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The Permeation of Benzodiazepines through Synthetic Membranes[1)]

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The permeation of chlordiazepoxide, diazepam, oxazepam, lorazepam, nitrazepam, and nimetazepam through a dimethylpolysiloxane membrane and that of chlordiazepoxide, oxazepam, and nitrazepam through an ethylene-vinyl acetate copolymer membrane were investigated using a newly designed permeation cell. Large permeability values of diazepam and nimetazepam were attributed to their large partition coefficients. Large lag time values of chlordiazepoxide, oxazepam, and nitrazepam through the silicone membrane were rationalized by adsorption of these drugs on silica filler within the silicone membrane. Thus permeation data of these drugs can be interpreted on the basis of their physical properties.

Keywords——permeation profile; benzodiazepines; silicone membrane; ethylene-vinyl acetate copolymer membrane; permeation cell; permeability; partition coefficient; lag time; adsorption; silica filler

A number of benzodiazepines are being introduced for anxiolytic, hypnotic, and anti-epileptic purposes in recent years.[3)] Since therapy by these drugs often involves a long-term administration, controlled release preparations would have a potential merit over conventional dosage forms. One of the means of moderating the delivery of drugs is the use of various membranes.[4)] This approach requires a detailed knowledge of permeation characteristics of these drugs, but the availability of such data is still very limited. Earlier studies on membrane permeation of benzodiazepines cover only the effects of excipients on permeation of chlordiazepoxide[5)] and diazepam.[6)]

In order to bridge the gap, the authors have examined the permeation behaviors of six benzodiazepines through synthetic membranes. This communication deals with the interpretation of the permeation data of these drugs on the basis of their physical properties.

1) Pharmaceutical Studies on Benzodiazepines, Part I. This work has been presented at 96th and 97th Annual Meeting of Pharmaceutical Society of Japan, April, 1976 in Nagoya and April, 1977 in Tokyo, respectively.
2) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan.*
3) L.S. Goodman and A. Gilman (eds.), "The Pharmacological Basis of Therapeutics," 5th ed., Macmillan, New York, N.Y., 1975, pp. 124—125, 189—193, and 216—218.
4) R.W. Baker and H.K. Lonsdale, *CHEMTECH.*, **5**, 668 (1975).
5) E.G. Lovering, D.B. Black, and M.L. Rowe, *J. Pharm. Sci.*, **63**, 1224 (1974).
6) E.G. Lovering, C.A. Mainville, and M.L. Rowe, *J. Pharm. Sci.*, **65**, 207 (1976).

Experimental

Materials——Chlordiazepoxide, diazepam, oxazepam, lorazepam, nitrazepam, and nimetazepam were generously supplied by pharmaceutical companies and were used as received after characterization by differential scanning calorimetry. Phosphate salts ($Na_2HPO_4 \cdot 12H_2O$ and $NaH_2PO_4 \cdot 2H_2O$) and acids (HCl and H_2SO_4) were of reagent grade (Wako Pure Chemical Industries, Osaka). Silicone oil (360 Medical Fluid, 20 centistokes, Dow Corning, Midland, Michigan), isopropyl myristate (Tokyo Kasei Kogyo Co., Tokyo), and *n*-amyl acetate (Wako Pure Chemical Industries) were used for partition studies.

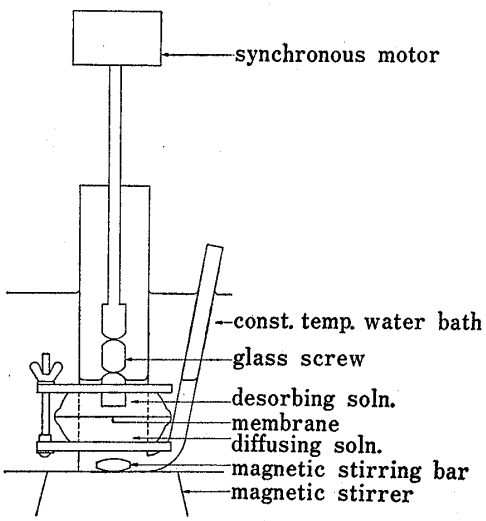

Fig. 1. Schematic Drawing of a Permeation Cell

Permeation Cell——A newly designed glass permeation cell as shown in Fig. 1 was constructed from greaseless joints 40 mm in internal diameter (Kokura Glass Industries, Kitakyushu). Dimethylpolysiloxane sheeting (Silastic, non-reinforced, Dow Corning, Midland, Michigan) in a labeled thickness of 5 mil (127 μm) or ethylene-vinyl acetate copolymer membrane (Evaflex, vinyl acetate content of 17%, Mitsui Polychemical, Tokyo) was secured between the joints with a Teflon O-ring by an aluminum circular holder with tightening screws. The cell was immersed in a constant-temperature water bath (Thermo Unit, C-600, Taiyo Kagaku Kogyo Co., Tokyo) maintained at 30.0±0.1°. A Teflon magnetic stirring bar (20 mm long) in the bottom compartment of the cell was rotated at a rate of 650±10 rpm by a synchronous stirrer (High Magmix Stirrer, Mitamura Riken Kogyo Inc., Tokyo) placed under the bath while a glass stirrer shaft with a screw shaped end (15 mm in diameter) in the top compartment was rotated by means of a synchronous motor (Labo-Stirrer, LS-15, Yamato Scientific Co., Tokyo) at a rate of 300±10 rpm.

Permeation Studies——A suspension of a drug in 0.05 M phosphate buffer, pH 7.4 was prepared by agitating an excess amount of the drug in the buffer by a magnetic stirrer at 30.0° overnight. Acid solutions, used as a desorbing solution to effect ionization of the permeant drug in order to maintain the sink condition, were also prewarmed. A hydrochoric acid solution (0.1 N) was used for the majority of the study whereas 1 N H_2SO_4 was employed for the permeation study of oxazepam and lorazepam because of their low pK_{a_1} values.[7]

After rinsing the diffusion cell, 45 ml portion of the suspension was poured into the bottom compartment and 20 ml portion of the acid solution was pipetted into the top compartment. Both magnetic stirring and motor-driven agitation were started immediately after filling both compartments.

At predetermined time intervals, the desorbing solution in the top compartment was sampled and its ultraviolet absorbance was quickly measured (model 100-50 double-beam spectrophotometer, Hitachi Manufacturing Co., Tokyo) at a wavelength of maximum absorbance for chlordiazepoxide. For diazepam, oxazepam, lorazepam, nitrazepam, and nimetazepam, in which ring-opening reactions in acid environment were noticeable even at 30°,[8] the wavelengths of isosbestic points in the spectra of the drugs and their open-ring products were used for the assay. The solution was returned to the top compartment immediately after the measurement of absorbance.

Measurement of Solubility and Partition Coefficient——Solubility at 30.0° was determined after filtration through sintered glass disks of the drug suspensions prepared for permeation studies.

In order to measure partition coefficients of the drugs between silicone oil, isopropyl myristate, or *n*-amyl acetate and the aqueous buffer solution, test tubes containing the drug solution and organic solvent were immersed in a water bath maintained at 30.0° and agitated by means of Teflon magnetic bars (10 mm long) placed in the test tubes. Sixty minute equilibration time was allowed for and the concentration of the drug remaining in the aqueous phase following centrifugal separation (3000 rpm for 10 min) of two layers was determined spectrophotometrically.

Results and Discussion

The permeation profiles of the six benzodiazepines through the silicone membrane presented in Fig. 2 indicate the presence of lag time which varied considerably among the drugs.

7) J. Barrett, F.W. Smyth, and I.E. Davidson, *J. Pharm. Pharmacol.*, **25**, 387 (1972).
8) M. Nakano, N. Inotsume, N. Kohri, and T. Arita, *Int. J. Pharm.*, in press.

The lag time obtained by extrapolation of the stedy-state portion of the curve to the time axis was as long as 8.9 hr for chlordiazepoxide and very short for diazepam.

Markedly delayed permeation of a molecule through the silicone membrane, which is known to contain a dispersed silica phase in a continuum of dimethyl polysiloxane polymer,[9] can be attributed to the small diffusivity of the molecule within the membrane due to (1) large molecular size of a permeant, (2) specific interaction of a permeant with the polymer matrix, and (3) adsorption of a permeant onto the dispersed silica surface.

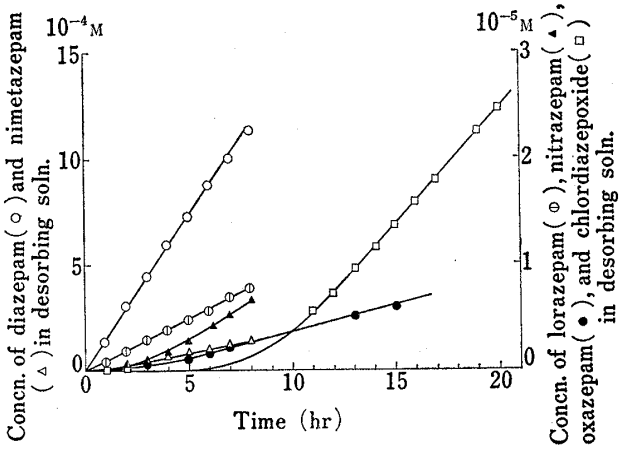

Fig. 2. Permeation Profiles of Six Benzodiazepines from Their Suspensions in 0.05 M Phosphate Buffer, pH 7.4 through 127 μm Silicone Membrane (area= 12.6 cm^2) to the Acidic Desorbing Solution (sink, volume=20 ml) at 30.0°

Even if the molecular size does not vary significantly among six drugs, there have been found the large difference in lag time. Thus, Reason 1 is not applicable in this case. Reason 2 may also be ruled out here because no evidence has been found to support a specific interaction between chlordiazepoxide, oxazepam or nitrazepam and silicone oil as can be seen in their partition coefficient values shown in Table I. Reason 3 is probable since siliceous fillers

Table I. Solubility in 0.05 M Phosphate Buffer, pH 7.4, Partition Coefficient between Silicone Oil and 0.05 M Phosphate Buffer, pH 7.4, Permeability through the Silicone Membrane, and Lag Time through 5 mil Silicone Membrane, of Six Benzodiazepines at 30.0°

Benzodiazepines	Solubility 10^{-4} M	Partition Coeff.	Permeability 10^{-8} cm^2 sec^{-1}	Lag time hr
Chlordiazepoxide	3.2	0.091	4.7	8.9
Diazepam	1.9	8.3	440	0.0
Oxazepam	2.0	0.050	1.6	2.8
Lorazepam	2.6	0.048	2.6	0.09
Nitrazepam	1.6	0.048	4.5	2.3
Nimetazepam	1.6	1.2	68	0.17

were reported to adsorb drugs.[9,10] For example, the influence of silica gel on the permeation of sarin through white petrolatum was reported by Finger *et al.*,[10] and the influence of the physical adsorption on flux through dimethylpolysiloxane membranes containing fumed silica was reported more recently by Flynn and Roseman.[9]

Lag time observed here may then be rationalized by the adsorption of drugs on a filler within the silicone membrane since the silicone membrane employed is known to contain 20—30% fumed silica filler.[9] Thus benzodiazepines which partition from the diffusing solution into the silicone membrane is adsorbed by the filler dispersed within the membrane and the diffusion through the membrane is impeded depending upon the adsorbability of each drug on the filler. A separate study showed that benzodiazepines are adsorbed strongly on

9) G.L. Flynn and T.J. Roseman, *J. Pharm. Sci.*, **60**, 1788 (1971).
10) K.F. Finger, A.P. Lemberger, T. Higuchi, L.W. Busse, and D.E. Wurster, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 569 (1960).

silica under nonpolar environment as is the case in the silicone membrane. Detailed studies on adsorbability of benzodiazepines on the fumed silica and other solid surfaces will be published elsewhere.[11]

The above supposition that adsorption be responsible for large lag time observed was further confirmed by extremely short lag time observed in the permeation of benzodiazepines through a fillerless membrane. Permeation profiles of benzodiazepines through an ethylene-vinyl acetate copolymer membrane are presented in Fig. 3. Nitrazepam, oxazepam, and chlordiazepoxide which exhibited large lag time through the silicone membrane permeated through the ethylene-vinyl acetate membrane without noticeable lag time.

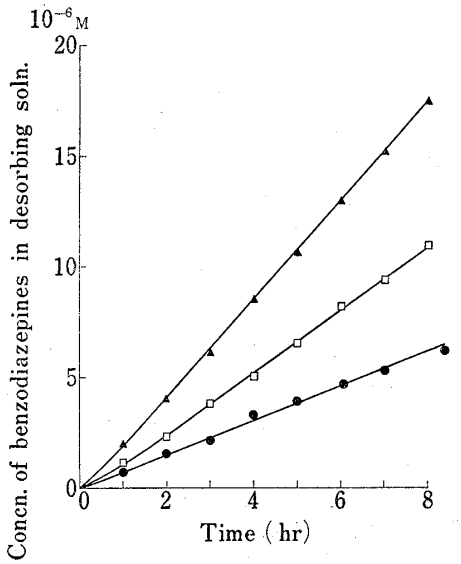

Fig. 3. Permeation Profiles of Nitrazepam (▲), Chlordiazepoxide (□), and Oxazepam (●) from Their Suspensions in 0.05 M Phosphate Buffer, pH 7.4 through 18 μm Ethylene-Vinyl Acetate Membrane (area=12.6 cm^2) to the Desorbing Solution (0.1 N for the first two drugs and 1 N H_2SO_4 for oxazepam, volume=20 ml) at 30.0°

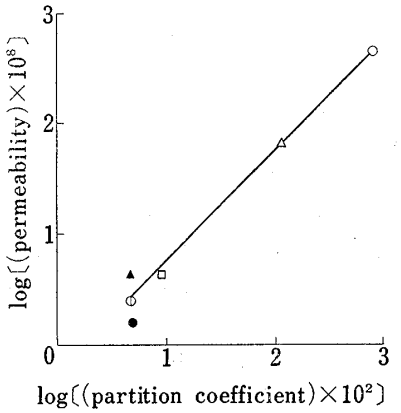

Fig. 4. Correlation of Permeability through the Silicone Membrane at 30.0° with (silicone oil/0.05 M phosphate buffer, pH 7.4) Partition Coefficient

○, diazepam; △, nimetazepam; □, chlordiazepoxide; ▲, nitrazepam; ⊙, lorazepam; and ●, oxazepam.

Permeability values tabulated in Table I were calculated from slopes of the steady-state portion of the permeation curve (Fig. 2) and solubility C_s of each drug in the diffusing solution (the second column, Table I) employing the following equation applicable to the permeation of the drug from the saturated solution to the sink at steady-state under the condition that the effect of diffusion through a stagnant layer neighboring the membrane on the overall permeability be negligible,[12]

$$\left(\frac{dC}{dt}\right)_{ss} = \frac{DAK}{lV}C_s = \frac{PA}{lV}C_s$$

where $(dC/dt)_{ss}$=rate of an increase in drug concentration in the desorbing solution at steady-state, D=diffusivity in the membrane, A=available area of the membrane, K=partition coefficient of a drug between the membrane and the diffusing solution, l=thickness of the membrane, V=volume of the desorbing solution, and $P=DK$.

11) N. Kohri, M. Nakano, and T. Arita, to be published.
12) M. Nakano, K. Juni, and T. Arita, *J. Pharm. Sci.*, **65**, 709 (1976).

Since permeability is the product of diffusivity and partition coefficient, it may be correlated with partition coefficient if diffusivity may be considered to be of the same order of magnitude among analogous drugs. Thus oxazepam, which is N^1-desmethyl-3-hydroxydiazepam, has much smaller partition coefficient and consequently much smaller permeability than diazepam. In the same way, nitrazepam, which is N^1-desmethylnimetazepam has smaller partition coefficient and smaller permeability than nimetazepam. The relationship between permeability and partition coefficient shown in Fig. 4 illustrates a good correlation of permeability with partition coefficient supporting the supposition that diffusivity do not differ significantly among five benzodiazepines. The result also indicates that the liquid silicone oil may be employed in place of the polymerized silicone elastomer when relative values for partition coefficients are needed.

It should be pointed out that permeability of nitrazepam through the ethylene-vinyl acetate membrane (11×10^{-9} cm^2sec^{-1}, Fig. 3) has been found to be 3 times greater than that of chlordiazepoxide (3.6×10^{-9} cm^2sec^{-1}, Fig. 3) although permeability values of nitrazepam and chlordiazepoxide through the silicone membrane are comparable (4.5×10^{-8} and 4.7×10^{-8} cm^2sec^{-1}, respectively, Table I). Since ethylene-vinyl acetate is ester whereas silicone (dimethylpolysiloxane) is ether, the above observation may be accounted for by greater affinity of nitrazepam toward an ester-type membrane than chlordiazepoxide. Thus two organic solvents belonging to ester-type solvents were employed to compare relative affinity of nitrazepam and chlordiazepoxide toward these solvents. Partition studies of these drugs between isopropyl myristate and 0.05 M phosphate buffer, pH 7.4 showed that the partition coefficient of nitrazepam (28) is some 3 times greater than that of chlordiazepoxide (9.9). Similarly partition coefficient between *n*-amyl acetate and 0.05 M phosphate buffer, pH 7.4 was 270 for nitrazepam and 87 for chlordiazepoxide. Thus nitrazepam seems to have more affinity toward esters than chlordiazepoxide. Since permeability is defined as product of partition coefficient and diffusivity, larger partition coefficient of nitrazepam than that of chlordiazepoxide is associated with larger permeability of nitrazepam through the ethylene-vinyl acetate membrane.

Thus it may be concluded that permeation behaviors of benzodiazepines from their suspensions through membranes can be rationalized by their aqueous solubility, (membrane/diffusing solution) partition coefficient and adsorbability on the filler within the membranes. This finding may be helpful in designing a proper membrane for controlled release of drugs.

Since this work forms the first part of a series of studies on benzodiazepines,[1] some of the conclusions drawn in the present investigation can be refined by further studies on chemical stability in acid solutions,[8] adsorbability on solid surface,[11] and effects of additives on permeability of benzodiazepines.

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