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67. Hisashi Nogami, Jun Hasegawa, and Manabu Hanano : Studies on Ointments. I. A New Simplified Determination Method for Percutaneous Absorption of Ointments.

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As a method of evaluating ointment bases, the absorption of medicaments from ointments through the intact skin has been the subject of many investigations. The quantity absorbed is usally estimated by the measurement of concentration of the drugs in urine, blood, or some tissues, but these methods are indirect and occasionally useless. It takes much time for the collection of the urine and some portions of the medicaments are metabolised in animals or human being, so that the determination methods of drugs normally used for simple solutions are not suitable. Accurate quantity of medicaments absorbed cannot be obtained from the concentration of drugs in the blood, since it is impossible to know the whole volume of blood in the body. Therefore, only the relative changes of absorption can be learned from medicinal concentration in the blood. It is unusual to estimate the absorption from tissue concentrations. Some medicaments are rapidly metabolized by the action of enzymatic systems in the body. For example, by the oral administration of diphenhydramine in man, only about 3% is recovered in the original form from the urine. After all, these methods are not direct methods of determining absorption through the skin.

Wild[1] estimated the absorption of drugs through the intact skin from the weight of an ointment that remained on the skin. However, it seems that his experiments were not so accurate, since he did not consider secretion through the skin. Hediger[2] and Bürgi[3] determined the percutaneous absorption from the decrease of medicinal solution, which was in a glass bell fixed on animal skin. These are improved and direct methods of measuring absorption, but not easily adapted to humans. Furthermore, the structure of the animal skin is different from that of mankind, so the results obtained from these animal experiments are hard to apply to man. It is necessary to establish a direct method measuring percutaneous absorption that can easily be applied in human subjects.

The object of the present study is to propose a new method by which the percutaneous absorption is estimated from the decrease of drugs in the ointment that remained on the skin. Salicylate was used as the reagent, on which were many reports[4~6] on absorption through the skin. These were added to four kinds of ointment bases. The ointment was applied on the femoral region of a healthy adult male in the perfectly closed conditions. After a lapse of a definite period of time, the ointment was taken off and the decrease of salicylate was determined. Simultaneously, the urine was collected for 24 hours after the application of the ointment and the recovery of salicylate in the urine was compared with the decrease in the ointment. Previously, different results of determination of salicylic acid and its metabolized products in the urine were obtained by the same procedure without volume of urine tested. There-

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1) R. B. Wild : Brit. Med. J., **2**, 161(1911).
2) S. Hediger : Klin. Wochschr., **7**, 1553(1928).
3) E. Bürgi : Schweiz. Med. Wochschr., **18**, 433(1938).
4) F. Dacommum : Pharm. Acta Helv., **25**, 25(1950).
5) J. W. Brown, W. O. Scott : J. Pharmacol., **50**, 32(1954).
6) J. W. Brown, W. O. Scott : *Ibid.*, **50**, 373(1954).

fore, statistical examination was made on whether the above experience was correct or not.

From the results obtained, the present method was proved to give the absolute value of absorption through the intact skin by an easy procedure.

Experimental

Ointment—Four kinds of ointment bases used for this study were yellow petrolatum (J. P. VI), hydrophilic ointment (J. P. VI), simple ointment (J. P. VI), and absorption ointment. The composition of the latter two ointment bases are shown in Table I. Salicylic acid or sodium salicylate was added to each of the four ointment bases to make 5.00 $^{w}/_{w}$% exactly as salicylic acid and kept in containers.

Table I. Composition of the Ointments

Simple ointment		Absorption ointment	
White wax	33.0 g	White petrolatum	44.0 g
Olive oil	67.0 g	Stearyl alcohol	15.0 g
	100.0 g	*Emalgen #408	2.0 g
		**Emasol #310	2.0 g
		Water	q.s.
			100.0 g

* Emalgen #408 : An emulsifying agent. Polyoxyethylene lauryl ether. $C_{12}H_{25}O(CH_2OCH_2)_5CH_2OH$

** Emasol #310 : An emulsifying agent. Polyoxyethylene oleyl ether. $C_{18}H_{35}O(CH_2OCH_2)_7CH_2CH_2OH$

Application of Ointment— About 750 mg. each of the above eight ointments was spread on a polyethylene film to the same thickness, using a rubber frame, whose internal area was 2.5×2.5 cm. The area of polyethylene film was 4×4 cm. and its weight was exactly weighed previously, so that an accurate weight of the ointment could be obtained. These two pieces of the ointment were applied on both the femoral regions of four healthy adult male* and fastened with 8×8 cm. adhesive plaster, the center of which was covered with 6×6 cm. linen cloth. The tested man was urinated before applicantion of ointments and the urine was collected for 24 hrs. After a definite time, ointment was removed.

Duration of Application—The duration of the application of ointments was 0, 8, and 16 hrs. Zero hour represents the controls of the removal and the ointment was well pressed for 30 secs. after application. As shown in Table III, the removal of the ointment was not complete and residue was added to the quantity of the ointment absorbed.

Preparation of Testing Material—Adhesive plaster, linen cloth, and polyethylene film were removed from the skin, and ointment remaining on the skin was repeatedly wiped off with cotton, soaked in 1% solution of sodium laurylsulfate. These were extracted completely with warm 1% NaOH solution, the extracts were combined, and made to 500 cc. with distilled water.

Colorimetric Determination of Salicylic Acid—2 cc. of 36% HCl and 30 cc. of ethylene dichloride (EDC) were added to 5 cc. of the test solution in a glass-stoppered test tube, shaken vigorously for 10 mins., and centrifuged for 5 mins. at 3,000 r.p.m. Aqueous layer was taken off, 20 cc. of EDC was transferred to another test tube, 10 cc. of distilled water and 0.25 cc. of iron test reagent (1% $Fe(NO_3)_3$ in 0.075N HNO_3) were added, shaken for 5 mins., and centrifuged for 3 mins. Upper aqueous layer was transferred to a 2-cm. cell and optical density was read, using an electrophotometer with S-51 filter.

Calculation formula : $Y=0.987X-54$

where Y is the amount of salicylic acid in μg. and X is the optical density×1,000.

Results of Determination of Salicylates in Ointments—The determination of salicylates in eight ointments gave the results shown in Table II. Each figure represents a mean value of 4 determinations (recovery, in %).

Table II. Determination of Salicylate in Ointment

Ointment base	Simple	Absorption	Hydrophilic	Yellow
Salicylic acid	97.4	99.8	97.8	97.5
Sodium salicylate	97.6	100.6	101.0	97.9

* Four men about 25 years old were chosen from the members of this laboratoy, who were recongnized to have no dermatological change in the femoral regions.

Colorimetric Determination of Salicylic Acid in Urine[7]—10 or 20 cc. of the urine was transferred to a glass-stoppered test tube, 4 or 8 cc. of 36% HCl was added, and refluxed for about 6 hrs. on a sand bath. After cooling, 30 cc. of EDC was added, shaken vigorously for 10 mins., and centrifuged for 5 mins. Upper aq. layer was discarded, 20 cc. of EDC was transferred to another test tube, 10 cc. of distilled water and 0.25 cc. of the iron test reagent were added, shaken for 5 mins., and centrifuged for 3 mins. The aqueous layer was transferred to a 3-cm. cell and colorimetrically determined as described previously.

Caluculation formula : $Y=0.656X-23$

where Y is the amount of salicylic acid in μg. and X is the optical density × 1,000.

Salicylic acid was added to the urine of a healthy adult male and determined. The results agreed well with the salicylic acid added and the difference of results was not recongnized between 10 and 20 cc. of tested urine.

Determination of Final Area of Ointment on the Skin—The viscosity of the ointment generally fall at body temperature, so the area of the ointment applied on the skin was changed with decrease of viscosity, and conditions of the man tested. A glass plate was placed above the linen cloth at about 5 mm. high, on which the figure of ointment was drawn. Then this figure was transferred on parchment paper and cut off. The weight of the parchment paper was recorded.

Results and Discussions

Original data are shown in Table III.

Amount of Percutaneous Absorption

Tables IV and V give the analysis of variance of the determinations obtained by both recovery and urinary excretion methods. The original data, however, were transformed as follows for the assumption of the same variance.

$$Y=\log_{10}\{(10+y)-1\}\times 100$$

Table III. Original Data of Percutaneous Absorption (in mg.)

Time	Base	Man	I				II				III				IV			
			R.H.		U.E.M.		R.H.		U.E.M.		R.H.		U.E.M.		R.H.		U.E.M.	
				a)	10	20		a)	10	20		a)	10	20		a)	10	20
0 hr.	Sal.Acid	Simp.	2.4				1.7				2.1				2.5			
		Abs.	0.2				1.5				1.0				0.8			
		Hyd.	1.0				2.3				1.9				1.8			
		Yel.	4.4				2.4				2.9				2.3			
	Na Sal.	Simp.	2.0				1.7				1.4				2.0			
		Abs.	−1.0				−0.6				−0.3				0.3			
		Hyd.	−0.8				1.5				1.8				1.5			
		Yel.	1.5				1.5				1.0				1.6			
8 hrs.	Sal.Acid	Simp.	24.8	11	16.0	13.1	20.8	8	15.0	12.4	21.6	23	13.9	14.1	11.4	0	16.2	14.8
		Abs.	37.1	15	25.7	21.3	27.0	2	25.4	20.0	29.3	19	20.0	18.1	26.1	11	23.6	14.3
		Hyd.	18.9	15	21.0	18.1	22.2	0	23.4	16.5	31.5	15	23.8	19.9	8.8	16	7.8	7.4
		Yel.	22.8	20	16.1	15.5	20.8	2	12.9	12.4	23.2	15	7.6	7.4	21.6	2.6	10.0	9.8
	Na Sal.	Simp.	4.9	8	6.2	4.6	4.0	23	4.4	4.2	6.5	13	6.1	6.2	4.1	10	2.9	2.9
		Abs.	4.2	1.9	7.5	5.8	5.8	23	5.6	4.7	6.5	24	2.4	5.9	7.1	24	6.9	6.6
		Hyd.	4.5	13	4.1	3.5	4.8	13	3.5	2.9	3.0	10	3.0	1.8	2.9	8	2.6	2.2
		Yel.	4.2	26	5.1	4.3	6.0	31	5.4	5.2	4.9	23	4.4	4.7	6.1	26	4.8	5.0
16 hrs.	Sal.Acid	Simp.	40.0	16	37.5	35.7	29.2	6	27.2	23.6	30.5	13	28.9	20.7	32.5	15	23.0	27.9
		Abs.	43.3	28	51.8	51.5	39.2	26	45.4	43.5	32.9	15	29.0	31.1	34.7	15	31.0	23.3
		Hyd.	17.4	27	18.5	15.0	23.0	24	18.0	17.8	26.6	15	23.5	23.3	25.4	16	21.0	12.7
		Ynl.	32.8	27	33.7	31.2	30.5	24	20.2	13.7	39.2	30	23.7	23.3	30.1	30	28.8	29.0
	Na Sal.	Simp.	9.5	10	8.0	7.7	7.0	8	4.1	7.0	7.9	8	8.6	6.9	10.3	19	7.2	5.7
		Abs.	7.6	8	7.9	5.3	10.1	18	6.8	6.9	8.7	15	7.4	6.3	10.0	19	8.3	8.1
		Hyd.	6.7	22	2.7	4.5	15.0	2	12.8	12.2	6.8	11	4.1	4.1	7.5	15	3.0	2.3
		Yel.	8.9	32	8.4	7.1	4.1	15	2.6	2.2	7.0	40	7.1	6.4	7.0	40	7.1	6.9

R. H. : Recovery method. U. E. M. : Urinary excretion method.
a) : Area index : Weight of parchment paper in mg./100 mg.
Simp. : Simple ointment. Abs. : Absorption ointment.
Hyd. : Hydrophilic ointment. Yel : Yellow petrolatum.

7) J. B. Brodie, S. Udenfriend, A. F. Coburn : J. Pharmacol. Exptl. Therap., **80**, 114 (1944).

Table IV. Analysis of Variance Table of Absorption by Recovery Method

Factor	S. S.	d. f.	M. S.	[V]	F_0
Man	S_A= 121	3	40.3	$16\sigma_A^2+\sigma_E^2$	1.18
Treatment	S_B=21397	15	1426	$4\sigma_B^2+\sigma_E^2$	42.5**
Error	S_E= 1513	45	33.6	σ_E^2	
Total	S=23031	63			

Treatment : Duration of application and ointments.
Man : Man tested.
Standard error for treatments $\sqrt{34/2}=2.96$ (95% confidence limits)
$\pm 2.02\times 2.96=\pm 5.98$ (d. f.=45).
Δy for original data was calculated approximately as follows :

$$Y=f(y) \qquad \Delta y \fallingdotseq \Delta Y/f'(y) \qquad Y=\{\log_{10}(10+y)-1\}\times 100$$

$$\therefore\ \Delta y \fallingdotseq \pm\frac{(10+y)\times 5.98}{100\times 0.434} \qquad (0.434=\log_{10}e)$$

Table V. Analysis of Variance Table of Absorption by Urinary Excretion Method

Prim. F.	S. S.	d. f.	M. S.	[V]	F_0
Man (H)	S_H= 420	3	140	$2\times 16\sigma_H^2+2\sigma_V^2+\sigma_E^2$	1.31
Treat. (V)	S_V=35926	15	2395	$2\times 4\sigma_V^2+2\sigma_V^2+\sigma_E^2$	29.2**
Error (a)	S_{Ea}= 3507	45	82	$2\sigma_V^2+\sigma_E^2$	
Total (p)	S_p=40051	63			
Sec. F.					
Treat. (A)	S_A= 126	1	126	$4\times 16\sigma_A^2+\sigma_E^2$	9.62**
Int. VA	S_{VA}= 313	15	21	$4\sigma_{VA}^2+\sigma_E^2$	1.62
Error (b)	S_{Eb}= 624	48	13	σ_E^2	
Total (s)	S_s=1063	64			

where Y is the transformed value and y is the original deta in mg.

As seen in Tables IV and V, the treatment was highly significant and it might be concluded that there is a great difference between the duration of application and the kind of ointment used. Furthermore, as seen in Table V, the percutaneous absorption of salicylate was different from 10 cc. and 20 cc. of urine, being 14.0 and 12.6 mg. in a mean value, respectively, and highly statistically significant. The recovery of salicylic acid from the urine, however, agreed with the amount added as described previously, and such a difference was not recongnized. It is doubtful which was the accurate estimate of percutaneous absorption, but there was no way to compare these values with the absolute evaluation of absorption, 17.01 mg. It was thought that there was no room for doubt about the determination of urinarily excreted salicylic acid, since its determination procedure and excreted metabolite had beed reported in many literatures.[8,9] This difference described above cannot fully be explained with extraction

Table VI. Mean Values of Treatment

	period	0 hr.	8 hrs.		16 hrs.	
	Base	R. M.	R. M.	U. E. M.	R. M.	U. E. M.
Sal. Acid	Simp.	2.2±0.7	19.7±4.1	14.4±3.5	33.1±6.0	28.0±5.5
	Abs.	0.9±0.7	29.9±5.8	21.0±4.6	37.6±6.9	38.4±7.8
	Hyd.	1.8±0.7	20.4±4.1	10.2±3.0	23.1±4.6	18.7±4.1
	Yel.	3.0±0.7	22.1±4.4	11.5±3.2	33.2±6.0	25.9±5.8
Na Sal.	Simp.	1.8±0.7	4.9±2.1	4.7±2.1	8.7±2.5	6.3±2.3
	Abs.	−0.4±0.7	5.9±2.2	6.2±2.3	9.1±2.6	7.1±2.5
	Hyd.	1.0±0.7	3.8±2.0	3.0±1.8	8.8±2.5	5.7±2.3
	Yel.	1.4±0.7	5.4±2.1	4.7±2.1	6.8±2.3	5.9±2.3

R. M. : Recovery method. U. E. M. : Urinary excretion method.
±=95% confidence limits.

8) E. M. Kapp, A. F. Coburn : J. Biol. Chem., **145**, 549(1942).
9) Clutmak-Mann : Biochem. J. (London), **37**, 246(1943).

efficiency of EDC or inhibitors of color development. In this paper, a significant differerence was pointed out but further investigations were not carried out.

The relationship between absorptions due to the kind of ointment used and testing period determined from the recovery method are shown in Table VI and Fig. 1.

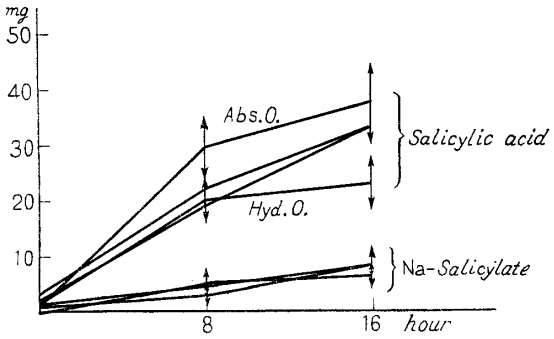

Fig. 1.
Effect of Treatment on Absorption through the Skin (from the recovery method)

Salicylate was absorbed from all the ointments used and absorption was affected by the following factors in that order : Kind of salicylate (free acid or sodium salt), duration of application, and ointment base. These results agreed with the lipid theory[10] and some other reports.[11]

Salicylic acid was absorbed far easier than its sodium salt. The absorption increased with the duration of application, but the behavior of hydrophilic ointment was different from other ointment bases. From these results, it may be concluded that the influence of this ointment was less than others and the difference between 8 and 16 hours was not very marked. These results would be caused by the change of emulsion states due to pH fluctuation.

Comparison of the Recovery Method with the Urinary Excretion Method

Fig. 2 presents a regression diagram, in which y_1 and y_2 represent the respective values from the recovery method and the urinary excretion method ($n=64$, every determination value was transformed). The regression formula was as follows :

$$y_1-38.9=1.074(y_2-33.8)$$

Both dotted lines show the 95% confidence limit of regression line. The coefficient of correlation was 0.9767 and highly significant ($n=64$).

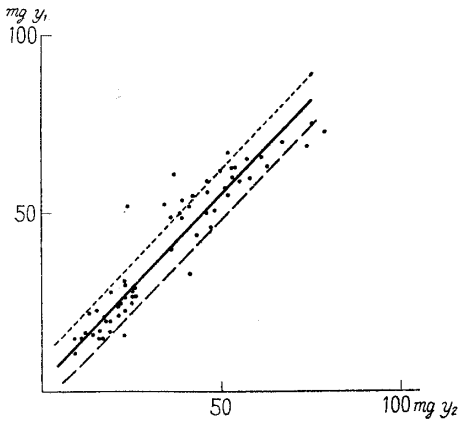

Fig. 2.
Regression Diagram of y_1 and y_2
y_1=Values from recovery method.
y_2=Values from urinary excretion method.

As shown in Fig. 2 and the regression formula, the total average of absorption estimated from recovery and urinary excretion methods was 38.9 (original data, 17.01 mg.) and 33.8 (13.71 mg.), respectively, giving smaller absorption by the urinary excre-

10) S. Rothman : J. Lab. Clin. Med., **28**, 1305(1943).
11) M. Nothmann, M. wolff : Klin. Wochschr., **12**, 302(1933).

tion method. y_1 and y_2 were highly correlated and regression coefficient was 1.074 which is almost nearly 1.00. The best estimate of the error of both determination methods are residual sum of the squares as shown in Tables IV and V. These values were 34 for recovery method, and 63 and 56 for the urinary excretion method determined from 10 and 20 cc. of urine. F-Values (ratio of variance) were $63/34=1.835$ and $56/34=1.647$, respectively, so that it may be concluded that the error of recovery method was smaller than that from the urinary excretion method, since F-values were significant at 5% level.

Influence of Applied Area

The area of the ointment applied was measured as described previously, but the coefficient between the applied area and percutaneous absorption was 0.2147 and compared with statistical tables, this value was not significant. For this reason, it was concluded that the influence of the area applied was negligible in this experiment.

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Summary

(1) The percutaneous absorption of salicylate was determined with simple procedure by the estimation of the amount of drugs remaining on the skin (recovery method).

(2) Absorption from each ointment determined by both recovery and urinary excretion methods was in parallel.

(3) The determination error of the recovery method was significantly smaller than that of the urinary excretion method.

(4) Salicylic acid was absorbed very well through the skin, but sodium salicylate was absorbed only silghtly and there was a great difference between the two.

(5) The influence of ointment bases was not remarkable, but when the ointment containing salicylic acid was applied, absorption from hydrophilic ointment was less than that from other ointment bases.

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