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Studies on Percutaneous Absorption of Drugs. III.[1] Percutaneous Absorption of Drugs through Damaged Skin[2]

Mitsunori Washitake, Tateki Yajima, Toshio Anmo,[3a] Takaichi Arita, and Ryohei Hori[3b]

Research Laboratory, Taisho Pharmaceutical Co., Ltd.[3a] *and Faculty of Pharmaceutical Sciences, University of Hokkaido*[3b]

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1) The percutaneous absorption through the damaged skin lacking *stratum corneum* greatly increased as compared with those of the intact skin, and the initial absorption pattern which was distinctly recognized in the intact skin almost disappeared in the damaged skin, and the absorption of the drugs occurs by the simple diffusion of a first order rate from the start of the experiment.

2) The absorption of the drugs in the intact skin are almost negligible at pH 6.0 where the most of the drugs ionized, however, in the damaged skin, the absorption of drugs are distinctly found.

3) The amounts of the drugs reserved in the intact skin attained to a definite amount reserved after the lapes of a given time, and are depicted by the plateau pattern. On the other hand, the amount reserved in the damaged skin varied the pattern which has a peak in early time, and the peak of the amount reserved in the skin increased as increasing of the drug concentration of test solution.

4) The amount reserved in the skin and the amount of the drugs decreased from test solution in the damaged skin decreased as decreasing of the fraction of the unionized form of the drugs, and it was recognized that the peak of amount reserved in the skin has a tendency to reach late.

5) After test solution was removed from the applied area, the amounts of both drugs reserved in the damaged skin are almost disappeared within 4 hours, and the ability as a cutaneous reservoir of drugs was missing in the damaged skin.

In the previous studies,[1,4] the percutaneous absorption of both salicylic acid and carbinoxamine from the aqueous solution by the intact skin of male guinea-pigs was investigated, and the factors involved in percutaneous absorption from the applied condition of drugs on the skin were clarified by measuring the amount reserved in the skin and the loss amount of drugs from the recirculating solution. Further, it was suggested that the *stratum corneum* has a high ability as a reservoir of the drugs and plays an important part in the absorption of drugs through the skin. In the percutaneous absorption, the factor controlling the absorption rate is generally dividable into the applied condition on the skin and the structure of the skin. As for the skin structure, the *stratum corneum* is hitherto pointed out to be an important tissue as a barrier controlling the percutaneous absorption.

Rein[5] reported that electrophysiologic barrier, that is, electrical double layers through which neither cation nor anion is permeable, is situated between the *stratum corneum* and the malpighian layer. MacKee, *et al.*[6] pointed out that this barrier exists in the three portions,

1) Part II: M. Washitake, T. Yajima, T. Anmo, T. Arita, and R. Hori, *Chem. Pharm. Bull.* (Tokyo), **20**, 2429 (1972).
2) This work was presented at the 91th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April, 1971.
3) Location: *a) 34-1, Takata 3-chome, Toshima-ku, Tokyo; b) Kita-12-Jo, Nishi-6-chome, Sapporo.*
4) T. Arita, R. Hori, T. Anmo, M. Washitake, M. Akatsu, and T. Yajima, *Chem. Pharm. Bull.* (Tokyo), **18**, 1045 (1970).
5) H. Rein, *Z. Biol.*, **81**, 125 (1925).
6) G.M. MacKee, M.B. Sulzberger, F. Herrmann, and R.L. Baer, *J. Invest. Dermat.*, **6**, 43 (1945).

that is the lowest portion of the *stratum corneum*, the *stratum lucidum* and the *stratum granulosum*. Szakall[7,8] reported that the barrier corresponds to the lowest portion of the *stratum corneum*. Concerning topical anesthetics, antihistaminics, *etc.*, Monash[9] proved that the barrier can be got rid of by removal of the outer half to two-thirds of the *stratum corneum* using the stripping method, and pointed out that this barrier consists of the whole layer of the *stratum corneum*, however, greater part of this barrier is formed of the outer half to two-thirds of the *stratum corneum* rather than of the lowest portion of the *stratum corneum*. Thus it has been reported by many authors that the percutaneous absorption is easily performed by damaging the *epidermis*, but the papers concerning quantitative comparison of the amount of drugs absorbed through the skin lacking the *stratum corneum* with that through the intact skin has been scarcely found.

In this study, in order to make clear the relationship between the structure of skin and the percutaneous absorption, the absorption experiments using the damaged abdominal skin of guinea-pigs from which *stratum corneum* was removed by the stripping method were carried out using the recirculating method described in the previous report. And the amount reserved and the absorption rate of the drugs by the damaged skin were compared with those of the intact skin.

Experimental

Drugs——In a similar manner as the previous report,[4] salicylic acid (pK_a: 3.0) was used as an acidic drug whereas carbinoxamine (pK_a: 8.9) was employed as a basic drug. They were dissolved in Sörensen isotonic buffer solutions 250, 500, 1000 μg/ml.

Absorption Experiments——Male guinea-pigs weighing 300 to 450 g were anesthetized with a 25% urethane aqueous solution injected intra-peritoneally. After they were fixed on their backs, the hair of abdominal skin was cut with an electric hair clipper, and the *startum corneum* of abdominal skin was removed by the stripping method, that is, the method for removing the skin surface by stripping off the cellophan adhesive tape (width: 38 mm, length: 100 mm, Nichiban K.K.) previously sticked on the skin to be removed. A glass vessel for recirculating test solutions, which is shown in the previous report,[4] was pasted on the damaged skin with α-cyanoacryrate, and test solutions were applied on the damaged skin by introduction into the glass vessel. The solution were then recirculated continuously. After the lapse of a given time, a part of the test solution was pipetted and the amount of drug absorbed was calculated from the concentration of drugs remaining in the recirculating solution. In a similar way as the previous report, the applied area of abdominal skin of 2.25π cm^2 was standardized and temperature of test solution, room temperature and recirculating flow rate were controlled to $31\pm1°$, $23\pm2°$ and 15 ± 5 ml/min, respectively. The initial volume of the solution was set at 25 ml.

Experiment on Cutaneous Reservoir of Drugs——In the above mentioned experiment, male guinea-pigs were killed immediately after recirculation of test solution for a given time, and the damaged abdominal skin of the applied area was wiped several times cleanly with absorbent cotton soaked in water in order to remove the test solution. And then the skin of the applied area was isolated up to the *corium*, and the drug in the skin was determined by the analytical method as described in the previous report.

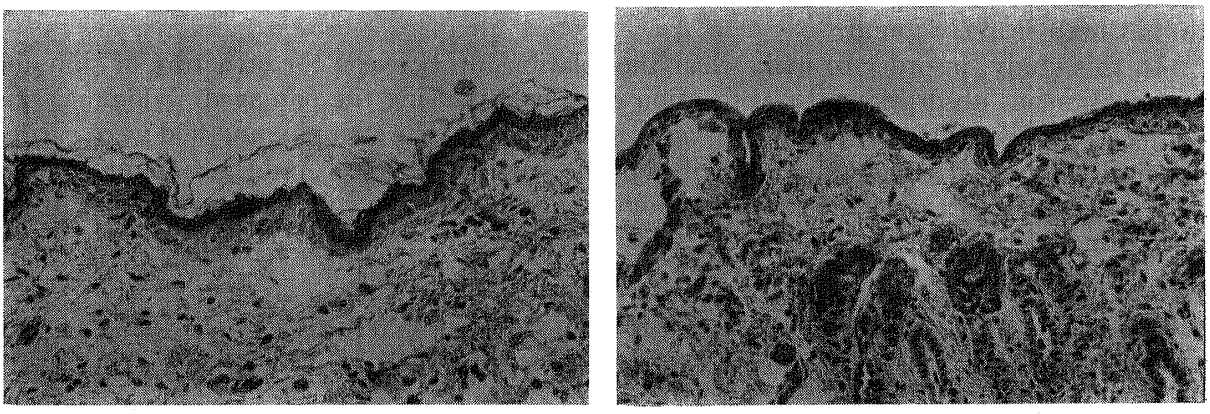
Time-Course of the Disappearance of Drugs from Cutaneous Reservoir——After 1 hour of recirculation according to the above mentioned method, the skin of the applied area was wiped several times cleanly with absorbent cotton soaked in water. The tested animals were killed after 0.5, 1, 2, 4 and 24 hours, respectively, and the skin of the applied area was isolated up to the *corium*, and then the amount of drugs remaining in the skin was determined.

Analytical Methods——Both salicylic acid and carbinoxamine in test solutions were determined according to the double extraction method as described in the previous report.[4] Otherwise, both drugs in the skin were also determined by a similar method to that described in the previous report.[1]

Examination of Experimental Conditions——1) Examination of Conditions for Removing the *Stratum Corneum*: The adhesive tape used for removing the *stratum corneum* was cellophan adhesive tape (width: 38 mm, length: 100 mm, Nichiban K.K.). Many papers[10–13] have been reported how many times repetition

7) A. Szakall, *Fette u. Seifen.*, **53**, 399 (1951).
8) A. Szakall, *Arch. Dermat. Syphil.*, **194**, 376 (1952).
9) S. Monash, *J. Invest. Dermat.*, **29**, 367 (1957).
10) H. Pinkus, *J. Invest. Dermat.*, **19**, 431 (1952).
11) A.L. Lorincz, *J. Invest. Dermat.*, **28**, 275 (1957).
12) T. Treger, *J. Soc. Cosmetic Chemists*, **13**, 145 (1962).
13) M. Mizoguchi, *Nihonhifukagakukai Zasshi*, **80**, 213 (1970).

of the stripping technique was required for satisfactorily removing the *stratum corneum*. In order to find the necessary repetition numbers, the authors observed microscopically change of the tissue by 5 to 25 times stripping. The results showed that 50%, 90% and approximately 100% of the *stratum corneum* was removed by 5, 10, and 15 times stripping, respectively. Figure 1 compares the intact abdominal skin of guinea pigs with the stripped skin by 20 times strippings, and shows that the *stratum corneum* of the skin surface was satisfactorily removed by 20 times strippings. Fig. 2 and Fig. 3 show the relationship between the number of strippings and the amount of drugs decreased from the recirculating solutions of both salicylic acid and carbinoxamine in 500 μg/ml, at pH 6.0. Thus, it was found that the amount of drugs absorbed did not increase by 15 times strippings or more, and the dispersion between the data was small in the range from 15 to 25 times strippings. The number of stripping was, therefore, set at 20 times for further test.



Intact skin (×250)

Stripped skin by 20 times strippings (×250)

Fig. 1. Optical Micrograph of Gross-sections through the Abdominal Epidermis of Guinea Pig, stained with Hematoxylin and Eosin

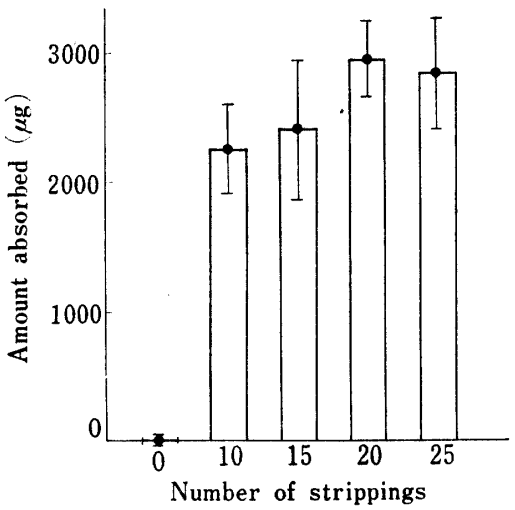


Fig. 2. Effect of Number of Strippings on Percutaneous Absorption of Salicylic Acid

test solution: pH 6.0, 500 μg/ml

: mean ± standard error of three to six values (0—6 hr)

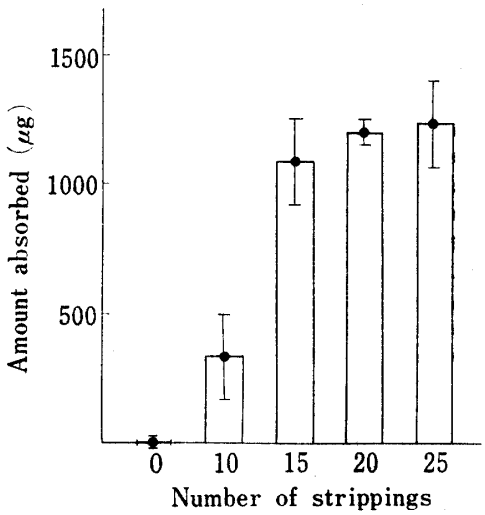


Fig. 3. Effect of Number of Strippings on Percutaneous Absorption of Carbinoxamine

test solution: pH 6.0, 500 μg/ml

: mean ± standard error of three to six values (0—6 hr)

2) Change of Test Solution Volume: So far as the intact skin is concerned, the absorption of water is negligible, however, it is necessary to check the absorption of water from the damaged skin lacking *stratum corneum*. At the start of this experiment, volume of test solution was therefore compared with that of test solution after 6 hours of recirculation. Consequently, it was found that the absorption of water by the damaged skin was also negligible.

Result and Discussion

Percutaneous Absorption of Drugs by the Damaged Skin

In Fig. 4 and Fig. 5 each decreasing rate from the recirculating solutions of salicylic acid at pH 3.0 and carbinoxamine at pH 9.0 in an initial concentration of 500 μg/ml was plotted as the time proceeds comparing the intact skin with the damaged skin. The amounts of both drugs absorbed by the damaged skin were much more than those by the intact skin. In the case of the damaged skin, the absorption rate of drugs from the recirculating solutions was 79.4%, about 10 times that of intact skin, in salicylic acid and 36.9%, about 3 times, in carbinoxamine after 6 hours in recirculation of test solutions. Furthermore, the initial absorption pattern which the drug concentration in the test solution decreased linearly after the lapse of the initial hour was distinctly recognized in the intact skin, but this pattern was almost disappeared in the damaged skin, and the drug concentration in the test solution decreased linearly from the start of the experiment. As the causes leading to these results, the removal of the *stratum corneum* was firstly considered to be an important part in the initial absorption pattern, and as the second, the increasing of absorption amount by damaging the barrier is also considerable reasonably. Because the initial absorption amount comes to be negligible by this remarkable increasement.

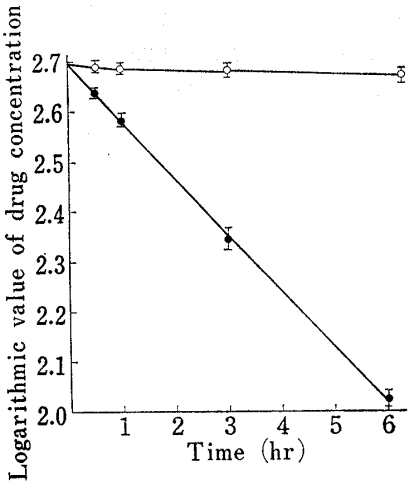


Fig. 4. Logarithmic Plot of Salicylic Acid Remaining in the Recirculating Solution

test solution: pH 3.0, 500 μg/ml
—○—: intact skin
—●—: damaged skin
⊕ : mean±standard error of three to six experiments

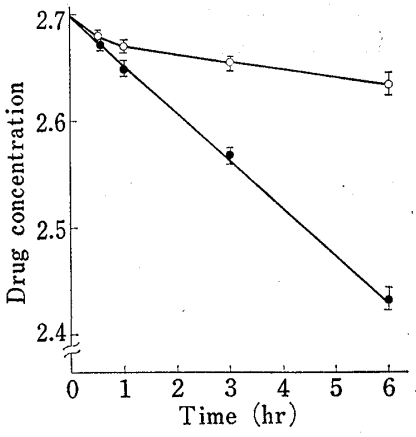


Fig. 5. Logarithmic Plot of Carbinoxamine Remaining in the Recirculating Solution

test solution: pH 9.0, 500 μg/ml
—○—: intact skin
—●—: damaged skin
⊕ : mean±standard error of three to six experiments

Nextly, the recirculating experiment was conducted under the various drug concentrations and various pH values in test solution in order to observe the influences by the applied conditions of drugs on the decreasing rate of drug from the recirculating solution.

The Effect of Drug Concentrations on Percutaneous Absorption——In Fig. 6 is plotted the drugs absorbed from the recirculating solution after 6 hours at the drug concentration of 250, 500 and 1000 μg/ml and at pH 3.0 and pH 9.0 for salicylic acid and carbinoxamine, respectively. Number of experiments in the respective drug concentration are 3—6, and the range of variation shows the standard error. The rates of absorption of both drugs from the recirculating solutions were independent of the drug concentration in the above concentration range. Thus it may be considered that the percutaneous absorption from the damaged skin occurs by the simple diffusion as far as in above range.

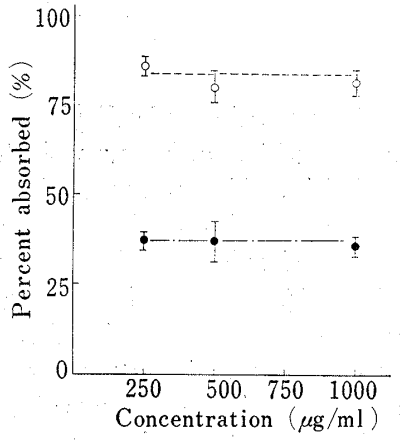


Fig. 6. Effect of Drug Concentration on Percutaneous Absorption by the Damaged Skin

○: salicylic acid, pH 3.0
●: carbinoxamine, pH 9.0
⊥●⊥: mean±standard error of three to six values of percent absorbed (0—6 hr)

The Effect of pH Values on Percutaneous Absorption——The plots of the absorption rate of salicylic acid and carbinoxamine against the pH values after 6 hours' recirculation are shown in Fig. 7 and Fig. 8, where the concentration of each drug is 500 μg/ml, and the pH values of test solution are varied at 2, 3, 4, 5 and 6 for salicylic acid, and at 6, 7, 8, 9 and 10 for carbinoxamine. While the absorption of salicylic acid and carbinoxamine in the intact skin are almost negligible at pH 6.0 where the most of both drugs are ionized, absorption rate is 20%/6 hr for salicylic acid, and 9.3%/6 hr for carbinoxamine in the damaged skin. These results suggest that the ionized form of both drugs are absorbed by the damaged skin. The absorption rates of both drugs by the damaged skin differ from those of the intact skin, and it was not recognized a good relationship between the absorption rates and the fraction of unionized form of the drugs. However, the absorption rates of both drugs increased as increasing of the fraction of unionized form of both drugs, except for pH 2.0 and pH 10.0. These results suggest that the preferential absorption of the unionized form of drugs is also presented in the damaged skin.

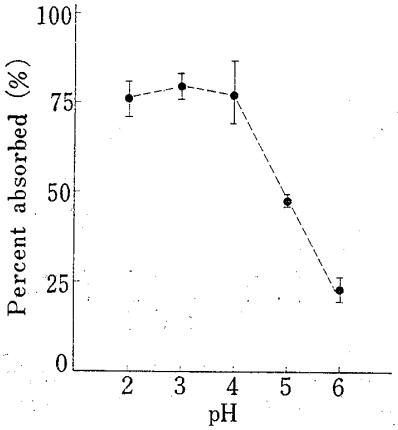


Fig. 7. Percutaneous Absorption Rate of Salicylic Acid through the Damaged Skin from Test Solutions of Various pH (after 6 hours)

drug concentration: 500 μg/ml
⊥●⊥: mean±standard error of three to six experiments

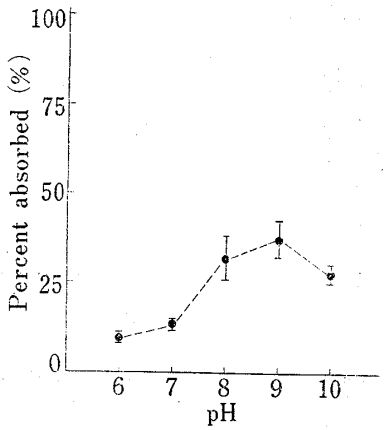


Fig. 8. Percutaneous Absorption Rate of Carbinoxamine through the Damaged Skin from Test Solutions of Various pH (after 6 hours)

drug concentration: 500 μg/ml
⊥●⊥: mean±standard error of three to six experiments

Time-Course of Cutaneous Reservoir of Drugs by the Damaged Skin

In Fig. 9 and Fig. 10, each plot of amount of salicylic acid and carbinoxamine reserved in the skin against the time progress was plotted comparing the intact skin with the damaged skin, where the concentration of each drug is 500 μg/ml, and the pH values of test solution are at 3.0 for salicylic acid and at 9.0 for carbinoxamine, and the recirculating times of test solution are varied at 0.5, 1.0, 3.0, 4.5 and 6.0 hours. The amounts of both drugs reserved in the intact skin attained to a definite amount reserved after the lapse of a given time, and are depicted by the plateau pattern. On the other hand, the amounts reserved in the damaged skin are depicted by the pattern which has a peak in early time after 0.5 to 1.0 hour from the

start of the experiment. As the causes leading to these results, it is firstly considered that the percutaneous absorption amounts of drugs are increased and the drug concentrations of test solution are rapidly decreased also because of the removement of the *stratum corneum* playing an important part in the percutaneous absorption and the cutaneous reservoir of drugs. As the second, it is considered that the amounts of drugs reserved in the skin are rapidly decreased as decreasing of the drug concentrations of test solution.

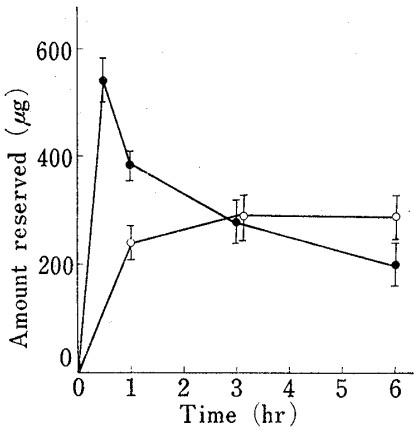


Fig. 9. Pattern of Amount of Salicylic Acid reserved in Skin

test solution: pH 3.0, 500 μg/ml
○: intact skin
●: damaged skin
⊕: mean ± standard error of three to six experiments

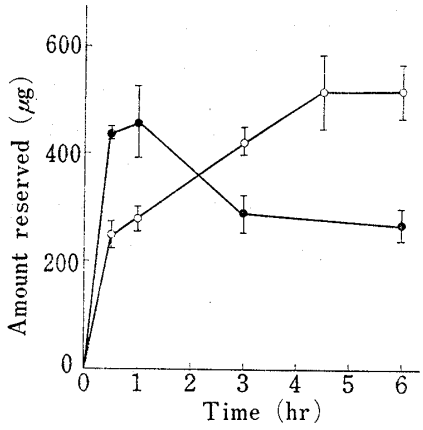


Fig. 10. Pattern of Amount of Carbinoxamine reserved in Skin

test solution: pH 9.0, 500 μg/ml
○: intact skin
●: damaged skin
⊕: mean ± standard error of three to six experiments

Nextly, the experiment on cutaneous reservoir of drugs was conducted under the various drug concentrations and various pH values in test solution in order to observe the influences by the applied conditions of drugs on the pattern of the amount reserved in the skin.

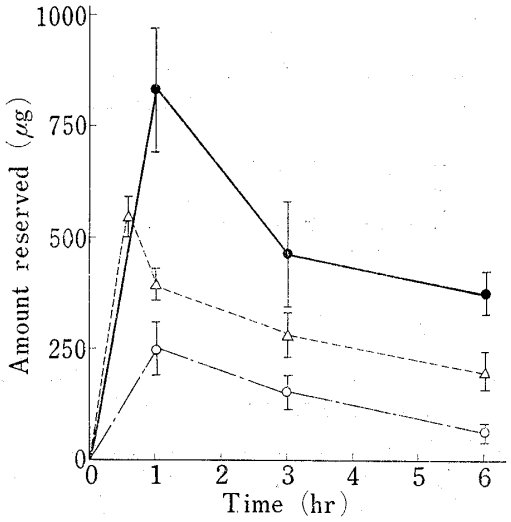


Fig. 11. Effect of Concentration of Salicylic Acid on Amount reserved by the Damaged Skin

test solution: pH 3.0, ○: 250 μg/ml
△: 500 μg/ml, ●: 1000 μg/ml
⊕: mean ± standard error of three to six experiments

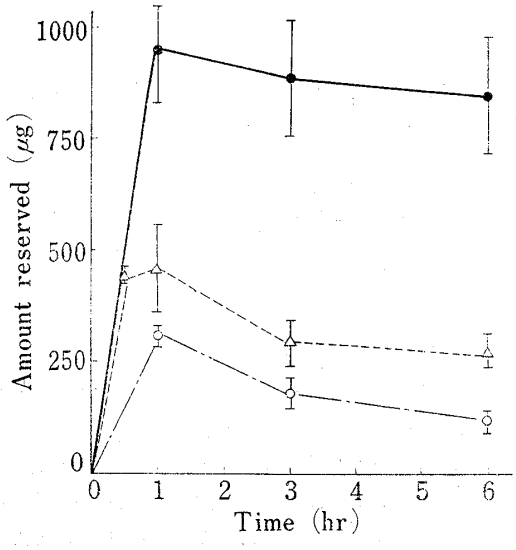


Fig. 12. Effect of Concentration of Carbinoxamine on Amount reserved by the Damaged Skin

test solution: pH 9.0, ○: 250 μg/ml
△: 500 μg/ml ●: 1000 μg/ml
⊕: mean ± standard error of three to six experiments

The Effect of Drug Concentrations on Cutaneous Reservoir——In Fig. 11 and Fig. 12, each plot of the amount of salicylic acid and carbinoxamine reserved in the skin against the time progress was plotted as the time proceeds, where the pH values of test solution are at 3.0 and 9.0 for salicylic acid and carbinoxamine, respectively, and the drug concentrations are varied 250, 500 and 1000 μg/ml. In the respective concentration of drugs, similar sharped patterns were obtained with respect to the amounts of both drugs reserved, and each peak of the amount reserved in the skin increased as increasing of the drug concentration.

The Effect of pH Values of Test Solution on Cutaneous Reservoir——In Fig. 13 and Fig. 14, each plot of the amount of salicylic acid and carbinoxamine reserved in the skin against the time progress was plotted as the time proceeds, where the concentration of each drug is 500 μg/ml, and the pH values of test solution are varied at 3.0, 5.0 and 6.0 for salicylic acid, and at 6.0, 7.0 and 9.0 for carbinoxamine. When the pH values of test solution are raised from 3.0 to 6.0 for salicylic acid, and lowered from 9.0 to 6.0 for carbinoxamine on the contrary, the peak of the amount reserved became lower and broader as decreasing of the fraction of unionized form of both drugs. Further, it was recognized that the time required to reach the peak have a later trend.

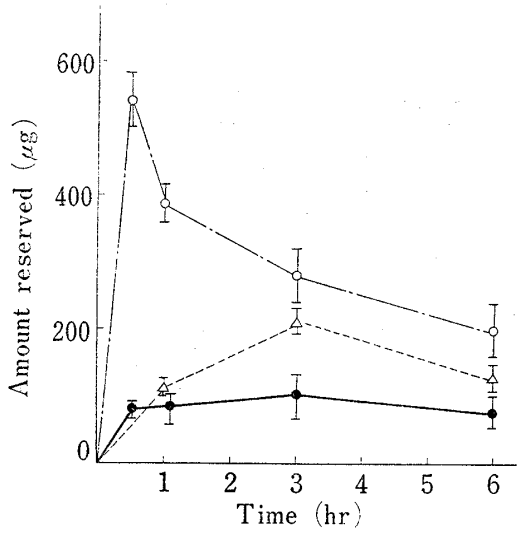


Fig. 13. Effect of pH on Amount of Salicylic Acid reserved by the Damaged Skin

drug concentration: 500 μg/ml
○: pH 3.0, △: pH 5.0, ●: pH 6.0
⊕: mean ± standard error of three to six experiments

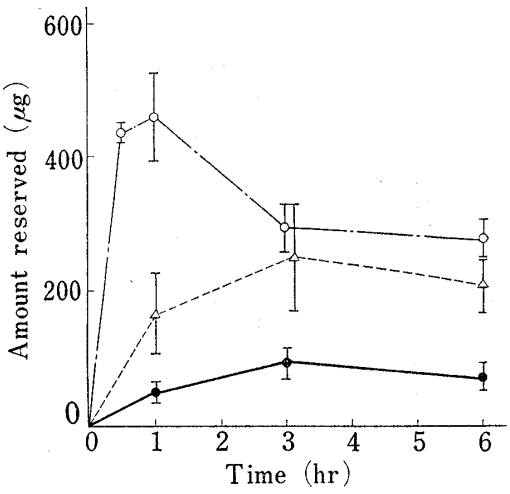


Fig. 14. Effect of pH on Amount of Carbinoxamine reserved by the Damaged Skin

drug concentration: 500 μg/ml
●: pH 6.0, △: pH 7.0, ○: pH 9.0
⊕: maen ± standard error of three to six experiments

Time-Course of the Disappearance of the Drugs from Cutaneous Reservoir

The time-course of the disappearance pattern of salicylic acid and carbinoxamine from the amount reserved in the skin after one hour recirculation are shown in Fig. 15 and Fig. 16, where the concentration of each drug is 500 μg/ml, and the pH values of test solution are at 3.0 for salicylic acid, and at 9.0 for carbinoxamine. The amounts of both drugs reserved in the skin rapidly decreased to a half to one-thirds, one-thirds to one-fifth and, less than one-tenth of initial amount at a half hour, one hour and two hours respectively. After 4 hours, only trace amount was found. In the case of the intact skin, as described in the previous paper,[1] the amounts of both drugs reserved in the skin decreased to a half to one-thirds of initial value after 24 hours, respectively. And the amount of salicylic acid reserved in the skin decreased to one-thirds, one-fourth of initial amount after 5 days and 13 days, respectively. On the other hand, the amount of carbinoxamine reserved in the skin decreased to

one-tenth of initial amount after 5 days, and was still slightly detected after 13 days. Contrary to the case of the intact skin, the amounts of both drugs reserved in the damaged skin are disappeared within 4 hours, and the ability as a cutaneous reservoir of drugs was missing. The results are proved that the ability as a cutaneous reservoir of drugs was missing by the removal of the *stratum corneum* was considered to be important part in the cutaneous reservoir of drugs. Vickers[14] and Scoggins[15] reported that topically applied corticosteroids are not reserved in the diseased skin whose *stratum corneum* was damaged, particulary in the skin of *psoriasis vulgaris*. Kukita and Matsuzawa[16] proved that the disappearance time of the drug from cutaneous reservoir of *psoriases vulgaris* was faster than that of the intact skin by the applied of the radioactive corticosteroids cream.

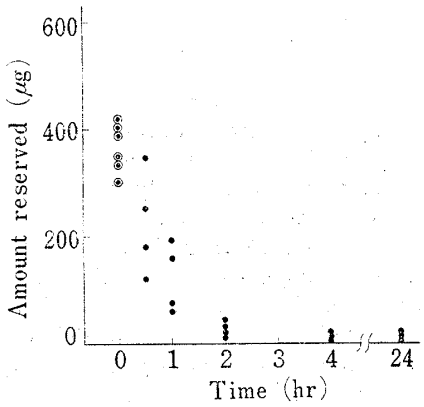


Fig. 15. Time-Course of the Disapperance of Salicylic Acid reserved in Skin

test solution: pH 3.0, 500 μg/ml
⊙: initial amount reserved
●: amount reserved after leaving for a certain period of time

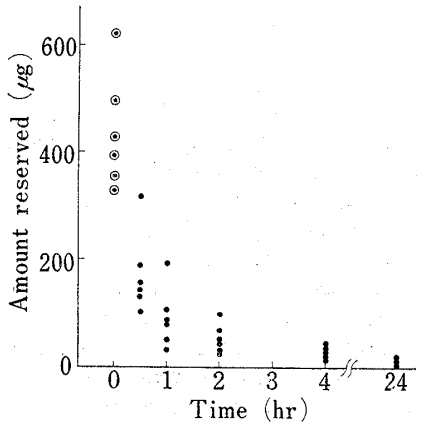


Fig. 16. Time-Course of the Disapperance of Carbinoxamine Reserved in Skin

test solution: pH 9.0, 500 μg/ml
⊙: initial amount reserved
●: amount reserved after leaving for a certain period of time

Our experimental results agreed with those of the reports described above, and it was proved that the surface structure of the skin, particularly the *stratum corneum*, has greatly effects upon the cutaneous reservoir of drugs.

14) C.F.H. Vickers, *Arch. Dermat.*, **88**, 20 (1963).
15) R.B. Scoggins, "American Academy of Dermatology," Chicago, 1963.
16) A. Kukita and T. Matsuzawa, *Hifu Rinsyo*, **24**, 139 (1970).