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Permeation of Diclofenac Salts through Silicone Membrane: a Mechanistic Study of Percutaneous Absorption of Ionizable Drugs

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To understand the mechanism of percutaneous absorption of ionizable drugs, diclofenac salts (DM: M = Li, Na, K, NH_4, $N(Me)_4$) as hydrophilic drugs were compared to free diclofenac acid (DH), a hydrophobic drug. DNa showed the highest permeability coefficient among DMs. The experimental diffusion coefficient through the silicone membrane (*Dm*) was compared with the calculated diffusion coefficient for an ion pair molecule (Dm_{pair}) of DM. This may suggest that the existence of the lipid pathway of the DMs is the same as the DH.

Keywords diclofenac salt; silicone membrane; ion-pair; lipid pathway; permeability

It has been postulated that there are two types of pathway for drugs to permeate through the skin: hydrophobic drugs mainly penetrate through the skin by a lipid pathway, whereas hydrophilic drugs do so by a pore pathway.[1] If this is true, the hydrophilic drug cannot penetrate through silicone membrane. To examine this concept we selected diclofenac salts (DM: M = Li, Na, K, NH_4, $N(Me)_4$) as hydrophilic drugs and studied their permeation through the silicone membrane in comparison with the free diclofenac (acid, DH) as a hydrophobic material. DNa, an anti-inflammatory drug, is water soluble but readily penetrates through the abdominal skin in rats *in vitro* and *in vivo*.[2-5] We have also reported on the experimental partition coefficient in octanol–water,[6] the calculated partition coefficient,[7] and the ionic behavior of DH and DMs in an ethanol-aqueous solution.[8] Previous studies have suggested the possibility that DH and DMs permeate in the state of ion pairs. In this paper, we studied the hydrophobic features of DMs by measuring the DM permeation through a silicone membrane, and report the possible existence of a lipid pathway for DMs by absorption through the skin.

Experimental

Materials DH was obtained by recrystallization two times from DNa (Sigma Chemical Co., St. Louis, MO, U.S.A.) in an ethanol-aqueous solution and an acidic state with the addition of hydrochloric acid. Potassium and lithium hydroxides, ammonia water and tetramethyl ammonium chloride were of guaranteed reagent grade (Wako Pure Chemical Industries, Ltd.). Other chemicals used were all of analytical grade. Distilled water was used throughout the experiments. Non-reinforced polydimethylsiloxane sheeting (Dow Corning, Midland, MI, U.S.A.) Silastic 500-1 (0.0127 cm thick) was used for the diffusive barrier, and Silastic 500-5 (0.0508 cm thick) was used to aid the absorption of DH and DMs into the membrane. The membranes were presoaked in water to remove extractables.

Preparation of DMs About 7 g of DH was dissolved in 500 ml of Mili Q water. The DMs were prepared by neutralizing an aqueous solution of the acid by addition of an equivalent amount of the selected base. The final product was slowly crystallized from the solution or recrystallized after solvent evaporation. The DMs were then filtered and dried *in vacuo*. The ratios of C, H and N in the DH and DMs dictated the coincident values which were within 0.3% of the calculated ones based on elemental analysis. The yields were 71.05% for DLi, 83.13% for DK and 86.77% for $DN(Me)_4$.

The melting points (*Tm*) were determined by differential scanning calorimetry (DSC-8230B, Rigaku Co., Tokyo) and are shown in Table I. Each sample was heated at a rate of 10 °C/min from room temperature to each melting point plus 60 °C, as reported previously.[8] The DNH_4 obtained in precipitation was not pure enough, therefore, it was prepared in the solution simply by adding an equivalent amount of ammonia water.

Determination of Solubility in Water and Isopropylmyristate Ten ml of water or isopropylmyristate (IPM) was added to an amount of each salt to ensure saturation at 50 °C. After the temperature was reduced to 25 °C, the excess DH and DMs crystallized. The system was left to equilibrate for one week at 25 ± 0.05 °C. The samples were centrifuged, then filtered (Ekicrodisc, 0.2 μm, Gelman Sciences Japan, Ltd., Japan), and assayed spectrophotometrically (Ubest-30, Japan Spectroscopic Co., Ltd., Tokyo, Japan).

Determination of Solubility in Membrane The membrane solubilities and membrane-solvent partition coefficients for DH and DMs were determined by the following method. The determinations were performed in quadruplicate. Accurately weighed samples (0.25—0.28 g) of the silicone membranes were placed into saturated aqueous solutions of the DH and DMs. After the membranes (0.0508 cm, thick) were maintained at 25 ± 0.05 °C for one week (preliminary studies had shown that this was sufficient time to attain equilibrium), they were removed from the aqueous solutions and carefully rinsed with water to remove adhering solute. Complete extraction of DH and DMs from the membrane was achieved by immersion of the membrane in 5 ml of an ethanol–water (2 : 3, w/w) mixture and sonication for 15 min at approximately 50 °C. The UV analysis of the ethanol-aqueous solution extract yielded the total amount of solute present in the membrane.

Calculation of the Partition Coefficient The apparent partition coefficients were defined by two ways: one as the ratio of the equilibrium concentration of solute in the membrane to that in the water (*Kapp*), and the other as the ratio of the equilibrium concentration of solute in the IPM to that in the water (*Kipm*).

Calculation of the Nonionized Fraction The nonionized fraction of DH and DMs (*Fu*) was calculated from diclofenac pK_a (4.16) and the pH of drug suspension using the Henderson-Hasselbalch equation:

$$Fu = 1/[1 + \mathrm{antilog}(\mathrm{pH} - \mathrm{p}K_a)]$$

Permeation Studies Horizontal diffusion cells maintained at 25 °C were used in the permeation studies. The donor and receptor solvent was 6 ml of the same water. Suspensions of DH and DMs were used as donor solutions. The available area of the silicone membrane for permeation was 0.968 cm^2 and its thickness was 0.0127 cm. Twenty μl of the receptor solution was sampled. The concentration of DH and DMs in the sample was determined with a Shimadzu LC 6A high performance liquid chromatograph (detector, SPP-6AV, YMC A-302 column; mobile phase consisted of methanol–water, 75 : 25, v/v with 1% (w/w) of phosphoric acid; flow rate, 1 ml/min; range 0.01; analyzed at 254 nm) equipped with a data processor, Chromatopac C-R6A. These studies were generally performed in quadruplicate.

Calculation of the Permeability Coefficient Steady-state flux *J* is a function of both the apparent permeability coefficient *Papp* and the solubility for DH and DMs in water (*Cw*), in the case of suspensions in the donor aqueous solution. The experimental *Papp* value was calculated as:

$$\begin{aligned} Papp &= J/Cw = Fu\cdot Pu + (1-Fu)\cdot Pi \\ &= Km\cdot Dm\cdot Fu/h + Km'\cdot Dm'\cdot(1-Fu)/h \end{aligned} \qquad (1)$$

where Pu and Pi are the intrinsic permeability coefficients of nonionized and ionized form of DH and DMs, respectively, Km is the intrinsic partition coefficient of nonionized form of DH and Km' is that of ionized form of DM, Dm and Dm' are the diffusion coefficients of nonionized form of DH and ionized form of DMs, respectively, and h is the thickness of the membrane.

The Dm and Dm' values could be found by determining Pu and Pi at low pH and high pH where the fraction of nonionized and ionized form Fu and $(1-Fu)$, respectively, are equal to unity. The simple mechanism can then be quantitatively tested with DH and DMs under known pH conditions for which Fu in the donor solution can be calculated. The pH of the donor aqueous solution where DH and DMs are saturated is 5.21 for DH, 7.92 for DLi, 7.70 for DNa, 8.04 for DK and 7.17 for $N(Me)_4$. The pK_a value of DNa is 4.16 at 25 °C[6]; therefore, D is ionized more than 99.9% in the donor aqueous suspension of DMs except for DH.

Results

Melting Point, Solubility and Partition Coefficient

Table I shows the molecular weight, the melting point, *Cw* values, the solubility for DH and DMs in the membrane (*Cp*) and in IPM (*Cipm*), and *Kapp* and *Kipm* values. The log *Km* and log *Km'*, and log *Kipm* showed very similar values.

The *J*, log *P* and log *Dm* values obtained using Eq. 1 are shown in Table II.

The log *Km* and log *Km'* values are dependent on the log *Cw* values, showing a good linear relationship ($r=0.995$) (Fig. 1). The *Cw* value is dependent on the *Tm* values (Fig. 2).

Figure 3 shows the cumulative amount of DH and DMs which permeated across the silicone membrane from their aqueous suspensions, against time. Steady state permeation was achieved immediately. The *J* values were cal-

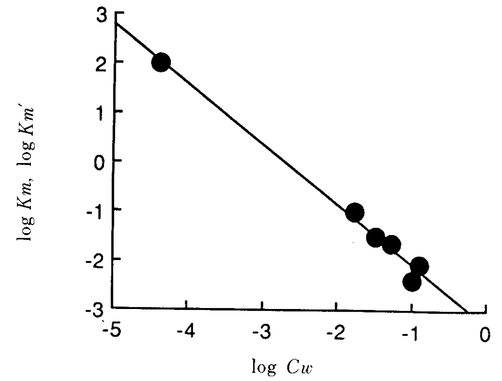

Fig. 1. Relationship between log *Km* and log *Km'*, and log *Cw*

Line (–) was determined by linear least-squares method using experimental mean values. $r=0.995$.

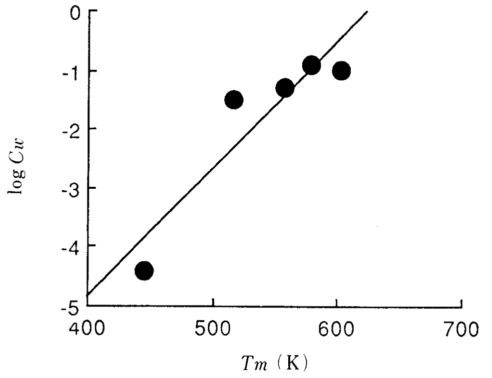

Fig. 2. Relationship between log *Cw* and *Tm*

Line (–) was determined by linear least-squares method using experimental mean values. $r=0.920$.

Table I. Physicochemical Properties of Diclofenac Salts

DM	M.W. g/mol	Tm[a] (°C)	Cw[b] (mmol/g)	Cp[c] (μmol/g silicone)	$Cipm$[d] (μmol/g)	log $Kapp$[e]	log $Kipm$[f]
DH	296.15	171.6	$4.08\pm0.68\times10^{-5}$	4.230 ± 0.357	18.6	2.016	2.659
DLi	302.08	330.0	$0.103\ \pm0.005$	0.422 ± 0.015	0.84	−2.388	−2.089
DNa	318.13	284.7	0.0527 ± 0.002	1.208 ± 0.265	1.25	−1.640	−1.625
DK	334.24	305.3	$0.126\ \pm0.004$	1.066 ± 0.116	0.49	−2.073	−2.410
DNH_4	313.18	—	0.0169 ± 0.005	1.668 ± 0.103	—	−1.006	—
$DNMe_4$	369.29	242.6	0.0324 ± 0.002	0.991 ± 0.016	0.31	−1.514	−2.019

a) DSC determination. *b*) Solubility in water. *c*) Solubility in silicone membrane. *d*) Solublity in isopropyl myristate. *e*) $Kapp=Cp/Cw$. *f*) $Kipm=Cipm/Cw$.

Table II. Flux, Permeability and Diffusion Coefficient of Diclofenac and Its Salts Suspension

DM	J[a] (μg/s·cm²)	pH	Fu[b]	log $Kapp$[c]	log Km[d] log Km'	log $Papp$[e] (cm²/s)	log Pu[f] log Pi	log Dm[g] log Dm'
DH	3.805 ± 0.454	5.21	0.0988	2.016	3.021	−4.058	−3.053	−7.010
DLi	3.689 ± 0.634	7.92	0.0002	−2.388	−2.388	−7.482	−7.482	−6.991
DNa	11.764 ± 4.241	7.60	0.0004	−1.640	−1.640	−6.710	−6.710	−6.967
DK	6.426 ± 1.084	8.04	0.0001	−2.073	−2.073	−7.373	−7.373	−7.196
DNH_4	0.562 ± 0.074	8.04	0.0001	−1.006	−1.006	−7.530	−7.530	−8.421
$DNMe_4$	6.688 ± 0.924	7.17	0.001	−1.514	−1.514	−6.809	−6.809	−7.191

a) Data are the mean of three or four experiments ± S.D. *b*) *Fu* was calculated using a pK_a value of 4.16 (determined at 25 °C). *c*) *Kapp* was determined from Cp/Cw in the saturated DH and DMs aqueous solutions at 25 °C. *d*) *Km* and *Km'* denote the partition coefficient of the nonionized and ionized form, calculated from the ratio $Kapp/Fu$ and $Kapp/(1-Fu)$, respectively. For DMs, as *Fu* values were neglected compared with unity, (*Kapp*) was equal to *Km'*. *e*) $Papp=J\cdot10^{-3}/(Cw\cdot3600\cdot A)$, *A* is the area of the membrane. *f*) *Pu* denotes the permeability coefficient of the nonionized form and was calculated from $Pu=Papp/Fu$. For DMs, as *Fu* values were neglected compared with unity, *Papp* was equal to *Pi*. *g*) $Dm=Pu\cdot h/Km$ for DH, $Dm'=Pi\cdot h/Km'$ for DMs, $h=0.0127$ cm.

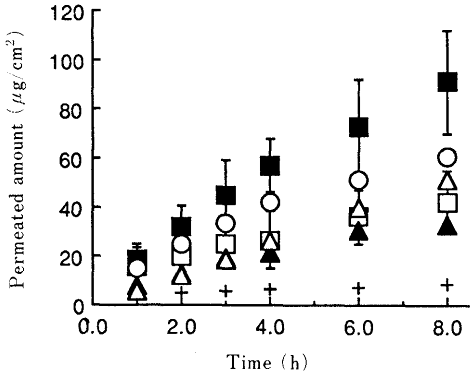

Fig. 3. Permeation of Diclofenac Salts through the Silicone Membrane

□, DH; ▲, DLi; ■, DNa; ○, DK; +, DNH_4; △, $DN(Me)_4$.
Each value represents the mean ± S.D. ($n=3$—4).

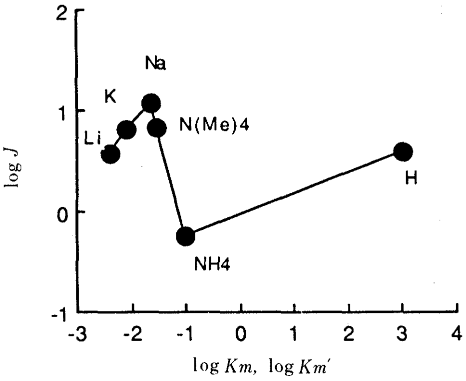

Fig. 4. Relationship between log J and log Km, and log Km'

culated from the slope of the linear portion in Fig. 3. The J value of DNa was the highest among DH and DMs. DH is the acid form and is hydrophobic, but the J value of the DH was not higher than that of DNa.

The relationship between log J and log Km is shown in Fig. 4. The log J value of DNa was the maximum among the DMs.

Discussion

Relationship between *Km* and *Cw* Values The log Km and log Km', and log $Kipm$ showed very similar values, indicating that a specific interaction between the membrane and the penetrant could be ruled out. Therefore, it is reasonable to assume that DMs adsorbed on the silicone membrane without specific polymer-penetrant interaction.

The relationship between log Km and log Km', and log Cw in Fig. 1 is linear ($r=0.995$). The log Cw values of DMs are dependent on the Tm values. The results indicate that the log Km values depend on the log Cw values, which, in turn, depend on the Tm values. Therefore, the log Km and log Km' values may have a linear relationship with the Tm values. In this experiment, log Km value of DH was much higher than log Km' values of DMs; therefore, it may be not appropriate to examine the linearity of log Km and Tm values.

If the solid drug is the same as the crystalline drug, the ideal solubility of the drug expressed as its mole fraction, X_i, is[9]:

$$\log X_i \simeq -\Delta H\cdot(Tm'-T)/(2.303\cdot \mathbf{R}\cdot Tm'\cdot T) \quad (2)$$
$$= -\Delta S\cdot(Tm'-298)/1364$$
$$= -\Delta S\cdot(Tm-25)/1364 \quad (2')$$

where Tm' and Tm are the melting temperatures (K) and (°C), respectively; ΔH is the heat of fusion of the drug; $\mathbf{R}$ is the gas constant; T is the experimental temperature (25 °C); and ΔS is the entropy of fusion. The real aqueous solubility of a crystalline drug (X_w) differs from the ideal solubility (X_i). When a drug is suspended in water, the activity of dissolving the drug in the water (a_w) is generally the same as that of a solid drug (a_c),[10] and it is generally expressed as $\gamma_w X_w = a_w = a_c = X_i$. Here γ_w is the activity coefficient for the drug in water. It is supposed that the solubility of DMs in water indicates a linear relationship with the Tm values as shown in Fig. 2. This means that the thermodynamic activity of the suspension of DH and DMs (a_w) depends on the Tm values. The relationship between the solubility of DMs in water and the Tm values is linear because the values of ΔS for the different counter ions are similar. It is difficult to measure the thermodynamic activity of DH and DMs in the donor suspension; therefore, the Tm values can be used as an indicator of thermodynamic activity of DH and DMs.

Comparison of *Dm′* with *Dm*$_{\text{pair}}$ Values As shown in Eq. 1, the Km and Km', and Dm and Dm' values are the practical factors that influence membrane permeability. The Dm value of DH and Dm' values of DMs are needed to evaluate the Pu and Pi values. The size of the D ion has already been reported while measuring the conductivity of DH and DMs[8]; hence, the diffusion coefficient in medium (D) can be calculated.[11] From the Stokes-Einstein formula, the D value for the uncharged sphere particles can be shown as[11]:

$$D = \mathbf{k}T/(6\pi\eta r) = B\cdot \mathbf{k}T \quad (3)$$
$$B = 1/(6\pi\eta r) \quad (3')$$

where $\mathbf{k}$ is Boltzmann's constant; B is mobility; r is the radius of the particle and η is the viscosity of the medium. The B values between the D anion and the M cation were averaged because the mobility of one anion is affected by another cation.[12] Therefore, the diffusion coefficients of the D and M ions in the medium are the same value ($D_D = D_M$) and are shown in water as:

$$Dw = Dw_D = Dw_M$$
$$= \mathbf{k}T\cdot 2B_D B_M/(B_D + B_M)$$
$$= \mathbf{k}T/[3\pi\eta_w(r_D^s + r_M^s)] \quad (4)$$

where the subscripts w, D and M indicate water and the D and M ions, respectively; r_D^s and r_M^s mean the hydrodynamic radius of the D and M ions in water; η_w is 0.8903×10^{-3} kg/(m·s)[13] and the ($Dw_D = Dw_M$) value is expressed as Dw.

An ion pair could produce a different flux. Pardo *et al.* indicated that the flux of physostigmine salicylate was higher than that of physostigmine across the skin.[14] Mazzenga and his colleagues reported that the flux and partition coefficient of salts of zwitterions increased compared with the parent compound.[15,16] Hence, if the D and M ions produce an ion pair, the diffusion coefficient of the ion pair molecule of DM (Dm_{pair}) in the membrane might be shown as:

$$Dm_{\mathrm{pair}}=\boldsymbol{k}T/(6\pi\eta_{\mathrm{si}}r_{\mathrm{DM}}) \tag{5}$$

where η_{si} is the viscosity of the silicone membrane and r_{DM} is the radius of the ion pair molecule of DM. The r_{DM} value is calculated based on the assumption that each ion constitutes one sphere ion and the volume of an ion pair is one sphere. This is expressed as:

$$r_{\mathrm{DM}}=(r_{\mathrm{D}}^{\mathrm{c}\,3}+r_{\mathrm{D}}^{\mathrm{c}\,3})^{1/3} \tag{6}$$

where r^{c} is the crystalline radius. The r^{c} value was used for an ion pair in the membrane because dehydration of the ion is assumed to occur. The radius of the *D* ion obtained from the conductivity method is 0.427 nm, as reported previously[8]; this is almost the same value as that calculated by the atomic or group contribution method.[17] This result suggests that the *D* ion is not hydrated in water; therefore, $r_{\mathrm{D}}^{\mathrm{c}}$ is equal to $r_{\mathrm{D}}^{\mathrm{s}}$. The r_{DM} values were calculated using $r_{\mathrm{D}}^{\mathrm{s}}$ and $r_{\mathrm{M}}^{\mathrm{c}}$ values[8] in Eq. 6.

The r_{DM}, *Dw* and Dm_{pair} values are listed in Table III. The η_{si} value is not known but if the *Dm'* values of DMs are equal to the Dm_{pair} values in Eq. 5, η_{si} value could then be calculated using Eq. 5 and r_{DM} values as shown in Table III (50.04 for DLi, 47.03 for DNa and 61.08 for DK). The average for the η_{si} values was 53.66 kg/(cm·s). Using this value in Eq. 5, the Dm_{pair} value was calculated back as shown in Table III. The Dm_{pair}/Dw values were compared with the experimental Dm'/Dw values to examine the possibility of an ion pair in DMs permeation. The values of the calculated Dm_{pair}/Dw and experimental Dm'/Dw are plotted against the crystalline radius of the M ion $r_{\mathrm{M}}^{\mathrm{c}}$ in Fig. 5.

TABLE III. Radius of D and M Ions and the Calculated Diffusion Coefficients of Diclofenac Salts in Water and Silicone Membrane

DM	$r_{\mathrm{D}}^{\mathrm{s}}$ nm	$r_{\mathrm{M}}^{\mathrm{s}}$ [a] nm	$r_{\mathrm{M}}^{\mathrm{c}}$ [a] nm	r_{DM} [b] nm	log *Dw* [c] cm²/s	log Dm_{pair} [d] cm²/s
DH	—	—	−0.004	0.423	−5.236	−7.010[e]
DLi	0.426	0.240	0.09	0.427	−5.434	−7.021
DNa	0.427	0.180	0.116	0.430	−5.393	−7.024
DK	0.558	0.130	0.152	0.562	−5.448	−7.140
DNH_4	—	0.130	0.148	0.562	−5.448	−7.140
$DNMe_4$	—	0.204	0.347	0.600	−5.492	−7.168

a) Data from ref. 8. *b*) Radius of ion pair DM; $r_{\mathrm{DM}}=(r_{\mathrm{D}}^{\mathrm{s}3}+r_{\mathrm{M}}^{\mathrm{c}3})^{1/3}$, $r_{\mathrm{D}}^{\mathrm{s}}$ is 0.427 nm. *c*) Diffusion coefficient of D and M ions in water, $Dw=\boldsymbol{k}T/[3\pi\eta_{\mathrm{w}}(r_{\mathrm{D}}^{\mathrm{s}}+r_{\mathrm{M}}^{\mathrm{s}})]$, $\eta_{\mathrm{w}}=0.8903\times10^{-3}$ kg/(m·s).[13] *d*) Diffusion coefficient of DM ion pair in silicone membrane; $Dm_{\mathrm{pair}}=\boldsymbol{k}T/(6\pi\cdot\eta_{\mathrm{si}}\cdot r_{\mathrm{DM}})$, if *Dm'* in Table II, is equal to Dm_{pair}, η_{si} is obtained ăt 50.04 for DLi, 47.03 for DNa, 61.08 for DK and 56.48 for $DNMe_4$. The average of the η_{si} value was determined at 53.66 kg/(m·s). Dm_{pair} value was calculated using each r_{DM} and η_{si} value. *e*) log *Dm* value in Table II.

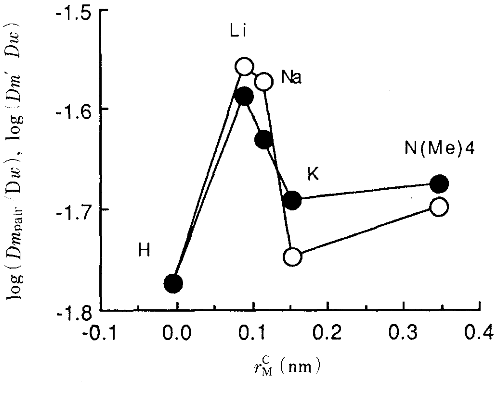

Fig. 5. Relative Calculated Dm_{pair}/Dw and Experimental Dm'/Dw Values Plotted against the Crystalline Radius of the M ion $r_{\mathrm{M}}^{\mathrm{c}}$

●, calculated Dm_{pair}/Dw values; ○, experimental Dm'/Dw values.

The relationship of the Dm_{pair}/Dw values to $r_{\mathrm{M}}^{\mathrm{c}}$ clearly correlates with that of the experimental Dm'/Dw values except in the case of NH_4. The log *Pi* value of DNH_4 did not correlate with log *Km'* in Table II, therefore, DNH_4 is not shown in Fig. 5. It was not previously known that DNH_4 has special behavior. This might be related to DNH_4 not being obtained in precipitate.

Lipid Pathway of DMs The silicone membrane has no aqueous pores, therefore, there is no possibility for the DMs to penetrate by a polar pathway. The permeation of DH and DMs through the membrane might be viewed as a mechanism depending on the partition, *i.e.*, a lipid pathway, since DMs penetrate through silicone membrane like DH. The penetrant is distributed to the membrane first, and then the DMs form an ion pair near the membrane and distribute it. Therefore, we have two hypotheses: 1) the DM permeates through the membrane in an ion pair state; 2) the DM changes to DH in the membrane and permeates through it as DH. If the second hypothesis is true, the diffusion coefficient values of DMs should be almost the same as those of DH. However, the experimental *Dm'* values of DMs are different, and the diffusion coefficient value of DNa is the highest among the DH and DMs (Table II). It might thus be possible to theorize that the DMs penetrated through the membrane in an ion pair state.

The underlying mechanism of the increased *J* value of DMs may require the interaction of the D^- and M^+ ions. DMs are dissolved in water in an ionic state but the D and M ions should be dehydrated for penetration into the membrane. The D ion may not be hydrated; therefore, the dehydration of the M ion could be the critical point for penetration through the membrane. Conradi *et al.* reported that the process of dehydration of a peptide was important for the solute to enter the biological membrane.[18] This result coincides with ours. The *J* value of DNa is the highest among the DH and DMs, this may be because the Na ion uses lower energy for dehydration of all compared to other M ions. This difference in dehydration behavior between Na and K ions was supported by the results of the partition coefficient; that is, DNa showed higher partition coefficient values than DK in the partition from water to octanol.[16]

This study demonstrates the possibility that a lipid pathway exists in the permeation of diclofenac salts. This would be useful as a method of percutaneous absorption, where the flux of hydrophilic drugs increases and changes the counter ions.

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