Drug Permeation through Membranes V: Interaction of Diazepam with Common Excipients

E. G. LOVERING ˣ, C. A. MAINVILLE, and M. L. ROWE

Abstract □ The effects of common tablet excipients on the permeation of diazepam through polydimethylsiloxane membranes and on the turnover time of goldfish were studied. The permeability coefficient decreased and the turnover time increased in the presence of talc, polysorbate 80, and, possibly, fumed silicon dioxide, but these parameters were unaffected by lactose, microcrystalline cellulose, and starch.

Keyphrases □ Permeation—diazepam through polydimethylsiloxane membranes, effect of excipients □ Diazepam—permeation through polydimethylsiloxane membranes, effect of excipients □ Polydimethylsiloxane—membranes, permeation of diazepam, effect of excipients □ Membrane permeation—diazepam through polydimethylsiloxane, effect of excipients

Tablet and capsule excipients are known to influence the absorption of drugs from the GI tract. For example, low blood levels of phenytoin in epileptic patients were associated with a formulation containing calcium sulfate (1), and significant differences in the plasma level of dicumarol in dogs were reported with six of 10 excipients investigated (2).

Drug–excipient interactions can sometimes be detected by experiments *in vitro*. Thus, the dialysis rate of prednisolone was found to depend upon the drug formulation (3). The effect of excipients and other materials on the permeation of amobarbital (4), phenylbutazone (5), chlordiazepoxide (6), and chlorpromazine (7) through polydimethylsiloxane also was reported.

There is little direct evidence relating drug–excipient interactions observed *in vitro* to pharmacological effects observed *in vivo*. Therefore, the effects of excipients on the permeation of diazepam through polydimethylsiloxane membranes and on the turnover time of goldfish in a diazepam solution was examined.

EXPERIMENTAL

Permeation—Cells similar to those employed by Garrett and Chemburkar (8) were used, except that the 5-mil polydimethylsiloxane membranes were attached with silicone adhesive. The cells were placed in a beaker containing a solution of the drug buffered at pH 7 (phosphate buffer) and thermostated at 37.0°. The desorbing fluid within the permeation cells was dilute hydrochloric acid, pH 1.2.

The solutions were pumped continuously through the sample cells of a spectrophotometer set at 284 nm, and the absorbance was read at appropriate time intervals. Beer's law calibration curve, made with NF diazepam reference standard, yielded a molar absorptivity of 13,260. Permeation rates were obtained from the steady-state slope of graphs of moles of drug in the desorbing solution plotted against time.

Turnover Time—Diazepam solutions, about 2.1×10^{-4} mole/liter, were prepared for turnover time experiments by dissolving the drug in 25 ml of 0.5 *N* hydrochloric acid, neutralizing with 0.5 *N* sodium hydroxide, diluting to 2 liters, and adjusting the pH to 7.0. Excipients were added before the final pH adjustment.

Turnover time experiments were carried out by placing each fish in 250 ml of the diazepam solution at 25°. Each fish and each solution were used only once. The end-point was taken as that time when the fish failed to right itself within 1–2 sec after being turned over with a glass rod.

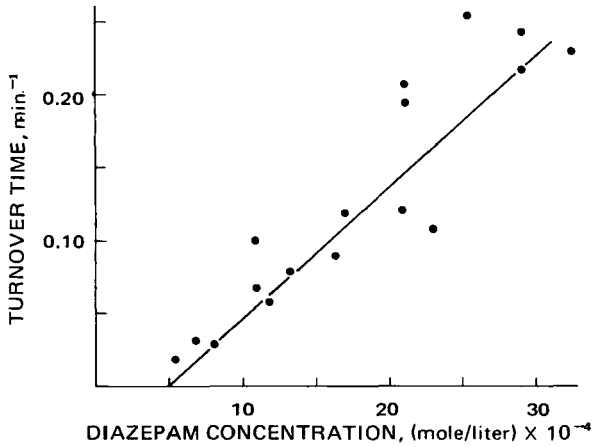

Figure 1—*Reciprocal turnover time of goldfish* versus *diazepam concentration.*

Some experiments were carried out using pairs of fish, one member of the pair being placed in a solution of the drug without excipient and the other being placed in the drug–excipient solution under test. The results were expressed as a ratio of drug–excipient turnover time to drug turnover time. This procedure reduced variations due to interbatch differences in the fish and in operator judgment of the turnover time.

The excipients were tested for toxicity by immersing fish in solutions or suspensions containing only the excipient at concentrations higher than those encountered under test conditions. After several hours the fish were removed to distilled water and observed for 24 hr. No adverse effects were noted.

Materials—Diazepam was used as received[1]. Other materials used were talc USP[2], starch USP[2], polysorbate 80[2], lactose USP[3], fumed silicon dioxide[4], and microcrystalline cellulose[5]. Goldfish (*Carassius auratus*), 3–6 cm in length, were obtained locally in batches of 50 or 100 fish.

RESULTS AND DISCUSSION

Characteristics of *In Vitro* System—The permeability coefficient, *P*, was calculated from:

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

where (dq/dt) is the rate at which drug permeates the cell membrane under steady-state conditions, l is the membrane thickness, A is its surface area, and C is the drug concentration outside the permeation cell. Steady-state conditions were achieved by an excess of drug outside the cell and a low pH within the cell. At low pH, diazepam is in the ionic state and the concentration of unionized drug is virtually zero. Ionized drug molecules are unlikely to permeate polydimethylsiloxane membranes.

Permeability coefficients were calculated from data obtained over a diazepam concentration range from 0.98×10^{-5} to $6.08 \times$

[1] Courtesy of Hoffmann–La Roche Ltd., Montreal, Quebec, Canada.
[2] Fisher Scientific Co., Montreal, Quebec, Canada.
[3] Courtesy of Peebles Products Ltd., Cornwall, Ontario, Canada.
[4] Courtesy of Cabot Carbon Corp., Boston, Mass.
[5] PH101, courtesy of FMC Corp., Marcus Hook, Pa.

Table I—Apparent Permeability Coefficients in Talc Slurry

Concentration of Talc, g/liter	Concentration, C_0, of Drug, moles/liter $\times 10^5$	Apparent Permeability[a] Coefficient, $(cm^2/sec) \times 10^6$	Equilibrium Concentration of Drug, mg/100 ml
1.5	5.303	1.58 ± 0.09	1.181
4.0	5.794	1.25 ± 0.06	1.021
6.0	5.303	1.06 ± 0.03	0.792
10.0	6.005	0.85 ± 0.04	0.720
20.0	5.478	0.62 ± 0.02	0.479

[a] Mean of three determinations ± *SD*.

10^{-5} mole/liter. The permeability coefficient was independent of concentration over this range, the mean being $(2.02 \pm 0.12) \times 10^{-6}$ cm^2/sec for 20 individual experiments. The permeability coefficient was independent of the pH of the drug solution over the 4.8–7.6 range.

Excipient interactions were investigated by adding excipient, either in solution or as a slurry, to the drug solution and calculating the permeability coefficient from the experimental permeation rate, dq/dt. If a drug–excipient interaction occurs, the concentration of free drug decreases and the permeability coefficient also appears to decrease. All excipient experiments were carried out at pH 7, and no pH correction was made as in previous studies (5, 6).

Characteristics of *In Vivo* System—To ensure that the behavior of goldfish toward diazepam was consistent with that observed toward other drugs (9, 10), the effect of changes in drug concentration on the time to the loss of the righting reflex was determined. The results were plotted as mean reciprocal turnover time *versus* concentration (Fig. 1). Each point represents the mean of experiments on four, six, or eight fish. In agreement with theoretical predictions and previous observations (11, 12), the results fall on a straight line. The minimum concentration for pharmacological effect was about 5×10^{-4} mole/liter of diazepam.

Lactose—The permeability coefficients of diazepam at lactose concentrations of 7.5, 40, and 80 g/liter were $(1.97 \pm 0.21) \times 10^{-6}$, $(1.94 \pm 0.04) \times 10^{-6}$, and $(1.87 \pm 0.06) \times 10^{-6}$ cm^2/sec, respectively (means of three experiments). These values were not significantly different from the permeability coefficient of the drug alone.

The turnover time of goldfish was not affected by lactose concentrations up to 40 g/liter. Turnover times were 5.61 ± 0.96, 6.08 ± 1.71, and 5.58 ± 1.18 min (means of eight fish) for lactose concentrations of 4, 8, and 40 g/liter, respectively, and a drug concentration of 2.111×10^{-5} mole/liter.

Lactose, at the concentrations investigated, appears to have no effect on the permeability coefficient of diazepam or on the turnover time of goldfish.

Microcrystalline Cellulose—Permeability coefficients calculated from measurements made in the presence of 2.5, 10.0, and 20.0 g/liter of microcrystalline cellulose were $(1.92 \pm 0.22) \times 10^{-6}$, $(1.98 \pm 0.07) \times 10^{-6}$, and $(1.87 \pm 0.12) \times 10^{-6}$ cm^2/sec, respectively (means of three experiments). These values were not significantly different from the permeability coefficient of diazepam alone.

Turnover times due to 2.111×10^{-5} mole/liter of diazepam in microcrystalline cellulose slurries of 5, 10, and 20 g/liter were 5.52 ± 1.15, 5.28 ± 0.83, and 5.65 ± 0.84 min, respectively (means of eight goldfish). There was no significant difference among these means at the 95% confidence level. Paired experiments, in which one fish was placed in a solution containing 20 g/liter of microcrystalline cellulose and drug and the other was placed in a solution of drug, showed no significant difference in turnover times: 7.31 ± 1.3 and 7.17 ± 1.3 min, respectively (means of four fish).

The permeability coefficient and the turnover time were not detectably influenced by microcrystalline cellulose.

Starch—Permeability coefficients of diazepam in the presence of 0.75, 3.75, 7.00, and 15.00 g/liter of starch were $(2.00 \pm 0.09) \times 10^{-6}$, $(1.88 \pm 0.11) \times 10^{-6}$, $(1.83 \pm 0.10) \times 10^{-6}$, and $(1.85 \pm 0.10) \times 10^{-6}$ cm^2/sec, respectively (means of three experiments). The values were not significantly different from the permeability of drug alone.

Talc—The permeability coefficient of diazepam appears to decrease if talc is slurried into the diazepam solution (Table I), prob-

Table II—Paired Turnover Times Using Talc

Concentration of Talc, g/liter	Number of Pairs of Fish	Mean Turnover Time, min: Drug	Mean Turnover Time, min: Drug and Excipient	Ratio of Turnover Time
2	4	6.10 ± 1.10	6.15 ± 1.43	1.01
4	8	6.21 ± 1.28	10.02 ± 3.02	1.61[a]
6	8	6.21 ± 1.28	8.56 ± 1.97	1.38[a]
6	4	8.02 ± 0.87	13.93 ± 1.16	1.75
8	8	6.21 ± 1.28	13.55 ± 3.13	2.18[a]
10	4	5.50 ± 0.82	14.42 ± 1.23	2.36
10	8	6.21 ± 1.28	15.05 ± 1.89	2.42[a]

[a] Experiments done on the same batch of fish but not using the paired technique.

Table III—Apparent Permeability Coefficients in Fumed Silicon Dioxide

Concentration of Fumed Silicon Dioxide, g/liter	Concentration, C_0, of Drug, moles/liter $\times 10^5$	Apparent Permeability Coefficient[a], $(cm^2/sec) \times 10^6$	Equilibrium Concentration of Drug, moles/liter $\times 10^5$
0.5	5.443	1.98 ± 0.07	5.335
2.0	6.040	1.81 ± 0.04	5.412
3.5	5.724	1.80 ± 0.10	5.101
8.0	5.338	1.24 ± 0.04	3.277

[a] Mean of three determinations ± *SD*.

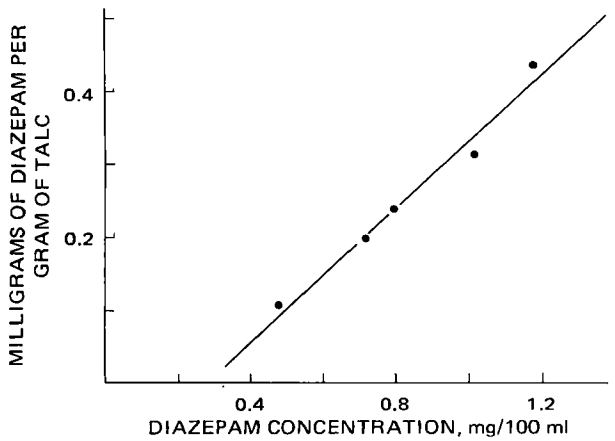

Figure 2—*Equilibrium ratio of drug to talc plotted against the concentration of drug.*

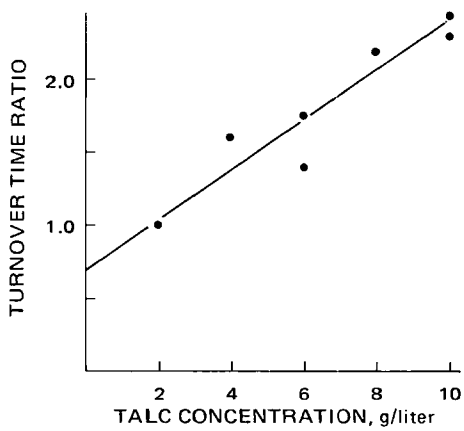

Figure 3—*Ratio of turnover times, drug in talc slurry to drug, plotted against the concentration of talc.*

Table IV—Paired Turnover Times Using Fumed Silicon Dioxide

Concentration of Fumed Silicon Dioxide, g/liter	Number of Pairs of Fish	Mean Turnover Time, min		Ratio of Turnover Times
		Drug	Drug and Excipient	
2	4	5.77 ± 1.28	6.39 ± 0.98	1.14 (NS)[a]
5	4	5.10 ± 0.71	7.75 ± 1.69	1.50
7	4	7.99 ± 2.02	10.14 ± 4.98	1.14 (NS)
7	4	6.56 ± 0.78	10.73 ± 0.98	1.66
10	4	6.17 ± 0.76	10.29 ± 1.51	1.65
10	4	8.88 ± 2.20	10.43 ± 1.42	1.22 (NS)

[a] NS = not significant.

ably due to drug adsorption on the talc surface. The apparent decrease in the permeability coefficient can be used to calculate the equilibrium concentration of drug in solution, C, from:

$$C = C_0 \frac{P_a}{P} \qquad \text{(Eq. 2)}$$

where C_0 is the initial drug concentration prior to the addition of talc, P_a is the permeability coefficient measured in the presence of talc, and P is the permeability coefficient uninfluenced by the excipient. A plot of drug adsorbed per gram of talc *versus* the equilibrium concentration of drug in solution (Fig. 2) indicates that the amount of drug adsorbed increases linearly with the equilibrium concentration of drug.

The results of *in vivo* experiments using the paired fish technique were expressed as the ratio of the turnover times with and without talc slurried into the drug solution. The initial drug concentration was held constant (Fig. 3). The experimental data are given in Table II.

Talc is usually present in formulations only to the extent of a few percent, although amounts over 40% have been observed. The data suggest that the interaction between talc and diazepam would be important in cases where formulations high in talc content were taken under conditions of low fluid content in the GI tract. Such conditions occur, and prudence suggests that excessive amounts of talc should not be used in formulating diazepam.

Fumed Silicon Dioxide—A modest reduction in the permeability coefficient was observed with the addition of increased quantities of fumed silicon dioxide to the diazepam solution (Table III).

Table V—Apparent Permeability Coefficients in Polysorbate 80 Solution

Concentration of Polysorbate 80, g/liter	Apparent Permeability Coefficient[a], $(cm^2/sec) \times 10^6$	Concentration, C_0, of Drug, moles/liter $\times 10^5$	Equilibrium Concentration of Drug, moles/liter $\times 10^5$
1.00	1.51 ± 0.07	5.092	3.806
1.75	1.25 ± 0.03	5.689	3.520
4.00	0.92 ± 0.04	4.916	2.239
10.00	0.62 ± 0.10	5.549	1.703

[a] Mean of three determinations ± *SD*.

Table VI—Mean Turnover Times Using Polysorbate 80

Concentration of Polysorbate 80, g/liter	Turnover Time[a], min	Significant Difference[b] (95% Confidence)
0.0000	6.21 ± 1.28	
0.0056	4.74 ± 0.51	S
0.0127	3.90 ± 0.91	S
0.0283	4.19 ± 0.70	S
0.0444	4.51 ± 0.95	S
0.0573	4.52 ± 0.60	S
0.0828	6.23 ± 1.23	NS
0.1081	7.08 ± 2.07	NS
0.2017	4.72 ± 1.77	NS

[a] Mean of eight fish ± *SD*. [b] Between turnover time at zero concentration of polysorbate 80. S = significant, and NS = not significant.

Experiments carried out using the paired goldfish technique suggest the possibility of a weak effect at concentrations over 5.0 g/liter of fumed silicon dioxide (Table IV). Unpaired experiments at 1.0, 2.0, 5.0, and 10 g/liter of fumed silicon dioxide yielded turnover times of 5.61 ± 0.81, 7.26 ± 1.01, 6.64 ± 0.46, and 7.15 ± 2.19 min, respectively (means of eight fish). The differences between the effects of 1.0 g and of 2.0 and 5.0 g were significant at the 95% level, but the difference between 1.0 and 10 g was not.

There may be a slight interaction between diazepam and fumed silicon dioxide. However, at the concentrations used in formulation manufacture, usually less than 1%, the interaction is unlikely to affect absorption adversely.

Polysorbate 80—The permeation rate of diazepam through polydimethylsiloxane membranes declined as the concentration of polysorbate 80 was increased up to 10 g/liter (Table V). The effect was probably due to partitioning of the drug between the aqueous phase and the micelles of the surfactant. The partition coefficient was calculated to be about 60, using a method described previously (4).

Polysorbate 80 at concentrations in the region of the critical micelle concentration (CMC) is known to increase the absorption of certain drugs by goldfish and to retard absorption at higher concentrations (13, 14). The results (Table VI) with diazepam are in general agreement with these findings. In the concentration range straddling the CMC of 0.014 g/liter (15), there was a significant decrease in the turnover time; but at a concentration above 0.0573 g/liter and up to at least 0.2017 g/liter of surfactant, there was no significant difference at the 95% level of confidence. The concentration ranges of the *in vitro* and *in vivo* experiments do not overlap because of the toxic effect of higher surfactant concentrations on the fish. The results suggest that low concentrations of polysorbate 80 may increase the rate of diazepam absorption.

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[x] To whom inquiries should be directed.

Effect of Dimethyl Sulfoxide on Permeability of Human Skin *In Vitro*

J. P. ASTLEY and M. LEVINE [x]

Abstract □ A diffusion flow cell is described for the continuous monitoring of skin permeability. The technique was used to study the permeability behavior of human skin subsequent to treatment with dimethyl sulfoxide. Such treatment produced an increased penetration rate of tritiated water, which was dependent upon the time of exposure and the concentration of dimethyl sulfoxide applied. Removal of the solvent resulted in partial recovery of barrier capacity. Skin, incubated *in vitro* in growth medium containing dimethyl sulfoxide, survived only at very low concentrations. Degeneration occurred after a few days in 4.5% dimethyl sulfoxide and much sooner at higher concentrations.

Keyphrases □ Dimethyl sulfoxide—effect on growth and permeability of human skin *in vitro*, diffusion flow monitoring cell described □ Permeability—human skin *in vitro*, effect of dimethyl sulfoxide □ Diffusion flow cell—description, continuous monitoring of skin permeability *in vitro* □ Skin, human—effect of dimethyl sulfoxide on growth and permeability *in vitro*

There are numerous reports concerning the enhancement of skin permeability by dimethyl sulfoxide (I) but very little agreement regarding the extent or degree of reversibility of this effect. High concentrations of I *in vivo* caused striking increases in permeability, which were almost completely reversible upon removal (1). Measurement of *in vivo* water vapor loss found an 11- to 17-fold permeability increase subsequent to contact with I, and the effect was partially reversible (2); other results (3) indicated a 25-fold increase in penetration of hexapyronium bromide *in vivo* after exposure to 50% I, with no mention of reversibility.

In vitro work has yielded more quantitative data. A maximum of a 66-fold permeability increase was found upon exposure to I, but the degree of reversibility was variable (4). With I–water mixtures, there was no effect until 60–70% I was applied (5). A 90-fold increase observed with 100% I was not reversible. Concentrations in excess of 70% I were necessary to produce appreciable enhancement of picrate-ion penetration, and such concentrations reduced the lag time from 5 to 2 hr (6). Subsequently it was demonstrated that the effect was not reversible (7).

In the present study, the effect of varying exposure times and concentrations of I on the permeability to tritiated water was investigated. Preliminary experiments revealed a rapid recovery of a fraction of the initial barrier capacity upon removal of I. Therefore, a continuous diffusion flow cell was developed to follow the permeability behavior in a more quantitative manner.

An *in vitro* system (8) was used to study the effect of I on the growth and maturation of human epidermis and thus to relate toxicity levels to the concentrations that enhance penetration.

Table I—Permeability of Human Skin to Tritiated Water Subsequent to Storage[a] at −20°

Sample	Storage Time at −20°			
	Zero	1 Month	3 Months	6 Months
1	3.2 ± 0.1[b]	2.3 ± 0.7	4.3 ± 0.2	3.4 ± 0.4
2	3.8 ± 0.1	3.4 ± 1.0	4.3 ± 0.3	3.2 ± 0.9
3	4.1 ± 0.2	1.8 ± 0.5	4.5 ± 0.4	3.4 ± 0.9
4	3.5 ± 0.3	2.4 ± 0.6	5.9 ± 3.0	2.6 ± 0.6

[a] Permeability coefficients × 10^7 (cm/sec). [b] Errors are quoted as ±95% confidence limits.

EXPERIMENTAL

Skin Samples—Excised thigh or lower leg skin (nominally 0.38 mm thickness) was obtained from amputated limbs using a battery-operated dermatome[1]. Before excision, the skin was cleaned by swabbing with 0.5% chlorhexidine in 70% ethanol, followed by 50% ethanol.

Strips of skin were rolled in sterile gauze, moistened with Hank's balanced salt solution, and stored in screw-capped containers at −20° until required for use. Such storage did not significantly affect the permeability of the skin (Table I).

Skin for growth studies was stored at 4° until required for use. This storage was without effect upon subsequent behavior (9).

Organ Culture—Explants were incubated by the method described by Levine (8) and harvested at 2, 4, 8, 11, 17, and 22 days after 24 hr in medium labeled with thymidine-6-T[2], 1 μCi/ml.

Dimethyl sulfoxide in growth medium[3] was tested at concentrations of 0, 0.9, 4.5, and 9% I.

Histology and Autoradiography—The methods used were described previously (8).

Diffusion Cells—*Glass Diffusion Cell*—The design and use of this type of cell were described previously (9). The "initial" permeability of the skin specimen is determined by filling the dermal compartment with a phosphate buffer medium (pH 7.0) and applying to the epidermal side a known volume of the same medium containing tritiated water[4] at a concentration of 80 μCi/ml.

[1] Davol Ltd., Providence, R.I.
[2] Radiochemical Centre, Amersham, Bucks, England.
[3] Eagle's Minimal Essential Medium plus 10% fetal bovine serum.
[4] Radiochemical Centre, Amersham, Bucks, England.