and

$C_{\text{PSP}} = 0.37\, J_V - 0.65.$

Interpolating to the mol wt of glucose we have $J_{SD} = C_{(\text{glucose})}c_1 = (0.43J_V - 0.34)c_1$. From Fig. 5 we see that when $J_V = 10$ ml hr^{-1} and $c_1 = 0.023$ mM ml^{-1} then $J_T = 0.93$ mM hr^{-1} whence $J_A = 0.93 - 0.12 - (0.43 \times 10 - 0.34) \times 0.023 = 0.73$ mM hr^{-1}. This value may be compared with the mean value of 0.75 mM hr^{-1} found by Barry, Matthews and Smyth [4] in everted sacs of comparable weight. The contribution of solvent drag to glucose transport is small relative to active transport under the conditions of our experiments where luminal concentrations of glucose do not exceed 30 mM (Fig. 6 or 8). However, the flux of glucose (or other nutrients) by solvent drag through paracellular channels is proportional to luminal concentration, c_1, and normal values of c_1 may exceed 300 mM after a meal. Under these conditions solvent drag through paracellular channels may account for the major fraction of intestinal absorption of glucose and amino acids as described in the Discussion section below.

3. Rates of Back Diffusion. $Js_{diffusion}$

At low rates of fluid absorption such as those produced by addition of osmotically active solute (ferrocyanide) to the lumen, the paracellular back diffusion of glucose may not be neglected; this is because active transport into the intercellular spaces may raise the concentration, c_2', of glucose at the distal ends of the junctional channels to values that greatly exceed the lumen concentration, thus creating a concentration gradient for diffusion in the direction opposite to solvent drag. The magnitude of this back diffusional flux at any given rate of fluid absorption may be estimated as follows from the data of Fig. 5 and Table 1

$$J_{S\text{diffusion}} = J_T - J_M - J_A - C_{\text{glucose}}c_1 = J_T - 0.12 - 0.73 - (0.43J_V - 0.34) \times 0.023.$$

Figure 6 shows the components of transmucosal glucose flux which could account for the dependence of flux on rate of fluid absorption as determined experimentally (Fig. 5 and [24]). When J_V is reduced to 2 ml hr^{-1} the diffusional back flux is 0.4 mM hr^{-1} or more than 50% of the rate of active transport from lumen toward blood.

Back-diffusion of glucose from lateral spaces to lumen also occurs after inhibition of active glucose transport by phlorizin ([6] and M. Lucas, *personal communication*).

4. Concentration of Glucose in Absorbate and Its Contribution to the Driving Force for Fluid Absorption

The concentration of glucose in mixed absorbate (paracellular + transcellular) is $c_2 = (J_T - J_M)/J_v$. At normal rates of fluid absorption ($J_v = 8$ ml hr^{-1}) and taking $c_1 = 23$ mM as in our experiments, the value of J_T is 0.85 mM hr^{-1} (Fig. 5) whence $c_2 = (0.85 - 0.12)/8 = 0.091$ mM ml^{-1} (91 mM) or approximately 4× luminal concentration. To this must be added equimolecular (or 2× equimolecular) Na^+ cotransported with the glucose at the apical surfaces and extruded with anion at basolateral surfaces. Even with an overall membrane osmotic reflection coefficient of 0.2 or less, this concentration gradient is sufficient to account for normal rates of fluid absorption as shown in Fig. 2. It could also contribute to the high tissue Na^+ and osmolality found in intestinal villi in the presence of glucose [30].

IV. Discussion

A. Absorption of Glucose

It has been known for more than 50 years [3, 35] that rate of absorption of glucose from the small intestine is more or less proportional to concentrations up to 300 mM or more. The optimal concentration for maximal rate of absorption in rats, cats and rabbits is said to be 750 mM [37]. According to Cummins [13] the absorption of glucose from human intestine is almost proportional to luminal concentrations up to 500 mM. In reviews by Parsons and Prichard [47], Crane [10, 11] and by Fordtran and Ingelfinger [21] there are numerous references to apparent K_ms in vivo of 100 mM or more. An illustrative example from the literature is reproduced here as Fig. 7 because of its importance to our present thesis that solvent drag is a major route for absorption of nutrients after a normal meal. Figure 7 shows that in normal human subjects the rates of absorption of glucose or galactose are roughly proportional to luminal concentrations from 28–280 mM (0.5–5 g/100 ml) and that absorption of fructose is 90% of glucose absorption at a concentration of 250 mM. The authors state that "K_m" for glucose is 150 mM and offer no explanation for the fructose data. More recent work by Thomson [55] on isolated intestinal epithelia of rabbits shows that the K_m of the active component of transport is 2–3 mM

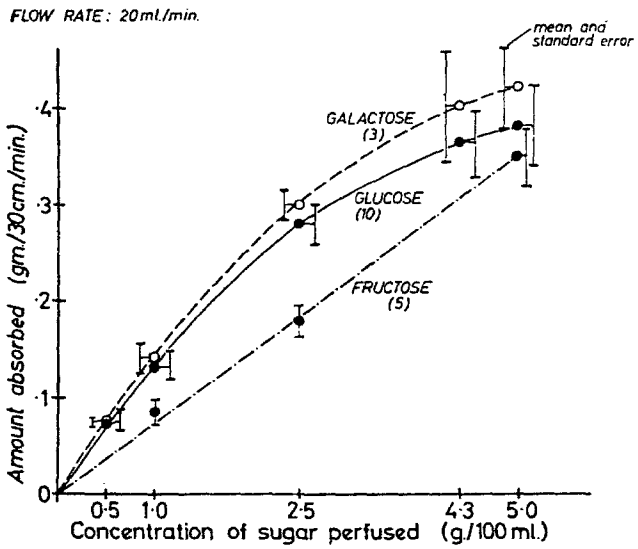

Fig. 7. Absorption of glucose, galactose and fructose in normal subjects. Figures in brackets indicate number of subjects in each group. [Reprinted by permission from *Clin. Sci.* **27**:371–379, copyright 1964, The Biochemical Society, London]

but that transport continues to increase rapidly in proportion to concentration above 10 mM. Similar results have been described by Bolufer et al. [6] who used galactose in perfused segments of rat duodenum or jejunum. Such data are not easily explained in terms of molecular mechanisms for carrier mediated transport in which the affinity constant is of the order of 5 mM; they are consistent, however, with the values for coefficients of solvent drag, paracellular channel dimensions, fraction of fluid flow through paracellular channels and glucose transport summarized in Table 1 and Figs. 3, 5 and 6 of the present paper. In our experiments the glucose concentration in luminal perfusate was always less than 30 mM so that the contribution of solvent drag to total glucose flux was small as illustrated in Fig. 6. After feeding, however, the concentration of glucose in chyme from the small intestine of rats is in the range 50–500 mM [35]. Similar values have been reported for human intestinal chyme [7, 21]. According to our results, the contribution of solvent drag to glucose absorption at a concentration of 300 mM and a normal J_v of 8 ml (per 10 ml intestinal contents) is given by

$$J_S = Cc_l = (0.43J_v - 0.34)c_l$$
$$= (0.43 \times 8 - 0.34) \times 0.3 = 0.96 \text{ mm hr}^{-1}$$

as compared with 0.73 mM hr^{-1} for active transport. Figure 8, based on our present results, shows the relative contributions to glucose absorption of solvent drag and active transport at physiological ranges of luminal concentration and at a moderate rate of fluid absorption (8 ml hr^{-1} per 10 ml intestinal contents in rat). Diffusional backflux of glucose at this rate of fluid absorption is negligible (Fig. 6). The data of Holdsworth and Dawson [28] and of Thomson [55] indicate that the proportion of glucose transport due to solvent drag is even greater in humans and in rabbits than in rats. The role of active transport in transfer from lumen to blood may be of more importance for the absorption of nutrients from low concentrations in the lower jejunum or ileum. From the point of view of energetics it is obvious that mass transport of glucose by solvent drag is far more efficient than by active transport, especially at the high concentrations of glucose normally found in the duodenum and upper jejunum after feeding. The only energy required for passive transport by solvent drag is that used in overcoming viscous resistance to fluid flow; it is otherwise independent of solute concentration or flux rate. The efficiency, expressed as mols transported per calorie, increases almost in proportion to concentration or flux rate.

Absorption of amino acids was not investigated in these experiments; nevertheless, it follows from the data on creatinine and glucose that absorption of amino acids and small, incompletely hydrolyzed peptides by solvent drag through paracellular channels will be at least as important as it is for glucose. It has been pointed out repeatedly that products of protein digestion are absorbed in the form of incompletely hydrolyzed peptides as well as in the form of free amino acids [26, 27]. For most common amino acids, as for glucose, the "K_m" for active transport determined in everted sacs is in the range 1–10 mM [see Wiseman [59] for review). However, the concentrations of amino acids [41] or small peptides [1] in intestinal chyme following a normal meal far exceed the concentrations which saturate the active transport systems. In accompanying papers on impedance [44] and ultrastructure [36] we show that amino acids, like glucose, trigger the opening of intercellular junctions for transport by solvent drag.

B. Role of Diffusion in Paracellular Transport

Our experiments indicate that steady-state diffusion of inert hydrophilic solutes from lumen to lateral spaces through intercellular channels is normally insignificant at all luminal concentrations. The coefficients of osmotic drag through the junctions are so close to unity for all small, inert, uncharged solutes that no significant concentration gradients for diffusion can be maintained across the junctions. In the case of actively transported solutes such as glucose

or amino acids, the steady-state concentrations at the distal ends of intercellular junctions and in total absorbate will always be higher than luminal concentrations, thus providing a concentration gradient for osmotic flow on the one hand and for back-diffusion on the other. Our data and their interpretation indicate that this gradient is sufficient to provide a significant force for fluid absorption but is normally too small to cause significant back-diffusion through the area per unit path length of the junctions.

Significant back-diffusion of glucose through the junctions can, however, be demonstrated under special, abnormal experimental conditions such as (i) when absorption of fluid is prevented by adding an osmotically active solute to the lumen without interfering with active transport as in Figs. 5 and 6 or (ii) under nonsteady-state conditions by blocking active transport and fluid absorption while concentration of glucose in lateral spaces is still high [6]. Under normal conditions, however, our results indicate that the dimensions of intercellular channels are such that solvent drag, rather than diffusion, is the predominate mechanism for paracellular transport.

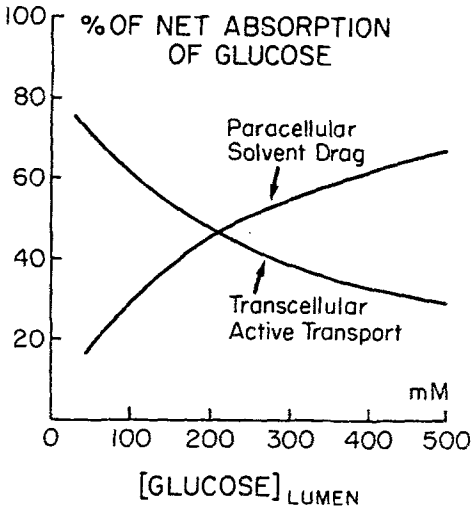

Fig. 8. Proportions of total glucose absorption carried by active transport and solvent drag in perfused rat intestine when fluid absorption is 80% of intestinal volume per hour (normal absorptive state). Values are calculated as in Part D, paragraphs 1–3. The contribution of solvent drag exceeds that of active transport at concentrations above 250 mM

C. Coefficients of Solvent Drag in a Composite Membrane

Equation (1) describing diffusion and solvent drag through porous membranes was derived for membranes in which flow of both solvent and solute take place through the same channels. This is *not* the case for organized membranes such as endothelia or epithelia, and there have been numerous modifications of Eq. (1) to predict composite coefficients of solvent drag or osmotic reflection in membranes having a distribution of channel sizes such that solvent but not solute can traverse some of the channels. The problem has recently been reviewed in detail by Curry [16].

The treatment of this problem given in the present paper is new, and we believe it avoids an erroneous assumption made implicitly in previous treatments, namely that the concentration of solute at the downstream side of the membrane is uniform. In endothelia, where water flow emerging from capillary plasma membranes is everwhere within a few micrometers of solutes and water passing through interendothelial slits this assumption may be valid, although there is no strong evidence for it. However, in epithelia it is improbable that the concentrations of solutes emerging from zonnulae occludens are equal to their average concentration in total absorbate as assumed, for example, by Whittembury et al. [58]. In order to solve the problem it is necessary to determine c_2' at the distal end of the solute channel. This we have done from the clearance data and the theoretical development of Eqs. (3) to (7). The concentrations, c_2', of inert solutes determined in this way are all much closer to c_1 than are the average concentrations in total absorbate, which is diluted by fluid containing no solute at a point far from the distal ends of the intercellular junctions. This treatment of the problem should be applicable to the general case of composite biological membranes in which concentrations of solutes emerging from permeant channels differ from their concentrations in mixed fluid on the distal side of the membrane as a whole.

D. Clearance of Large Inert Solutes

The relatively large clearances of PSP, PEG_{4000}, and inulin at high rates of fluid absorption came as a surprise and indeed we originally added these substances on the supposition that they would not be absorbed and could be used as reference markers for absorption of the muramyl dipeptides we were investigating. The assumption that PEG or inulin do not penetrate the epithelium is based largely on perfusion of the intestinal lumen with glucose-free salt solutions [7, 48] in fasted animals or man. Under these circumstances the rate of fluid absorption and the clearances of all inert solutes from luminal fluid are greatly reduced as shown in the present paper (Table 2). In the presence of glucose, however, sev-

eral previous investigators have noted that inert markers ranging in size from sorbitol to inulin can penetrate the epithelium of isolated intestinal segments or flat sheets [17, 39, 40, 53]. The possibility exists, therefore, that activation of the glucose transport system might not only increase rate of fluid absorption but in addition increase the width of intercellular channels, thus increasing permeability to large solutes and promoting absorption of nutrients by solvent drag. In accompanying papers on electrical impedance [44] and ultrastructure [36] of intestinal epithelial we provide evidence that this latter mechanism does indeed exist and plays an important role in determining the permeabilities summarized in Table 1 of the present paper.

E. Coefficient of Osmotic Flow: Role of Na-Coupled Solute Transport in Fluid Absorption

The fact that intestinal epithelium is impermeable to ferrocyanide (mol wt 212) while it simultaneously allows the passage of PEG or inulin suggests that intercellular channels bear a high negative charge. Ferrocyanide passes rapidly through glomerular membranes [5, 56] or through peripheral capillary membranes [45]. Our measurements of L_p, based on reduction of fluid absorption by ferrocyanide, are considerably larger than those found by previous investigators who used salts [14] or mannitol [22, 54] as the osmotically active solute on the assumption that their osmotic reflection coefficients are 1.0. The value of L_p which we have found using ferrocyanide (Figs. 1 and 2, Table 1), combined with our estimate of glucose concentration in the absorbate, suggests that active glucose transport may provide the principal driving force for normal intestinal absorption of fluid. This would account for the well-known stimulation of fluid absorption by Na-coupled solute transport.

The question as to whether glucose or other actively transported substrates are required for absorption of fluid is a controversial one which is central to understanding the mechanism of absorption of nutrients. Several investigators [14, 17, 25, 53] state that in vitro preparations of intestinal epithelia fail to transport fluid from mucosa to serosa in the absence of glucose. On the other hand, Barry et al. [4] found that everted sacs immersed in glucose-free media can continue to absorb fluid for 20–30 min. This discrepancy may be resolved, at least in part, by the presence of residual glucose in tissue fluids. In excised intestine there is sufficient residual glucose in tissue fluids to last for 15–20 min (*see* Section B of Ref. 44) and data from everted sacs are usually obtained during the first 20–30 min after immersion in glucose-free solutions [4]. In the case of intact preparations, such as described in the present paper, there is a constant supply of glucose from plasma (5–6 mM) to epithelial cells via facilitated passive transport through basolateral membranes. Since K_m for glucose transport is less than 5 mM it is not unreasonable to suppose that the steady-state supply from blood is sufficient to keep the apical Na-coupled glucose transport system running at a low rate, even though no detectable glucose may appear in the luminal perfusate. Residual absorption of fluid in the absence of mucosal glucose may therefore be associated with a residual supply of glucose from tissue stores (in vitro) or from blood (in vivo). In any case it appears that sodium and glucose together provide most or all of the osmotic force for fluid absorption, thus providing an efficient mechanism for mass transport of all hydrophilic nutrients (including glucose itself) by solvent drag through intercellular channels which admit passage of solutes of mol wt 5000 Daltons or more.

We thank Ms. Tanya Atagi for help with some of the experiments and Drs. T. H. Wilson and J. L. Madara for many helpful discussions. The work was supported, in part, by a Pilot Study Grant from the Harvard Digestive Diseases Center NIH Grant #1-P30-AM-34854.

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Received 11 March 1987; revised 20 August 1987