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Absorption of Salicylic Acid through the Oral Mucous Membrane of Hamster Cheek Pouch¹⁾

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Ointments containing salicylic acid were applied to the cheek pouch of the hamster, and the influence of the type of base on drug absorption at the mucous membrane of the oral cavity was examined. Clear differences in absorption were found: emulsion base water-soluble base oleaginous base. Furthermore, the absorption of salicylic acid into the blood was closely related to drug retention in the tissue of the mucous membrane. The results of *in vitro* membrane permeation experiments corresponded very well with the results of the absorption experiments *in vivo*.

Keywords—absorption experiment; oral mucous membrane; hamster cheek pouch; oral dosage form of ointment; salicylic acid; blood concentration; retention in tissue; absorption parameters; *in vitro* membrane permeability

Only a few reports on the absorption of drugs from the oral cavity membrane, *e.g.* by Walton,^{3,4)} Makiyama,⁵⁾ Katz,⁶⁾ Aoki,⁷⁾ *etc.*, have appeared since the early work of Karmel⁸⁾ in 1873.

The existing methods for measuring the absorption from the oral membrane are to administer drugs dropwise into the oral cavity, to use an oral suppository⁷⁾ in animals in which the esophagus has been ligated by an operation,^{7,9)} and so on. However, none of these methods is straightforward. In this study, therefore, a method for measuring oral membrane absorption was devised, and the differences of absorption due to the use of different bases as drug carriers were examined. Furthermore, in order to investigate the validity of *in vitro* results, an *in vitro* membrane permeation experiment was performed using a method previously reported by the authors.¹⁰⁾

Experimental

Dosage Forms—The bases used in the experiments were prepared by emulsification with a kneader following the formula shown in Table I. Salicylic acid of reagent grade was added to the bases at a concentration of 2%.

Administration of Drugs—Male Golden hamsters weighing 95–105 g were anesthetized with ether after fasting for 24 hr and were used for the experiments. The inside of the cheek pouch of each hamster was cleaned with a tampon, and 1 g of oral dosage form ointment was administered through a syringe with

- 1) This work was presented in part at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1977.
- 2) Location: a) *Tajima-100, Odawara, Kanagawa 256, Japan*; b) *Ebara-2-4-41, Shinagawa-ku, Tokyo 142, Japan*.
- 3) R.P. Walton, *J. Pharmacol. Exptl. Therap.*, **54**, 53 (1935).
- 4) R.P. Walton, *J. Am. Med. Assoc.*, **124**, 183 (1944).
- 5) M. Makiyama, *J. of the Jap. Stomatological Soc.*, **2**, 1 (1953).
- 6) M. Katz and M. Barr, *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 419, 472, 476 (1955).
- 7) D. Aoki, *Yakuzaigaku (The Arc. of Prac. Pharm. of Japan)* **18**, 236 (1958).
- 8) J. Karmel, *Dent. Arch. Klin. Med.*, **12**, 466 (1873).
- 9) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.*, **13**, 861 (1965).
- 10) M. Tanaka, H. Fukuda, and T. Nagai, *Chem. Pharm. Bull.*, **26**, 9 (1978).

TABLE I. Formulae of the Ointment Bases

	Absorption ointment	Hydrophilic ointment	Macrogol ointment	White petrolatum	No. 1	No. 2	No. 3
White petrolatum	40.0	25.0	—	98.0	36.4	35.0	36.4
Cetyl alcohol	18.0	—	—	—	9.1	10.0	9.1
Stearyl alcohol	—	22.0	—	—	—	—	4.5
Hexadecyl alcohol	—	—	—	—	2.3	5.0	—
Oleyl alcohol	—	—	—	—	2.3	—	—
Lanolin	—	—	—	—	4.5	5.0	4.5
Beeswax	—	—	—	—	4.5	—	4.5
Sorbitan monooleate	5.0	—	—	—	3.2	5.0	3.2
Sorbitan monostearate	—	—	—	—	1.4	—	1.4
Propyleneglycol	—	12.0	—	—	—	—	—
Sodium lauryl sulfate	—	1.5	—	—	—	—	—
Macrogol 400	—	—	49.0	—	—	—	—
Macrogol 4000	—	—	49.0	—	—	—	—
Salicylic acid	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Aq.pur.	35.0	37.5	—	—	34.4	38.0	34.4

a sound needle of 1.5 mm inner diameter so as to reach the inside tip of the cheek pouch. To prevent swallowing of the ointment, the cheek pouch was fixed with Muchel forceps.

We confirmed that swallowing of the drug had not occurred by quantitative analysis of the stomach contents 6 hr after administration of the drug.

Blood Collection—The hamsters were anesthetized with ether at 0.5, 1.0, 1.5, 2.0, 3.0 and 5.0 hr after administration of the drug, and the blood was collected from the inferior vena cava after laparotomy.

Quantitative Analysis of Salicylic Acid in Blood—Each blood sample was separated by centrifugation for 30 minutes at 3000 rpm to obtain the plasma sample. After leaving the plasma to stand in a cool place, fibrin was mechanically removed with a glass capillary. Next, 0.25 ml of 6 N HCl was added to 1 ml of this plasma, and after precipitation of protein, 15 ml of 1,2-dichloroethane was added. The mixture was shaken for about 15 minutes, and centrifuged at 3000 rpm. To 10 ml of 1,2-dichloroethane extract thus obtained, 0.25 ml of iron reagent¹¹⁾ was added, followed by 3 ml of distilled water, and the color was developed by shaking for 10 minutes. The supernatant liquid was measured by colorimetry at 530 nm against a similarly treated control solution.

Quantitative Analysis of Residual Salicylic Acid in the Cheek Pouch—The residual base in the cheek pouch of each hamster after collecting the blood was removed and homogenized. Two hundred mg of sample base was weighed exactly and salicylic acid was extracted with 1,2-dichloroethane. The extracted salicylic acid was analyzed colorimetrically with iron reagent as described for the blood concentration measurement.

Quantitative Analysis of Salicylic Acid in the Tissue of the Cheek Pouch—The cheek pouches of hamsters were cut off after the residual base had been removed, and washed with 70% alcohol. The exact weight (350–400 mg) of cheek pouch tip was weighed, and 3 ml of 2 N NaOH was added. After heating the cheek pouch for 30 minutes on a water bath at 80°, 2 ml of 3 N HCl was added to neutralize the solution, then a further 1 ml of 6 N HCl was added. Fifteen ml of 1,2-dichloroethane was added to the solution, then the mixture was shaken for about 20 minutes and centrifuged at 3000 rpm. After adding iron reagent¹¹⁾ to the extract thus obtained, the supernatant solution was measured by colorimetry at 530 nm against a similarly treated control solution.

Calculation of Various Constants from the Blood Concentration—If the absorption and elimination process of the administered drugs is analyzed according to a one component model, the blood concentration C can be expressed as

$$C = A(e^{-K_{el} \cdot t} - e^{-K_a \cdot t}) \quad (1)$$

where A is a constant, K_a is the absorption rate constant and K_{el} is the elimination rate constant.^{12,13)} A , K_a and K_{el} were experimentally obtained by the simulation method of Ouchida.¹³⁾ A can also be expressed as $K_a \cdot D \cdot f / (K_a - K_{el}) \cdot V$, where D , f and V are the dose, absorption ratio and distribution volume, respectively.¹⁴⁾

11) One gram of $\text{Fe}(\text{NO}_3)_2 \cdot 9\text{H}_2\text{O}$ was dissolved in 0.07 N nitric acid to make 100 ml.

12) J.G. Wagner and C.M. Metzler, *J. Pharm. Sci.*, **56**, 658 (1967).

13) A. Ouchida, E. Mizuta, and T. Shima, *J. Takeda Res. Lab.*, **32**, 522 (1973).

14) M. Hanano, "Bioavailability Research Materials," Japan Medical Center, Tokyo, 1976, p. 27.

$D \cdot f/V$ was calculated from A , K_a and K_{el} for various bases. The area under the blood concentration curve (AUC) for 5 hr was obtained using the trapezoidal rule, and X_a/V was calculated from (2)

$$X_a/V = Ct + K_{el} \int_0^t C dt \quad (2)$$

where X_a is the total absorption concentration, Ct is the blood concentration at time t , and $\int_0^t C dt$ represents AUC until time t .¹⁴⁻¹⁶⁾

In Vitro Membrane Permeation Experiment—Following the method previously reported by the authors,¹⁰⁾ a Millipore filter membrane treated with olive oil was set in a glass cell, and on applying a fixed quantity of an ointment sample to the upper part of the model membrane, the quantity of salicylic acid that passed into the liquid in compartment B was measured. The results were plotted against time, and the membrane permeation constant was calculated from the slope of the plot.

Results and Discussion

Differences in Absorption from Various Bases

The blood concentration curves of salicylic acid after the administration of ointments prepared with water-soluble type, emulsion type (o/w and w/o type) and oleaginous bases are shown in Fig. 1. The results of calculation for each ointment are listed in Table II. There

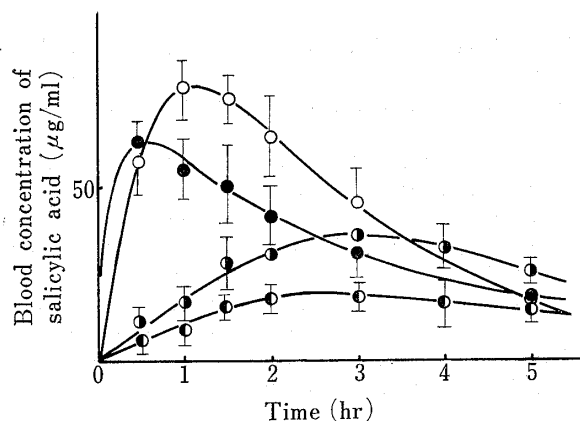


Fig. 1. Blood Concentration Curves of Salicylic Acid for Different Ointments

○ absorption ointment, ● hydrophilic ointment,
 ◐ macrogol ointment, ◑ white petrolatum.

Each symbol represents the mean of 5 determinations with different animals. Bars indicate the standard error.

was not a large difference in the time course of blood concentration of salicylic acid between the hydrophilic and the water absorbing ointments (both emulsion type bases), and the maximum blood concentration was attained in about 30—60 minutes for both. On the other hand, in water-soluble and oleaginous bases, it took about 3 hr to attain the maximum blood concentration.

The absorption rate constant of emulsion type base was larger than those of oleaginous and water-soluble bases, *i.e.*, the absorption of salicylic acid from emulsion type base is faster than from other bases. The differences of absorption from various bases were clarified by comparing values of AUC , $D \cdot f/V$ and X_a/V . If the distribution volume of salicylic acid V in the hamster is

constant, $D \cdot f/V$ and X_a/V are measures of the absorption of salicylic acid from the bases, since the quantity administered was constant.

The absorption from emulsion type base was particularly good, as might be expected, since the release of drug from the bases is accelerated by the surface active agent, resulting in an increase of the transport of drug to the surface of the mucous membrane, an enhancement of membrane permeability, and so on. The AUC of white petrolatum was as small as 1/2—1/3 compared with the other bases, as shown in Table II, indicating poor release of salicylic acid. The results obtained in this experiment for white petrolatum base correspond well with those already reported for rectal absorption.¹⁷⁾

When comparing the $D \cdot f/V$ and X_a/V values of various bases, $D \cdot f/V$ for Macrogol ointment showed a higher value than X_a/V , because AUC within five hours was used for the calculation of X_a/V .

15) J.G. Wagner and E. Nelson, *J. Pharm. Sci.*, **52**, 610 (1963).

16) J.G. Wagner and E. Nelson, *J. Pharm. Sci.*, **53**, 1392 (1964).

17) T. Takubo and S. Tsuchiya, and M. Miura, *Yakuzaigaku*, **31**, 292 (1971).

TABLE II. Various Constants Calculated from the Blood Concentration Curves and Physical Properties

	η_{app} (poise)	F (dyne/cm ²)	K (min ⁻¹)	K_a (hr ⁻¹)	K_{el} (hr ⁻¹)	AUC	X_a/V	$D \cdot f/V$
Absorption ointment	2.09×10^5	8.53×10^3	3.88×10^{-4}	2.92	0.37	235	102	106
Hydrophilic ointment	2.15×10^5	1.41×10^4	3.18×10^{-4}	5.13	0.36	182	81	79
Macrogol ointment	3.97×10^4	7.85×10^3	9.03×10^{-5}	0.36	0.30	145	74	87
White petrolatum	1.76×10^5	8.38×10^3	5.70×10^{-5}	0.56	0.33	70	34	38
No. 1	2.59×10^5	1.38×10^4	4.23×10^{-4}	1.87	0.32	336	141	147
No. 2	6.93×10^4	3.42×10^3	4.55×10^{-4}	1.66	0.37	459	216	208
No. 3	9.42×10^5	2.77×10^4	3.18×10^{-4}	1.93	0.30	254	106	105

η_{app} (apparent viscosity) and F (yield value) were obtained by the method of one of authors.¹⁹⁾

Retention of Salicylic Acid in the Cheek Pouch

The changes in the concentration of salicylic acid in each oral dosage form of ointment in the cheek pouch after administration are shown in Fig. 2. The decrease of salicylic acid combined with emulsion bases such as absorption ointment and hydrophilic ointment was large, while the decrease of salicylic acid combined with oleaginous base was small. Clearly the release of salicylic acid from emulsion bases was superior to that from oleaginous base.

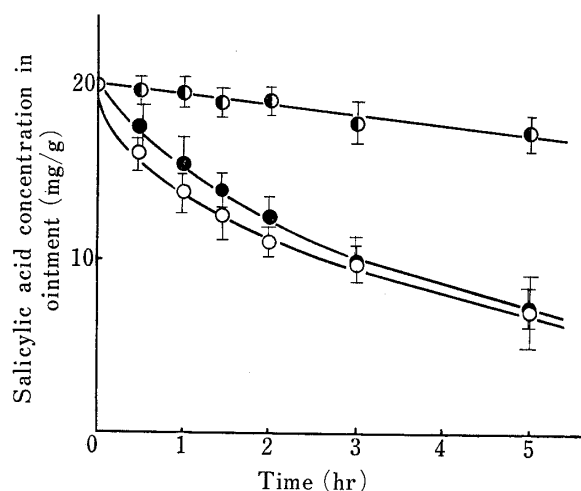


Fig. 2. Decreases of Salicylic Acid Content in Different Ointments after Administration

○ absorption ointment, ● hydrophilic ointment, ● white petrolatum.
Each symbol represents the mean of 3 determinations with different animals.

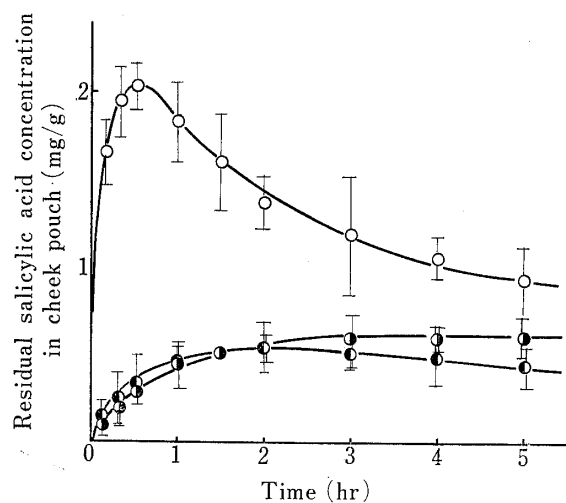


Fig. 3. Changes in the Concentration of Salicylic Acid in the Tissue of Hamster Cheek Pouch after Administration of Ointments

○ absorption ointment, ● macrogol ointment, ● white petrolatum.
Each symbol represents the mean of 5 determinations with different animals.

However, the losses of salicylic acid from the bases were larger than the total quantities found in the blood, and it can be presumed that some salicylic acid was retained in the tissue. As the mucous membrane of the oral cavity has a substantial lipid content, it was considered that salicylic acid released from an oral dosage form of ointment dissolved in the lipid of the mucous membrane of the oral cavity, and migrated to the blood through the mucous membrane. It is likely that same retention of salicylic acid occurs in the tissue of the cheek pouch.

Figure 3 shows the change in concentration of salicylic acid in the tissue of the cheek pouch after applying the emulsion, the water-soluble and the oleaginous base.

Salicylic acid was detected in the tissue of the cheek pouch at relatively high concentration. Furthermore, the quantities detected clearly differed according to the kind of base, being in the order emulsion, water-soluble and fatty oil base; this order is the same as that of the blood concentration curves. In particular, the quantity detected in the case of absorption ointment reached a maximum at 30 minutes, followed by gradual decrease. In view of these results, it seems clear that salicylic acid released from ointment was partly retained in the tissue of the cheek pouch, and that the retained salicylic acid was subsequently released into the blood.

In the case of w/o type emulsion base, *i.e.*, absorption ointment, the changes in blood and tissue concentrations when various concentrations of salicylic acid were used are shown in Figs. 4 and 5.

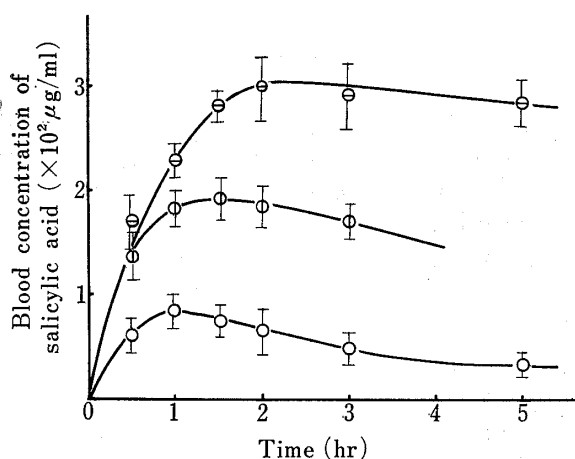


Fig. 4. Blood Concentration Curves of Salicylic Acid after Administration of Absorption Ointments containing Different Concentrations of Salicylic Acid

○ 2%, ⊕ 3% and ⊖ 5%.
Each symbol represents the mean of 5 determinations.

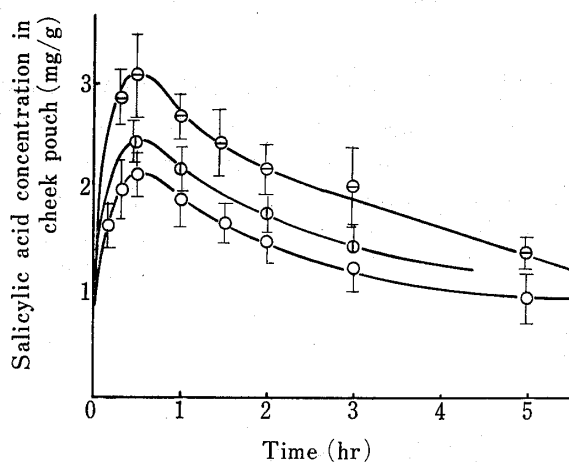


Fig. 5. Changes in the Concentration of Salicylic Acid in Hamster Cheek Pouch after Administration of Absorption Ointments containing Different Concentrations of Salicylic Acid

○ 2%, ⊕ 3% and ⊖ 5%.
Each symbol represents the mean of 5 determinations.

As is clear from the figures, the absorption of salicylic acid gradually increased, as the concentration of salicylic acid increased. Furthermore, the quantity retained in the tissue increased similarly. The curves in Fig. 4 and Fig. 5 are significantly different at the 1% and 5% levels, respectively. However, damage to the oral mucous membrane at higher salicylic acid concentration cannot be ruled out as an explanation for the results in Figs. 4 and 5.

It appears that the mucous membrane acts as a storage depot in the absorption of salicylic acid in the oral cavity, but further studies are required.

Physical Properties of the Bases and Relationship between *in Vitro* Membrane Permeation and Absorption through the Mucous Membrane of the Oral Cavity

Table II shows the membrane permeability constant (K) of each base measured using a Millipore filter membrane infused with olive oil,¹¹⁾ the physical properties of each base determined according to one of the authors¹⁸⁾ and the constants calculated from the blood concentration. Bases No. 1, 2, and 3 used here represent w/o type emulsion bases.

Since the *in vitro* membrane permeability constants K obtained by the author's previous method¹⁾ were in the order emulsion base, water-soluble base and fatty oil base, they correspond

18) M. Tanaka and J.M. deMan, *J. of Texture Studies*, 2, 306 (1971).

very well with the order of values of AUC , $D \cdot f/V$ and X_a/V calculated from the blood concentration. No relationship was observed between the physical properties of a base and various constants, but in the case of emulsion base, particularly w/o type base, as the apparent viscosity of the base increases the values of membrane permeability, AUC and X_a/V decrease. That is, when the viscosity of the base became high, the absorption of salicylic acid became poor. It is considered that this occurs because salicylic acid diffuses readily in a base with low viscosity, and is readily released, and equally its diffusion is retarded in a base with high viscosity. The absorption of salicylic acid at the mucous membrane of the oral cavity differed considerably according to the type of base, but it was also influenced by the physical properties. Furthermore, it was observed that the membrane permeability constant *in vitro* corresponded very well with various constants determined *in vivo*, particularly with AUC and X_a/V , expressing the drug absorption. Thus, membrane permeability constant determined *in vitro* appears to be available as a criterion of drug absorption at the mucous membrane.