

Intestinal Transport of Weak Electrolytes

Evidence in Favor of a Three-Compartment System

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ABSTRACT A study has been made of the transmural fluxes of benzoic, phenylacetic, and pentanoic acids, benzylamine, hexylamine, and D-amphetamine across rat jejunum incubated in vitro. The M to S fluxes of the weak acids were greater than their corresponding S to M fluxes, and the S to M fluxes of the weak bases were larger than their M to S fluxes. These patterns of asymmetric movements were observed when the transmural electrical potential difference was clamped at 0 mV, and when the pH values of the mucosal and serosal fluids were identical. The effects of a weak acid on the fluxes of other weak electrolytes were qualitatively similar when the effector weak acid was added to the mucosal fluid, and when it was added to the serosal fluid. But the effects of a weak base on the fluxes of other weak electrolytes were dependent upon its location, and the interactions observed when the effector weak base was added to the mucosal fluid were qualitatively different than those seen when it was added to the serosal fluid. The interactions between weak electrolytes could readily be explained in terms of the function of a system of three compartments in series, in which the pH of the intermediate compartment is greater than that of the bulk phases. But these observations could not be explained in terms of an analogous system involving an intermediate compartment of low pH, or in terms of a carrier mediated system. The transport function of the three-compartment system can be described in the form of an equation, and it is found that a pH difference of less than 0.5 unit may explain our observations on weak electrolyte transport.

The mechanisms involved in the intestinal transport of weak electrolytes have been the subject of several previous investigations. Some studies have demon-

strated the importance of dissociation and pH, and have suggested that weak electrolyte absorption is largely dependent on diffusion of the compound in the nonionized form [for example see 18]. Other studies have revealed properties such as saturation kinetics [2, 4], that are reminiscent of carrier mediated processes, and have suggested the possibility of membrane carrier systems for the transport of weak electrolytes in the intestine. In 1955 Hogben [11] proposed an additional possibility for the mechanism of weak electrolyte transport in epithelia. The system consists of three aqueous compartments arranged in series, and the pH of the intermediate compartment is different than that of the bulk phases. It has been suggested that this system may play a role in the transport of weak electrolytes across the wall of the small intestine [6, 12], but empirical support for this suggestion was not developed. The present paper is concerned with studies of the transmural fluxes of weak electrolytes across the wall of rat jejunum *in vitro*. These studies show that weak electrolyte transport in this tissue cannot be explained in terms of transmural gradients of pH or electrical potential, and observations on the interactions between weak electrolytes indicate that a version of the three-compartment system is an appropriate model for the mechanism of weak electrolyte transport in the intestine. In addition, the parameters of weak electrolyte transport in the three-compartment system are defined, and it is shown that the values of these parameters that are required to account for our observations can be suggested to reflect properties of the intestinal wall.

METHODS

The animals used were male albino rats of the Wistar strain weighing 180–250 g, and were allowed food and water *ad libitum* to the time of experiment. The animals were anesthetized with Nembutal (70 mg/kg, intraperitoneal), the abdomen opened, and the small intestine washed out with 0.9% saline at room temperature. The length of small intestine from the distal end of the ligament of Treitz to the ileocecal junction was stripped from the mesentery, and placed in chilled saline. Three or four segments, approximately 3 cm in length, were taken from the middle of the combined jejunum and ileum, and opened along the antimesenteric border. The sheets of tissue were mounted in flux chambers based on those described by Schultz and Zalusky [19]. The exposed area of tissue was 1 cm² and the volume of incubation saline perfused through each half of the chamber was 20 ml. 1 μ Ci of radioactive solute was added to one reservoir and the appearance of tracer in the other reservoir estimated. Fluxes were calculated using the formula given by Schultz and Zalusky [19]. Preliminary experiments showed that both mucosal to serosal (M to S) and serosal to mucosal (S to M) fluxes were independent of time after an initial period of 20 min (see Fig. 1). The period 30–40 min after the addition of tracer was routinely used for estimation of fluxes in these experiments.

Some observations were made on the electrical correlates of weak electrolyte transport. In these experiments a pair of saturated KCl/agar bridges were inserted into the

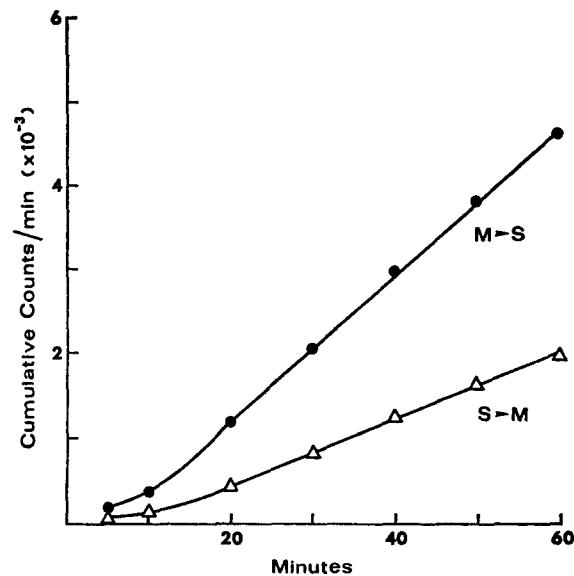


FIGURE 1. Results of typical experiments with the flux chamber preparation. In these experiments tissue from the midregion of rat small intestine was perfused with bicarbonate saline containing 1 mM benzoic acid. Tracer was added to either mucosal or serosal reservoirs and its appearance in the other compartment estimated. It should be noted that the fluxes were constant in time after an initial period of 20 min.

chamber so that the tips rested about 3 mm from each surface of the tissue. The outer ends of these bridges (1 mm-ID) were connected to matched calomel half-cells the potentials of which did not differ by more than 0.5 mV. The output of these cells was fed to an Orion pH/electrometer (Orion Research, Inc., Cambridge, Mass., model 801, input impedance $10^{14} \Omega$) for estimation of the transintestinal electrical potential difference (PD). A second pair of saturated KCl/agar bridges were inserted into the chamber approximately 5 cm from the surfaces of the tissue. These bridges were also connected to a pair of matched calomel half-cells, and this system was used to apply current from a variable source to the chamber. Estimates of tissue resistance, and transintestinal PD when current was applied from the external source, were corrected for the resistance of the saline between the PD measuring bridges by the graphical method of Clarkson and Toole [7]. With this preparation the values of tissue resistance ($90\text{--}100 \Omega\text{-cm}^2$) and short circuit current ($30\text{--}40 \mu\text{A}/\text{cm}^2$) were similar to those found using intact intestine in comparable conditions [3, 7], and it is considered that the interpretation of these studies is not complicated by a significant "edge effect."

The incubation saline used in most experiments was buffered with bicarbonate and equilibrated with 95% O_2 /5% CO_2 . This saline had the following composition in meq/l: Na^+ , 143; K^+ , 6; Ca^{2+} , 5; Mg^{2+} , 2.4; Cl^- , 128; SO_4^{2-} , 2.4; H_2PO_4^- , 1; HCO_3^- , 25. The pH of this saline was 7.30 ± 0.05 . In some experiments the pH of the saline was decreased to 6.20 by decreasing the concentration of bicarbonate to 1 mM, and increasing the concentration of chloride to maintain the same concentration of sodium.

Radioactive tracers for the transported solutes were obtained from commercial

sources and were found to be at least 98% pure by thin layer chromatography [23]. Radioactivity was estimated in undiluted samples of the perfusion saline with the liquid scintillation fluid described by Bray [5], and quenching was monitored by the channels ratio method using an external standard. The counting conditions were such that the standard error of the net count rate was less than 2% of the count rate for all samples.

RESULTS

Transintestinal Fluxes of Weak Acids and Weak Bases

Table I shows the results of a series of experiments in which the fluxes of benzoic, phenylacetic, and pentanoic acids, benzylamine, hexylamine, and D-amphetamine across rat intestine were estimated. In all of these experiments the M to S flux of a weak electrolyte was significantly different than its S to M flux. The M to S fluxes of the weak acids were greater than the corresponding S to M flux, and the S to M flux of a weak base was larger than its M to S flux.

TABLE I

Fluxes of weak acids and weak bases. Sheets of intestine obtained from the midregion of the combined jejunum and ileum of rat intestine were mounted in flux chambers and perfused with bicarbonate buffered saline (pH 7.30) containing 1 mM weak electrolyte. M to S and S to M fluxes were determined in separate experiments in the period 30–40 min after the start of the perfusion. Results are means \pm SEM, with the number of experiments given in parentheses. Fluxes are expressed as *n*-moles of weak electrolyte moving per square centimeter of tissue per hour. Flux ratios were calculated from the mean fluxes.

Weak electrolyte	Fluxes		
	M to S	S to M	Ratio
Benzoic acid	52 \pm 3 (5)	21 \pm 3 (5)	2.47
Phenylacetic acid	56 \pm 4 (5)	23 \pm 2 (5)	2.33
Pentanoic acid	49 \pm 3 (5)	24 \pm 3 (5)	2.05
Benzylamine	20 \pm 3 (5)	41 \pm 3 (5)	0.49
Hexylamine	18 \pm 2 (5)	35 \pm 4 (5)	0.51
D-amphetamine	17 \pm 3 (5)	35 \pm 2 (5)	0.49

Effect of Transmural PD on Fluxes of Benzoic Acid and Benzylamine

Table II shows the results of experiments in which the fluxes of benzoic acid and benzylamine were estimated at three values of the transmural PD. In addition to studies in the unclamped condition, when the mean spontaneous PD was 2.5 mV, the table includes observations made when the PD was clamped at 0 mV, or at 25 mV. No significant variations in the fluxes of the weak electrolytes were observed when these changes were made in the PD.

TABLE II

Effect of transmural PD on fluxes of benzoic acid and benzylamine. Conditions of incubation and expression of results similar to experiments described in Table I, but in these experiments the transmural PD was recorded, and in some cases the PD was altered by application of current from an external source. The lower part of the table gives values of flux ratios that were calculated from the conditions used in these experiments, and using Eq. A6 from the Appendix.

PD (mV)	Benzoic acid fluxes			Benzylamine fluxes		
	M to S	S to M	Ratio	M to S	S to M	Ratio
0	59±4 (5)	24±3 (6)	2.46	16±2 (5)	36±5 (5)	0.44
2.5±0.1 (23)	50±3 (6)	19±3 (6)	2.63	19±3 (5)	40±2 (6)	0.47
25	55±2 (5)	24±3 (5)	2.29	18±2 (6)	42±4 (6)	0.43

Calculated flux ratios

	(P^i/P^o)					(P^i/P^o)				
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
2.5	1.09	1.09	1.08	1.05	1.01	0.92	0.92	0.95	0.99	1.00
25	2.53	2.51	2.36	1.67	1.11	0.42	0.45	0.64	0.92	0.99

The table also gives values of flux ratios that were calculated from Eq. A6 (see Appendix). The assumptions involved in making these calculations, and the relations between the calculated flux ratios and those observed in the experiments are considered in the Discussion.

Effects of Glucose and Galactose on Fluxes of Benzoic Acid and Benzylamine

Table III shows the effects of addition of 10 mM glucose or galactose to the incubation saline on the fluxes of benzoic acid or benzylamine and on the value of the spontaneous PD. In this series of experiments 10 mM mannitol was added to the saline in the control condition to ensure that the osmolarity of the saline was the same in all of the experiments in the series. Glucose and galactose produced similar changes in the transmural PD, but had opposite effects on the fluxes of the weak electrolytes. The M to S flux of benzoic acid was increased in the presence of glucose but decreased in the presence of galactose, and the S to M flux of benzoic acid decreased when glucose was added to the saline but increased in the presence of galactose. Similarly, the M to S flux of benzylamine was decreased, and the S to M flux was increased by the addition of glucose, but in the presence of galactose the M to S flux of the weak base was increased and the S to M flux was decreased.

Effects of pH on Fluxes of Benzoic Acid and Benzylamine

Table IV shows that no significant variations in the pH of the incubation saline occurred during the course of the experiment even when the pH values of the mucosal and serosal fluids differed by 1.1 unit. The table also shows that

TABLE III

Effects of glucose and galactose on fluxes of benzoic acid and benzylamine. Conditions of incubation and expression of results as described in Table I, but in these experiments mannitol, glucose, or galactose (10 mM) were present in the incubation saline in addition to the weak electrolyte.

	Addition to saline		
	Mannitol	Glucose	Galactose
Benzoic acid transport			
M to S flux	49±3 (6)	79±4 (5)	32±3 (7)
S to M flux	19±2 (5)	13±1 (6)	28±3 (5)
Flux ratio	2.58	6.06	1.14
PD	2.1±0.1	8.3±0.2	8.0±0.1
Benzylamine transport			
M to S flux	21±2 (5)	14±1 (6)	34±3 (7)
S to M flux	53±4 (5)	71±4 (5)	44±2 (7)
Flux ratio	0.40	0.20	0.77
PD	1.9±0.1	7.9±0.1	8.3±0.1

TABLE IV

Effects of pH on fluxes of benzoic acid and benzylamine. Conditions of incubation and expression of results as described in Table I, but in these experiments the pH of the incubation saline was altered by changing the concentration of bicarbonate. The table includes flux ratios calculated from the pH values of the incubation salines, using Eq. A7 from the Appendix.

Initial mucosal pH	7.30	6.20	6.20	7.30
Final mucosal pH	7.30±0.01	6.21±0.01	6.22±0.01	7.30±0.01
Initial serosal pH	7.30	6.20	7.30	6.20
Final serosal pH	7.31±0.01	6.20±0.02	7.28±0.02	6.20±0.02
Benzoic acid transport				
M to S flux	57±4 (5)	107±8 (6)	118±10 (5)	47±3 (6)
S to M flux	24±3 (6)	35±3 (6)	18±4 (6)	31±2 (5)
Ratio of observed fluxes	2.40	3.06	6.66	1.51
Calculated flux ratio	1.00	1.00	12.48	0.08
Benzylamine transport				
M to S flux	23±2 (5)	11±2 (6)	14±3 (6)	23±3 (6)
S to M flux	55±3 (5)	28±3 (5)	61±4 (6)	31±3 (5)
Ratio of observed fluxes	0.42	0.39	0.23	0.74
Calculated flux ratio	1.00	1.00	0.08	12.45

the fluxes of benzoic acid and benzylamine were markedly dependent upon the pH value of the compartment of origin of the flux. For example, the M to S flux of benzoic acid increased, and that of benzylamine decreased when the pH of the mucosal fluid was decreased to 6.2. Qualitatively similar changes in the S to M fluxes of benzoic acid and benzylamine were observed when the serosal pH was decreased. In contrast, no such relation could be described between the fluxes of these weak electrolytes and the pH of the trans compartment.

The changes in the fluxes of benzoic acid and benzylamine that were observed when the pH of the saline was altered, suggest that the movements of the nonionized forms of these compounds contribute significantly to the observed fluxes. Table IV includes flux ratios that were calculated using Eq. A7 from the Appendix which describes the movements of a weak electrolyte across a barrier that is impermeable to the ionized form. No correlation was found between the observed and calculated values of the flux ratios.

Interactions between Weak Electrolytes

Table V shows the results of experiments that examined the effects of one weak acid on the fluxes of another. In these studies, and in those described below with weak bases, the compound the fluxes of which are estimated is

TABLE V

Interactions between weak acids. Conditions of incubation and expression of results as described in Table I, but in some experiments a second weak acid was added either to the mucosal fluid or to the serosal fluid. The second weak acid is referred to as the effector acid and its concentration was 5 mM. The transported weak acid was present in both mucosal and serosal fluids and its concentration was 1 mM.

[Effector acid]	Fluxes of transported weak acid								
	Benzoic		Phenylacetic			Pentanoic			S to M
	M to S	Ratio	S to M	M to S	Ratio	S to M	M to S	Ratio	
None	58±2 (17)		23±2 (15)	53±3 (12)		25±2 (14)	45±2 (15)		20±2 (15)
		2.52			2.12			2.25	
Benzoic/mucosal	—		—	39±2 (15)*		33±1 (13)‡	33±2 (15)‡		27±2 (12)§
					1.18			1.22	
Benzoic/serosal	—		—	43±1 (11)‡		31±2 (12)§	38±2 (13)§		27±1 (10)‡
					1.39			1.41	
Phenylacetic/mucosal	42±3 (18)*		33±2 (15)‡	—		—	37±2 (12)‡		29±2 (14)‡
		1.27						1.28	
Phenylacetic/serosal	41±2 (14)*		35±3 (12)§	—		—	34±3 (15)‡		30±2 (14)‡
		1.17						1.13	
Pentanoic/mucosal	47±2 (16)*		35±2 (12)‡	41±2 (10)‡		35±3 (18)‡	—		—
		1.34			1.18				
Pentanoic/serosal	50±2 (12)‡		30±1 (13)‡	45±2 (11)§		33±2 (14)‡	—		—
		1.67			1.37				

Significance of difference from flux in control condition:

* $P < 0.001$

‡ $P < 0.01$

§ $P < 0.05$

called the *transported* compound, and the compound whose effect is under investigation is called the *effector* compound. In all of these experiments the effector compounds were added either to the mucosal fluid or to the serosal fluid, but were not added to both fluids in the same experiment.

Table V shows that both M to S, and S to M fluxes of a transported weak acid were altered in the presence of an effector weak acid, and that the pattern of changes in the fluxes of the transported weak acid was independent of the location of the effector weak acid. Both when the effector weak acid was present in the mucosal fluid, and when it was added to the serosal fluid, the M to S flux of the transported weak acid was decreased and the S to M flux was increased. In contrast, in the studies of interactions between weak bases (Table VI) the pattern of changes in the fluxes of a transported weak base was found to be dependent upon the location of the effector weak base.

TABLE VI

Weak base fluxes. Design of experiments, conditions of incubation and expression of results as described in Table V, but weak bases were used for transported (1 mM) and effector (5 mM) compounds.

Effector base	Fluxes of transported weak base								
	Benzylamine			Hexylamine			D-amphetamine		
	M to S	Ratio	S to M	M to S	Ratio	S to M	M to S	Ratio	S to M
None	18±2 (12)		40±2 (15)	20±2 (18)		35±2 (18)	19±1 (16)		35±2 (14)
		0.45			0.57			0.54	
Benzylamine/mucosal	—		—	14±2 (14)*		41±2 (16)*	12±2 (15)‡		43±1 (15)‡
					0.34			0.28	
Benzylamine/serosal	—		—	27±2 (18)*		28±2 (15)*	26±2 (14)‡		25±2 (15)‡
					0.96			1.04	
Hexylamine/mucosal	12±1 (16)*		48±2 (15)‡	—		—	11±1 (16)§		45±2 (16)‡
		0.25						0.24	
Hexylamine/serosal	27±2 (15)‡		33±2 (12)*	—		—	25±2 (18)*		25±1 (18)§
		0.82						1.00	
D-amphetamine/mucosal	10±1 (15)‡		51±2 (15)§	12±1 (15)‡		42±2 (16)*	—		—
		0.20			0.29				
D-amphetamine/serosal	26±2 (15)‡		31±2 (16)‡	26±2 (18)*		25±1 (18)§	—		—
		0.84			1.04				

Significance of difference from flux in control condition:

* $P < 0.05$

‡ $P < 0.01$

§ $P < 0.001$

When an effector weak base was added to the mucosal fluid, the M to S flux of the transported weak base was decreased and the S to M flux increased. But in those experiments in which the effector weak base was present in the serosal fluid, the M to S flux of the transported weak base was increased and the S to M flux was decreased.

Table VII shows that interactions were observed between a weak acid and a weak base, and some similarities between these interactions and those described above were noted. Thus the changes in the fluxes of the transported weak base were not dependent upon the location of the effector weak acid.

TABLE VII

Interactions between a weak acid and a weak base. Design of experiments, conditions of incubation and expression of results as described in Table V, but benzylamine (5 mM) was used as an effector in the studies of benzoic acid transport, and benzoic acid (5 mM) was used in the studies of benzylamine transport.

Effector compound	Fluxes of transported weak electrolyte					
	Benzoic acid			Benzylamine		
	M to S	Ratio	S to M	M to S	Ratio	S to M
None	62±2 (14)	2.95	21±2 (12)	20±1 (15)	0.44	45±2 (13)
Benzylamine/mucosal	76±3 (12)*	5.85	13±1 (10)‡	—	—	—
Benzylamine/serosal	41±3 (10)*	1.32	31±2 (14)‡	—	—	—
Benzoic acid/mucosal	—	—	—	27±2 (17)‡	0.71	38±2 (15)§
Benzoic acid/serosal	—	—	—	30±2 (18)*	0.88	34±2 (16)*

Significance of difference from flux in control condition:

* $P < 0.001$

‡ $P < 0.01$

§ $P < 0.05$

Both when benzoic acid was added to the mucosal fluid, and when it was added to the serosal fluid, the M to S flux of benzylamine was increased and the S to M flux decreased. In contrast, the actions of the effector weak base on the fluxes of the transported weak acid were dependent upon the location of the effector weak base. When benzylamine was present in the mucosal fluid the M to S flux of benzoic acid was increased, and the S to M flux was decreased. But when benzylamine was added to the serosal fluid the M to S flux of benzoic acid was decreased, and the S to M flux was increased.

Effects of Weak Electrolytes on Fluxes of Galactose and Urea

Table VIII shows that the fluxes of the actively transported hexose, galactose, and those of the passively transported solute, urea, were not altered by the

TABLE VIII

Effects of weak electrolytes on fluxes of galactose and urea. Conditions of incubation and expression of results similar to experiments described in Table I, but in these experiments transport of galactose (1 mM) or urea (1 mM) was studied and weak electrolytes (5 mM) were added to the incubation saline in some experiments.

Addition to saline	Transported compound			
	Galactose		Urea	
	M to S	S to M	M to S	S to M
None	177±9 (12)	20±3 (10)	52±3 (10)	51±4 (10)
Benzoic acid	181±10 (10)	19±2 (10)	51±2 (11)	49±4 (10)
Phenylacetic acid	173±7 (10)	21±3 (9)	54±3 (12)	50±2 (9)
Pentanoic acid	187±9 (10)	24±2 (10)	55±5 (10)	51±3 (10)
Benzylamine	185±12 (10)	18±2 (10)	48±4 (10)	52±3 (10)
Hexylamine	167±15 (12)	24±4 (10)	51±3 (10)	54±4 (10)
D-amphetamine	180±9 (10)	21±2 (10)	47±5 (8)	53±3 (10)

presence of weak electrolytes in conditions similar to those of the experiments described above. In all of these experiments, the M to S flux of galactose was substantially greater than its S to M flux, and these fluxes were not altered significantly in the presence of weak electrolytes. The M to S, and S to M fluxes of urea were not significantly different in any of the conditions used, and did not change significantly when weak electrolytes were added to the saline.

DISCUSSION

Transmural Gradients and Weak Electrolyte Transport

In the preliminary experiments described in Table I, unequal M to S and S to M fluxes were observed with all of the weak electrolytes used in this study. The M to S fluxes of the weak acids were greater than the corresponding S to M fluxes, and the S to M flux of a weak base was larger than its M to S flux. The studies of the effects of a transmural PD on weak electrolyte fluxes (Table II) suggested that the asymmetric patterns of weak electrolyte movement did not reflect the influence of transmural gradients of electrical potential. The patterns of weak electrolyte movements were the same when the transmural PD was clamped at 0 mV and in the unclamped condition, and the fluxes of benzoic acid and benzylamine did not change significantly when the transmural PD was increased to 25 mV. Equations describing weak electrolyte transport in a two-compartment system are given in the Appendix, and Eq. A6 indicates that the range of values for transmural PD used in the experiments described in Table II, should have produced substantial alterations in the observed fluxes if the movements of the ionized forms in response to a gradient of electrical potential had contributed significantly

to the total movement. Examination of Eq. A6 shows that the ratio (P^i/P^{ni}) is an important determinant of the effect of a transmural PD on weak electrolyte transport. This ratio reflects the ability of the barrier to discriminate between the ionized and nonionized forms of the transported weak electrolyte. It is suggested that a value of unity for the permeability ratio represents the upper limit of interest in the present study. This value would reflect the properties of a barrier through which solute movements are limited by molecular weight or size, and where charge or lipophilic characteristics are not significant determinants. A ratio equal to unity may be representative of weak electrolyte movements through long, narrow channels, such as the intercellular spaces which are believed to form an important restriction to solute movements linked to fluid transport [9]. Cell membranes are generally considered to be less permeable to the ionized forms of weak electrolytes than to their nonionized forms [for example see 8], and permeability ratios less than unity may be appropriate to these barriers. Table II includes flux ratios that were calculated from Eq. A6 using values of pH, pK_a , and PD appropriate to the experiments described in the table, and using a series of values of decreasing magnitude for the permeability ratio. The values of the flux ratios calculated for a PD of 25 mV are comparable to those observed in the experiments when the values of the permeability ratio used in the calculations were greater than 10^{-3} in the case of benzoic acid, or greater than 10^{-2} in the case of benzylamine. But the calculations also show that for such values of the permeability ratio, substantial changes in the flux ratio should have been observed when the PD was changed from 2.5 mV to 25 mV. Since no significant changes in the fluxes of benzoic acid or benzylamine were observed when this alteration was made in the transmural PD, it can be suggested that the correlation between the observed and calculated flux ratios in some conditions is fortuitous, and that the movements of the ionized forms of these weak electrolytes in response to transmural gradients of electrical potential is a small fraction of the observed fluxes. In contrast, the studies of the effects of pH on weak electrolyte fluxes suggest that the movements of the nonionized forms of benzoic acid and benzylamine contribute significantly to the observed fluxes. The fluxes of the weak acid were increased, and those of the weak base were decreased, when the pH of the compartment of origin of the flux was decreased. But in no case could the results of these experiments be described solely in terms of the bulk phase pH values. The conclusion that the passive movements of the ionized forms of benzoic acid and benzylamine are small relative to the total observed movements allows Eq. A7 from the Appendix to be applied to the results of the experiments described in Table IV. No correlation between the observed and calculated flux ratios was obtained, and in some experiments the transport of the weak electrolytes occurred in the opposite direction to that predicted from Eq. A7. For example, in the

experiments using a mucosal pH of 7.3 and a serosal pH of 6.2, the ratio of the observed fluxes of benzoic acid is greater than unity, and the flux ratio calculated from Eq. A7 is less than unity. Similarly in these conditions, the flux ratio predicted from Eq. A7 for benzylamine is greater than unity, but the ratio of the observed fluxes is less than unity. Also in the experiments in which the pH gradient was consistent with the directions of spontaneous transport of weak electrolytes (mucosal pH lower than serosal pH), the observed flux ratios were closer to unity than those predicted from Eq. A7, indicating that the intestinal wall may modify the effects of transmural pH gradients even when such gradients are arranged to reinforce the spontaneous transport.

In summary, the experiments discussed above show that the intestinal transport of the weak electrolytes used in these studies cannot be described in terms of a model system of two compartments in which weak electrolyte movements are determined by transmural gradients of electrochemical potential. These experiments suggest that the permeability of the intestinal wall to the ionized forms of the weak electrolytes is small, and that the movements of the nonionized forms may contribute significantly to the observed fluxes.

Three-Compartment System for Weak Electrolyte Transport

A more complex possibility for the mechanism of intestinal transport of weak electrolyte is illustrated in Fig. 2. The system consists of three compartments in series, and it is shown in the Appendix that the transport function of this system can be described by an expression of the form:

$$J_{MS}/J_{SM} = \frac{(1 + R_I 10^{\alpha_M})(1 + R_{II} 10^{\alpha_X})}{(1 + R'_I 10^{\alpha_X})(1 + R'_{II} 10^{\alpha_M})} \quad (1)$$

In this expression (J_{MS}/J_{SM}) is the ratio of transmural fluxes from mucosal to serosal, and from serosal to mucosal sides. The terms α_M and α_X are the differences between the pK_a of the weak electrolyte and the pH value of the bulk phases, or of the intermediate compartment. In the case of a weak acid $\alpha = (\text{pH} - \text{pK}_a)$, and for a weak base $\alpha = (\text{pK}_a - \text{pH})$. R_I , R'_I , R_{II} and R'_{II} are terms relating the parameters of movement of the nonionized and ionized forms of the transported weak electrolyte at the barrier represented by the subscript. In the absence of electrical potential differences at the barriers $R_I = R'_I = (P_I^i/P_I^{n_i})$, and $R_{II} = R'_{II} = (P_{II}^i/P_{II}^{n_i})$. But when a PD exists at a barrier the permeability ratio is modified by a term related to the PD, and the R terms appropriate to that barrier are not equal.

The transmural PD of rat intestine can be represented as the sum of two components in series [1, 15]. These component PD's are usually considered to be associated with the luminal and abluminal membrane complexes of the epithelial cells, and Eq. 1 can be used to evaluate the roles of these membrane

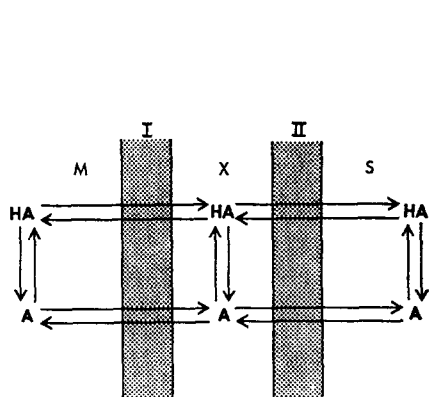


FIGURE 2

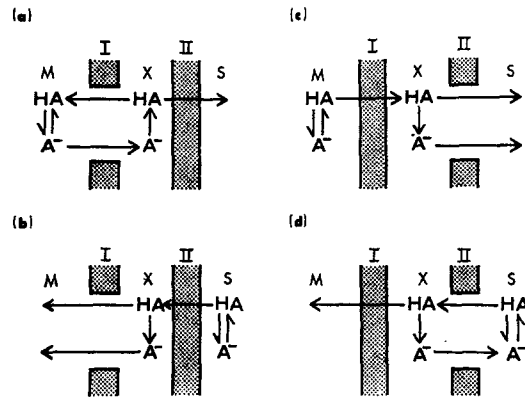


FIGURE 3

FIGURE 2. Three-compartment model for weak electrolyte transport. The system consists of three aqueous compartments, M, X, and S, arranged in series and separated by two barriers, I, and II. In the generalized form of the model system shown here, both barriers are considered to be permeable to both ionized and nonionized forms of the transported weak electrolyte, but the properties of the two barriers may be different. The bulk phase compartments, M and S, have identical compositions, and the movements of the weak acid shown in the figure may be influenced by the presence of electrical potential differences at the barriers, and by a difference between the pH values of the intermediate compartment and the bulk phases.

FIGURE 3. Influence of an effector weak acid on the pH of the intermediate compartments of two versions of the three-compartment system for weak electrolyte transport. In the system shown at the left of the figure, the pH of the intermediate compartment is lower than that of the bulk phases, and barrier II is considered to be impermeable to the ionized form of the weak acid. In the system shown at the right of the figure, barrier I is impermeable to anion, and the pH of the intermediate compartment is greater than that of the bulk phases. The arrows in these diagrams show the net movements that occur when a weak acid is added either to the mucosal bulk phase, or to the serosal bulk phase. Examination of the diagrams shows that these movements are related to net dissociation or association reactions within the intermediate compartment, and it is suggested that these reactions may alter the pH of this compartment. For further discussion see text.

PD's in weak electrolyte transport. In conditions similar to those used in our experiments, it has been reported that the PD at the luminal membrane of rat intestinal epithelial cells is approximately 10 mV, cell interior negative, and that the PD at the abluminal side of the cell is approximately 12 mV, cell interior negative [1, 15]. It will be noted that the PD at the luminal membrane does not have the appropriate polarity for the observed directions of weak electrolyte transport, but the PD at the abluminal membrane does have an appropriate orientation. Thus as a first step in the evaluation of the possible roles of these PD's, we will assign a low value (10^{-5}) to the permeability ratio for the luminal membrane, and a high value (10^0) to the perme-

ability ratio for the abluminal membrane. These assumptions make the influence of the PD at the luminal membrane negligible, and emphasize the influence of the PD at the abluminal membrane. The system described by these assumptions is similar to systems that have been suggested to play a role in the transport of bicarbonate in pancreas [20], kidney [25], and choroid plexus [31]. The assumption that one of the component barriers of the intestinal wall is relatively impermeable to the ionized forms of the transported weak electrolytes is consistent with the finding that alterations in the transmural PD did not change the fluxes of weak electrolytes, and the suggested arrangement of the two sets of permeability properties is consistent with the suggestion that the luminal membrane of intestinal cells is less permeable to hydrophilic solutes than the abluminal membrane [14]. Using the values described above for permeability ratios and membrane PD's, and if it is assumed that the intracellular pH is equal to that of the bulk phases, Eq. 1 can be used to test the possibility that the transport of weak electrolytes can be accounted for solely in terms of the PD's associated with the membranes of the epithelial cells. In fact the values of the flux ratios obtained for such calculations (1.52 in the case of benzoic acid and 0.64 in the case of benzylamine) are substantially closer to unity than the flux ratios observed in the experiments (more than 2.4 in the case of benzoic acid and approximately 0.45 in the case of benzylamine). It should be emphasized that the values of the permeability ratios used in these calculations were chosen to maximize the influence of the PD at the abluminal side of the epithelial cells. If the permeability ratio of the luminal membrane is greater than 10^{-5} , or if the permeability ratio of the abluminal membrane is less than 10^0 , the calculated values of the flux ratios are closer to unity. In addition, the assumption that the pH value of the intracellular space is equal to that of the extracellular environment probably cannot be justified. In most tissues that have been investigated, the intracellular pH is lower than the pH of bathing medium [27], and use of values for pH_x that are lower than that of pH_M also changes the calculated flux ratios toward unity. Thus these considerations indicate that the magnitudes of the PD's associated with the membranes of rat intestinal epithelial cells are inadequate to account for our observations on weak electrolyte transport. Larger PD's have been demonstrated at the membranes of the epithelial cells in the intestines of some other species [for example see 17]. It may be that membrane PD's play a role of major importance in the transport of weak electrolytes across the intestines of other species, and these considerations do not preclude the possibility that the membrane PD's exert some influence on weak electrolyte transport in rat intestine. But it is clear that, if the transport of weak electrolytes in rat intestine is to be described in terms of a three-compartment system, the driving force represented by the membrane PD's is inadequate to account for the observed asymmetries

in weak electrolyte movements, and an additional or alternative driving force must be considered. For this reason we will temporarily disregard the possible effects of PD's at the barriers in the evaluation of the alternative forms of the three-compartment system discussed below, and we will assume for these purposes $R_I = R'_I = (P_I^i/P_I^{n_i})$, and $R_{II} = R'_{II} = (P_{II}^i/P_{II}^{n_i})$.

In most experiments the ratio of observed fluxes (J_{MS}/J_{SM}) was greater than unity in the case of weak acids, and less than unity in the case of weak bases. Eq. 1 suggests two possible combinations of parameters that lead to flux ratios similar to those observed in the experiments. One of these can be represented as $\text{pH}_x < \text{pH}_M$ and $(P_I^i/P_I^{n_i}) > (P_{II}^i/P_{II}^{n_i})$, and the other version is a mirror image of this which can be represented as $\text{pH}_x > \text{pH}_M$ and $(P_I^i/P_I^{n_i}) < (P_{II}^i/P_{II}^{n_i})$. In both systems the driving force for weak electrolyte transport is related to the difference between the pH values of the intermediate compartment and the bulk phases, and the vectorial characteristics of transport are determined by the relative permeability properties of the two barriers. The low pH version of this system ($\text{pH}_x < \text{pH}_M$) is similar to that previously proposed to account for the transport of weak acids in rat intestine [6, 11], but the high pH version ($\text{pH}_x > \text{pH}_M$) does not appear to have been given consideration in previous work. In fact the results of the studies of interactions between weak electrolytes are readily explicable in terms of the high pH version, and the low pH version provides an incomplete explanation of these observations.

The studies of the effects of weak electrolytes on the fluxes of galactose and urea are pertinent to the interpretation of the interactions between weak electrolytes. It is well established that the transport of galactose is dependent upon the energy metabolism of the intestinal epithelial cells [21]. Thus the finding that the fluxes of galactose were not altered in the presence of weak electrolytes indicates that the weak electrolytes did not interfere with the metabolism of the epithelial cells, and that the interactions between weak electrolytes cannot be ascribed to alterations in the level of energy metabolism. The fluxes of galactose also reflect the permeability properties of the intestinal wall, and the lack of change in galactose fluxes when weak electrolytes were added suggests that the weak electrolytes did not alter the permeability properties of the tissue. The latter suggestion is substantiated by the studies of the effects of weak electrolytes on the fluxes of urea. Urea has been shown to be passively transported in the intestine, and both diffusion through lipoid barriers, and movements through aqueous channels are believed to contribute significantly to the total transport process [13]. The finding that the fluxes of urea were not changed by the addition of weak electrolytes again suggests that the permeability properties of the system were not altered in the conditions used.

Eq. 1 shows that the parameters of transport of the versions of the three-

compartment system under consideration are the relative permeabilities of the barriers, and the pH of the intermediate compartment. Since the experiments with galactose and urea provided no evidence to support the suggestion that the weak electrolytes produced changes in the permeability properties of the tissue, an explanation of the results of the interaction studies should be sought in terms of alterations in the pH of the intermediate compartment.

The process of net transport of weak electrolyte from one bulk phase to the other of the three-compartment system involves net dissociation, or association reactions within the intermediate compartment. These reactions result in the net formation or removal of hydrogen ions, and may change the

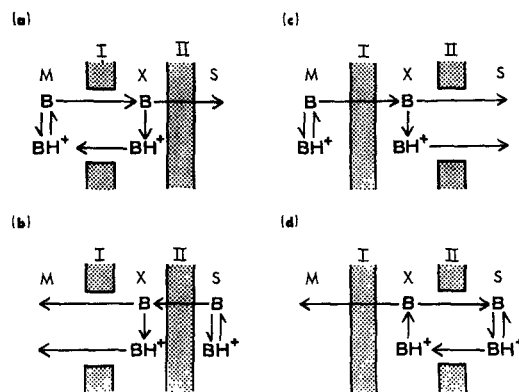


FIGURE 4. Influence of an effector weak base on the pH of the intermediate compartment of the two versions of the three-compartment system for weak electrolyte transport. The arrows show the net movements that occur when a weak base is added either to the mucosal fluid, or to the serosal fluid, and allow predictions to be made concerning changes in the pH of this compartment in these conditions. For further discussion see text.

pH of the intermediate compartment. It will be recalled that the studies of interactions between weak electrolytes involved the addition of an effector weak electrolyte either to the mucosal fluid, or to the serosal fluid, and Figs. 3 and 4 provide a basis for the description of the changes in the pH of the intermediate compartments of the two versions of the system under consideration, which may be expected to be associated with these conditions. For example, addition of an effector weak electrolyte to the bulk phase adjacent to the barrier that is relatively impermeable to the ionized form (Figs. 3 b, 3 c, 4 b, and 4 c) establishes a gradient for net transmural movement through the intermediate compartment. Since the barrier separating the bulk phase containing the effector and the intermediate compartment is relatively impermeable to the ionized form, the major form of the effector

compound entering the intermediate compartment is the nonionized form. Part of this nonionized effector will become ionized within the intermediate compartment and alter the value of pH_x . An effector weak acid added to the serosal bulk phase of the low pH version of the system (Fig. 3 *b*), or to the mucosal bulk phase of the high pH version (Fig. 3 *c*), will be expected to decrease pH_x , and an effector weak base in these situations (Figs 4 *b* and 4 *c*) will be expected to increase pH_x . When an effector weak electrolyte is present in the bulk phase adjacent to the ion permeable barrier (Figs. 3 *c*, 3 *d*, 4 *a*, and 4 *d*) both ionized and nonionized forms may move across the barrier, and the net movements that occur are dependent upon the steady-state distributions of the effector between the bulk phase to which it is added, and the intermediate compartment. A basis for describing these steady-state distributions is given in the Appendix (Eq. A9) and shows that the distribution reflects the pH difference between the two compartments. In the case of a weak acid the concentration of the nonionized form is greater, and that of the anion is less in the compartment of lower pH. For a weak base the concentration of the ionized form is greater, and that of the nonionized form is less in the compartment of lower pH. An effector weak acid present in the mucosal bulk phase of the low pH version of the model (Fig. 3 *a*) is distributed so that net movement of anion occurs from the bulk phase into the intermediate compartment, and nonionized acid moves out of the intermediate compartment. The net result of these movements is the removal of hydrogen ions from the intermediate compartment, and pH_x is increased. When an effector weak acid is added to the serosal bulk phase of the high pH system (Fig. 3 *d*) the distribution is such that the effector enters the intermediate compartment in the nonionized form while anion moves back into the serosal bulk phase, and pH_x is decreased. Similar considerations show that addition of an effector weak base to the mucosal bulk phase of the low pH version (Fig. 4 *a*) will increase pH_x , and that addition of an effector weak base to the serosal bulk phase of the high pH version (Fig. 4 *d*) will decrease pH_x .

The effects of these changes in pH_x on the flux ratios of transported weak electrolytes can be evaluated with the aid of Eq. 1, and the results of these considerations are summarized in Table IX. It will be noted that the two versions of the model system show different patterns of response to the presence of effector weak electrolytes. The influence of an effector weak acid on the transport functions of the low pH system are dependent upon the location of the effector, but the influence of an effector weak acid on the transport function of the high pH system are the same when the effector is added to the mucosal bulk phase, and when it is added to the serosal bulk phase. In the case of weak base effectors the situation is reversed. The influence of an effector weak base in the mucosal bulk phase of the low pH version of the model is qualitatively similar to the influence of an effector weak base present

TABLE IX

Influences of effector weak electrolytes on pH_x in two versions of the three-compartment model system for the transport of weak electrolytes, and on the flux ratios of weak electrolytes transported in these systems. For discussion of the mechanisms that may be involved in eliciting these changes see text. This table includes a summary of the results of the experiments shown in Tables V, VI, and VII for comparison.

Effector	Effect on pH_x	Effect on flux ratio of transported weak acid	Effect on flux ratio of transported weak base
Low pH system			
Mucosal weak acid	Increased	Decreased	Increased
Serosal weak acid	Decreased	Increased	Decreased
Mucosal weak base	Increased	Decreased	Increased
Serosal weak base	Increased	Decreased	Increased
High pH system			
Mucosal weak acid	Decreased	Decreased	Increased
Serosal weak acid	Decreased	Decreased	Increased
Mucosal weak base	Increased	Increased	Decreased
Serosal weak base	Decreased	Decreased	Increased
Experimental observations			
Mucosal weak acid		Decreased	Increased
Serosal weak acid		Decreased	Increased
Mucosal weak base		Increased	Decreased
Serosal weak base		Decreased	Increased

in the serosal bulk phase of this system. But the influence of an effector weak base on the transport function of the high pH version is dependent upon the location of the effector. Another interesting feature of these considerations is that the influence of an effector weak base is not always the opposite of the influence of an effector weak acid. The influence of an effector weak base in the mucosal bulk phase of the low pH version of the model, or in the serosal bulk phase of the high pH version, is qualitatively similar to the influence of an effector weak acid in these situations. This means that the studies with weak base effectors provide additional pattern-identifying information, rather than simply confirming studies with effector weak acids.

Table IX includes a summary of the results of the experiments on interactions between weak electrolytes described in Tables V, VI, and VII. It should be noted that the description "increased" or "decreased" is applied to changes in flux ratios that were calculated from the means of the observed fluxes. Since the M to S, and S to M fluxes were determined in separate experiments, we have not attempted to provide statistical analysis of the ratios. But in all cases the suggested changes in the flux ratios are associated with statistically significant, and reciprocally related changes in both M to S, and S to M fluxes. Thus the suggestion that a flux ratio was increased in some conditions is based on the observation that the M to S flux was significantly increased, and the S to M flux was significantly decreased. Conversely, a

suggested decreased flux ratio is associated with a significantly decreased M to S flux, and a significantly increased S to M flux.

Comparison of the results of the interaction experiments with the patterns suggested by consideration of the alternative versions of the model system, shows that the observed pattern of results is the same as that expected of the high pH version of the model. It is recognized that the two versions of the model system yield the same predictions in some situations, but the influences of a serosal effector weak acid, and a mucosal effector weak base are distinctive, and the studies of weak electrolyte transport in these conditions are entirely consistent with the predictions of the high pH version of the model system, and provide no evidence in favor of the low pH version.

The studies of interactions between weak electrolytes also are pertinent to the evaluation of some other possible mechanisms of weak electrolyte transport. For example, the demonstration of competitive inhibition and counter transport has been suggested to be characteristic of carrier mediated mechanisms [29]. The interactions observed between weak bases, and the influence of an effector weak acid in the mucosal fluid on the fluxes of a transported weak acid could be described in these terms. But the finding that an effector weak acid in the serosal fluid decreased the M to S flux, and increased the S to M flux of a transported weak acid, could not be explained in this way, and the proposal of carrier mediated processes for transport of weak acids and weak bases does not provide an explanation for the interactions between the two types of weak electrolyte. Interactions between weak acids and weak bases in intestinal transport have been described previously [24], and it has been suggested that these interactions reflect the formation of a lipophilic, diffusible complex between a weak acid and a weak base [24, 26]. Formation of such a complex would suggest that the flux of a transported compound down the gradient of effector concentration would be increased, and that the flux in the opposite direction may be decreased. The changes in the fluxes of a weak acid in the presence of a weak base, and the influence of a mucosal effector weak acid on the fluxes of a weak base, are consistent with these suggestions. But the changes in the fluxes of a weak base when an effector weak acid was present in the serosal fluid, could not be explained in this way, and the proposal of formation of a diffusible complex does not explain the influence of one weak acid on the fluxes of another weak acid, or the interactions between weak bases.

In summary, some of the observations described above are capable of explanation in terms of more than one mechanism, but the version of the three-compartment model that includes an intermediate compartment of high pH is the only mechanism considered that provides a single explanation of all of our observations, and we conclude that this system should be con-

sidered as a primary working hypothesis for the mechanism of intestinal transport of weak electrolytes.

It is of interest to consider briefly the possible locations of the intestinal analog of the intermediate compartment of the model, and evaluate some of the parameters of the system. The model suggests that the intermediate compartment is separated from the mucosal fluid by a barrier that is relatively impermeable to the ionized forms of the transported weak electrolytes, and from the serosal fluid by a barrier that is significantly permeable to the ionized forms. This arrangement of permeability properties is similar to the arrangement of permeabilities that has been suggested [14] to characterize the luminal and abluminal membranes of intestinal epithelial cells. But the proposal of the intracellular space as the analog of the intermediate compartment of the model would require that the intracellular pH was greater than that of the extracellular environment. In most tissues the intracellular pH is less than that of the extracellular space [27], and our own observations on the intracellular concentrations of weak electrolytes indicate that the intracellular pH of intestinal cells *in vitro* is less than that of the bathing medium (Jackson and Shiau, unpublished observations). In addition, the proposal of the abluminal membrane of the epithelial cells as an analog of the ionpermeable barrier of the model, suggests that the PD at this barrier should be an important determinant of weak electrolyte transport. The experiments with glucose and galactose were an attempt to demonstrate the influence of the PD at the abluminal cell membrane. These hexoses produce similar changes in the membrane PD [1, 15], but exhibited opposite effects on the transport of weak electrolytes. The mechanisms involved in the actions of the hexoses are not known, but these effects cannot be explained in terms of changes in the PD at the abluminal membrane.

The lateral intercellular space of the epithelium is another possibility for the analog of the intermediate compartment of the model. This space has been suggested to play the dual roles of equilibrating compartment and restriction to diffusion in fluid transport [9], and it is conceivable that it may fulfill similar functions in weak electrolyte transport. Movement of solute from the mucosal fluid into the lateral interspace may involve both cellular and extracellular routes [10]. For present purposes we will consider the cellular element as a single barrier whose permeability properties are determined largely by the cell membranes and which, in consequence, exhibits the ability to discriminate between the ionized and nonionized forms of weak electrolytes. It has been argued that the tight junctions located at the luminal ends of the lateral channels are the major resistance to solute movement via the extracellular route, and that these structures are the locus of the discrimination between cations and anions that is a characteristic of the extracellular channels [10]. The ability of these structures to discriminate between

ionized and nonionized species is not known, and it may be that the permeability properties of the tight junctions are appropriate to those of the first barrier in the three-compartment system. But even if the tight junctions do not discriminate between the ionized and nonionized forms of weak electrolytes, the transcellular route of access to the lateral interspace is mainly permeable to the nonionized form of a weak electrolyte and, since the area available for movement of the nonionized form via the transcellular route is substantially greater than the area available for solute movement through the tight junctions, it is reasonable to assume that the complex barrier represented by the parallel array of cellular elements and tight junctions will be characterized by a permeability ratio (P^i/P^{n^i}) less than unity. In contrast, it has been pointed out that the regions of the lateral interspaces below the tight junctions are unlikely to represent the locus of discriminatory permeability properties [10], and it seems reasonable to assume that the permeability ratio appropriate to the basal regions of the lateral interspace is unity. It has been shown that the pattern of acid base metabolism in rat jejunum includes secretion of hydrogen ions to the mucosal side and movement of metabolic anions, such as lactate and bicarbonate, into the serosal fluid [16, 30]. Since the metabolic anions are not accompanied by hydrogen ions their movements may be expected to increase the pH in the lateral interspace, and it can be seen that this space can be suggested to have permeability properties and a pH value that are qualitatively appropriate to the intermediate compartment of a three-compartment system that performs net transport of weak acids towards the serosal side and of weak bases in the opposite direction. Table X shows the results of calculations based on these considerations. Eq. 1 was solved for pH_x using values for flux ratios and transepithelial PD observed in our experiments, and using values for the permeability ratios as discussed above. Although it seems reasonable to suggest that the permeability ratio of the first barrier (the epithelial cell-tight junction complex) is less than unity, the absolute value of the ratio appropriate to this barrier is not known. Accordingly we have used a series of values of decreasing magnitude in the calculations, and the table shows that the values of pH_x obtained are markedly dependent upon the value of this permeability ratio. In the case of benzoic acid, use of values greater than 10^{-4} yields negative numbers which do not have realistic significance, but when values of ($P_1^i/P_1^{n^i}$) less than 10^{-4} are used in the calculations the values of pH_x required to account for the observed flux ratios are less than 0.5 unit greater than the pH of the bulk phases. In the case of benzylamine real values of pH_x were obtained in all of the calculations. These values of pH_x appeared to approach a limiting value, approximately 0.35 unit greater than the pH of the bulk phases as the value of the ratio ($P_1^i/P_1^{n^i}$) was decreased. Thus these calculations suggest that our observations on the transport of weak

TABLE X

Calculated values for the pH of the intermediate compartment of a three-compartment system. The values of pH_X given in this table were calculated from Eq. A8 in the Appendix using data derived from experiments described in the text. For the purposes of the calculation it has been assumed that $\text{pH}_M = 7.30$, $(P_{II}^i/P_{II}^{n_i}) = 10^0$, PD at barrier II = 0 mV, and PD at barrier I = 2.5 mV. For further details see Discussion.

$(P_{II}^i/P_{II}^{n_i})$	pH X calculated from experiments with benzoic acid ($\text{pK}_a = 4.2$, flux ratio = 2.5)	pH X calculated from experiments with benzylamine ($\text{pK}_a = 9.33$, flux ratio = 0.45)
10^{-1}	<i>nr</i>	8.55
10^{-2}	<i>nr</i>	7.82
10^{-3}	<i>nr</i>	7.68
10^{-4}	7.79	7.65
10^{-5}	7.71	7.65

nr indicates no real solution to equation

electrolytes could be explained by a relatively modest difference between the pH values of the fluid in the lateral intercellular channels and that of the bathing media. But the calculations also show that the permeability properties of the system are critical parameters, and a conclusion concerning the acceptability of the system as a description of the mechanism for intestinal transport of weak electrolytes will be dependent upon the evaluation of these parameters.

APPENDIX

Weak Electrolyte Fluxes in a Two-Compartment System

The system under consideration consists of a single barrier separating two large, well mixed compartments, M and S , containing aqueous solutions of a monovalent weak electrolyte. The weak electrolyte is partially dissociated and the aqueous phases contain both ionized and nonionized forms. It is assumed that the reversible dissociation reaction occurs very much more rapidly than the movements of the nonionized and ionized forms of the weak electrolyte through the barrier, and that the two forms of the weak electrolyte do not interact during their passage through the barrier. Thus the fluxes of the weak electrolyte through the barrier can be considered as the sum of the independent fluxes of the nonionized and ionized forms of the weak electrolyte, and the unidirectional fluxes of the weak electrolyte can be described by these relations:

$$J_{MS} = J_{MS}^{n_i} + J_{MS}^i, \quad \text{and} \quad J_{SM} = J_{SM}^{n_i} + J_{SM}^i, \quad (\text{A1})$$

where J_{MS} and J_{SM} are the unidirectional fluxes of the weak electrolyte from compartment M to compartment S , and from compartment S to compartment M , respectively; and the J^{n_i} and J^i terms refer to the component unidirectional fluxes of the nonionized and ionized forms of the weak electrolyte. The unidirectional fluxes of the nonionized

form of the weak electrolyte are determined by the concentration of the nonionized form in the compartment of origin of the flux, and the properties of the barrier. These unidirectional fluxes may be described by expressions of the form:

$$J_{MS}^{ni} = P^{ni}[\text{NI}_M], \quad \text{and} \quad J_{SM}^{ni} = P^{ni}[\text{NI}_S], \quad (\text{A2 } a)$$

and the unidirectional fluxes of the ionized form are given by [28]:

$$J_{MS}^i = \frac{P^i[\text{I}_M]\xi}{(1 - e^{-\xi})}, \quad \text{and} \quad J_{SM}^i = \frac{P^i[\text{I}_S]\xi e^{-\xi}}{(1 - e^{-\xi})}, \quad (\text{A2 } b)$$

where P^{ni} and P^i are the permeabilities of the barrier to the nonionized and ionized forms of the weak electrolyte, respectively; $[\text{NI}_M]$, $[\text{NI}_S]$, $[\text{I}_M]$, and $[\text{I}_S]$ are the concentrations of the nonionized and ionized forms in the compartments indicated by the subscripts; and $\xi = zF(\psi_M - \psi_S)/RT$, in which z is the valence of the ionized form, F is the Faraday, $(\psi_M - \psi_S)$ is the electrical potential difference across the barrier, and R and T have the usual thermodynamic significance.

Combination of Eqs. A1, A2 *a*, and A2 *b* allows an expression to be written that describes the ratio of weak electrolyte fluxes in the two-compartment system:

$$J_{MS}/J_{SM} = \frac{P^{ni}[\text{NI}_M] + \left(\frac{P^i[\text{I}_M]\xi}{(1 - e^{-\xi})} \right)}{P^{ni}[\text{NI}_S] + \left(\frac{P^i[\text{I}_S]\xi e^{-\xi}}{(1 - e^{-\xi})} \right)}. \quad (\text{A3})$$

The concentrations of the nonionized and ionized forms of a weak electrolyte are related by the Henderson-Hasselbalch equation, and for present purposes this relation may be written in the form:

$$[\text{I}] = [\text{NI}]10^\alpha, \quad (\text{A4 } a)$$

in which $\alpha = (\text{pH} - \text{pK}_a)$ in the case of a weak acid, and $\alpha = (\text{pK}_a - \text{pH})$ in the case of a weak base. The total concentration of weak electrolyte ($[C]$) is the sum of the concentrations of the nonionized and ionized forms. Thus if $[\text{NI}]$ is added to both sides of Eq. A4 *a*, on rearrangement we obtain:

$$[\text{NI}] = \frac{[C]}{1 + 10^\alpha}, \quad (\text{A4 } b)$$

and a similar expression can be written in terms of the concentration of the ionized form:

$$[\text{I}] = \frac{[C]}{1 + 10^{-\alpha}}. \quad (\text{A4 } c)$$

Expressions of the form of Eqs. A4 *b* and A4 *c* can be used to substitute for

the concentration terms in Eq. A3, and when these substitutions are made, on simplification and rearrangement we obtain:

$$J_{MS}/J_{SM} = \frac{\left(\frac{[C_M]}{1 + 10^{\alpha_M}}\right) \left[1 + \left(\frac{P^i}{P^{ni}}\right) \left(\frac{\xi 10^{\alpha_M}}{1 - e^{-\xi}}\right)\right]}{\left(\frac{[C_S]}{1 + 10^{\alpha_S}}\right) \left[1 + \left(\frac{P^i}{P^{ni}}\right) \left(\frac{\xi e^{-\xi} 10^{\alpha_S}}{1 - e^{-\xi}}\right)\right]} \quad (\text{A5})$$

Eq. A5 is a general expression describing the ratio of weak electrolyte fluxes in a two-compartment system. In the context of the experiments described in this paper, $[C_M] = [C_S]$ in all conditions, and in those experiments in which $\text{pH}_M = \text{pH}_S$, Eq. A5 can be simplified to give:

$$J_{MS}/J_{SM} = \frac{1 + \left(\frac{P^i}{P^{ni}}\right) \left(\frac{\xi 10^\alpha}{1 - e^{-\xi}}\right)}{1 + \left(\frac{P^i}{P^{ni}}\right) \left(\frac{\xi e^{-\xi} 10^\alpha}{1 - e^{-\xi}}\right)}, \quad (\text{A6})$$

in which α applies to both aqueous compartments. Also if it can be shown that the ratio (P^i/P^{ni}) is so small that the fluxes of the ionized form of the weak electrolyte are a small fraction of the total observed movement, Eq. A5 may be simplified to give:

$$J_{MS}/J_{SM} = \frac{1 + 10^{\alpha_S}}{1 + 10^{\alpha_M}}. \quad (\text{A7})$$

Weak Acid Fluxes in the Three-Compartment System

The system under consideration is shown in Fig. 2 in the text. Solomon [22] has shown that the transmural unidirectional fluxes in a system of three compartments in series can be expressed as functions of the unidirectional fluxes of solute at each of the barriers in the system, and that a flux ratio expression for a system of the type shown in Fig. 2 would take the form:

$$J_{MS}/J_{SM} = \frac{(J_{MX} J_{XS})}{(J_{XM} J_{SX})}.$$

In the case of a weak electrolyte this expression becomes:

$$J_{MS}/J_{SM} = \frac{(J_{MX}^{ni} + J_{MX}^i)(J_{XS}^{ni} + J_{XS}^i)}{(J_{XM}^{ni} + J_{XM}^i)(J_{SX}^{ni} + J_{SX}^i)}.$$

The unidirectional fluxes of the nonionized and ionized forms of the weak electrolyte can be substituted from expressions of the form of Eq. A2 *a* and A2 *b*, and concentration terms replaced using expressions similar to A4 *b* and A4 *c*. Then if it is assumed that $[C_M] = [C_S]$ and $\text{pH}_M = \text{pH}_S$, the resulting expression can be simplified and rearranged to give:

$$J_{MS}/J_{SM} = \frac{\left[1 + \left(\frac{P_I^i}{P_I^{ni}}\right)\left(\frac{\xi_I}{1 - e^{-\xi_I}}\right)10^{\alpha_M}\right]\left[1 + \left(\frac{P_{II}^i}{P_{II}^{ni}}\right)\left(\frac{\xi_{II}}{1 - e^{-\xi_{II}}}\right)10^{\alpha_X}\right]}{\left[1 + \left(\frac{P_I^i}{P_I^{ni}}\right)\left(\frac{\xi_I e^{-\xi_I}}{1 - e^{-\xi_I}}\right)10^{\alpha_X}\right]\left[1 + \left(\frac{P_{II}^i}{P_{II}^{ni}}\right)\left(\frac{\xi_{II} e^{-\xi_{II}}}{1 - e^{-\xi_{II}}}\right)10^{\alpha_M}\right]}, \quad (\text{A8})$$

in which the subscripts to the permeability and electrical potential terms denote the barrier to which the term is appropriate.

Steady-State Distributions of Weak Electrolytes in the Three-Compartment System

We require to describe the steady-state distribution of a weak electrolyte in those situations shown in Figs. 3 and 4 in which the weak electrolyte is added to one bulk phase only. We will assume that the concentration of the weak electrolyte is constant in the compartment to which it is added, and that the concentration in the trans bulk phase is negligible during the period of observation.

A steady state may be defined as a condition in which the concentration of weak electrolyte within the intermediate compartment does not change with time, and the sum of the movements of the nonionized and ionized forms of the weak electrolyte into the intermediate compartment is equal to the sum of the movements from the intermediate compartment into the bulk phases. For example, if a weak electrolyte is added to the mucosal bulk phase of the system shown in Fig. 2, the steady-state condition may be described by an expression of the form:

$$J_{MX}^{ni} + J_{MX}^i = J_{XM}^{ni} + J_{XM}^i + J_{XS}^{ni} + J_{XS}^i.$$

If it is assumed that no significant differences in electrical potential exist at the barriers in the system, the unidirectional fluxes in the above expression can be replaced by the products of concentrations and permeabilities, and the steady-state expression takes the form:

$$P_I^{ni}[NI_M] + P_I^i[I_M] = P_I^{ni}[NI_X] + P_I^i[I_X] + P_{II}^{ni}[NI_X] + P_{II}^i[I_X].$$

The concentration terms for the ionized forms of the weak electrolyte in this expression can be substituted using expressions of the form of Eq. A4 *a*, and when these substitutions are made, on simplification and rearrangement, we obtain:

$$[NI_X]/[NI_M] = \frac{1 + \left(\frac{P_I^i}{P_I^{ni}}\right)10^{\alpha_M}}{1 + \left(\frac{P_I^i}{P_I^{ni}}\right)10^{\alpha_X} + \left(\frac{P_{II}^{ni}}{P_I^{ni}}\right)\left[1 + \left(\frac{P_{II}^i}{P_{II}^{ni}}\right)10^{\alpha_X}\right]}. \quad (\text{A9})$$

We are particularly interested in the application of Eq. A9 to those situations in which the weak electrolyte is added to the bulk phase adjacent to the ion permeable barrier, as shown in Figs. 3 *a*, 3 *d*, 4 *a*, and 4 *d*. In these situations the ratio $(P_{II}^i/P_{II}^{n_i})$ is very small relative to $(P_I^i/P_I^{n_i})$. Then provided that the ratio $(P_{II}^{n_i}/P_I^{n_i})$ is not very large, Eq. A9 indicates that the concentration of the nonionized form of a weak acid will be greater in the compartment of lower pH, and that the concentration of the nonionized form of a weak base will be greater in the compartment of greater pH. Similar considerations can be applied to the distributions of the ionized forms of weak electrolytes, and show that the concentration of the ionized form of a weak acid is greater in the compartment of higher pH, and that the concentration of the ionized form of a weak base is greater in the compartment of smaller pH.

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