Gastrointestinal Absorption of the Strongly Acidic Drug Proxicromil

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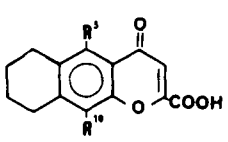
Abstract □ Gastrointestinal absorption of the strongly acidic drug proxicromil has been studied with respect to its physical organic chemistry. The lipophilicity of the drug above pH 6 in octanol–buffer partition experiments is dependent on ion pair formation. Similar trends were demonstrated for the *in vitro* partition of the compound into GI tissue. The absorption of the compound from the perfused GI tract of rats *in vivo* was not consistent with classical un-ionized drug absorption theories and indicated the operation of other processes, especially ion pair formation, as major mechanisms of proxicromil absorption.

Keyphrases □ Proxicromil—GI absorption, lipophilicity, ion pair formation □ Absorption, GI—proxicromil, lipophilicity, ion pair formation

The transfer of acidic drugs across membranes has usually been ascribed to the un-ionized form (1). Many acidic drug substances possess only a weak acidic function which allows a significant proportion of the un-ionized drug to be present at physiological pH. The oral anti-allergy agent proxicromil (FPL 57787, I) (2) and related compounds [FPL 55365(II) and FPL 57579(III)] are of considerable interest since the strong acidity of I (pK_a 1.93) (3) causes the proportion of the drug present as the un-ionized species at physiological pH to be very small. Despite this, the compound has been demonstrated to be well absorbed in a number of animal species and in humans (>60%) (4). The compound also crosses other membrane systems, since it is transferred into the milk from the plasma when administered to lactating albino rats (5). Since the processes involved in the passage of the drug are not adequately explained by the sole passage of un-ionized drug, alternative explanations must be sought.

Several works on GI absorption refer to ion pair partitioning (for a review, see Ref. 6) as a possible mechanism of ionized drug absorption. Ion pairing forms a neutral species by electrostatic attraction between oppositely charged ions in solution. Houston and Wood (6), in their monograph on the GI absorption of drugs, state that there is no *a priori* reason why any molecular entity, regardless of ionic status, should not be absorbed if it possesses adequate partitioning characteristics. We have therefore examined the partitioning characteristics of proxicromil and related compounds from buffer systems into octanol. The partitioning of proxicromil into GI tissues has also been examined. Similarly, we have investigated the absorption of the drug in the albino rat using a perfused intestine system.



I: R^5 = OH, R^{10} = $CH_2CH_2CH_3$
II: R^5 = H, R^{10} = H
III: R^5 = H, R^{10} = $CH_2CH_2CH_3$

EXPERIMENTAL SECTION

Chemicals—[^{14}C]Proxicromil (9.67 mCi/g) and unlabeled proxicromil, prepared in-house, had a radiochemical and chemical purity of 99%. The octanol[1] used had been specially purified for the determination of partition coefficient of drugs. For physicochemical and *in vivo* studies, McIlvaine's (7) citrate–phosphate buffer (for pH 2.2–6.4) and modified Sörensen's (7) phosphate buffer (NaH_2PO_4 replaced KH_2PO_4) (for pH 7.4–8.2) had the sodium ion concentration adjusted to 0.155 M by the addition of NaCl, where necessary. Krebs–Ringer bicarbonate buffer was modified to exclude Ca^{2+} and Mg^{2+} since these ions were found to cause precipitation of proxicromil. All other reagents[2] were of analytical reagent grade.

Solubility Determination—The aqueous solubility of proxicromil was measured (single determinations) in aqueous buffers at a series of pH values covering the range 0–7. Successive aliquots (1 mg) of the compound were added to the buffer (2 mL) at 20°C until saturation was indicated by the presence of undissolved material, for up to 3 h. The pH was checked, and the sample was filtered (cellulose filter, 0.45 μm)[3] and assayed spectrophotometrically for proxicromil (360 nm)[4].

Physicochemical Measurements—All distribution coefficients were measured by the shake-flask method (8). Results are reported as the mean of at least three determinations, together with the observed spread. Compounds were partitioned between 1-octanol saturated with buffer and the same aqueous phase saturated with 1-octanol; the concentration of compound in the organic phase was analyzed (UV spectrophotometry at 360 nm)[4] before and after equilibration. Volume ratios of the two phases were selected to give a reduction of ~50% in the UV absorbance of the solution after equilibration. Equilibration was carried out by shaking at room temperature for 1 h, followed

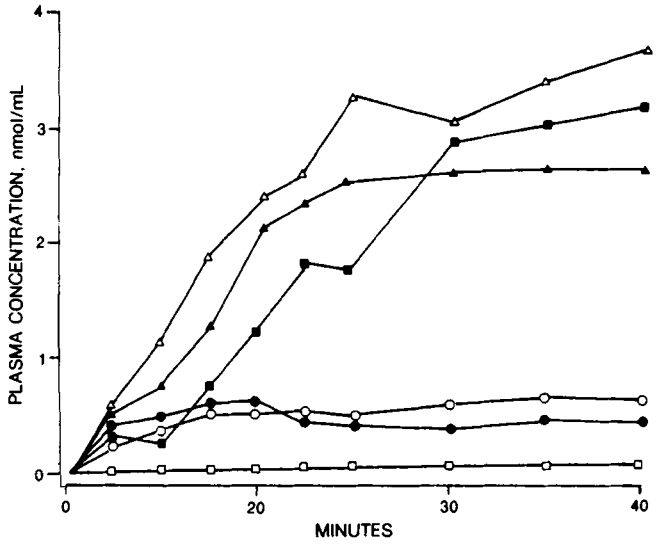


Figure 1—*Plasma concentrations of radioactivity (equivalent to proxicromil) after injection of [^{14}C]proxicromil into separate regions of the rat gut. Key: (△) duodenum; (▲) jejunum; (■) upper ileum; (○) lower ileum; (●) colon; (□) stomach and cecum. The stomach and cecum show very similar negligible absorption, and the results are represented therefore as a single point; n = 4 for each tissue.*

[1] Spectrograde; Fisons Scientific Apparatus, Loughborough, Leicestershire, U.K.
[2] BDH Ltd, Poole, Dorset, U.K.
[3] Millipore (UK) Ltd, London.
[4] Model SP1700; Pye Unicam, Cambridge, U.K.

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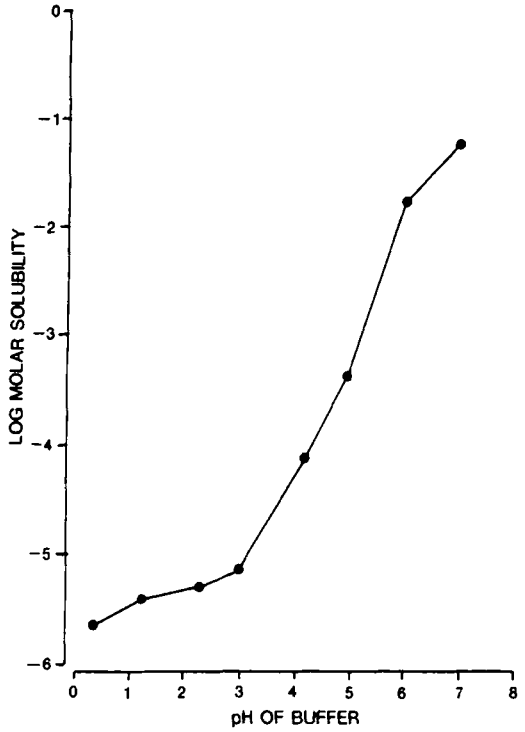

Figure 2—*Aqueous solubility of proxicromil with respect to buffer pH (see text).*

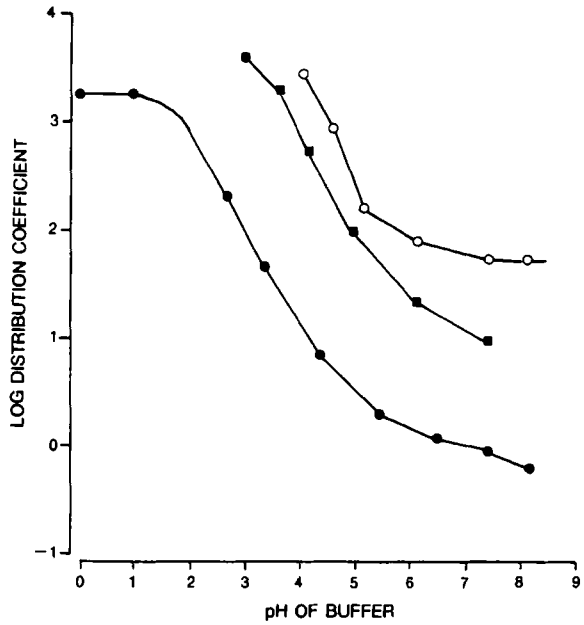

Figure 3—*Distribution coefficient (log* D*) of II (●), III (■), and proxicromil (I) (○) with respect to buffer pH. Variation of log* D *was* < ±*0.03 of the mean.*

by centrifugation[5] at 2000 rpm for 1 h. The distribution coefficient (D) was calculated from the ratio of the overall concentration of compound in the ionized and undissociated states between the two phases (9).

Measurements of distribution coefficients were made over a wide pH range for II (pH 0-8.2), III (pH 3-8.2), and I (pH 3-8.2). Control of the aqueous phase pH was achieved using the buffers described above adjusted to a sodium ion concentration of 0.155 M. Experiments at pH 0-2 employed dilute hydrochloric acid with added sodium chloride (0.155 M). Values of log $D > 4$ could not be measured to an acceptable accuracy owing to practical limitations of the spectrophotometric method.

The sodium content in the octanol phase was measured in proxicromil distribution experiments at various aqueous phase pH values between 4.0 and 8.2, by atomic emission spectrometry[6]. Sodium content results are given as the average value from two experiments at each pH value. Control experiments were carried out under the same conditions, but omitting the proxicromil (I), to estimate background sodium levels in the octanol. All test solutions contained 4 mL of octanol made up to 50 mL with methanol. Where the volume of octanol available was smaller (pH 5-8.2), additional octanol was added in order to keep the overall solvent composition constant.

The effect of cationic concentration on the octanol distribution coefficient of proxicromil was measured at a constant pH of 7.4 in a dilute phosphate buffer system (0.02 M) containing varying concentrations of either sodium chloride or potassium chloride (0.02-0.24 M).

Experiments with Everted Gut Segments—Male Wistar rats[7] were killed by a blow to the head, and the jejunal section of the small intestine removed. This section (between 3 and 18 cm from the pylorus) was everted over a glass rod and cut into 2-3-mm segments (10). Three single segments from three separate rats were incubated together in 25-mL conical flasks at 37°C in Krebs-Ringer bicarbonate buffer (2 mL) containing the appropriate concentration of [^{14}C]proxicromil. Experiments were carried out in triplicate at various pH values between 3.5 and 8.5.

The flasks were aerated for 30 s with oxygen-carbon dioxide (95:5), sealed with rubber turnover closures[8], and shaken at 37°C for the determined period of time in a shaking water bath. At the completion of the incubation the tissue was removed, rinsed twice in 0.9% NaCl solution, blotted, and weighed.

After incubation, segments were placed in aqueous sodium hydroxide (0.5 mL, 1 M) in scintillation vials. The containers were then heated in an oven to ≤80°C to speed up tissue digestion, which was continued until a uniform solution was obtained. The contents were allowed to cool to room temperature and then acidified with an equal volume of aqueous hydrochloric acid (2 M), and Bray's scintillation fluid was added (5 mL). Aliquots of incubation medium (0.2 mL) were also taken for radioactivity determination. Radioactivity was measured in a scintillation counter[9], and counting efficiency was determined using channel ratios for quench correction.

Experiments with Gut *In Situ*—Male CR/CD rats[10] (weight range, 240-280 g; fed *ad libitum*) were anesthetized with pentobarbital solution administered *via* an indwelling needle in the caudal vein. Following an incision in the throat, the carotid artery was exposed and cannulated with polyethylene tubing. For experiments to investigate the site of absorption, a midline incision was made and the GI tract was ligated in each animal with surgical thread to isolate either the stomach, duodenum, jejunum, upper ileum, lower ileum,

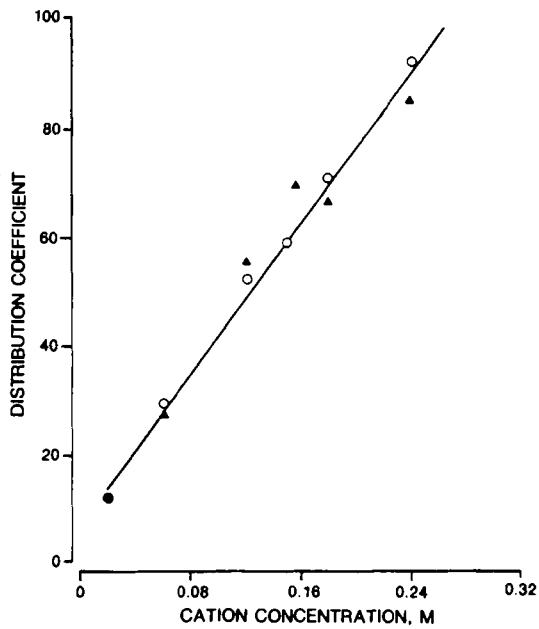

Figure 4—*Relationship between the distribution coefficient (*D*) of proxicromil and the cation concentration of Na^+ (○) or K^+ (▲). Variation in* D *was* < ±*2.5% of the mean value in all cases.*

[5] Model Mistral 4L; MSE Instruments, Crawley, U.K.
[6] Model 703; Perkin-Elmer.
[7] Hatfield Polytechnic.
[8] Suba-Seal, Barnsley, U.K.
[9] Model CM2700; Tracerlab Services Ltd., Twickenham, U.K.
[10] Charles River, Manston, Kent, U.K.

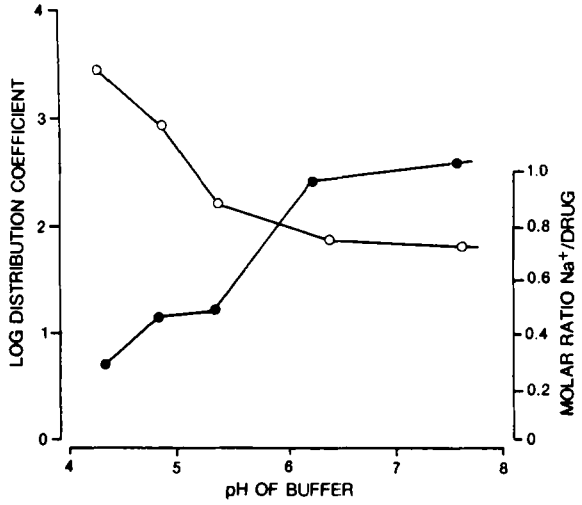

Figure 5—*Relationship between the distribution coefficient (log D) of proxicromil (○) and the ratio of Na^+ to proxicromil ions (●) present in the octanol phase with respect to pH.*

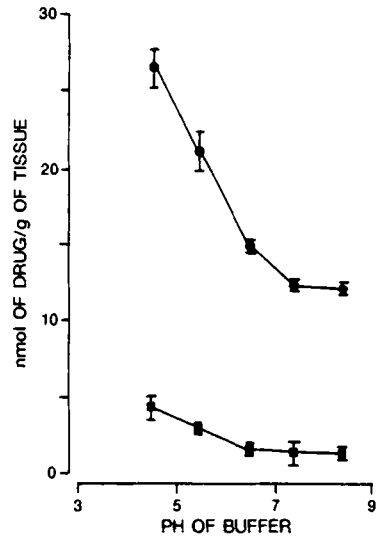

Figure 6—*Partitioning of proxicromil into everted jejunal segments with respect to pH. Incubation of the segments was conducted in proxicromil-buffer solutions for 30 min at concentrations of 1.0 mM (●) and 0.1 mM (■); n = 3 for each point.*

colon, or cecum. Proxicromil was administered as a bolus injection (0.2 mL) into the isolated segment in modified Sörensen's phosphate buffer, sodium ion concentration 0.155 M. Arterial blood samples were collected from the carotid artery at intervals for 60 min.

The absorption of the drug was investigated after single-pass perfusion at various pH values. For these experiments a midline incision was made and a 20-cm length of silicone rubber tubing (2 mm i.d.) was inserted ventrally into the duodenum 2 cm from the stomach pylorus. The end inserted into the duodenum had a number of turns of surgical thread glued to the extreme end which, in conjunction with ligation of the duodenum, secured the tubing. The other end of the tubing was adapted to take standard syringe fittings. A similar length of tubing was inserted dorsally into the jejunum and secured in an identical manner to give a 10-cm section of perfused gut. All perfusion solutions were maintained at 37°C. The gut was then flushed to remove debris by gentle perfusion of saline (5 mL) administered *via* a syringe, followed by flushing with buffer (5 mL) at the respective pH of investigation. Perfusion of the buffer was continued for a further 10 min at a steady rate of 1 mL/min. After this, a solution of [^{14}C]proxicromil (1 mM; 0.6 μCi/mL) dissolved in the same buffer was rapidly flushed through the intestine (5 mL) and then perfused at a rate of 1 mL/min for 20 min. Blood samples were collected into heparinized capillary tubes at 4-min intervals from the carotid vein cannula. The pH of the eluant buffer was monitored throughout the experiment.

In certain experiments the perfusion was performed using the segmented flow method of Winne (11). Total rate of buffer perfusion remained at 1 mL/min. Plasma was obtained by centrifugation of blood at 2500×*g* for 10 min. Radioactivity present in plasma was obtained by mixing the samples with liquid scintillation cocktail[11] followed by liquid scintillation counting. Counting efficiency was determined by the external standard channels ratio method.

RESULTS AND DISCUSSION

Site of Absorption—To determine if the absorption of proxicromil observed in animals was from the stomach or from other regions of the GI tract, a bolus of [^{14}C]proxicromil was injected into various regions of the gut, and blood samples were collected at intervals. Rapid absorption was observed from the duodenum, jejunum, and ileum, while much slower absorption occurred from the lower ileum and colon and very little absorption from the stomach and cecum (Fig. 1). Since little or no absorption occurred from the stomach, it is likely that proxicromil is absorbed in intact animals from regions of the GI tract in which the pH is approximately neutral. As little proxicromil is absorbed in the stomach *in vivo*, where the compound would be substantially in its un-ionized state, the aqueous solubility of the compound at various pH values was investigated since lack of solubility would limit absorption. Also the partitioning of the compound was determined in the octanol-aqueous buffer system over the same range of pH. Emphasis was given to the higher pH values corresponding to those of the region of the GI tract where absorption had been observed.

Solubility and Partitioning—The aqueous solubility of proxicromil is strongly pH dependent (Fig. 2). The insolubility at low pH reflects the low water solubility of the compound when its carboxyl group is undissociated (HA). At pH values above the pK_a (1.93) (3), solubility in water increases rapidly reflecting the abundant water solubility of the ionized compound (A^-). The lack of absorption of proxicromil from the stomach is paralleled by its lack of solubility and, hence, low availability at gastric pH.

The relationship between pH and partition was studied for I-III (Fig. 3). The partition coefficient (P) refers to the ratio of concentrations of the undissociated compound (HA) between the two liquid phases (Eq. 1). The distribution coefficient (*D*) represents the overall ratio of concentrations of proxicromil in the octanol and aqueous phases regardless of ionization (9) (Eq. 2). Equation 2 is expanded from its usual form (9) to show the possibility of the sodium salt as potentially contributing to the concentration of proxicromil in the octanol phase:

$$P = \frac{[HA]_{oct}}{[HA]_{aq}} \qquad \text{(Eq. 1)}$$

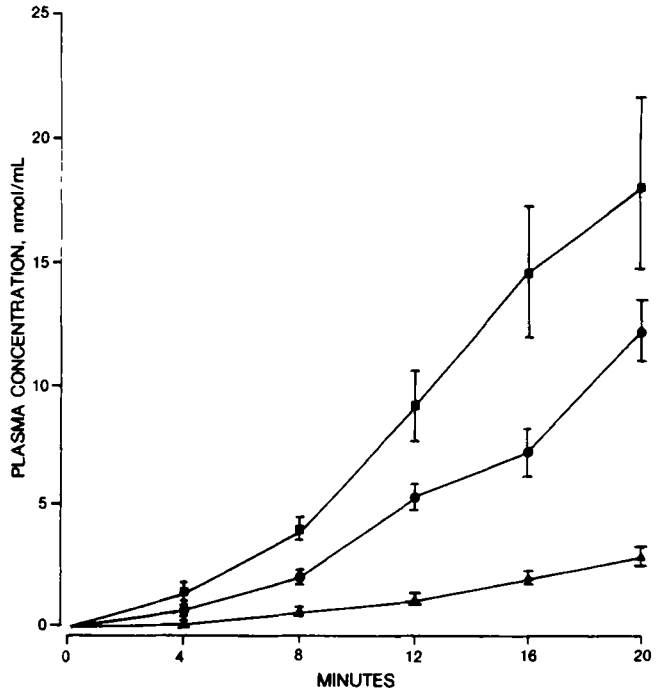

Figure 7—*Plasma concentrations of radioactivity (equivalent to proxicromil) during perfusion of the small intestine of the rat with [^{14}C]proxicromil-buffer solutions at pH 5 (■), 7 (●), and 9 (▲) (see text); n = 6 for each point.*

[11] Fisofluor mpc; Fisons Scientific Apparatus, Loughborough, Leicestershire, U.K.

Table I—Influence of pH of Perfusing Buffer on Proxicromil Absorption Expressed as Absorption Relative To pH 6.98

pH of Eluant Buffer	Absorption Ratio Calculated Using: Un-ionized Drug[a]	$AUC_{0\text{-}20\ min}$[b]	Plasma Conc. at the End of Infusion[b]
8.93 ± 0.02	0.01	0.17	0.17
6.98 ± 0.01	1.00	1.00	1.00
5.23 ± 0.01	56	1.42	1.24

[a] Expected ratio if un-ionized drug was the sole form of drug absorbed. [b] Ratio calculated from perfusion experiments. See text and Fig. 7.

$$D = \frac{[HA]_{oct} + [Na^+A^-]_{oct}}{[HA]_{aq} + [A^-]_{aq}} \quad \text{(Eq. 2)}$$

The relationship of log D to pH was measured for II over the pH range 0–8. For the other two compounds, however, log D could not be obtained below pH 3 owing to limitations of the spectrophotometric analysis. At pH values ≤5, all three compounds decrease in log D, their behavior being described by the pH-partition relationship:

$$\log\left(\frac{P}{D} - 1\right) = pH - pK_a \quad \text{(Eq. 3)}$$

Substitution of the data for II (Fig. 3) into Eq. 3 yields a pK_a value of 1.65 ± 0.1, in agreement with other reports (3).

The proportion of undissociated proxicromil in the water phase falls from 0.0676 at pH 5.1 to ~0.0001 at pH 8.0 (calculated from dissociation constant pK_a = 1.93). Using Eq. 3, it can be calculated that the observed value of log D of 2.17 at pH 5.1 should fall to −0.83 at pH 8.0. In contrast to this, however, it was found that at higher pH the distribution coefficient became progressively independent of pH. This region was examined in more detail to identify the factors which now control the distribution ratio. At pH 7.4 a linear relationship was established between observed distribution coefficient (D) and concentration of potassium or sodium ion in the aqueous phase (Fig. 4). This is parallel to the findings on the lipophilic base chlorpromazine (12), where added counterions such as chloride (Cl^-) also produced a linear relationship to distribution coefficient. This is consistent with ion pair partition, whose extent is related to the product of proxicromil ion and sodium ion concentrations in the aqueous phase. Scheme I indicates a series of equilibria which are consistent with the observed behavior. Proxicromil anion entry into the organic phase is shown as a distribution rather than a partition ratio (9), since it is a complex effect dependent in magnitude on factors including, for example, counterion concentration.

$$\begin{array}{ccc} [HA]_{oct} & & [Na^+A^-]_{oct} \\ \Big\downarrow\uparrow P_{HA} & & \Big\downarrow\uparrow \\ [HA]_{aq} & \overset{K_a}{\rightleftharpoons} & [A^-]_{aq} \end{array}$$

Scheme I

Analysis of the octanol layer for sodium ion using atomic emission analysis showed the concentrations of proxicromil (UV spectrophotometry) and sodium (atomic emission) to be in 1:1 molar agreement in distribution experiments at pH 7.4. Similar experiments at a series of lower pH values (Fig. 5) showed the penetration of sodium into the octanol to be significant only at pH > 5. Below this pH, the ratio of sodium ion to proxicromil fell as conventional pH-partition of the undissociated compound (HA) became dominant. This evidence for ion pair partition was supplemented by analysis for sodium ion in octanol equilibrated with phosphate buffer containing sodium ion (0.155 M) but no proxicromil. The penetration of sodium ions into the octanol thus was insignificant, despite its high concentration in the aqueous phase, unless proxicromil anion was available.

Effect of pH on Absorption—The experiments utilizing octanol were reproduced using gut segments *in vitro*. Uptake of [^{14}C]proxicromil into jejunal segments was studied at 0.1 and 1.0 mM concentrations of proxicromil at various pH values. The results are shown in Fig. 6 and demonstrate that at pH < 6 the lower the pH, the greater the partitioning into the tissue. At pH >6 the uptake was approximately constant. Similar trends were shown at incubation times up to 90 min. The partitioning of proxicromil into gut tissue, therefore, closely follows the characteristics observed for the partitioning of the compound into octanol with pH-dependent partitioning at low pH and a constant partitioning of the drug at pH > 6. The change in behavior for the compound takes place in both octanol and in gut tissue at pH ~6 and very far from the value of the pK_a of the drug (1.93).

The effect of buffer pH on proxicromil absorption *in vivo* was studied using *in situ* perfused gut loops. Figure 7 illustrates the plasma concentrations of radioactivity present in plasma after perfusion of the gut of anesthetized animals with [^{14}C]proxicromil dissolved in buffers of varying pH. The plasma concentrations are increased by decreasing pH. Taking either peak plasma concentration or areas under the plasma curve ($AUC_{0\ 20\ min}$ as measured by the trapezoidal method) (Table I), absorption can be shown to be increased at a nominal pH 5 and decreased at a nominal pH 9 from that at pH 7. These increases are much smaller than would be expected if un-ionized drug was the sole determinant since over the pH range evaluated (5.23–8.93), there is an ~5000-fold change in free drug concentration. The absorption at pH 5.0 is not limited by the unstirred layer (13), since in experiments in which this layer was deliberately disrupted by alternating buffer perfusion with air using the method of Winne (11), there was no significant change in proxicromil absorption.

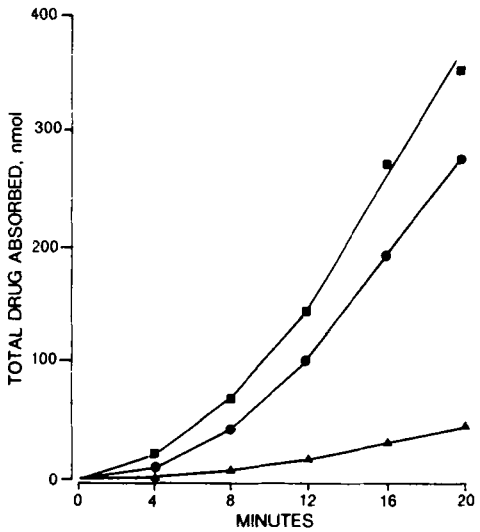

Figure 8—*Cumulative total absorption of radioactivity (equivalent to proxicromil) during perfusion of the small intestine of the rat with [^{14}C]-proxicromil–buffer solutions at pH 5 (■), 7 (●), and 9 (▲) (see text).*

The shape of the plasma curve, however, indicates that absorption is not defined by a single rate constant, since the zero-order input of drug, by perfusion, to the intestine should result in a curve resembling the classical curves following intravenous infusion. Calculation of clearance in the rat (0.3 mL/min) and volume of distribution (17 mL) from intravenous infusion experiments[12] allow calculation of the actual amount absorbed using the classical approach of Loo and Riegelman (14). Figure 8 illustrates the total amount absorbed with time during gut perfusion experiments.

The data can be interpreted by a model in which the mucosal tissue itself represents a compartment (6) in proxicromil absorption. The model is defined by Scheme II:

$$\text{proxicromil in lumen} \xrightarrow{k_0} \text{proxicromil in mucosa}$$

$$\xrightarrow{k_1} \text{proxicromil systemically absorbed}$$

Scheme II

The constant k_0 represents the zero-order absorption of the drug from the constant perfusion system and is a hybrid of the actual absorption rate constant and the perfusion rate. The constant k_1 represents the rate constant for the passage of drug from the mucosa to the circulatory system. Individual zero-order rates of absorption (k_0) were calculated by methods analogous to those used for intravenous infusion experiments (15). The linear portion of the curve for each pH value in Fig. 8 (12–20 min) represents the zero-order rate of absorption, yielding approximate values of 30, 20, and 4 nmol/min at pH 5, 7, and 9, respectively. The linear portion of the curves at each pH value can be extrapolated to give an intercept value from which k_1 can be calculated (0.14 min^{-1}).

CONCLUSIONS

The partitioning characteristics demonstrated in octanol and jejunal segments can partly be reproduced *in vivo* in the perfused intestine. While the perfusion results could be affected by a number of other factors, it is likely that at pH values > 6 ion pair formation operates to allow distribution of the

[12] Unpublished results.

compound into lipoidal phases. This process is likely to occur in the intestine as demonstrated both *in vitro* and *in vivo*. Since absorption of the compound occurs from regions of the GI tract which have pH values >6, it is suggested that ion pair formation with naturally abundant cations such as sodium plays a significant role in the absorption of proxicromil.

An alternative explanation for the GI absorption is that there is a difference between bulk and surface pH. The data of Lei *et al.* (15) suggest the real surface pH might be lower than that expected from bulk pH. To overcome surface pH effects in the intestinal perfusion experiments, we have equilibrated the gut for 10 min by perfusion of buffer at the appropriate pH at 1 mL/min. Under these conditions, surface pH and bulk pH should have tended toward an equilibrium (16), the true pH of which would be very close to that of the measured eluant buffer (see Table I). The linear rate of absorption at each pH indicated in Fig. 7 also demonstrates that an equilibrium has been achieved, since a decreasing rate of absorption would be expected at the lower pH values as the surface pH gradually increased. The significance of a surface pH of 6.5 in explaining absorption of acidic compounds is also of much greater relevance for compounds such as *n*-butyric acid (pK_a 4.9) (16) than proxicromil (pK_a 1.93).

The ion pair behavior described here is in marked contrast to the normally held concept (17). Proxicromil possesses intrinsic lipophilicity and requires only charge neutralization to partition into lipoidal phases. Endogenous ions such as sodium and potassium readily form such neutral lipophilic ion pair complexes. This can be contrasted to the facilitation of partitioning of a nonlipophilic drug by the incorporation of a lipophilic counterion, the concept of which has led Jonkman and Hunt (17) to consider ion pair absorption as more fiction than fact.

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Relationship Between Molecular Structure and Cytochrome P_{450}–Metabolic Intermediate Complex Formation, Studied with Orphenadrine Analogues

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Abstract □ Complexation of ferrous cytochrome P_{450} by metabolic intermediates formed during NADPH-catalyzed metabolism of compounds structurally related to orphenadrine was studied. This so-called metabolic intermediate complexation was determined in rat liver microsomes, obtained from phenobarbital-pretreated rats, at 455 nm using 33 μM of the orphenadrine derivatives. Using secondary amine derivatives with various *N*-alkyl substituents, a parabolic relationship between the logarithm of percentage of cytochrome P_{450} complexation and hydrophobic fragmental constant was observed. The derivative with a bulky tertiary butyl group, however, was devoid of metabolic intermediate-complexing activity. This indicates that steric factors besides lipid solubility may govern the complexing activity; also substitution at the phenyl group affects metabolic intermediate complex formation.

Keyphrases □ Cytochrome P_{450}—complexation with metabolic intermediates, orphenadrine analogues □ Orphenadrine—analogues, metabolic intermediate Complexation with cytochrome P_{450} □ Complexation—metabolic intermediate, orphenadrine analogues with cytochrome P_{450}

Several types of nitrogenous compounds have been shown to undergo cytochrome P_{450}-catalyzed metabolic conversions leading to metabolic intermediates that complex with cytochrome P_{450} (1–3). This complexed cytochrome P_{450} is inactive. Metabolic intermediate complex formation may therefore inhibit metabolic reactions (4). Among the nitrogenous compounds which elicit this complex formation, there are several classes of therapeutically important drugs.

Amphetamine derivatives form a metabolic intermediate complex with hepatic microsomal cytochrome P_{450} *in vitro*, but not *in vivo* (4, 5). With regard to amphetamine analogues, relationships between molecular structure and *in vitro* complexation have been described (4, 6). Recently, metabolic intermediate complex formation produced by macrolide antibiotics have gained much interest (7, 8). With a series of macrolide antibiotics, structural features which are important for the formation of metabolic intermediate complexes have been determined (9). However, with regard to compounds related to proadifen (like propoxyphene, desipramine, and orphenadrine) which produce such complexation both *in vitro* and *in vivo* (10, 11), little is known about the relationship between molecular structure and the ability to evoke complexes

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