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Studies on Percutaneous Absorption of Drugs. II.[1] Time-Course of Cutaneous Reservoir of Drugs

MITSUNORI WASHITAKE, TATEKI YAJIMA, TOSHIO ANMO,[2a] TAKAICHI ARITA, and RYOHEI HORI[2b]

Research Laboratory, Taisho Pharmaceutical Co., Ltd.[2a] *and Faculty of Pharmaceutical Sciences, Hokkaido University*[2b]

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1) Time-course of cutaneous reservoir of both salicylic acid and carbinoxamine showed same pattern as those of decreasing from the recirculating solution in the initial time, and the cutaneous reservoir attained to a definite amount after the lapse of a given time.

2) This definite amount reserved was different with the variation of the drug concentration and the pH values in test solution, and increased as increasing of the fraction of the unionized form of both drugs.

3) The amount decreased from test solution was almost negligible at pH 6.0 where the most of both drugs ionized, however, the amount reserved was slightly detected in the skin.

4) The diffusion of drug into the external area of applied skin was negligible, and the drug permeability through the skin was restricted to the applied area.

5) After test solution was removed from the applied area, the disappearance of drug from the cutaneous reservoir was traced quantitatively during 13 days, and it was cleared that considerable amounts of both drugs reserved remained for a long time.

6) Studying on the reservoir part of drugs, it was recognized that the skin surface, particulary the *stratum corneum*, had a high ability of the drug reservoir.

In the previous report, the authors established a new experimental method of percutaneous absorption using the recirculation method, and made clear that the percutaneous absorption of drugs from the aqueous solution occurs by simple diffusion according to a first-order process after the lapse of a certain initial time and preferential absorption of unionized form of the drug is remarkable. Though the amount of drugs decreased from the recirculating solution was discussed with regard to percutaneous absorption in the previous report, the behavior of drugs in the skin was not discussed.

However, as the skin is thicker than the membrane of other organs, it is not negligible that the skin has the function of cutaneous reservoir with the transport ability of the drug.

In fact, it is considered in a certain disease of skin that the effect of medication is more influenced by the drug reservoir in the diseased part than the effect of percutaneous absorption, and so it is more important that the behavior of drugs in the skin is cleared for topical medicine such as ointment. The result that topically applied drug is reserved in the skin was recognized by Guillot[3] in his experiment using salicylic acid. He reported that topically applied salicylic acid was excreted in the urine for several days, though salicylic acid injected intradermally was quite excreted within 24 hours.

Recently, several studies on cutaneous reservoir of drugs have been reported in regard to corticosteroids mainly. Malkinson and Ferguson[4,5] found that radioactive corticosteroid

1) Part I: T. Arita, R. Hori, T. Anmo, M. Washitake, M. Akatsu, and T. Yajima, *Chem. Pharm. Bull.* (Tokyo), **18**, 1045 (1970).
2) Location: *a) 34-1, Takada-3-chome, Toshima-ku, Tokyo; b) Kita-12-jo, Nishi-6-chome, Sapporo.*
3) H. Guillot, *J. Physiol.* (Paris), **46**, 31 (1954).
4) F.D. Malkinson and E.H. Ferguson, *J. Invest. Dermatol.*, **25**, 281 (1955).
5) F.D. Malkinson and E.H. Ferguson, *J. Invest. Dermatol.*, **28**, 211 (1957).

was reserved for 6 days by measurement of radioactivity on the skin or in the 17-ketosteroid fraction of the urine after the application of radioactive corticosteroid ointment.

Vickers[6] proved that triamcinolone acetonide was reserved in the *stratum corneum* for 2 weeks using vasoconstrictive activity. Further, Kukita, *et al.*[7] recognized that coritcosteroids were reserved for 7 days by the experiment on the distribution and the cutaneous reservoir of radioactive corticosteroid using radioautographic method. Most of these studies were conducted using the qualitative evaluation methods such as excretion in the urine, vasoconstrictivity, and radioautography, however, few study using the quantitative evaluation methods of cutaneous reservoir of drugs was reported.

In order to clarify the behavior of drugs in the skin, this time the authors measured drugs in the skin —*stratum corneum*, *stratum lucidum*, *stratum granulosum*, *stratum spinosum*, *stratum basale* and *corium*— under the applied area as the time proceeds after the recirculating method was performed in a similar way as the previous report, and investigated the diffusion of drugs into the external area of the applied skin. Further, it was traced the time-course of the disappearance of the drugs from cutaneous reservoir after removal of test solution, while, the reservoir part of drugs was investigated by measurement of drugs in the *stratum corneum* of the applied area which was removed with cellophan tape.

Experimental

Drugs——In a similar way as the previous report, both salicylic acid (p*K*a: 3.0) as an acidic drug and carbinoxamine (p*K*a: 8.9) as a basic drug dissolving in Sörensen isotonic buffer solutions were used.

Experiment on Cutaneous Reservoir of Drugs——Using the experimental conditions and the recirculation apparatus of a similar way as the previous report, male guinea pigs were killed immediately after recirculating of test solution for a given time, and the abdominal skin of the applied area was fitst wiped cleanly several times with absorbent cotton soaked in water.

And then the skin of the applied area was isolated to the corium, and after the isolated skin was sliced with a scissors, the drug in the skin was determined by analytical method as described later. Otherwise, the amount of drugs decreased from the recirculating solutions was tested by the similar method as the previous report.

Diffusion of Drugs into the External Area of the Applied Skin——In the above mentioned experiment, 1 cm wide area of external circumference of the applied skin was removed in a similar way, and the amount of drugs recovered from this skin was defined as the amount diffused.

Time-Course of the Disappearance of Drugs from Cutaneous Reservior——In the above mentioned experiment, after 6 hours in recirculation, test sloution was removed, and the skin of the applied area was wiped cleanly with absorbent cotton soaked in water. After the guinea-pigs of which the skin of the applied area was covered with polyethylene porous cap for preventionfrom dirty were breeded for a given time, the skin of the applied area was isolated to the *corium*, and then the amount of drugs remained in the skin was determined.

Studies on Reservior Part of Drugs——After 6 hours in recirculation in a similar way as the above mentioned experiment, guinea-pigs were killed immediately, and the skin of the applied area was wiped cleanly. The *stratum corneum* of the applied area was removed by 10 times' repetition of the stripping technique, that is, the technique for removing the skin surface by stripping off the cellophan tape sticked on the skin to be removed. This skin free from the *stratum corneum* was further isolated to the *corium*. Each drug content in the *stratum corneum* thus stripped and in the skin excluding the *stratum corneum* was determined in order to calculate the proportion of drug in the *stratum corneum* to that in all skin isolated to the *corium*.

Analytical Methods——In the chemical determination of drug contents in the skin, some obstacles from skin are considerable. Many experiments for removing obstacles against the determination brought us the suitable method having a satisfactory result as described below. According to this method, determination value of drug can be obtained with a high reproducibility.

Carbinoxamine: The mixture of isolated skin in slice and 20 ml of 0.2N HCl in a glass-stoppered test tube was shaken for 20 min, and homogenized for 45 min in an Universal Homogenizer (Nihon Seiki Seisakusho Co., Ltd.). The homogenized solution was further added with 20 ml of $CHCl_3$, shaken and centrifuged. To 10 ml of aqueous layer transfered to another glass-stoppered test tube, 2 ml of 2N NaOH and 20 ml of cyclohexane were added, and the mixture was shaken and centrifuged. The mixture of 10 ml

6) C.F.H. Vickers, *Arch. Dermatol.*, **91**, 657 (1965).
7) A. Kukita, T. Matsuzawa, and K. Yamada, *Japanese J. Dermatol.*, **78**, 889 (1968).

of cyclohexane layer transfered to another glass-stoppered test tube and 10 ml of 0.1N HCl was shaken and centrifuged. And then, carbinoxamine in aqueous layer was determined using ultraviolet absorption spectrum at 264 mμ as described in the previous report.

Salicylic Acid: As mentioned above, the mixture of the sliced skin and 20 ml of 0.1N NaOH in a glass-stoppered test tube was shaken for 20 min, and homogenized for 45 min. Further, the homogenized solution thus obtained was added with 20 ml of $C_2H_4Cl_2$, shaken and centrifuged. One ml of aqueous layer was diluted to 50 ml with 0.1N NaOH, and then salicylic acid in aqueous layer was determined by the fluorometry using excitative wavelength at 305 mμ, emissive wavelength at 401 mμ with a MPF-2A form Hitachi fluorometric-photometer. As shown Fig. 1, calibration curve of salicylic acid was described with a good linear relationship in the range of this concentration.

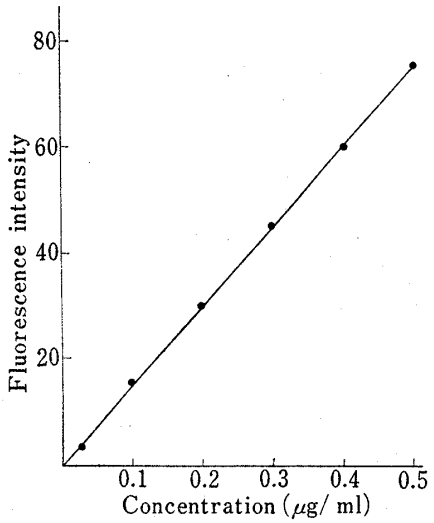

Fig. 1. Calibration Curve of Salicylic Acid

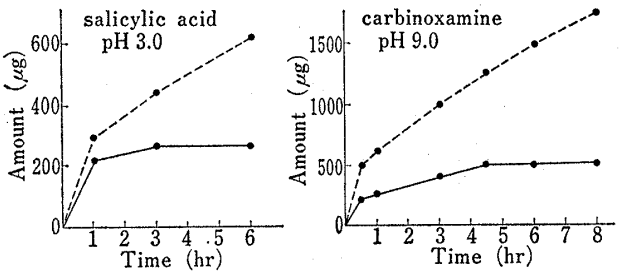

Fig. 2. Pattern of Percutaneous Absorption of Drugs

drug concentration: 500 μg/ml
---●---: amount decreased
—●—: amount reserved

Result and Discussion

Time-Course of Cutaneous Reservoir of Drugs

Fig. 2 shows that the amount reserved in the skin and the amount decreased from the recirculating solutions of both salicylic acid of pH 3.0 and carbinoxamine of pH 9.0 in a concentration of 500 μg/ml were plotted as the time proceeds. The amount decreased which was expressed with the dotted line increased as changing of the drug concentration in the recirculating solutions after the lapse of a certain initial time as described in the previous report. The amount reserved which was expressed with the full line increased as the same pattern as amount decreased from the recirculating solutions in the initial time, and attained to a definite amount reserved after the lapse of a given time.

The variation of the drug concentrations and the pH values in test solution were studied to observe how they influenced on this pattern of the amount reserved and a definite amount reserved.

Fig. 3 and Fig. 4 show that the amount reserved in the variation of the drug concentrations, 250, 500, and 1000 μg/ml of both salicylic acid of pH 3.0 and carbinoxamine of pH 9.0 were plotted as the time proceeds. Number of experiments in the respective time are 3—6, and the range of vairation shows the standard error. The amount of salicylic acid reserved attained to a definite value after 3 hours in the respective concentration, and increased as increasing of the drug concentrations, and these results show a similar tendency as the relation between the drug concentration and the amount decreased which was described in the previous report. On the other hand, the amount of carbinoxamine reserved attained to a definite value somewhat later than those of salicylic acid, that is, it took carbinoxamine 4.5—6.0 hours to attain the definite value, however, it was recognized that the amount reserved similarly increased as increasing of the drug concentrations.

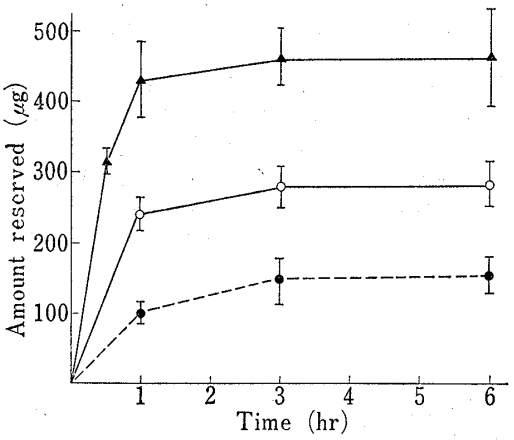

Fig. 3. Effect of Concentration on Amount reserved in Skin

test solution: salicylic acid, pH 3.0
—▲—: 1000 μg/ml
—○—: 500 μg/ml
---●---: 250 μg/ml
: mean±standard error

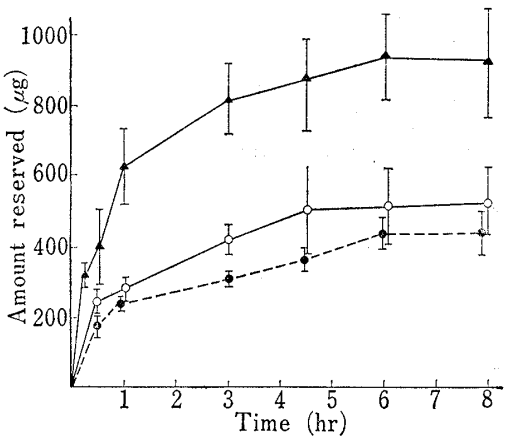

Fig. 4. Effect of Concentration on Amount reserved in Skin

test solution: carbinoxamine pH 9.0
—▲—: 1000 μg/ml
—○—: 500 μg/ml
---●---: 250 μg/ml
: mean±standard error

The plots of the reserved amount of salicylic acid and carbinoxamine against the time progress were shown in Fig. 5 and Fig. 6, were plotted as the time proceeds respectively, where the concentration of each drug was 500 μg/ml, and the pH values of test solutions were varied at 2, 3, 4, and 6 for salicylic acid, and at 6, 8, 9, and 10 for carbinoxamine. In respective pH values, the amounts of both drugs reserved show a similar pattern as those of variance of the drug concentrations, and increased as increasing of the fraction of unionized form of drugs. Similar tendency was previously reported as to the amounts of drugs decreased. Meanwhile, the amount decreased from the recirculating solutions was almost negligible at pH 6.0 where the most of both drugs ionized, however, the amount reserved was slightly recognized in the skin. The results suggest that the ionized form of drug slightly participates in the cutaneous reservoir.

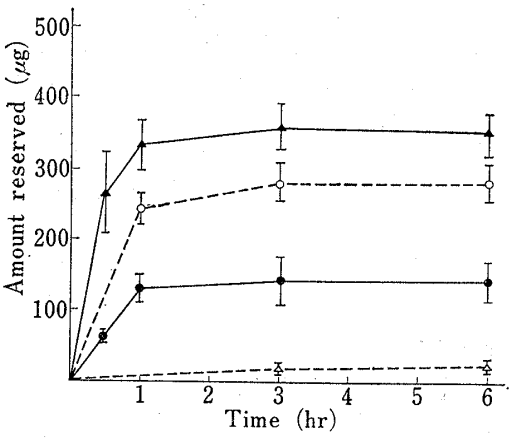

Fig. 5. Effect of pH on Amount reserved in Skin

test solution: salicylic acid, 500 μg/ml
—▲—: pH 2.0 —●—: pH 4.0
---○---: pH 3.0 ---△---: pH 6.0
: mean±standard error

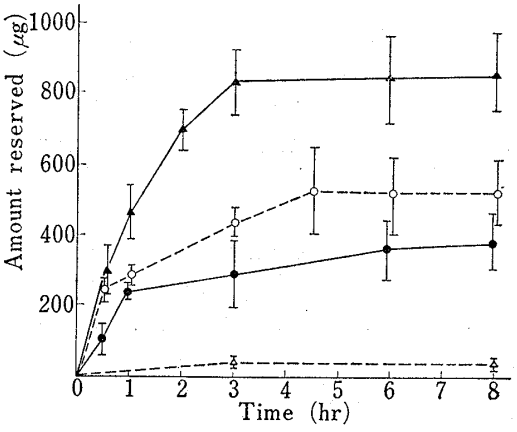

Fig. 6. Effect of pH on Amount reserved in Skin

test solution: carbinoxamine, 500 μg/ml
—▲—: pH 10.0 —●—: pH 8.0
---○---: pH 9.0 ---△---: pH 6.0
: mean±standard error

Diffusion of Drugs into the External Area of the Applied Skin

In order to regard the amount of drug recovered from the skin of the applied area as the amount reserved, it is necessary to determine the amount of drugs diffused into the external area of the applied skin. For this purpose, the skin of external circumference in 1 cm-wide of the applied skin was isolated to the *corium* at each interval of time, and the drug recovered from this isolated skin was defined as the amount diffused. Table I shows the time-course of the diffused amount of both salicylic acid and carbinoxamine together with the amount recovered from the external area of the applied skin at the start. The amount of both drugs diffused into the external area of the applied skin may be almost negligible, for the reason made evident in Table I. The reports on the diffusion of drug into the external area of the applied skin are very few. Using the deficiency rat of Vitamin A, Sobel, *et al.*[8] reported that topically applied Vitamin A improved only the applied area. Few amount of diffusion of drug into the external area of the applied skin agreed with the results of this experiment in spite of different drugs.

Table I. Diffusion of Drugs into the External Area of the Applied Skin

No.	Salicylic acid (pH 3.0, 500 μg/ml)				Carbinoxamine (pH 9.0, 500 μg/ml)			
	0 hr	1 hr	3 hr	6 hr	0 hr	3 hr	6 hr	8 hr
1	7.3 μg	13.4 μg	8.7 μg	10.0 μg	5.0 μg	16.6 μg	11.0 μg	13.8 μg
2	13.4	13.4	14.0	16.0	10.0	6.4	7.6	13.4
3	9.3	23.4	11.3	9.4	6.2	12.6	12.2	12.6
4	14.0	15.4	22.0	14.7	5.0	16.6	18.4	11.0
5	18.0	13.4		11.4	10.0			11.0
Mean	12.4	15.8	14.0	12.3	7.2	13.1	12.3	12.4

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

Fig. 7 and Fig. 8 show the time-course of the behavior of disappearance from cutaneous reservoir of both salicylic acid of pH 3.0 and carbinoxamine of pH 9.0 in a concentration of 500 μg/ml. The amount of salicylic acid reserved decreased less than one-half of initial value in one day, and further gradually tended to decrease. But 1/5—1/6 of initial value still remained after 13 days. On the other hand, the amount of carbinoxamine reserved was more rapidly decreased at 1/3 of initial value in one day than those of salicylic acid, and decreased at 1/10 of its after 5 days, however, amount of carbinoxamine reserved was still slightly detected after 13 days. Reports on cutaneous reservoir of drugs showed that salicylic acid[3] was reserved for several days and corticosteroids[4–7] were reserved for 6—14 days. These reports were qualitatively judged such as excretion in the urine, vasoconstrictivity and autoradiography, on the other hand, the report which determined directly drug in the skin has not been published to date. The results which were quantitatively studied using both salicylic acid and carbinoxamine in this experiment, show clearly that considerable amount of drugs were reserved in the skin for a long time though these phenomenon of reservoir somewhat differ with variety of drugs, and the importance of cutaneous reservoir in the process of percutaneous absorption was recognized.

Study on the Reservoir Part of Drugs

After 6 hours in recirculation of test solution, the skin surface of the applied area was removed with cellophan tape by 10 times' repetition. Most of the *stratum corneum* and a part of hair were removed by these strippings of 10 times. Table II shows the amount of the

8) A.E. Sobel, J.P. Parnell, B.S. Sherman, and D.K. Bradley, *J. Invest. Dermatol.*, **30**, 315 (1958).

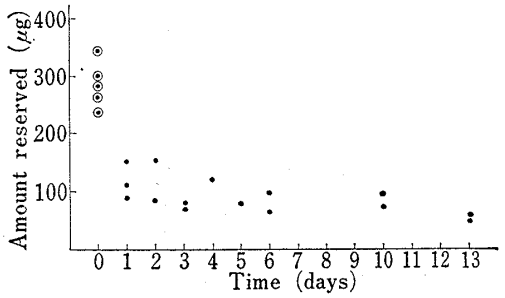

Fig. 7. Disappearance of Salicylic Acid in Skin

application: pH 3.0, 500 μg/ml, 6 hr
⊙: initial amount reserved
●: amount reserved after leaving for a certain period of time

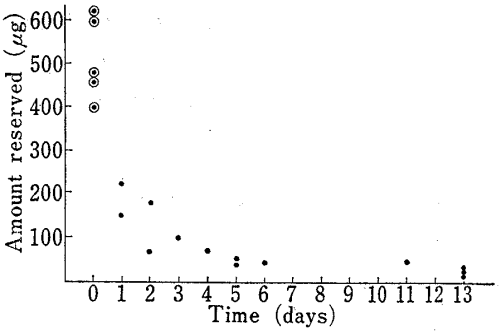

Fig. 8. Disappearance of Carbinoxamine in Skin

application: pH 9.0, 500 μg/ml, 6 hr
⊙: initial amount reserved
●: amount reserved after leaving for a certain period of time

drug detected in the *stratum corneum* removed with cellophan tape in comparison with the amount reserved in all skin isolated to the *corium*. About 65% of salicylic acid and about 60% of carbinoxamine were found in the *stratum corneum* removed with cellophan tape. The results of this experiment show that most of the amount reserved exists in the skin surface, particulary in the *stratum corneum*.

Table II. The Proportion of the Amount Removed with Cellophan Tape in Comparison with Total Cutaneous Amount of Drugs

No.	Salicylic acid (pH 3.0, 500 μg/ml)				Carbinoxamine (pH 9.0, 500 μg/ml)			
	Amount removed with cellophan tape (A)	Amount in stripped skin (B)	Total cutaneous amount (A+B)	$\frac{A}{A+B}$	Amount removed with cellophan tape (A)	Amount in stripped skin (B)	Total cutaneous amount (A+B)	$\frac{A}{A+B}$
1	193.9 μg	93.6 μg	287.5 μg	67.4 %	218.8 μg	316.8 μg	535.6 μg	40.8 %
2	150.4	100.0	250.4	60.0	307.0	248.0	555.0	55.3
3	173.8	80.9	254.7	68.2	288.0	210.6	498.6	57.7
4	175.8	91.6	267.4	65.7	500.0	108.0	608.0	82.2
Mean	173.5	91.5	265.0	65.3	328.4	220.9	549.3	59.0

Stoghton[9] and Carr, *et al.*[10] reported on the reservoir part of drugs. After each radioactive corticosteroid was topically applied to the intact skin, the *stratum corneum* of the applied area was removed with Scotch tape, and it was proved that the reservoir part of corticosteroid was in the *stratum corneum* by measuring the radioactivity in the *stratum corneum* removed. Using autoradiography method, Kukita, *et al.*[11,12] reported that the reservoir part of topically applied corticosteroid on the intact *axillae* skin and the scalp skin was recognized in the *stratum corneum* and the wall of *folliculus pili*. Trager[13] reported on the barrier layers of percutaneous absorption, and pointed out that the high barrier ability as the mechanism for inhibition of permeability through the skin and the inhibitor of diffusion through the *epidermis* exist in the *stratum corneum*, and suggested the existence of drug re-

9) R.B. Stoughton, *Arch. Dermatol.*, **91**, 657 (1965).
10) R.D. Carr and R.G. Wieland, *Arch. Dermatol.*, **94**, 81 (1966).
11) A. Kukita and T. Matsuzawa, *Japanese J. Dermatol.*, **77**, 742 (1967).
12) A. Kukita, T. Matsuzawa, and K. Yamada, *Hifu Rinsyo*, **11**, 122 (1969).
13) R.T. Tregear, *J. Soc. Cosmetic Chemist*, **13**, 145 (1962).

servoir in the *stratum corneum*. Our experimental results agree approximately with those reports, and it was proved that the surface structure of the skin together with the physicochemical properties of the drugs have great effects upon the cutaneous reservoir and the absorption of drugs from test solution.

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