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Transport of Heterocyclic Acids Across Rat Small Intestine *in Vitro*

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Summary. A study has been made of the steady-state fluxes of barbituric acid, six of its substituted derivatives, and 5,5-dimethyloxazolidinedione (DMO) across the wall of rat jejunum *in vitro*. For each of the compounds tested the mucosal (*M*) to serosal (*S*) flux was significantly larger than the *S* to *M* flux. Both *M* to *S* and *S* to *M* fluxes increased linearly with concentration, and the transport of one acid was not influenced by the presence of a tenfold greater concentration of a second heterocyclic acid. The fluxes decreased as the pH of the incubation saline was increased, but neither the *M* to *S*, nor the *S* to *M* fluxes could be described in terms of simple nonionic diffusion. It was found that the relation between the flux ratios of the transported acids and their pK_a values could be described by an equation derived from consideration of the transport of a weak acid in a series three compartment system, and it has been concluded that the three compartment system provides a good working hypothesis for the mechanism of heterocyclic acid transport across rat jejunum. It was found that the best fit of the theoretical curve to the experimental data was obtained when the ratio of permeabilities to the ionized and nonionized forms of a weak acid at one of the barriers was assigned the value 5×10^{-1}. It is suggested that this value may be characteristic of a noncellular restriction to diffusion, such as a layer of connective tissue, and substantiates previous suggestions that the intermediate compartment of the intestinal three compartment system is a component of the sub-epithelial extracellular space.

Previous studies [4, 5, 6] have suggested that the mechanism for transport of some simple, monofunctional weak electrolytes across rat jejunum *in vitro* may be described in terms of a series three compartment system, in which the driving force for transport is represented by a difference between the pH values of the intermediate, and end compartments, and where the vectorial characteristics of transport are determined by differences in the permeability properties of the two barriers separating the adjacent compartments. Investigation of a theoretical model of this system [3] suggests that it may be broadly applicable to

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the transport of a wide variety of weak electrolytes. However, certain characteristics, in particular the permeability properties of the barriers, appear to be critical determinants of the ability of the system to effect net transport of weak electrolytes. Since it is to be anticipated that these critical properties will vary significantly from one weak electrolyte to another, the suggestion that the transport of a particular compound, or group of compounds, may be described in terms of the general model requires empirical justification. In the study described here we have investigated the transport of several barbiturates and other heterocyclic acids. The results of these studies suggest that the model may be applicable to a pharmacologically significant group of compounds, and provide quantitative information concerning the properties of the transport system.

Materials and Methods

Male albino rats of the Wistar strain, weighing 150–175 g, were allowed food and water *ad libitum* to the time of experiments. Under Nembutal anesthesia (70 mg/kg i.p.) the entire small intestine from the distal end of the ligament of Treitz to the ileo-cecal junction was washed free of contents *in situ* with 0.9% saline. After washing, the intestine was manually stripped free of the mesentery and rinsed by immersion in chilled saline. Two segments, approximetely 5 cm in length, were cut from the mid-region of the isolated small intestine, opened along their antimesenteric borders, and mounted in Lucite flux chambers as described previously [6]. Each hemichamber was perfused *via* a gas lift system with 20 ml of incubation saline maintained at 37 °C. The transported weak acid was added in equal concentrations to both mucosal and serosal reservoirs, 1 μCi of a ^{14}C-labelled tracer was added to one reservoir at the start of the experiment, and fluxes calculated from the rate of appearance of tracer in the other reservoir. Preliminary experiments showed that a steady-state was established 10–15 min after addition of tracer to the system, and that the steady-state was maintained for at least 120 min. A flux was usually calculated from the mean increase in tracer in the *trans* reservoir during five or six consecutive 10-min intervals, starting 20 min after addition of tracer to the *cis* reservoir. It should be noted that the *n* values given in the text indicate the number of pieces of tissue studied, and do not reflect the number of observations made on each piece of tissue. In some experiments the transmural electrical potential difference was determined. In these experiments matched calomel half cells were connected to the reservoirs by means of saturated KCl/2% agar bridges. The voltage difference between the half cells was evaluated with a digital multimeter (Model 160, Keithley Instruments, Cleveland, Ohio).

The incubation saline was of the following composition in mEquiv/liter: Na^+, 147; K^+, 2.5; Ca^{2+}, 3; Mg^{2+}, 2; Cl^-, 125; SO_4^{2-}, 2; phosphate, 2.5; HEPES, 25. This saline was equilibrated with 100% O_2 before and during the course of the experiment. In most experiments the pH of the saline was 7.50 ($\pm$0.02), but in some experiments the pH was adjusted to 6.5 or 8.5. In most experiments the weak acids were dissolved directly in the incubation saline, but poorly soluble compounds, such as hexobarbital, were taken into solution in a small volume of ethanol which was then dispersed in the incubation saline. Preliminary experiments showed that the small volumes of ethanol used in this procedure did not influence the transport of readily soluble weak acids, or of galactose,

suggesting that the experiments with poorly soluble acids were not influenced by the presence of ethanol.

^{14}C-labelled tracers for barbituric acid, barbital, phenobarbital, pentobarbital, amobarbital, and secobarbital were obtained from California Bionuclear Corp., and ^{14}C-labelled tracers for hexobarbital and 5,5-dimethyloxazolidinedione (DMO), were obtained from New England Nuclear.

Results

Steady-State Transmural Fluxes

Table 1 shows the results of a series of experiments in which were estimated the steady-state transmural fluxes in 1 mM solutions of the eight heterocyclic acids included in this study. For each of the compounds tested the *M* to *S* flux was significantly greater than the corresponding *S* to *M* flux, but the magnitudes of the mean fluxes, and the ratios of the opposed transmural fluxes varied signficantly from one compound to another. The *M* to *S*, and *S* to *M* fluxes did not vary in parallel. For example, the *M* to *S* fluxes of DMO and hexobarbital were similar to each other, and were substantially larger than the *M* to *S* fluxes of most of the other compounds included in these experiments. However, although the *S* to *M* flux of hexobarbital was large, the corresponding flux of DMO was smaller than the *S* to *M* fluxes of most of the other compounds included in the study. Thus the ratio of the transmural fluxes did not vary regularly with the magnitudes of the transmural fluxes.

The initial pH of the salines used in these experiments was adjusted to 7.50 in all cases, and estimates of the pH values of the mucosal

Table 1. Transintestinal fluxes of heterocyclic acids

Compound	pK_a	J_{ms}	J_{sm}	n	J_{ms}/J_{sm}
1. Barbituric acid	4.0[a]	41±3	14±1	11	2.93
2. DMO	6.1[b]	143±9	38±2	8	3.76
3. Phenobarbital	7.3[a]	111±7	44±3	8	2.50
4. Barbital	7.8[a]	78±3	52±7	9	1.50
5. Amobarbital	7.8[a]	113±4	78±3	8	1.45
6. Secobarbital	7.9[a]	120±5	74±3	8	1.62
7. Pentobarbital	8.0[a]	105±4	69±3	8	1.52
8. Hexobarbital	8.2[a]	146±14	116±3	9	1.26

Units of fluxes are nmoles/cm^{2}hr. J_{ms} is the flux from the mucosal, to the serosal fluid, and J_{sm} is the flux in the opposite direction. pK_a values from [1][a] or [8][b]. Results are means ±SEM for the number of experiments given under *n*.

and serosal fluids taken at the end of the experiments showed that the pH did not change significantly during the course of the incubation. In the steady-state conditions in which the transmural fluxes were estimated, the transmural electrical potential difference (PD) averaged 0.8 mV mucosal side negative, and this value appeared to be independent of the presence of the heterocyclic acids.

Effects of Concentration

Table 2 shows the effects of concentration on the fluxes of barbituric acid and two of its derivatives. In all cases the fluxes increased continuously with concentration over the 100-fold range included in these experiments. For any particular flux, the ratio of the flux and the concentration did not vary significantly from one concentration to another, indicating that the relations between the fluxes and concentration could be represented by straight lines. In addition, the calculated intercepts of these lines were not significantly different from zero in all cases. Thus, these studies provided no evidence to suggest the existence of a rate-limiting component in the transport of the heterocyclic acids.

Effects of Analogues

Table 3 shows that the fluxes of one barbituric acid derivative were not influenced by the presence of a structural analogue. In these experiments the concentration of the second acid added to the system was ten times greater than that of the acid the fluxes of which were estimated, and the compounds used included two acids that are well transported (barbituric acid and phenobarbital: flux ratios > 2) and one acid the transport of which is less asymmetric (barbital: flux ratio < 1.5). Thus, the lack of interactions observed in these experiments suggests that the heterocyclic acids do not share a common rate-limiting step in their transport.

Effects of pH

Table 4 shows that the fluxes of barbituric acid and several of its derivatives varied with the pH of the incubation saline. Both the M to S, and S to M fluxes of the weak acids decreased as pH increased,

Table 2. Effects of concentration on fluxes of heterocyclic acids

Transported acid		Concentration (mM)					Calculated intercept
		0.05	0.1	0.5	1.0	5.0	
Barbituric	J_{ms}	1.8 ±0.1	4.1 ±0.3	20 ±1.6	38 ±4	217 ±18	−1.8±1.6
	J_{ms}/C	0.036±0.002	0.041±0.003	0.040±0.003	0.038±0.004	0.043±0.004	
	J_{sm}	0.7 ±0.1	1.4 ±0.2	8 ±1	13 ±2	87 ±11	−1.3±1.2
	J_{sm}/C	0.014±0.002	0.014±0.002	0.016±0.002	0.013±0.002	0.017±0.002	
Phenobarbital	J_{ms}	5.7 ±0.3	10.1 ±0.8	48 ±6	111 ±9	507 ±42	1.6±1.3
	J_{ms}/C	0.114±0.006	0.101±0.008	0.096±0.012	0.111±0.009	0.101±0.008	
	J_{sm}	2.3 ±0.2	3.8 ±0.3	21 ±1	44 ±2	227 ±16	−0.8±0.6
	J_{sm}/C	0.046±0.004	0.038±0.003	0.042±0.002	0.044±0.002	0.045±0.003	
Barbital	J_{ms}	3.8 ±0.3	8.1 ±0.7	43 ±2	78 ±6	407 ±37	−0.3±0.6
	J_{ms}/C	0.076±0.006	0.081±0.007	0.086±0.004	0.078±0.006	0.081±0.007	
	J_{sm}	2.7 ±0.3	5.1 ±0.3	23 ±1.3	56 ±4	263 ±21	−0.1±0.7
	J_{sm}/C	0.054±0.006	0.051±0.003	0.046±0.003	0.056±0.0004	0.053±0.004	

Results are means ±SEM for five experiments. Units of fluxes are nmoles/cm^{2}hr, and units of ratios of fluxes and concentrations are cm hr^{-1}. The intercepts given in the table were calculated by a linear regression analysis.

Table 3. Effects of structural analogues on transport of heterocyclic acids

Addition	Transported acid (0.1 mM)					
	Barbituric acid		Phenobarbital		Barbital	
	J_{ms}	J_{sm}	J_{ms}	J_{sm}	J_{ms}	J_{sm}
None	4.3±0.2	1.4±0.1	11 ±1	4.1±0.2	8.1±0.8	5.7±0.3
1 mM barbituric acid	–	–	9.7±1	4.0±0.1	8.9±1	6.2±0.2
1 mM phenobarbital	4.0±0.3	1.5±0.1	–	–	8.8±0.6	5.7±0.4
1 mM barbital	4.3±0.2	1.3±0.2	11 ±0.8	3.8±0.2	–	–

Table 4. Effects of pH on barbiturate fluxes

Transported acid		pH 6.5		pH 7.5		pH 8.5	
		J_{ms}	J_{sm}	J_{ms}	J_{sm}	J_{ms}	J_{sm}
Barbituric	Observed flux	6.2±0.4	2.8±0.3	4.4±0.4	1.5±0.2	3.2±0.2	0.8±0.1
	Calculated flux	44	15			0.4	0.15
Phenobarbital	Observed flux	16 ±2	6.2±0.5	11 ±1.3	4.3±0.3	9.5±0.8	2.9±0.2
	Calculated flux	24	10			1.6	0.7
Barbital	Observed flux	10 ±1.2	6.5±0.4	8.3±0.6	5.6±0.3	6.8±0.6	4.4±0.3
	Calculated flux	12	8			2.1	1.4
Pentobarbital	Observed flux	14 ±1	7 ±0.6	9.8±0.7	6.1±0.4	8.9±0.7	5.7±0.6
	Calculated flux	13	7.8			3.1	1.9
Hexobarbital	Observed flux	16 ±2	12 ±1.3	15 ±1.1	13 ±0.8	12 ±0.8	9.1±0.8
	Calculated flux	18	15			6	5.2

Results are means ±SEM for five experiments in each case. Values for calculated fluxes at pH 6.5 or 8.5 were computed from observed fluxes at pH 7.5 using the formula given in the text.

suggesting that the changes in the fluxes may reflect variations in the concentrations of the nonionized forms of the acids. The possibility that the effects of pH could be ascribed solely to changes in concentration of nonionized acid was tested in the following way: Jackson *et al.* [6] have shown that the unidirectional, diffusive flux (J) of the nonionized form of a weak acid through a simple barrier is related to the concentration of the acid (C) and the pH in the compartment of origin of the flux by an expression of the form:

$$J = P^{ni}C/[1 + 10^{(pH\text{-}pK_a)}]$$

where P^{ni} is the permeability of the barrier to the nonionized form of the acid. Thus if the nonionized form of an acid is the only permeant species, and assuming that P^{ni} is independent of pH, the fluxes J_1 and J_2 at the pH values pH_1 and pH_2 are related by the expression

$$J_2 = J_1[1 + 10^{(pH_1 - pK_a)}]/[1 + 10^{(pH_2 - pK_a)}]$$

Table 4 includes values of fluxes at pH 6.5 and 8.5 that were calculated from the observed fluxes at pH 7.5 using this expression, and these may be compared with the experimentally determined fluxes at pH 6.5 and 8.5. In most cases agreement between calculated and observed fluxes was poor. The observed fluxes at pH 8.5 were always substantially larger than the calculated values and, in the cases of barbituric acid and phenobarbital at pH 6.5, the observed fluxes were significantly smaller than the calculated values. The calculated and observed fluxes at pH 6.5 were similar in the cases of barbital, pentobarbital, and hexobarbital. However, the pK_a values of the latter group of acids are all greater than 7.5, and both the calculated and the observed fluxes of these acids at pH 6.5 were similar in magnitude to the fluxes observed at pH 7.5. Thus the similarities between the magnitudes of the calculated and observed fluxes of barbital, pentobarbital, and hexobarbital at pH 6.5 may be a reflection of the insensitivity of these fluxes to pH at values below 7.5, and does not provide unequivocal support of the nonionic diffusion model on which the calculations were based. In all of the situations in which the calculated fluxes were significantly different than the observed fluxes at pH 7.5, the observed fluxes at pH 6.5 or 8.5 were significantly different than the corresponding calculated values. Thus the nonionic diffusion model does not provide a generally satisfactory explanation of the effects of pH on the transmural fluxes of heterocyclic acids, and it may be concluded that neither the *M* to *S*, not the *S* to *M* fluxes of these compounds can be described in terms of diffusion of undissociated acid through a simple barrier.

Discussion

Fig. 1 shows a model of the mechanism for weak electrolyte transport proposed in a study of the movements of several monocarboxylic acids and primary amines across the wall of rat jejunum *in vitro* [6]. The system consists of three aqueous compartments arranged in series and separated by the barriers *I* and *II*. The forces influencing the movements

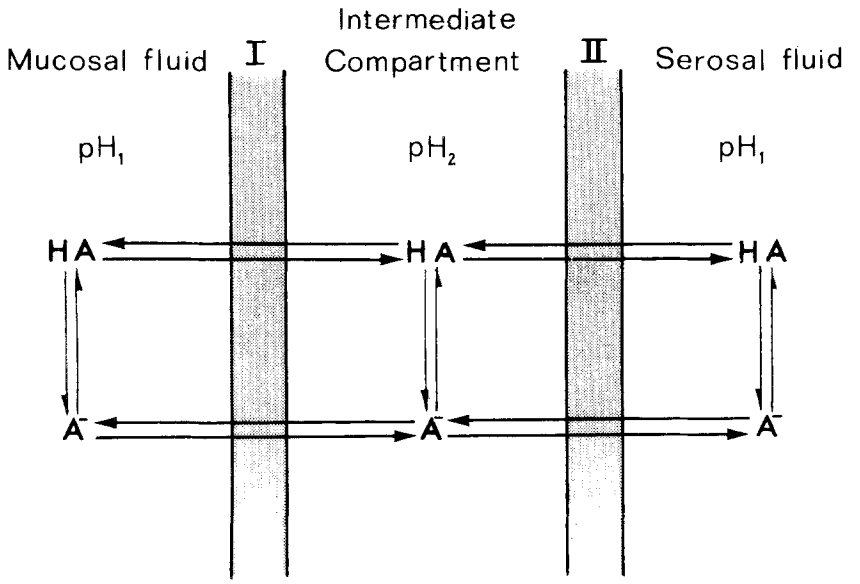

Fig. 1. Three compartment model for the mechanism of intestinal transport of weak electrolytes. The arrows represent the unidirectional fluxes of nonionized acid (*HA*) and anion (*A*) which contribute to the exchanges of weak acid between the end compartments

of weak electrolytes in this system may include a difference between the pH values of the intermediate and end compartments, and electrical potential differences at the barriers.

The jejunal transport of monocarboxylic acids and primary amines was found to be related with the acid-base metabolism of the tissue [5], but was not altered by variations in the transmural PD in the range 0–25 mV [6]. These observations suggested that the principal driving force for intestinal transport of weak electrolytes may be associated with a difference between the pH values of the intermediate and end compartments, and Jackson *et al.* [6] showed that the transport of a weak acid in this type of system is described by an expression of the following form:

$$\frac{J_{ms}}{J_{sm}} = \frac{\left[1+\left(\frac{P_I^i}{P_I^{ni}}\right)10^{(pH_1-pK_a)}\right]\left[1+\left(\frac{P_{II}^i}{P_{II}^{ni}}\right)10^{(pH_2-pK_a)}\right]}{\left[1+\left(\frac{P_I^i}{P_I^{ni}}\right)10^{(pH_2-pK_a)}\right]\left[1+\left(\frac{P_{II}^i}{P_{II}^{ni}}\right)10^{(pH_1-pK_a)}\right]} \quad (1)$$

where P_I^i and P_{II}^i are the permeabilities to the ionized form of the acid at the barriers *I* and *II* respectively, and P_I^{ni} and P_{II}^{ni} are the corre-

sponding permeabilities to the nonionized acid; pH_1 and pH_2 are the pH values of the end compartments and the intermediate compartment, respectively; and pK_a is the negative logarithm of the dissociation constant of the transported acid. The permeability ratios, (P_I^i/P_I^{ni}) and (P_{II}^i/P_{II}^{ni}), describe the abilities of the barriers to discriminate between the ionized and nonionized forms of a compound, and examination of Eq. (1) shows that the conditions for net transport $(J_{ms}/J_{sm} \neq 1)$ of a weak acid in the system may be summarized as $pH_2 \neq pH_1$, and $(P_I^i/P_I^{ni}) \neq (P_{II}^i/P_{II}^{ni})$. Two types of observations suggest that the intestinal version of the three compartment system is one in which the pH of the intermediate compartment is greater than that of the bulk phases: (i) studies of interactions between concurrently transported weak electrolytes revealed a unique pattern that was distinctive of the high pH version of the three compartment system [6]; and (ii) jejunal transport of weak electrolytes was found to be well correlated with the activity of a serosally directed alkalinizing component of intestinal acid-base metabolism, and was independent of the luminally directed acidifying component [5].

The anatomical correlates of the barriers and intermediate compartment in the intestinal three compartment system have not been identified, but the following observations are pertinent to the development of a proposal for these structures: (i) the vectorial characteristics of intestinal weak electrolyte transport ($J_{ms}/J_{sm} > 1$ for carboxylic acids and $J_{ms}/J_{sm} < 1$ for primary amines [6]) requires an organization of permeability properties that may be summarized as $(P_I^i/P_I^{ni}) < (P_{II}^i/P_{II}^{nn})$ in a system in which the pH of the intermediate compartment is greater than that of the bulk phases; (ii) the barriers to weak electrolyte movement associated with the intestinal wall include structures, such as unstirred water layers or layers of connective tissue, that are poorly discriminatory for the ionized and nonionized forms of weak electrolytes, as well as cell membranes that are more permeable to the ionized form of a weak electrolyte than to its ion; (iii) study of a mathematical model of the three compartment system [3] has shown that significant asymmetries in weak electrolyte transport may only be observed if the permeability properties of the two barriers differ by several orders of magnitude, and this degree of asymmetry can readily be conceived in terms of qualitative differences in the natures of the barriers, rather than quantitative differences between similar structures such as two cell membranes in series. For these reasons it has been suggested that the most discriminatory barrier of the intestinal system (barrier *I*) includes the epithelial layer, and that the least discriminatory barrier (barrier *II*) may be associated with a noncellular restriction

such as the sub-epithelial connective tissue. This proposal for the structural organization of the intestinal three compartment system was supported by a study of the electrical correlates of weak electrolyte transport in the intestine [4].

The experiments described in the present paper demonstrated asymmetric transport of eight heterocyclic acids across the wall of rat jejunum *in vitro*. The following observations suggest that the asymmetries in heterocyclic acid transport may reflect the function of the three compartment system discussed previously in connection with the transport of monocarboxylic acids and primary amines:

1) *Polarity of transport.* For each of the heterocyclic acids included in our experiments the M to S flux was significantly larger than the corresponding S to M flux, indicating net transport in the M to S direction, and this polarity is identical with that observed in the earlier studies on jejunal transport of carboxylic acids [5, 6]. The vectorial characteristics of weak acid transport in the three compartment system are determined by the relative discriminatory abilities of the two barriers. Since in the intestinal system the polarity of permeability properties is believed to be associated with qualitative differences in the natures of the two barriers rather than quantitative variations in homologous structures, the pattern of asymmetry presented by the intestinal system should not vary from one weak acid to another, and the finding that the polarity of heterocyclic acid transport is the same as that of the carboxylic acids is consistent with the suggestion that the mechanism of carboxylic acid transport also influences the transmural movements of heterocyclic acids.

2) *Concentration dependence and (lack of) interactions between analogues.* The range employed in the studies of the effects of concentration on heterocyclic acid fluxes was limited by solubility considerations. Within the range used the fluxes were directly proportional to concentration, and the calculated intercepts of the line relating flux and concentration were not significantly different from zero in all cases. Thus these experiments provided no evidence that the transport of the heterocyclic acids is restricted by interaction with a limited number of specific membrane components, and these observations are consistent with the proposal that the movements of the heterocyclic acids are diffusive. This proposal was supported by the finding that concurrently transported acids did not interact in their transports. Interactions between concurrently

transported weak electrolytes were observed in studies of the transport of carboxylic acids and primary amines [6]. Indeed, the particular mechanism proposed to account for the transport of these compounds was based largely upon demonstration of a characteristic pattern of interactions. It was suggested that the interactions observed in the earlier studies derived from modifications in the pH of the intermediate compartment associated with the movement of a weak electrolyte through the system, and two factors may have contributed to our failure to demonstrate a comparable pattern of interactions in the present studies of heterocyclic acid transport. First, most of the acids used in the present study are relatively weak with pK_a values close to the pH of the incubation saline, and the capacity of the heterocyclic acids to modify the pH of their environment is much smaller than that of the stronger electrolytes employed in the previous studies. Second, in the present experiments the well-poised HEPES buffer was used in contrast to less stable bicarbonate/CO_2 system employed in the earlier experiments, and the lack of interactions found in the studies of heterocyclic acid transport may be associated with the improved buffering characteristics of the system. In summary, the lack of interactions between concurrently transported compounds observed in the present study contra-indicates a carrier-mediated mechanism, but is not inconsistent with the three compartment system for weak electrolyte transport.

3) *Quantitative correlation of data with model.* In Fig. 2 weak acid flux ratios have been plotted against pK_a. The individual data points included in the figure were taken from Table 1, and the lines drawn through these points were calculated using Eq. (1). In making these calculations, pH_1 was made equal to the pH of the bulk phases in the studies of heterocyclic acid fluxes (7.5), and pK_a was varied in the range 4 through 8.5. The values used for pH_2, (P_I^i/P_I^i) and (P_{II}^i/P_{II}^{ni}) were chosen by an iterative procedure to fit the calculated curve to the empirical data. It was found that a satisfactory fit was obtained in the case of a single, unique set of values for the three determinants, and this curve is illustrated by the solid line drawn through the data points in the figure. In constructing this curve the determinants were assigned the following values: $pH_2 = 8.1$; $(P_I^i/P_I^{ni}) = 5 \times 10^{-5}$; and $(P_{II}^i/P_{II}^{ni}) = 5 \times 10^{-1}$. Small variations in the values of any of these determinants led to significant changes in the position or profile of the resulting curve, and a poor fit to the experimental data. For example, values of (P_I^i/P_I^{ni}) one-half order of magnitude greater (curve *a*) or smaller

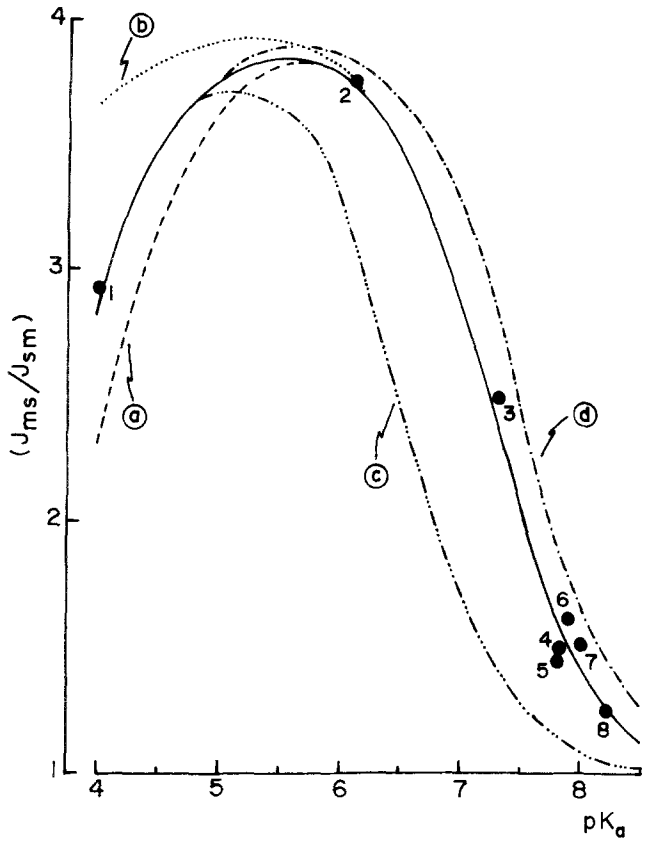

Fig. 2. Relations of flux ratios and pK_a. The individual data points (•) are taken from Table 1, and the lines were calculated from Eq. (1) using the numerical values for the determinants given in the text

(curve *b*) than 5×10^{-5} changed the profile of the curve at low values of pK_a, and the resulting curves were significantly separated from data point 1, (barbituric acid). Similarly, values of (P^{i}_{II}/P^{ni}_{II}) one-half order of magnitude smaller (curve *c*) or greater (curve *d*) than 5×10^{-1} shifted the position of the curve at high pK_a values so that all of the data points lay on one side of the resulting line. The figure does not include lines illustrating the influence of pH_2, but variations in this determinant of as small as 0.05 unit on either side of 8.1, produced changes in the profile of the curve and excluded two, or more data points from the fit. Thus the fit of the empirical data to the theoretical model appears to be highly characteristic, and cannot be ascribed to a broad catholicity of the model.

In summary, the qualitative characteristics of heterocyclic acid transport across rat jejunum *in vitro* are consistent with the proposal that the three compartment system described previously in connection with the transport of carboxylic acids and primary amines may also influence the movements of heterocyclic acids, and a mathematical model of the three compartment system provides a good quantitative description of the relation between the flux ratios of the heterocyclic acids and their pK_a values. For these reasons we conclude that the three compartment system provides a good working hypothesis for the mechanism of heterocyclic acid transport across rat jejunum *in vitro*.

It has been suggested [4, 6] that barrier *II* of the intestinal three compartment system may be an extracellular restriction such as a layer of connective tissue, and it was proposed that the permeability properties of this barrier are determined mainly by diffusional pathlength considerations which are similar for the ionized and nonionized forms of a weak electrolyte so that the permeability ratio characteristic of barrier *II* is close to unity. The present studies showed that the calculated curve obtained using $P^i_{II}/P^{ni}_{II}=1$ did not provide a satisfactory fit of the experimental data, and that the line of apparent best fit required that barrier *II* should be assigned a modest but significant discriminatory property ($P^i_{II}/P^{ni}_{II}=5\times10^{-1}$). However, this finding does not necessarily require reconsideration of the morphological basis of intestinal weak electrolyte transport proposed previously. Biochemical studies have shown that connective tissue consists largely of polyanionic macromolecules [7]. The fixed negative charges at the surfaces of these molecules may be expected to exert some restriction on the diffusion of negatively charged solutes through the interstices of the tissue, and to provide some discrimination between undissociated acid and anion. In this context it is of interest to note that an analysis of electrolyte transport in the intestine [2] suggested the existence of a sub-epithelial barrier bearing negative fixed charged groups that exhibited a small degree of discrimination between cations and anions. Thus the present studies of weak electrolyte transport are consistent with the functional organization suggested in studies on the intestinal transport of strong electrolytes, and support the proposal that the intermediate compartment of the weak electrolyte transport system is a component of the sub-epithelial extracellular space.

The value for the permeability ratio at barrier *I* obtained in the present studies ($P^i_I/P^{ni}_I=5\times10^{-5}$) confirmed the suggestion made in earlier work [6] that the value of this determinant would be equal to, or less than 10^{-4} for weak acids. Studies of a mathematical model of the

three compartment system [3] have shown that electrical potential differences, of magnitudes comparable to those seen in the intestine, exert a negligible influence on weak electrolyte movements at barriers characterized by permeability ratios smaller than 10^{-3}. Thus the finding that the permeability ratio at barrier *I* of the intestinal system is as low as 5×10^{-5} supports the suggestion that the principal driving force for weak acid transport can be described in terms of a difference between the pH values of the intermediate compartment and the bulk phases, and that electrical potential differences play a minor role in the intestinal transport of weak acids. However, the present studies have provided little additional information concerning the nature of barrier *I* in the intestinal system, or of the physiological properties that determine weak acid permeabilities at this barrier. Jackson and Kutcher [4] have suggested that barrier *I* may be a complex structure consisting of the whole epithelial layer in which the ratio P^{i}/P^{ni} represents the relative permeabilities of the extracellular shunt channel to anions, and of the transcellular channel to the nonionized forms of weak acids. If this proposal is correct the present studies suggest that the permeability of the cellular element to the nonionized form of a weak acid should be more than four orders of magnitude greater than the permeability of the extracellular shunt to the anion, but we have no independent data that allows this proposal to be evaluated.

Studies of intestinal acid-base metabolism [5] have shown that the tissue readily generates pH differences between mucosal and serosal bulk phases of 0.5 unit in suitable conditions of incubation. Thus the suggestion that the pH of a component of the sub-epithelial extracellular space is maintained at a level 0.6 unit greater than that of the bulk phases is not inconsistent with the known properties of the tissue. In a previous study it was suggested that the development of a compartment of high pH within the intestinal wall may be associated with the serosally directed transport of bicarbonate [6]. The present studies showed that transport of weak acids may be observed in the absence of exogenous bicarbonate and CO_2. This observation does not preclude the possibility that the transport of weak electrolytes is dependent upon the handling of bicarbonate derived from cellular metabolism, but suggests that other asymmetric components of intestinal acid-base metabolism, such as cation-coupled hydrogen ion movements, may require consideration for the mechanism of development of the distinctive pH in the intermediate compartment of the intestinal three compartment system for weak electrolyte transport.

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