Drug Permeation through Membranes I: Effect of Various Substances on Amobarbital Permeation through Polydimethylsiloxane

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Abstract ☐ The effect of some excipients, nutrients, surfactants, and adsorbents on the permeation of amobarbital through polydimethylsiloxane was measured. At a pH of 5, the permeability coefficient, P, of amobarbital is $(7.70 \pm 0.34) \times 10^{-8}$ cm.^{2} sec.$^{-1}$. The rate of drug transfer across the membrane depends on pH because only the unionized species is eligible for transfer. If P is calculated from the actual concentration of unionized drug, then transfer is not a function of pH. Excipients typical of those used in Canadian formulations of amobarbital or its sodium salt have no significant effect on the coefficient of permeability. Bovine albumin has no effect, but the coefficient is depressed by skim milk, perhaps due to binding of the drug. The drug is strongly adsorbed by charcoal. The permeation coefficient is reduced by anionic, cationic, and nonionic surfactants in a way that is consistent with the concept of drug partition between the aqueous and surfactant phases present when surfactant micelles form in water. After dissolution, the permeability coefficients of all Canadian amobarbital preparations tested were found to be identical.

Keyphrases ☐ Drug permeation through membranes—effect of excipients, nutrients, surfactants, and adsorbents on amobarbital permeation through polydimethylsiloxane ☐ Amobarbital, permeation through polydimethylsiloxane membranes—effect of excipients, nutrients, surfactants, and adsorbents, permeability coefficients of 14 formulations ☐ Permeation, amobarbital through polydimethylsiloxane membranes—effect of excipients, nutrients, surfactants, and adsorbents ☐ Surfactant effect—amobarbital permeation through polydimethylsiloxane membranes ☐ Excipient effect—amobarbital permeation through polydimethylsiloxane membranes

While always a matter of concern (1), bioavailability and drug absorption have become topics of enlarged interest because of the economically motivated need to compare the effectiveness of different formulations of the same drug. Drug absorption can be envisaged as a two-step process: disintegration and dissolution in the GI tract followed by permeation through the gut wall into the blood. Both of these processes can be modeled *in vitro*. Extensive *in vitro* dissolution studies were carried out and led to USP (2) and NF (3) dissolution specifications for certain drugs.

Drug permeation measurements, while not used as a means to control and evaluate formulations, have been applied to the study of interactions between drugs and other materials liable to affect absorption from the GI tract. The theoretical aspects of drug absorption were discussed by Suzuki *et al.* (4) and Wagner (5), while *in vitro* experimental work with animal membranes was reported by a number of workers (6–8). Synthetic membranes are of value in studying the interactions of drugs that cross the gut wall primarily by a partition–diffusion mechanism, where the rate of transfer across the membrane depends mainly on the membrane–aqueous phase partition coefficient and the solubility of drug in the membrane. If permeation rates of a series of similar drugs through a synthetic membrane are arranged in

rank order, one would expect to find the same order of permeation rate *in vivo*, provided no significant interaction between drug and membrane takes place. This behavior is exhibited by the barbiturates (9).

In comparison to animal membranes, synthetic membranes are uniform, reproducible, easy to handle, stable, and cheap. For convenient, reproducible rates of permeation, the membranes should be elastomeric and well above their glass temperature. The material used should be noncrystalline at the temperature of the experiment to avoid variations in permeability due to changes in the degree of crystallinity. The latter depends upon the temperature and rate of crystallization, molecular weight, molecular weight distribution, degree of branching, and other factors (10). Variations in membrane permeability may also arise from the presence of filler, plasticizer, and changes in the degree of cross-linking (11). While a number of synthetic polymers have been tried for drug interaction studies (9), most workers choose polydimethylsiloxane, probably because it is one of the most permeable, nondialysis types of elastomers known (11). The effect of high surface area silica filler, present in commercially available polydimethylsiloxane membranes, was examined by Most (12) and Flynn and Roseman (13). Filler adds to the difficulty of membrane characterization and may lead to variation in the coefficient of permeability between different specimens of polydimethylsiloxane. Drugs and drug interactions studied by this technique include barbiturates, phenylalkylamines (9), aminophenones (14, 15), chlorpromazine (16), and various organic molecules (17).

This paper reports the effect of typical excipients, endogenous substances, adsorbents, emulsifiers, and foods on the permeation of amobarbital through polydimethylsiloxane and rat intestinal membranes. The work is part of an extensive program of biopharmaceutical studies involving the economically most important drugs sold in Canada.

EXPERIMENTAL

Permeation Apparatus—Steady-state permeation coefficients were measured using a cell of the type described by Garrett and Chemburker (18), modified by placing a magnetic stirring bar inside the cell. The cell was placed in a beaker containing a buffered solution of the drug, and both the cell and beaker, stirred magnetically, were thermostated at $37 \pm 1.0°$. The cell membranes, made of polydimethylsiloxane, were 0.014 cm. thick and contained about 30 parts of silica filler (13). During an experiment the cell contained borate buffer solution at pH 10 and the beaker contained about 160 ml. of sodium amobarbital solution at a concentration in the region of 3.0×10^{-3} M. Under these conditions, steady-state diffusion was reached about 1 hr. after beginning the experiment. The permeation coefficient did not change when the same membrane was used in successive experiments.

Table I—Permeability Coefficients Calculated from Measurements Made over a Range of pH Values

pH	Concentration of Unionized Drug, C_0, $M \times 10^3$	Apparent Permeation Coefficient[a], P_a, (cm.^{2} sec.$^{-1}$) $\times 10^8$	Actual Permeation Coefficient[b], P, (cm.^{2} sec.$^{-1}$) $\times 10^8$
6.03	2.428	8.22	8.35
6.98	2.906	6.59	7.46
7.05	2.867	6.24	7.21
7.51	2.204	5.11	7.36
		5.29	7.62
		5.17	7.45
7.52	1.927	5.24	7.61
		5.34	7.75
		5.26	7.64
7.61	2.002	4.92	7.69
7.85	1.725	3.62	7.16
8.03	1.301	2.87	7.12
8.38	0.825	1.66	7.17
		Mean	7.51 ± 0.33

[a] Calculated from the total drug concentration, C. [b] Calculated from C_t, the concentration of unionized drug.

Analysis—The desorbing borate solution was pumped continuously through the cells of a UV spectrometer, and readings at 240.5 nm. were taken manually at appropriate time intervals. The time correction due to the external loop was less than 10 sec. and was neglected. Calibration of the spectrometer was with USP grade sodium amobarbital. Beer's law was obeyed. The extinction coefficient was 9830 in borate buffer containing 3.092 g. l.$^{-1}$ boric acid, 3.782 g. l.$^{-1}$ potassium chloride, and sufficient sodium hydroxide to yield a final pH of 10.00 (about 1.75 g. l.$^{-1}$).

Intestinal Preparation—Intestinal segments were obtained from fasted, male, Sprague-Dawley rats, weighing 240–280 g., after killing by decapitation or carbon dioxide. Ten-centimeter segments of the proximal jejunum under a tension of 20 g. were mounted, non-everted, in a system of glass and polyethylene tubing, which permitted continuous flow of fresh 2.0 × 10^{-3} M sodium amobarbital in Krebs–Ringer bicarbonate buffer (19) at a pH of 7.4 through the segment to discard (20). Drug transfer across the intestine was into 50 ml. of serosal solution of the buffer without the drug. All solutions, at 37 ± 1°, were continuously oxygenated by bubbling an oxygen-carbon dioxide (95:5) mixture through them. Two-milliliter aliquots were taken at 10-min. intervals for analysis and replaced by fresh buffer solution.

Drug Extraction and Analysis—Blank experiments made in the absence of drug showed that some substance which absorbs strongly in the UV is extracted from the intestinal segments under the experimental conditions, making a direct UV analysis impossible. For analysis, the serosal aliquot pH was lowered to 3.2 by adding 1 ml. of 0.05 N hydrochloric acid. The drug was extracted into 10 ml. chloroform by shaking overnight at room temperature. Eight milliliters of the chloroform layer was shaken for 30 min. with 2 ml. 0.45 N sodium hydroxide. About 76% of the drug was recovered by this procedure. Absorption in the aqueous layer was read at 255 nm., and the drug concentration was read from a calibration curve.

Materials—The following were used: sodium amobarbital USP[1]; lactose USP[2]; calcium sulfate dihydrate[3]; acacia USP[4]; starch USP[4]; calcium stearate, technical[5]; talc, fine powdered, acid purified[6]; activated charcoal[7]; bovine albumin, fraction V[6]; skim milk, household; caffeine USP[8]; sodium lauryl sulfate USP[4]; cetrimonium chloride[8]; polysorbate 80[9]; sodium cholate[10]; and gelatin BP[6].

[1] May and Baker.
[2] Merck.
[3] Analar, British Drug House.
[4] Fisher.
[5] E. H. Sargent.
[6] British Drug House.
[7] Darco G-60, Anachemia.
[8] Eastman.
[9] Tween 80, Fisher.
[10] Sigma Chemical.

Table II—Effect of Excipients on the Permeability Coefficient of Amobarbital

Excipient	Concentration[a], g. l.$^{-1}$	Permeability Coefficient, P, (cm.^{2} sec.$^{-1}$) $\times 10^8$
Lactose	3.4	7.23
	10.5	7.45
	41.3	7.83
	Mean	7.50 ± 0.31
Calcium sulfate dihydrate	1.8	7.34
	1.8	7.10
	10.6	7.73
	39.8	7.71
	Mean	7.47 ± 0.31
Acacia	5.0	7.23
Gelatin	5.0	7.05
	10.2	7.36
	42.8	7.88
	Mean	7.42 ± 0.42
Corn starch	4.0	7.12
	10.8	7.32
	41.2	7.25
	Mean	7.23 ± 0.10
Calcium stearate	2.4	7.77
	2.4	7.54
	Mean	7.65 ± 0.16
Talc	3.0	7.02
	5.2	6.81
	10.6	6.72
	20.0	6.91
	Mean	6.87 ± 0.13

[a] In drug solution at a pH of 5.

RESULTS AND DISCUSSION

Permeability Coefficient—The effect of various substances on the permeation of amobarbital through a polydimethylsiloxane membrane is expressed in terms of a permeability coefficient, P:

$$P = kD \qquad \text{(Eq. 1)}$$

where k is a constant for a given set of experimental conditions and D is the diffusion coefficient. Permeation refers to the transfer of drug from solution on one side of the membrane to solution on the other side, whereas diffusion is movement of drug within the membrane. If the concentration of drug in the membrane at the absorbing side is c and it is zero at the desorbing side and if steady-state conditions prevail, then the quantity of drug, q, transferred across the membrane at time t is:

$$q = \frac{Dc}{l}\left(t - \frac{l^2}{6D}\right) \qquad \text{(Eq. 2)}$$

where l is the thickness of the membrane (21). The concentration of drug in the membrane at the surface in contact with the solution is proportional to C_0, the concentration of unionized drug in solution and to the conditions of the experiment. Thus:

$$c = kC_0 \qquad \text{(Eq. 3)}$$

and Eq. 2 can be written as:

$$q = \frac{DkC_0}{l}t - \frac{kC_0 l}{6} \qquad \text{(Eq. 4)}$$

The experimental results were plotted as q versus t. Upon reaching the steady state, the slope, dq/dt, is given by DkC_0/l. Substitution into Eq. 1 yields:

$$P = \frac{(dq/dt)l}{C_0 A} \qquad \text{(Eq. 5)}$$

where A is the area of membrane in contact with the drug solution and dq/dt is the rate of drug transfer.

Table III—Effect of Charcoal on the Permeability Coefficient

Charcoal Concentration[a], g. l.$^{-1}$	Apparent Permeability Coefficient[b], P_a, (cm.^{2} sec.$^{-1}$) × 10^8	Permeability Coefficient[c], P, (cm.^{2} sec.$^{-1}$) × 10^8
1.0	5.56	—
—	6.34	—
1.8	4.09	6.95
4.5	1.57	8.14
6.8	0.68	7.58
16.7	0.00	—
	Mean	7.55 ± 0.59

[a] The solution pH was 5. [b] Calculated from the original concentration of amobarbital before mixing with charcoal. [c] Calculated from the concentration of amobarbital measured spectroscopically in the supernatant liquid after mixing with charcoal.

Dependence of Permeability on pH—The permeability coefficient of amobarbital going from an acetate buffer at pH 5.00 ± 0.02 was found to be 7.70 × 10^{-8} cm.^{2} sec.$^{-1}$, with a standard deviation of ±0.34 cm.^{2} sec.$^{-1}$ based on 10 determinations. The rate of drug transfer across the membrane is pH dependent because only the nonionized form is soluble in the membrane and hence eligible for transfer. The concentration of nonionized drug in solution, C_0, is given by:

$$C_0 = C\{1 + \exp[2.303 (pH - pKa)]\}^{-1} \qquad (Eq. 6)$$

where C is the total concentration of drug substance, pH is the pH of the drug solution, and K_a is the ionization constant of the drug. Taking pKa = 7.865 for amobarbital (22), only 0.1% of the drug is ionized at a pH of 5. Permeability coefficients obtained from measurements at pH values in the range where C_0 changes rapidly with pH are given in Table I. The apparent permeability coefficient, P_a, is calculated from Eq. 5 using C, the total concentration of amobarbital present in the system, and reflects the effect of pH on drug transfer. The coefficient P, calculated from the actual concentration of unionized drug, is obtained from Eq. 5. The permeability coefficient P is (7.51 ± 0.33) × 10^{-8} cm.^{2} sec.$^{-1}$ and does not vary significantly over the pH range examined. This constancy indicates the only effect of pH on permeability is the effect on concentration of unionized drug.

Effect of Excipients and Charcoal—The effect of a number of substances, commonly present as excipients in Canadian formulations of amobarbital, on the permeability coefficient was measured. The results (Table II) show these substances to have no significant effect on the permeability coefficient.

The consequence of adding activated charcoal to a drug solution

Table IV—Effect of Some Nutrients on the Permeability Coefficient

Additive	pH	Concentration, g. l.$^{-1}$	Permeability Coefficient[a], P, (cm.^{2} sec.$^{-1}$) × 10^8
Bovine albumin	5.00	2.0	7.69
	5.00	19.6	7.22
	7.51[b]	4.5	7.02
	7.51[b]	10.7	7.32
	7.51[b]	15.7	6.20
	7.44[b]	31.0	6.80
Skim milk	5.00	38.7	6.34
	5.00	85.6	5.50
	7.30[b]	43.0	7.07
	7.15[b]	80[c]	6.58
	7.01[b]	127	5.45

[a] Calculated from C_0, the concentration of unionized drug. [b] Measured pH after dissolving drug and additive in phosphate buffer at pH 7.40. [c] This is the approximate concentration of reconstituted skim milk.

Table V—Effect of Sodium Lauryl Sulfate on Amobarbital Permeation[a]

Weight of Surfactant, W_m, g./l.	Apparent Permeability Coefficient, P_a, (cm.^{2} sec.$^{-1}$) × 10^8	Total Drug, Q, (moles) × 10^3	$\frac{Q}{C_w W_w}$, (1 g.$^{-1}$) × 10^3	W_m/W_w, × 10^3
24.39	1.36	2.433	5.81	25.1
18.52	1.67	2.821	4.72	18.9
6.17	3.21	2.612	2.42	6.22
0.81	6.65	2.559	1.16	0.81
0.81	6.73	2.559	1.16	0.81

[a] Drug–surfactant solution at pH 5.

is shown in Table III. This material presumably adsorbs drug, thereby reducing the solvent concentration and hence the coefficient of permeability calculated on the basis of the original drug concentration (23). If the actual concentration of drug is used to calculate the coefficient, it should be, within experimental error, the same as that calculated for drug with no additive, namely (7.70 ± 0.34) × 10^{-8} cm.^{2} sec.$^{-1}$. This is the case with P = (7.55 ± 0.59) × 10^{-8} cm.^{2} sec.$^{-1}$ in the presence of charcoal. There is a good correlation (r^2 = 0.92) between the weight and, hence, surface area of the activated charcoal and the apparent permeability coefficient. The result suggests that administration of activated charcoal might be of value in the early stages of amobarbital poisoning.

Effect of Nutrients—The effect of bovine albumin and skim milk on the permeation of amobarbital is reported in Table IV. There appears to be little, if any, interaction between amobarbital and bovine albumin, at least under the conditions of these experiments. There is a clear interaction with skim milk, but if the interaction occurs to the same extent *in vivo*, it is not of such magnitude as to have much effect on the absorption of the drug by the body. An attempt was made to study the effect of caffeine on the permeation coefficient, but the membrane was found to be permeable to caffeine. The latter absorbs at 240.5 nm., interfering with the amobarbital analysis. The permeation coefficient of caffeine is (2.8 ± 0.5) × 10^{-8} cm.^{2} sec.$^{-1}$.

Surfactant Effects—Drug in an aqueous system containing surfactant micelles is expected to partition between the aqueous phase and the micellar, oil, phase. The total quantity of drug in the system, Q, is given by:

$$Q = C_m V_m + C_w V_w \qquad (Eq. 7)$$

where C_m and C_w are the concentrations in the micellar and water phases, respectively, and V_m and V_w are the corresponding volumes. Substituting weight for volume in Eq. 7 and rearranging yield:

$$\frac{Q}{C_w W_w} = \frac{K_p}{\rho_m} \cdot \frac{W_m}{W_w} + \frac{1}{\rho_w} \qquad (Eq. 8)$$

where ρ_m and ρ_w are the densities of the micellar and water phases, respectively, and K_p is the partition coefficient of drug between the micellar and aqueous phases: $K_p = C_m/C_w$. Equation 8 can be plotted as a straight line with slope K_p/ρ_m and intercept $1/\rho_w$. In Eq. 8, Q and W_m are known directly from the concentrations of drug

Table VI—Effect of Cetrimonium Chloride on Amobarbital Permeation[a]

Weight of Surfactant, W_m, g./l.	Apparent Permeability Coefficient, P_a, (cm.^{2} sec.$^{-1}$) × 10^8	Total Drug, Q, (moles) × 10^3	$\frac{Q}{C_w W_w}$, (1 g.$^{-1}$) × 10^3	W_m/W_w, × 10^3
19.09	1.06	2.865	7.51	19.52
14.35	1.33	2.907	5.89	14.60
9.09	1.84	2.670	4.25	9.20
4.02	4.02	2.893	2.56	4.05
1.24	5.58	2.608	1.40	1.24
0.45	7.12	2.012	1.08	0.45

[a] Drug–surfactant solution at pH 5.

Table VII—Effect of Polysorbate 80 on Amobarbital Permeation[a]

Weight of Surfactant, W_m, g./l.	Apparent Permeability Coefficient, P_a, (cm.² sec.⁻¹) × 10⁸	Total Drug, Q, (moles) × 10³	$\frac{Q}{C_w W_w}$, (1 g.⁻¹) × 10³	W_m/W_w, × 10³
80.6	1.62	2.907	5.20	88.0
70.2	1.68	3.089	5.29	75.7
60.0	1.85	3.554	4.46	64.0
42.3	2.46	2.900	3.28	44.3
37.5	2.38	2.617	3.38	39.1
28.1	2.73	2.900	2.91	29.0
14.5	4.09	2.338	1.91	14.7
3.06	6.24	2.898	1.24	3.07

[a] Drug–surfactant solution at pH 5.0.

and surfactant in the solution and W_w is obtained by taking the density of the surfactant to be unity and the change in volume on mixing with water to be zero. This approximation introduces an error of less than 1 % for the surfactants and concentrations used in this study. The concentration of drug in the aqueous phase, C_w, was calculated from the known permeation coefficient (7.70 × 10⁻⁸ cm.² sec.⁻¹) and the measured apparent permeation coefficient, P_a. This assumes that the membrane was unaffected by surfactant and that the only effect of the latter was to remove drug from aqueous solution by incorporation into micelles. For sodium cholate studied in the region of pH 7.5, the aqueous phase concentration of nonionized drug was calculated from P_a, which was used to calculate the total aqueous concentration, C_w, from Eq. 6. Results for the four surfactants studied are presented in Tables V–VIII. The data for each surfactant were analyzed according to Eq. 8. The slope, intercept, and K_p obtained in each case are given in Table IX, along with the coefficient of correlation r.

The experimental results are adequately described by Eq. 8 and are consistent with the concept that partitioning of amobarbital between the aqueous and micellar phases takes place. Equation 8 is valid only above the CMC, which is about 2.5, 0.4, and 0.1 g. l.⁻¹ for sodium lauryl sulfate (24), cetrimonium chloride (24), and polysorbate 80 (25), respectively. Below the CMC, the surfactant has little effect on the permeation rate. This presumably means that there is little if any complexing at a pH of 5 between amobarbital and the surfactants studied. The results also indicate that there is little interaction between the surfactant and the membrane. Generally the type of interaction that could occur would be inbibing of the low molecular weight surfactant by the membrane. The swelling of synthetic elastomers by organic molecules is well known and may lead to substantial increases in the rate at which permeant molecules traverse the membrane (10, 21). The extent of imbibation depends upon the solubility of the low molecular weight species in the membrane, solubility usually being greatest for materials of similar chemical structure. The structures of the surfactants examined here and that of polydimethylsiloxane are quite different and little swelling would be anticipated, as is borne out by the results. The structure of animal membranes is closer to that of typical surfactants. Perhaps it is the dissolution of the surfactant in these membranes that

Table VIII—Effect of Sodium Cholate on Amobarbital Permeation[a]

Weight of Surfactant, W_m, g./l.	Apparent Permeability Coefficient, P_a, (cm.² sec.⁻¹) × 10⁸	Total Drug, Q, (moles) × 10³	$\frac{Q}{C_w W_w}$, (1 g.⁻¹) × 10³	W_m/W_w, × 10³
74.8	2.41	3.222	3.109	81.1
39.8	3.77	2.779	1.957	41.6
21.1	5.27	2.733	1.392	21.6
11.8	6.15	2.479	1.185	12.0
4.7	6.75	3.040	1.077	4.7

[a] Drug–surfactant solution at pH ∼ 7.5.

Table IX—Surfactant Slopes, Intercepts, and Partition Coefficients Calculated from Eq. 8

Surfactant	$K\rho_m^{-1}$, 1 g.⁻¹	ρ_w^{-1}, (1 g.⁻¹) × 10³	r^2	Partition Coefficient, K
Sodium lauryl sulfate	0.191	1.07	0.99	191
Cetrimonium chloride	0.334	1.05	0.99	334
Polysorbate 80	0.049	1.27	0.97	49
Sodium cholate	0.029	0.76	0.99	29

Table X—Effect of Sodium Lauryl Sulfate on Transfer of Amobarbital across Rat Intestine

Concentration of Sodium Lauryl Sulfate, g. l.⁻¹	$\bar{R}^a$
0.00	1.17 ± 0.09
0.01	1.15 ± 0.22
0.30	1.29 ± 0.22
10	0.49 ± 0.07
20	0.36 ± 0.06
40	0.34 ± 0.04
80	0.19 ± 0.05

[a] $\bar{R}$ is the ratio of the average drug transfer rate without surfactant to the average transfer rate in the presence of surfactant.

leads to enhanced *in vivo* absorption of certain drugs as, for example, absorption of secobarbital by goldfish (25, 26).

Results obtained for the transfer of amobarbital across the rat intestine are given in Table X. The quantity, R, is the ratio of transfer rate in the presence, and the absence, of surfactant in the same segment of gut, following the crossover procedure described by Reuning and Levy (6). Results from two or more segments were averaged to obtain $\bar{R}$. At surfactant concentrations greater than the CMC, the transfer rate clearly decreases as the surfactant increases. Results below the CMC are insufficiently precise to permit one to say whether there is a surfactant effect or not.

The partition coefficients, K_p, in Table IX indicate that amobarbital is most soluble in sodium lauryl sulfate and cetrimonium chloride. Both of these compounds have alkyl chains which might be expected to be highly compatible with the nonpolar side chains of amobarbital. There is also a good possibility of reaction between cetrimonium ion and barbiturate ion leading to a decrease in concentration of nonionized drug available for transfer. Polysorbate 80 contains repeating polar groups, while cholic acid has a dense ring structure of perhaps limited compatibility with amobarbital.

Amobarbital Formulations—The permeability of amobarbital in 14 Canadian formulations was measured. Each dosage form was stirred in phosphate buffer at 37° for about 2 hr., and the pH and concentration of the solution were measured before measuring the permeation rate. For all formulations the average coefficient of permeability was (6.87 ± 0.26) × 10⁻⁸ cm.² sec.⁻¹ with a range of 6.50 to 7.29 × 10⁻⁸ cm.² sec.⁻¹. Thus, from the point of view of *in vitro* permeability, all formulations are equivalent.

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Distribution of Dibenzoxazepines Bearing the Carboxamide or Other Side Chains in Ocular and Other Tissues of Dogs

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Abstract ☐ The distribution of four substituted dibenzoxazepines in tissues of dogs was examined 7–14 days after oral administration to intact dogs and 7 hr. after intravenous administration to dogs with externalized bile ducts. 4-[3-(7-Chloro-5,11-dihydrodibenz-[b,e][1,4]oxazepin-5-yl)propyl]-1-piperazine ethanol hydrochloride, its trifluoromethyl analog, and 5-[(2-dimethylamino)ethyl]-5,11-dihydrodibenz[b,e][1,4]oxazepine maleate were present in organs in greater concentrations than in blood, particularly in the brain, liver, lungs, and melanin-containing portion of the eye consisting of the combined retina, choroid, and sclera. These same compounds were bound to various extents to melanin granules of beef eyeball *in vitro*. 7-Chloro-5,11-dihydrodibenz[b,e][1,4]oxazepine-5-carboxamide, which bears a carboxamide substituent, was neither localized in any tissues of dogs, relative to concentrations in blood, nor bound to melanin granules *in vitro*. It is concluded that the presence of the carboxamide side chain alters the affinity of 7-chloro-5,11-dihydrodibenz[b,e][1,4]oxazepine-5-carboxamide for tissues, especially those containing melanin.

Keyphrases ☐ Dibenzoxazepines, carboxamide and other side chains—tissue distribution, affinity for melanin, dogs ☐ Carboxamide-substituted dibenzoxazepines—tissue distribution in dogs, affinity for melanin ☐ Tissue distribution—dibenzoxazepines, melanin affinity, dogs ☐ Melanin tissue distribution—dibenzoxazepines, dogs ☐ Ocular tissue distribution—dibenzoxazepines, dogs

It is generally accepted that substituted phenothiazines can adversely affect the eye and skin when large amounts are ingested chronically (1–3). Some compounds, like chlorpromazine, produce primarily opacities of the lens and cornea, whereas others, like thioridazine, can produce a loss of vision by their effect on the

retina. Because of these past findings, studies were conducted with some substituted dibenzoxazepines that have exhibited CNS activity in animals (4, 5). The results of these studies show that the presence of a carboxamide side chain alters the localization of the compound and/or its metabolites in the tissues of dogs, including the melanin-containing portion of the eye, as well as the binding of the parent molecules to melanin granules of beef eyeball *in vitro*.

METHODS AND MATERIALS

Purity and Specific Activity of Compounds—The radioactive compounds studied had the following chemical names, radiochemical purities, and specific activities, respectively: I, 4-[3-(7-chloro-5,11-dihydrodibenz[b,e][1,4]oxazepin-5-yl)propyl]-1-piperazine-$^{14}C_2$-ethanol hydrochloride, 96%, 21.6 μc./mg.; II, 4-[3-(7-(trifluoromethyl)-5,11-dihydrodibenz[b,e][1,4]oxazepin-5-yl)propyl]-1-piperazine-$^{14}C_2$-ethanol hydrochloride, 99%, 24.9 μc./mg.; III, 5-[(2-dimethylamino)ethyl-1,2-$^{14}C_2$]-5,11-dihydrodibenz-[b,e][1,4]oxazepine maleate, 99%, 6.9 μc./mg.; and IV, 7-chloro-5,11-dihydrodibenz[b,e][1,4]oxazepine-5-^{14}C-carboxamide, 99%, 5.0 μc./mg.

Surgical Preparation of Dogs—Purebred male beagles were anesthetized with 30 mg./kg. of sodium pentobarbital administered intravenously. A catheter was inserted into the bladder for the collection of urine. The radial vein was then cannulated, and infusion of the following solution was begun at the rate of 3 ml./min.: mannitol (100 g.), potassium dihydrogen phosphate (200 mg.), potassium hydrogen phosphate (900 mg.), sodium pentobarbital (25.5 mg./kg. of body weight), and sufficient water to make 2 l. Mannitol was included to ensure an adequate flow of urine. A midline incision was made, and the entrance to the gallbladder was clamped at its juncture with the common bile duct. A polyethylene