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Fluid Recirculation in *Necturus* Intestine and the Effect of Alanine

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Abstract. Fluid absorption by *Necturus* small intestine has been studied using radiolabeled dextrans as molecular probes of the paracellular pathway under voltage-clamped conditions. Fluxes of H^3-dextrans of MW up to 20K were followed in both directions between mucosal (M) and serosal (S) baths by fractionating those that passed the epithelium as a function of molecular radius. Consideration of the unstirred layers in the baths and the surface geometry rules out any contribution made by solute polarization. The geometry of the paracellular system was measured by light microscopy, TEM and SEM, and values were used in conjunction with a program that calculates convective-diffusive coupling in the tight junctions, intercellular spaces and subepithelium in series. The results indicate that the net fluxes are due to the convection of fluid through two opposing paracellular fluid circuits with different size selectivity, resulting in net absorption at small radii.

Alanine at 20 mM stimulates fluid and salt uptake by a factor of 2. Its effect on the two convective components is to increase the *M* to *S* flux and decrease the *S* to *M*. The selectivities are not significantly different from those before alanine treatment. The volume absorption predicted from the net probe fluxes is very close to that measured gravimetrically across the epithelium.

Key words: *Necturus* intestine — Epithelium — Alanine — Junctions — Dextran probes — Fluid transport

Introduction

The problem of fluid transport in the small intestine has received a great deal of attention over the last few decades but although the phenomena of volume absorption and secretion have been separately addressed there is no clear picture of the role of recirculation in fluid movement. There are in fact several questions about the overall process that require clarification. (i) Are absorption and secretion separate modes of transfer by epithelia cells i.e., do enterocytes absorb *or* secrete and are switched from one mode to the other? (ii) Are these modes confined to different cells localized in different regions of the epithelium? (iii) What is the general mechanism of fluid movement?

The general consensus would seem to be that absorption is the 'normal' mode of fluid transfer but that fluid secretion can occur under special conditions; these can be certain normal states of the intestine [8, 9] or disease states [2] which represent extremes of secretion; when secretion occurs it seems to involve intestinal crypts and is associated with Cl ion secretion by certain cells only [17] although absorption also occurs in crypts [10] and, depending on the epithelium, both may apparently occur in the villi [5]. However, when nutrients are being absorbed it would seem that a considerable measure of recirculation must be taking place. The absorption of 1 mole of glucose or alanine, for example, by a Na-linked transporter in the apical membrane would require at least 1 mole of Na and 6 liters of water under isotonic conditions in mammals and 10 liters in amphibia. These quantities of Na and water are not ingested and could only be made available through recirculatory flows of both.

In earlier studies of this problem Visscher and coworkers [6,16] proposed that there were two 'fluid circuits' operating across the intestinal wall to transfer salt and water. The mechanism generating the flows was not discussed. Subsequently, it became obvious that ions and water could follow separate routes and that such a scheme was premature [7]. As to the mechanism of fluid movement, a model of local osmosis is widely believed to represent the process either through the cell membranes [15] or over the junctions [11]. A problem with

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local osmotic theories of recirculation is that two differently oriented osmotic gradients have to be set up between interspace and mucosal bath within a short distance of each other.

In *Necturus* gallbladder epithelium it has been shown that absorption occurs by a paracellular transport of salt and water and not by local osmotic action [13]. Extension of these ideas goes a long way to explain the lack of correlation between the rate of fluid absorption measured in *Necturus* intestinal epithelium and that of the Na pump when the fluid transfer rate is modulated by the presence of alanine [14].

We have used here the technique of measuring paracellular convection with radio-dextrans which has been used previously on *Necturus* gallbladder epithelium to define the paracellular pathways in operation during net fluid absorption. Fluxes of a homologous series of dextran oligomers of increasing radius have been measured in both directions, before and after stimulation of absorption by alanine. In the same tissue light and electron microscopy has been employed to investigate the geometry of the epithelium and define the size of the pathways in operation during net fluid absorption.

Although the pattern of dextran permeation could be more difficult to interpret if fluid flow in more than one direction were present, it was considered that the results could be interpreted in the light of those obtained from *Necturus* gallbladder epithelium. This analysis has revealed a convective paracellular channel with a selectivity which is linearly dependent on radius and which is responsible for fluid flow from mucosa to serosa [4].

Materials and Methods

The measurements of bidirectional radiolabeled dextran fluxes was carried out in the same experimental setup which was used for measurement of the ion fluxes (*see* companion paper [14]). The two sets of flux studies are complimentary and were carried out under the same conditions, and only the salient features are described here.

Dextran Characteristics

The characterization of dextran molecules as quasi-spherical molecular probes and the techniques for fractionating them on a soft-gel column, with the determination of their effective hydrodynamic (Stokes-Einstein) radii, has been described in detail previously [13].

Dextrans (T10-molecular weight range 0–20K) were obtained from Pharmacia (Uppsala) and tritiated by Amersham International (Aylesbury, UK) by a tritium-palladium process. Calculation of the Scheraga-Mandelkern β-factor from physical data for dextrans gives a mean value of 2.156 equivalent to an axial ratio of 2–3. We therefore assume that the radius of gyration is close to the Stokes-Einstein (SE) radius and that the molecules are effectively quasi-spherical in aqueous solution.

These molecules were routinely fractionated by size exclusion chromatography on Sephadex G25 from which dextrans elute according to a fractionation index given by a fitted Ogston curve

$$K_{av} = \exp[-\pi L(r + r_s)^2] \quad [1]$$

where the fiber length per unit volume of gel $L = 2.21 \times 10^{11}$ m/cm^3, the fiber radius $r = 0.417$ nm, and the dextran radius is r_s. Dextrans used in experiments could thus be fractionated and the activity of different radii determined both before and after passing through the intestinal epithelium.

Dextran Fluxes

Sections of *Necturus* small intestine were stripped and adjacent pieces mounted in cassettes as previously described. The tissue area exposed in the chambers was ~0.3 cm^3. The hemichambers were stirred with magnetic bars monitored with Hall effect transistors at a high rate. The epithelium was voltage-clamped with a resistance-compensated circuit to eliminate electro-osmotic flows and ensure conformity with the conditions under which the ion fluxes were measured. The clamp current was also used to monitor the preparation and assess the effects of added alanine. Fluxes were measured at 20° by adding H^3-labeled dextrans in saline (Na 100, K 3.6, Ca 1, Mg 1, phosphate 3.6 mM, pH 7.2, gassed with pure oxygen) to the mucosal or serosal side of the tissue. After 60 min the sink hemichamber was emptied for analysis of the transported dextrans and refilled with saline. Alanine was then added to both sides to a concentration of 20 mM and the flux collected again after 60 min. The dextran concentrations were in the micromolar range and did not contribute to the osmotic pressure. The flux during an experimental period was about 1% of the source dextran and so the source hemichamber was not diluted; it was sampled at the end of the experiment.

Unstirred Layers

There are two unstirred layers to be considered, one in the baths and the other within the subepithelium. The stirring velocity within the dimensions of the hemichamber resulted in a calculated Reynolds number of 8000 or greater [13] i.e., there was clearly a turbulent regime, and consequently USL effects in the baths were considered to be negligible.

In the subepithelium, which is essentially unstirrable, the gradients are incorporated into the program SPACES (*see* Computer Modelling) and appear as a natural consequence of the calculated flux through the junctions, lateral intercellular spaces and subepithelium in series.

Flux Analysis

Dextrans were fractionated on Sephadex G25 (Pharmacia) and the radioactivity of the eluted fractions determined by an in-line flow scintillation detector (Canberra Packard). The spectrum of activity *v.* K_{av} (about 50 points per 0.1 K_{av}) was put through a software routine that interpolated activity values at exactly 0.01 K_{av} steps thus enabling two spectra to be aligned and compared, and converted the K_{av} to SE-radius (r_s) by using Eq. (1). The low activity M_j (from the sink hemichamber) was then divided by the high activity M_i (source hemichamber) and using the hemichamber volume V and the flux period t this was converted to a plot of specific unidirectional dextran flux J_{ij}/C_i at each radius

$$J_{ij}/C_i = (M_j/M_i)V/At \quad [2]$$

Dextrans are inert carbohydrate polymers which are not transported by any known membrane system and are widely considered to be markers of the free space or paracellular system. The only way they can show

net transport is by being convected. The flux J_{ij} through a channel is given in terms of convection-diffusion theory by

$$J_{ij} = v\phi_f \frac{C_i \exp(v\phi_f L/D\phi_s) - C_j}{\exp(v\phi_f L/D\phi_s) - 1} \quad [3]$$

where v is the fluid velocity, D the diffusion coefficient and L the length. ϕ_f and ϕ_s are convective and diffusive drag factors. If the net flux J_{net} is the difference between the two undirectional specific fluxes then

$$J_{net} = J_{ij}/C_i - J_{ji}/C_j = v\phi_f \quad [4]$$

The passive fluxes through any channels (including leak pathways) cancel out and the net flux is a measure of the convection velocity v through the channel modified by a drag factor ϕ_f which falls as the dextran radius increases. It has been shown in *Necturus* gallbladder that ϕ_f is a linear function of radius.

Volume Flows

Fluid is absorbed from the lumen when bathed with saline only and this absorption is enhanced when alanine is added to the baths. The rate of fluid transfer was measured gravimetrically as described in a companion paper [14].

Tissue Morphology

Necturus intestine was stripped and stretched as specimens used for flux studies. Tissue was fixed in 1.5% glutaraldehyde plus 0.5% paraformaldehyde buffered in 100 mM Hepes at pH 7.4. This tissue was subsequently used for scanning electron microscopy (SEM), transmission electron microscopy (TEM) and light microscopy.

SEM

After aldehyde fixation the tissue was dehydrated in ethanol and critical point dried in CO_2. It was then shadowed with carbon and gold, viewed and photographed in a Philips XL30SEG SEM.

TEM

After aldehyde fixation the tissue was post-fixed in 1% OsO_4 in the same buffer, dehydrated in ethanol and embedded in epoxy resin. Thin sections were stained in lead citrate and uranyl acetate, viewed and photographed in a Philips CM100 TEM.

Light Microscopy

Tissue was processed as for TEM, semi-thin sections were photographed unstained using phase contrast on a Zeiss Standard microscope. Sections were also stained with methylene blue and basic fuchsin for coloured phtography. Cell circumferences, cell density and cell heights were determined by elementary stereology and used to calculate the linear extent of junction and interspace per unit area of epithelium.

Computer Modelling

A program SPACES representing the serially concatenated elements between the stirred mucosal and serosal baths (junctions, lateral spaces (*lis*) and subepithelium) was set up and solutions obtained for the contribution of junctional diffusion to the net dextran flux, using measured values of the dimensions. This employs the same equations and computational approach used previously for work on *Necturus* gallbladder epithelium [13]. Flows across the elements were calculated with a one-dimensional convection-diffusion equation (CDE-Eq. 3) in which the diffusion coefficients, convective terms and local concentrations were modified with appropriate drag and steric factors that are functions of radius.

Briefly, the velocity gradient in the interspace must be quasi-linear with length due to the (assumed) high osmotic permeability required to effect *lis* osmotic flow. For $m \rightarrow s$ solute flow, at any given radius, a rate of diffusion over the junction is determined by assuming a head concentration at the junctional end of the *lis.* Using this flow rate the concentration at the end of the interspace can be calculated using a CDE applied to the subepithelium. A CDE for the *lis* is then back-integrated to yield a revised *lis* head concentration, and successive iterations of this procedure yield a consistent flux through the whole system. The procedure is also performed for $s \rightarrow m$ flow and the net fluxes can then be calculated as the difference between these undirectional fluxes at every solute radius (Eq. 4).

Results and Discussion

Epithelial Structure

When stripped and mounted for experimental work *Necturus* intestine does not have a conventional structure with villi and crypts but shows flat areas with widely spaced parallel ridges of subepithelial connective tissue which are about twice the normal thickness (80 μm); on the ridges the cells have a greater height, 95 μm as opposed to 56 μm on the flats. At intervals along the flats there are nests of apparent stem cells as seen in *Amphiuma* intestine [18]. Key elements of the epithelial geometry used in the calculations are collected in Table 1.

Net Fluxes

Net fluxes as a function of radius were obtained as the difference between two datasets representing mean MS and SM undirectional fluxes (as shown in Fig. 1 for MS data without alanine). These are shown in Figs. 2 and 3 for experiments with and without alanine present. It can be seen that the results are broadly similar and have a common pattern which is quite distinctive. The existence of significant positive or negative net fluxes indicates the existence of a mechanism responsible for transport *via* the paracellular pathway to which dextrans are confined. In as much as they are not being transported across membranes by a conventional pump, and some form of convection is implied, we refer to this mecha-

Table 1. Cell parameters used for calculating junctional diffusion

	Area fraction	Width (μm)	Depth (μm)	Linear extent (cm/cm^2 epi)
tj (tight junction)	1.0	$12–20 \times 10^{-4}$	0.34	1030
lis (interspace)	1.0	1.0	75.5	1030
s-3 (sub-epithelium)	0.2		80	

All the dimensions are taken from the material prepared as described in Materials and Methods. The *tj* width is difficult to measure with TEM; the values used here are the cutoff dimensions for dextrans of 6Å (*MS* fluxes) or 10Å (*SM* fluxes). The diffusion coefficient of small solutes in *Necturus* subepithelium is a fifth that in free solution, which is modelled here as a restricted area parameter of 0.2.

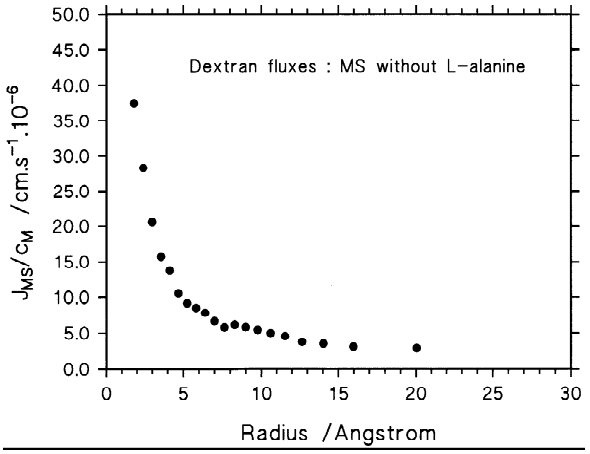

Fig. 1. The unidirectional specific flux of labeled dextrans from mucosal to serosal bath as a function of dextran size ($n = 8$). Individual points are eluates (5 ml averaged) from the chromatographic separation, converted to molecular radii by the relevant Ogston equation (*see* Materials and Methods).

nism as transport through a convective channel. In Fig. 1, there is a greater flux at all dextran radii than shows up on the net flux curves (Figs. 2 and 3) indicating that there are routes for permeation parallel to that of the convective channel which are purely diffusive in nature. These have been analysed in detail previously [13] but are not further analyzed here.

There are three sections to each set of results in the Figs. 2 and 3: (i) Above a radius of r_2 Å the flux is very small, not significantly different from zero, indicating that no molecules greater than this size can traverse the convective paracellular channels. This must represent an absolute cutoff at a radius r_2 for the largest of such channels. (ii) Between r_1 and r_2 Å the net fluxes are negative, with a distinct minimum at r_1, which indicates a convection from serosal to mucosal bath i.e., a secretion of molecules into the lumen. (iii) At even smaller radii below r_1 Å the fluxes become positive, exhibiting a very steep relationship between J_{net}/C and radius i.e., absorption from the lumen finally predominates at the smallest radii. The two sections (ii) and (iii) of each plot show pronounced linear characteristics (*see* Figs. 2 and 3).

The existence of positive and negative regions within a single flux curve that depend on the radius of the probe can only mean that there are two convective channels which are operating in opposite directions. The pattern of probe fluxes indicates that there must be fluid recirculating across the epithelium between the two baths within the area of tissue chosen for analysis i.e., less than 0.3 cm^2. The possibility of intestinal absorption being due to the balance of recirculatory flows has often been suggested in the literature but has never been put onto a clear quantitative basis. Here it is possible to do this using size selectivity as the differentiating parameter.

If there are two opposing convective channels which discriminate against probe molecules on account of their size, they apparently do this as a linear function of radius, having cutoff points at different radii. It is therefore possible to resolve the separate channels on the basis of these effects, in the following way (Figs. 2 and 3): the lowest point (radius r_1) is the cutoff of the flux through the MS channel; the zero point (radius r_2) is the cutoff of the flux through the SM channel. The data points lying between r_1 and r_2 therefore represent the backflux (secretory) from serosa to mucosa i.e., the properties of the SM channel. This is fitted well by a linear regession which can be extrapolated to the radius of water (Fig. 4). Subtraction of this line from all data points below r_2 yields the characteristic of the forward or absorptive flux i.e., the properties of the MS channel from the radius of water to its cutoff point at r_1 (Fig. 4). The flow rates of water and the cutoff radius of the two channels derived from these flux experiments, with and without alanine, are shown in Table 2 and as regression lines in Figs. 4*a* and *b*.

Net Paracellular Water Flows

If the curves of Figs. 2 and 3 are overall flows i.e., the sum of two convective channel flows it is possible to calculate the flows of water giving rise to the convection and compare this with the observed flows. Section (iii)

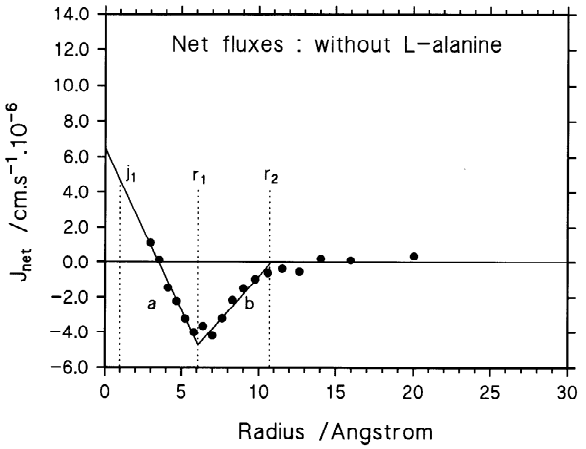

Fig. 2. Net dextran fluxes without alanine ($n = 8$). r_1 is the intersection of the first linear regression *a* ($r^2 = 0.99$) with the second *b* ($r^2 = 0.94$) and represents the cutoff point of the MS convective channel. Likewise r_2 is the intersection of the second linear regression *b* with the zero axis, and represents the cutoff point of the SM channel. j_1 is the value of the net flux at the water radius (1 Å).

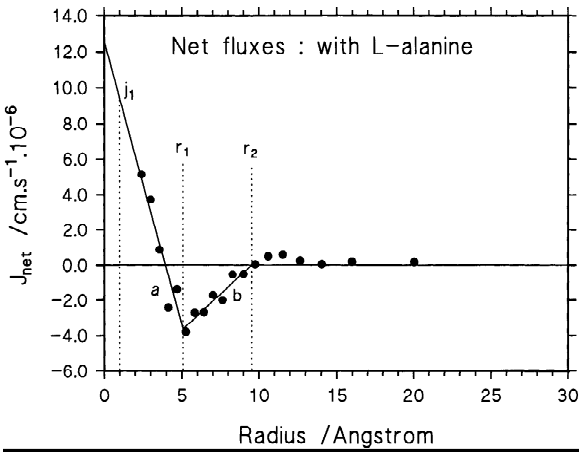

Fig. 3. Net dextran fluxes with alanine at 20 mM ($n = 8$). r_1 is the intersection of the first linear regression *a* ($r^2 = 0.92$) with the second *b* ($r^2 = 0.94$) and represents the cutoff point of the MS convective channel after alanine. Likewise r_2 is the intersection of the second linear regression *b* with the zero axis, and represents the cutoff point of the SM channel after alanine. j_1 is the value of the net flux at the water radius (1 Å).

of the data plots between 0 and r_1 has an intercept of 6.5 × 10^{-6} cm/sec without alanine and 12.6 × 10^{-6} with alanine. These intercepts may be interpreted as being due to the net volume flow through the convective channels. As the probe radius increases, both steric and hydrodynamic drag effects come into play to decrease the flux of the probes i.e., the net flux approaches zero. However, when the probe radius approaches zero these effects, which are nonlinear functions of radius, disappear, revealing the maximum rate of convection. Strictly, this would hold for a fluid that was a perfect continuum but water is particulate with a radius of about

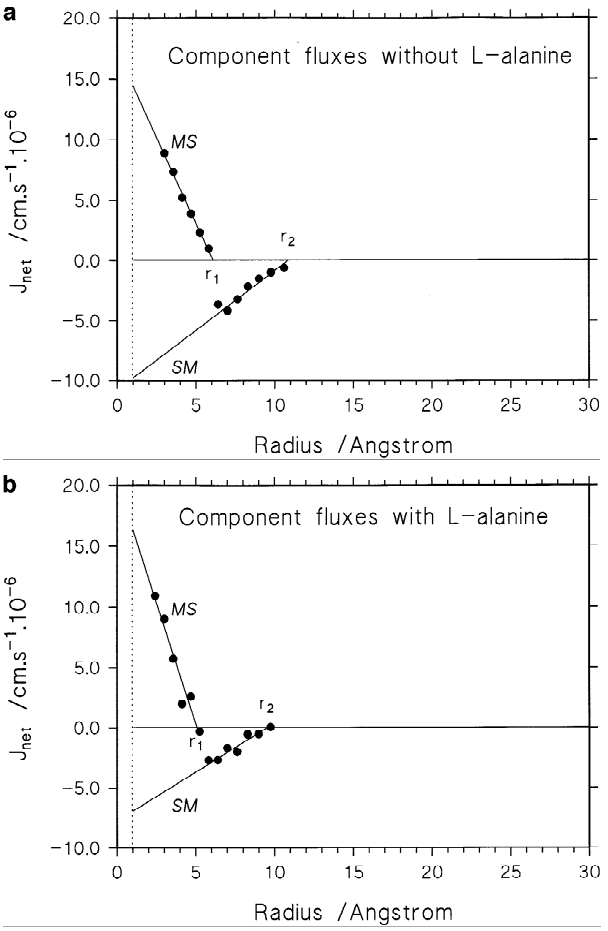

Fig. 4. The two linear components of the net fluxes between 0 and r_2 (*a*) from Fig. 2 and (*b*) from Fig. 3. *MS* − absorption, *SM* − secretion.

1 Å and so the rate of fluid transfer should be given by the net flux at a probe radius of this magnitude. It should correlate with the rate of fluid transfer measured independently under these conditions, and this is indeed the case. The fluid transfer rates measured on this tissue were 4.21 ± 0.4 × 10^{-6} (cm/sec) without alanine and 9.59 ± 1.64 × 10^{-6} (cm/sec) with alanine ($n = 11$). The extrapolated rates obtained from the dextran plots at 1 Å (j_1 in Figs. 2 and 3) are 4.65 ± 0.33 × 10^{-6} (cm/sec) without alanine and 9.40 ± 1.4 × 10^{-6} (cm/sec) with alanine.

This agreement between the flow of water that is required to explain the dextran fluxes, which are confined to the paracellular system, and the measured flow, before and after stimulation, is an indication that the water is following a paracellular path (Table 2).

Junctional Flows

Concentration Polarization Effects

When water is drawn through an epithelium and leaves certain solutes behind i.e., the pathways are selective to

Table 2. Properties of the fluid circuits in *Necturus* intestine

		Dextran analysis			*Direct flow measurements*
		Convective flow rate ($cm^3/cm^2 \cdot sec \times 10^{-6}$)	Cutoff (Å)	Net Flow $\mu L/cm^2 \cdot hr$	$\mu L/cm^2 \cdot hr$
MS	*Ringer*	14.43	6.09	16.74 ± 1.4	15.15 ± 1.2
SM		9.79	10.82		
MS	*Ringer* + 20 mM	16.34	5.08	33.84 ± 5.9	34.52 ± 5.0
SM	L-alanine	6.94	9.53		

The intercepts of the two fluid channel curves obtained by regression analysis are shown in Fig. 4. The net absorption rates at 1 Å determined by extrapolation from Figs. 2 and 3 (± alanine) represent a doubling of convective transport although the calculated % change in the rates of each circuit is smaller. The 95% confidence on the cutoff points is estimated to be ~0.7 Å. The gravimetrically measured fluid transfers are shown in the final column and are not significantly different.

some extent, there is the possibility of concentration polarization. Solutes accumulate at the epithelial face and there is a concentration gradient created across the structure in the direction of the fluid flow. Depending upon the permeability of the epithelium there will be a diffusive flow which appears as an apparent convective component. This effect has been invoked to dispose of solvent drag effects in epithelia [1], but the magnitude of the effect has rarely been determined, and this is all important. In a study of water absorption by rat colonic epithelium [10] FITC-dextran, present in the luminal bath, was seen to accumulate in the crypts which were considered to be the sites of water absorption. This of itself does not indicate the mechanism of water transport but shows that concentration polarization can occur at the epithelial face if there is no stirring, as would be the case in a crypt. *Necturus* intestinal preparations have no crypts and the stirring regime in the baths is certainly turbulent, so that concentration polarization is not considered to be present here to any extent [12, 13]. Furthermore, the pattern of net flux (Figs. 2 and 3), being positive at some radii and negative at others, cannot be generated by concentration polarization in any comprehensible manner. Thus the programs run to determine convective-diffusive coupling within the paracellular system begin with the boundary condition that there are no unstirred-layers at the epithelial interfaces.

Junctional Convection

In treating this problem previously in *Necturus* [13] the contribution of 'interspace osmosis' to the net dextran flux was calculated in some detail. The result was that junctional diffusion could not account for more than 10% of the apparent convection, whilst junctional convection accounted for 100% of it. In gallbladder the junctions are quite wide: the cutoff for the convection channel is at a molecular radius of 77 Å and this corresponds well with the separation between membranes in the tight-junctional complex (*tj*) seen with TEM. In *Necturus* intestine the junctions are apparently far 'tighter'. The data analyzed here shows that the apparent convection channels cut off at a molecular radius of between 5–11 Å and this also corresponds with the appearance of the *tj* complex by TEM: there is close contact between the membrane leaflets in transverse sections without the wide space seen in gallbladder *tj* sections. The membranes are closely opposed enough for it to be impossible to determine the junctional width accurately from TEM.

The calculations were performed with the program SPACES using the structural data given in Table 1 and the flow rates and cutoffs from Table 2. Probe fluxes across the junction were described by simple diffusion. At a junction half-width of 11 Å, the cutoff point for SM flow (Table 2) the calculated net flux at the smallest radius is 28% of that observed, while at a half-width of 6 Å, the cutoff for MS flow, it is 14%. These percentages are low and cannot be raised by slightly changing the geometry of the system. The controlling parameter is in fact the restricted area for diffusion across the junctions, due to their narrowness (which controls the size selectivity) and their small cross-section as compared to that of the interspaces. As this is a constant feature of all epithelia, the correlation between convection at small probe radius and the measured water flow is a clear indication that water is traversing the junctions.

The same calculations, performed with SPACES but using a CDE across the junctions (Eq. 3) with the convection set equal to the observed fluid flow rates, predicts the net fluxes correctly, as might be expected. In other words, virtually 100% of the fluid circuits are crossing the epithelium by a paracellular as opposed to a cellular route.

There is the possibility that the fluid with its solutes is being transferred by some form of transcytosis but this is highly unlikely. As the cutoff point for dextran fluxes is about 11 Å this sets an upper limit to the radius of the vesicles and it is difficult to see how lipid vesicles could have such a great curvature. Furthermore, the number required to transport the fluid would entail prohibitively high rates of membrane turnover.

Junctional Osmosis

If virtually all the fluid is crossing the junctions there remains the possibility that a fraction of this is doing so by transjunctional osmosis. The widening of the junctional pathway by nutrients applied to the mucosal bath has been proposed as the mechanism underlying the stimulation of absorption [11]; increasing the hydraulic conductance of the junctions is considered to enhance the osmotic flow, so drawing in nutrients by solvent drag.

A simple calculation will suffice to make it clear that this mechanism cannot account for more than a minute fraction of the water uptake by epithelia [3]. The fraction of water crossing the junctions by osmosis can be calculated using the following dimensions of junction and interspace of *Necturus* intestinal epithelium given below (Table 1). The P_f of the junctions calculated as a slit, at a maximum width of 20 Å, a linear extent of ~10^3 cm/cm^2 epithelium and a depth of ~0.4 μm, lies between 10^{-6}–10^{-5} cm/sec. That of the membranes, in common with those of most epithelia, lies between 10^{-3}–10^{-2} cm/sec. The flow over the junctions, driven by a solute whose reflexion coefficient is 1.0 could not be more than 1/1000th of any osmotic flow rate. The actual reflexion coefficient of a set of junctional channels 12–20 Å wide to the driving osmolytes, Na and Cl ions, can be calculated to be substantially less than 1.0. This shows that junctional osmosis can only make a negligible contribution to paracellular fluid flow.

Fluid Circuits

The linearity of the bidirectional flows with radius is similar to that found in *Necturus* gallbladder epithelium and we interpret it in a similar way [4]. Convective drag is a highly nonlinear phenomenon, and so it beomes necessary to choose a convective model in which drag is avoided. This is one in which micro-domains containing water and solute molecules are transported across the junction by mechano-contractile elements of the *tj* complex. Molecules which initially enter these domains do so subject to size selection i.e., by molecular sieving, but when transfer is effected all molecular species within the domain, water and solutes, are transported at the same rate; there is therefore no separation by differential drag effects. Furthermore, the linearity implies sieving through a slit pore because only this geometry generates linearity; cylindrical pores, for example, generate nonlinearity.

The results show that there is a circulation of fluid which is stimulated by alanine although it is imbalanced towards net absorption. Reference to Table 2 also shows that the cutoff points in the two directions are different but do not change in a statistically significant manner with alanine, indicating that the flow rate through the circuits is modulated but the selectivity, and by implication the structure of the pathways, remains unaltered.

It is apparent that the stimulation of uptake caused by alanine is due to an increase of the MS fluid circuit and a concomitant decrease in the SM circuit. These changes are not large fractions of the pre-existing rates, but together they add up to a rise in the net absorptive rate to about 200%. The changes in the two limbs of the circuit are in opposite directions and it is not ruled out by the data that these changes may be about equal in magnitude. If this is so, it would be consistent with a scheme in which fluid flow in the junctions of some cells is reversed such that flow in the MS limb gains in magnitude at the expense of the SM limb. It is not yet clear that this is the case however, and changes of direction remain only a possibility. The existence of two opposed pathways implies that the tissue is structurally heterogeneous. The scale of this heterogeneity is not yet clear, but the recirculation is on a small scale because the area of tissue on which the fluxes were measured was less than 0.3 cm^2. The opposing limbs of the fluid circuit may be confined to the junctions of adjacent cells, or adjacent cell groups.

It is quite possible that various treatments would alter the MS:SM ratio in favor of either complete absorption or drastic secretion, such as occurs in disease states. We suggest that a function of the Na-alanine transporter may be that of regulating the paracellular flow. The precise sequence of cellular events which bring about these shifts is not clear but future work should determine whether directional fluid transfer by the junctions is a general property of all cells or is confined to specialized cells in the epithelium.

Relation to Salt Transport

In a companion paper it has been concluded that the salt and water transport in *Necturus* intesting cannot be described as simple interspace osmotic coupling driven by Na pumping, and that the apparent effect of alanine is to stimulate a paracellular mechanism of fluid secretion which transfers sodium and volume at a faster rate than could be predicted from changes in the activity of the Na pump. The paracellular convective flows found here are in agreement with this conclusion and the flows are of the correct magnitude. As the radii of the major ions, Na and Cl, are smaller than the cutoff radius of either fluid circuit limb it is virtually certain that such a mechanism would transfer ions as well, and if the pathway is charged then such a process will give rise to charge flow i.e., a current. The process would be analogous to a ‘forced’ electro-osmosis which generates a streaming current, with the difference that the fluid is propelled not by pressure but by motile elements of the junctional complex.

Such a model would not require that different groups

of cells have radically different orientation of pumps and transporters to bring about absorption or secretion, but that the differences lie in the control of the tight junctional complexes. It also removes the need for differently oriented osmotic gradients that would have to exist side-by-side over small distances within the epithelium to bring about concomitant absorption and secretion.

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