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58. Manabu Hanano : Studies on Percutaneous Absorption. III.[1] Kinetics of Percutaneous Absorption.

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The factors determining the rate of biological absorption have been established gradually from investigations on kinetics of absorption through various tissues. In the studies on theophylline salts and its derivatives, Nelson[2] determined the concentration of the chemicals in blood stream of human being and the solution rate of the medicines, and discussed the result assuming that the permeation of drugs through the gastrointestinal tract was far faster than the dissolution of the powder administered. Riegelman and his co-worker[3] discovered that the diffusion in the suppository was a rate determinant in the absorption of radioactive iodine.

In the percutaneous absorption, however, the velocity of absorption may not be so large, since the intact skin is a barrier against penetration of substance, and it may be considered that rate determinant process is the passing of drugs through tissues of the skin.

The effects of the concentration and pH-values of the vehicle on the percutaneous absorption were investigated in the present studies, and the kinetics of absorption through intact skin was discussed and treated from the standpoint of physical diffusion. The equations derived from this point of view were proved to agree sufficiently with the results obtained experimentally with salicylic and benzoic acids.

MacKee, *et al.*[4] found that percutaneous penetration of lipid-soluble substances follows the route of pores of the skin→lipid in hair follicle and sebaceous gland→body fluid. According to this theory, the drug applied to the skin must dissolve and then permeate into the lipid, and it is easily understood that the penetration of drugs depends on their solubility in the lipid. It may, however, be considered that the absorption is possible by other routes, because some kinds of lipid-insoluble substances are also absorbed.[5] The quantity of lipid-insoluble substances penetrated is generally small and it is evident that the MacKee's route is principal one of medicinal absorption.

Chemical reactions or adsorptions may occur when some kinds of substances pass along the route. However, in the simplest case, passage of a substance is caused by diffusion. This case was handled for the fundamental studies of percutaneous absorption in the present report.

The following assumptions and definitions may be suitable for diffusion along the route. The route consists of three different media, i.e. vehicle, lipid, and body fluid. The concentration of a substance in the vehicle layer is homogeneous, since it was shaken always during the application in this experiment. There exists a relatively quiet zone of fluid in the interface between the vehicle and the lipid, and a considerable part of the total movement of substances in the vehicle may occur by diffusion.

For example, the same film of liquid as this layer was assumed by Noyes and Whitney[6] in the theory of dissolution of crystals. This part of the vehicle is defined

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1) Part II : This Bulletin, **6**, 249(1958).
2) E. Nelson : J. Am. Pharm. Assoc., Sci. Ed., **47**, 607(1957).
3) S. Riegelman, W. Crowell : *Ibid.*, **47**, 115, 123, 127(1958).
4) G. MacKee, M. Sulzberger, F. Herrmann, R. Baer : J. Invest. Dermatol., **6**, 43(1945).
5) H. Nogami, J. Hasegawa, M. Hanano : This Bulletin, **4**, 347(1956).
6) A. Noyes, W. Whitney : J. Am. Chem. Soc., **19**, 930(1897).

as the diffusion layer of a vehicle. The lipid layer scarcely moves during the measurement of absorption and passage of a drug may depend only on its diffusion through it. The concentration of a drug in the circulating body fluid may be negligible, because the amount absorbed is small and the drug is always excreted. There is the diffusion layer of body fluid in the border area between lipid and body fluid, as described before with the vehicle.

In a steady state, the amount of a substance passing through each diffusion layer may be expressed as the function of concentration, the permeability coefficient, and the contact area :

$$W=UA\Delta C$$

where W is the amount that passes, U the permeability coefficient, A the contact area, and ΔC the difference in the concentration between the two layers. Shape of the actual layer may be so complex that the area cannot be measured, but UA can be calculated from the quantity of the medicine passing. It may be convenient that the area of medicinal application is introduced into this theory. Then, the contact area of each layer is defined to be equal to the area applied, A, and the diffusion resistance is expressed by the reciprocal of the permeability coefficient per unit area. When only the quantity passing is observed, the direction of diffusion can be treated as one dimension.

A scheme of the diffusion layer which consists of above assumptions and definitions is shown in Fig. 1.

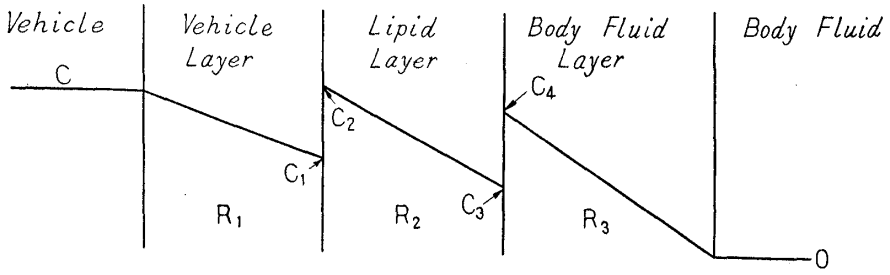

Fig. 1. Schematic Diffusion Layer

R_1=Diffusion resistance of vehicle layer
R_2=Diffusion resistance of vehicle layer
R_3=Diffusion resistance of vehicle layer
C=Drug concentration in vehicle at time t
C_1, C_2, C_3, C_4=Drug concentration of each portion at the same time t
A=Mean contact area

The equation expressing the amount of permeation could be derived from the model shown in Fig. 1 as follows : When the diffusion is in a quasi-steady state, the rate of diffusion, q, may be expressed by Fick's law :

$$q=A(C-C_1)/=A(C_2-C_3)/R_2=A(C_4)/R_3 \quad (1)$$

When each apparent partition coeffcient, m_{12} and m_{34}, is independent of C, the following equations may be expressed at each border area :

$$m_{12}C_1=C_2, \quad m_{34}C_3=C_4 \quad (2)$$

From Eqs. (1) and (2), the equation may be expressed :

$$q=AC/(R_1+R_2/m_{12}+R_3/m_{12}m_{34}) \quad (3)$$

This denominator is defined as over-all resistance, R.

$$q=AC/R \quad (4)$$

When the volume of vehicle is V, the initial concentration of drug C_0, the initial quantity of drug in vehicle Q_0, and the quantity absorbed till time t, Q, the concentration of a drug in the vehicle, C, may be expressed as follows :

$$C=(Q_0-Q)/V \quad (5)$$

by differentiation :

$$(-1/V)(dC/dt)=dQ/dt=q \quad (6)$$

from Eqs. (4) and (6) :

$$dC/dt=-AC/RV \quad (7)$$

This equation is integrated in terminal condition :

$$C/C_0=\exp.(-At/RV) \quad (8)$$

where C/C_0 represents the decrease of concentration.

$$ln\ C/C_0=-At/RV$$

In Eq. (8), A/RV is independent of C and t, and, therefore, this equation indicates the rate of decrease of the concentration to follow a first-order course.

The absorption of a lipid-soluble and both weak acid and base may largely be influenced by the pH value of a vehicle, since only the molecular form of the drug is soluble in lipid, and the dissociate type is not. Therefore, the equilibrium between the concentration in a vehicle and lipid is dependent only on the degree of dissociation, a.

$$m_{12}(1-a)C_1=C_2 \quad (9)$$

From Eq. (9), the over-all resistance, R, may be rewritten as follows :

$$R=R_1+R_2/m_{12}(1-a)+R_3/m_{12}m_{34}(1-a) \quad (10)$$

If R_1 is negligible, the following may be derived from Eqs. (8) and (10).

$$ln\ (C/C_0)=-(1-a)At/RV \quad (11)$$

Eq. (11) may express the effect of pH on absorption, because the degree of dissociation can be calculated from the following equation of pH value of vehicle and pKa of a drug:

$$\mathrm{pH}=\mathrm{pKa}+log\ a/(1-a)$$

Experimental

Material—Salicylic acid was dissolved in distilled water to make 0.1% and 0.2% solutions. The buffer solution containing salicylic acid was prepared by dissolving Na salicylate in Clark-Lubs' buffer solutions of pH values 1.5, 2.3, 3.0, and 4.0, in a concn. of 0.1% as salicylic acid. Na Benzoate was dissolved in a concn. of 0.2% as acid in Clark-Lubs' buffer solutions with pH values of 2.5, 3.5, 4.0, and 5.0. pH-Values were measured by the Beckman pH-meter model-G immediately after preparation.

Experimental Design—The experiment was designed using completely randomized designs with four repetitions with aqueous solution of salicylic acid and with two repetitions for three men with the buffer solution. The same design for five men was used with benzoic acid.

Application—Each solution was applied on the forearm of a healthy adult male, as described in the preceding report.[1] The durations of application for aqueous solution of salicylic acid were 2, 4, 6, and 8 hr., and 16 hr. for all buffer solutions.

Measurement—Salicylic acid was determined by colorimetric method using iron reagent as described before.[1] Ultraviolet absorption was determined in $CHCl_3$ solution of BzOH. The recovered solution of the drug was diluted to 25 cc. with distilled water, 1 cc. of 36% HCl and 10 cc. of $CHCl_3$ were added to the solution in a glass-stoppered test tube, shaken, and centrifuged. Aqueous layer was removed and the $CHCl_3$ layer transfered to a cell and its optical density was read using a spectrophotometer at 275 mμ.

Results and Discussion

Absorption of Salicylic Acid from Aqueous Solution

Table I gives the decrease in the rate of absorption from 0.1% and 0.2% aqueous solutions of salicylic acid.

Table I. Absorption of Salicylic Acid from Aqueous Solution

(hr.)	0.1% solution C/C_0				mean	0.2% solution C/C_0					mean
2	0.838	0.730	0.713	0.740	0.755	0.755	0.728	0.735	0.765	0.793	0.755
4	0.740	0.670	0.668	0.663	0.685	0.715	0.735	0.713	0.668	—	0.708
6	0.628	0.588	0.560	0.588	0.591	0.593	0.655	0.660	0.558	—	0.617
8	0.518	0.515	0.525	—	0.519	0.505	0.538	0.565	0.580	—	0.547

Fig. 2 presents the relation between the logarithm of the mean decrease rate and the duration applied.

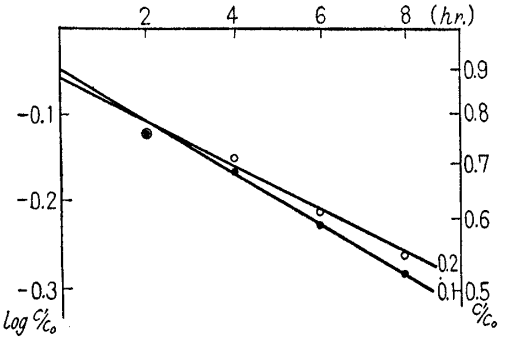

Fig. 2.
Relation between Rate of Decrease and Duration of Application

As seen in Fig. 2, the evident regression was found between these values by assuming the same variance at the decrease rates observed. The regression formula was determined by weighted least-square method, and the mean squares of external error, σ(ext), and the mean squares of internal errer, σ(int), were calculated by the deviation and the replication as listed in Table II.

Table II. Statistical Values calculated from Table I
Regression formula : $\log(C'/C_0)=a-bt$
t=Period of application (hrs.)

	0.1% solution		0.2% solution	
	$a=-0.051$	$b=0.029$	$a=-0.057$	$b=0.025$
σ(int)	0.0093	$\phi=22$	0.0093	$\phi=22$
σ(ext)	0.00015		0.00047	
σ_a	0.00020		0.00016	
σ_b	0.000009		0.000007	

As seen in Table II, there was no significant difference between σ(ext) and σ(int) in both 0.1% and 0.2% solutions, so that the regression was statistically significant. The rate of decrease was independent of the initial concentration, because significant difference was not found between these regression formulae.

In this experiment, the decrease rates of concentration were measured by the recovering method, so that a correction of recovering ratio must be added to the equation obtained from the values observed, C'. The corrected equation may be shown by common logarithm as follows :

$$\log C'/C_0 = \log K - At/2.303RV \qquad (12)$$

where C' is the observed value of concentration and K, the recovering ratio. The experimental data fitted well into this equation and it was considered that the assumption described above was established experimentally.

Absorption of Salicylic Acid from Buffer Solution

Table III gives the decrease rate observed by absorption from various buffer solutions for 16 hours.

Table III. Absorption Ratio C/C_0 of Salicylic Acid from Buffer Solution

pH		1.62	2.31	3.16	4.00
1-a		0.958	0.822	0.395	0.086
men	m_1	0.293	0.375	0.623	0.815
		0.235	0.353	0.560	0.835
	m_2	0.413	0.480	0.703	0.923
		0.370	0.408	0.625	0.810
	m_3	0.293	0.373	0.558	0.783
		0.135	0.290	0.518	0.815

These data evidently do not show the same variance, i.e. the smaller decrease rate

gives a larger error. Then, each value was transformed for the assumption of the same variance as follows :

$$y=\sqrt{1-C'/C_0}$$

where y is a transformed value.

Table IV gives the analysis of variance at the transformed value, i.e. the square root of the absorption ratio.

Table IV. Analysis of Variance of Table III at the transformed value $y=\sqrt{1-C'/C_0}$

Factor	s. s.	d. f.	m. s.
pH	0.6707	3	0.2236
Man	0.0285	2	0.0143
pH × man	0.0078	6	0.0013
Error	0.0360	12	0.0030
Total	0.7430	23	

As seen in Table IV, the difference of pH value or kind of buffer solution was evidently statistically significant. The relation between the decrease rate observed and the value of (1−a) calculated from pH and pKa is given in Fig. 3.

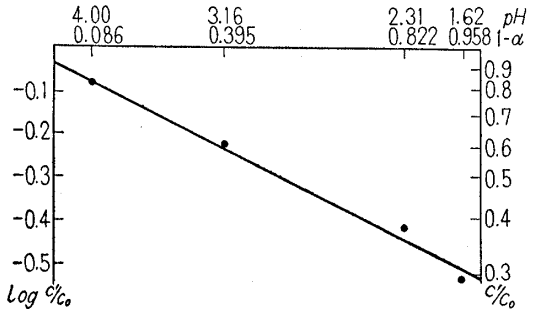

Fig. 3.
Relation between Rate of Decrease and Degree of Dissociation of Salicylic Acid

Several statistical values calculated by the assumption of the same variance of $\sqrt{1-C'/C_0}$ are listed in Table V.

Table V. Statistical Values of Table III at the transformed value $y=\sqrt{1-C'/C_0}$

Regression formula : $\log(C'/C_0)=-0.034-0.507(1-a)$

σ(int) : 0.0030 $\phi=13$ σ(ext) : 0.00178
$\sigma \log K$: 0.00067 $\sigma\, 16A/2.303RV$: 0.00155

As seen in Table V, there was no significant difference between σ(ext) and σ(int), so that the regression is statistically significant.

From the correction of recovery, Eq. (11) is expressed as follows :

$$\log C'/C_0 = \log K - 16A(1-a)/2.303RV \qquad (13)$$

Therefore, it may be established that this equation evidently fits experimental data.

Absorption of Benzoic Acid from Buffer Solutions

Table VI gives the decrease rate observed by the absorption of benzoic acid from various buffer solutions during 16 hours.

Table VI. Absorption Ratio of Benzoic Acid from Buffer Solution

pH	2.64	3.67	4.29	5.17
$1-a$	0.972	0.764	0.431	0.093
m_1	0.248	0.310	0.525	0.895
m_2	0.353	0.445	0.640	0.813
m_3	0.325	0.405	0.673	0.900
m_4	0.313	0.465	0.710	0.893
m_5	0.415	0.538	0.530	0.855

Table VII gives the analysis of variances calculated by the assumption of the same variance of $\sqrt{1-C'/C_0}$.

Table. VII. Analysis of Variance of Table VI

Factor	s. s.	d. f.	m. s.
pH	0.625	3	0.2083
Man	0.012	4	0.0030
Error	0.034	12	0.0028
Total	0.671	19	

As seen in Table VII, only the pH value is statistically significant. Fig. 4 presents the relation between the decrease rate observed and the value of (1—a).

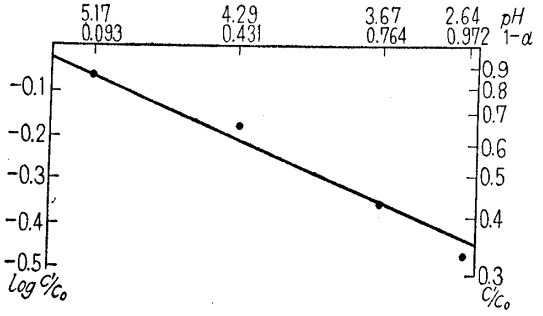

Fig. 4.
Relation between Rate of Decrease and Degree of Dissociation of Benzoic Acid

Various statistical values calculated by the assumption of the same variance of $\sqrt{1-C'/C_0}$ are listed in Table VIII.

Table VIII. Statistical Value of Table VI

Regression formula : $\log (C'/C_0) = -0.017 - 0.437(1-a)$

σ(int) : 0.0028	σ(ext) : 0.0022
$\sigma \log K$: 0.00010	$\sigma\, 16A/2.303RV$: 0.00128

As established by these results, the regression is statistically significant and Eq. (13) may fit evidently the experimental data in the case of benzoic acid as well as salicylic acid.

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Summary

1) A diffusion model was presented on the percutaneous absorption from lipid-insoluble vehicle and the equation was derived in regard to the decrease in the rate of concentration of a drug applied :

$$C/C_0 = \exp(-At/RV)$$

where C_0 is the initial concentration of a drug, C the concentration at time t, R the over-all resistance, V the volume of vehicle, and A the area applied. The relation between the rate of decrease and the degree of dissociation of a drug was also expressed from the same model :

$$C/C_0 = \exp(-At(1-a)/RV)$$

where a is the degree of dissociation.

2) Both equations were proved to be conformable to the absorption of salicylic and benzoic acids from the aqueous and buffer solutions through the intact human skin.

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