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ACKNOWLEDGMENTS AND ADDRESSES

Received February 27, 1974, from the *College of Pharmacy, University of Kentucky, Lexington, KY 40506*
Accepted for publication October 9, 1974.

Presented to the APhA Academy of Pharmaceutical Sciences, San Diego meeting, November 1973.

The authors thank Mrs. G. H. Tan, Mr. R. E. Johnson, and Dr. W. T. Lipscomb for their technical assistance, Dr. Waugh and Dr. McCutcheon of the Department of Physiology for the use of equipment, and Mr. Dan Chilko of the West Virginia University Computer Center for help with the computer program.

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In Vivo Method for Monitoring Polysorbate 85 Effect on Epidermal Permeability

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Abstract □ An *in vivo* method of monitoring the rate of water desorption from human forearms, using "dry" nitrogen gas passed over approximately 1 cm^2 of skin was investigated with the aid of a commercial electrolytic moisture analyzer. The assembled apparatus was used to evaluate the differences in water loss rates from treated and untreated (control) forearms following surfactant application. The changes in the differences were also monitored after cessation of treatment, *i.e.*, during the healing process. The apparatus provided an accurate, rapid, and painless method of monitoring relative water loss rates and, as such, could prove a useful tool in routine testing in experimental dermatology and cosmetology. The results confirm the earlier finding from an *in vitro* method with excised rabbit skin that the tested surfactant increases the permeability of the epidermis.

Keyphrases □ Permeability, epidermal—*in vivo* method for monitoring effect of polysorbate 85, relative water loss rates □ Epidermis—permeability, *in vivo* method for monitoring effect of polysorbate 85, treated and untreated human epidermis □ Polysorbate 85—effect on epidermal permeability, *in vivo* method for determining relative water loss rates □ Water loss rates—human epidermis, *in vivo* method

An *in vitro* method (1) proved satisfactory for quantitating the water content and rate of water desorption from 1.0-cm^2 samples of excised rabbit skin. The method could be applied to human skin; however, the excision of the skin sample, although virtually painless, is not readily accepted by most human subjects. In addition, an *in vitro* method can monitor the desorption of only a finite amount of water and may be, at best, unpredictably extrapolatable to the *in situ* conditions where the supply of water is essentially inexhaustible.

It is well known (2–8) that surfactants generally increase the permeability of the skin, which can easily be studied by measuring the rate of the transepidermal water loss. Accurate quantitative *in vivo* measurements from human forearms have been reported (9–13). The actual water loss under defined conditions has been determined for areas as small as 0.1 mm^2, although at least 1 mm^2 should be the minimum area considered "a representative sample for forearm skin generally" (12). These quantitative methods required considerable effort to minimize the instrumental baseline. The purpose of this study was to design a relative method that requires less stringent ambient and instrumental manipulation and, therefore, is more practical and rapid.

EXPERIMENTAL

An electrolytic moisture analyzer[1] provided the nucleus for the assembled apparatus. The "heart" of the instrument is a horizontal glass "cell" containing two platinum wires across which there is a potential difference of 75 v dc. The medium between these wires is phosphorus pentoxide, which is converted into phosphoric acid following the introduction of water into the cell by the carrier gas. Upon completion of the circuit, the water is electrolyzed and the phosphorus pentoxide is regenerated. There is a direct relationship between the amount of water in the gas and the current used. The instrument is so designed that there is a direct readout on the instrument meter in parts per million of water for a specific gas flow. The signal to the meter is connected to a recorder[2], so a continuous graph of moisture content *versus* time is obtained.

The carrier gas, ultra high purity nitrogen (containing less than 3 ppm of water), was passed *via* Teflon tubing (1 mm i.d., 4 mm o.d.) into a sampling "cup" sealed against the skin of the forearm by 400 g weight. The "dry" gas inlet was so positioned as to direct the stream of gas onto the skin surface, where it readily picked up the surface water; the "wet" gas exited from the cup *via* Teflon tubing into the instrument. The sampling cup was turned down from a 2-cm diameter brass rod, 3 cm in length. It was plastic coated to decrease porosity and heat transfer. The apparatus was in operation for several hours prior to each day's use to remove moisture that had permeated the apparatus, so that a constant baseline could be achieved.

As a test of the reproducibility of the apparatus, readings were taken on symmetrical sites on 20 subjects' left and right forearms. Prior to their participation in the study, the subjects' arms were visually inspected to ensure that no dry skin condition or other dermatological irregularities existed. The subjects were university

[1] MEECO, model W, type SPR, Manufacturers Engineering & Equipment Corp., Warrington, PA 18976
[2] Coleman 165.

Table I—Tests for the Reproducibility of Readings

Subject (Sex)	Variation[a], %
E.K. (f)	0
O.M. (f)	0
S.R. (f)	0
K.R. (m)	0
C.P. (m)	0
J.I. (f)	0
T.N. (f)	0
D.F. (m)	3.3
S.M. (f)	3.6
S.L. (m)	3.6
K.J. (m)	3.8
D.K. (m)	3.8
E.F. (f)	3.9
K.D. (f)	5.0
F.M. (m)	5.6
L.R. (m)	5.9
M.M. (m)	6.6
J.W. (f)	6.9
C.N. (f)	8.3
S.L. (f)	9.0

[a] The transepidermal water loss was measured from symmetrical sites on untreated left and right forearms of each subject, and the difference in readings is expressed as percent variation.

staff and students ranging in age from 19 to 45 years, with the majority being in the early 20's (12 females and eight males). Dense hair on the forearms of one male subject was clipped with a hair clipper[3] 1 day prior to commencement of readings.

Of the initial 20 subjects used to establish the limits of reproducibility, six (five females and one male) continued on to the evaluation of surfactant application effects. They applied the ointment base (petrolatum USP) to one forearm for the control, and the other forearm was treated with the same base containing 10% polysorbate 85[4].

The preparations were applied liberally (~0.5 g) twice daily and massaged into forearms for 10 sec. Readings were taken (prior to and during the treatment) on treated and then control forearms (order randomly varied), and the difference between the readings was expressed as a percentage of the control value. For this reason, no absolute units had to be calculated since the results were expressed as percentage difference in rates of water loss between the two sites.

One hour prior to readings, the subjects' forearms were gently washed with mild soap and warm water and patted with a tissue to remove excess water. The bare forearms were air dried to the laboratory environment while the subjects relaxed.

RESULTS AND DISCUSSION

At the commencement of the readings, the initial pen response prescribed a peak, the height of which varied as did the amount of water in the stratum corneum (which varies as does ambient temperature, humidity, physical and mental activity of the subject, blood volume, breathing rate, the ingestion of certain drugs, *etc.*). During the next 8–10 min, the peak declined to a line parallel to the baseline. This straight portion of the graph shows a constant rate of transepidermal water loss and, as such, is an indication of the "barrier function" of the epidermis.

Thirty-five percent of the readings performed on the left and right untreated forearms were identical to each other; *i.e.*, the difference in final constant rates of water loss rates was 0%. The remainder of the subjects' readings did not vary from each other by more than 9% (Table I). Thus, the limit of reproducibility of the assembled apparatus (due to environmentally, instrumentally, physiologically, and psychologically mediated variations during the time required to perform a pair of readings) was established.

When readings were performed on treated and control forearms, in every case there was a substantial increase in the rate of transepidermal water loss from the surfactant-treated arm with respect to the control arm (Table II). There was a definite correlation between the duration of treatment and an increased rate of transepidermal water loss. In half of the cases, 9 days of treatment resulted in a difference in the rate of water loss of around 100%.

Table II—Effect of Surfactant Treatment on Rate of Transepidermal Water Loss

	Subject					
	K.R.	S.R.	J.I.	K.D.	C.N.	R.S.
Day	Increase of Transepidermal Water Loss, %					
0	0	0	0	0	8.3	0
1				5.0		
2						12
3	19		10		13	30
4		66	26			
5	33					72
6				54	53	
7	52		64			
8						
9	93	100	75			100

It is readily evident that it would be impossible to reproduce consistently all of the variables present during the first hours, much less days, of readings. However, with the method's built-in controls, and by reading the control and treated forearms (order randomly varied) as close together in time as possible, the differences in readings should be due almost entirely to surfactant application.

All readings were performed in an isolated room with a relatively constant temperature of about 20° (peaks of sweat may appear on the graph above 25°). Slight fluctuations in the room's humidity and temperature during the 0.5 hr or so required for the two readings constituting a set of data did not have any noticeable effect on readings.

One subject was treated with surfactant in (ointment) base on the left arm and with base on the right arm for 9 days. The surfactant induced an increase in transepidermal water loss such that the difference in rates was 100% by the 9th day, whereupon treatment was ceased (Fig. 1). Sixteen days after cessation of treatment, while the difference was still about 12%, treatment was reinstated in a crossover fashion; *i.e.*, the control arm now became the treated arm. Since the new control arm still had a greater transepidermal water loss at the time of crossover, initially the difference in readings showed a negative value. After 3 days, the effects of the surfactant became so evident that the difference became positive and continued to increase at a greater rate than it had in phase one of the experiment (prior to crossover). Within 9 days of the crossover, the difference in rates again approached 100% and treatment was ceased. Differences again diminished, rapidly at first and then at a declining rate. By monitoring the difference in water loss rate for up to 6 weeks after cessation of treatment (and having the subject wash arms daily with mild soap and water), the declining differences in readings indicated the recovery of barrier function, which is due to the healing process.

The rate of the regeneration process is very high in the early stages. Figure 1 indicates that a 50% regain of the barrier function occurred in less than 4 days after cessation of treatment (after cessation of continuous injury), while the next 50% reparation (resulting in 0% difference in rates) required another 3–4 weeks. The time required for total regeneration of the barrier function (14) after cessation of surfactant application indicates that the full barrier function returns only with the presentation of new stratum corneum cells (unaffected by surfactant) to the surface of the epidermis.

The kinetic microhygrometric technique employed does not simulate normal environmental conditions of the epidermis. However, the method is so designed that the effectiveness of the treated forearm's epidermis in preventing excessive water loss under specific conditions can be evaluated with respect to control epidermis under the same conditions. Since the portion of the values due to sweating could be kept virtually constant by maintaining fairly constant ambient conditions (especially temperature) and psychic state, it was not deemed necessary to introduce any type of drug into the body to inhibit sweating.

To prevent the escape of carrier gas and the entrance of ambient

[3] Oster model A2-22, blade ANG-RA.
[4] Tween 85, Atlas Chemical Industries, Wilmington, Del.

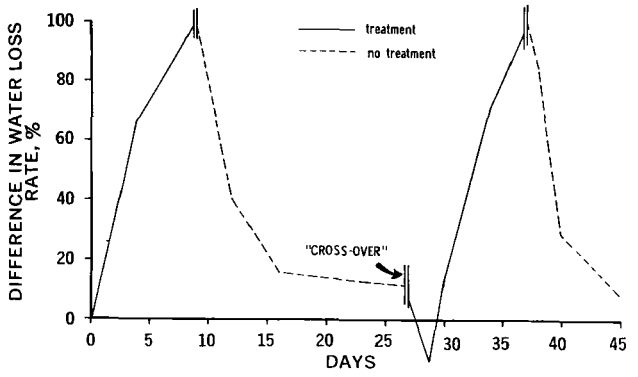

Figure 1—*Effects of surfactant treatment as measured by the percent difference in water loss rate between treated and untreated skin* versus *time. Details concerning the crossover period are given in the text.*

moisture, the cup was pressed to the skin with a weight of 400 g; this weight was determined experimentally. On untreated skin, a weight of about 200 g would be sufficient to seal the rim of the cup against normal skin. However, after several days of treatment with surfactant, the skin surface became more irregular and some 400 g was required to effect a seal, undoubtedly due to "a type of crust formation, shown microscopically to consist of necrotic and sloughing epidermis" (15).

CONCLUSION

The present study and other reports (1–8) indicate that surfactants can affect epidermal permeability. Today, with increasing exposure to epidermal insult from physical, chemical, and energy sources, the regenerating process of the epidermis not infrequently approaches overextension. Insidiously, even some of the preparations designed to improve the quality or appearance of the skin usually contain chemicals (to improve the formulation's appearance, stability, or penetration) that may actually further damage the tissue. There is, therefore, a real need for a convenient, rapid method for monitoring changes in the barrier function of the epidermis, resulting from dermatological and cosmetic preparations.

Paradoxically, damage to the barrier will cause increased water loss through the skin and the result may be "dry skin"; *i.e.*, the water content of the stratum corneum is decreased. This increased water loss through the skin will occur slightly following the application of substances that cause the removal of surface lipids (16). Substances that also have a destructive action on the barrier structure will effect a dramatic rise in the skin permeability.

Blank (17) showed that water is the only plasticizer of the stratum corneum. Accurate measurements of the actual water content of the stratum corneum, however, would provide no specific data because the water content of the stratum corneum varies (nonlinearly) with ambient humidity (11) and temperature, as well as with many other physiological and psychological conditions. A quantitative figure for the water binding capacity would be of great value in the assessment of the effects of topical applications with respect to the presence (or absence) of the yet to be determined "critical amount" of the stratum corneum's plasticizer.

The method in this paper measures the relative efficiency of the barrier function, which is dependent upon the presence of at least the critical amount of water in the stratum corneum. This method is not a quantitative measure of the *amount* of water but is a measure of its *activity*.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 3, 1974, from the *College of Pharmacy, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 3J5.*
Accepted for publication October 9, 1974.
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