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Biopharmaceutical Studies on the Effect of Some Topical Vehicles on Human Skin[1)]

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The effects of some essential vehicles in cosmetics or topical preparations for transepidermal water loss (TWL) and skin surface lipids were investigated on human skin in relation to their physicochemical properties.

The test vehicles were isopropyl myristate (IPM), liquid paraffin 70cs (LPC70) and glycerol.

The results revealed newer biopharmaceutical aspects concerning the mode of vehicles against skin surface as follows.

1) The degree of occlusive effect for TWL in the closed experimental system was graded in the following order: glycerol>LPC70>IPM.

2) The removal effect for sebaceous squalene and epidermal cholesterol was found to be in the following order: IPM>LPC70>glycerol.

3) The occlusive effect of vehicles on TWL was suggested to be inversely related to the removal effect of vehicles for skin surface lipids.

4) Vehicle viscosity seemed to be one of the physicochemical regulating factors for occlusive nature of vehicles against TWL.

5) The *in vitro* water loss test seemed to be meaningful for a better understanding of the vehicle effect on TWL.

6) The importance of the removal effect on epidermal cholesterol that reflected TWL increase was suggested in the evaluation of vehicles against human skin surface barrier properties.

Keywords——vehicle effect; TWL; skin surface lipids; viscosity; *in vitro* water loss; TWL occlusion; lipid removal effect; epidermal cholesterol-barrier properties

There have been many investigations on the vehicle role in topical medicaments with respect to percutaneous absorption of drugs.[3)]

Cosmetological functions, such as protective, emollient and cleansing actions are mainly caused by the effect of the vehicle itself on the hydroregulatory and barrier system of the skin surface.[4)] In recent years, much attention has been focused on the effect of surfactants, penetration enhancers and oily vehicles *etc.* for the skin hydroregulatory system.[5)]

However, *in vivo* studies of vehicles directly on the human skin surface have not given any satisfactory basis on the optimum use and formulation of cosmetics and external preparations. In the previous papers,[6)] transepidermal water loss (TWL) and skin surface lipids

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3) *a*) B. Idson, *J. Pharm. Sci.*, **64**, 901 (1975); *b*) M. Katz and B.J. Poulsen, "Concepts in Biochemical pharmacology," ed. by, B.B. Brodie and J.R. Gillette, Springer-Verlag., New York, 1971, pp. 130—141.
4) J.S. Jellinek, "Formulation and Function of Cosmetics," Wiley-Interscience, New York, 1970, pp. 206—361.
5) *a*) K.J. Ryan and M. Mezei, *J. Pharm. Sci.*, **64**, 671 (1975); *b*) H. Oishi, Y. Ushio, K. Narahara, and M. Takehara, *Chem. Pharm. Bull.* (Tokyo), **24**, 1765 (1976); *c*) R.E. Dempski, J.D. Demarco, and A.D. Marcus, *J. Invest. Dermatol.*, **44**, 361 (1965); *d*) H. Baker, *ibid.*, **50**, 283 (1968); *e*) K. Okamoto and T. Kondo, *Yakuzaigaku*, **29**, 207 (1969); *f*) D.H. Powers and C. Fox, *J. Soc. Cosmet. Chem.*, **10**, 109 (1959); *g*) M.M. Rieger and D.E. Deem., *ibid.*, **25**, 253 (1974); *h*) F. Tranner and G. Berube, *Cosmetics and Toiletries*, **93**, March, 81 (1978).
6) *a*) T. Abe, *Jap. J. Derm.*, **86**, 815 (1976); *b*) T. Abe, *Chem. Pharm. Bull.* (Tokyo), **26**, 1659 (1978).

were shown to be two important parameters for the normal human skin surface barrier and hydroregulatory properties.

Therefore, the effects of typical cosmetological vehicles on these two parameters were studied in connection with their physicochemical properties.

This paper describes novel quantitative aspects concerning interrelations between some physicochemical properties of vehicles and vehicle effects on these two parameters. Also, the importance of the removal effect of the vehicles on epidermal cholesterol is discussed in the evaluation of vehicle effects on human skin surface barrier properties.

Experimental

Vehicles——Test vehicles were isopropyl myristate (IPM), liquid paraffin 70cs (LPC70) and glycerol. All vehicles used were of the grade described by the Japanese Standards of Cosmetic Ingredients.

***In Vitro* Experiments**——From the previous experiments,[6] human skin surface temperature was shown to be near 30°. Therefore, the following *in vitro* experiments were carried out at 30°.

Viscosities of vehicles were measured with a Brookfield type viscometer and specific gravities were measured with a hydrometer.

Using a water diffusion cell devised by the authors (Fig. 1), the *in vitro* water loss value through the vehicle layer was measured. Ten and 20 μl of the vehicles were applied to the paper (on an area 3.14 cm^2). Each cell was placed in a dessicator and the assembly was thermostated at 30°. The water loss value was measured gravimetrically.

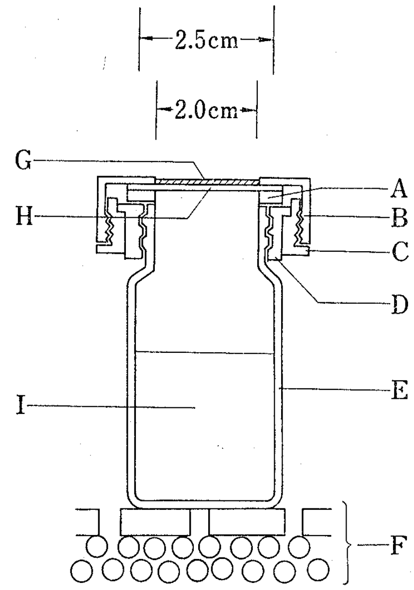

Fig. 1. Sectional Diagram of Water Diffusion Cell

A: packaging (gum),
B: paper holding ring (stainless, 凹),
C: paper holding ring (stainless, 凸),
D: buffer ring (plastic),
E: glass cell,
F: dessicator ($CaCl_2$),
G: vehicles,
H: paper (Ostrich tracing paper),
I: water (15 ml).
Each connection part was hermetically sealed with petrolatum.

***In Vivo* Experiments**——The mode of acute action of vehicles against TWL and skin surface lipids was investigated on the forearm skin of healthy adults.

First, TWL was measured on the right forearm skin (7 cm^2 in area). This TWL was defined as control TWL (TWLco) in this experiment. Simultaneousely, the skin surface temperature was measured with a thermister thermometer. Casual lipids were collected on the left forearm skin (7 cm^2 in area).

After this procedure, 20 μl of vehicle (2.9 μl/cm^2) was placed on the same site of the right forearm and TWL was measured simultaneousely. This TWL was defined as charged TWL (TWLch). One hour after application, the vehicle on the skin was removed by Toyo filter paper No. 6 and TWL was measured. This TWL was defined as discharged TWL (TWLdi). Skin surface lipids were collected and the lipid sample obtained was defined as residual lipids.

Replacement lipids were obtained 2 hr after the collection of casual lipids on the left forearm skin.

Subjects——Thirty four healthy adults aged 19 to 52 years were used in this study. They were divided into three groups corresponding to the three test vehicles.

TWL Measurements——TWL was measured by the electrohygrometric method previously reported.[6] TWL values were expressed as mg/cm^2/hr.

Determination of Squalene and Free and Total Cholesterols——The collection method of skin surface lipids was the same as previously reported.[6] Gas chromatographic quantitative analytical procedures for squalene and free and total cholesterols in previous publications[6] were used on the lipid samples obtained.

The retention times corresponding to the main peak of the tested vehicles were shown to be 0.36 min for IPM and 1.0 min for LPC70. In the case of glycerol, no peak appeared in a column of 5% silicone SE-30 on chromosorb G-DMCS. The retention time of squalene was 4.8 min and that of cholesterol was 9.3 min. Therefore, no overlapping hindrance by tested vehicles was found in the determination of squalene and cholesterols.

Environmental Conditions——*In vivo* experiments were carried out at 20—22° and 60—70% R.H.

Results and Discussion

Viscosity and Specific Gravity of Vehicles

The data at 30° are listed in Table I.

In Vitro Water Loss Value of Vehicles

The results are shown in Table I. Calculated vehicle thickness was 32 μm for 10 μl application (3.2 μl/cm²) and 64 μm for 20 μl application (6.4 μl/cm²). Vehicle weights calculated by the specific gravity are also shown in Table I.

However, in these application ranges, no marked difference in the resistance for *in vitro* diffusional water loss was observed. LPC70 and IPM suppressed water loss and glycerol prompted water loss when compared with controls.

These results are analogous to what other workers obtained by various *in vitro* experiments.[5b,c,g,h,)]

Table I. Physicochemical Properties of Vehicles

Vehicle	Viscosity (cp)	Specific gravity (g/ml)	Vehicle volume (μl/cm²)	Vehicle[a)] thickness (μm)	Vehicle[b)] weight (mg/cm²)	*In vitro* water loss value (mg/cm²)[c)] 8 hr after	24 hr after	48 hr after
IPM	5.2	0.844	3.2	32	2.7	15.0±0.2	46.6±0.2	78.2± 3.1
			6.4	64	5.4	16.0±0.3	48.0±1.6	83.4± 2.6
LPC70	15.9	0.828	3.2	32	2.6	12.4±0.5	34.8±3.1	74.4± 6.0
			6.4	64	5.3	13.2±0.6	37.1±3.1	75.5±11.6
Glycerol	432.5	1.255	3.2	32	4.0	23.3±1.0	66.3±1.6	95.5± 9.0
			6.4	64	8.0	19.7±0.8	53.8±4.2	96.7± 5.3
Control (paper only)						16.5±0.2	51.0±1.0	93.0± 3.0

Measurements were performed at 30°.
a) Vehicle thickness was calculated on the basis of the applied area (3.14 cm²) and applied volume (10 and 20 μl) of vehicle.
b) Vehicle weight was calculated on the basis of vehicle volume and specific gravity of vehicle.
c) Data are expressed as mean values and standard deviations of three measurements.

Changes in TWL Values after Vehicle Application

As shown in Table II, significant differences compared to control values were found in the following cases.

In the LPC70 applied group, the value of TWLch was significantly lower than that of TWLco ($p<0.02$). In the glycerol applied group, TWLch value was significantly lower than TWLco value ($p<0.001$).

In the relative comparison of the vehicle effect on TWL, the percentage ratios of TWLch/TWLco and TWLdi/TWLco are also shown in Table II.

Table II. TWL Values and Ratios after Vehicle Application

Vehicle	No. of subjects	TWL (mg/cm²/hr)[a)] TWLco	TWLch	TWLdi	Ratio (%)[b)] TWLch/TWLco	TWLdi/TWLco
IPM	12	0.208±0.026	0.221±0.030	0.215±0.040	106.3	103.4
LPC70	12	0.227±0.031	0.190±0.034[c)]	0.222±0.033	83.7	97.8
Glycerol	10	0.209±0.057	0.087±0.040[d)]	0.207±0.059	41.6	99.0

Average skin temperature was 30.5° in the IPM applied group, 30.9° in the LPC70 applied group and 30.8° in the glycerol applied group at the control site.
a) Data are expressed as mean values and standard deviations.
b) Percentage ratios were calculated on the basis of the mean values of TWLco, TWLch and TWLdi.
c) $p<0.02$; significantly different from control value.
d) $p<0.001$; significantly different from control value.

Changes in Skin Surface Lipid Values after Vehicle Application

The results are shown in Table III. Statistical comparison was carried out between each lipid component in casual lipids and that in residual lipids.

In the IPM applied group, the free cholesterol value of residual lipids was significantly less than that of casual lipids ($p<0.02$). In addition, the total cholesterol value was significantly lower in residual lipids than in casual lipids ($p<0.01$). In the LPC70 applied group, a significant decrease in free cholesterol value was observed ($p<0.02$).

TABLE III. Skin Surface Lipid Values after Vehicle Application

Vehicle	Casual lipids (μg/cm²)			Residual lipids (μg/cm²)			Replacement lipids (μg/cm²)[a]		
	Sq.	F.Ch.	T.Ch.	Sq.	F.Ch.	T.Ch.	Sq.	F.Ch.	T.Ch.
IPM (12)[b]	0.60± 0.38	1.62± 0.83	3.16± 1.96	0.35± 0.21	0.89± 0.47[c]	1.18± 0.61[d]	0.30± 0.52	0.49± 0.33	0.67± 0.47
LPC70 (12)	0.67± 0.59	1.44± 0.37	2.42± 1.80	0.45± 0.53	0.96± 0.45[e]	1.49± 0.57	0.44± 0.57	0.59± 0.41	0.70± 0.50
Glycerol (10)	0.50± 0.28	1.35± 0.42	1.95± 0.39	0.36± 0.30	1.14± 0.53	1.50± 0.80	0.32± 0.30	0.26± 0.24	0.53± 0.24

Data are expressed as mean values and standard deviations.
Sq.: squalene, F.Ch.: free cholesterol, T.Ch.: total cholesterol.
a) Replacement lipids for 2 hr.
b) Number of subjects.
c) $p<0.02$; significantly different from casual lipids.
d) $p<0.01$; significantly different from casual lipids.
e) $p<0.02$; significantly different from casual lipids.

To compare the relative vehicle effect on skin surface lipids, the removal ratios (%) were calculated on the basis of the mean amounts of lipid components in the residual, casual and replacement lipids listed in Table III. The results are shown in Table IV.

TABLE IV. Removal Ratios of Vehicles for Casual and Replacement Lipids

Vehicle	Ratio for casual lipids (%)[a]			Ratio for replacement lipids (%)[b]		
	Sq.	F.Ch.	T.Ch.	Sq.	F.Ch.	T.Ch.
IPM	41.7	45.1	62.7	83.3	149.0	295.5
LPC70	32.8	31.3	38.4	50.0	76.3	132.9
Glycerol	28.1	15.6	23.1	43.8	80.8	84.9

Sq.: squalene, F.Ch.: free cholesterol, T.Ch.: total cholesterol.
Removal ratio (%) was calculated on the basis of the mean values of lipid component in casual lipids (A), residual lipids (B) and replacement lipids (C) in Table III.
a) Removal ratio (%) was calculated by the following formula: $(A-B)/(A)\times 100$.
b) Removal ratio (%) was calculated by the following formula: $(A-B)/(C)\times 100$.

The Effect of Vehicles for TWL

The TWLch/TWLco ratio is generally used as an indication of vehicle occlusivity for TWL during a period of its application.[5b,e,f] From the clinical experiments on xerotic skin, Kligman confirmed that the vehicle occlusivity is one of the important factors of emollient or moisturizing effects.[7]

As shown in Table II, glycerol and LPC70 were superior to IPM in the occlusive effect. This result in the closed experimental system is in fairly good agreement with the earlier reports by Okamoto *et al.*[5e] in the diffusion system. Thus, the degree of occlusivity *in vivo* was graded in the following order: glycerol>LPC70>IPM.

7) A.M. Kligman, *Cosmetics and Toiletries*, 93, April, 27 (1978).

On the other hand, the TWLdi/TWLco ratio is prefarably employed as an indication of barrier suppression of the *stratum corneum* induced by vehicles.[5a,b,d] The TWLdi/TWLco and TWLch/TWLco ratios above 100 were found only in the case of IPM application (Table II). Thus, the degree and duration of barrier suppression by IPM was found to be greater than that by LPC70 and glycerol. However, the effect of IPM on the *stratum corneum* of the human skin does not seem to be as intense as in the case of rat skin where the TWLdi/TWLco ratio has been shown to be 266.[5b] This must be an evidence for the difference of barrier efficacy between human *stratum corneum* and rat *stratum corneum*.

The Relationship between the Effect of Vehicles for TWL and Their Physicochemical Properties

As illustrated in Fig. 2, the TWLch/TWLco ratio of vehicles was shown to be inversely related to the logarythmic viscosity of vehicles. Thus, the vehicle viscosity seemed to be one of the physicochemical regulating factors for the vehicle occlusivity against TWL.

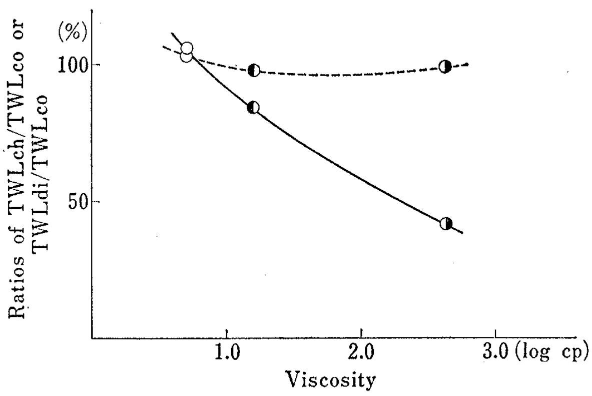

Fig. 2. Relationship between Viscosity of Vehicles and Ratios of TWLch/TWLco or TWLdi/TWLco of Vehicles

IPM: ○, LPC70: ◐, glycerol: ◑.
TWLch/TWLco: ——, TWLdi/TWLco: ----.

On the other hand, the occlusive order of vehicles on TWL did not completely agree with that for *in vitro* water loss: LPC70> IPM>glycerol as shown in Table I. The stimulation of water loss *in vitro* by glycerol seems to indicate that glycerol has a drawing action on water from the deeper layer of the skin to the skin surface.[5f,g,h] The observed intense occlusion *in vivo* by glycerol is assumed to be due to the given properties and its ability to hold water in contact with the skin in the non-diffusional experimental system.

Thus, *in vitro* water loss test seems to be useful for a better understanding of the vehicle effect on TWL.

The Effect of Vehicles on Skin Surface Lipids

It is generally accepted that squalene is regarded as a marker of sebaceous lipids, while cholesterol as a marker of epidermal lipids.[6,8,9]

Table IV indicates that IPM removed an equivalent of 62.7% of the total cholesterol in casual lipids. Thus, the removal effect for epidermal cholesterol was found to be in the following order: IPM>LPC70>glycerol. Similarly, the order of the removal effect for sebaceous squalene of casual lipids was as follows: IPM>LPC70>glycerol.

Replacement lipids are regarded as the recovery lipids of the skin itself for 2 hr especially in this experiment. It is noteworthy that IPM removed about 3 times the epidermal cholesterol and 83.3% of sebaceous squalene in replacement lipids. LPC70 removed about one half of the sebaceous squalene and 1.3 times the epidermal cholesterol corresponding to the replacement lipids. Glycerol showed the lowest removal effect on both epidermal cholesterol and sebaceous squalene.

Furthermore, it was characteristic that the removal effect of the vehicles was higher for epidermal cholesterol than for sebaceous squalene. As mentioned above, the present findings provide a new quantitative aspect of vehicle effects on human skin surface lipids.

8) R.E. Kellum, *Arch. Derm.*, **95**, 218 (1967).
9) N. Nicolaides, "Epidermis," ed. by, W. Montagna and W.C. Lobitz, Jr., Academic Press, New York, 1964, pp. 511—538.

These observations may have important implications with respect to the formulations of cleansing or protecting preparations.

The Relationship between the Effect of Vehicles on Skin Surface Lipids and Their Physicochemical Properties

The above interesting vehicle effects on skin surface lipids were considered in connection with their physicochemical properties.

As shown in Figs. 3 and 4, the removal ratio of vehicles for epidermal cholesterol was shown to be inversely related to the logarythmic viscosity of vehicles both in casual and replacement lipids. However, the relation between the removal ratio of vehicles for squalene and vehicle viscosities were not as remarkable as in the case of cholesterols.

Thus, vehicle viscosity was assumed to be one of the physicochemical regulating factors for the removal effect of vehicles for skin surface lipids.

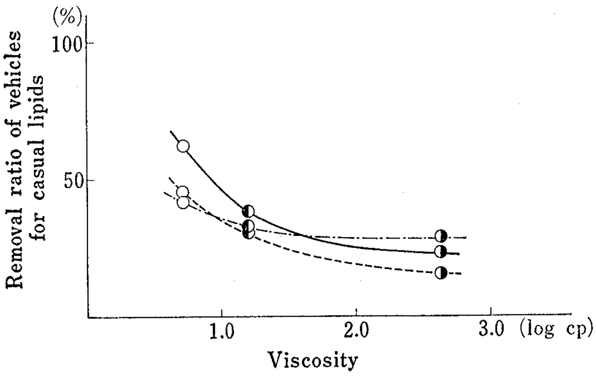

Fig. 3. Relationship between Viscosity of Vehicles and Removal Ratio of Vehicles for Casual Lipids

IPM: ○, LPC70: ◐, glycerol: ◑.
Squalene: —·—, free cholesterol: ----,
total cholesterol: ——.

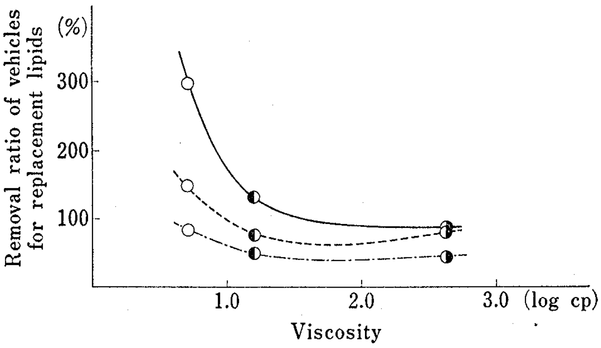

Fig. 4. Relationship between Viscosity of Vehicles and Removal Ratio of Vehicles for Replacement Lipids

IPM: ○, LPC70: ◐, glycerol: ◑.
Squalene: —·—, free cholesterol: ----,
total cholesterol: ——.

The Relationship between the Effect of Vehicles on TWL and on Skin Surface Lipids

As mentioned above, the order of occlusivity of vehicles *in vivo* was shown to be inversely related to that of removal effect of vehicles on skin surface lipids.

The increase of TWL, *i.e.* the lowering of barrier properties of the *stratum corneum* by IPM was found to be especially related to its removal effect for epidermal cholesterol, one of the membrane lipids of the *stratum corneum*.[9] From these results, it is suggested that IPM has a possibility of altering the interrelationship of water and lipids regulating the epidermal water barrier functions. These results support the previous findings of epidermal cholesterol as a barrier for TWL in normal adult and child skin.[6]

Since cosmetological preparations are used frequently, the barrier depression in a single application is assumed to be linked to undesirable repeated effects. Thus, the solvent effect is suggested to be one important factor in the evaluation of vehicles against human skin surface barrier properties. In conclusion, the present findings indicate newer quantitative aspects concerning the mode of the three important cosmetological vehicles against human skin surface. The present experimental system is considered to be useful in further biopharmaceutical studies of various vehicles.

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