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Measurement *in Vivo* of Transepidermal Moisture Loss

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Synopsis—A technique is described for evaluating the effect of a cosmetic product on the rate of TRANSEPIDERMAL MOISTURE LOSS in human subjects. The thickness of product necessary to reduce the rate to zero is calculated and defined as the OCCLUSIVE THICKNESS, a significant and inherent property of the product, independent of human subject variation.

Introduction

Loss of water from the skin is an important physiological function. Blank (1) has stated that the water content of the stratum corneum is probably the prime factor in determining its softness and flexibility, making the measurement of the effect of a cosmetic product on the moisture loss rate important to the cosmetic chemist.

A large number of methods has been developed to measure the moisture loss *in vivo*. Noninstrumental methods such as the desiccator method of Powers and Fox (2) and the cold trapping of vapor from a stream of gas passing over the surface of the skin have been used with a limited degree of success. Thiele and Schutter (3) have described an instrumental approach utilizing a salt crystal method. Later, these same authors presented a critical review concerning an improved salt crystal method and electrolytic methods (4). Electrolytic methods were also

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used by Spruit and Malten (5, 6). Spruit (7) also applied a thermal conductivity cell to *in vivo* measurements. Baker and Kligman (8) used electrohygrometry to make moisture loss measurements.

This paper is mainly concerned with a description of our method for isolating and measuring *in vivo* the transepidermal diffusion loss.

Experimental and Results

Description of Apparatus

Figure 1 shows a general flow diagram of the apparatus which is used to measure moisture loss. Prepurified compressed air having a dew point of −59.5°C is utilized as a carrier gas. Suitable pressure reduction equipment is used to reduce the pressure to 5 psig. Stainless steel tubing (⅛ in. o.d.) and Swagelok®* fittings were used to connect all units. The flow of the gas was split into two streams and the flow rate in each stream was adjusted by a NUPRO®† fine metering valve. Each stream then flows through a 4 × 4 cm Sage Instrument Probe‡ where the moisture is swept from the surface of the skin into the gas stream. The stream passes through the sensing chamber of a Cambridge Systems Model 990 Thermoelectric Dew Point Hygrometer§ where the amount of moisture present is measured utilizing the dew point principle. The gas exits the device through a flow meter which allows the operator to detect abnormalities in the gas flow.

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† Nupro Co., Cleveland, Ohio.
‡ Sage Instruments, Inc., White Plains, N. Y.
§ Cambridge Systems, Inc., Newton, Mass.

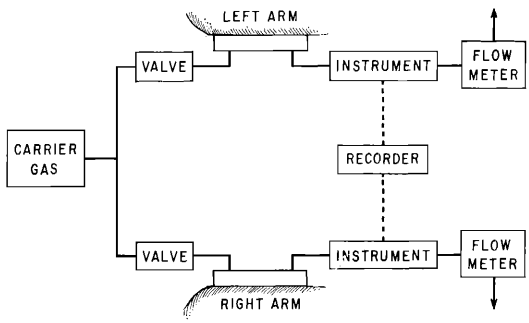

Figure 1. Schematic for apparatus used to make *in vivo* moisture measurements

Each dew point instrument is attached to one side of a two-pen recorder. An electronic calibration unit is also attached to equalize the electrical response of the two instruments.

The range of moisture levels which may be measured is determined by the flow rate. A rate of 50 ml/min allows measurements of 0 to 10 mg/cm²/hr with a 50% scale reading of 0.3 mg/cm²/hr.

Standardization of Apparatus

The Sage Instrument Probe is replaced with a gas chromatographic type injector port. Standardization is accomplished by leaking water at several different rates into the gas stream utilizing a radiometer automatic syringe microburet fitted with a 2-μl gas chromatographic syringe. Standard response graphs are plotted and used to interpret the instrumental response.

Environmental Precautions

Water measured by the apparatus in *in vivo* experiments could come from three sources. These sources are listed in Table I along with the orders of magnitude of each effect. Eccrine sweating is the largest effect. It is discontinuous and varies greatly in amount. Baker and Kligman (8) utilized pharmacologically induced anhydrosis. Not being so equipped we have attempted to eliminate eccrine sweating by maintaining the thermal and emotional environment below threshold values. Subjects are conditioned to a 19.4–20.0°C room for 20 min and then tested at this temperature. Testing is conducted in an isolated area. While not absolutely guaranteeing anhydrosis, eccrine sweating is controlled out of our measurement within practical limits.

Table I

Basic Mechanisms by which Water Is Lost through Skin

Mechanism	Rate (mg/cm²/hr)	Duration
Eccrine sweating	32–48	Discontinuous
Transepidermal diffusion	0.2–0.3	Continuous
Stratum corneum desorption of normal hydrated skin by a stream of dry gas	0.6–0.7	10–15 min

Stratum Corneum Desorption

During exposure of the surface of normal skin to the dehydrating influence of a stream of dry air, some stratum corneum desorption of the

normally hydrated skin takes place. This effect is superimposed on the measurement of the rate of transepidermal moisture loss. An experiment in which the normal hydration level was raised slightly by soaking the arm in water for 5 min prior to making the measurement was carried out to find out how long it would take to bring the two different arms to the same level of dehydration. The results, shown in Fig. 2, coincided with our finding that about 10 to 15 min were required to reach equilibrium when dry gas is used on the normally hydrated arm. To prevent stratum corneum desorption from causing an error in the rate of transepidermal diffusion loss, we wait 15 min after putting the sampling cells into place before determining the response.

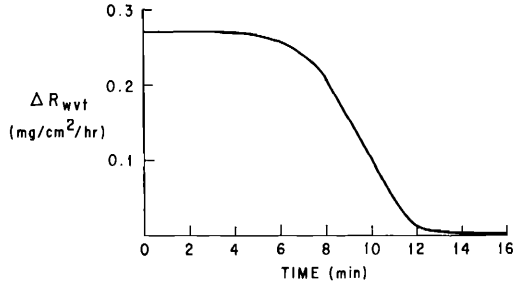

Figure 2. Desorption of hydrated stratum corneum. Difference in moisture loss rate (ΔR_{wvt}) between an experimentally hydrated and a normal arm is shown as a function of time

Influence of Age, Sex, Height, and Weight

A brief survey was made of 14 subjects from 19 to 48 years of age, both sexes, and all types of complexions and builds. The results (Table II) ranged from 0.2 to 0.46 mg/cm²/hr with a mean of 0.31 and a standard deviation of ±0.04 mg/cm²/hr. No correlation with age or physical characteristics was noted in this study.

Sample Thickness Determination

In order to test products for their effect on the rate of transepidermal moisture loss, one must know the thickness or weight per unit area placed on the subject's skin. A 6 × 6 cm area of the medial forearm is marked off. From 100 to 300 mg of sample are placed onto a flat stainless steel spatula and weighed. The sample is then evenly dispersed over the 36 cm² with even strokes. Excess sample remains on the spatula which is reweighed. Knowing the weight (W) in grams and the area (A) in cm²,

Table II
Transepidermal Diffusion Loss Rates for Several Subjects[a]

Subject	Age	Sex	Height (inches)	Weight (lb)	Transepidermal Diffusion Loss (mg/cm²/hr)
1	19	F	64	115	0.31 ± 0.04
2	21	F	66	122	0.20 ± 0.04
3	23	F	62	100	0.28 ± 0.04
4	23	F	63	118	0.34 ± 0.04
5	23	F	67	145	0.25 ± 0.04
6	24	F	65	135	0.21 ± 0.04
7	24	F	66	142	0.46 ± 0.02
8	27	M	74	172	0.28 ± 0.04
9	30	M	66	165	0.22 ± 0.04
10	31	M	71	170	0.39 ± 0.06
11	37	F	59	95	0.26 ± 0.04
12	45	M	75	195	0.37 ± 0.04
13	46	M	72	178	0.24 ± 0.04
14	48	F	62	150	0.45 ± 0.07

[a] Results were obtained on the medial untreated forearm.

the weight per unit area may be calculated. Knowing the specific gravity (sp gr) at skin temperature, one can calculate the film thickness (d) in millimeters according to eq 1.

$$d = \frac{10\,W}{\text{sp gr} \times A} \tag{1}$$

Occlusive Thickness

In the past, examination of a product for its effect on the transepidermal diffusion loss rate has been to determine the rate before use (B) and the rate after use (C), and to calculate the per cent reduction ($100\,n$) according to eq 2.

$$100\,n = \frac{B - C}{B} \times 100 \tag{2}$$

The average per cent reduction is then presented as a characteristic of the product. In our work it is necessary to use volunteer subjects all of whom have different base rates. For the same thickness of film widely different per cent reductions were obtained requiring use of the same subject for comparing products as well as requiring reproducible sample application. For this reason, an experimental approach was desired which would measure a property of the product independent of human subject variables.

The fractions reduced (n) for five thicknesses of white petrolatum (normally marketed product) were determined on four different subjects. The results are shown in Fig. 3 for two of these subjects. Figure 3 shows a plot of thickness *vs.* fraction reduced. These data can be linearized by replotting on log-log paper. When this is done, Fig. 4 results. If the lines are extrapolated to the $1/n = 1$ intercept, it can be observed that they intersect. The thickness of the film at which $1/n = 1$ is the occlusive thickness, represented as (S). It has as its dimension millimeters and represents the theoretical thickness of products necessary to reduce the rate of transepidermal moisture loss to zero.

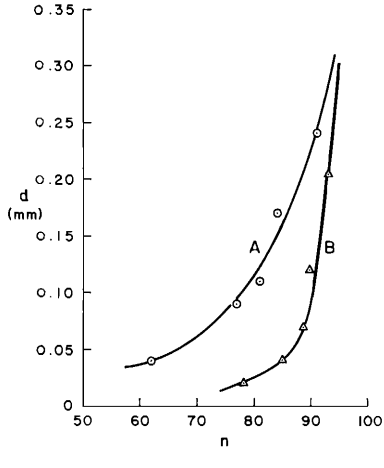

Figure 3. The fraction reduction (n) of transepidermal moisture loss for 2 subjects is shown as a function of the thickness (d) of a layer of white petrolatum applied to the surface of the skin

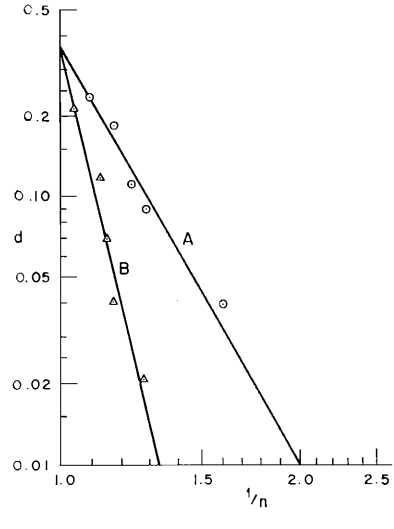

Figure 4. The thickness (d) in mm of white petrolatum is shown as a function of the reciprocal of the fraction reduced ($1/n$) on a log-log scale

Table III shows the results obtained for the occlusive thickness and the per cent reduction of the transepidermal moisture loss by a 0.1-mm film of white petrolatum on each of four subjects.

If the results are calculated as the per cent reduction of transepidermal moisture loss achieved by the application of a uniform film of product, the results vary widely from subject to subject because the subjects have different base rates. By contrast, when occlusive thickness is calculated, essentially the same result is obtained from each subject, despite the variation in base rate.

Table III
Results obtained for Occlusive Thickness and Per Cent Reduction of White Petrolatum

Subject	Subject Base Rate ($mg/cm^2/hr$)	Reduction (%) by 0.1-mm Film of White Petrolatum	Occlusive Thickness (mm) of White Petrolatum
A	0.39	77.5	0.38
B	0.24	90.2	0.38
C	0.32	83.0	0.40
D	0.19	93.0	0.39

Table IV
Occlusive Thickness of White Petrolatums of Different Oil Contents

Sample	Oil Content (%)	Occlusive Thickness (mm)
A	52.8	2.31
B	57.0	1.35
C	61.7	0.74
D	66.1	0.39
E	70.4	0.22
F	74.6	0.13

Occlusive Thickness and Oil Content of White Petrolatum

The occlusive thickness technique was applied to a comparison of white petrolatum samples of different oil content to determine if varying the oil content would give products of different occlusivity. Six white petrolatums of different mineral oil content were prepared as shown in Table IV. All of these samples meet the standards of the USP monograph for white petrolatum with the exception of sample A which had a consistency slightly harder than the minimum penetration specified. Four thicknesses for each sample were examined on each of three subjects. The reciprocal of the fraction reduced ($1/n$) was calculated and the occlusive thickness (S) was determined for each sample. The results are shown in Table IV and Fig. 5. The occlusive thickness decreases logarithmically with an increase in mineral oil content.

Conclusions

Occlusivity, an important property of cosmetic products used on the skin, is evaluated *in vivo* by a new, reproducible approach which eliminates subject-to-subject and experiment-to-experiment variables. Char-

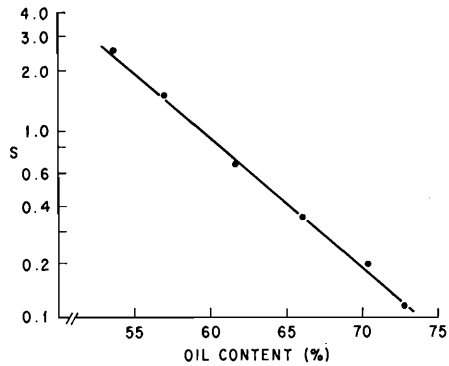

Figure 5. The occlusive thickness (S) of white petrolatums of different oil contents is shown as a function of the oil content in a semilog plot

acterizing a product by calculating an "occlusive thickness" permits comparison of the effect of products on transepidermal moisture loss.

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