Intestinal Transport of Weak Electrolytes

Determinants of Influx at the Luminal Surface

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ABSTRACT The determinants of weak electrolyte influx into everted segments of rat small intestine have been studied. Preliminary experiments showed that the observed influxes could be described as unidirectional, diffusional fluxes of the nonionized compound uncomplicated by a parallel ionic component. It is shown that the determinants of weak electrolyte influx in this situation may be described in terms of the resistance of the unstirred layer to movement from the bulk phase to the cell surface, the degree of ionization of the weak electrolyte at the cell surface, and the cellular permeability to the nonionized weak electrolyte. Quantitative considerations indicated that the unstirred layer was totally rate-limiting in the cases of some poorly ionized, or highly permeant compounds, but the unstirred layer was not totally rate limiting for most of the compounds studied. Calculation of cellular permeabilities for the nonionized forms of weak electrolytes required assumptions to be made concerning the pH value in the surface fluid layer. A uniform set of permeability data including both weak acids and weak bases was obtained only when it was assumed that the pH in the surface fluid layer was equal to that in the bulk phase, and it was concluded that these studies do not support the concept of a microclimate of distinctive pH at the epithelial surface as a determinant of weak electrolyte transport.

In 1959 Hogben et al. (5) found that the steady-state distributions of weak electrolytes across the wall of the small intestine could not be described in terms of a two-compartment, pH partition model. A possible explanation of this discrepancy was based on the observation that the acid-base metabolism of the intestinal epithelium is asymmetric (16, 17). Hogben et al. (5) proposed that the secretion of hydrogen ions into the luminal fluid leads to the formation of a region of low pH at the epithelial surface. In this situation the distribution of a weak electrolyte between ionized and nonionized forms in the surface "microclimate" is different from the distribution in the luminal bulk phase, and Hogben et al. (5) suggested that the transmural distributions of weak electrolytes are determined by the concentrations of the permeant, nonionized species at the epithelial surface. The experiments described here were intended to test the suggestion that the intestinal transport of weak electrolytes is influenced by a region of distinctive pH at the luminal surface of the epithelium. Comparison

of the unidirectional influxes of several weak acids and weak bases across the brush border of rat small intestine in vitro indicates that the pH at the epithelial surface is not significantly different from that in the bulk phase. In addition, these experiments have provided information concerning the determinants of weak electrolyte permeation in the intestine.

METHODS

Influx Experiments

Male rats of the Wistar strain, weighing 180–200 g, were allowed food and water ad libitum to the time of experiment. The animals were anesthetized with Nembutal (70 mg/kg ip, Abbott Laboratories, N. Chicago, Ill.), and the abdomen was opened by a midline incision. The entire small intestine, from the distal end of the ligament of Treitz to the ileocecal junction, was rinsed out *in situ* with 0.9% NaCl, and manually separated from the mesentery. From each small intestine segments approximately 10 cm in length were cut from the distal end (ileum) and the mid-region (jejunum). The segments were everted onto glass rods (4 mm in diameter), tied in place with ligatures at each end, and immersed in a bath of oxygenated incubation saline at 37°C for 20 min. The rods bearing the tissue were then transferred individually to an influx chamber consisting of a jacketed glass cylinder approximately 20 mm in diameter and 150 mm long. The fluid in the influx chamber was vigorously stirred by means of a stream of gas bubbles entering through a scintered glass disc (porosity 40–60 μm) at the base of the chamber, and the rate of gas flow was maintained at 500–600 ml/min during the influx period. The temperature of the saline in the influx chamber was maintained at 37°C by perfusion of the water jacket from a thermostatically controlled reservoir. The saline in the influx chamber contained a weak electrolyte together with a ^{14}C-labeled tracer and 1 mM mannitol together with a ^{3}H-labeled tracer. The tissue was usually held in the influx chamber for a period of 1 min and was then removed, blotted on moist filter papers, and divided into three equal segments. Each segment was weighed, and one segment from each group of three was dried in an oven overnight at 90°C. The dry weight/wet weight ratio of this segment was used to calculate the dry weights of the other two segments in its group which were extracted overnight for estimation of tissue radioactivity. The extraction procedure varied with the nature of the weak electrolyte. In the case of weak acids each piece of tissue was extracted in 2 ml of barium hydroxide (4.5% wt/vol) and was neutralized with an equal volume of 5% zinc sulphate. Weak bases were extracted into 2 ml of 7% copper sulphate which was neutralized with 2 ml of 10% sodium tungstate. Tests showed that these procedures yielded recoveries of the ^{14}C and ^{3}H tracers used in our experiments that did not differ significantly from 100%. The ^{14}C and ^{3}H contents of the neutral aqueous extracts of the tissues and diluted samples of the saline in the influx chamber were estimated in a liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif., model LS-230) using a proprietary cocktail (Yorktown Research, Inc., S. Hackensack, N. J., formula TT-21). Quenching was monitored by the channels ratio method using an external standard, and all values were converted to disintegrations per minute before calculation of results. The influx of a weak electrolyte (J) was calculated according to the formula

$$J = \frac{\text{Total tissue } ^{14}\text{C} - ^{14}\text{C in } ^{3}\text{H mannitol "space"}}{^{14}\text{C specific activity in saline}}.$$

The saline used in the pre-incubation and in the influx chamber was of the following ionic composition in meq/liter: Na^+, 147; K^+, 2.5; Ca^{2+}, 3; Mg^{2+}, 2; Cl^-, 125; SO_4^{2-}, 2; phosphate, 2.5; HEPES, 25. In addition, 10 mM glucose was routinely added to the saline

which was equilibrated with 100% O_2 before and during the course of the experiment. In most experiments the pH values of the preincubation saline and the saline in the influx chamber were both adjusted to 7.40 (±0.02), but in a few experiments other values were used for the pH value of the saline in the influx chamber.

In some experiments the unstirred layer thickness of the preparation was determined using the method described by Diamond (3). In these experiments the end of a saturated KCl/agar bridge was tied inside the intestinal segment at the time of its eversion, and a similar bridge was placed into the incubation saline. The two bridges were connected to matched calomel half cells, the output of which was fed to a millivoltmeter and pen recorder (Keithley Instruments, Inc., Cleveland, Ohio, Models 160 and 370, respectively). To determine unstirred layer thickness a quantity of solute, sufficient to give a concentration of 50 mM in the incubation bath, was dissolved in a small volume of saline and was rapidly added to the bath while the transmural electrical potential difference was recorded. The unstirred layer thickness (d) was calculated from the formula:

$$d = (Dt/0.38)^{0.5},$$

where D is the coefficient of diffusion for the solute used to induce the potential, and t is the half time of the change in potential.

Partition Studies

Partion in two-phase aqueous/organic solvent (heptane or chloroform) systems was determined for all of the weak electrolytes used in the influx experiments. 1 ml of an aqueous solution of a weak electrolyte, containing between 0.1 and 5 μCi of ^{14}C tracer, was mechanically shaken with 3 ml of an organic solvent for 1 h at room temperature in glass stoppered tubes. The tubes were centrifuged (15 min at 1500 g), the phases were separated, and aliquots of each phase were assayed in a liquid scintillation spectrometer. The aqueous phases were counted in the TT-21 cocktail described above, and the organic extracts were counted in a cocktail consisting of 4 g/liter of 2,5-diphenyloxazole and 0.05 g/liter of *p*-bis-[2-(5-phenyloxazoyl)] benzene in toluene. All counts were converted to disintegrations per minute before concentration ratios were calculated. In many cases the aqueous phase used in the partition studies was identical with the incubation saline used in the flux experiments, but for a few poorly lipid-soluble weak electrolytes, such as acetate or 2-amino-propane, the aqueous phase consisted either of 1 N sulfuric acid or 1 N sodium hydroxide. These strongly acidic or basic solutions were used to drive the weak electrolyte into its non-ionized form and increase the concentration in the organic phase.

The tracers used in the experiments were obtained from commercial sources. All of the weak electrolyte tracers were found to contain small quantities of contaminants and purification procedures are described in the text.

RESULTS

Partition of Weak Electrolytes in Two-Phase Systems

Preliminary studies showed that, in the conditions of our experiments, the partition of weak electrolytes between a well-buffered aqueous phase and chloroform or heptane achieved a constant value within 15 min of mechanical shaking, was not markedly influenced by temperature in the range of 5–20°C, and was independent of weak electrolyte concentration in the aqueous phase in the range 0.01–5 mM, of ionic strength in the aqueous phase in the range 0.05–9 μ, and of the chemical composition of the buffer in the aqueous phase.

The partition of weak electrolytes was markedly influenced by variations in

the pH value of the aqueous phase. Some representative data are plotted in Fig. 1 according to the format suggested by the considerations given in the Appendix. The solid points and lines included in the figure show the relation between the partition ratio (r) and the function $1/(1 + 10^{\alpha})$ using tracers obtained from the manufacturers without additional purification. The log-log plot of r against $1/(1 + 10^{\alpha})$ was linear over much of the range, but partition tended to become

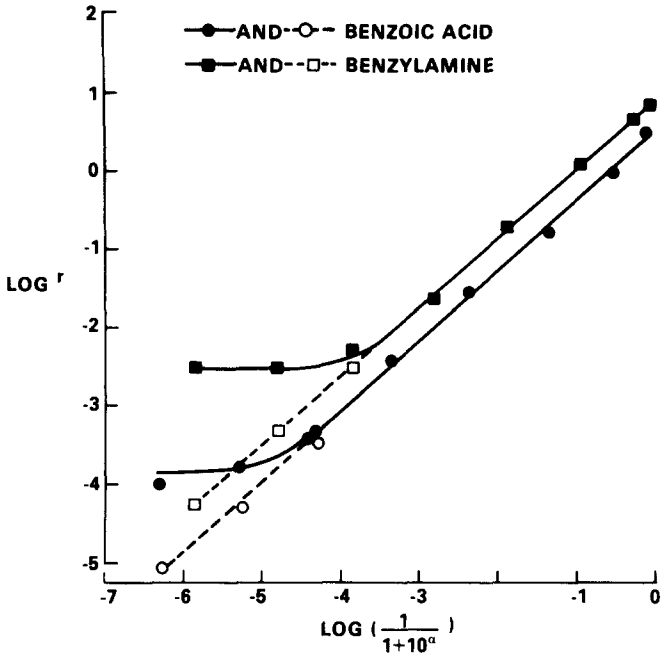

FIGURE 1. Relation between degree of ionization and partition of weak electrolytes in chloroform:aqueous system. 1 ml of a well-buffered 1 mM solution of a weak electrolyte containing a ^{14}C-labeled tracer was shaken with 3 ml of chloroform at room temperature for 1 h. Partition values (r) were calculated as the ratio of the concentration in the organic and aqueous phases. The figure shows the data obtained when the pH of the aqueous phase was varied in the range 3.5–10.5, and values of r have been plotted against the function $1/(1 + 10^{\alpha})$ as suggested by the considerations outlined in the Appendix. In the case of a weak acid $\alpha = (pH\text{-}pK_a)$, but for a weak base $\alpha = (pK_a\text{-}pH)$. Thus, the degree of ionization decreases from left to right in the figure. Solid symbols and lines represent data obtained using tracers purchased from commercial sources without further purification. Open symbols and broken lines represent data obtained using tracers purified by repetitive extraction with heptane.

independent of the degree of ionization at high degrees of ionization (left side of Fig. 1). To explain these deviations, the possibility was considered that the tracers contained small quantities of contaminants, the partition of which did not vary with pH, and to test this suggestion the tracers were dissolved in 1 N sulfuric acid or 1 N NaOH so that they existed in solution almost entirely in the ionized form. These highly ionized solutions were extracted several times with

heptane. It was found that the substantial quantities of radioactivity appeared in the heptane layer during the first two or three heptane extractions, but a stable low level was achieved after four or five extractions. When the purified tracer from the repetitively extracted aqueous phase was used to examine the influence of aqueous phase pH on partition, the partition ratio was found to be linearly related with the function $1/(1 + 10^{\alpha})$ over the whole range of pH values studied, and the data obtained with purified tracers is represented by the open symbols and dashed lines shown in Fig. 1. Essentially similar results were obtained with the other weak electrolyte tracers used in our experiments. These observations may be interpreted by proposing that the partition of weak electrolytes is directly proportional to the concentration of the nonionized form in the aqueous phase, and that the tracers used in our experiments contained small quantities of contaminants which are not weak electrolytes and the partition of which does not vary with aqueous pH. The concentrations of these contaminants were usually so small that their influence was only observed when the pH values were such that the concentrations of the nonionized forms of the weak electrolytes were less than 1% of the total concentration of weak electrolyte in solution. However, it should be noted that the pK_a values of most of the weak electrolytes included in our experiments are such that the influence of the contaminants would be significant at the pH of the incubation saline used in the influx experiments (7.4), and it can be calculated that the contaminants may increase the apparent concentrations of the nonionized forms of weak electrolytes at physiological pH values by factors in the range 2–20-fold. For this reason all tracers were routinely purified by serial extraction with heptane from aqueous solutions in which the weak electrolytes were highly ionized until the counts appearing in the organic phase fell to a low, constant value. Usually between five and seven extractions were necessary to give a tracer the partition of which varied with pH so that a log-log plot of r against $1/(1 + 10^{\alpha})$ gave a straight line, and all subsequent experiments were conducted with tracers purified by extraction to meet this criterion. It should be noted that the considerations outlined in the Appendix indicate that the straight line log-log plot of r against $1/(1 + 10^{\alpha})$ means that the ionized forms of the weak electrolytes did not contribute significantly to the observed partitions. Accordingly, the partitions of the weak electrolytes have been characterized as k^{ni} values calculated from Eq. A1 in the Appendix.

Fig. 2 is a plot of $\log k^{ni}_{CHCl_3}$ against $\log k^{ni}_{Hept}$ for all of the weak electrolytes included in our experiments, and shows that the two variables were well correlated. The parameters of the calculated regression line for the weak acids were not significantly different than those of the weak bases. In general, many of the k^{ni} values were close to, or less than unity, and the values determined with the nonpolar solvent, heptane, were usually more than one order of magnitude smaller than the values determined with the more polar solvent, chloroform. These observations indicate that the nonionized forms of most of the weak electrolytes included in these experiments should be considered to be polar, hydrophilic species. However, the range of values covers several orders of magnitude, and a significant number of compounds were included with k^{ni} values greater than unity. It is of interest to note that the k^{ni} values of weak

bases were usually greater than those of acids of comparable molecular form. Thus, the k^{ni}_{Hept} value for decylamine is more than one order of magnitude greater than that of decanoic acid, and the ratio of k^{ni}_{Hept} values for 2-aminopropane and propionic acid are also greater than 20. Similarly, k^{ni}_{Hept} for benzylamine is greater than that of benzoic acid, although the difference in this case is less than one order of magnitude.

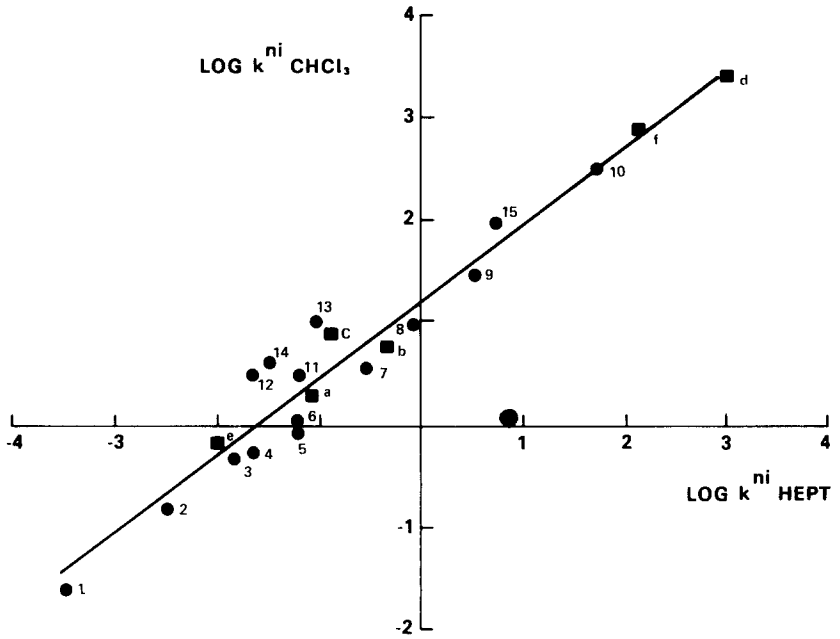

FIGURE 2. Relations between partitions in chloroform:aqueous and heptane: aqueous systems for weak electrolytes. Well-buffered 1 mM solutions of weak electrolytes with ^{14}C-labeled tracers were extracted with either chloroform or heptane as described in Methods. Partition values (k^{ni}) were expressed as the ratio of the concentration of weak electrolyte in organic phase and the concentration of its nonionized form in the aqueous phase. Each point shown in the figure is the average of three separate determinations. The numbers or letters to the right of each point identify individual compounds according to the key given in Table III. The line drawn through the points in the figure is a regression line calculated for all of the data. The parameters of the lines calculated separately for weak acids and weak bases were as follows:

$$\text{weak acids } \log k^{ni}_{CHCl_3} = 0.76(\pm 0.07)\log k^{ni}_{Hept} + 1.24(\pm 0.11)$$

$$\text{weak bases } \log k^{ni}_{CHCl_3} = 0.72(\pm 0.05)\log k^{ni}_{Hept} + 1.22(\pm 0.09)$$

In that mannitol was used as a marker in the influx experiments described below, an attempt was made to determine partition values for this compound. The concentrations of mannitol which appeared in the organic phases in these experiments were always so small that accurate evaluation was not possible. From the data obtained, it can be calculated that the chloroform/water concentration ratio for mannitol is less than 10^{-6} and, by analogy with the weak

electrolyte data, it is probable that the heptane/water ratio for mannitol is substantially less than the chloroform/water value.

Weak Electrolyte Influx into Intestinal Epithelium

Fig. 3 shows the results of a series of experiments in which was examined the effect of time of exposure in the influx chamber on the uptake of several weak electrolytes into everted rat jejunum in vitro. The figure shows that the uptakes were linearly related with time for at least 2 min and, in most cases, for up to 5 min of incubation. It was concluded that the uptakes observed during the 1-min period of exposure used in most of our experiments represented a unidirectional flux that was not complicated by a significant backflux.

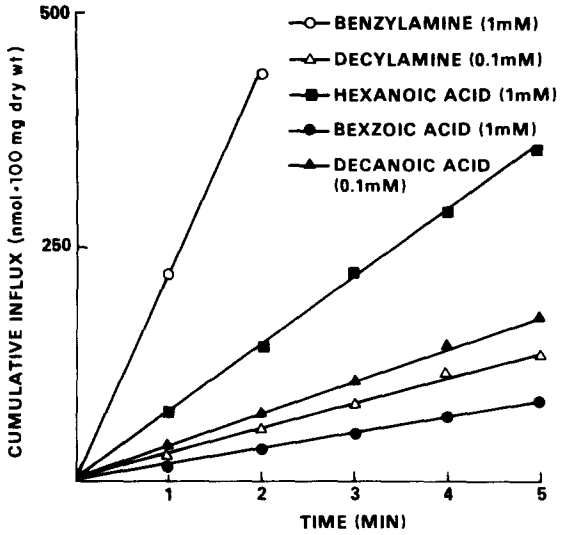

FIGURE 3. Effect of time of exposure on influx of weak electrolytes. Everted segments of rat jejunum were preincubated for 20 min in a buffered saline solution equilibrated with 100% O_2 at 37°C. The segments were then transferred to a bath of the same saline containing a weak electrolyte for the time indicated on the horizontal axis, and the uptake of the weak electrolyte into the tissue was determined. In most cases the concentration of the weak electrolyte was 1 mM, but for decanoic acid and decylamine, 0.1 mM solutions were used. Results are means of five determinations.

Fig. 4 shows the results of experiments on the effects of concentration on the influxes of several representative weak electrolytes. In all cases the relation between influx and concentration could be described by a straight line which passed through the origin, suggesting that the influxes of these weak electrolytes were not restricted by saturable, rate-limiting processes in the conditions of these experiments. Table I shows that the influx of one weak electrolyte was not altered in the presence of a 10-fold greater concentraton of a second weak electrolyte, indicating that the determinants of the influx of one weak electrolyte were independent of those regulating the influxes of analogous compounds.

Table II includes the results of a series of experiments on the effects of pH

on the influx of some representative weak electrolytes. Preliminary experiments using the nonelectrolyte, heptanol, suggested that the permeability of the tissue was increased at pH values below 7.5, but the influx of heptanol was constant in the range of pH values, 7.5–8.5, and studies on the influx of weak electrolytes were confined to this range. The influx of octanoic acid decreased, and that of c-hexylamine increased as the pH of the incubation saline was increased from 7.4 to 8.4, suggesting that the influxes of the weak electrolytes may be determined by the concentrations of their nonionized forms. To test this possibility the ratios of influx and nonionized weak electrolyte concentration in the incubation saline were calculated. The data included in the table shows that

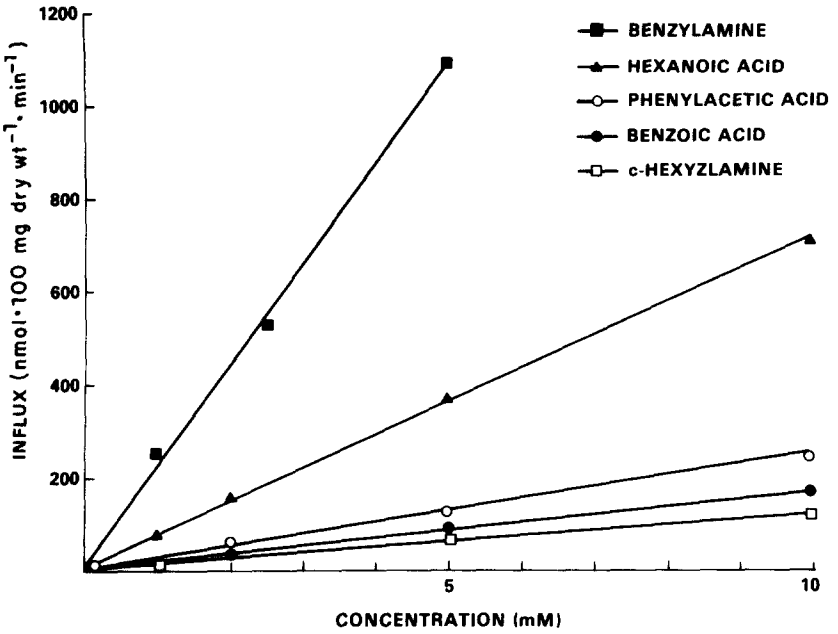

FIGURE 4. Effect of concentration on influx of weak electrolytes. Protocol for experiments similar to that described in Fig. 3, but in these experiments a 1-min influx period was used in all cases, and the concentrations of the weak electrolytes were changed from one experiment to another. Results are means of five determinations.

these ratios did not vary significantly from one pH value to another indicating that the influxes of the weak electrolytes were directly proportional to the concentrations of their nonionized forms.

Table III gives the influxes in jejunum and ileum for the 15 weak acids and six weak bases included in this study. In the case of jejunum no influx of acetate was observed in the conditions of these experiments, but a significant uptake of propionate was observed, and the rate of influx increased progressively with chain length for the series of aliphatic monocarboxylic acids included in these experiments. A significant influx of acetate was observed in the studies of ileal tissue but, although the ileal influx of aliphatic acids increased with chain

TABLE I

INTERACTIONS BETWEEN WEAK ELECTROLYTES DURING INFLUX

Second weak electrolyte (10 mM)	Influx			
	Pentanoic acid	Benzoic acid	c-Hexylamine	Benzylamine
	nmol·100mg dry wt^{-1}·min^{-1}			
None	20±2	19±1	11±1	208±15
Pentanoic acid	–	21±1	9±1	218±21
Benzoic acid	22±1	–	9±2	221±13
c-Hexylamine	20±1	17±2	–	211±17
Benzylamine	18±2	19±1	12±2	–

Everted segments of rat jejunum were incubated according to the protocol described in Methods, and the influxes of weak electrolytes from 1 mM solutions were determined. In some experiments a second weak electrolyte was present in the incubation saline at a concentration of 10 mM. Results are means ±SE of five experiments.

TABLE II

EFFECT OF pH ON INFLUXES OF OCTANOIC ACID AND c-HEXYLAMINE

	pH		
	7.4	7.9	8.4
Octanoic acid			
Influx	145±12	60±8	15±2
Ratio of influx and nonionized concentration	47±5	61±10	49±8
c-Hexylamine			
Influx	12±3	41±6	152±17
Ratio of influx and nonionized concentration	22±6	24±3	28±2

Everted segments were incubated according to the protocol described in Methods, but in some experiments, the pH values of the pre-incubation and incubation salines were adjusted to 7.9 or 8.4. Ratios of influx and the concentration of the nonionized form of a weak electrolyte were calculated from the formula $J(1 + 10\alpha)/C$, where J is the observed influx, C is the concentration of the weak electrolyte, and α = pH-pK_a for the weak acid, but α = pK_a-pH in the case of the weak base. Results are means ±SE of five experiments.

length, the influx of decanoate observed in this tissue was substantially less than that seen in the jejunal studies. In both regions of the intestine the range of influx values for the aliphatic acids covered two orders of magnitude, and the values obtained with branched chain acids did not differ significantly from those of the corresponding straight chain isomers. The influxes of aromatic and heterocyclic acids, and of the weak bases were generally within the range of values seen in the studies of the aliphatic acids.

Physical Properties of the Influx Preparation

Table IV gives the results of a series of unstirred layer determinations using urea, mannitol, sodium chloride, or sodium propionate as the test solutes. In general the values obtained in studies on jejunum did not differ significantly from those seen in experiments on ileum, and the values did not vary systematically with the solute used. Accordingly, the data obtained in these experiments have been pooled, and the average of all of these determinations

TABLE III

INFLUXES OF WEAK ELECTROLYTES IN JEJUNUM ILEUM

Compound	pK_a	Influx	
		Jejunum	Ileum
		nmol·100mg dry wt^{-1}·min^{-1}	
Weak acids			
Acetic	4.75*	0±2(12)	3±1(7)
Propionic	4.87*	6±1(9)	9±1(9)
Butyric	4.81*	12±1(6)	11±1(8)
iso-Butyric	4.84*	10±1(8)	13±2(7)
Pentanoic	4.82*	22±1(9)	18±1(6)
iso-Pentanoic	4.77*	21±2(6)	20±1(8)
Hexanoic	4.88*	73±4(9)	29±3(6)
Heptanoic	4.89*	81±6(6)	50±3(8)
Octanoic	4.89*	147±11(8)	56±6(9)
Decanoic	4.90*	361±22(10)	158±12(6)
Benzoic	4.19*	17±3(6)	15±1(8)
Phenylacetic	4.28*	24±2(6)	15±2(7)
Diphenylacetic	3.94*	14±2(6)	7±1(7)
Phenobarbital	7.30‡	321±32(6)	317±28(6)
Hexobarbital	8.20‡	348±27(5)	327±33(5)
Weak bases			
2-Amino propane	10.63*	6±1(8)	4±1(9)
c-Hexylamine	10.66*	12±1(6)	6±1(8)
Benzylamine	9.33*	220±18(7)	155±14(6)
Decylamine	10.64*	264±21(6)	65±4(8)
Naloxone	6.93§	286±31(8)	263±22(8)
Methadone	8.25§	272±14(8)	259±31(8)

Steady-state unidirectional influxes of weak electrolytes were determined according to the protocol described in Methods. In most experiments the concentration of the weak electrolyte in the incubation saline was 1 mM, but for the poorly soluble compounds, decanoic acid and decylamine, 0.1 mM solutions were used. To facilitate comparison between the data obtained with the poorly soluble compounds with those of the other weak electrolytes, the observed influxes of decanoic acid and decylamine have been increased by a factor of 10. Results are means ±SE with the number of observations given in parentheses.

* pK_a value taken from Ref. 6.

‡ pK_a value taken from Bush (1).

§ pK_a value taken from Taylor (11).

was used in calculations involving unstirred layer thickness. This average value was 1.55×10^{-2} cm.

The flux data summarized in Table III were normalized by reference to the dry weight of the tissue. For some purposes it was found useful to relate tissue mass and the area of a planar surface covering the tissue. To facilitate this conversion six pieces of intestine were mounted on rods and incubated for 20 min, and the diameter of the preparation was estimated with calipers. A segment of known length was cut from the rod and dried overnight at 90°C. The cylindrical surface area was calculated from the observed diameter and length, and normalized by reference to the dry weight of the tissue. The conversion factor given by this procedure was 16.19 (±0.17) cm²/100 mg dry wt in the case of the jejunum, and 15.40 (±0.18) cm²/100 mg dry wt for ileal tissue. The difference between these two values was statistically significant ($P < 0.01$).

DISCUSSION

Model for Weak Electrolyte Influx

The main objective of the experiments described here was to examine the possibility that the intestinal transport of weak electrolytes is influenced by a region of distinctive pH at the luminal surface of the epithelial cell layer. The rationale for these experiments may be described in terms of the model system shown in Fig. 5. The system consists of a series arrangement of three compartments: the mucosal fluid bulk phase; a layer of fluid at the epithelial surface which is poorly mixed with the mucosal fluid bulk phase; and the cellular compartment. It is suggested that a weak electrolyte penetrates the cellular

TABLE IV

UNSTIRRED LAYER THICKNESS ESTIMATES IN JEJUNUM AND ILEUM

Solute	Jejunum		Ileum	
	t	*d*	*t*	*d*
	s	*cm×10²*	*s*	*cm×10²*
Urea	7.0±0.6	1.78±0.16	4.2±0.5	1.38±0.18
Mannitol	7.2±0.5	1.33±0.10	8.5±0.6	1.44±0.10
Sodium chloride	4.3±0.3	1.51±0.11	5.3±0.3	1.68±0.11
Sodium propionate	8.5±0.8	1.76±0.16	6.2±0.5	1.50±0.11
Pooled data		1.60±0.12		1.50±0.10
				1.55±0.11

Unstirred layer thicknesses were estimated from the temporal characteristics of the change in transmural PD observed when a quantity of solute sufficient to give a final concentration of 50 mM was added to the incubation saline. The formula used to calculate the unstirred layer thickness was $d = (Dt/0.38)^{0.5}$, where d is the unstirred layer thickness in cm, D is the diffusion coefficient for the solute used to induce the change in PD, and t is the half time of the change in potential. Values of D for the solutes used in these experiments were taken from data given by Sallee and Dietschy (10). Results are means ±SE for six experiments.

compartment in the nonionized form, and that the influx of a weak electrolyte is proportional to the concentration of its nonionized form in the surface fluid layer. Weak acids and weak bases behave oppositely with respect to the influence of pH on the distribution between ionized and nonionized forms. If the pH in the surface fluid layers is lower than that in the bulk phase, the distribution of a weak acid will be shifted toward the nonionized form and the influx will be enhanced, but the distribution of a weak base will favor the ionized form and depress influx. This means that permeability values calculated from observed influxes and nonionized concentration in the bulk phase will be exaggerated in the case of a weak acid and underestimated in the case of a weak base. Conversely, if the pH in the surface fluid layer is greater than that of the bulk phase, the permeabilities of weak bases will be overestimated and those of weak acids will be depressed. In our experiments we have determined the influxes of weak acids and weak bases in two regions of the small intestine. The acid-base metabolism of the jejunum is characterized by luminal acidification, but in the ileal region the secretion of bicarbonate is associated with luminal alkalinization

(7, 17). In terms of the microclimate hypothesis for weak electrolyte transport, these patterns of acid-base metabolism suggest that the apparent permeability of the jejunum to weak acids may be exaggerated while that of weak bases is underestimated, and that the opposite pattern may be observed in the ileum. Thus, the objective of our experiments was to compare the apparent permeabilities of weak acids and weak bases in the jejunum and ileum, and to identify systematic differences.

Validation of Model

The interpretation of our data in terms of the model shown in Fig. 5 is dependent upon a number of assumptions. These assumptions and their justifications may be summarized as follows:

(*a*) The measured influxes represent uptake into the cellular compartment. Mannitol was used to correct for weak electrolyte present in extracellular compartments of the tissue. Mannitol was chosen for this purpose, in preference

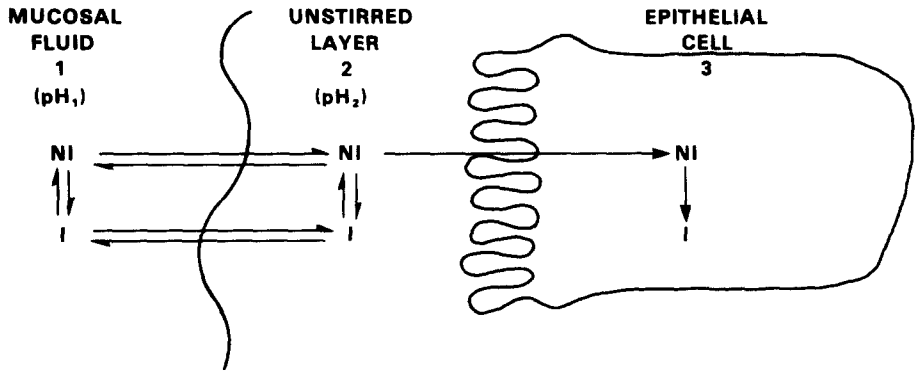

FIGURE 5. Model for weak electrolyte influx into everted segments of rat small intestine. For further description, see Discussion.

to large solutes, such as inulin or polyethylene glycol, because the diffusion coefficient for mannitol is intermediate in the range of values appropriate to the weak electrolytes included in this study. (Using the semiempirical relation of Wilke and Change [14], the diffusion coefficients for mannitol, acetic acid, and decanoic acid are 4.07×10^{-4}, 8.07×10^{-4}, and 3.51×10^{-4} $cm^2 \cdot min^{-1}$, respectively). In contrast, the diffusion coefficients of inulin or polyethylene glycol are below the range representative of the weak electrolytes included in our experiments, and use of these markers may have introduced a systematic error. (Using the relation $DM^{0.5} = 7.62 \times 10^{-3}$ $cm^2 \cdot min^{-1}$ (15) gives a value of $D = 1.21 \times 10^{-4}$ $cm^2 \cdot min^{-1}$ for a solute of mol wt 4000 daltons). It is possible that some of the weak electrolyte present in the tissue at the end of the incubation may have penetrated the lateral intercellular channels between the epithelial cells by way of the junctional complex. The geometry of these spaces suggested that this component of the extracellular weak electrolyte would be small relative to that present in the adherent mucosal fluid which presumably includes saline trapped between the villi. In addition, it was considered that penetration of

small solutes into the intercellular channels in the small intestine can be described mainly in terms of diffusion in a continuous aqueous environment, and that determination of the mannitol space would provide a correction for the small amount of weak electrolyte present in the intercellular channels, as well as the weak electrolyte present in the adherent mucosal fluid. The possibility that the use of mannitol as an extracellular marker may have introduced an error associated with penetration of mannitol into the cellular compartment was discounted by the observation that the chloroform/water partition ratio for mannitol was at least four orders of magnitude smaller than that of the least permeable weak electrolyte (acetate) included in our experiments. Inasmuch as permeation in this system varies directly with the chloroform/water partition ratio (see Fig. 6), it was concluded that mannitol provides a satisfactory estimate of extracellular space in studies of cellular penetration through lipophilic channels.

(*b*) The observed influxes were steady-state unidirectional fluxes and were diffusional in nature. The quantity of weak electrolyte penetrating the tissue increased linearly with time at least up to 2 min in the case of the more highly permeant species, and in most cases for as long as 5 min. If efflux of weak electrolyte from the tissue back into the mucosal fluid had complicated these experiments, it would have been expected that the slopes of the lines relating tissue solute uptake and time would decrease as the tissue solute level increased. In that the tissue solute increased as a straight line function of time, we conclude that the uptakes observed during the first minute of incubation can be described as unidirectional influxes. The studies of the effects of concentration on weak electrolyte influx, and of the effects of one weak electrolyte on the influx of a second compound, provided no evidence of a saturable rate-limiting step in the influx process and supported the description of weak electrolyte influx in terms of simple diffusion.

(*c*) The weak electrolytes penetrated the epithelial cells predominantly in the nonionized form. For many of the compounds included in our experiments, the concentration of the ionized form is several orders of magnitude greater than that of the nonionized form in solutions of physiological pH, and the possibility was considered that diffusion of the ionized form contributed significantly to the observed cellular influx. The experiments on the effects of pH on weak electrolyte influx were performed to test this possibility. The selection of the weak electrolytes studied in these experiments was influenced by a number of considerations. In particular, we sought to avoid the possibility that the observed influx was restricted by diffusion in the surface fluid layer rather than by permeation at the cell membrane. The relative significances of the unstirred layer and the brush border membrane as determinants of weak electrolyte influx are discussed in more detail in a later section. For the purposes of the experiments on the effects of pH, our choice of weak electrolytes was influenced by the findings of Sallee and Dietschy (10) that the unstirred layer was not rate-limiting for the influx of fatty acids of chain length less than 10 carbons in a system with slightly thicker unstirred layer characteristics than those found in our studies. Accordingly, we used compounds the fluxes of which were smaller than those of decanoic acid, i.e., the compounds were

chosen for permeabilities that were smaller than that of the compound suggested by Sallee and Dietschy (10) to be the limiting case for membrane limited influx. In addition, the selection of compounds for study in the pH experiments was influenced by the observation that the permeability of the tissue, as judged from heptanol influx, appeared to increase at pH values below 7.4. For this reason, only the effect of increasing pH was studied. Because the influx of a weak acid is decreased, and that of a weak base is increased at high pH, the choice of acids was limited to those compounds the fluxes of which could be estimated with precision when decreased by a factor of 10, and the choice of bases was limited to those compounds the fluxes of which at pH 8.4 did not exceed the flux of decanoic acid at pH 7.4 so that the weak base influx was unlikely to be unstirred layer-limited at high pH. The objective of the pH experiments was to test the possibility that the influx of weak electrolytes could be described simply in terms of a flux of the nonionized form and was not complicated by a parallel movement of the ionized form. Accordingly, the compounds selected for study in these experiments were chosen from among the more highly ionized compounds. Thus, the concentration of the ionized form was maximized and varied by less than 1% in the range of pH values used in these experiments. In this situation it would be expected that the ratio of the observed flux and the concentration of nonionized weak electrolyte would decrease as the nonionized concentration increased if movement of the ionized form contributed significantly to the observed flux of weak electrolyte. In fact, the observed fluxes of octanoic acid and c-hexylamine were directly proportional to the concentrations of their nonionized forms, and the ratios of the observed fluxes and the nonionized concentrations did not vary significantly over a 10-fold range of nonionized concentrations. We conclude that the observed influxes can be described in terms of a flux of the nonionized form uncomplicated by a parallel ionic component. It should be noted that the relations between influx of a weak electrolyte and the concentration of its nonionized form in the bulk phase do not necessarily mean that the nonionized concentrations at the epithelial surface were equal to those in the bulk phase, i.e., that the pH values at the epithelial surface and in the bulk phase were equal. The observed relations between influx and bulk phase pH would be explained if the conditions at the epithelial surface changed in parallel with those in the bulk phase. In this situation a change in the pH of the incubation saline which produced a 10-fold change in the nonionized concentration in the bulk phase would alter the pH at the epithelial surface so that the nonionized concentration in that region also changed by a factor of 10, but the two concentrations may be different. Thus, these experiments justify the assumption that weak electrolytes penetrate the epithelial cells predominantly in the nonionized form, but provide no indication concerning conditions in the fluid layer at the epithelial surface.

Determinants of Weak Electrolyte Influx

For the reasons summarized above we consider that the model system shown in Fig. 5 provides a satisfactory basis for discussion of the weak electrolyte influxes observed in our experiments. A quantitative approach to the description of weak electrolyte fluxes in this system is given in the Appendix. This shows that

the influx of a weak electrolyte (J_{23}^{ni}) is related to the properties of the system by an expression of the form:

$$J_{23}^{ni} = \frac{C_1}{\frac{1 + 10^{\alpha_2}}{P^{ni}} + \frac{d}{D}}, \quad (1)$$

where C_1 is the total concentration (ionized + nonionized) of the weak electrolyte in the bulk phase; $\alpha_2 = pH_2\text{-}pK_a$ in the case of a weak acid, but for a weak base $\alpha_2 = pK_a\text{-}pH_2$, and in these expressions pH_2 refers to the pH value of the fluid layer at the epithelial surface and pK_a is the logarithm of the reciprocal of the dissociation constant for the weak electrolyte; d is the thickness of the fluid layer at the epithelial surface; and D is the diffusion coefficient for the weak electrolyte in free solution.

The denominator of the right hand side of Eq. 1 may be regarded as a resistance term evaluating the factors that restrict the influx of weak electrolytes. One component of this term, d/D, represents the influence of the surface fluid layer to diffusion of weak electrolyte from the bulk phase to the epithelial surface, and corresponds to the unstirred layer restriction discussed in previous studies (10, 13, 15). The second component, $(1 + 10^{\alpha})/P^{ni}$, is concerned with the movement of weak electrolytes from the surface fluid layer into the epithelial cells. In this expression the term α reflects the distribution of the weak electrolyte between the ionized and nonionized forms. In general, when α is positive and greater than unity, the weak electrolyte exists mainly in the ionized form, and small or negative values of α indicate small degrees of ionization. Thus, the term, $(1 + 10^{\alpha})/P^{ni}$, reflects the interaction between the permeability of the epithelial cells to the nonionized form of the weak electrolyte and the degree of ionization of the compound at the epithelial surface as determinants of the movement of weak electrolyte through the epithelial cell membrane. The delineation of the components of the resistance term in this way is useful for the insight it provides into the factors that determine the relative significances of the unstirred layer and membrane terms in the total resistance to weak electrolyte influx. The values of the weak electrolyte influxes observed in our experiments covered a range of more than two orders of magnitude. Because all of these data referred to the same bulk phase concentration, this means that the variation in weak electrolyte influxes reflect variations in the resistance term. The thickness of the unstirred layer, d, is independent of the penetrating solute, and although the diffusion coefficient varies from one solute to another, the values of D appropriate to the solutes included in our experiments cover a range of less than threefold (see Table V). Thus, much of the variation in influx from one weak electrolyte to another may be ascribed to variations in the term $(1 + 10^{\alpha})/P^{ni}$. Examination of the form of Eq. 1 allows description of two extreme cases for the location of the principal resistance to weak electrolyte influx, and inasmuch as variations in the term $(1 + 10^{\alpha})/P^{ni}$ were the main source of variation in the observed fluxes, these extreme cases may be characterized by reference to the properties of weak electrolyte molecules included in this term:

(*a*) The first extreme case is described by the relation $(1 + 10^{\alpha})/P^{ni} \gg d/D$. In this situation the rate limiting step in the influx process is the movement of the weak electrolyte from the surface fluid layer into the epithelial cells. The characteristics of weak electrolyte molecules that may contribute to this situation include poorly permeant nonionized forms (low values of P^{ni}) and high degrees of ionization in the surface fluid layer (large, positive values of α).

(*b*) In the second extreme case the rate limiting step is the diffusion of weak electrolyte through the surface fluid layer. This situation is described by the relation $(1 + 10^{\alpha})/P^{ni} \ll d/D$ which indicates that the influx of weak electrolytes the nonionized forms of which are highly permeant (high values of P^{ni}), or that are poorly ionized in the surface fluid layer (small or negative values of α) may be unstirred layer-limited.

The relation between the significance of the unstirred layer as a component of the total resistance to weak electrolyte influx and the permeability of the epithelial cells was described by Sallee and Dietschy (10) in studies on the determinants of influx of aliphatic monocarboxylic acids into intestinal epithelium. These studies showed that permeability increased progressively with chain length, and that the unstirred fluid layer became totally rate-limiting in the case of fatty acids containing 12 or more carbon atoms. The considerations summarized above show that the significance of the unstirred layer may also vary with the acidic strength of weak electrolytes. Values of α will be larger for acids of low pK_a or for bases of high pK_a, than for weaker, less well dissociated compounds. Inasmuch as α occurs in Eq. 1 as an exponent, this means that the term $(1 + 10^{\alpha})/P^{ni}$ may change by several orders of magnitude with variations in acidic strength, and that the influxes of well ionized weak electrolytes are less likely to be restricted by diffusion in the surface fluid layer than are the influxes of poorly ionized compounds.

The aqueous diffusion term, d/D, included in Eq. 1 may be evaluated from the unstirred layer thickness determined by the osmotic method, and values of D calculated from the semi-empirical equation of Wilke and Chang (14). The values of d/D calculated in this way may be subject to two important errors: (*a*) Westergaard and Dietschy (13) have suggested that the unstirred layer thickness estimated by the osmotic method in small intestine in vitro may be greater than the thickness of the fluid layer that is functionally significant as a restriction to the uptake of solute into the epithelial cells in that solute transport processes apparently are not uniformly distributed throughout the epithelial layer and are associated mainly with the cells located on the upper part of the villus. (*b*) Values of the diffusion coefficient calculated from the equation of Wilke and Chang (14) have the units $cm^2 \cdot min^{-1}$, but the complexity of the epithelial surface in the intestine precludes the expression of fluxes in terms of epithelial surface area, and the observed fluxes were normalized by reference to the dry weight of the tissue. To allow examination of the role of the unstirred layer as a determinant of the observed fluxes, we have estimated a conversion factor which relates the surface area of a hypothetical plain cylinder that just covers the tissue in the influx chamber and the dry weight of the tissue. This conversion factor approximates the area of the outer surface of the unstirred layer covering a piece of tissue of 100 mg dry wt provided that it may be

assumed that the surface of the unstirred layer can be represented as a smooth cylinder. If the surface is not smooth but contains irregularities or undulations, the ratio of surface area and tissue dry weight will be underestimated.

A value for the unstirred layer resistance referred to tissue dry weight may be calculated as d/DS_c), where d is the unstirred layer thickness estimated by the osmotic method, D is the diffusion coefficient calculated from the Wilke-Chang equation, and S_c is the surface area of the cylinder covering tissue of 100 mg dry wt. Although the values of d and D used in these calculations are independent of the region of intestine, S_c varied significantly from one region to another, and the calculated unstirred layer resistances are different for jejunum and ileum. Because d may overestimate the thickness of the unstirred layer that is functionally significant as a resistance to weak electrolyte influx, and S_c is a minimal estimate for the conversion factor, the values of the unstirred layer resistance must be regarded as maximal estimates. The units of the unstirred layer resistance calculated in this way are $cm^{-3} \cdot min \cdot 100$ mg dry wt, and an empirical resistance in the same units may be calculated as the ratio of weak electrolyte concentration and the observed influxes (C/J). Table V compares the calculated unstirred layer resistance with the values derived from the experiments on weak electrolyte influxes. In most cases the empirical resistance was found to be substantially larger than the calculated resistance of the unstirred layer indicating that the movement of weak electrolyte from the surface fluid layer into the epithelial cells was the rate-limiting step in the influxes observed in most of our experiments. However, in some cases the calculated unstirred layer resistance was approximately equal to the empirical resistance, suggesting that the influxes observed in these experiments may have been limited by diffusion in the surface fluid layer. In particular, the empirical resistances for influx of decanoic acid in the jejunum and the influxes of phenobarbital, hexobarbital, methadone, and naloxone in both jejunum and ileum were very close to the values of the unstirred layer resistances calculated for these solutes. It was interesting to note that equivalence between the empirical resistance and the calculated unstirred layer resistance was observed with the most highly permeant aliphatic acid and the weakest electrolytes included in the experiments. Thus, the finding that the influxes of these compounds may have been limited by diffusion in the surface fluid layer is consistent with the suggestion made above that the unstirred layer term is expected to be most significant in the cases of highly permeant and poorly ionized compounds. Significantly, the empirical resistances were never substantially smaller than the calculated values of the unstirred layer resistance. It was pointed out above that the assumptions used in calculating the unstirred layer resistances made these values maximal estimates. If the assumptions had introduced significant errors into the calculations, it may have been expected that the calculated unstirred layer resistances would have been larger than the empirical resistances in the cases of solutes the influxes of which were unstirred layer-limited. Thus, the finding that the calculated unstirred layer resistances were comparable to, and never larger than, the empirical resistances for those weak electrolytes, which may be expected to be the most susceptible to unstirred layer limitation, suggests that the calculated unstirred layer resistances were not subject to large errors.

Weak Electrolyte Partition in Two Phase Systems

The main objective of our experiments was to compare the apparent permeabilities of weak acids and weak bases, and the organic solvent: aqueous partition values were evaluated to provide a basis of comparison of the permeability data for the two classes of weak electrolyte. The preliminary studies on the effects of pH on weak electrolyte partition showed that the equilibrium concentration of

TABLE V

COMPARISON OF CALCULATED RESISTANCE TO WEAK ELECTROLYTE INFLUX ASSOCIATED WITH THE UNSTIRRED LAYER AND EMPIRICAL RESISTANCE DETERMINED FROM DATA GIVEN IN TABLE III

		Resistances			
		Jejunum		Ileum	
Compound	D	Unstirred layer	Empirical	Unstirred layer	Empirical
	$cm^2 \cdot min^{-1} \times 10^4$	$cm^{-3} \cdot min \cdot 100$ mg dry wt			
Weak acids					
Acetic	7.40	1.29	–	1.36	3.33×10^2
Propionic	6.25	1.53	1.67×10^2	1.61	1.11×10^2
Butyric	5.48	1.75	8.33×10^1	1.84	0.09×10^1
iso-Butyric	5.48	1.75	1.00×10^2	1.84	7.69×10^1
Pentanoic	4.92	1.95	4.55×10^1	2.05	5.56×10^1
iso-Pentanoic	4.92	1.95	4.76×10^1	2.05	5.00×10^1
Hexanoic	4.49	2.13	1.37×10^1	2.24	3.45×10^1
Heptanoic	4.15	2.31	1.23×10^1	2.43	2.00×10^1
Octanoic	3.87	2.47	6.80×10^0	2.60	1.79×10^1
Decanoic	3.43	2.79	2.77×10^0	2.93	6.33×10^0
Benzoic	4.85	1.97	5.88×10^1	2.08	6.67×10^1
Phenylacetic	4.43	2.16	4.17×10^1	2.27	6.67×10^1
Diphenylacetic	3.41	2.81	7.14×10^1	2.95	1.43×10^2
Phenobarbital	3.41	2.81	3.11×10^0	2.95	3.15×10^0
Hexobarbital	3.35	2.86	2.87×10^0	3.00	3.06×10^0
Weak bases					
2-Amino propane	6.25	1.47	1.67×10^2	1.54	2.50×10^2
c-Hexylamine	4.91	1.95	8.33×10^1	2.05	1.67×10^2
Benzylamine	4.98	1.92	4.55×10^0	2.02	6.45×10^0
Decylamine	3.45	2.78	3.79×10^0	2.92	1.54×10^1
Naloxone	2.83	3.38	3.50×10^0	2.56	3.80×10^0
Methadone	2.56	3.74	3.68×10^0	3.93	3.86×10^0

The values of the calculated resistance of the unstirred layer were evaluated from the formula d/DS_c, where d is the unstirred layer thickness from Table V, D is the diffusion coefficient of the solute calculated from the equation of Wilke and Chang (14), and S_c is the area of a plain cylinder which just covers the tissue in the influx chamber. Empirical resistances were evaluated as C/J, where C is the concentration of weak electrolyte in the incubation saline, and J is the observed influx as given in Table III.

a weak electrolyte in the organic phase was directly proportional to the concentration of its nonionized species in the aqueous phase. For this reason, the partition properties of weak electrolytes in two phase systems have been characterized in terms of the k^{ni} values which represent the ratios of the equilibrium concentrations of weak electrolyte in the organic phase and the

nonionized concentration in the aqueous phase. These k^{ni} values are independent of the pH of the aqueous phase and are analogous to the partition coefficients of nonelectrolytes that have proved useful correlates of nonelectrolyte permeability (4, 12). The values of k^{ni} given here are in excellent agreement with data obtained in previous studies using the same organic solvents and where the degree of ionization in the aqueous phase was taken into account; for example, compare our $k^{ni}_{CHCl_3}$ values for aliphatic acids through C_6 with the data of Davis et al. (2). However, it should be noted that, in many previous studies of weak electrolyte partition, the degree of ionization in the aqueous phase was not considered (e.g., Wartiovaara and Collander [12]), and our data are not comparable with those given in such studies. The solvents used in the partition studies were chosen for their low miscibilities with water and represent a wide range of molecular polarities. The $k^{ni}_{CHCl_3}$ and k^{ni}_{Hept} values of weak electrolytes were found to be well correlated, and the parameters of the relation for the weak bases were very similar to those of the relation for the weak acids.

Weak Electrolyte Permeabilities

Previous work has provided extensive documentation for relations between partition of nonelectrolytes in two phase systems and the permeability of biological membranes to these compounds, and it has been suggested that these correlations indicate that partition and permeation are determined by the same set of intermolecular forces (4). By extrapolation of this concept, our observation that a single relation may be used to approximate the partitions of both weak acids and weak bases in two very different solvent systems means that it is a reasonable assumption that a single relation will approximate the correlation between partition in one of these solvent systems and intestinal permeability for the two classes of weak electrolytes; i.e., inasmuch as the relation between the partitions in the two solvent systems does not discriminate between weak acids and weak bases, it is probable that the two classes of weak electrolytes will not be distinguished in their relations between permeability and partition.

Values of P^{ni} can be calculated from Eq. 1 using the data given in Tables III and V when arbitrarily chosen values are assigned to pH_2. Because α is defined differently for weak acids and weak bases, the values of P^{ni} calculated from Eq. 1 will be distorted in opposite directions if the value assigned to pH_2 is different than the pH of the surface fluid layer in the conditions of our experiments. Thus, the pH of the surface fluid layer may be identified as the value of pH_2 that yields a consistent set of values of P^{ni} for both acids and bases, and from the rationale given above, the consistent set of values of P^{ni} will be indicated as that which exhibits a correlation with partition that does not discriminate between acids and bases. In Fig. 6, values of P^{ni} calculated from the jejunal influx data are plotted against the corresponding chloroform:aqueous partition data. Three values for pH_2 were tried in these calculations. The values used were 5.3 as suggested by the calculations of Hogben et al. (5), 6.0 as suggested by the microelectrode studies of Lucas et al. (9), and the pH of the incubation saline used in our experiments, 7.4. Fig. 6 shows that the permeabilities were well correlated with the chloroform:aqueous partition data, and the correlation coefficients were close to unity in all cases. The slopes of the lines describing the

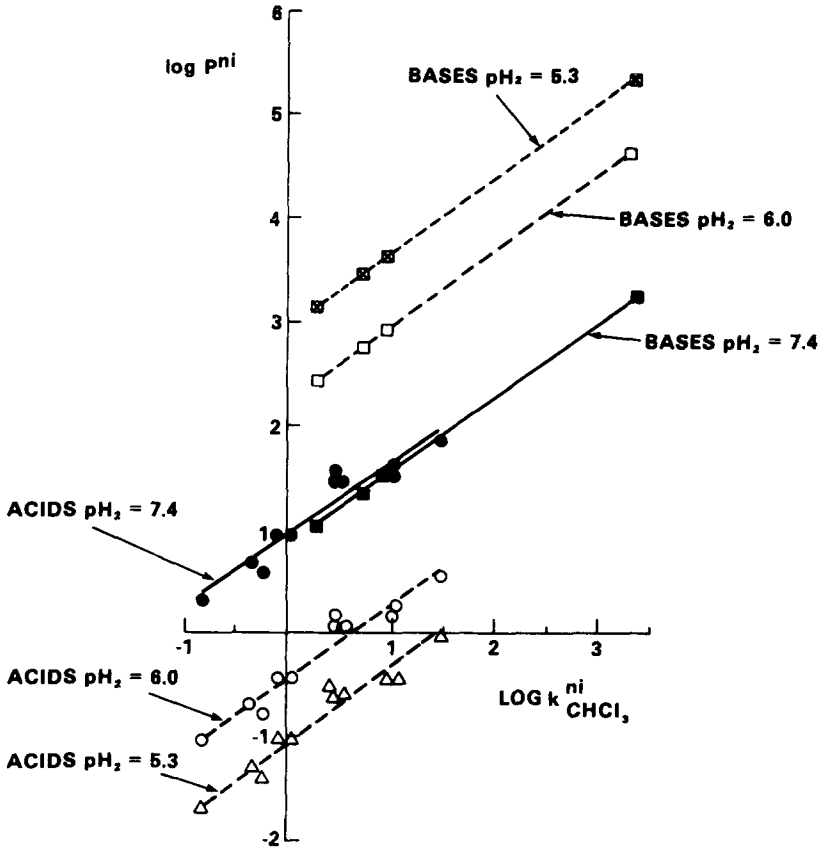

FIGURE 6. Relations between values of P^{ni} calculated from jejunal influx data and $k^{ni}_{CHCl_3}$. Values of P^{ni} were calculated using Eq. 1 in the text, and the data for jejunal influx and unstirred layer resistance given in Tables III and V. pH_2 was assigned values of 5.3, 6.0, or 7.4. The lines drawn in the figure are the calculated regression lines for the relation $\log P^{ni} = b(\log k^{ni}_{CHCl_3}) + a$. The parameters of the lines shown were as follows:

		b	a	Correlation coefficient
pH_2=5.3	Weak acids	0.69±0.06	−1.05±0.04	0.97
	Weak bases	0.72±0.01	2.95±0.02	1.00
pH_2=6.0	Weak acids	0.71±0.06	−0.43±0.04	0.97
	Weak bases	0.72±0.01	2.25±0.02	1.00
pH_2=7.4	Weak acids	0.71±0.07	0.94±0.05	0.97
	Weak bases	0.72±0.02	0.85±0.03	1.00

relations shown in the figure did not change with the value of pH_2 used in the calculations, and the slopes of the lines describing the weak acid data were not significantly different than those derived from the studies with weak bases.

However, the intercepts varied markedly with the value assigned to pH_2. The intercepts of the lines calculated for weak acids when the values 6.0 or 5.3 were assigned to pH_2 are significantly different from the lines calculated for the weak bases at these values of pH_2, but the intercepts of the weak acid and weak base lines at $pH_2 = 7.4$ were not significantly different. Thus, the relation between jejunal permeability and organic solvent:aqueous partition did not discriminate between acids and bases when the value assigned to pH_2 was equal to that of the incubation saline used in these experiments, and we conclude that the pH of the fluid at the jejunal epithelial surface is not significantly different from that of the luminal bulk phase in the conditions of our experiments. Essentially, the same conclusion derives from the studies on the ileal influx of weak electrolytes. The experiments with ileal tissue were included in this study because the pattern of acid-base metabolism in ileum is different from that of jejunum (7, 17), and it was considered possible that the two regions of intestine may exhibit different patterns of relations between weak acid and weak base permeabilities. However, the literature contains no suggestions for the pH of the surface fluid layer specifically related to ileal tissue. Thus, in making calculations of ileal permeabilities, we have used values of pH_2 one-half unit and one full unit greater than the pH of the incubation saline to take into account the possibility that the secretion of bicarbonate may be associated with the formation of a high pH microclimate at the epithelial surface, and the results of these calculations are included in Fig. 7 together with the data obtained when pH_2 was assigned a value equal to the pH of the incubation saline. As in the case of the jejunum, the ileal permeabilities were found to be well correlated with the chloroform: aqueous partition values, and the correlation coefficients were close to unity in all cases. It should be noted that the data on weak acid influx in the ileum covers a broader range than that of the jejunum in that a significant influx of acetate was observed in the ileum but not in the jejunum, and since the ileal permeability to decanoic acid was such that the ileal influx of this compound was not unstirred layer-limited, as it was in the studies of jejunum. As in the case of the jejunum, the slopes of the lines did not change with the value assigned to pH_2, and the slopes of the lines calculated for the weak bases were not significantly different from those calculated for the weak acids. Again, the intercepts varied with pH_2 and only when pH_2 was assigned a value equal to the pH of the incubation saline did the weak acid and weak base relations overlap. When pH_2 was assigned values one-half unit, or one full unit greater than the pH of the incubation saline, the corresponding weak acid and weak base relations were significantly separated on the P^{nl} axis. Thus, a consistent set of data covering both weak acid and weak base permeabilities was obtained only when it was assumed that the pH at the epithelial surface was equal to that in the bulk phase, and we conclude that the pH in the fluid layer at the ileal epithelial surface was not significantly different than that in the incubation saline.

In summary, the experiments described above indicate that the pH value that is a determinant of weak electrolyte influx into intestinal epithelial cells is equal to the pH in the mucosal fluid bulk phase, and our experiments have provided no support for the concept of a superficial region of distinctive pH different from that of the bulk phase as a significant determinant of weak electrolyte

influx. The proposal of the microclimate hypothesis was based on observations on asymmetries in weak electrolyte transport (5) and has received support from studies employing pH microelectrodes (9). It is of interest to consider the earlier observations supporting the microclimate hypothesis in the light of our present findings. Jackson et al. (8) have pointed out that the microclimate hypothesis proposed by Hogben et al. (5) is a special case of a general series three-compartment system in which the driving force for weak electrolyte transport is associated with a difference between the pH values of the intermediate and end

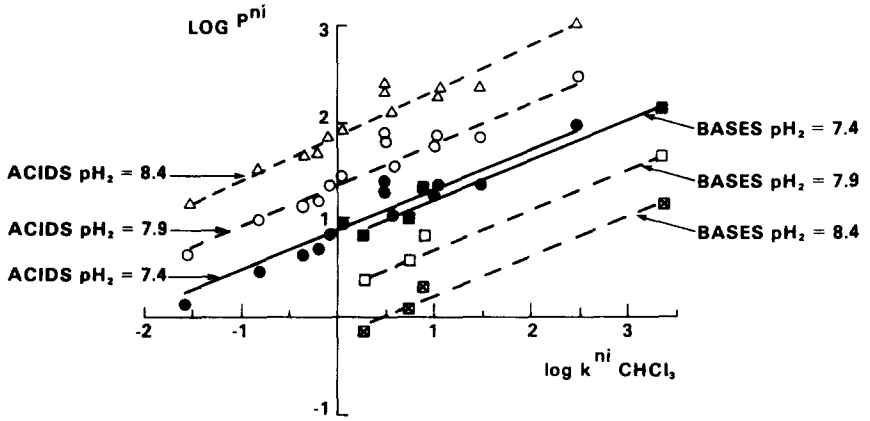

FIGURE 7. Relations between values of P^{ni} calculated from ileal influx data and $k^{ni}_{CHCl_3}$. Values of P^{ni} were calculated using Eq. 1 in the text and the data for ileal influx and unstirred layer resistance given in Tables III and V. pH_2 was assigned values of 7.4, 7.9, or 8.4. The lines drawn in the figure are the calculated regression lines for the relation $\log P^{ni} = b(\log k^{ni}_{CHCl_3}) + a$. The parameters of the lines shown were as follows:

		b	a	Correlation coefficient
pH_2=7.4	Weak acids	0.44+0.04	0.87±0.04	0.96
	Weak bases	0.41+0.05	0.79±0.09	0.99
pH_2=7.9	Weak acids	0.44±0.04	1.37±0.04	0.96
	Weak bases	0.40±0.05	0.30±0.09	0.99
pH_2=8.4	Weak acids	0.44±0.04	1.87±0.04	0.96
	Weak bases	0.41±0.06	−0.18±0.10	0.99

compartments, and that an alternative version in which the intermediate compartment is located in the subepithelial extracellular space also requires consideration. Studies on interactions between concurrently transported weak electrolytes (8) and on the relations between weak electrolyte transport and asymmetries in intestinal acid-base metabolism (7) favor the subepithelial compartment version over the superficial microclimate version. Thus, the finding that the pH at the epithelial surface was not different from the pH in the mucosal fluid bulk phase does not preclude the possibility that the asymmetries

in weak electrolyte transport are associated with asymmetries in intestinal acid-base metabolism. The present findings suggest that the hydrogen ions secreted to the luminal side of the jejunal epithelium do not accumulate at the epithelial surface and diffuse rapidly into the bulk phase, but this suggestion carries no implications for dispersal of anionic components of intestinal acid-base metabolism from the subepithelial extracellular space. It is readily conceivable that the diffusion of bicarbonate and lactate through the subepithelial layers may be restricted by the connective tissue layer so that the metabolic anions may accumulate and establish the compartment of high pH suggested by Jackson et al. (8). A region of low pH was demonstrated in the studies of Lucas et al. [9] as a pH-sensitive microelectrode was advanced toward jejunal epithelium, but this region could not be visualized by use of indicator dyes. Lucas et al. (9) concluded that the low pH region was not superficial and probably represented fluid trapped in the intervillus space. However, Westergaard and Dietschy (13) have shown that the uptake of solutes including fatty acids into intestinal epithelium occurs predominantly at the top of the villus and that the cells located on the lateral and basal regions of the villus play only a minor role in this respect. In this situation the cells exposed to the low pH environment described by Lucas et al. (9) are not the cells predominantly concerned with the uptake of solutes, and our finding that the pH value that is a determinant of weak electrolyte uptake is equal to the pH of the bulk phase is consistent with the finding of Lucas et al. (9) that the low pH microclimate could not be detected as a superficial region.

APPENDIX

Weak Electrolyte Partition in Organic Solvent:Aqueous Systems

The objective is to characterize the determinants of weak electrolyte partition in a system of two immiscible phases in contact and at equilibrium. Phase 1 is an aqueous solution the pH of which is maintained by a buffer system that is independent of the partitioned weak electrolyte. At equilibrium the concentration of weak electrolyte in this phase is C_1, and the distribution of the weak electrolyte between the ionized and nonionized forms is described by the Henderson-Hasselbalch equation in the form:

$$\frac{[I_1]}{[NI_1]} = 10^{\alpha_1},$$

where $[I_1]$ is the equilibrium concentration of ionized weak electrolyte in the aqueous phase; $[NI_1]$ is the corresponding concentration of nonionized weak electrolyte; and, $\alpha_1 = pH_1\text{-}pK_a$ in the case of a weak acid, but $\alpha_1 = pK_a\text{-}pH_1$, if the compound is a weak base. Phase 2 is an organic solvent. We will assume that both the ionized and nonionized forms of the weak electrolyte may enter the organic phase, and that the two forms of the weak electrolyte partition between the organic, and aqueous phases independently. Thus, the following relations are applicable to the equilibrium condition:

$$[NI_2] = k^{ni}[NI_1] \text{ and } [I_2] = k^{i}[I_1],$$

where $[NI_2]$ and $[I_2]$ are the concentrations of the nonionized and ionized forms,

respectively, of the weak electrolyte in the organic phase at equilibrium, and k^{ni} and k^{i} are the partition coefficients. The equilibrium concentration ratio of weak electrolyte, r, is the ratio of the sums of the concentrations of the ionized and nonionized forms in each of the phases, i.e.,

$$r = \frac{[NI_2] + [I_2]}{[NI_1] + [I_1]}.$$

In this expression the organic phase concentrations can be expressed in terms of the aqueous phase concentrations using the partition functions given above, and the aqueous concentration of the ionized form can be expressed in terms of the nonionized form by use of the Henderson-Hasselbalch equation. When these substitutions are made we obtain on simplification:

$$r = \frac{k^{ni} + k^{i}10^{\alpha_1}}{1 + 10^{\alpha_1}},$$

which indicates that a log-log plot of r against $1/(1 + 10^{\alpha_1})$ will be curvilinear with slope increasing as α_1 increases. If k^{i} is negligibly small this expression reduces to

$$r = \frac{k^{ni}}{1 + 10^{\alpha_1}}, \tag{A1}$$

which indicates that a log-log plot of r against $1/(1 + 10^{\alpha_1})$ will give a straight line. Thus, the finding that a log-log plot of r against $1/(1 + 10^{\alpha_1})$ yields a straight line indicates that the partition of the weak electrolyte can be described simply in terms of the distribution of the nonionized form, and k^{ni} may be calculated as $r(1 + 10^{\alpha_1})$.

Weak Electrolyte Permeation

The objective is to characterize the determinants of weak electrolyte influx in the system illustrated in Fig. 5 in the text. This system may be described as an arrangement of three compartments in series: the well-mixed mucosal fluid bulk phase (compartment 1); a fluid layer at the epithelial surface which mixes poorly with the bulk phase (compartment 2); and, the cellular compartment (compartment 3). The mucosal fluid bulk phase contains a weak electrolyte, but the pH of this solution is maintained by a separate, well-poised buffer system, the concentration of which is substantially greater than that of the weak electrolyte under study so that the pH of the bulk phase is independent of the weak electrolyte. The buffer system may penetrate compartment 2 and regulate the pH there. However, the continuous secretion of products of acid-base metabolism from the epithelial cells may alter the distribution of the buffer between its associated and dissociated forms, so that the pH in compartment 2 may be different than that in the bulk phase. The value of pH_2 will be determined by the relative rates of exchange of buffer constituents between compartments 1 and 2, and of the secretion of products of epithelial acid-base metabolism. For present purposes we will assume that a steady state is established in which the pH of compartment 2 is constant in time, is independent of

the movements of the weak electrolyte under investigation, and may be different than the pH of the mucosal fluid bulk phase.

Both the nonionized and the ionized forms may contribute to the exchanges of the weak electrolyte between the bulk phase and compartment 2, but only the nonionized form may penetrate the cellular compartment. It is assumed that the conditions in the cellular compartment are such that the concentration of nonionized species in this compartment remain at a negligibly low level. This may be because the volume of the compartment is large, or because the pH is such that the weak electrolyte is converted to its ionized form and trapped. In either case, the movement of the weak electrolyte across the cellular membrane can be represented as a unidirectional flux of its nonionized form. It will be assumed that this flux is directly proportional to the concentration of nonionized weak electrolyte in compartment 2 ($[NI_2]$), and since $[NI_2]$ is related to the concentration of weak electrolyte in the surface fluid layer (C_2) by a form of the Henderson-Hasselbalch equation,

$$[NI_2] = \frac{C_2}{1 + 10^{\alpha_2}},$$

we may write for the influx:

$$J_{23}^{ni} = \frac{P^{ni} C_2}{1 + 10^{\alpha_2}}, \tag{A2}$$

where J_{23}^{ni} is the flux of weak electrolyte into the cellular compartment, and P^{ni} is the permeability of the cell membrane to the nonionized weak electrolyte. If it is assumed that pH_2 is constant in time, Eq. A2 indicates that the demonstration of a steady-state influx indicates that C_2 does not change during the period of observation. In this situation the sum of the fluxes of weak electrolyte into compartment 2 must be equal to the sum of the fluxes leaving the compartment, i.e.,

$$J_{12}^{ni} + J_{12}^{i} = J_{21}^{ni} + J_{21}^{i} + J_{23}^{ni}. \tag{A3}$$

The exchanges of weak electrolyte between compartments 1 and 2 are diffusional fluxes and, because the electrical potential difference between the unstirred layer and the bulk phase may be assumed to be negligible, each of these fluxes may be described by an expression of the following general form:

$$J_{mn}^{q} = \frac{D_q [Q_m]}{d}, \tag{A4}$$

where J_{mn}^{q} is the flux of component q from compartment m to compartment n; D_q is the diffusion coefficient for q in free solution; d is the distance through which the movement occurs; and $[Q_m]$ is the concentration of q in compartment m. The masses of the ionized and nonionized forms of weak electrolytes are very similar, and a common value of D may be applied to the fluxes of both forms. When substitutions of the forms given in Eqs. A2 and A4 are made for the fluxes included in Eq. A3, and keeping in mind that the sum of the concentrations of the nonionized and ionized forms in a compartment is the

concentration of the weak electrolyte in that compartment, on simplification and rearrangement we obtain:

$$C_2 = \frac{C_1}{1 + \dfrac{d\,P^{ni}}{D(1 + 10^{\alpha_2})}}.$$

This expression may be used to substitute for the concentration term in Eq. A2 to give:

$$J_{23}^{ni} = \frac{C_1}{\dfrac{1 + 10^{\alpha_2}}{P^{ni}} + \dfrac{d}{D}}. \tag{A5}$$

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