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Drug Permeation through Egg Shell Membranes[1,2]

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In order to establish an *in vitro* method for determining drug release from ointments, the drug permeability and the drug release from ointments were investigated using egg shell membrane and isopropyl myristate (IPM)-containing membrane, and the results were compared with the *in vivo* results.

The results of the permeation of salicylic acid at various pH values showed that egg shell membrane behaved as a dialysis membrane in the same way as cellulose membrane, while IPM-containing egg shell membrane behaved as a partition membrane in the same way as polyamide lipoid membrane. In the release experiments with betamethasone 17-valerate from various ointments, the release from hydrophilic ointment was substantial, followed by that from absorption ointment, and that from white vaseline was the least. These results were correlated with those of *in vivo* vasoconstrictor assay. Although macrogol ointment showed high drug release, since it changed into a solution due to the diffusion of water from receptor solution, the vasoconstrictor activity was lower than those of the other ointments.

Keywords——egg shell membranes; drug permeation through membrane; salicylic acid; drug release from ointments; betamethasone 17-valerate; vasoconstrictor activity; *in vitro*/*in vivo* comparison

It is important to establish an *in vitro* method for studying drug release from ointments, in order to select the vehicles that enhance the availability of drugs in ointments. The permeation of drugs through various artificial membranes, such as collagen membrane[4] and oil-saturated membrane filter,[5] has been investigated. In addition, many studies have been reported on drug release from ointments using silicon rubber membrane,[6] cellulose membrane,[7] membrane filter,[8] and excised skin of humans[9] and animals.[10] However, the results obtained with

1) Studies on Drug Release from Ointment, Part I.
2) This work was presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April, 1978.
3) Location: *1-403 Yoshino-cho, Omiya-shi, Saitama-ken, 330, Japan.*
4) M. Nakano, A. Kuchiki, and T. Arita, *Chem. Pharm. Bull.*, **24**, 2345 (1976).
5) M. Tanaka, H. Fukuda, and T. Nagai, *Chem. Pharm. Bull.*, **26**, 9 (1978).
6) F. Bottari, G. Di Colo, E. Nannipieri, M.F. Saettone, and M.F. Serafini, *J. Pharm. Sci.*, **63**, 1779 (1974); M. Nakano and N.K. Patel, *ibid.*, **59**, 985 (1970).
7) J.W. Ayres and P.A. Laskar, *J. Pharm. Sci.*, **63**, 1402 (1974).
8) M. Takehara and M. Koike, *Yakugaku Zasshi*, **97**, 770 (1977).
9) J. Ostrenga, C. Steinmetz, and B. Poulsen, *J. Pharm. Sci.*, **60**, 1175 (1971).
10) V. Shahi and J.L. Zatz, *J. Pharm. Sci.*, **67**, 789 (1978).

these artificial membranes do not always reflect percutaneous absorption and the experiments using excised skin showed rather poor reproducibility.

Therefore, in this study, egg shell membrane was used, since it consists mainly of keratin, like the stratum corneum. Moreover, in order to increase its similarity to the stratum corneum, which is considered to be a barrier to percutaneous absorption, isopropyl myristate (IPM) was added as an oil component to the egg shell membrane. Salicylic acid, the molecular species of which varies with pH, was chosen as a model drug, and the permeation results were compared with those using cellulose membrane and polyamide lipoid membrane, which have been widely investigated.[11]

The releases of betamethasone 17-valerate from typical ointments were measured using these egg shell membranes and artificial membranes. These results were compared with those for percutaneous absorption, as estimated by the vasoconstrictor activity of betamethasone 17-valerate released from each ointment.

Experimental

Materials——Salicylic acid used was of reagent grade and betamethasone 17-valerate was of pharmacopoeial grade (B.P. '73). Hydrophilic ointment, absorption ointment, and macrogol ointment were prepared according to J.P. IX, and betamethasone 17-valerate was dissolved or suspended in these ointment bases and white vaseline with 0.12% (w/w). Other materials used were of reagent grade.

Membranes——Egg shell membrane was prepared as follows: a whole chicken egg was soaked in 0.5 N HCl solution. The outer calcareous shell was dissolved, then a part of the egg shell membrane was cut off and the inner contents were removed. The membrane obtained was thoroughly washed in distilled water, stored in a refrigerator, and used within a week.

IPM-containing egg shell membrane was prepared as follows: the wet egg shell membrane was soaked in IPM in a Petri dish under a vacuum overnight to exchange water in the membrane for IPM. IPM adhering to the surface of the membrane was washed mildly in distilled water, so that the membrane was partly rehydrated. As a result, membrane containing water and IPM uniformly was obtained, as shown in Table I. The IPM content of the membrane was determined spectrophotometrically at 212 nm after extraction with methanol.

Cellulose membrane (36/32 type, Union Carbide Corp., Chicago) and Desaga Resomate II polyamide lipoid membrane (Desaga Co., Ltd., Heidelberg) were used after washing in distilled water. The thicknesses of these membranes were measured with a dial-type thickness gauge (Ozaki Factory Co., Ltd., Japan).

Drug Permeation through Membranes——The permeation of salicylic acid through these membranes was measured using the two-compartment diffusion cell shown in Fig. 1. The exposed area of the membrane was 7.02 cm^2. The diffusion cell assembly was placed in a thermostated water bath at 30°. Salicylic acid was dissolved to a concentration of 500 μg/ml in Sørensen buffer solution. The preheated solution (20 ml) was added to the donor compartment and drug-free buffer solution (300 ml) to the receptor compartment. At suitable intervals, 5 ml of receptor solution was removed and 5 ml of buffer solution was added to the receptor compartment. The sample solution was diluted with 0.1 N HCl solution and the concentration of salicylic acid was determined spectrophotometrically at 302 nm.

Partition Coefficient of Salicylic Acid with Membranes——Several sheets of each membrane were shaken with pH 1.9 and 7.0 buffer solutions (5 ml) containing 50 μg/ml of salicylic acid for 3 hours at 30°. From the change in the concentration of salicylic acid in the buffer solution, the partition coefficient, defined as the concentration of salicylic acid in wet membrane (μg/g) divided by the concentration of salicylic acid in the buffer solution (μg/ml), was calculated.

Drug Release from Ointment——The experiments on betamethasone 17-valerate release from ointments were performed using the cell shown in Fig. 2. The cell was shaken horizontally at a rate of 70 cpm in a shaker (Iwashiya, K. Sawada Co., Ltd., Tokyo) at 30°.

At suitable intervals, 10 ml of sample solution was removed from the receptor cell, and replaced with 10 ml of distilled water. Betamethasone 17-valerate in the sample solution was extracted with 10 ml of chloroform, then 7 ml of the chloroform layer was evaporated to dryness and the residue was dissolved in 0.5 ml of methanol. The amount of betamethasone 17-valerate in this solution was determined by high performance liquid chromatography using a Hitachi 633 liquid chromatograph with a 50 cm × 2.1 mm i.d. stainless steel column packed with Hitachi gel #3010. Other conditions were as follows: eluent, 0.2% NH_4OH

11) R. Withington and J.H. Collett, *J. Pharm. Pharmacol.*, **25**, 273 (1973); H.-W. Dibbern and G.H. Scholz, *Arzneim.-Forsch.*, **19**, 1140 (1969).

Table I. Water and IPM Contents in IPM-containing Egg Shell Membrane

No.	Water content (%)	IPM content (%)
1	49	20
2	52	20
3	50	22
Mean	50	21

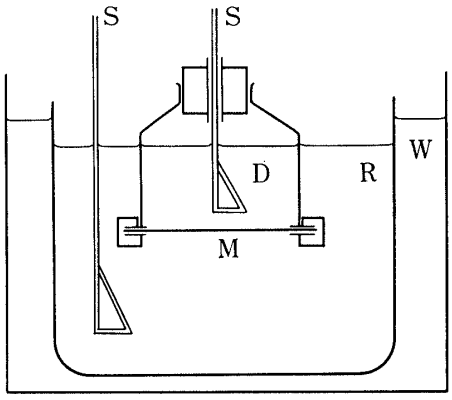

Fig. 1. Apparatus used for Diffusion Experiments

D, donor solution (20 ml); R, receptor solution (300 ml); M, membrane (7.02 cm²); W, water bath (30°); S, stirrer (360 rpm).

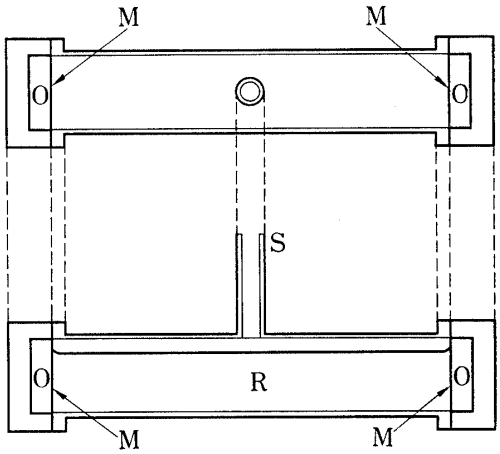

Fig. 2. Apparatus used for Drug Release Experiments from Ointment

O, ointment (1 g × 2); R, receptor solution (50 ml of distilled water); M, membrane (3.14 cm² × 2); S, sampling hole.

in methanol; flow rate, 0.8 ml/min; detector, UV at 254 nm; temperature, ambient; sensitivity, 0.02 AUFS; injection volume, 30 μl.

Vasoconstrictor Study——The method used was an adaptation of the vasoconstrictor assay described by Pepler *et al.*[12] About 50 mg of each ointment was applied at random to two sites on the flexor surface of each forearm of six healthy volunteers, using adhesive plaster for patch tests (Torii Pharm. Co., Ltd., Tokyo). The total number of test sites was 24 per ointment.

At 4 hours after the application of the ointments, the plaster was removed and the site was wiped with gauze containing 70% ethanol. Then, at 6, 8, and 24 hours after application, the blanching of the test site was graded as showing no blanching, slight blanching, distinct blanching, or very distinct blanching (scored as 0, 1, 2, and 3, respectively). The average score of each ointment was obtained by dividing the total score by the number of test sites.

Results and Discussion

Permeation of Salicylic Acid through Egg Shell Membranes

The permeation rate of drug from the donor solution to the receptor solution through the membrane is expressed by

$$\left(\frac{dQ}{dt}\right)_{d\to r} = -k(C_d - C_r) \qquad \text{Eq. 1}$$

where C_d and C_r are the concentrations of the drug in the donor and receptor solutions, respectively. Equation 1 can be integrated to give Eq. 2.[13]

$$\ln\left(1 - \frac{V_d + V_r}{V_r} \cdot \frac{Q_r}{Q_{tot}}\right) = -P \cdot A_m\left(\frac{1}{V_d} + \frac{1}{V_r}\right)t \qquad \text{Eq. 2}$$

12) A.F. Pepler, R. Woodford, and J.C. Morrison, *Br. J. Derm.*, **85**, 171 (1971).
13) R.J. Scheuplein, *J. Invest. Derm.*, **45**, 334 (1965).

where A_m is the area of the membrane, and V_d and V_r are the volumes of the donor and receptor solutions, respectively. Q_r is the amount of drug that has permeated to the receptor solution at time t, and Q_{tot} is the total amount of drug. The permeability constant (P) is given by

$$P = \frac{D_m(PC)}{X_m} \qquad \text{Eq. 3}$$

The diffusion coefficient (D_m) of the drug in the membrane can be calculated with Eq. 3 from measurements of the partition coefficient (PC) and the thickness (X_m) of the membrane.

When the left-hand side of Eq. 2 for the permeation of salicylic acid through egg shell membranes was plotted as a function of time, straight lines were obtained as shown in Fig. 3. The leakage of IPM from the membrane was thus not sufficient to affect the permeability rate during the experiment. To confirm this, a drug-free experiment was done and the IPM content of the membrane after stirring for 2 hours was measured. It was 19% (SD=±2%, n=3), which was nearly equal to the value of 21% of the initial membrane, as shown in Table I. The permeability constant was calculated from the slope of this regression line. Under the conditions of this experiment, the amount of drug distributed to the membrane was negligible.

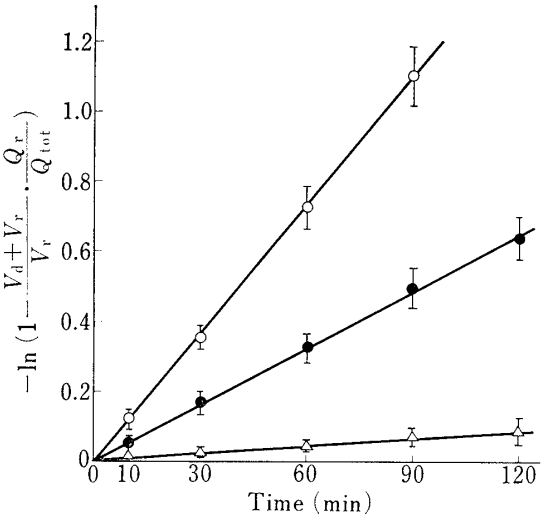

Fig. 3. Permeability of Salicylic Acid through Egg Shell Membrane at pH 1.9 (●), and through the IPM-containing Membrane at pH 1.9 (○) and at pH 7.0 (△) at 30°

The data are means±S.D. for 5 experiments.

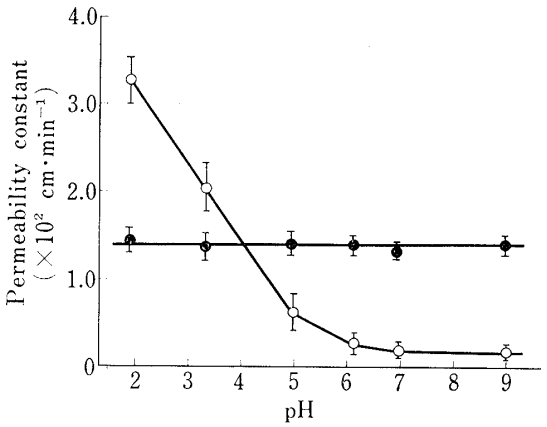

Fig. 4. Relationships between pH and the Permeability Constants of Salicylic Acid through Egg Shell Membrane and the IPM-containing Membrane at 30°

●, egg shell membrane; ○, IPM-containing egg shell membrane.
The data are means±S.D. for 5 experiments.

Table II. The Permeability of Salicylic Acid through Various Membranes

Membrane	Thickness ($\times 10^3$ cm)	pH 1.9			pH 7.0		
		Permeability constant ($\times 10^2$ cm/min)	Partition coefficient	Apparent diffusion coefficient ($\times 10^5$ cm^2/min)	Permeability constant ($\times 10^2$ cm/min)	Partition coefficient	Apparent diffusion coefficient ($\times 10^5$ cm^2/min)
Egg shell membrane	8.2	1.42	4.40	11.6[a]	1.36	—[b] (1)	11.2[a]
Cellulose membrane	3.3	0.912	—[b] (1)	3.01[a]	0.825	—[b] (1)	2.72[a]
IPM-containing egg shell membrane	7.9	3.25	17.5	1.41	0.201	—[b]	—[b]
Polyamide lipoid membrane	1.7	1.47	27.5	0.09	0.165	—[b]	—[b]

a) Calculated on the assumption that the partition coefficient is unity.
b) Could not be measured.

The permeability constants, partition coefficients, and apparent diffusion coefficients at pH 1.9 and 7.0, and the thicknesses of the membranes are summarized in Table II. The relationships among pH, the permeability constants of salicylic acid through the egg shell membrane and the IPM-containing membrane are shown in Fig. 4.

The permeability constant of egg shell membrane was independent of pH, in the same way as that of cellulose membrane. Therefore, the permeation of salicylic acid through egg shell membrane (which is composed of fibrous keratin) is considered to take place through the water channels of the membrane, *i.e.*, the vacant spaces in the membrane. However, salicylic acid was distributed into the membrane at pH 1.9, but not at pH 7.0, as shown in Table II. The distribution of salicylic acid into the membrane at pH 1.9 may be due to adsorption on the fibrous keratin, which should not affect the permeability rate through the water channels of the membrane. The permeability constant of the membrane was larger than that of the cellulose membrane, although the thickness was about three times that of the cellulose membrane, as shown in Table II. The egg shell membrane was, therefore, considered to be more porous

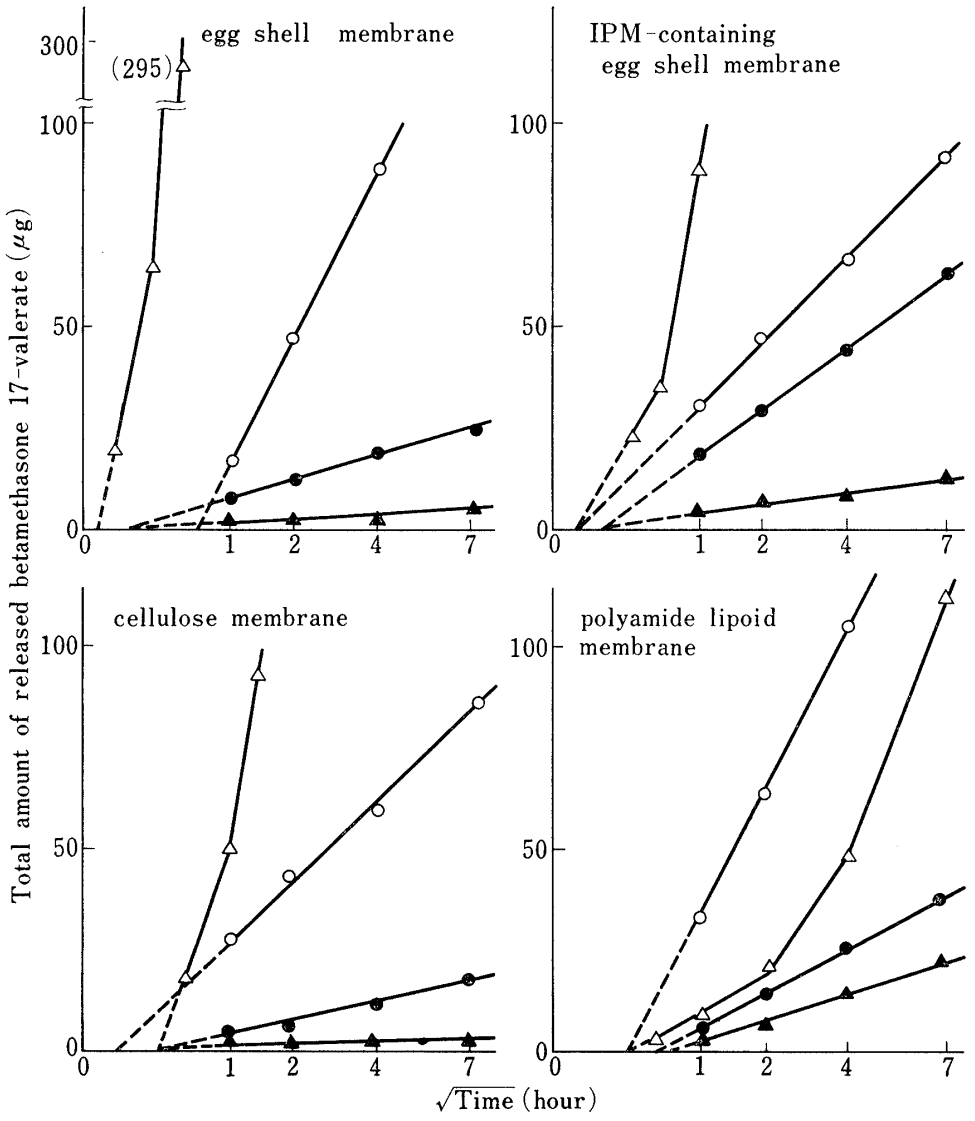

Fig. 5. Release of Betamethasone 17-Valerate from Ointments through Various Membranes at 30°

○, hydrophilic ointment; ●, absorption ointment; ▲, white vaseline; △, macrogol ointment. The data are means of 3 experiments.

than the cellulose membrane. The apparent diffusion coefficients of these membranes were calculated on the assumption that the partition coefficient is unity.

The permeability constant of the IPM-containing membrane was dependent on the degree of dissociation of salicylic acid (pK_a=3.0). The permeation rate of the free form of salicylic acid through this membrane was about twice that of the intact membrane, though the apparent diffusion coefficient decreased to about one-eighth, as shown in Table II. For the ionized form, 21% incorporation of IPM brought about an 85% reduction of the permeability relative to that of the intact membrane. The permeation of salicylic acid through the IPM-containing membrane may be due to its distribution into the IPM phase as a main access route for the free form. The ionized form is not likely to be distributed to the oil phase and may diffuse through the unoccupied water channels of the membrane. The permeability constant of salicylic acid in IPM-containing membrane was considerably larger than that in excised skin, reported by Takehara *et al.*[14]

Release of Betamethasone 17-Valerate from Ointments

The release experiments on betamethasone 17-valerate from various ointments were carried out using the egg shell membrane, IPM-containing membrane, cellulose membrane, and polyamide lipoid membrane.

It is known that the amount of drug released from ointment is approximately proportional to the square root of time.[15] When the amounts of betamethasone 17-valerate released from ointments were plotted *versus* $\sqrt{t}$, straight lines were obtained except in the case of macrogol ointment, as shown in Fig. 5.

In the case of the egg shell membrane, betamethasone 17-valerate was released most quickly from macrogol ointment. The release rates from hydrophilic ointment, absorption ointment, and white vaseline decreased in that order. Further, in the cases of the IPM-containing membrane and cellulose membrane, the release rates showed the same pattern for all ointments examined. However, in the case of polyamide lipoid membrane, the drug was released most quickly from hydrophilic ointment. The release rate of macrogol ointment was slow up to one hour and then accelerated.

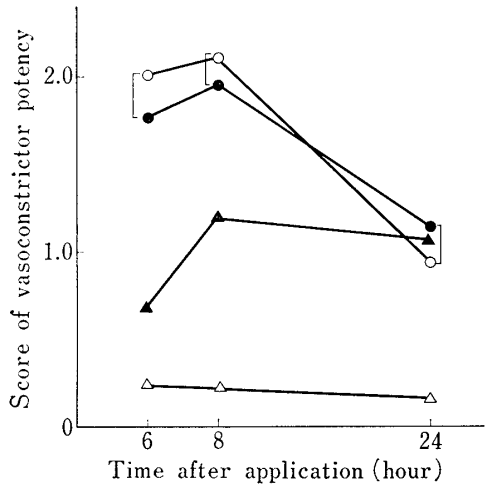

Fig. 6. Vasoconstrictor Potencies of Betamethasone 17-Valerate in Various Ointments

○, hydrophilic ointment; ●, absorption ointment; ▲, white vaseline; △, macrogol ointment.
The symbol ⊏ indicates that the difference in scores between ointments in this group is not statistically significant ($p < 0.05$).

In the experiment with macrogol ointment, it was observed that water in the receptor cell diffused to the ointment side through the membrane, and the macrogol ointment changed into a solution. The degree of solution formation in the case of the egg shell membrane was the highest of all the membranes. On the other hand, in the case of polyamide lipoid membrane, such a solution was hardly formed up to about one hour, followed by increasing solution formation. Thus, one reason for the fast drug release from macrogol ointment seems to be an increase in the rate of drug diffusion in the macrogol ointment due to the formation of a solution.

Vasoconstrictor Activities of Various Ointments

The human vasoconstrictor activities of various ointments containing betamethasone 17-valerate were examined and the results were compared with those for *in vitro* release.

14) M. Takehara, T. Nakagawa, Y. Ushio, K. Narahara, and H. Oishi, *Chem. Pharm. Bull.*, **24**, 1779 (1976).
15) T. Higuchi, *J. Soc. Cosm. Chem.*, **11**, 85 (1960).

The degree of vasoconstrictor activity of each ointment is shown in Fig. 6. The order of vasoconstrictor activities at 6 and 8 hours after application was hydrophilic ointment>absorption ointment>white vaseline>macrogol ointment. The order of the vasoconstrictor activities of these ointments, except for macrogol ointment, agreed with that of their *in vitro* release, as shown in Fig. 5. The vasoconstrictor activity of macrogol ointment was the lowest of all, but the *in vitro* release was the fastest of all. The reason for this was considered to be that macrogol ointment changed into a solution in the release experiment, and so the drug was released quickly, while in the case of *in vivo* study, in which the influence of diffused water is small, the affinity of betamethasone 17-valerate for the macrogol base may be high, in the same way as with salicylic acid,[16] so that the percutaneous absorption seems to be suppressed.

Accordingly, in order to establish an *in vitro* method for determining drug release from ointment which correlates with the percutaneous absorption, it is necessary to avoid the diffusion of water from the receptor cell through the membrane in the case of drug release from a water-soluble vehicle such as macrogol.

16) H. Nogami and M. Hanano, *Chem. Pharm. Bull.*, **6**, 249 (1958).