Chem. Pharm. Bull. 26(6)1659—1665(1978)

UDC 612.014.1 : 547.922.03

Studies on Skin Surface Barrier Functions. Transepidermal Water Loss and Skin Surface Lipids during Childhood[1]

Takashi Abe

Cosmetic Laboratory, Kanebo Ltd.[2]

(Received September 1, 1977)

Since there are few reports dealing with the barrier efficiency of normal skin during childhood, *in vivo* experiments were carried out on 40 healthy Japanese children of both sexes aged between 3 to 12 years.

Two important parameters for barrier properties: transepidermal water loss (TWL) value and the skin surface lipid value, were measured by the original methods devised during the course of our studies. Statistical analysis was employed to understand interrelations between those two parameters.

The results revealed the following characteristic barrier properties of child skin surface. 1) Inverse correlations were found between TWL values and total lipid values in the forearm skin. Analogous correlations were also observed between TWL values and cholesterol values. However, there were no correlations between TWL values and squalene values. 2) The amount of cholesterol was significantly higher than the amount of squalene. 3) No statistical significance with respect to age, sex or weight of the children and the observed TWL, total lipid, squalene or cholesterol values was found in this study. 4) TWL measurements suggested that the barrier efficiency of the *stratum corneum* of child skin is nearly the same as that for adult skin as previously reported. 5) It was confirmed that the amount of total lipids or squalene is significantly smaller and the cholesterol amount slightly higher in children than in adults.

Keywords——skin surface barrier functions; normal child skin; transepidermal water loss; skin surface lipids; squalene; cholesterol; barrier efficiency; lipid pattern; barrier role of epidermal lipids

One of the most important functions of the human skin is to act as a two-way barrier, preventing the loss of water, electrolytes and other body constituents and barring noxious or unwanted molecules from the external environment.[3] This barrier function has an obvious relevance to the study of percutaneous absorption, protection against chemical poisons and the practical problems of cosmetics or topical preparations.[4-6]

Although there have been many reports concerning barrier properties of normal adult skin,[7] no comparable reports have been found on barrier efficiency of normal child skin. Recently, it has become essential to investigate the barrier integrity reflected by ages during

1) This paper was presented at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1977.

2) Location: *3-28, Kotobuki-5-chome, Odawara, Kanagawa.* Part of this work was carried out at the Department of Dermatology, Tokai University, *Bohseidai, Isehara, Kanagawa.*

3) H. Baker, "Textbook of Dermatology," 2nd ed., ed. by A. Rook, Blackwell Scientific Publications, Oxford, 1972, pp. 249—255.

4) R.T. Tregear, "Physical Functions of Skin," Academic Press, London, 1966, pp. 1—52.

5) 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. 117—174.

6) I.H. Blank and R.J. Scheuplein, *Br. J. Derm.*, **81**, (Supplement 4), 4 (1969).

7) *a*) H. Baker and A.M. Kligman, *Arch. Derm.*, **96**, 441 (1967); *b*) K. Grice, H. Sattar, M. Sharrat, and H. Baker, *J. Invest. Derm.*, **57**, 108 (1971); *c*) K. Grice, H. Sattar, and H. Baker *ibid.*, **58**, 343 (1972).

childhood for the optimum use of topical preparations in the cosmetological[8] and pharmaceutical[9] domains.

Therefore, the *in vivo* barrier properties were investigated using two different parameters, transepidermal water loss (TWL) and skin surface lipid values on the forearm skin of 40 healthy children. The TWL values were measured as indicators of barrier integrity of the horny layer. Total lipid, squalene and cholesterol values of the skin surface were measured for the discussion of barrier functions of skin surface lipids against TWL.

This paper deals with the relationships between total lipid, epidermal cholesterol and TWL values in the subjects whose sebaceous gland activities were proved to be lower than those of adults.

Experimental

Subjects——The 40 healthy child subjects were of both sexes (14 females and 26 males) ranging in ages from 3 to 12 years and weights from 13.4 to 44.5 kg.

Measurement Site——Skin surface lipids were collected on the ventral side of the right forearm and TWL was measured on the other side.

Measurement of TWL——TWL was measured by resistance hygrometry using the methods described previously.[10] The apparatus was essentially composed of a hygrometer unit, recorder and measuring glass cell devised by the authors.[10] The hygrometer sensor and coupled thermister thermometer were installed in the cell. Measurements were performed with our new equipment on the forearm, by attaching the open side of the cell tightly to the skin surface.

The ambient temperature and relative humidity in the environmental chamber were maintained at 22—23° and 50—60% respectively. The skin surface temperature was measured with a thermister thermometer.

Under these experimental conditions, TWL was calculated according to the following formula:

$$\text{TWL (mg/cm}^2\text{/hr)} = \frac{(\text{RH10–RHi}) \times D^{\text{T}}\,(\text{mg/l}) \times V\,(\text{l}) \times 6\,(\text{hr})}{100 \times S\,(\text{cm}^2)}$$

where RHi is the initial relative humidity (30±3%), RH10 is the relative humidity after 10 min of measurement, D^{T} is the density of saturated vapor at the air temperature in the cell, V is the volume of the cell (0.035 l) and S is the measured area (7 cm²).

Measurement of Total Lipids——Casual lipids were extracted three times with acetone (total volume of acetone: 15 ml, extraction time: 5 min) by the cup method[10,11] (7 cm² in area). Replacement lipids for 2 hr were obtained by the same method at the collection site of casual lipids. The solutions of skin surface lipids thus obtained were filtered through a glass filter and then concentrated under reduced pressure. The oily residue was transferred to a weighing bottle and dried under a vacuum to a constant weight. The dried substance was measured gravimetrically and this value was regarded as the amount of total lipids.

Quantitative Analysis of Squalene and Free and Total Cholesterols——Squalene (a marker of sebaceous lipids) and cholesterol (a marker of epidermal lipids) were both analyzed by gas–liquid chromatography (GLC).[10] Tetratriacontane ($C_{34}H_{70}$) was used as the internal standard.

At first, squalene and free cholesterol were analyzed by a sample prepared by dissolving the dried residue in 0.5 ml of hexane containing 0.01% (w/v) $C_{34}H_{70}$. After the hexane solution was evaporated to dryness under reduced pressure, the residue was hydrolyzed under reflux with 2 ml of 1 N ethanolic KOH solution for 1.5 hr.

The resulting solution was diluted with 2 ml of water and then taken up with 0.5 ml of hexane to obtain a lipid component. Analogous analysis by GLC of the hexane solution gave total free cholesterol expressed as total cholesterol.

Each analytical value was obtained by the calibration curves of the peak-height ratio against relative concentration of squalene and cholesterol.

GLC conditions were as follows: instrument used: Hitachi GC-163; column: a 1 m×3 mm stainless column packed with 5% silicone SE-30 on chromosorb G-DMCS; temperatures: column oven (255°), injection

8) *a*) M.S. Ehrensberger, *Cosmetics and Perfumery*, **88**, 43 December (1973); *b*) R. Masuda, *Fragrance Journal*, **5**, No. 2, 84 (1977); *c*) U.D. Hoppe, H.J. Kopplow, and H.S. Kreysel, *J. Soc. Cosmet. Chem.*, **28**, 103 (1977); *d*) A.S. Ramons and B.F. Bower, *J. Am. Med. Assoc.*, **207**, 368 (1969).

9) *a*) R. Konishi, *Yakkyoku.*, **22**, 271 (1971); *b*) S. Tomisaka, *ibid.*, **24**, 1 (1973); *c*) K. Dietel, "Die Haut und ihre Erkrankungen im Kindesalter," VEB Georg Thieme., Leipzig, 1976, pp. 38—44.

10) T. Abe, *Jap. J. Derm.*, **86**, 815 (1976).

11) M. Ohkido, H. Hanaoka, and I. Matsuo, *Jap. J. Clin. Dermat.*, **25**, 535 (1971).

port (290°) and detector (290°); detection: FID (H_2; 1.2 kg/cm², air; 1.0 kg/cm²); and carrier gas; N_2 (64 ml/min). Retention times were as follows: squalene (4.8 min), cholesterol (9.3 min) and $C_{34}H_{70}$ (20.6 min).

Results

TWL Values for Child Skin

The mean value and standard deviation of TWL for child forearm skin was found to be 0.228±0.034 mg/cm²/hr. Child TWL values ranged from 0.142 to 0.284 mg/cm²/hr. There was no significant difference between the TWL value of females (0.234±0.026 mg/cm²/hr) and males (0.225±0.037 mg/cm²/hr) as shown in Table I.

No correlations between weight ($p>0.10$) or age ($p>0.10$) of the child and TWL values were found in the ranges studied.

Total Lipid Values for Child Skin

The replacement lipid value (25.1±9.4 μg/cm²) for 2 hr was about one half of the casual lipid value (46.0±16.4 μg/cm²). No significant difference was observed between females and males both in casual and replacement lipid values as shown in Table I. There were no correlations between weight ($p>0.10$) or age ($p>0.10$) of the child and lipid values.

Table I. Mean Values and Standard Deviations of TWL and Casual and Replacement Lipids

	Total[a] ($n=40$)	Male ($n=26$)	Female ($n=14$)
Casual lipids : Total lipids (μg/cm²)	46.0±16.4[b]	45.4±17.6	46.3±15.3
Replacement lipids[c] : Total lipids (μg/cm²)	25.1±9.4	24.8±10.3	25.8±7.5
TWL (mg/cm²/hr)	0.228±0.034	0.225±0.037	0.234±0.026

n : number of subjects.
room conditions : temperature-22—23° and relative humidity- 50—60%.
average skin temperature : 30.5°.
a) range of ages: 3—12 years, mean±S.D. of ages: 6.6±2.4 years.
range of body weights : 13.4—44.5 kg, mean±S.D. of body weights : 22.9±7.8 kg.
b) mean±S.D.
c) replacement lipids for 2 hr.

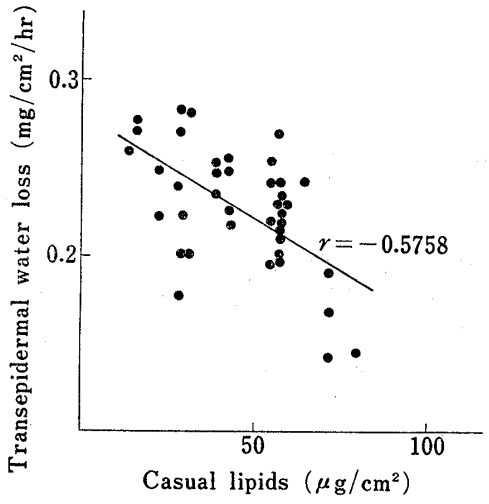

Fig. 1. Relationship between Casual Lipids and Transepidermal Water Loss

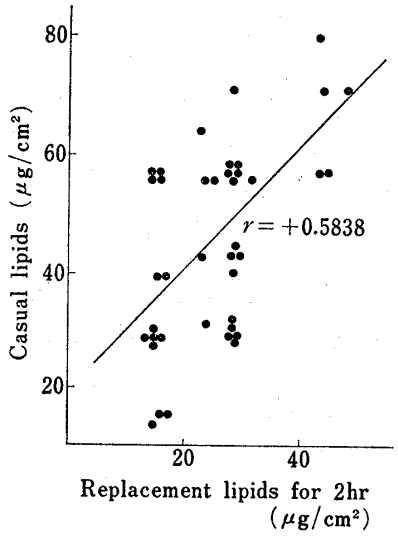

Fig. 2. Relationship between Casual and Replacement Lipids

The Relationship between Total Lipids and TWL

The relationship between casual lipids (x) and TWL (y) was considered on the basis of each value of the individual subjects. In a statistical analysis, an inverse correlation was found in this case. The regression equation was $y=-0.0012x+0.2820$ (r: -0.5758, $p<0.01$) as shown in Fig. 1. On the other hand, there was no appreciable correlation between replacement lipids and TWL ($p>0.10$).

The Relationship between Casual and Replacement Lipids

To evaluate the significance of the amount of replacement lipids, the relationship between replacement lipids (x) and casual lipids (y) was examined statistically. As shown in Fig. 2, one regression line was obtained. The regression equation was $y=1.0119x+20.3331$ (r: $+0.5838$, $p<0.01$).

Squalene, Free and Total Cholesterol Values of Child Skin

The results are shown in Table II. The cholesterol amount was much higher than that of squalene in both casual and replacement lipids. The quantitative ratio of free cholesterol to total cholesterol was found to be about 7/10 in both casual and replacement lipids. There was no significant difference between females and males for each lipid component. Furthermore, no significant correlations were observed between the amount of each lipid component and age ($p>0.10$) or weight ($p>0.10$) in this experiment.

Table II. Mean Values and Standard Deviations of Squalene, Free and Total Cholesterol in Casual and Replacement Lipids

Casual lipids	Total[a] ($n=40$)	Male ($n=26$)	Female ($n=14$)
Squalene ($\mu g/cm^2$)	0.31 ± 0.25[b]	0.30 ± 0.25	0.29 ± 0.09
Free cholesterol ($\mu g/cm^2$)	2.13 ± 1.38	2.14 ± 1.52	2.10 ± 1.04
Total cholesterol ($\mu g/cm^2$)	2.84 ± 1.74	2.83 ± 1.75	2.93 ± 1.76

Replacement lipids[c]	Total ($n=40$)	Male ($n=26$)	Female ($n=14$)
Squalene ($\mu g/cm^2$)	0.030 ± 0.041	0.032 ± 0.047	0.030 ± 0.018
Free cholesterol ($\mu g/cm^2$)	0.46 ± 0.33	0.38 ± 0.23	0.61 ± 0.41
Total cholesterol ($\mu g/cm^2$)	0.63 ± 0.46	0.59 ± 0.47	0.70 ± 0.43

n : number of subjects.
room conditions : temperature-22—23° and relative humidity- 50—60%.
average skin temperature: 30.5°.
TWL : 0.228 ± 0.034 mg/cm²/hr.
a) range of ages : 3—12 years, mean ± S.D. of ages: 6.6 ± 2.4 years.
range of body weights : 13.4—44.5 kg, mean ± S.D. of body weights: 22.9 ± 7.8 kg.
b) mean ± S.D.
c) replacement lipids for 2hr.

The Relationship between Each Lipid Component and TWL

To understand interrelations between skin surface lipids and TWL in more detail, a statistical analysis between each lipid value (x) and TWL value (y) was performed.

Inverse correlations were found in three cases. The regression equations were as follows: free cholesterol of casual lipids (x)-TWL (y); $y=-0.0105x+0.2501$ (r: -0.4311, $p<0.01$), total cholesterol of casual lipids (x)-TWL (y); $y=-0.0070x+0.2478$ (r:-0.3658, $p<0.02$) and total cholesterol of replacement lipids (x)-TWL (y); $y=-0.0312x+0.2470$ (r:-0.4160, $p<0.01$). Figures 3, 4 and 5 show these results clearly.

No appreciable correlation was observed between TWL and squalene of casual and replacement lipids and free cholesterol of replacement lipids.

The Relationship between Squalene and Casual Total Lipids

A correlation was observed between squalene (x) and total lipids (y) indicating a regression equation: $y=29.86x+36.84$ ($r:+0.4465$, $p<0.01$) (see Fig. 6).

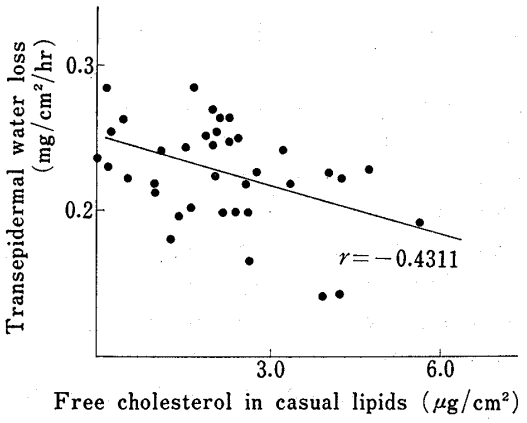

Fig. 3. Relationship between Free Cholesterol in Casual Lipids and Transepidermal Water Loss

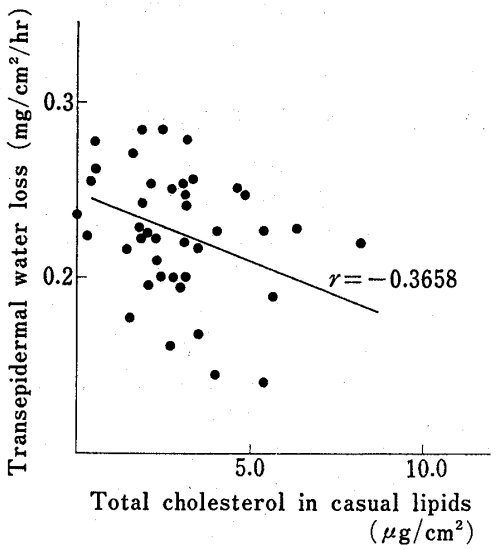

Fig. 4. Relationship between Total Cholesterol in Casual Lipids and Transepidermal Water Loss

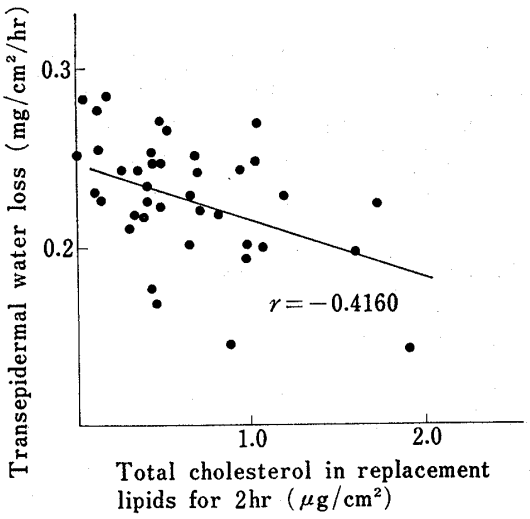

Fig. 5. Relationship between Total Cholesterol in Replacement Lipids and Transepidermal Water Loss

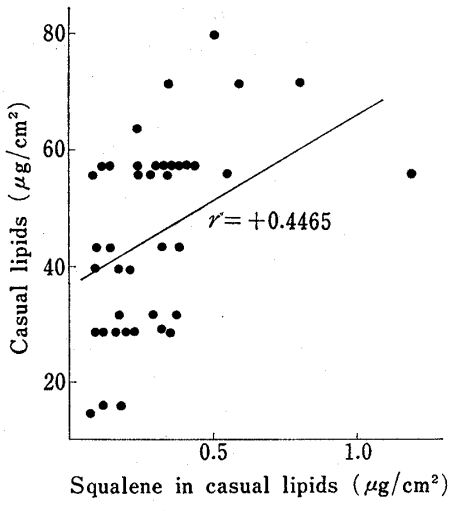

Fig. 6. Relationship between Squalene and Total Lipids in Casual Lipids

Discussion

Barrier Efficiency of The Horny Layer of Child Skin

It is generally accepted that the measurement of outward trans-epidermal water diffusion provides an useful parameter of the barrier functions of the *stratum corneum*.[7a,12] From

12) *a*) H. Baker, *J. Invest. Derm.*, **50**, 283 (1968); *b*) K.J. Ryan and M. Mezei, *J. Pharm. Sci.*, **64**, 671 (1975); *c*) H. Oishi, Y. Ushio, N. Narahara, and M. Takehara, *Chem. Pharm. Bull.* (Tokyo), **24**, 1765 (1976).

this concept, Wildnauer[13] *et al.* suggested that some morphological or physiological differences exist between newborn (12 to 234 hr) and adult epidermis which influenced TWL.

Interestingly, the present TWL data for child forearm skin (0.228 ± 0.034 mg/cm^2/hr) are almost equal to the data of comparable adult forearm skin (0.238 ± 0.022 mg/cm^2/hr) as shown in our previous reports.[10] Thus, the *stratum corneum* of child skin seems to have nearly the same efficient physical barrier as that of adult skin.

It is also notable that no marked difference between sexes was observed.

Skin Surface Lipids of Child Skin

It is well known that skin surface lipids originate from sebaceous glands and epidermis.[14] The sebaceous glands biochemically synthesize squalene, which is excreted on the skin surface without decomposition.[15] Squalene is regarded as a marker of sebaceous lipids.[10,16] On the other hand, cholesterol and phospholipids are the membrane components of the cellular or subcellular units of the epidermis.[17] These lipid components are not contained in sebaceous lipids and can therefore be used as markers for epidermal lipids.[10,16a,18]

The present results reveal that the amount of cholesterol is significantly higher than in the squalene of the child forearm skin. These observations are in good agreement with the earlier reports by Boughton *et al.*[19] In addition, it is also noteworthy that the free cholesterol content is much higher than the esterified cholesterol content in this experiment.

Comparison of the present results in children with the previous results[10] in adults is of interest. The amount of casual lipids in children ($n=40$; 46.0 ± 16.4 μg/cm^2) is significantly smaller than that in adults ($n=40$; 60.1 ± 14.6 μg/cm^2) ($p<0.01$). Furthermore, the amount of squalene ($n=40$; 0.31 ± 0.25 μg/cm^2) shows a significantly lower value than that for adults ($n=21$; 0.74 ± 0.61 μg/cm^2) ($p<0.01$). It is also remarkable that the amount of total cholesterol in child skin ($n=40$; 2.84 ± 1.74 μg/cm^2) is almost equal to that of adult skin ($n=18$; 2.44 ± 1.44 μg/cm^2) ($p>0.10$).

From these results, the following conclusions can be drawn. In child forearm skin, sebaceous lipids are present in smaller and variable amounts, while epidermal lipids are present in relatively constant and larger amounts. Therefore, total lipids are the sum of the epidermal and sebaceous lipids, and the small amount of sebaceous lipids reflects the decline of the amount of total lipids in child skin. The reason for this is that the secretory activity of sebaceous glands in child skin is immature.

These tendencies may have some relation to vulnerability to the dissolving of skin surface lipids by topical preparations such as soap and cleansing cosmetics.

The Barrier Role of Skin Surface Lipids for TWL in Child Skin

Figure 1 indicates that TWL becomes smaller with an increase in the amount of casual lipids (and *vice versa*) on the normal skin surface. Similar interrelations between TWL and free or total cholesterol values are also remarkable as shown in Fig. 3—5. On the other hand, the lack of correlation between squalene and TWL values may reflect some other biological significance of sebaceous lipids.

These findings clearly demonstrate that the barrier action of skin surface lipids for outward transepidermal water diffusion may depend on the relative constancy of the amount of epi-

13) R.H. Wildnauer and R. Kennedy, *J. Invest. Derm.*, **54**, 483 (1970).
14) M. Ohkido, *Metabolism and Disease*, **11**, 1023 (1974).
15) R.E. Kellum, *Arch. Derm.*, **95**, 218 (1967).
16) *a*) M. Ohkido, I. Matsuo, and T. Abe, "Biology and Disease of the Hair," ed. by T. Kobori and W. Montagna, University of Tokyo Press, Tokyo, 1975, pp. 575—579; *b*) H. Hanaoka, M. Ohkido, Y. Hattori, T. Murata, and T. Arai, *Jap. J. Derm.*, **81**, 259 (1971).
17) N. Nicolaides, "Epidermis," ed. by, W. Montagna and W.C. Lobitz, Jr., Academic Press, New York, 1964, pp. 511—538.
18) M. Ohkido, K. Suzuki, I. Sugihara, and N. Mizuno, *Acta. dermatovenerol.*, **54**, 223 (1974).
19) B. Boughton, R.M.B. Mackenna, V.R. Wheatley and A. Wormall, *Biochem. J.*, **61**, 32 (1957).

dermal lipids during childhood. It is also noteworthy that sebaceous glands lipids do not seem to contribute to these barrier properties. In spite of different quantitative lipid patterns during childhood, the importance of epidermal cholesterol in the prevention of water loss was proved to be essentially unchanged in the case of adult skin.[10]

In conclusion, our studies revealed that epidermal cholesterol, one of the membrane lipids of the *stratum corneum*, also takes part in barrier functions against transepidermal water loss in the living human skin surface.

These barrier properties of the human skin surface may have important implications with respect to the following studies: the effect of vehicles in topical preparations for the skin, the influence of U.V. irradiation on skin, influence of ambient temperature and humidity on the state of the skin surface, *etc.*

Acknowledgement The author wishes to thank Dr. M. Ohkido, Professor of Dermatology, Tokai University School of Medicine, for his kind revision of this paper and staff members of our laboratory for their technical assistance.