[Chem. Pharm. Bull. 29(8)2330—2337(1981)]

Mechanism of Indomethacin Partition between *n*-Octanol and Water

Toshio Inagi,*,[a] Toyojiro Muramatsu,[a] Hidetaka Nagai,[a] and Hiroshi Terada[b]

Tokyo Research Laboratories, Kowa Co., Ltd.,[a] Noguchi-cho, Higashimurayama-city, Tokyo, 189, Japan and Faculty of Pharmaceutical Sciences, University of Tokushima,[b] Shomachi-1, Tokushima, 770, Japan

(Received December 5, 1980)

To examine the fundamental process of the transfer of indomethacin (IND) to the biological membrane, the partition coefficient between *n*-octanol and water was measured at various pH values or in the presence of various cations by the shaking-flask method at 25°C.

The apparent partition coefficient decreased with increase in pH and the true partition coefficient of unionized IND was determined to be 1.20×10^4, while that of ionized IND was 1.72×10^{-1}. The IND anion was found to be readily transferred to the hydrophobic region by forming ion-pair complexes with cations in the aqueous phase. The ion-pair complex of IND with the ammonium ion was transferred to the organic phase more readily than that with the sodium or potassium ion.

In this study, a method for quantitative analysis of the ion-pair partition of an unstable drug at alkaline pH was established under conditions where more than two cationic species were present.

Keywards——partition coefficient; indomethacin; anti-inflammatory drug; partition; ion-pair partition; ion-pair partition coefficient; ion-pair formation constant; extraction constant

Indomethacin (IND: molecular structure, Chart 1) is a well-known nonsteroidal anti-inflammatory agent, and is effective against severe rheumatoid arthritis, ankylosing spondylitis, osteoarthritis of large joints, and other inflammations.

Recently, we reported that the percutaneous absorption of IND through guinea-pig skin decreased with increase in the pH of the medium. However, the amount of IND absorbed was much greater than expected from the value calculated on the basis that only unionized IND is absorbed, especially at pH above pK_a.[1] This suggests that the ion-pair complexes of IND anion with cations in the medium are transferred to the skin, just as ion-pair complexes of various compounds (*e.g.* methyl orange[2] and 2,4-dinitrophenol[3]) are moved to hydrophobic regions. Thus, a study on the partition and the ion-pair partition of IND between organic solvent and water should help to clarify the molecular mechanism of percutaneous absorption of IND.

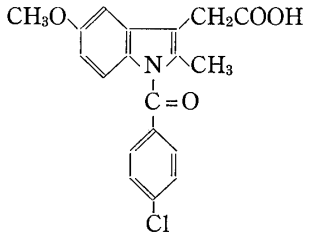

Chart 1. Structure of IND

The present study was intended to elucidate the mechanism of IND partition at both acidic and alkaline pH, by measuring the partition coefficient of IND between *n*-octanol and water under various conditions. *n*-Octanol is regarded as the organic solvent most nearly reflecting the properties of biomembranes.[4]

Experimental

Materials——IND used was of J.P. grade. Since it was thin-layer chromatographically pure, it was used without further purification. *n*-Octanol was a special-grade product of Wako Pure Chem. Ind., Osaka, and was distilled 3 times before use. The other reagents used were standard commercial products.

Measurement of Partition Coefficient——A glass-stoppered centrifuge tube, containing 1 ml of *n*-octanol solution of IND (1.02×10^{-2}—6.00×10^{-4} M) and 5 ml of phosphate buffer was gently shaken in a water

both at 25±0.1°C for 18 h. The concentration of IND in the aqueous phase after partition equilibrium was determined spectrophotometrically or gas-chromatographically as described below. The concentration of IND in the *n*-octanol phase was determined according to Eq. (1), where C and V represent the concentration of IND and the volume, respectively, subscripts o and w represent the *n*-octanol and aqueous phases, respectively, and C_{in} indicates the initial concentration of IND in the *n*-octanol phase,

$$C_o = (C_{in}V_o - C_wV_w)/V_o \quad (1)$$

Determination of Solubility in H_2O——An excess of IND was shaken with McIlvaine's buffer at various pH values for 18 h at 25°C. This suspended IND was filtered off through a glass-wool-tipped pipet and then the concentration of IND in the filtrate was determined spectrophotometrically as described below.

Determination of IND in the Aqueous Phase——1) By Spectrophotometry: The concentration of IND in the aqueous phase (above 10^{-6} M) was determined from the absorption at 320 nm in a Hitachi spectrophotometer, model 124.

2) By Gas Chromatography: When the concentration of IND was very low (less than about 10^{-6}M), IND was determined by using an electron-capture detector gas chromatograph (Shimadzu gas chromatograph, model 4BM-ECD). IND in the aqueous phase was extracted with 1,2-dichloroethane, then 1,2-dichloroethane was evaporated off under a stream of N_2 gas. The butyl ester of IND was obtained by adding carbonyl-diimidazole, triethylamine and butanol to the residue. This sample was analyzed by gas chromatography with the amyl ester of IND as an internal standard.

Results and Discussion

1. Effect of pH on Partition Coefficient

The effect of the initial concentration of IND on the partition coefficient was measured over a concentration range of 1.00×10^{-4} to 2.80×10^{-3} M between pH 2.0 and 9.0. The apparent partition coefficient P' ($=C_o/C_w$: the ratio of the concentration of IND in the *n*-octanol to that in the aqueous phase) was independent of the initial concentration of IND at any pH (Fig. 1), indicating that IND was not associated in the *n*-octanol or aqueous phase.

Assuming that only unionized IND is responsible for the partition between *n*-octanol and H_2O, the apparent partition coefficient (P') can be expressed as follows:

$$P' = C_o/C_w = [AH]_o/([AH]_w + [A^-]_w) \quad (2)$$

where AH, A^- and [ ] represent unionized IND, ionized IND and the concentration, respectively. Eq. (2) can be written as:

$$\log P' = \log P_{AH} - \log(1 + K_a/(H^+)) \quad (3)$$

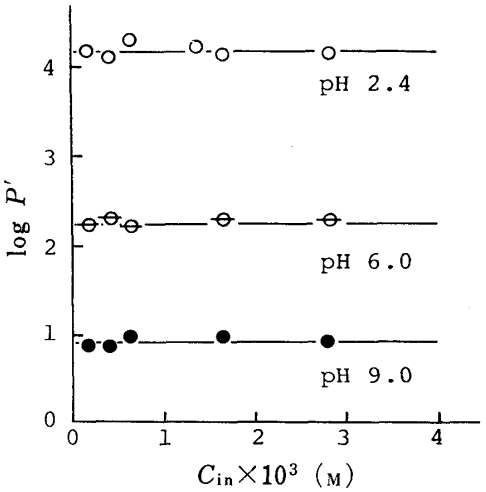

Fig. 1. Relationship between log P' and Initial Concentration of IND (C_{in})

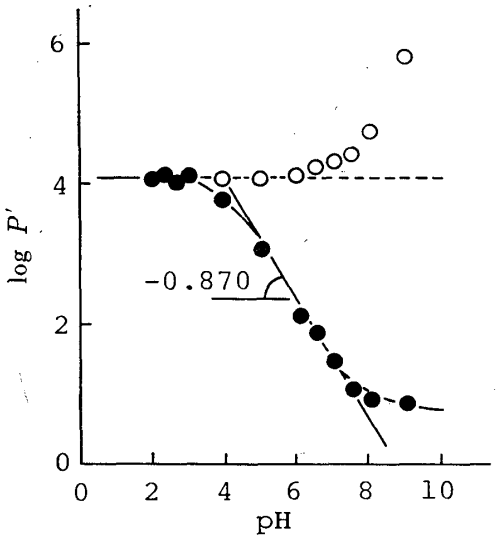

Fig. 2. pH Dependence of log P' of IND

●: log P' determined by partition experiments.
○: log P_{AH} determined from log P' (●) according to Eq. (3).

where (H^+) is the activity of H^+ in the aqueous phase, K_a is the acid dissociation constant and P_{AH} is the partition coefficient of unionized IND ($=[AH]_o/[AH]_w$). According to Eq. (3), P' is pH-dependent, and P' changes with pH as shown in Eqs. (4) and (5).
When $pH \ll pK_a$,

$$\log P' = \log P_{AH} \tag{4}$$

and when $pH \gg pK_a$,

$$\log P' = -pH + (pK_a + \log P_{AH}) \tag{5}$$

According to Eqs. (4) and (5), P' in the region of $pH \ll pK_a$ is P_{AH} itself, and log P' should decrease linearly with a slope of -1 as the pH increases in the region of $pH \gg pK_a$. Thus, if the relations expressed by Eqs. (4) and (5) hold over a wide range of pH, it can be concluded that only the unionized form of IND is transferred from the aqueous to the organic phase.

Fig. 2 shows the changes in the apparent partition coefficient of IND with pH. The pH of the aqueous phase was kept constant with McIlvaine's buffer which is available in the range of pH 2 to pH 9. Below pH 3, log P' took a nearly constant value of 4.08. According to Eq. (4), this value should be log P_{AH} of IND, since the pK_a of IND was determined to be 4.3 from the relation in Eq. (6), as shown in Fig. 3, and this value is in good agreement with that of Fuwa *et al.*[5] In Eq. (6), S_0 is the solubility of the neutral form of IND (solubility below pH 3), and S is the apparent solubility of IND at various pH values.

$$\log (S-S_0)/S_0 = pH - pK_a \tag{6}$$

Log P_{AH} of IND was calculated on the basis of the additive character of the partition coefficient using π and log P values[6] according to Eq. (7).

$$\begin{aligned} \log P_{AH} &= \pi_{CH_2COOH} + \pi_{OCH_3} + \pi_{C_1} + \pi_{CH_3} + \pi_{C_6H_6} + \pi_{CO} + \log P_{indole} \\ &= -0.61 - 0.02 + 0.71 + 0.5 + 2.13 - 0.43 + 2.14 \\ &= 4.42 \end{aligned} \tag{7}$$

The calculated value coincided well with the experimental one.

In Fig. 2, log P' decreases almost linearly with increase in pH in the region higher than pH 5, but the value of the slope (-0.870) was less than that predicted by Eq. (5). In addition, the value of log P_{AH}, calculated according to Eq. (3) based on P' at a given pH, was always greater than 4.08 (dotted line in Fig. 2), and the difference between P_{AH} determined from

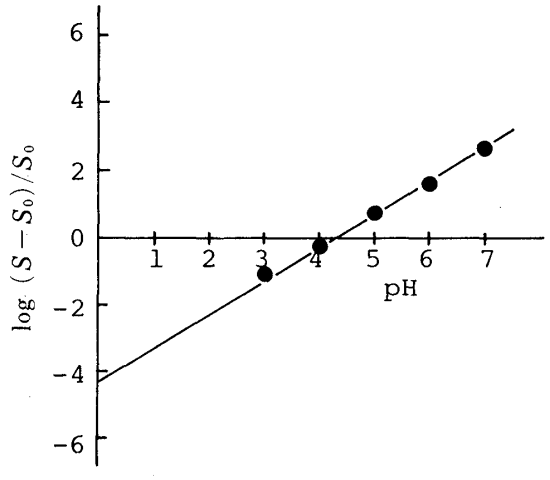

Fig. 3. Relationship between log $(S-S_0)/S_0$ and pH

S: apparent solubility of IND.
S_0: solubility of the neutral form of IND.

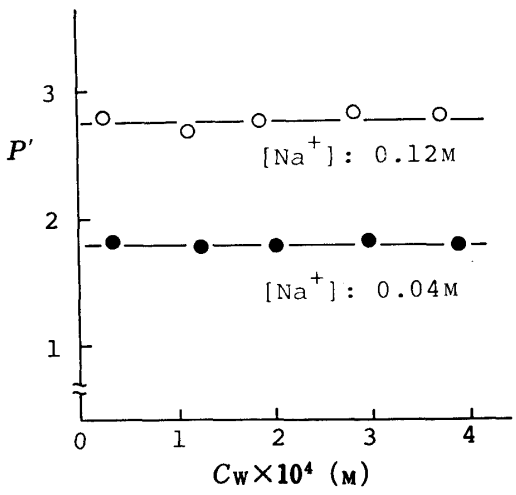

Fig. 4. Dependence on Sodium Concentration of the Apparent Partition Coefficient

P' at acidic pH and that from P' at higher than pH 5 became greater with increase in pH (shown as (○) in Fig. 2). Thus, the partition process of IND does not involve only the unionized species being transferred from the aqueous phase to the organic phase.

2. Effect of Counter Ion on the Partition Coefficient

From the above results, it seems possible that ionized IND is also transferred to the *n*-octanol phase. When the ionized species participates in the transfer to the organic phase, the ion-pair complex formed between ionized IND and a cation is expected to move into the hydrophobic region more readily than ionized IND alone.

To see whether the ionic form of IND is transferred to the organic phase by forming an ion-pair complex, the effects of various cations on the partition of IND were examined at a fixed pH. Fig. 4 shows the effect of NaCl on the partition of IND. The apparent partition coefficient (P') was determined by adding various amounts of NaCl (0.02—0.10 M) to 0.01 M $NaH_2PO_4 \cdot 2H_2O$ and 0.01 M NaOH buffer, pH 8.3, used as the aqueous phase. In Fig. 4, P' increases with increase in the concentration of sodium ion ($[Na^+]$), but at constant $[Na^+]$, P' is constant with any concentration of IND in the aqueous phase, suggesting that IND forms an ion-pair complex between ionized IND and Na^+, but that there is no association between IND molecules in the *n*-octanol or aqueous phase.

Assuming that all the species of IND which exist in the aqueous phase (unionized, ionized and ion-pair complex of IND with cation (X^+)) are transferred from the aqueous phase to the *n*-octanol phase, the partition of IND can be depicted as shown in Fig. 5. In this case, the apparent partition coefficient can be expressed as follows:

$$P' = \frac{[AH]_o + [A^-]_o + [A^-X^+]_o}{[AH]_w + [A^-]_w + [A^-X^+]_w} \tag{8}$$

where A^-X^+ is the ion-pair complex between ionized IND and a cation X^+. Eq. (8) is transformed into Eq. (9), from the relations in Eqs. (10)—(13), when association equilibria between A^- and H^+ and between A^- and X^+ in the organic phase can be ignored. This equation is fundamental

$$P' = \frac{P_{AH}K_{AH}(H^+) + P_{A^-} + P_{AX}K_{AX}[X^+]_w}{1 + K_{AH}(H^+) + K_{AX}[X^+]_w} \tag{9}$$

$$P_{A^-} = [A^-]_o/[A^-]_w \tag{10}$$

$$P_{AX} = [A^-X^+]_o/[A^-X^+]_w \tag{11}$$

$$K_{AH} = \frac{[AH]_w}{[A^-]_w(H^+)} = 1/K_a \tag{12}$$

$$K_{AX} = \frac{[A^-X^+]_w}{[A^-]_w[X^+]_w} \tag{13}$$

for partition where transfer of all the molecular species of IND is taken into account in the absence of intermolecular association of IND.[3] Under conditions where almost all IND is ionized, the terms of $K_{AH}(H^+)$, $P_{AH}K_{AH}(H^+)$ and P_{A^-} in Eq. (9) can be ignored, and thus Eq. (14) is obtained:

$$\frac{1}{[X^+]_w} = \frac{P_{AX}K_{AX}}{P'} - K_{AX} \tag{14}$$

According to Eq. (14), P_{AX} and K_{AX} can be determined from the intercept on the Y-axis and the slope of the straight line in a plot of $1/P'$ against $1/[X^+]_w$, respectively.

However, when a drug is unstable at alkaline pH and considerable amounts of ionized species are transferred to the hydrophobic phase, Eq. (14) cannot be used. In this case, P_{A^-}, P_{AX} and K_{AX} can be determined as follows. According to Eq. (9), P' should increase linearly with increase in $[X^+]_w$ under conditions where (H^+) is kept constant, since in the region

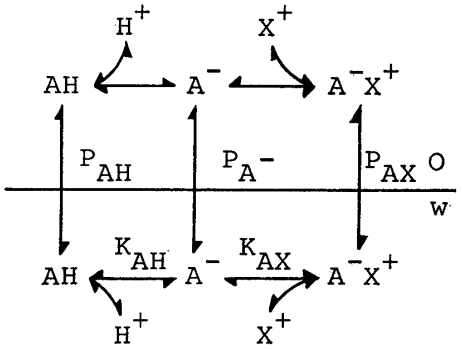

Fig. 5. Proposed Partition Mechanism of IND in the Presence of Cation (X^+)

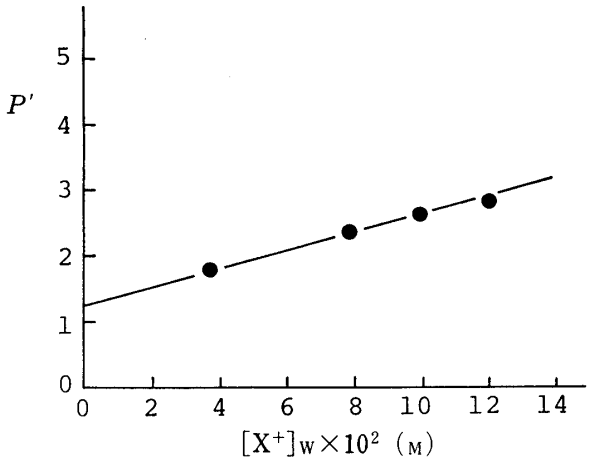

Fig. 6. Relationship between P' and $[X^+]_w$
In this case $X^+=Na^+$.

of $[X^+]_w \ll\{1+K_{AH}(H^+)\}/K_{AX}$, Eq. (9) can be expressed as Eq. (15), Then, as $[X^+]_w$ increases,

$$P' = \frac{P_{AX}K_{AX}}{1+K_{AH}(H^+)}[X^+]_w + \frac{P_{AH}K_{AH}(H^+)+P_{A^-}}{1+K_{AH}(H^+)} \tag{15}$$

increase of P' becomes more gradual, and finally P' reaches a constant value.[3] The intercept on the Y-axis determined by extrapolation of the linear portion in the plots of P' *vs.* $[X^+]_w$ is denoted as P'_0. From the relation of Eq. (15), P'_0 is expressed by Eq. (16).

$$P'_0 = \frac{P_{AH}K_{AH}(H^+)+P_{A^-}}{1+K_{AH}(H^+)} \tag{16}$$

Eq. (16) can be transformed to Eq. (17).

$$P'_0 = K_{AH}(P_{AH}-P'_0)\cdot(H^+)+P_{A^-} \tag{17}$$

Therefore the partition coefficient of the ionized species (P_{A^-}) can be determined as the intercept on the Y-axis in the linear relation between P'_0 and $(P_{AH}-P'_0)\cdot(H^+)$.

Using the experimental data in Fig. 4, plots of P' *vs.* $[X^+]_w$, and P'_0 *vs.* $(P_{AH}-P'_0)\cdot(H^+)$ were found to be linear as shown in Figs. 6 and 7. From Fig. 7, the value of P_{A^-} of IND was determined to be 0.172. If Q and R are defined as in Eqs. (18) and (19), respectively, Eq. (9) can be expressed as Eq. (20).

$$Q = P_{AH}K_{AH}(H^+) + P_{A^-} \tag{18}$$

$$R = 1 + K_{AH}(H^+) \tag{19}$$

$$\frac{RP'-Q}{P'[X^+]_w} = \frac{P_{AX}K_{AX}}{P'} - K_{AX} \tag{20}$$

Thus, the ion-pair complex formation constant (K_{AX}) and the ion-pair partition coefficient (P_{AX}) can be determined from plots of $(RP'-Q)/P'[X^+]_w$ *vs.* $1/P'$ as expected from Eq. (20) (Fig. 8). P_{AX} and K_{AX} (in this case, $X=Na^+$, thus, these values are expressed as P_{ANa} and K_{ANa}, respectively) were determined to be 1.45 $\times 10$ and 1.16, respectively.

Next, the ion-pair partitions of ionized IND with various cations such as NH_4^+ and K^+, were measured in the presence of constant amounts of (H^+) and $[Na^+]$. In these experiments, $[Na^+]$ and pH were kept constant at 0.02 M and 8.3, respectively, with 0.01 M $NaH_2PO_4\cdot 2H_2O$–0.01 M NaOH buffer. On addition of NH_4Cl to the buffer solution of IND (initial concentration of IND: 1.4×10^{-4} M), P' increased linearly as the amount of NH_4Cl increased, as shown in Fig. 9. Thus, it is concluded that ionized IND can form an ion-pair complex with NH_4^+. Denoting Na^+ as X^+, and the "second cation" NH_4^+ as Y^+, P' of IND can be expressed by Eq. (21).

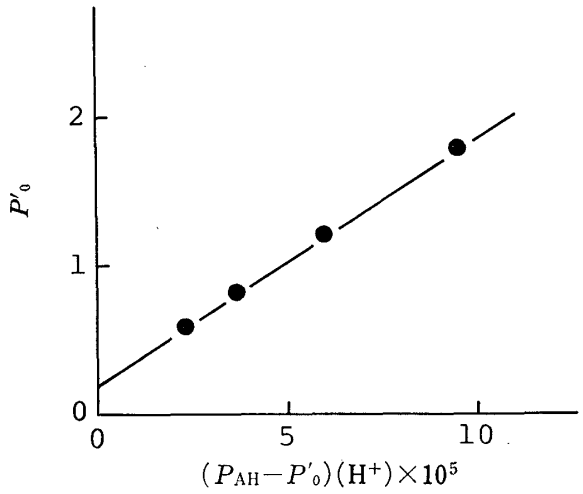

Fig. 7. Relationship between P'_0 and $(P_{AH}-P'_0)$ (H^+) according to Eq. (17)

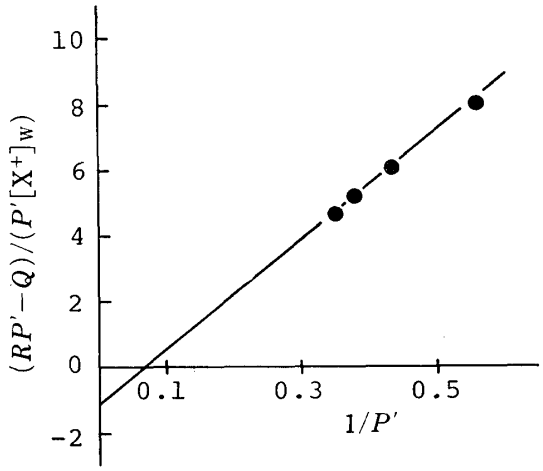

Fig. 8. Relationship between $(RP'-Q)/(P'[X^+]_w)$and $1/P'$ according to Eq. (20)
In this case $X^+=Na^+$.

$$P' = \frac{[AH]_o+[A^-]_o+[A^-X^+]_o+[A^-Y^+]_o}{[AH]_w+[A^-]_w+[A^-X^+]_w+[A^-Y^+]_w} \tag{21}$$

where A^-Y^+ is the ion-pair complex between ionized IND and the "second cation" Y^+. Eq. (21) can be simplified when P' is measured at a fixed value of (H^+) and $[X^+]$ in the presence of various amounts of Y^+ as follows:

$$P' = \frac{S+P_{AY}K_{AY}[Y^+]_w}{T+K_{AY}[Y^+]_w} \tag{22}$$

where P_{AY}, K_{AY}, S and T are defined as follows:

$$P_{AY} = [A^-Y^+]_o/[A^-Y^+]_w \tag{23}$$

$$K_{AY} = [A^-Y^+]_w/[A^-]_w[Y^+]_w \tag{24}$$

$$S = P_{AH}K_{AH}(H^+) + P_{A^-} + P_{AX}K_{AX}[X^+]_w \tag{25}$$

$$T = 1 + K_{AH}(H^+) + K_{AX}[X^+]_w \tag{26}$$

By a treatment similar to that used for the derivation of Eq. (20), Eq. (21) provides Eq. (27).

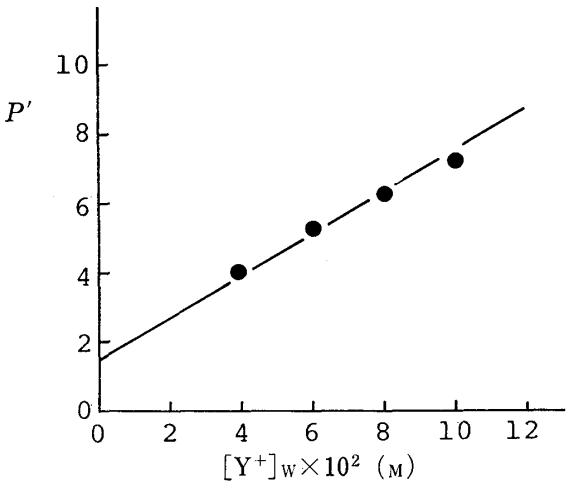

Fig. 9. Relationship between P' and $[Y^+]_w$
In this case $Y^+=NH_4^+$.

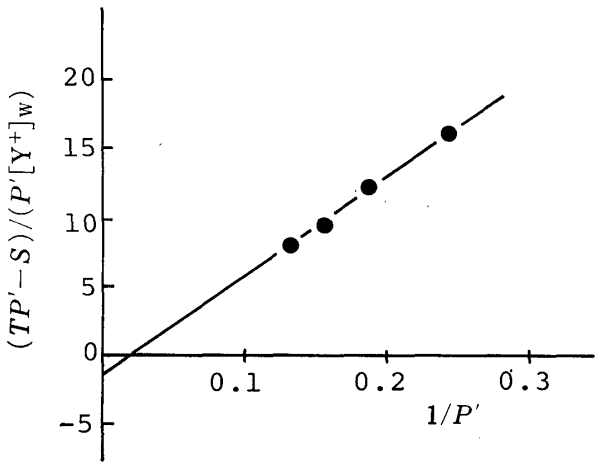

Fig. 10. Relationship between $(TP'-S)/(P'[Y^+]_w)$ and $1/P'$ according to Eq. (27)
In this case $Y^+=NH_4^+$.

$$\frac{TP'-S}{P'[Y^+]_w} = \frac{P_{AY}K_{AY}}{P'} - K_{AY} \quad (27)$$

Using the results of the ion-pair partition of ionized IND with NH_4^+ in the presence of H^+ and Na^+ shown in Fig. 9, linear plots of $(TP'-S)/P'[Y^+]_w$ *vs.* $1/P'$ are obtained, as shown in Fig. 10. Thus, the values of P_{ANH_4} and K_{ANH_4} were determined to be 4.97×10 and 1.45, respectively. Therefore, Eq. (27) was confirmed to be suitable for the determination of the constants which characterize the ion-pair partition, even in the presence of various cations including H^+. The ion-pair partitions of IND with K^+ and triethanolamine (TEA) were also carried out using 0.01 M $NaH_2PO_4\cdot2H_2O$–0.01 M NaOH buffer at pH 8.3 as the aqueous phase. By a treatment similar to that with NH_4^+, the ion-pair complex constants (P_{AY} and K_{AY}) were determined and these values are summarized in Table I. Table I shows that the hydrophobicity of the unionized IND is about 7×10^4 times greater than that of ionized IND and about 2.4×10^2, 7.9×10^2, 8.3×10^2 and 2.6×10^3 times greater than those of the ion-pair complexes of ionized IND with NH_4^+, TEA, Na^+ and K^+, respectively, The affinity of ionized IND for H^+ is about 2.2×10^3, 7.3×10^3, 1.2×10^4 and 1.5×10^4 greater than the affinities for K^+, TEA, NH_4^+ and Na^+, respectively. Thus, unionized IND can readily be partitioned mostly to the *n*-octanol phase and the order of extractability ($E_{AY}=P_{AY}K_{AY}$) of the ion-pair complexes of IND with cations (Y^+) is in the order $NH_4^+>K^+\geqq TEA>Na^+$. This order is in good accordance with the view of Motomizu *et al.*[7]: the ion-pair complex of ionized IND (a water structure-former ion) with NH_4^+ (a water structure-promotor ion) moves to the *n*-octanol phase more easily than that with a water structure-breaker ion (K^+) or with a water structure-former ion (Na^+).

Furthermore, the total concentration of IND in *n*-octanol after partition equilibrium (C_o) is shown by Eq. (28), when the conter ion Y^+ is present in the aqueous phase. The concentration ratio of various species of

$$C_o = [AH]_o + [A^-]_o + [A^-Y^+]_o \quad (28)$$

IND in the *n*-octanol phase can be calculated from the values of P_{AY} and K_{AY} as follows. From Eqs. (10), (12), (23) and (24), Eq. (28) can be rewritten:

$$C_o = (P_{AH}K_{AH}(H^+)+P_{A^-}+P_{AY}K_{AY}[Y^+]_w)[A^-]_w \quad (29)$$

Therefore, the relative concentrations of the various species of IND in the *n*-octanol can be expressed as Eqs. (30)—(32): (ref. 3)

$$[AH]_o = P_{AH}K_{AH}(H^+)/Z \quad (30)$$

$$[A^-]_o = P_{A^-}/Z \quad (31)$$

$$[A^-Y^+]_o = P_{AY}K_{AY}[Y^+]_w/Z \quad (32)$$

where Z is,

$$Z = P_{AH}K_{AH}(H^+) + P_{A^-} + P_{AY}K_{AY}[Y^+]_w \quad (33)$$

Fig. 11 shows the relative concentrations of various molecular species of IND in the *n*-octanol phase, in the case of the ion-pair partition with TEA as a function of pH in the aqueous phase when 0.1 M TEA is present. The percentage of unionized IND decreases abruptly above biological pH (about 6—7) and that of the ion-pair complex of IND with TEA increases as the pH increases (especially at biological pH), but the percentage of the ionized IND increases only slightly with increase in pH (the percentage of ionized IND is 4.7% even at pH 12). Thus, the ion-pair complex of IND with cation (Y^+) is the most important species in the partition process of IND, especially in the region of $pH\gg pK_a$.

Recently, we reported that the percutaneous absorption of IND through guinea-pig skin decreased as the pH of the vehicle increased; this suggests that the unionized IND was mainly absorbed through the skin. However, the amount of percutaneous absorption of IND was much greater than that expected from pH-partition theory at about biological pH.[1] In

Table I. Summary of the Formation of Ion-Pair Complexes

A-Y$^+$	Constants		
	P_{AY}	K_{AY}	E_{AY}*
A-Na$^+$	1.45×10	1.16	1.68×10
A-K$^+$	4.61	7.75	3.57×10
A-NH$_4^+$	4.97×10	1.45	7.21×10
A$^-$·TEA	1.51×10	2.33	3.52×10
AH	1.20×10^4	1.70×10^4	2.04×10^8
A$^-$	1.72×10^{-1}	—	—

* E_{AY} is the extraction constant of the ion-pair complex of IND with cation (Y$^+$), and is the product of P_{AY} and K_{AY}.

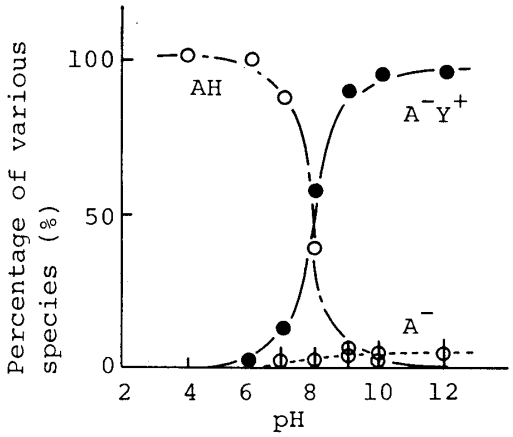

Fig. 11. Relative Concentrations of the Various Species of IND in the *n*-Octanol Phase

In this case Y$^+$=TEA.

view of the results of the present study, it can be concluded that the high absorption of IND is due to the formation of an ion-pair complex of IND with organic amine added in the vehicle.

References and Notes

1) T. Inagi, T. Muramatsu, H. Nagai and H. Terada, *Chem. Pharm. Bull.*, **29**, 1708 (1981).
2) H. Terada and T. Inagi, *Membrane*, **2**(1), 63 (1977).
3) H. Terada, K. Kitagawa, Y. Yoshikawa and F. Kametani, *Chem. Pharm. Bull.*, **29**, 7 (1981).
4) C. Hansch and W.J. Dunn, III., *J. Pharm. Sci.*, **61**, 1 (1972); H. Terada, *Kagaku No Ryoiki, Zokan*, **122**, 205 (1979).
5) T. Fuwa, R. Iga, M. Hanano, H. Nogami and M. Kashima, *Yakugaku Zasshi*, **91**, 1223 (1971).
6) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
7) S. Motomizu, T. Iwachido and K. Toei, *Bunseki*, **4**, 38 (1980).