Drug Permeation through Membranes III: Effect of pH and Various Substances on Permeation of Phenylbutazone through Everted Rat Intestine and Polydimethylsiloxane

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Abstract □ The permeation rate of phenylbutazone through membranes of polydimethylsiloxane and everted rat intestine was measured over a range of pH values. The classical pH-partition hypothesis was not obeyed. Permeability coefficients were calculated using a modification of Higuchi's general equation relating pH and permeation rate. The effect of various substances on the permeability coefficient was determined. Phenylbutazone permeation rates in one or both membranes decrease in the presence of gelatin, methylcellulose, polyvinylpyrrolidone, skim milk, albumin, sodium lauryl sulfate, polysorbate 80, and cetrimonium bromide. Permeation rates through everted intestine were enhanced by bile salt concentrations below the CMC and by porcine mucin, while rates through polydimethylsiloxane were unaffected. Substances having no apparent effect were: lactose, starch, talc, kaolin, calcium chloride, acacia, calcium sulfate, sucrose, sodium saccharin, cholesterol, ferrous sulfate, and magnesium stearate.

Keyphrases □ Permeation—phenylbutazone through polydimethylsiloxane and everted rat intestine, effect of pH and formulation excipients □ Phenylbutazone—permeation through polydimethylsiloxane and everted rat intestine, effect of pH and formulation excipients □ Membranes, polydimethylsiloxane and everted rat intestine—permeation of phenylbutazone, effect of pH and formulation excipients on permeation rate and coefficient

It is well known that the choice of excipients and the manufacturing process can have a profound effect on the bioavailability of drugs in solid oral dosage forms. Formulation changes in established drugs may lead to important changes in their physiological behavior, sometimes with serious consequences, as shown by recent events concerning diphenylhydantoin (1) and digoxin (2). As far as it can be controlled by the drug manufacturer, bioavailability depends primarily on the dissolution characteristics of the formulation and on the possible interaction of excipients with the drug or the GI membranes.

Part of the bioavailability program being carried out in these laboratories concerns both dissolution behavior and the possible interaction of drugs with excipients and other materials. This paper deals with the effect of various excipients, nutrients, and endogenous materials on the permeation of phenylbutazone through polydimethylsiloxane and everted rat intestine. The work is aimed at detecting possible interactions which would have an adverse effect on bioavailability and is a continuation of the previous permeability studies of amobarbital (3).

EXPERIMENTAL

Permeation through Polydimethylsiloxane—Steady-state permeation rates were measured at 37° using cells similar to those described by Garrett and Chemburkar (4). The cells were equipped with polydimethylsiloxane membranes 0.014 cm thick. In use, they were filled with borate buffer, pH 10, and immersed in a beaker of phenylbutazone solution. The solutions both inside and outside the cell were stirred magnetically. The drug solutions were buffered with citrate or phosphate and ranged in concentration from 2×10^{-5} to 5×10^{-4} mole liter^{-1}. These conditions lead to steady-state permeation because the total amount of drug in the beaker is large compared to that permeating into the cell and the pH of 10 within the cell completely ionizes the drug, thereby preventing back-permeation. Excipients and other materials were added to the drug solution to form either a solution or a slurry. The phenylbutazone content of the desorbing borate buffer was measured by pumping the solution continuously through a spectrometer set at 265 nm and taking readings at appropriate time intervals. A Beer's law calibration curve was prepared from USP phenylbutazone reference standard, yielding a molar extinction coefficient of 21,050.

Everted Rat Intestine Permeation—Male Sprague–Dawley rats, weighing 180–250 g, were fasted 24 hr prior to the experiment, with water allowed as desired. The rats were sacrificed with ether, a method reported to prolong the structural integrity of the intestine (5). The entire small intestine was removed; after discarding the first 15 cm of the proximal end, two intestinal segments, 12 cm in length, were cut and everted on a glass rod according to the method of Crane and Wilson (6). Each everted intestinal segment (A in Fig. 1) was ligated to glass cannulas (B and C), mounted in an adjustable clamp (D) and extended until kink-free. The assembly was placed in a test tube (E) containing 50 ml of mucosal drug solution and a sintered-glass plug (F) through which a 95% oxygen–5% carbon dioxide stream was bubbled during the experiment. The entire apparatus was controlled at 37° in a bath (K). The desorbing serosal solution at pH 7.2 was placed in a 50-ml syringe (G) from which, after passing through a preheater (H), it entered the everted segment at point B. During operation, the drug was allowed to perfuse into the serosal fluid for 5 min. The sample was removed from the segment and collected at point J by pushing it through the segment with fresh serosal fluid from the syringe. A volume of 2.5 ml was sufficient to empty and rinse the segment.

The apparatus was used to measure permeation rates from mucosal solutions at different pH's and the ratio of drug permeation rates with and without an excipient or other material (7). This was done by measuring the perfusion rate of the drug for 50 min, after which the drug solution in the test tube (E) was replaced by a solution of drug and excipient and the rate measurements were continued. Two segments from each rat were run simultaneously. The order in which the mucosal solutions were presented to the intestinal segments was reversed in the case of the second segment. This allowed detection of nonreversible membrane changes brought on by substances added to the drug solution or by deterioration of the membrane. All solutions were prepared in isotonic sodium phosphate buffers (8). The 2.5-ml serosal samples taken at 5-min intervals were diluted to 5 ml and analyzed spectroscopically for phenylbutazone at 265 nm. Correction for background absorption was established by five experiments with mucosal buffer containing no drug. Compounds leached from the membrane give an average absorbance of 0.028 ± 0.004 for a 5-min period of contact with the membrane.

Materials—The following were used: phenylbutazone[1], dibasic calcium phosphate[2], calcium chloride[2], cholesterol[2], polysorbate

[1] Butazolidin, Ciba-Geigy.
[2] Fisher Scientific.

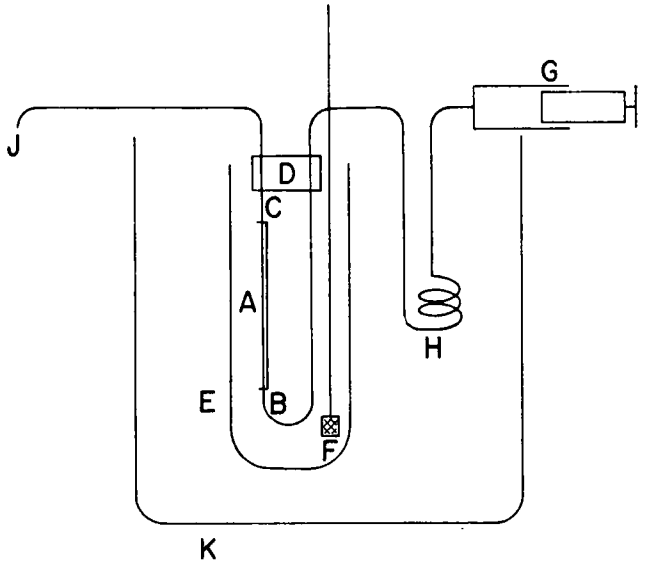

Figure 1—*Schematic diagram of the everted intestine permeation apparatus. Key: A, the everted segment; B and C, glass cannulas; D, clamp; E, 50-ml test tube; F, sintered-glass bubbler; G, syringe; H, mucosal solution preheater; J, sample collecting point; and K, thermostated bath.*

80[2], acacia USP[2], sodium lauryl sulfate USP[2], magnesium stearate USP[2], starch USP[2], ferrous sulfate[3], calcium sulfate dihydrate[3], bovine albumin[3] (fraction V), egg lecithin[3] (95–100%), polyvinylpyrrolidone[3], methylcellulose 400 cps[3], gelatin[3], sodium riboflavin-5′-phosphate dihydrate[3], talc[3] (fine powdered, acid purified), sodium taurocholate[3], sodium tauroglycocholate[3], sodium deoxycholate[3], cetrimonium bromide[3], porcine mucin[4], sodium cholate[4], lactose USP[5], skim milk (household), sodium glycocholate[6], egg albumin[7], silica[8], lecithin[9] (90% bovine), and activated charcoal[10]. The polydimethysiloxane membranes[11], 0.014 cm thick, contained a silica filler.

RESULTS AND DISCUSSION

Permeation through Polydimethylsiloxane—The permeability coefficient, P, is a measure of drug transfer from bulk solution on one side of the membrane to bulk solution on the other side. In a well-defined system, P is the product of the drug partition coefficient, K_p, and the diffusion coefficient, D_m, within the membrane. The polydimethylsiloxane membranes used here, however, contain silica filler which affects both K_p and D_m. Furthermore, $P = K_pD_m$ only if drug diffusion through the aqueous diffusion layers on either side of the membrane is not rate limiting. For these reasons, the P value reported here is not a characteristic of the phenylbutazone–polydimethylsiloxane system. It is valid only for the particular membrane and apparatus used in the experiments described in this paper.

Under steady-state conditions, P can be calculated from:

$$P = \frac{(dq/dt)l}{AC} \qquad \text{(Eq. 1)}$$

where dq/dt is the rate at which drug permeates the membrane under steady-state conditions; l and A are membrane thickness and surface area, respectively; and C is the concentration of unionized drug outside the permeation cell. C is relatively large and remains essentially unchanged throughout the experiment, while the unionized drug concentration within the diffusion cell is held at zero by suitable choice of pH. This choice of conditions permits steady-state diffusion.

Effect of pH on Permeation through Polydimethylsiloxane—The permeation rate of ionizable drugs is highly dependent on the pH of the drug solution. Before permeability coefficients obtained from experiments at different pH's can be calculated and compared, the relationship between pH and the coefficient must be known. The concentration of unionized drug was obtained from:

$$C = C_0[1 + \exp 2.303(\text{pH} - \text{pKa})]^{-1} \qquad \text{(Eq. 2)}$$

where C_0 is the total drug concentration and pKa = 4.5. Permeation rates were measured over the 2.30–8.04 pH range, and P was calculated from Eqs. 1 and 2. The results (Fig. 2) show that P, labeled the apparent permeability coefficient, is pH dependent contrary to the pH-partition principle (9) and previous experience with this technique (3). The pH dependence of P may be due to use of an incorrect pKa value or to more complex pH effects than are implied by the pH-partition principle. It is highly improbable that ionic permeation occurs in polydimethylsiloxane rubber or that its properties are pH dependent because of the very low solubility of ions in the membrane.

The pKa of phenylbutazone, determined by the spectroscopic method, is reported to be 4.5 (10). In addition, the solubility behavior of phenylbutazone calculated from this pKa (11) is in agreement with the experimentally determined solubility (12, 13). The spectroscopic determination of the pKa was repeated, giving substantially the same result as that already reported. In this study, therefore, the pKa was taken as 4.5.

According to some reports (14–16), the pH-partition theory is a special case of a more general theory of pH effects on drug permeation. From Eqs. 19, 35a, and 36 in the Suzuki *et al.* (14) paper, for an acidic drug:

$$\frac{dq}{dt} = -V\frac{dC_0}{dt} = \frac{AD_aC_0}{h}\left[\frac{1}{\left(1 + \frac{K_a}{[H^+]_m}\right)\frac{D_al}{hD_mK_p} + 1}\right] \qquad \text{(Eq. 3)}$$

where V is the volume of the bulk drug solution; D_a and D_m are the diffusion coefficients of the drug in water and in the membrane, respectively; h is the thickness of the aqueous diffusion layer; $[H^+]_m$ is the hydrogen-ion concentration at the surface of the membrane; K_p is the partition coefficient of the unionized drug between the membrane and the aqueous solution; and t is time.

Rearranging Eq. 3 yields:

$$P = D_mK_p = \frac{(dq/dt)l}{AC_0}\left\{[1 + \exp 2.303(\text{pH} - \text{pKa})] + \frac{1}{T}\right\} \qquad \text{(Eq. 4)}$$

where:

$$T = \frac{D_al}{K_phD_m} \qquad \text{(Eq. 5)}$$

In a well-buffered solution, the pH at the membrane surface would not differ substantially from that in the bulk of the solution. Hence, $[H^+]_m = [H^+]$. If T is large, the T^{-1} term in Eq. 4 becomes negligible and Eq. 4 reverts to the relationship predicted by the pH-partition hypothesis (Eqs. 1 and 2). The permeation of amobarbital and other drugs (3) was described by the pH-partition hypothesis. If T is not large, as was the case in this work, it cannot be ignored and Eq. 4 must be used to describe the effect of pH on the permeability coefficient. If T is taken as 0.063, permeability coefficients calculated from the data in Fig. 2 are independent of pH at the 95% level of confidence. On the basis of 29 experiments, $P = (4.88 \pm 0.35) \times 10^{-5}$ cm^2 sec^{-1}. The term T was estimated by writing Eq. 4 for two independent experiments, assuming P to be the same in each case, and solving for T. The fact that T^{-1} is not negligible indicates that the permeation rate is being influenced by the aqueous diffusion layer at the membrane surface.

Permeation through Everted Intestine—One difficulty encountered in permeation work in animal membranes is the large variability among individuals. Meaningful results are obtained only by repeating each experiment several times. For example,

[3] British Drug House.
[4] Sigma Chemical.
[5] Merck.
[6] K & K Laboratories.
[7] Miles.
[8] Cabosil M-5, Cabot Carbon.
[9] Nutritional Biochemicals.
[10] Darco G-60, Anachemia.
[11] Dow-Corning.

Table I—Effect of Excipients on Phenylbutazone Permeation

Excipient	Polydimethylsiloxane Membrane: Excipient Concentration, g liter^{-1}	Polydimethylsiloxane Membrane: Permeability Coefficient, P, (cm^2 sec^{-1}) × 10^5	Everted Rat Intestine: Excipient Concentration, g liter^{-1}	Everted Rat Intestine: Permeability Factor Ratio[a], P_f
Lactose	10.1	5.54	30	1.01, 0.97
	25.6	5.41	—	1.15
	53.0	5.33	—	—
Starch	4.5	5.18	30	0.98, 0.91
	22.6	5.26	—	—
Talc	1.9	5.24	—	—
	19.7	5.10	—	—
Kaolin	10.6	4.97	—	—
Magnesium stearate	2.1	5.40	10.6	0.92, 0.93
	16.4	4.75		—
Calcium chloride	0.6	4.94	—	—
	2.1	4.64	—	—
Acacia	2.8	4.82	—	—
	19.6	5.01	—	—
Fumed silica	0.5	4.62	—	—
	4.2	4.33	—	—
Gelatin	4.0	5.21	30	0.98, 0.91
	19.8	4.90	—	—
	40.0	3.67	30	1.04, 0.81
Methylcellulose	3.8	4.59	19.8	0.86, 0.74
	11.6	4.39	—	—
	18.9	3.52	—	—
Polyvinylpyrrolidone	5.5	3.72	19.1	0.58, 0.59
	12.5	3.36	—	—
	21.7	2.53	—	—
Calcium sulfate	10.1	5.10	18.7	1.01, 1.04
	40.2	4.58	—	—

[a] The permeability factor for drug from solutions of drug divided by the factor from solutions of drug and excipient.

Kaplan and Cotler (17), studying absorbability in a series of drugs, found it necessary to do from three to 41 runs, depending upon the drug, to obtain a satisfactory measurement of drug absorption. To reduce this source of variability, two rate measurements in the same intestinal segment were carried out and the ratio of the permeation rates was calculated (7). To determine the reproducibility of the ratio technique, two drug solutions, one half as concentrated as the other, were used. The rate of permeation of one solution was measured over an initial 50-min period followed by rate measurement of the second solution over the second 50 min. For eight segments the mean of the ratio was 0.51 ± 0.04. A ratio of 0.50 is predicted by Eq. 1. On the basis of these results, drug additive interactions were considered significant if the rate ratios were less than 0.90 or greater than 1.10. A ratio of unity indicates no interaction.

Effect of pH on Intestinal Permeation—The permeation of phenylbutazone through the everted rat intestine does not obey the classical pH-partition hypothesis, as is also the case with other drugs. Hogben *et al.* (18) attributed noncompliance with the pH-partition theory to the existence of an acidic zone at the lumen-epithelial border, with the acidity being maintained as long as the membrane cells live. Nogami and Matsuzawa (19, 20), on the other hand, attributed noncompliance to permeation of the intestine by drug ions. The concept of the pH static epithelial border was developed in connection with *in vivo* work using weak buffers and its applicability to *in vitro* work is questionable. Although the *in vitro* results of the present study can be interpreted on the basis of ion permeation, yielding a ratio of ionized to unionized permeation rates of about 0.48, this approach was not adopted because the results can be described more simply in terms of the general theory of pH effects of Suzuki *et al.* (14). This is not to say, however, that some permeation of ionized drug does not take place *in vitro*.

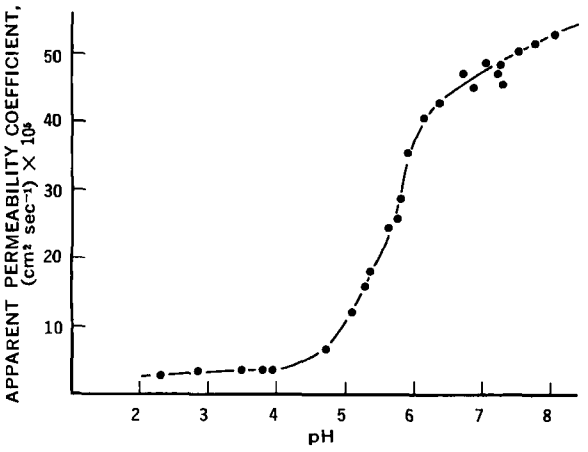

Figure 2—*Plot of apparent permeability coefficient,* P, versus *pH for phenylbutazone solutions.* P *was calculated from the pH-partition theory (Eqs. 1 and 2).*

The effect of varying pH over the 5.00–7.40 range on the permeation rate was measured in a number of intestinal segments. Two solutions were presented consecutively to the mucosal side of each segment; the solutions were usually of different drug concentration and pH, and the permeation rate was measured. Ratios of the rates were not calculated in the pH experiments. A permeability factor, P_f, defined as the permeability coefficient multiplied by the membrane area and divided by its thickness, was calculated from Eq. 4 by taking the area and the thickness as unity and $T = 1.26 \times 10^{-3}$. Other than cutting the segments to the same length for each experiment, no attempt was made to measure surface area or thickness of the intestinal segments. The factor T was found by the same procedure used for the polydimethylsiloxane membranes. Based on 22 experiments carried out on 11 intestinal segments, P_f was found to be 3.79 ± 1.00 cm^3 sec^{-1} with a coefficient of variation of 26%. Coefficients of this magnitude are typical of those observed in work with animal membranes. If ionic permeation can be neglected and if the pH at the membrane surface is the same as in the bulk solution, the value of T, small compared to that found for the polydimethylsiloxane cells, indicates that the permeation rate is controlled by the aqueous-mucus layer at the membrane surface.

Effect of Excipients—Excipients were dissolved or slurried in

Table II—Effect of Nutrients on Phenylbutazone Permeation

Nutrient	Polydimethylsiloxane Membrane: Nutrient Concentration, g liter^{-1}	Polydimethylsiloxane Membrane: Permeability Coefficient, P, (cm^2 sec^{-1}) × 10^5	Everted Rat Intestine: Nutrient Concentration, g liter^{-1}	Everted Rat Intestine: Permeability Factor Ratio[a], P_f
Sucrose	80.0	4.88	80.4	0.96, 0.99
Sodium saccharin	12.0	4.71	—	—
Skim milk powder	23.0	5.24	99.2	1.09, 0.76
	43.6	4.82	100.0	1.10, 0.83
	79.3	3.52	—	—
	100.0	2.70	—	—
Cholesterol	1.6	5.05	—	—
Egg lecithin	3.0	4.93	—	—
Egg albumin	0.5	4.70	—	—
Bovine albumin	2.1	3.43	5.0	0.74
	5.2	1.83	—	—
	8.0	1.14	—	—
Porcine mucin	3.5	4.79	19.8	1.39, 1.54
Ferrous sulfate			0.8	0.96, 0.96

[a] The permeability factor for drug from solutions of drug divided by the factor from solutions of drug and nutrient.

Table III—Effect of Surfactants on Phenylbutazone Permeation through Polydimethylsiloxane

Total Quantity of Drug, Q, (moles liter^{-1}) × 10^4	pH of Drug Solution	Weight of Surfactant, W_m, g liter^{-1}	Apparent Permeability Coefficient, P_a, (cm^2 sec^{-1}) × 10^5
	Sodium Lauryl Sulfate		
3.912	7.19	4.9	4.26
3.946	7.14	10.4	3.61
3.850	7.15	19.9	3.66
3.978	7.17	30.3	3.24
3.954	7.00	50.0	2.09
3.967	7.00	80.8	1.72
	Polysorbate 80		
4.017	7.26	2.1	4.60
4.011	7.28	11.7	3.04
4.297	7.27	25.5	2.03
3.935	7.13	33.4	1.27
	Cetrimonium Bromide		
4.497	7.30	0.104	4.21
3.746	7.35	0.200	2.51
4.284	7.29	0.297	1.72
3.863	7.28	0.477	0.78
3.978	7.29	0.903	0.00

phenylbutazone solutions, and the permeation rates through polydimethylsiloxane were measured (Table I). The permeability coefficients were compared to that of drug alone, (4.88 ± 0.35) × 10^{-5} cm^2 sec^{-1}, and any change greater than two standard deviations, *i.e.*, $p < \sim 0.05$, was considered to indicate a significant interaction between the drug and the excipient. The effect of excipient on the permeation rate through everted intestine was also evaluated. This was done by measuring the permeation rate of drug alone through a segment and then the permeation rate of the drug in the presence of the excipient, using the same segment. The results were expressed as the ratio of the permeability factor in the presence of excipient to the factor for drug alone (Table I). Ratios less than 0.90 were taken to indicate a significant interaction between drug and excipient.

The excipients examined are among those commonly present in Canadian formulations of phenylbutazone. No interaction was found between drug and lactose, starch, talc, kaolin, magnesium stearate, calcium chloride, acacia, fumed silica, or calcium sulfate. Gelatin in polydimethylsiloxane and methylcellulose and polyvinylpyrrolidone in both membranes interfere with permeation of the drug but only at concentrations much higher than would be encountered in an actual formulation. The permeability coefficient of phenylbutazone through polydimethylsiloxane is inversely proportional to the concentration of polyvinylpyrrolidone, with a correlation coefficient of −0.99. This relationship suggests a drug–excipient interaction rather than a viscosity effect, which would lead to a more complex dependence of the coefficient on the excipient concentration.

Effect of Nutrients—Permeation of drug in the presence of various nutrients was measured using polydimethylsiloxane, everted rat gut membranes, or both (Table II). Sucrose, sodium saccharin, cholesterol, ferrous sulfate, egg lecithin, and egg albumin were without effect at the concentrations examined. In the presence of dissolved skim milk powder, the permeability coefficient of phenylbutazone through polydimethylsiloxane was inversely proportional to the concentration of dissolved powder, with a correlation coefficient of −0.99. This indicates a physical interaction, possibly adsorption or binding, since a chemical reaction would probably go to completion in the presence of the large excess of milk powder. The results obtained with the intestinal segment are less clearcut, although it is evident that an interaction occurred.

Permeation of drug through both membranes is retarded if bovine albumin is present. Again the synthetic membrane results are inversely proportional to the albumin concentration (r = −0.98), suggesting a physical interaction such as adsorption or binding. Permeation of drug through polydimethylsiloxane was unaffected by porcine mucin, indicating that no appreciable interaction takes place. The permeation rate through the everted intestine was substantially increased, however, and must be the result of an interaction between the mucin and the surface or contents of the intestine rather than an effect involving the drug,

Table IV—Effect of Bile Salts on Phenylbutazone Permeation

Bile Salt	Polydimethylsiloxane Membrane: Bile Salt Concentration, g liter^{-1}	Polydimethylsiloxane Membrane: Apparent Permeability Coefficient, P_a, (cm^2 sec^{-1}) × 10^5	Everted Rat Intestine: Bile Salt, (moles liter^{-1}) × 10^2	Everted Rat Intestine: Rate Ratio: Drug[a] First	Everted Rat Intestine: Rate Ratio: Drug and Bile Salt First[b]
Sodium deoxycholate	0.52	4.93	—	—	—
	1.93	4.19	4.01	2.22	1.05
	4.0	4.48	—	—	—
	9.8	4.60	—	—	—
	16.4	4.08	—	—	—
	41.9	4.25	—	—	—
Sodium tauroglycocholate	19.7	3.30	—	—	—
	26.2	3.75	—	—	—
	50.5	3.35	—	—	—
	79.4	3.14	—	—	—
Sodium glycocholate	2.1	4.62	3.98	2.32	1.09
	9.2	4.37	—	—	—

[a] The permeation rate was measured for drug alone, after which the rate was measured for drug in solution with bile salt. The rate ratio is the former divided by the latter. [b] The permeation rate was measured for drug in solution with bile salt, after which the rate was measured for drug alone. The rate ratio is the latter divided by the former.

since mucin had no effect on the role of permeation through polydimethylsiloxane.

Effect of Surfactants—It was shown previously that the reduction in permeation rates observed in the presence of surfactants could be attributed to a reduction in the aqueous drug concentration occasioned by drug partition into surfactant micelles (3). This approach yields the relationship:

$$\frac{Q}{C_w W_w} = \left(\frac{K_p'}{\rho_m}\right)\frac{W_m}{W_w} + \frac{1}{\rho_w} \qquad \text{(Eq. 6)}$$

where Q is the total quantity of drug in the system; C_w is the quantity of drug in the aqueous phase; W_m and W_w are the weights of the micellar and water phases, respectively; and ρ_m and ρ_w are the respective densities. K_p' is the ratio of unionized drug in the micellar phase to that in the aqueous phase. From Eq. 6, K_p' can be determined from the slope of a plot of $Q/C_w W_w$ *versus* W_m/W_w. C_w was calculated from the expression:

$$C_w = \frac{P_a Q}{P} \qquad \text{(Eq. 7)}$$

where P_a is the experimentally determined, apparent permeability coefficient in the presence of the surfactant; P was previously shown to be (4.88 ± 0.35) × 10^{-5} cm^2 sec^{-1}. Equation 7 is based on the assumption that the decrease in P is due to the reduced concentration of free drug caused by partitioning into the micellar phase. The density of the surfactant was taken as unity, and it was assumed that water and surfactant volumes were additive upon mixing.

For permeation through polydimethylsiloxane, the data (Table III) yield a slope of 2.28 × 10^{-2} liter g^{-1} and an intercept of 0.99 × 10^{-3} liter g^{-1} (r = 0.98) for sodium lauryl sulfate. The slope and intercept obtained from experiments with polysorbate 80 were 8.39 × 10^{-2} liter g^{-1} and 0.72 × 10^{-3} liter g^{-1} (r = 0.96), respectively. The main effect of cetrimonium bromide is complexation or salt formation between the phenylbutazone anion and the surfactant cation, since very low concentrations of surfactant completely remove free drug from solution. It is not possible to calculate the partition coefficient from the cetrimonium bromide data, because the analysis does not distinguish between drug lost to micelles and to reaction. The partition of phenylbutazone between water and micelles of sodium lauryl surfate and polysorbate 80 yields partition coefficients, K_p', of 23 and 84, respectively.

In the rat intestine, cetrimonium bromide exhibits the drug interaction effect previously discussed. At low concentrations, the other surfactants discussed here are without apparent effect; at high concentrations, the membranes are attacked, becoming completely transparent.

Effect of Bile Salts—Bile salts are surfactants with a weak effect on the permeation of phenylbutazone through polydimethylsiloxane membranes (Table IV). Equation 6 was applied to sodium tauroglycocholate, yielding a slope of 3.9 × 10^{-3} liter g^{-1} and an intercept of 1.33 × 10^{-3} liter g^{-1} (r = 0.82). The partition coefficient was 3.9. This value, low compared to that of nonbiological surfactants, is a manifestation of the relatively poor solubility of phenylbutazone in micelles of these compounds. Changes in the permeability coefficient observed in studies of sodium deoxycholate and sodium glycocholate were not significant.

Permeation in everted rat intestine is appreciably enhanced by bile salts. The bile salt appears to interact with the membrane since the permeation rate through the segment is permanently increased after being exposed to bile salt at concentrations approximating those *in vivo*. The rate does not decrease when the solution of drug and bile salt is replaced by one containing the drug alone. Bile salts are observed to have similar effects on the permeation of salicylate (21), salicylamide (22), and riboflavin (23) through the everted rat intestine.

The apparent relationship (24) between bile salt concentration below the critical micelle concentration (CMC) and membrane permeability suggests that an equilibrium may exist between the concentration of bile salt in solution and that taken into the intestinal segments. Bile salt dissolved in the lipoidal cell wall membranes may be plasticizing the membranes, thereby enhancing their permeability. Plasticization results from the dissolution of low molecular weight compounds in high molecular weight materials. Physical properties, including permeability, undergo substantial changes. Insufficient evidence is currently available to determine whether the observed effect of bile salts on the membrane is plasticization. It is clear, however, that bile salts interact with membranes, making them more permeable. Bile salt molecules agglomerated into micelles do not appear to have much effect on the permeability of the membrane, although they undoubtedly play an important role in other aspects of the digestive process.

CONCLUSIONS

None of the excipients examined significantly affects the permeation of phenylbutazone when present in concentrations liable to be used in formulating the drug. Interactions with a number of other materials found in the GI tract occurred but, generally speaking, these materials cannot be avoided *in vivo*. The dependence of the permeability coefficient on T^{-1} (Eq. 4) is the first example of which the authors are aware involving a widely used drug in a synthetic rubber membrane, and it demonstrates the importance of the diffusion layer at the membrane surface. Further studies of the factors comprising T and their effect on the permeation of phenylbutazone are currently in progress. Comparable results were obtained from the experiments on polydimeth-

ylsiloxane and everted intestine only when materials not interacting with the membranes were under examination. If membrane interaction occurs, it is difficult to draw conclusions about possible interactions between the drug and the substance added to the drug solution.

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Surfactant Effects on Drug Absorption III: Effects of Sodium Glycocholate and Its Mixtures with Synthetic Surfactants on Absorption of Thiamine Disulfide Compounds in Rat

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Abstract □ The effects of surfactants, such as sodium glycocholate and its related compounds or mixtures, on the absorption and metabolism of the model compounds, *O*-benzoylthiamine disulfide and thiamine disulfide were evaluated by both *in situ* and *in vivo* experiments using rats. The *in situ* results indicated that, in the presence of a biosurfactant, the increasing or decreasing effect of a synthetic surfactant on drug absorption and metabolism could be canceled by a possible formation of new mixed micelles consisting of the drug and both surfactants. Results were confirmed by oral experiments using the drug-micellar solution in rats.

Keyphrases □ Surfactants (sodium glycocholate, sodium lauryl sulfate, and polysorbate 80)—effects on absorption and metabolism of thiamine disulfide compounds, rats □ Drug absorption (thiamine disulfide compounds)—effects of biosurfactants and synthetic surfactants, rats □ Absorption (thiamine disulfide compounds)—effects of biosurfactants and synthetic surfactants, rats □ Thiamine disulfide compounds—effects of biosurfactants and synthetic surfactants on absorption and metabolism, rats □ Sodium glycocholate—effects on absorption and metabolism of thiamine disulfide compounds, rats

When a drug is entrapped into micelles, the thermodynamic activity (1) of the drug is decreased and the absorption is thereby suppressed while the stability of the drug is increased. However, previous studies (2) indicated that the reaction rates of the thiol–disulfide exchange reaction between thiamine disulfide compounds and thiols were influenced (acceleration or inhibition) by sodium lauryl sulfate, polysorbate 80, and sodium glycocholate, depending on the concentrations of surfactants, the lipophilic character of the disulfide, and the thiols used. These findings suggest that the thermodynamic activity of drugs is not always decreased by the solubilization or interaction with surfactants. Another study (3), which included kinetic treatment of the drug–surfactant interaction, indicated that *O*-benzoylthiamine disulfide (I) interacted with the lauryl sulfate anion to form a 1:2 molar complex and that this complex is