Die ganze Menge des Verseifungsproduktes wurden in Hexan-Benzol (1:1) gelöst und über 130 g Al_2O_3 chromatographiert. Zum Eluieren wurde ein Gemisch von Hexan-Benzol (1:1) für folgende 4 Fraktionen verwendet: 700 ccm des Nr. 1 Ausflusses gaben keine Kristalle. Die Fraktion Nr. 2 von 250 ccm Ausfluß gab 280 mg Kristalle vom Schmp. 147~151°. Die Fraktion Nr. 3 von 350 ccm Ausfluß gab 410 mg Kristalle vom Schmp. 133~146°. Die Fraktion Nr. 4 von 200 ccm Ausfluß gab 190 mg Kristalle vom Schmp. 128~130°.

Beim weiteren Eluieren mit Benzol-MeOH (1:1) ließen sich für die Fraktion Nr. 5, 310 mg Kristalle vom Schmp. 105~120° liefern, welche nach Umkristallisation aus MeOH 130 mg Kristalle vom Schmp. 130~132° ergaben.

Aus der Fraktion Nr. 3 ergaben sich nach nochmaligem Chromatographieren mittels 50 g Al_2O_3, 160 mg Kristalle vom Schmp. 145~152°. Diese Kristalle und diejenigen aus der Fraktion Nr. 2 ließen sich vereinigen und nochmals mit 45 g Al_2O_3 chromatographieren, wobei 310 mg der Kristalle vom Schmp. 153~155° erhalten wurden. Diese Substanz zeigte nach dem nochmaligen Chromatographieren keine Schmp.-Erhöhung. Zur Mikroanalyse wurde diese Substanz (IVa) aus MeOH umkristallisiert und ergab Seidenfadenförmige Nadeln vom Schmp. 154~155°. $[\alpha]_D^{28.5}$ −63.1°(c=1.01, $CHCl_3$). $C_{27}H_{44}O$-Ber.: C, 84.31; H, 11.53. Gef.: C, 84.19; H, 11.42.

Acetat: Nadeln, Schmp. 107~108°. $[\alpha]_D^{29}$ −66.6°(c=1.01, $CHCl_3$). $C_{29}H_{46}O_2$-Ber.: C, 81.63; H, 10.87. Gef.: C, 81.66; H, 10.83.

Benzoat: Nadeln vom Schmp. 164.5~165.5°. $[\alpha]_D^{29.5}$ −33.0°(c=1.05, $CHCl_3$). $C_{34}H_{48}O_2$-Ber.: C, 83.55; H, 9.90. Gef.: C, 83.33; H, 10.13.

Kristalle aus der Fraktion Nr. 4 sowie aus der Fraktion Nr. 5 wurden vereinigt, mit Ac_2O-Pyridin acetyliert und das Acetat in das Tetrabromid übergeführt. Nach Reinigung und Dehalogenierung dieses Tetrabromides ließ sich das reine Acetat vom Schmp. 125~126° liefern. Ausbeute: 170 mg. Dieses Acetat (Vb) erwies sich nach Schmp., Mischprobe und IR-Spektrum als identisch mit dem natürlichen 22-Dehydrocholesterin-acetat.

Zusammenfassung

Das 3β-Acetoxy-20β-methylpregn-5-en-21-al (I) wurde über sein Enamin (II) in das 3β-Acetoxy-20α-methylpregn-5-en-21-al (III) verwandelt. Bei der Wittig-Reaktion von (III) ergaben sich das 20-Iso-22-dehydrocholesterin (IVa) und 22-Dehydrocholesterin (Va) in ungefähr gleicher Menge. Der Verfasser nahm also an, daß die Epimerisation der Methyl-Gruppe an C-20 dabei eintrat.

(Eingegangen am 10. October, 1960.)

UDC 612.386[615.7]-084

85. Hisashi Nogami and Tai Matsuzawa: Studies on Absorption and Excretion of Drugs. I.*1 Kinetics of Penetration of Acidic Drug, Salicylic Acid, through the Intestinal Barrier *in vitro*.

(*Faculty of Pharmaceutical Sciences, University of Tokyo**2)

Drug absorption from the gastrointestinal tract has been discussed by many investigators and in recent years theoretical considerations on the mechanism of drug absorption have become important for establishing the proper methods of drug administration.

Brodie,[1] Schanker,[2] and Hogben[3] studied drug absorption based on the thesis of

*1 Presented before the Kanto Local Meeting of the Pharmaceutical Society of Japan, Tokyo, June, 1960.

*2 Hongo, Tokyo (野上 寿, 松沢 兌).

1) B.B. Brodie, *et al.*: J. Pharm. and Pharmacol., **9**, 345 (1957).
2) L.S. Schanker, *et al.*: J. Pharmacol. Exptl. Therap., **123**, 81 (1958).
3) C.A.M. Hogben, *et al.*: *Ibid.*, **125**, 275 (1959).

Overton, the so-called lipid theory. They premised that the boundary between plasma and gastrointestinal tract might be essentially lipoidal in nature, which allowed preferential passage of drugs in their undissociated form, and concluded that the degree of dissociation of an organic electrolyte in solution appeared to be one of the most important factors determining its absorption from the gastrointestinal tract.

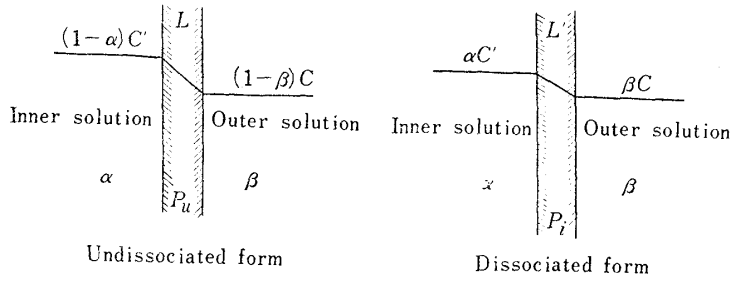
On the other hand, there are many reports concerning the passage of drugs in their dissociated form. Therefore, it seems that the hypothesis of Brodie is not enough to account for this phenomenon.

The present report describes the penetration of foreign organic compounds through the rat small intestinal barrier from the physicochemical standpoint. Satisfactory results obtained indicated that the penetration mechanism of drugs in both undissociated and dissociated forms was explainable by applying kinetic consideration.

In the present experiment, salicylic acid was selected as a representative of acidic drugs and its penetration mechanism was investigated.

Brodie and his co-workers studied the absorption of salicylic acid by circulating the buffered drug solution through the intestinal tract *in vivo* and from the results obtained at equilibrium state they suggested that salicylic acid was absorbed by a physical process, that is, a simple diffusion.

Hence, the following theoretical equations are introduced to the penetration of salicylic acid.



C': concentration of drug in inner solution
C: concentration of drug in outer solution
α: degree of dissociation of the drug at the pH value of inner solution
β: degree of dissociation of the drug at the pH value of outer solution

Fig. 1. Distribution of Drug between Inner and Outer Solutions separated by the Intestinal Barrier

Fig. 1 shows an imaginary scheme of the penetration layer in the intestinal barrier, in which L and L' represent each layer which determines the respective penetration rates of undissociated and dissociated forms of the drug. Of course, there may be cases where L equals L'. The permeability coefficients per unit length of the intestinal segment are respectively represented as P_u and P_i for the undissociated and dissociated forms. C is the concentration of drug which appeared in solution bathing the exterior of the intestine (outer solution), and C' the concentration of drug solution circulating through the lumen (inner solution). If α is the degree of dissociation of the drug in the inner solution and β the degree of dissociation in the outer solution, each concentration of the drug on the respective sides of intestinal barrier will be illustrated as shown in Fig. 1.

If the intestinal barrier is partially permeable to both the undissociated and dissociated forms of the drug, the fluxes in the two directions of the barrier, influx and efflux, are equal in the condition for the steady state. The flux of the undissociated form from inner to outer solution, q_u, is defined as follows:

$$q_u=\{(1-\alpha)C'-(1-\beta)C\}l\cdot P_u \qquad (1)$$

where l is the length of intestinal segment used.

Similarly, for the flux of the dissociated form,

$$q_i=(\alpha C'-\beta C)l\cdot P_i \qquad (2)$$

Then, overall flux of the drug, q, is given by

$$q=q_u+q_i=l\cdot[\{(1-\alpha)P_u+\alpha P_i\}C'-\{(1-\beta)P_u+\beta P_i\}C] \quad (3)$$

When the amount of drug in the intestinal barrier is assumed to be negligible, the total amount of drug in outer solution, Q, is expressed by

$$Q=V\cdot C \quad (4)$$

where V is each volume of the solution on the respective sides of the barrier.

Then,

$$q=\frac{dQ}{dt}=V\frac{dC}{dt} \quad (5)$$

If C_0 is the initial concentration of the drug in the inner solution,

$$C_o=C+C' \quad (6)$$

Substitution of Eq. (6) into Eq. (3) and then into Eq. (5) gives

$$\frac{V}{l}\cdot\frac{dC}{dt}=\{(1-\alpha)P_u+\alpha P_i\}(C_o-C)-\{(1-\beta)P_u+\beta P_i\}C$$
$$=\{(1-\alpha)P_u+\alpha P_i\}C_0-\{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i\}C \quad (7)$$

$$\frac{dC}{\{(1-\alpha)P_u+\alpha P_i\}C_o-\{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i\}C}=\frac{l}{V}dt \quad (7')$$

On integration of Eq. (7'), the equation becomes

$$\frac{ln[\{(1-\alpha)P_u+\alpha P_i\}C_o-\{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i\}C]}{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i}=-\frac{l}{V}t+I_o \quad (8)$$

where I_o is the constant of integration. Since $C=0$ in the case where $t=0$,

$$I_o=\frac{ln[\{(1-\alpha)P_u+\alpha P_i\}C_o]}{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i} \quad (9)$$

Therefore,

$$ln[\{(1-\alpha)P_u+\alpha P_i\}C_o-\{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i\}C]$$
$$=-\frac{l}{V}\{(2-\alpha-\beta)P_u+(\alpha+\beta)P_i\}t+ln[\{(1-\alpha)P_u+\alpha P_i\}C_0] \quad (10)$$

As Krebs-Ringer hydrogencarbonate solution was employed as the outer solution in the present study, $\beta\fallingdotseq 1$ for salicylic acid (pK=2.97). Therefore, Eq. (10) becomes

$$ln[\{(1-\alpha)P_u+\alpha P_i\}C_o-\{(1-\alpha)P_u+(1+\alpha)P_i\}C]$$
$$=-\frac{l}{V}\{(1-\alpha)P_u+(1+\alpha)P_i\}t+ln[\{(1-\alpha)P_u+\alpha P_i\}C_o] \quad (11)$$

If the overall permeability coefficients for undissociated and dissociated forms of the drug are represented as P_u' and P_i', respectively,

$$P_u'=l\cdot P_u, \quad P_i'=l\cdot P_i \quad (12)$$

On substitution of Eq. (12) into Eq. (11),

$$ln[\{(1-\alpha)P_u'+\alpha P_i'\}C_0-\{(1-\alpha)P_u'+(1+\alpha)P_i'\}C]$$
$$=-\frac{l}{V}\{(1-\alpha)P_u'+(1+\alpha)P_i'\}t+ln[\{(1-\alpha)P_u'+\alpha P_i'\}C_0] \quad (11')$$

In the special cases where $\alpha \fallingdotseq 0$ and $\alpha \fallingdotseq 1$, Eqs. (13) and (14) are obtained :

$$\alpha \fallingdotseq 0, \quad ln\left\{1-\left(\frac{P_u+P_i}{P_u}\right)\frac{C}{C_0}\right\}=-\frac{l}{V}(P_u+P_i)\cdot t \quad (13)$$

$$\alpha \fallingdotseq 1, \quad ln\left\{1-\frac{2C}{C_0}\right\}=-\frac{2\cdot l\cdot P_i}{V}\cdot t \quad (14)$$

In accordance with the above assumption, when the pH of the intestinal content is varied, the amount of salicylic acid appearing in the outer solution was determined as the function of t and the relationship between pH gradients and the degree of penetration was investigated.

Experimental

Animals——Male albino rats of Wister stock weighing 150~350 g. were used. The animals were taken off the stock diet about 20 hr. before experiment and were provided with plain water only. The animal was anesthetized by the intraperitoneal injection of pentobarbital sodium (6 mg./100 g. body weight).

Inner solution——Buffer solution for a warm-blooded animal, consisting of 0.263M citric acid and 0.123M Na_2HPO_4, and Krebs-Ringer hydrogencarbonate solution were used. To these was added salicylic acid to give a salicylic acid concentration of about 1000 or 500*3mg./L.

Outer solution——Krebs-Ringer hydrogencarbonate solution was employed as an outer solution in all experiments.

Determination of the pH values of inner and outer solutions was carried out with the Beckman Model G pH-meter before and after the experiment.

Circulation apparatus——A circulation apparatus described by Wiseman[4] and Smyth[5] was employed with minor modification.

As shown in Fig. 2, which for the sake of clarity shows only one loop of intestine instead of

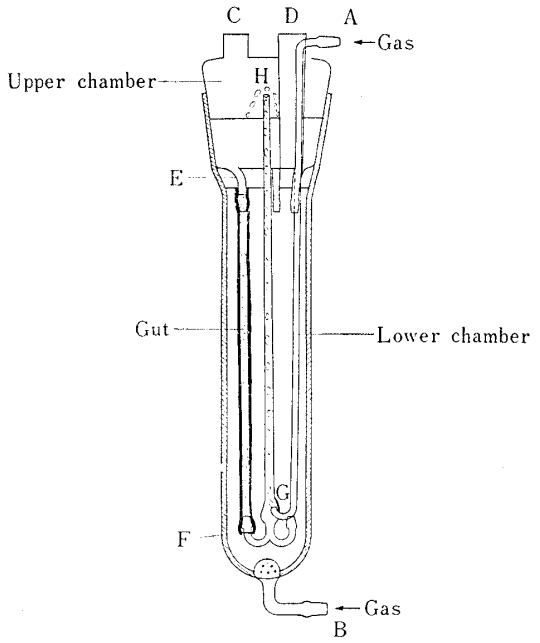


Fig. 2. Circulation Apparatus

A : Gas inlet
B : Gas inlet
C : Gas outlet
D : Gas outlet
E : Nippled projection
F : Nippled projection
G : Sealed-in gas outlet

*3 A solution containing 500 mg./L. of salicylic acid was employed in the case of rapid penetration.

4) G. Wiseman : J. Physiol., **120**, 63 (1953).
5) D.H. Smyth : *Ibid.*, **121**, 2P (1953).

the three normally used, this apparatus consists of two parts, an upper and a lower chamber. The upper chamber, which contains the inner solution and carries the intestinal segment, fits into the lower chamber by means of a ground-glass joint. The openings C and D can be used for taking out samples of the inner or outer solutions for analysis. A glass tube, G, has at its lower end three upturned nippled projections, F. In the floor of the upper chamber, there are three projections, E, each one being opposite its fellow on the lower end of the tube G. The distance between these projections is 15 cm. A rosette, B, is sealed into the lower end of the outer chamber. The gas mixture of 5% CO_2 and 95% O_2 entering the inlet A acts as a lift to raise the inner solution from the lower end of the intestine back to the reservior. Inner solution from the upper chamber flows down the gut and toward the end of glass tube H. Gas mixture enters by the rosette B and serves to oxygenate and to mix the outer solution. The apparatus is kept submerged in a water bath of 37°, to the level of the ground-glass joint.

Experimental procedure——The abdominal cavity was opened by a midline incision and the incisions were made at the duodenal-jejunal and ileo-caecal junctions. A cannula was tied into the upper end of the intestine and the gut between these incisions washed out from above with a warm Ringer solution. Then the intestine was cut free leaving about 0.2 cm. of mesentery margin attached to the gut. Blood contamination was removed by submerging the gut in warm distilled water and it was then rapidly removed to a Petri dish containing warm Ringer solution. The intestine was brought up to the upper projection and tied over the projection. The intestine was then cut to give a length which required slight streching to reach the lower projection, to which it was then tied. This procedure was made for all three intestinal samples. As each length of the segment was about 15 cm., about 45 cm. of intestine was used for the experiment. After the last intestinal segment had been tied in position, the upper chamber was fitted into outer chamber which contained 80 cc. of the outer solution, the apparatus was submerged in a water bath, and 80 cc. of the inner solution of 37° was poured into the upper chamber. Then the gas mixture was allowed to flow through A. As the inner solution always retained some residual Ringer solution, the samples of inner and outer solutions were taken out after 2 min. of the perfusion to find the initial concentration. This period was sufficient to allow mixing of the solutions and for establishment of steady state in the intestinal barrier. The samples of the inner and outer solutions were taken out at regular intervals and analyzed.

The following factors had to be considered before carrying out the experiment.

1) Physiological appearances of the intestine : As the intestine was apparently injured under the severe conditions such as pH 2.03 of inner solution, it was necessary to shorten the experimental period.

2) Constancy of the pH values : As the pH values of inner solution became slightly higher with lapse of time, the measurement had to be performed within the region in which the pH values remained constant.

3) Volume change of the inner and outer solutions : The initial volumes of the inner and outer solutions added to the circulation unit were 80 cc., respectively. When the final volumes were measured by drainage into 100-cc. graduated cylinder after the circulation had continued for 1 hr., the decrease of each volume was 1 and 2 cc., respectively. The volume change of the solutions, therefore, was negligible.

Analytical method——Salicylic acid was determined colorimetrically by the method described by Brodie, *et al.*,[6] as follows: A sample of 2 or 1 cc.[*4] is pipetted into a 50-cc. glass-stoppered bottle. 0.5 cc. of conc. HCl and 30 cc. of ethylene dichloride are added and shaken for 30 min. The mixture is centrifuged, the aqueous phase is removed by aspiration, and 20 cc. of the solvent phase is transferred to a 50-cc. glass-stoppered bottle containing 10 cc. of distilled water. To this solution 1 cc. of 1% $Fe(NO_3)_3$ in 0.07*N* HNO_3 solution is added, shaken for 15 min., and centrifuged. About 5 cc. of the colored aqueous phase is transferred to a cuvet and the optical density at 530 mμ is determined (Hitachi Spectrophotometer Model EPU-2 used).

Results and Discussion

The observed concentration ratio C/C_0 when the pH values of inner solution, that is, the degrees of dissociation of salicylic acid, were varied, is listed in the column of the observed value in Table I.

*4 When the solution containing 1000 mg./L. of salicylic acid was used, 1 cc. of the sample was taken and diluted to 2 cc. with the buffer used.

6) B.B. Brodie. *et al.* : J. Pharmacol. Exptl. Therap., **80**, 114 (1944).

Table I. Absorption of Salicylic Acid at Various pH Values

$$y(\%)=\frac{\text{Concentration of drug } (C) \text{ at any time } (t)}{\text{Initial concentration of drug } (C_o)}\times 100$$

pH (α)	y_1			y_2			y_3		
	t_1 (min.)	Observed value	Calcd. value	t_2 (min.)	Observed value	Calcd. value	t_3 (min.)	Observed value	Calcd. value
2.03 (0.103)	8	4.5	4.0	16	9.9	10.0	24	14.5	15.9
	8	1.0		16	8.7		24	13.5	
2.41 (0.215)	8	3.1	3.6	16	8.9	9.2	24	14.7	14.5
	8	2.5		16	8.4		24	13.9	
2.55 (0.275)	10	5.9	4.3	20	12.8	10.7	30	17.8	16.7
	10	4.3		20	12.4		30	16.8	
2.71 (0.350)	15	6.5	5.9	30	12.0	14.3	45	17.8	22.2
	15	6.3		30	9.6		45	13.7	
2.91 (0.470)	15	7.1	5.2	30	14.4	12.5	45	19.1	19.5
	20	8.4	6.7	30	11.9		40	15.5	17.6
3.18 (0.619)	10	1.8	2.8	20	6.9	6.9	30	13.8	11.0
	10	1.8		20	8.7		30	14.7	
3.27 (0.666)	10	1.7	2.6	20	6.7	6.4	30	13.3	10.3
	10	1.3		20	6.8		30	12.5	
	10			20	8.1		30	11.2	
	10	3.6		20	9.0		30	14.7	
3.31 (0.686)	10	1.1	2.5	20	5.2	6.2	30	11.8	11.3
	10	1.7		20	5.7		30	11.7	
	10	3.0		20	6.9		30	12.8	
	10	1.6		20	7.1		30	11.5	
3.60 (0.810)	15	2.1	2.9	25	6.1	5.9	35	10.4	8.9
	15	2.3		25	6.7		35	10.9	
	15	2.8		25	7.7		35	12.8	
3.72 (0.849)	10	0.7	1.8	20	5.5	4.3	30	10.5	7.0
	10	1.3		20	5.5		30	10.8	
	10	2.1		20	6.5		30	11.1	
3.77 (0.863)	10			20	5.5	4.2	30	9.9	6.7
	10			20	5.6		30	10.9	
3.82 (0.876)	10	2.7	1.7	20	5.3	4.0	30	9.5	6.6
4.45 (0.968)	15	2.4	1.8	30	6.6	4.4	45	10.2	7.1
	15	2.0		30	5.9		45	9.6	
	15	2.0		30	5.9		45	8.7	
	15	1.0		30	5.1		45	8.0	
	15	1.2		30	4.9		45	8.9	
6.70 (1.00)	40	4.8	4.3	60	7.4	7.4	80	9.7	10.6
	40	4.3		60	6.5		80	8.3	
	40	5.2		60	8.0		80	10.1	
	40	5.5		60	8.3		80	10.3	
	40	4.8		60	6.5		80	8.9	
	40	5.1		60	7.4		80	10.2	
	40	3.6		60	6.1		80	7.6	
	40	5.1		60	7.0		80	9.8	
	40	4.5		60	6.8		80	8.8	
	40	3.3		60	5.5		80	7.1	
	40	4.7		60	6.5		80	8.8	
	40	4.7		60	8.1		80	10.3	
7.39 (1.00)	40	3.8	4.3	60	5.9	7.4	80	9.2	10.6
	40	2.8		60	4.3		80	8.2	
	40	4.4		60	8.0		80	10.6	

In this table, y_1, y_2, and y_3 are the values of C/C_o at the time t_1, t_2, and t_3, respectively. From these results, the permeability coefficients, P_u and P_i, may be obtained from the procedure to be described below. By substituting the observed values into Eqs. (13) and (14) in the case of $\alpha \fallingdotseq 0$ and $\alpha \fallingdotseq 1$, respectively, these two parameters can be determined. However, if the degree of dissociation of salicylic acid becomes near zero, pH value of the inner solution will be about 0.97 ($\alpha \fallingdotseq 0.01$) and it is impossible to perform the

experiment. Therefore, applicability to Eq. (14) was initially examined by using the inner solution of pH 6.70, when $\alpha \fallingdotseq 1$. For this purpose, the experiment was carried out on twelve subjects. From the data obtained in the above experiment, the straight lines were obtained by plotting $\log(1-2C/C_o)$ as a function of t, and there was no marked difference between their slopes. An example of the results is shown in Fig. 3.

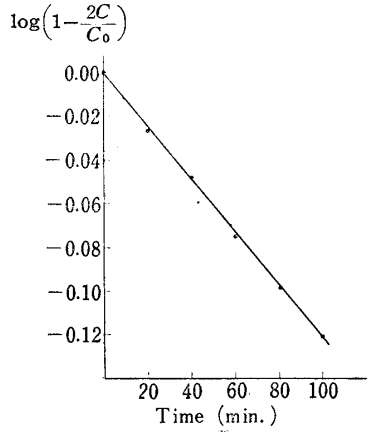


Fig. 3. Curve illustrating the Linear Relationship between the Logarithmic Function and Time at pH 6.70

As a result of calculation of P_i from Eq. (14), the mean value of P_i is 0.00233 (cc./cm./min.). Moreover, the same result as this was obtained for the alkaline inner solution. By substituting the P_i value into Eq. (11) and solving it graphically at several pH levels, the estimated value of P_u is 0.0125 (cc./cm./min.). By substituting these estimated values into Eq. (11) and using the respective mean values of y_1, y_2, and y_3 at all pH levels, two parameters, P_u and P_i, at each series of y were statistically determined by means of the method of least squares from all experimental data.[7] The values of P_u and P_i are given in Table II.

Table II. Values for P_u and P_i obtained by the Method of Least Squares (mean ±95% confidence limit)

	$P_u' \times 10\left(\frac{\text{cc.}}{\text{min.}}\right)$	$P_u \times 10^2\left(\frac{\text{cc.}}{\text{cm.}\cdot\text{min.}}\right)$	$P_i' \times 10\left(\frac{\text{cc.}}{\text{min.}}\right)$	$P_i \times 10^3\left(\frac{\text{cc.}}{\text{cm.}\cdot\text{min.}}\right)$
y_1	4.52±1.15	1.00±0.26	0.90±0.19	2.00±0.41
y_2	5.93±0.85	1.32±0.19	1.06±0.19	2.35±0.42
y_3	6.45±1.32	1.43±0.29	1.19±0.31	2.64±0.70
Mean	5.63	1.25	1.05	2.33

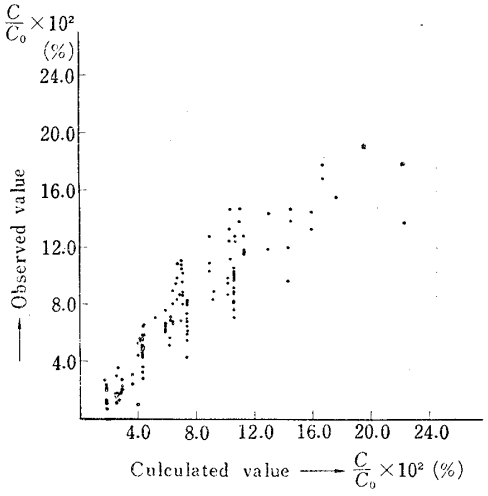


Fig. 4. Relationship between Observed and Calculated Values

7) W.E. Deming: "Statistical Adjustment of Data," (1946). John Wiley & Sons, Inc., New York.

The calculated values in Table I were calculated by using the values in Table II and by Eq. (11). A relationship between the observed and calculated values is illustrated in Fig. 4, which shows the obvious correlation between them.

Fig. 5 also shows the relationship between the theoretical curves which were calculated from Eq. (11) by using mean values of the parameters and observed values.

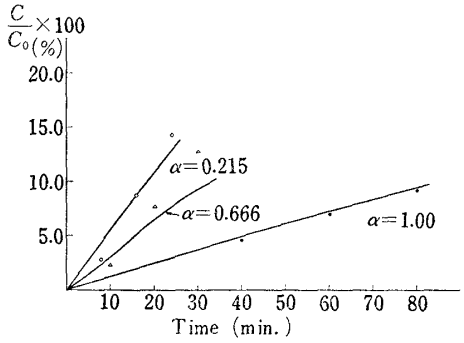


Fig. 5. Diagram illustrating the Relationship between the Theoretical Curves and the Observed Values

○ : $\alpha=0.215$
△ : $\alpha=0.666$
• : $\alpha=1.00$

Accordingly, it is evident from Table II and Fig. 4 that there is no significant difference between each value of the parameters obtained from y_1, y_2, and y_3. However, a tendency for both P_u and P_i to increase slightly with the lapse of time is recognized. This phenomenon may be due to the fact that salicylic acid is a toxic substance for living cells and causes physiological change in the intestinal tissue. However, it may be inevitable to produce an error to some extent in the case of biological reaction. In comparing the observed values with those calculated in Table I, it is seen that the difference between them becomes somewhat larger in the pH range in which both P_u and P_i take part in drug penetration. This difference may be due to the error of parameters which act on the data as a summation in such a pH range. For the purpose of inquiring into the cause of error closely and examining the propriety of the hypothesis advanced before, the following experiment was attempted.

From condition for the equilibrium state,

$$\frac{dC}{dt}=0$$

Therefore, from Eq. (7),

$$P_u(1-\alpha)C'+P_i\cdot\alpha C'-P_i\cdot C=0 \tag{15}$$

$$\frac{C}{C'}=\frac{P_u(1-\alpha)+P_i\cdot\alpha}{P_i} \tag{15'}$$

A transfer mechanism occurs durably and an equilibrium system is not accomplished until the concentration ratio described by Eq. (15′) is attained. If the experiment is executed under the condition of $P_u=0.0125$, $P_i=0.00233$, and $\alpha=0.863$ (pH=3.77), the equilibrium state is established at the concentration ratio of 1.598.

The concentration change in both inner and outer solutions was investigated by employing the outer solution containing 1.598 times salicylic acid as that in the inner solution. The results are given in Table III.

As seen in Table III, the salicylic acid concentration in the outer solution was kept nearly constant and only a very small amount of salicylic acid was transferred inversely into outer solution from the lumen. That is, the movement of salicylic acid took place against a concentration gradient. This phenomenon is probably due to either the estimated P_u is slightly smaller or the P_i is a little larger than the true values. At any

Table III. Results obtained from the Experiment at Equilibrium State

Exptl. No.	Concn. of inner solution C' (γ/cc.)		Concn. of outer solution C (γ/cc.)			
	0	30 (min.)	0	10	20	30 (min.)
1	307	262	493	493	497	506
2	307	273	496	496	497	507
3	309	267	491	491	493	510
4	309	274	487	487	489	490
5	305	266	487	487	490	493

rate, the amount transferred is so small that the results obtained by Eq. (14) and by the above experiment at equilibrium state conform to the assumption described before. Then, it is concluded that both undissociated and dissociated forms of salicylic acid are transferred across the intestinal barrier with each specific permeability coefficient and the ratio for P_u/P_i is about 6, which shows a more rapid penetration of the dissociated form than expected from the so-called lipid theory. It is natural, however, that these coefficients are specific for salicylic acid only and when the subject drug is changed, their values vary depending on the physical and chemical properties of that particular drug.

Finally, it is noteworthy that the results obtained from the present study do not always mean that the rate of absorption *in vivo* will be in the same proportion to that *in vitro*. There is then a question of what relationship there would be between the degree of absorption when salicylic acid is administered to an intact animal and the results obtanied here. Therefore, further investigation will be necessary for the elucidation of the relationship between *in vivo* and *in vitro* absorption.

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Summary

1. A penetration of salicylic acid through the rat small intestine was investigated from the standpoint of chemical kinetics *in vitro*.

2. Theoretical equations were derived from the assumption that the intestinal barrier was partially permeable to both the undissociated and dissociated forms of the drug.

3. From the data obtained, the respective permeability coefficients for the undissociated and dissociated forms of salicylic acid, P_u and P_i, were statistically determined. The estimated value of P_u was 0.0125 (cc./cm./min.) and that of P_i 0.00233 (cc./cm./min.).

4. As there was a little difference between the observed and the calculated values, an experiment at the equilibrium state was tried for the purpose of elucidating the cause of the difference.

5. The results indicate that salicylic acid is transferred across the intestinal barrier with specific coefficient for both forms of drug, P_u and P_i, that the ratio for P_u/P_i is about 6, and the relative rapid penetration of the dissociated form can be attributed to the above ratio.

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