

Notes

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Studies on Percutaneous Absorption of Drugs. I.¹⁾TAKAICHI ARITA, RYOHEI HORI,^{2a)} TOSHIO ANMO, MITSUNORI
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Studies on percutaneous absorption have been carried out by many investigators. However the experimental method has not yet been established mainly because of the structural complexity of skin and a number of factors influencing the absorption. Though Hanano³⁾ and Scheuplein⁴⁾ reported the kinetics of percutaneous absorption, reports showing quantitative views are few in number as compared with those of the intestinal absorption. The authors designed a new method for the investigation of the permeability of drugs through the intact skin using a guinea-pig. Tregear⁵⁾ reported that a guinea-pig gave results relatively similar to those in human beings. In this report the reliability of this method is discussed and using this, the effects of the pH values of the solutions on percutaneous absorption of both acid and basic drugs are investigated.

Experimental

Drugs—Both salicylic acid (pK_a 3.0) as an acid drug and 2-[*p*-chloro- α -(2-dimethylaminoethoxy)-benzyl]pyridine (Carbinoxamine; pK_a 8.9) as a basic drug were used. Salicylic acid was of reagent grade. Carbinoxamine was prepared by the vacuum distillation, bp 167–170° (1 mmHg). UV $\lambda_{max}^{H_2O}$ $m\mu$ (ϵ): 260 (4870).

Analytical Method—As shown in Chart 1, both salicylic acid and carbinoxamine were determined by spectrophotometry. Salicylic acid was determined by the method described by Kakemi,⁶⁾ *et al.* Carbinoxamine was determined by the extractive method using ultraviolet absorption spectrum at 264 $m\mu$. Calibration curve of it is shown in Fig. 1.

Determination of Partition Coefficients—A given amount of each drug was dissolved in 10 ml of the buffer solutions having various pH values. These solutions were added to 10 ml of the organic solvents, chloroform, ethyl ether and cyclohexane. The mixtures were shaken for a time (1–2 hours) at $31 \pm 1^\circ$ and after it reached the equilibrium state, the concentration of aqueous phase was determined by spectrophotometry and partition coefficients were calculated.

Absorption Experiments—Male guinea-pigs weighing 300 to 450 g were anesthetized with a 25% urethane aqueous solution by intraperitoneal injection. After they were fixed on their backs, the hair of abdominal skin was cut with an

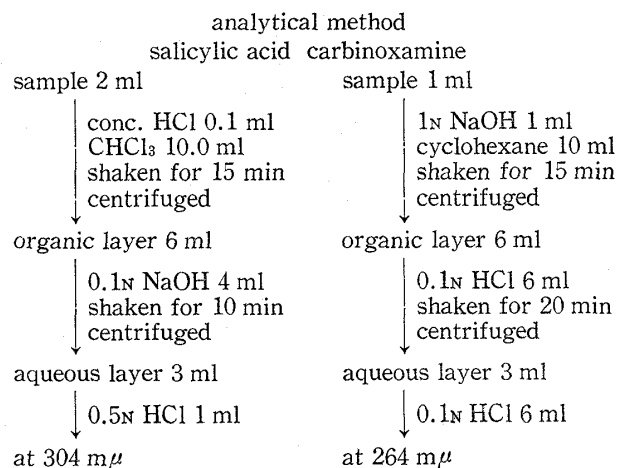


Chart 1

- 1) Presented before the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968.
- 2) Location: a) Kita-15-jo, Nishi-7-chome, Sapporo; b) 34-1, Takada-3-chome, Toshima-ku, Tokyo.
- 3) M. Hanano, *Chem. Pharm. Bull.* (Tokyo), **7**, 300 (1959).
- 4) R.J. Scheuplein, *J. Invest. Derm.*, **45**, 334 (1965).
- 5) R.T. Tregear, "Physical Function of Skin," Academic Press, 1966, p. 13.
- 6) K. Kakemi, T. Arita and H. Yamashina, *Yakuzaigaku*, **21**, 17 (1962).

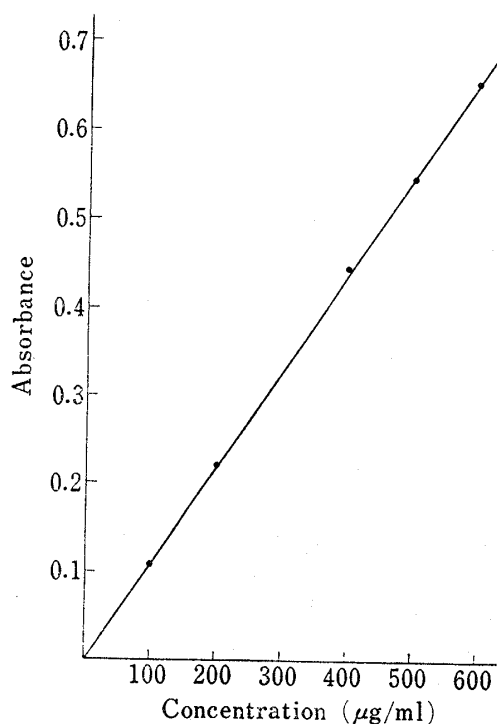


Fig. 1. Calibration Curve of Carbinoxamine

was calculated from the remaining concentration of the drug. Temperature of solution, room temperature and recirculating flow rate were controlled respectively $31 \pm 1^\circ$, $23 \pm 2^\circ$ and 15 ± 5 ml/min. It is generally accepted that the temperature on the skin is 2° or more below the bodily temperature.⁷⁾ Therefore the temperature of the solution was adjusted to $31 \pm 1^\circ$, which was 2° lower than the bodily temperature of the anesthetized guinea-pig. With one exception, the examination of the effect of drug concentration of the absorption rate, the experiments were carried out at $500 \mu\text{g/ml}$. The initial volume of the solution was set at 22 ml in order to permit 2 samplings of 1 ml each, thus leaving exactly 20 ml at the end of the experiments.

Examination of Experimental Conditions—1)

Recirculating Flow Rate: As shown in Fig. 3, at a flow rate of 0 ml/min, *i. e.*, in a stationary state, wide variation and lowness of the amount decreased were observed in comparison with those in a recirculated state. Also at a flow rate of 2–3 ml/min a slightly smaller amount decreased was found than in the more rapid flow rate. But in the more rapid flow rate of above 10 ml/min, every case showed the same amount decreased. Consequently it was concluded that the flow rate within the range of 10 to 20 ml/min had no effect on the absorption of drug from the buffer solution. It was assumed that differences of amount decreased between the stationary and recirculated state of solution were mainly caused by the difference in temperature of solution and the non-homogeneous concentration of the drug in the solution.

2) Temperature of Solution: From the results in Fig. 4 and Fig. 5, it was suggested that the absorption of carbinoxamine was more susceptible to the effect of temperature than that of salicylic acid. As was suggested by Scheuplein and Blank,⁸⁾ it was evident that the temperature of the solution had a great effect

electric hair clipper. The exposed skin was first wiped cleanly and cautiously with absorbent cotton soaked in ethyl alcohol, and then with one soaked in water. After 30 minutes lapsed, a glass vessel as showed

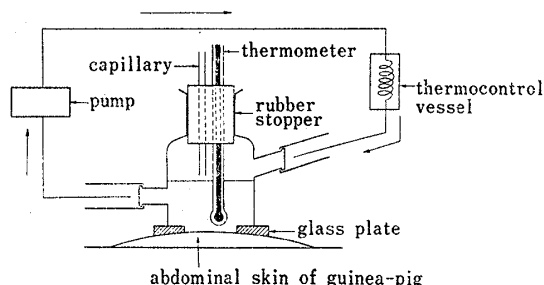


Fig. 2. Recirculation Apparatus

in Fig. 2 was applied to the abdominal skin using α -cyanoacrylate (Toa Gosei Co., Ltd.). The area of abdominal skin was standardized at $2.25 \pi \text{ cm}^2$. As shown in Fig. 2, the glass vessel was connected with a recirculating pump (Tokyo Kagaku Seiki Co., Ltd.) and a thermocontrol vessel by a teflon tube (the inside diameter 4 mm). Drug in each Sørensen isotonic buffer solution was applied to the intact abdominal skin by introducing it into the glass vessel and then continuously recirculating it.

After the lapse of a given time, a part of the solution was pipetted and the amount of the drug absorbed

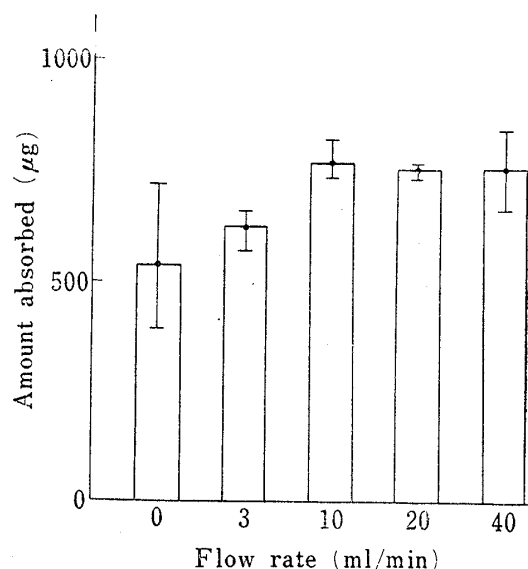


Fig. 3. Effect of Flow Rate on Percutaneous Absorption of Salicylic Acid

solution used: pH 3.0 $500 \mu\text{g/ml}$
 □ indicates the range of three values of amount absorbed (0–6 hr)

7) K. Ogata, "Physiology for Clinic," Asakura Shoten, 1967, p. 431.

8) R.J. Scheuplein and I.H. Blank, *J. Invest. Derm.*, **49**, 582 (1967).

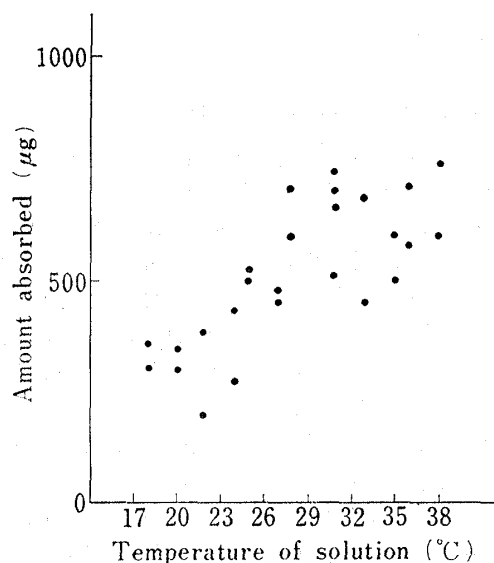


Fig. 4. Effect of the Temperature of the Solution on Percutaneous Absorption of Salicylic Acid

amount absorbed: 0—6 hr
solution used: pH 3.0 500 µg/ml

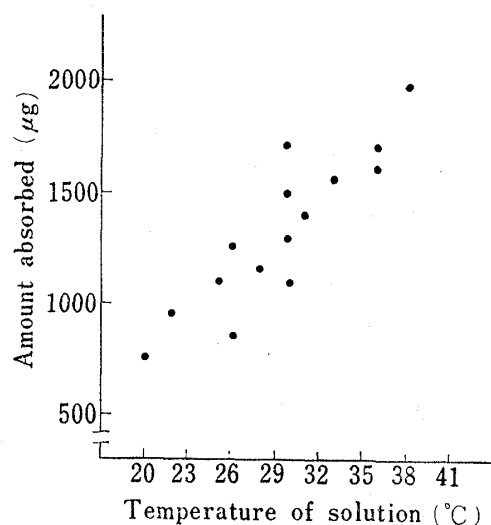


Fig. 5. Effect of the Temperature of the Solution on Percutaneous Absorption of Carbinoxamine

amount absorbed: 0—6 hr
solution used: pH 9.0 500 µg/ml

on the percutaneous absorption of drug. Therefore the temperature of solution had to be strictly controlled during the experimental period.

3) Change of Solution Volume: Using phenol red which was expected to be unabsorbed, the change of solution volume was examined.⁹⁾ After 6 hours in recirculation, no decrease in the concentration of phenol red was observed and therefore it was found that the absorption of water was negligible.

4) Skin Cleaning: Concerning with skin cleaning agents, besides ethyl alcohol, ethyl ether and a soap solution were selected, and the effects on the skin tissue and the variance of results of this use were investigated. It was observed that ethyl alcohol was most suitable. It was recently reported by Matoltsy¹⁰⁾ that among organic solvents ethyl alcohol had little effect on the barrier function of skin.

5) Tubing: Using a teflon tube, polyvinyl tube and silicone tube, blank experiments of the recirculation apparatus were carried out. When polyvinyl and silicone tubes were used, a decrease in the concentration of the drug was observed, but no decrease was observed with the teflon tube.

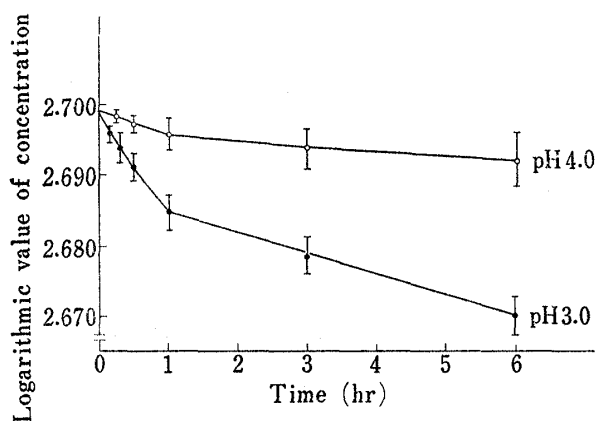


Fig. 6. Logarithmic Plot of Salicylic Acid Remaining in the Recirculating Solution

concn.: 500 µg/ml

●: mean ± S.D.

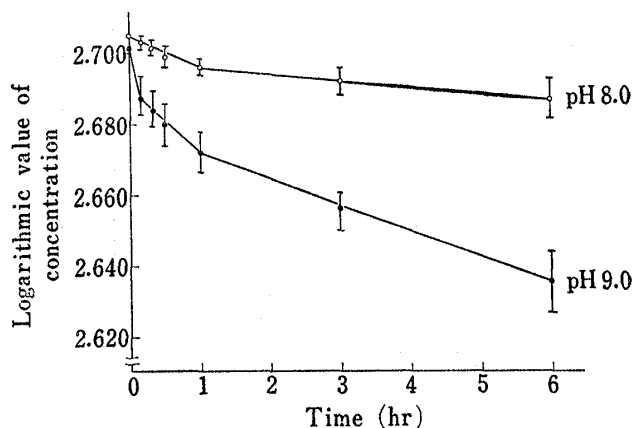


Fig. 7. Logarithmic Plot of Carbinoxamine Remaining in the Recirculating Solution

concn.: 500 µg/ml

●: mean ± S.D.

9) L.S. Shanker, P.A. Shore, B.B. Brodie and C.A.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **120**, 528 (1957).
10) A.G. Matoltsy, A.M. Downes and T.M. Sweeney, *J. Invest. Derm.*, **50**, 19 (1968).

Results and Discussion

Percutaneous Absorption of Drugs

It has been popularly accepted that there is a transient diffusion followed by a steady state diffusion in the process of percutaneous absorption.¹¹⁾ Therefore in the experiments samplings were made several times during the initial hour of experiment in order to know the outline of the absorption pattern of the drug. The results are shown in Fig. 6 and Fig. 7.

In both drugs it is recognized that the concentration of the solution decreases linearly after the lapse of the initial hour. From the results in Fig. 8, it is evident that both salicylic acid and carbinoxamine show a constant rate of absorption at each concentration.

These results suggest that the absorption of the drug occurs by the simple diffusion and its absorption rate does not depend on the concentration of the drug after the lapse of a certain initial time. Consequently in this report the initial hour is referred to as "the initial absorption time" and is excluded from the time quoted.

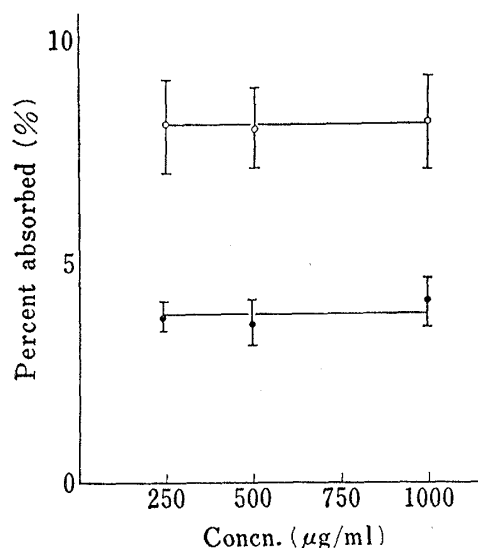


Fig. 8. Effect of Drug Concentration on Percutaneous Absorption

○: carbinoxamine pH 9.0
●: salicylic acid pH 3.0
⊥: mean ± S.D.

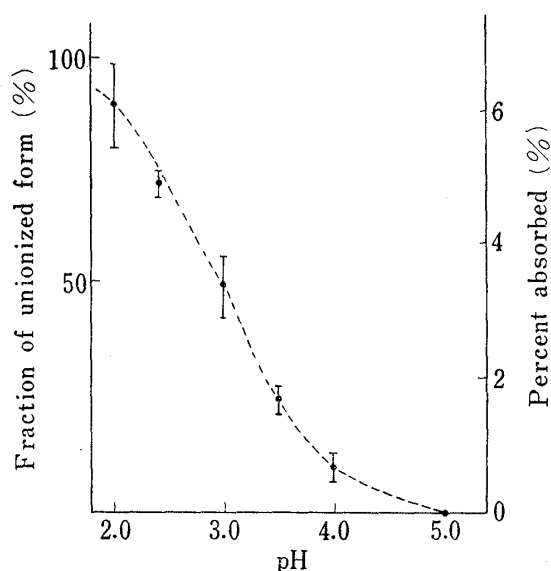


Fig. 9. Percutaneous Absorption of Salicylic Acid from the Solutions of Various pH

drug concn.: 500 µg/ml
-----: fraction of unionized form
⊥: percent absorbed of 1 to 6 hr (mean ± S.D.)

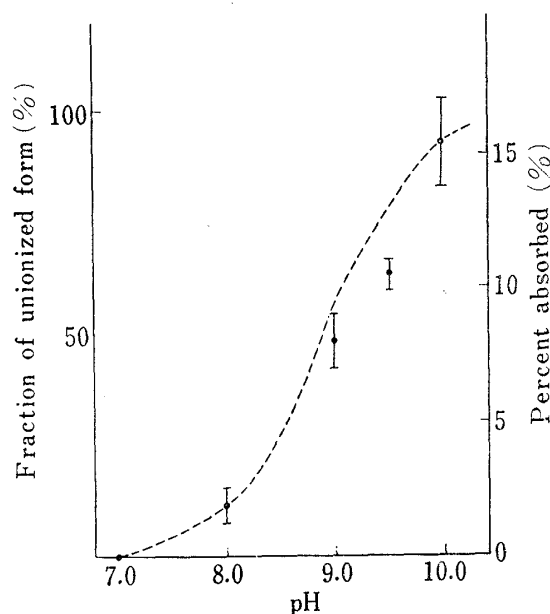


Fig. 10. Percutaneous Absorption of Carbinoxamine from the Solutions of Various pH

drug concn.: 500 µg/ml
-----: fraction of unionized form
⊥: percent absorbed of 1 to 6 hr (mean ± S.D.)

11) R.J. Scheuplein, *J. Invest. Derm.*, **48**, 79 (1967).

The Effect of pH Value of Solution on Percutaneous Absorption

The results are shown in Fig. 9 and Fig. 10, pH values are expressed on the axis of abscissa, the rate of absorption from 1 to 6 hour on the right side and the fraction of the unionized form on the left side of vertical axis.

In both salicylic acid and carbinoxamine the absorption rates of 1 to 6 hour are in accordance with the fraction of the unionized form which are expressed with the dotted line. This suggests that the preferential absorption of the unionized form of drug is remarkable.¹²⁾

Relation between Absorption Rate and Partition Coefficients

As shown in Table I, the partition coefficients of both drugs are larger in the pH range of the unionized form. They are in relation to the pH profile of rate absorbed. It is suggested that the lipid solubility of drugs gives an important indicator in percutaneous absorption¹³⁾ as same as in intestinal absorption.

TABLE I. Relation between Absorption and Partition Coefficients of Salicylic Acid and Carbinoxamine

Salicylic acid					
pH	F.U.F ^{a)} (%)	Percent absorbed (1—6 hr)	Partition coefficient (at 32°)		
			CHCl ₃	Ether	C ₆ H ₁₂
2	90.9	6.1 ± 0.6 ^{b)}	3.74	>100	0.135
3	50.0	3.3 ± 0.5	1.89	49.5	0.061
4	9.09	0.6 ± 0.2	0.35	13.0	0.006
5	0.99	0	0.04	1.6	0.004
Carbinoxamine					
pH	F.U.F (%)	Percent absorbed (1—6 hr)	Partition coefficient (at 32°)		
			CHCl ₃	Ether	C ₆ H ₁₂
7	1.2	0	>100	2.7	1.4
8	11.2	1.8 ± 0.6	>100	17.3	7.8
9	55.7	8.0 ± 1.0	>100	95.9	66.8
10	92.6	15.5 ± 1.8	>100	>100	>100

a) fraction of unionized form of the drug

b) mean ± S.D.

12) D.E. Loveday, *J. Soc. Cosmetic Chemists*, **12**, 224 (1961).

13) M. Katz and Z.I. Shaikh, *J. Pharm. Sci.*, **54**, 591 (1965).