

Water and Solute Movement in the Small Intestine of Patients with Sprue *

JOHN S. FORDTRAN,† FLOYD C. RECTOR, T. WARD LOCKLEAR, AND
MAYNARD F. EWTON

(From the Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas)

Summary. Water and electrolyte movement in the jejunum of normal subjects and patients with sprue was measured during perfusion with isotonic electrolyte solutions. Normal subjects absorbed water, sodium, and potassium. By contrast, in patients with sprue (seven with adult celiac sprue and one with tropical sprue) who had diarrhea and steatorrhea, these substances were secreted into the intestinal lumen. This indicates that the jejunal mucosa of these patients was in a secretory state with respect to water and electrolytes.

A method is presented for detecting abnormalities in the effective pore size in disease states. The method is based on the principle of restrictive diffusion and involves measuring the simultaneous diffusion rates of solutes of different molecular size. Since the method does not depend on measurement of water flow in response to osmotic pressure gradients, it can be used in disease states in which absorption and secretory processes involving water may be abnormal.

The ratio of urea to tritiated water diffusion in the jejunum of normal subjects averaged 0.8, compared to 0.2 in patients with sprue. This indicates a marked decrease in the effective pore size of the jejunal mucosa in sprue. This conclusion was strengthened by the finding that erythritol and L-xylose, which are somewhat larger solutes than urea, are essentially non-absorbable in small bowel involved with sprue.

Introduction

Malabsorption of water and electrolytes is a well-known feature of sprue. It is not clear, however, whether this is due simply to decreased mucosal surface area of small bowel mucosa, or whether specific alterations of permeability and transport processes are also involved. The development of intestinal perfusion techniques offers a means for studying this problem.

* Submitted for publication June 30, 1966; accepted November 3, 1966.

Supported by research grant AM-06506 and training grants T1-AM-5395 and T1-AM-5490 from the National Institutes of Health.

† Address requests for reprints to Dr. John S. Fordtran, Dept. of Internal Medicine, The University of Texas Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, Texas 75235.

The present experiments were designed to examine two aspects of the functional characteristics of the intestinal epithelium in sprue: first, the ability of the intestine to absorb water and electrolytes from an isotonic perfusion; and second, the permeability of the intestinal mucosa to water and nonelectrolytes. At the outset, we recognized that permeability to water and nonelectrolytes is likely to be deranged in sprue because of the marked decrease in the surface area that is characteristic of this disease. In addition, however, sprue may alter intestinal permeability by changing the length, size, or number of water-filled pores in the intestinal cell membranes.

In previous studies in normal subjects we measured effective pore size by comparing bulk water flow across the intestinal mucosa induced by

perfusion of solutions made hypertonic with solutes of different molecular size (1). Preliminary data indicated that this method is subject to large errors in sprue because of very low rates of water flow in response to osmotic pressure gradients, and because sprue mucosa was found to be in a secretory state with respect to water and electrolytes. We have therefore developed a different method for detecting abnormalities in pore size that is not affected by these technical problems. The method is based on the principle of restrictive diffusion [as developed by Renkin (2)] and involves measuring the simultaneous diffusion rates of solutes of different molecular radii. Thus, a membrane with a small effective pore size will readily discriminate between a small and a larger solute, so that the diffusion ratio of the large relative to the small solute will be low. If, on the other hand, the same two solutes are exposed to a membrane with a larger pore size, less discrimination will occur, and the solute diffusion ratio will be higher, and approach 1 as the size of the pore increases. If the diffusion of the different solutes is measured simultaneously, such variables as surface area, number of pores, pore length, variations in flow rate, and variations in intestinal motility will cancel out when results are expressed as a ratio. Under these conditions, the ratio will be a function principally of the effective size of the diffusion channels.

The present studies, conducted in patients with severe and moderate sprue, have demonstrated abnormalities of both intestinal transport processes and intestinal permeability that cannot be attributed to abnormalities of surface area or intestinal motility. We found that perfusion of isotonic electrolyte solutions elicits a secretory response in patients with sprue, in contrast to normal subjects, and that this abnormality is not corrected by adding glucose to the test solutions. In addition, the diffusion rates of L-xylose, erythritol, and urea are disproportionately reduced relative to the diffusion of tritiated water. This increased selectivity suggests that there is a marked decrease in the effective pore size of sprue mucosa.

Methods

Nine patients with adult celiac sprue and one with tropical sprue were studied. Data on manifestations of the disease, degree of steatorrhea and small bowel dilatation, xylose test, mucosal biopsy, and response to

gluten-free diet are given in Table I. Control subjects were normal volunteers, ages 21 to 70 years, without evidence of gastrointestinal disease.

In all subjects test solutions, containing a volume marker, polyethylene glycol (PEG) (6), were infused into the small intestine at 9 ml per minute through the proximal opening of a triple-lumen tube (1, 7) that had been previously checked fluoroscopically to ascertain its position. After a 30-minute equilibration period, perfusates were collected continuously for 1 hour at points 10 and 40 cm distal to the site of infusion, via the other openings of the tube. Water and solute movement within the test segment between the two collecting sites was determined by methods that have been previously described (1, 7).

Normal subjects and patients were studied sequentially with three test solutions, each designed to measure a specific aspect of water and solute movement. 1) The first period was used to measure net water and electrolyte absorption from an isotonic electrolyte solution containing 140 mEq per L of sodium, 5 mEq per L of potassium, 110 mEq per L of chloride, and 35 mEq per L of bicarbonate. In some experiments 20 mM glucose was included in the perfusion solution, and the concentrations of sodium and chloride were reduced slightly to maintain isotonicity with plasma.

2) The second test period was used to measure solute diffusion ratios. The theoretical background and principles of this technique are described in the Appendix. Test solutions contained 15 mM urea labeled with urea-¹⁴C, 15 mM L-xylose, and either 15 mM erythritol labeled with tritium, or 0.2 mc of tritiated water (THO). Sodium chloride was added to make the solution isotonic to plasma. The absorption rates of solutes of different molecular radii (THO, 1.5 Å; urea-¹⁴C, 2.3 Å; erythritol-³H, 3.2 Å; L-xylose, 3.5 Å) (8) were measured in the test segment between the proximal and distal collecting sites (7), and the diffusion ratio of solute pairs was determined by Equation 8 in the Appendix.

3) The third period was used to measure the filtration coefficient of the intestine, by a previously described method (1). Briefly, this involves measurement of net water movement into the intestinal lumen in response to perfusion of isotonic electrolyte solutions made hypertonic by addition of mannitol. In calculating the results in sprue patients, we subtracted net water movement during isotonic perfusion (determined from the first study period) from water movement observed during hypertonic perfusion in order to determine the amount of water flow in response to the osmotic gradient per se. A similar correction is not necessary in normal subjects since net water movement during perfusion with isotonic electrolyte solutions is negligible compared to the high rate of water flow in response to osmotic pressure gradients.

Additional studies on restrictive diffusion

The effect of antidiuretic hormone (ADH) on solute diffusion ratios in the normal intestine was studied by measuring diffusion rates during two sequential test

TABLE I
Clinical data on patients with sprue

Patients	Age and sex	Primary manifestations	Duration of illness	Degree of steatorrhea	Degree of dilatation of small bowel by X ray (3)	Xylose test Normal >5 (4)	Degree of abnormality by mucosal biopsy (5)	Response to gluten-free diet
Fle	30 F	Diarrhea, weight loss, lethargy	10 years	Severe	Mild	g/5 hours Not done	Severe	Dramatic improvement
Blu	57 M	Diarrhea, weight loss	>20 years with intermittent severe exacerbations	Severe	Moderate	2.4	Severe on 3 separate occasions	No response after 18 months; no response to folic acid; improved on adrenal steroid therapy
Cad	33 F	Diarrhea, weight loss, anemia	10 years	Severe	Moderate	2.5	Severe	Definite improvement
Flo	50 M	Diarrhea, weight loss	Several years	Severe	Mild	1.7	Severe	Dramatic improvement
Beh	40 M	Diarrhea, potassium deficiency, weight loss	7 months	Severe	Moderate	1.2	Severe	Dramatic improvement
Gum	55 M	Diarrhea, bleeding tendency, weight loss	Many years	Severe	Moderate	1.1, 1.0, 0.9	Severe	Dramatic improvement
Dic	48 F	Emaciation, diarrhea	>20 years	Severe	Marked	0.7	Severe	Diarrhea improved but patient died 1 month after study due to ileal ulcer with perforation and peritonitis
War	38 M	Fatigue, weight loss (12 pounds), megaloblastic and iron deficiency anemia	2 months	None	None	2.5, 2.9	Moderate	Definite improvement
San	30 F	Macrocytic anemia of pregnancy, osteomalacia	5 years	None	None	3.3	Moderate	Dramatic improvement
Vin	35 M	Diarrhea, weight loss, anorexia, contracted in the Philippines	8 months	Moderately severe	None	1.9, 1.6	Moderate	None after 2 months; complete remission on folic acid

periods. Before and during the first period the subjects were in a state of water diuresis, induced by infusion of water into the intestine 75 cm below the collection point of a double-lumen tube. In addition, 3% glucose in water was given intravenously at a rate of 600 ml per hour. Solute perfusion was not started until urinary osmolality was less than 100 mOsm per kg. After the first study period, infusion of water into the small intestine was discontinued, and the intravenous infusion was changed to 5% glucose in water with aqueous Pitressin. The latter was given at a rate of 200 mU per hour during the second test period, which was defined as starting 30 minutes after the Pitressin infusion was begun. Solute diffusion ratios were determined by comparing PEG and solute concentrations in the infused test solution and in the perfusate collected 25 cm distally (Appendix, Equation 8).

In vitro studies

a) *Cellulose dialysis tubing.* Solute diffusion ratios were measured in 15-cm lengths of Visking cellulose tubing. The tubing was tied at one end with silk suture, and 2 ml of normal saline solution containing radioisotopes of the solutes to be studied was injected into the bag. The other end was tied with silk and dipped into normal saline solution, blotted, weighed, and then returned to the saline bath. After 10 to 30 minutes, depending on the solutes being studied, the bags were re-

moved, blotted, and weighed, and then their contents were emptied into test tubes and subsequently counted. The ratio of the diffusion of solute pairs was calculated by methods described in the Appendix. The solutes used in these experiments were tritiated water, urea-¹⁴C, tritiated erythritol, and tritiated *d*-xylose.

b) *Toad bladder.* The effects of aqueous Pitressin (ADH) on solute diffusion ratio of tritiated water and urea-¹⁴C were studied with the technique described by Bentley (9). The bathing solution was that used by Bentley, and the incubation period was 2 hours. In three experiments, the bathing solution had no ADH during the first test period, and during the second period this hormone was added to the serosal media in a concentration of 5 mU per ml. In one experiment this order was reversed. Diffusion ratios were calculated as described in the Appendix.

Analytical methods

PEG was determined in duplicate by the method of Hyden (10). In our hands this method is reproducible within $\pm 2\%$ on duplicate testing. L-Xylose was measured by the method of Roe and Rice (11) and osmolality by freezing point depression; sodium and potassium were analyzed by flame photometer and tritium and ¹⁴C by counting in a Packard Tri-Carb liquid scintillation spectrometer.

Results

Net water and electrolyte movement during perfusion with isotonic electrolyte solutions

During perfusion with isotonic electrolyte solutions, normal subjects absorbed an average of 1,551 μl per hour per cm of water and 207 and 19 μEq per hour per cm of sodium and potassium, respectively. By contrast, as shown in Table II, all patients with celiac sprue and steatorrhea secreted both water and sodium into the intestinal lumen, and all but one secreted potassium. Mean water secretion was 2,328 μl per hour per cm, mean sodium secretion was 278 μEq per hour per cm, and mean potassium secretion was 14 μEq per hour per cm. The two patients with celiac sprue without steatorrhea showed essentially zero net water movement, but both absorbed sodium normally (205 and 172 μEq per hour per cm). One absorbed potassium, and the other demonstrated zero net potassium movement. The patient with tropical sprue secreted water, sodium, and potassium in amounts comparable to those seen in patients with celiac sprue with steatorrhea.

It has been previously shown that the presence

of glucose in the jejunum of normal subjects stimulates sodium absorption from electrolyte solutions (12). To see whether a similar effect occurs in sprue, we carried out additional studies on patients Vin, Dic, and Gum. The jejunum of these patients was perfused with an isotonic electrolyte solution containing 20 mM glucose. The presence of glucose did not affect the secretion of sodium, potassium, or water. The secretory rates were approximately the same with and without glucose in the perfusate.

Measurement of osmolality and sodium and potassium concentrations in fluid collected from both collection tubes in normal and sprue patients demonstrated that osmotic gradients and sodium and potassium concentration gradients between gut lumen and plasma were not present during these studies. Gross inspection of the fluid collected from the sprue patients did not reveal any differences from normal subjects. The perfusate of three sprue patients was analyzed for hexosamine,¹ and no differences were found between normal and sprue subjects.

¹ The analysis was performed by Dr. J. Donald Smiley.

TABLE II
*Net water and electrolyte movement, filtration coefficient, and urea/THO diffusion ratio in jejunum of patients with sprue and in normal subjects**

	Water	Sodium	Potassium	Diffusion ratio THO/urea	Filtration coefficient
	$\mu\text{l/hr/cm}$	$\mu\text{Eq/hr/cm}$	$\mu\text{Eq/hr/cm}$		$\mu\text{l/mOsm/hr/cm}$
Adult celiac sprue					
With diarrhea and steatorrhea					
Fle	+3,800	+480	+43		41
Blu	+1,880	+216	+14		8
Cad	+3,360	+448	- 2		4
Flo	+4,000	+443	+24	0.13	35
Beh	+1,600	+ 97	- 5	0.09	9
Gum	+ 800	+120	+ 6	0.20	17
Dic	+ 858	+139	+ 8	0.21	
Mean	+2,328	+278	+14	0.16	19
Without diarrhea or steatorrhea					
War	- 36	-172	0		161
San	0	-205	- 7	0.22	127
Tropical sprue					
Vin	+2,640	+381	+33	0.28	18
Normal (n = 10)	-1,551	-207	-19	0.82	135
(Range)	(-198--2,178)	(-30--300)	(-8--59)	(0.66-0.88)	(69-195)

* THO = tritiated water; - = absorption; + = secretion.

TABLE III
Restrictive diffusion in cellulose membranes (Visking)

	Unstretched tubing				Stretched tubing		
	No. ex- peri- ments	Average ratio	Range of ratio	Pore size A	No. ex- peri- ments	Average ratio	Pore size A
Urea/THO	6	0.7473	0.7000-0.7840	14.0	1	0.6319	9.7
Erythritol/urea	5	0.6429	0.6225-0.6734	12.0	2	0.4576	9.6
L-Xylose/urea	4	0.6368	0.6124-0.6603	14.8	1	0.4312	9.5
Average				13.6			9.6

Restrictive diffusion

A series of *in vitro* studies was performed to determine the sensitivity of the relative diffusion rates of different solutes to changes in pore size of artificial and biologic membranes. Cellulose dialysis tubing, with and without longitudinal stretching [which decreases effective pore size (13)], and the toad bladder with and without Pitressin were chosen for study.

Cellulose bags. The pore radius of Visking cellulose tubing was determined with three pairs of solutes, and the results are given in Table III. Pore radius, calculated by Renkin's (2) equation for restricted diffusion, ranged from 12.0 A with urea and erythritol to 14.8 A with urea and xylose. These results are in good agreement with those reported by Renkin (2).

Diffusion ratios were also measured in cellulose bags that had been stretched longitudinally; this procedure has been shown by Craig and Konigsberg to decrease the effective pore size of cellulose membranes (13). Results of four experiments in four different stretched bags are shown in Table III. It is evident that the diffusion ratio of each solute pair decreased, which is compatible with a

decrease in effective pore radius of the stretched bag.

Toad bladder. As shown in Table IV, ADH increased the diffusion of both THO and urea through the toad bladder. However, the percentage increase in urea diffusion was greater than that of THO. Consequently, the solute diffusion ratio in each of the four bladders was increased by ADH, indicating an increase of the effective pore radius.

These *in vitro* studies indicate that solute diffusion ratios are sensitive to changes in the effective pore size of membranes and suggest that the principle of restrictive diffusion may be usefully employed in studying permeability characteristics of the intestine.

Human small intestine

a) **Diffusion ratios of different solute pairs in the normal small intestine.** Three pairs of solutes were studied: THO and urea, urea and erythritol, and urea and L-xylose. Mean results in the jejunum (Table V) were as follows: urea/THO, 0.82; erythritol/urea, 0.50; L-xylose/urea, 0.31.

The reproducibility of this technique in individual subjects was assessed by measuring solute

TABLE IV
Solute diffusion in toad bladder

Bladder no.	No ADH*			ADH		
	THO ab- sorption† %	Urea ab- sorption %	Diffusion ratio urea/THO	THO ab- sorption %	Urea ab- sorption %	Diffusion ratio urea/THO
1	47.1	8.3	0.142	76.5	24.8	0.209
2	56.8	7.2	0.108	76.5	21.0	0.167
3	69.7	13.3	0.121	91.4	30.6	0.157
4	54.5	3.5	0.073	85.8	24.4	0.151

* ADH = antidiuretic hormone.

† The term absorption denotes disappearance of solute from the lumen of the toad bladder during a 1-hour study period.

TABLE V
Diffusion ratio for solute pairs in normal jejunum

	No. studies	Diffusion ratio	
		Mean	Range
Urea/THO	10	0.82	0.66-0.88
Erythritol/urea	8	0.50	0.42-0.57
L-Xylose/urea	14	0.31	0.21-0.38

diffusion ratios during two sequential test periods. The results, shown in Figure 1, demonstrate excellent agreement between the two test periods. Furthermore, it is evident that permeability of the small bowel mucosa, as measured by this technique, does not change during two perfusion periods.

To establish whether the blood level of ADH affects pore size of the human intestine, we measured solute diffusion ratios during sequential test periods, the first during water diuresis (urinary osmolality < 100 mOsm per kg), the second during intravenous infusion of ADH, at a rate of 200 mU per hour. That this amount of ADH was sufficient to exert a biologic effect was dramatically evident in each subject by a marked reduction in urine flow and an increase in urinary osmolality. The results in the intestine, shown in Figure 2, demonstrated no effect of ADH on solute diffusion ratios. Furthermore, ADH did not change the absolute rate of absorption of THO, urea, erythritol, or L-xylose. For instance, in the jejunum, where most of these studies were done, mean absorption rates before and after ADH were as follows: THO, 59.3 and 56.9%; urea,

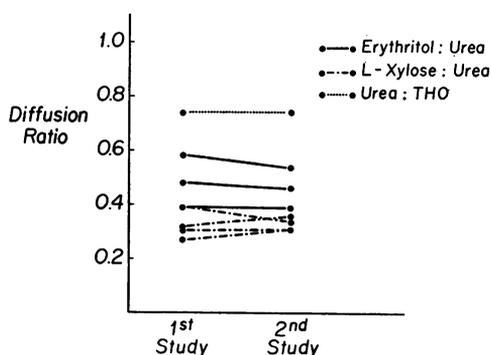


FIG. 1. REPRODUCIBILITY OF SOLUTE DIFFUSION RATIOS IN THE HUMAN SMALL INTESTINE DURING SEQUENTIAL TEST PERIODS. THO = tritiated water.

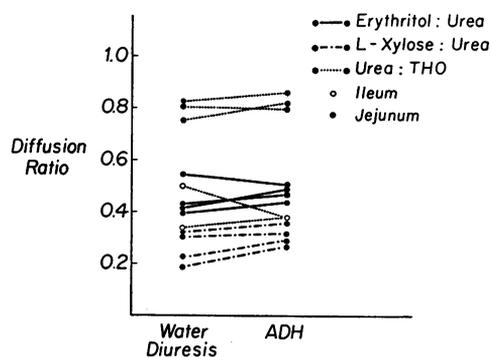


FIG. 2. EFFECT OF ANTIDIURETIC HORMONE (ADH), INFUSED INTRAVENOUSLY, ON SOLUTE DIFFUSION RATIOS IN THE HUMAN SMALL INTESTINE.

62.9 and 62.4; erythritol, 44.3 and 46.4; and L-xylose, 25.6 and 29.5. Likewise, ADH did not influence absorptive rates or diffusion ratios of these solutes in the ileum.

It proved impossible to accurately measure diffusion of L-xylose and erythritol in the ileum because absorption of these solutes was negligible in this area of the small intestine. This is in accord with our previous finding that the reflection coefficient of erythritol and xylose approaches 1.0 in the ileum (1). However, urea and THO were absorbed in the ileum to an extent that made it possible to compute diffusion ratios with this solute pair. The data in Table VI compare jejunal and ileal absorption rates of urea and THO and the diffusion ratio of this solute pair. It is evident that THO absorption was only slightly less in the ileum than in the jejunum (56 compared to 60%), but that urea absorption was markedly less in the ileum than in jejunum (20 compared to 52%). This selective decrease in ileal urea absorption caused the solute diffusion ratio to be much less in the ileum than in the jejunum.

Since we hoped to use solute diffusion ratios as a measure of intestinal permeability in diarrheal diseases, in which abnormalities of water movement are likely to occur, it was important to see whether variations in the direction and magnitude of water movement affect the diffusion ratios. Therefore, diffusion ratios in the jejunum were determined under experimental conditions in which net water movement was manipulated over a wide range by the addition of varying amounts of mannitol to the test solution. Although water movement varied from an absorption rate of 5,700 to a

TABLE VI
Absorption rates and solute diffusion ratios of THO and urea

	No. subjects	Urea absorption		THO absorption		Diffusion ratio	
		Mean	Range	Mean	Range	Mean	Range
Normal jejunum	10	52.2	44-64	60.1	51-74	0.82	0.66-0.88
Normal ileum	8	19.7	13-29	55.7	32-81	0.34	0.17-0.52
Sprue with steatorrhea (jejunum)	5	8.5	6-13	40.5	29-54	0.21	0.09-0.28
Sprue without steatorrhea (San, jejunum)	1	30.3		76.6		0.22	

secretion rate of 7,200 μl per hour per cm, mean flow rate in the intestinal segment was kept constant during all of these studies by methods that have been previously described (1). These results, given in Figure 3, demonstrate that absorption of THO and urea is accelerated by high rates of water absorption and depressed by high rates of wa-

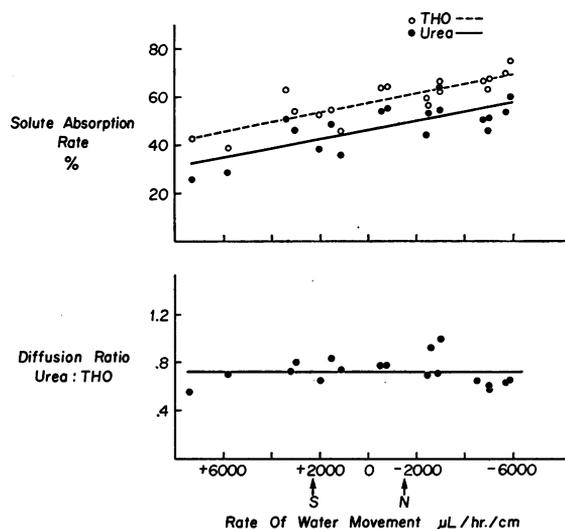


FIG. 3. EFFECT OF DIRECTION AND MAGNITUDE OF NET WATER MOVEMENT ON ABSORPTION OF UREA- ^{14}C AND THO (UPPER PANEL) AND ON THE UREA:THO DIFFUSION RATIO (LOWER PANEL) IN THE NORMAL JEJUNUM. Water movement was manipulated by addition of varying amounts of mannitol to test solutions, and mean flow rate during different rates and directions of water movement was controlled by methods previously described (1). Water absorption is indicated by a minus sign, secretion by a positive sign. Regression lines are calculated by the method of least squares. For comparative purposes, the mean water absorption or secretion rate during perfusion with isotonic electrolyte solutions is indicated by N for normal subjects and S for sprue patients.

ter secretion. The urea:THO diffusion ratio, however, is not altered by the rate or direction of water movement.

b) *Diffusion ratios in the jejunum of patients with sprue.* Calculation of diffusion ratios using xylose and erythritol was impossible in patients with sprue because the absorption rates of these solutes were negligible. Only the ratios of urea to THO gave interpretable results in these patients. Unfortunately, this was not recognized in advance, so that urea/THO diffusion ratios were not measured in patients Fle, Blu, and Cad.

Compared to the jejunum of normal subjects, patients with sprue had reduced absorption rates of both THO and urea (Table VI). However, urea absorption was reduced much more dramatically (8.5 compared to 52.2% in normals) than was THO absorption (40.5 compared to 60.1%). Thus, each patient with sprue who was studied with this solute pair had a marked reduction of the solute diffusion ratio. Even in San, who had sprue by mucosal biopsy but no diarrhea or steatorrhea, the diffusion ratio was markedly reduced, 0.22.²

Filtration coefficient

The filtration coefficient for normal subjects was determined by measuring water movement across the intestinal mucosa in response to hyper-

² The possibility that intestinal urease activity was modifying the apparent absorption rate of urea was excluded by incubating labeled urea in collected perfusate. After 2 hours of incubation at 37° C, there was no evidence of urea breakdown. Even if there had been greater urea breakdown in the patients with sprue, the result would be to falsely elevate the diffusion ratio and could not account for the observed differences between patients with sprue and normal subjects.

osmotic solutions containing mannitol. As shown in Table II, normal subjects had a mean filtration coefficient of $135 \mu\text{l}$ per mOsm per hour per cm (range, 69 to 195) in the upper jejunum. In patients with sprue a correction had to be made for the basal secretory state of the epithelium in this disease, which was documented above. When this correction was made, by subtracting basal secretion from water movement observed during perfusion with hyperosmotic solutions containing mannitol, very low filtration coefficients were calculated for each of the seven patients with steatorrhea. On the other hand, the two patients with celiac sprue without steatorrhea (both of whom had only a moderately severe mucosal lesion by jejunal biopsy) had filtration coefficients that fell within the normal range.

Discussion

These studies demonstrate several abnormal characteristics of the jejunal mucosa in sprue that probably contribute to water and electrolyte losses in this disease. First, during jejunal perfusion of patients who had severe celiac sprue by mucosal biopsy and who had diarrhea and steatorrhea clinically, water, sodium, and potassium were added to, rather than absorbed from, the perfusate. Similar results were noted in one patient with tropical sprue. Since the osmolality and ionic composition of the perfusate were the same as those of plasma, net water and electrolyte movement cannot be attributed to solute concentration gradients or to osmotic pressure gradients between intestinal contents and blood. These results therefore indicate that the jejunal mucosa of these patients is in a secretory condition with respect to water and electrolyte movement, the amount secreted being even greater than the amount of these substances that is absorbed in normal subjects. Addition of glucose to the perfusion solution, which in normal subjects stimulates jejunal absorption of sodium and water (12), had no demonstrable influence on the secretion of water and electrolytes in patients with sprue.

The specific cells involved in this secretory process are, of course, unknown. Trier has shown by electron microscopy that the undifferentiated crypt cells of normal jejunum secrete material into the crypt lumen in response to intravenous pilocarpine (14), and perhaps these cells, which

are relatively increased in sprue, are responsible for the water and electrolyte secretion we observed. However, Trier's histochemical studies suggested that the secretory granules of normal undifferentiated crypt cells were composed at least in part of a neutral polysaccharide-protein complex, whereas analysis of the intestinal perfusate in sprue failed to show a high concentration of hexosamine.

In contrast to the patients with severe celiac sprue by mucosal biopsy, two subjects with only a moderately severe mucosal lesion by biopsy who did not have diarrhea or steatorrhea absorbed sodium and potassium normally, although water absorption was not so rapid in these patients as in the normal subjects.

The secretory condition of the sprue intestine makes it difficult, if not impossible, to accurately appraise the intrinsic permeability characteristics of the jejunal mucosa in this disorder by techniques that were previously used in normal subjects (1). We therefore attempted to study permeability in sprue by measuring the relative diffusion rates of solutes of differing molecular radii, a method that has several technical advantages over the previous technique. For instance, measurement of relative diffusion rates requires only a single perfusion of an isotonic solution containing two solutes of different molecular radii, rather than two sequential perfusions with hypertonic fluids. This allows permeability measurements to be carried out in a shorter period of time and obviates the necessity of giving intravenous fluids to prevent shifts in blood volume, blood flow, and so on, secondary to gut perfusion with hypertonic fluids. In addition, studies carried out within a given intestinal segment are highly reproducible, as shown in Figure 1. This is almost certainly related to the fact that diffusion rates are measured simultaneously, and therefore variations in the length and area of the intestine exposed to the test solution, and variations in intestinal motility (as might occur during sequential perfusions), cannot influence the results. Finally, since this method is not dependent on the measurement of water flow in response to osmotic gradients, it can be used in disease states in which absorptive and secretory processes involving water and electrolytes may be abnormal, which would invalidate the previous method.

In vitro studies with cellulose membranes, and with the toad bladder, with and without ADH, indicate that changes in membrane permeability can be detected by measuring relative diffusion rates of different solutes. Furthermore, the markedly lower diffusion ratio of urea to THO in the normal ileum than in the jejunum is in excellent agreement with our previous study in normal subjects in which we found, by a different technique, that ileal pore radius is less than half that in the normal jejunum (1).

The principal difficulty associated with the use of diffusion ratios as a means of estimating the size of diffusion channels arises from the use of tritiated water as one of the solutes. In the jejunum the diffusion ratios of erythritol:urea and L-xylose:urea give a calculated effective pore size of about 9 Å, which closely approximates that obtained in our previous studies (1); by contrast, the urea:THO, erythritol:THO, and L-xylose:THO diffusion ratios all give a very large calculated effective pore size (greater than 20 Å). A similar discrepancy has been observed by Solomon and co-workers (8, 15) and by us (16) when the coefficient for diffusion of THO and the osmotic flow of water are compared in the intestine; calculated pore size by this method gives values of 30 to 40 Å, whereas calculations on the basis of reflection coefficients of nonlipid-soluble nonelectrolytes in both the rat and human give values of 4 to 9 Å. These observations indicate that diffusion of THO across the intestinal epithelium (but not across toad bladder or cellulose membranes) is restricted to a greater degree than would be predicted from its molecular size. Possibly this relates to the fact that diffusion coefficients of solutes in water within pores are not known and cannot be assumed to be equal to the diffusion coefficient in free solution, as is emphasized in the Appendix. Unfortunately, only the urea:THO diffusion ratio can be accurately measured in the lower small intestine of normal humans and in the jejunum of sprue patients because of the extremely low diffusion rates of erythritol and L-xylose in these areas of intestine. However, since the relative change in this diffusion ratio accurately reflects the known differences in the jejunum and ileum of normal subjects, in the toad bladder with and without ADH, and in cellulose membranes with and without stretching, its use to indicate di-

rectional changes in effective pore size in disease states seems justified.

In the jejunum of patients with sprue, we found that, compared to normal subjects, the absorption rate of THO was only moderately decreased, whereas the absorption of urea, xylose, and erythritol was markedly impaired. For instance, the ratio of urea to THO diffusion in normal subjects was 0.8, but in sprue it was only 0.2.

Although patients with sprue were secreting water into the lumen (against the direction of diffusion), whereas the normal subjects were absorbing water, the data in Figure 3 indicate that neither the direction nor magnitude of water flow alters the urea:THO diffusion ratio. Consequently, the markedly lower ratio in sprue patients cannot be attributed to directional differences in water movement and must represent some structural change in the membrane. As indicated in Equation 8 of the Appendix, a selective reduction in urea diffusion relative to tritiated water diffusion could be due either to a reduction in pore size or conceivably to an increase in structural orientation of water within the pores (i.e., more icelike). The effect of water structure on the relative diffusion rates of different solutes, however, has not been studied. In addition, it seems unlikely that water in pores will become more icelike without a concomitant change in pore size. Consequently, it seems most likely to us that the reduction in the diffusion ratio is due to a diminution in the average effective pore size of sprue mucosa, although the complexity of the small intestinal mucosa precludes the calculation of a precise dimension.

A decrease in average pore size might occur in at least two ways. First, if all the solute molecules penetrate the membrane exclusively through a uniform set of pores, a fall in the solute diffusion ratio from 0.8 to 0.2 would indicate a marked decrease in the radius of these aqueous channels. Since the rate of THO diffusion was only modestly decreased in sprue, this would suggest that the relatively large pores of the normal jejunum had been changed to a greater number of small pores in sprue, an event that would not produce a marked decrease in THO diffusion but which would significantly decrease the diffusion of the larger solutes and markedly restrict bulk water flow in response to osmotic pressure gradients.

A similar circumstance has been suggested in the toad bladder, where the action of ADH is pictured as reversibly making several small channels into one large pore (17). Second, if the jejunal barrier contains two sets of pores (either in the same membrane or in two adjacent membranes), one for THO diffusion and one for the diffusion of the larger solutes, our data are compatible with a decrease in the number of the larger pores while the smaller channels are left intact. From a functional point of view, however, this is the same as decreasing the size of the uniform set of pores.

A decrease in effective pore size of the jejunal mucosa in sprue would be expected to significantly influence absorptive processes in the involved areas of the gut. For example, water movement in response to osmotic pressure gradients (which is the major mechanism of water movement across biologic membranes) is approximately proportional to the fourth power of the radius of pores in a membrane. Water movement is, therefore, very sensitive to changes in pore size, and a decrease in pore radius of sprue mucosa would be expected to make a major contribution to the reduced filtration coefficient noted in these patients (Table II).³ In addition, passive movement of sodium chloride, which may be an important mechanism of salt absorption in the proximal human small intestine, would be expected to be markedly reduced in areas of the intestine where effective pore size is reduced. Finally, passive absorption of nonelectrolytes would be reduced in areas where membrane pore radius is decreased. For instance, absorption of xylose, which has a molecular radius of 3.5 Å, would be dependent to a large extent on the effective size of pores of the mucosal cell membranes. Small pore radii in sprue would, therefore, account for the fact that xylose absorption is almost always markedly decreased in this disease. It is interesting that the patient San in the present study absorbed xylose

³ The only exception to the association between diminished diffusion ratio and decreased filtration coefficient is in patient San, who had a urea/THO diffusion ratio of 0.22 but a normal filtration coefficient. It is noteworthy, however, that the absolute rate of THO diffusion was greater than normal (Table VI), suggesting that there might have been enough increase in the number of pores to counteract the effects of diminished pore size and thus maintain a normal filtration coefficient.

very poorly even though she had no diarrhea or steatorrhea. It seems likely that this was due to a decrease in the effective pore size of her jejunal mucosa, as evidenced by a urea/THO diffusion ratio of 0.22 (compared to 0.82 in normal subjects).

Taken *in toto*, these studies suggest that patients with celiac and tropical sprue, who have steatorrhea and diarrhea, secrete water and electrolytes and have a markedly reduced effective pore radius of jejunal mucosa compared to normal subjects. There is no reason to believe that these defects are interdependent. The two patients with "moderate" celiac sprue by biopsy who did not have diarrhea or steatorrhea did not secrete water and electrolytes, but, like those with steatorrhea, they had a marked decrease in effective pore radius.

One other observation of the present study deserves comment. When antidiuretic hormone (ADH) was given intravenously to normal subjects undergoing water diuresis, the diffusion ratio of urea/THO, erythritol/urea, and L-xylose/urea did not change. Furthermore, the absolute absorption rate of these solutes was not altered by the ADH infusion. These findings suggest that the permeability barrier of the small intestine is not under the control of the posterior pituitary gland.

Appendix

In a given length (l) of intestinal segment (from $l=0$ to $l=l$), perfused at a constant rate, a steady state will be achieved in which the luminal concentration of solute at any specified point is constant with time. However, the luminal concentration changes with respect to distance. The amount of substance diffusing per unit time (dN) out of a small segment (dl) of perfused intestine is given by the expression,

$$dN = -D \frac{A}{\Delta X} \Delta C dl, \quad [1]$$

where D is the diffusion coefficient of the solute in free solution, A is the effective diffusion area per unit length of intestine, ΔX is the membrane thickness, and ΔC the concentration difference of the solute across the membrane (luminal concentration - plasma concentration).

If movement of an isotope is measured, it can be assumed that the plasma concentration is always zero, and ΔC becomes equal to the luminal concentration (C). Since $C = N/V$, where N = amount of solute flowing by per unit time and V = volume flowing by per unit time,

Equation 1 becomes

$$dN = -D \frac{A}{\Delta X} \frac{N}{V} dl, \text{ which on rearrangement gives } [2]$$

$$\frac{dN}{N} = -D \frac{A}{\Delta X} \frac{dl}{V}. [3]$$

V is not constant along l , but changes in some indeterminate way as a function of length [$V(l)$], depending on absorption or secretion of fluid or mixing with secretions entering the test segment from higher in the intestine. This means that the integral of 3 is indeterminate.

$$\ln \frac{N^0}{N} = D \frac{A}{\Delta X} \int \frac{dl}{V(l)}. [4]$$

The diffusion rates for a single substance, therefore, cannot be determined with certainty. However, if two substances (N_1 , N_2) are incorporated into the perfusate so that the function [$V(l)$] is identical for both, the ratio of the two diffusion rates can be obtained:

$$\ln \frac{N_1^0}{N_1} = D_1 \frac{A_1}{\Delta X} \int \frac{dl}{V(l)}, \text{ and } [5]$$

$$\ln \frac{N_2^0}{N_2} = D_2 \frac{A_2}{\Delta X} \int \frac{dl}{V(l)}. [6]$$

The terms $\int dl/V(l)$ and ΔX are identical in Equations 5 and 6.

The effective diffusion areas (A_1 , A_2) for two water-soluble solutes depend not only on the number and size of the channels in the membrane available for diffusion, but also on the size of the diffusing molecule. Therefore, in a membrane with a fixed number and size of diffusion channels or pores, the effective diffusion area (A_1 , A_2 , and so on) is different for every solute. Dividing Equation 5 by 6 gives

$$\frac{\ln \left(\frac{N_1^0}{N_1} \right)}{\ln \left(\frac{N_2^0}{N_2} \right)} = \frac{A_1 D_1}{A_2 D_2}. [7]$$

The quantity of the two solutes entering (N_1^0 , N_2^0) and leaving (N_1 , N_2) the test segment can be calculated from their inflow and outflow concentrations and the concentration of a nonabsorbable volume marker (PEG). Therefore, Equation 7 becomes

$$\frac{\ln \left(\frac{[\text{PEG}]C_1^0}{[\text{PEG}]^0 C_1} \right)}{\ln \left(\frac{[\text{PEG}]C_2^0}{[\text{PEG}]^0 C_2} \right)} = \frac{A_1 D_1}{A_2 D_2}. [8]$$

Renkin (2) has derived equations for restricted diffusion through a membrane whereby the ratio A_1/A_2 for a given solute pair can be used to calculate equivalent pore size of the membrane. This calculation assumes that the diffusion coefficient (D_1 and D_2) through the water in the pore is the same as that in free solution. However, since water in the pore may not be in a fluid state (17), this assumption may be unjustified. For example, if water in pores were in a highly organized (ice-

like) state, the diffusion coefficients for all solutes would be reduced (18). Moreover, the diffusion coefficients for large solutes conceivably might be reduced disproportionately to that of smaller solutes, although this latter point has not been studied experimentally. In addition, the diffusion ratio for a solute pair diffusing across a complex membrane such as the intestinal mucosa will be a function of several different sets of pores in several different diffusion barriers rather than a single set of homogeneous pores. For these reasons, no attempt was made to assign a precise dimension to the pores. A change in the diffusion ratio, however, can be used to indicate directional changes. For example, if the diffusion rate of a large relative to the diffusion rate of a small solute were reduced, this would indicate either a decrease in the average effective pore size or possibly an increase in the state of organization of water filling the pores. From a functional point of view, however, either of these two changes would have the effect of increasing the selectivity of the membrane.

As indicated in the Methods, the experiments in the present study were performed by perfusing a length of intestine with a triple-lumen tube. The perfusion solution was infused through the most proximal opening, and samples were collected through two more distal openings. Analysis of the fluid collected from the most proximal collecting site permitted correction to be made for mixing with intraluminal contents. Thus, any change in the concentration of PEG and solute between the proximal and distal collecting sites must represent movement of solute and water across the wall of the intestine. However, as indicated in Equations 5, 6, and 7, the effect of mixing with intraluminal contents cancels out when the two diffusion rates are expressed as a ratio. For this reason, it is immaterial whether the concentration of the solute and PEG from the distal sample is compared with that in the proximal sample or that in the infused test solution.

Acknowledgments

We wish to express our appreciation to Drs. Robert M. Donaldson, Jerry Trier, Kermit Knudsen, Marshall Sparberg, Odell Hargrove, and Callum Bain for allowing us to study their patients. We are also grateful to Stephen Morawski and Charlotte Douglas for expert technical assistance and to Carolyn Arnold for her assistance in preparation of the manuscript.

References

1. Fordtran, J. S., F. C. Rector, M. F. Ewton, N. Soter, and J. Kinney. Permeability characteristics of the human small intestine. *J. clin. Invest.* 1965, **44**, 1935.
2. Renkin, E. M. Filtration, diffusion, and molecular sieving through porous cellulose membranes. *J. gen. Physiol.* 1954, **38**, 225.
3. Rubin, C. E., and W. O. Dobbins III. Peroral biopsy of the small intestine. A review of its diagnostic usefulness. *Gastroenterology* 1965, **49**, 676.

4. Fordtran, J. S., P. H. Clodi, K. H. Soergel, and F. J. Ingelfinger. Sugar absorption tests, with special reference to 3-O-methyl-d-glucose and d-xylose. *Ann. intern. Med.* 1962, 57, 883.
5. Laws, J. W., H. Shawdon, C. C. Booth, and J. S. Stewart. Correlation of radiological and histological findings in idiopathic steatorrhea. *Brit. med. J.* 1963, 1, 1311.
6. Jacobson, E. D., D. C. Bondy, S. A. Broitman, and J. S. Fordtran. Validity of polyethylene glycol in estimating intestinal water volume. *Gastroenterology* 1963, 44, 761.
7. Cooper, H., R. Levitan, J. S. Fordtran, and F. J. Ingelfinger. A method for studying absorption of water and solute from the human small intestine. *Gastroenterology* 1966, 50, 1.
8. Lindemann, B., and A. K. Solomon. Permeability of the luminal surface of intestinal mucosal cells. *J. gen. Physiol.* 1962, 45, 801.
9. Bentley, P. J. The effects of neurohypophyseal extracts on water transfer across the wall of the isolated urinary bladder of the toad *Bufo marinus*. *J. Endocr.* 1958, 17, 201.
10. Hyden, S. A turbidometric method for the determination of higher polyethylene glycols in biologic materials. *Ann. roy. agricul. Coll. Swed.* 1955, 22, 139.
11. Roe, J. H., and E. W. Rice. A photometric method for the determination of free pentoses in animal tissues. *J. biol. Chem.* 1948, 173, 507.
12. Malawer, S. J., M. F. Ewton, J. S. Fordtran, and F. J. Ingelfinger. Interrelation between jejunal absorption of sodium, glucose and water in man. *J. clin. Invest.* 1965, 44, 1072.
13. Craig, L. C., and W. Konigsberg. Dialysis studies. III. Modification of pore size and shape in cellophane membranes. *J. phys. Chem.* 1961, 65, 1966.
14. Trier, J. S. Studies on small intestinal crypt epithelium. II. Evidence for and mechanisms of secretory activity by undifferentiated crypt cells of the human small intestine. *Gastroenterology* 1964, 47, 480.
15. Curran, P. F., and A. K. Solomon. Ion and water fluxes in the ileum of rats. *J. gen. Physiol.* 1957, 41, 143.
16. Fordtran, J. S., F. C. Rector, Jr., T. W. Locklear, and M. F. Ewton. Unpublished observations.
17. Hays, R. M., and A. Leaf. The state of water in the isolated toad bladder in the presence and absence of vasopressin. *J. gen. Physiol.* 1962, 45, 933.
18. Eigen, M. The protonic charge transfer in hydrogen bonded systems in *Hydrogen Bonding*, D. Hadzi, Ed. New York, Pergamon, 1959, p. 429.

ANNOUNCEMENT OF MEETINGS

The American Federation for Clinical Research will hold its Twenty-fourth Annual Meeting in Atlantic City, N. J., in the Pennsylvania Room, Haddon Hall, on Sunday, April 30, 1967, at 9:00 a.m. Joint sectional meetings with The American Society for Clinical Investigation will be held on Sunday afternoon at Chalfonte-Haddon Hall, and additional meetings sponsored by The American Federation for Clinical Research will be held on Sunday evening.

The American Society for Clinical Investigation, Inc., will hold its Fifty-ninth Annual Meeting in Atlantic City, N. J., on Monday, May 1, at 9:00 a.m., in the Pennsylvania Room, Haddon Hall, and will join The American Federation for Clinical Research in simultaneous sectional meetings on Sunday afternoon, April 30, at Chalfonte-Haddon Hall.

The Association of American Physicians will hold its Eightieth Annual Meeting in Atlantic City, N. J., in the Pennsylvania Room, Haddon Hall, on Tuesday, May 2, at 9:30 a.m., and in the Vernon Room on Wednesday, May 3, at 9:30 a.m.