Effect of Surfactants on Epidermal Permeability in Rabbits

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Abstract □ The rate of water desorption of untreated and surfactant-treated depilated rabbit skin was determined to supplement previous data of surfactant-induced changes in biological membranes. The compounds applied were petrolatum USP alone (control) and petrolatum containing 10% polysorbate 85, 10% polyoxyethylene ether 96, or 10% sorbitan trioleate. Skin slices 0.4 mm. thick, were placed in a constant-temperature and constant-humidity chamber, and water-vapor loss was continuously recorded with an electric microbalance. The water desorption rate was invariably greater with surfactant-treated than with control skin. The results confirm earlier findings that the tested surfactants affect membrane structure, thereby probably increasing permeability.

Keyphrases □ Surfactants—effect on epidermal permeability in rabbits, water desorption rates □ Permeability, epidermal—effect of surfactants, rabbits □ Skin permeability, rabbits—effect of surfactants, water desorption rates

Nonionic surfactants are used in pharmaceutical formulation for various reasons, including the modification of an active ingredient's absorption. A surfactant may affect absorption of an orally administered drug by influencing the disintegration of a tablet and the solubility or dissolution of an active ingredient or by altering the rate of gastric emptying or intestinal transit. In the case of topically applied preparations, surfactant-induced dissolution or emulsification of active ingredients and changes in ointment viscosity may modify the absorption process; in addition, surfactants may act directly on biological membranes (1, 2). This and other theories of the mechanisms of surfactant effects on drug absorption were reviewed recently by Gibaldi and Feldman (3).

Interaction between surfactant molecules and membrane components (phospholipids, proteins, and water) could lead to changes in structure and, consequently, in membrane permeability—a result that may be the major factor in regulating absorption processes. Experiments with rabbit skin (4–6) showed that surfactant preparations change the content, composition, and biosynthesis rate of epidermal phospholipids; and, since phospholipids are major components of biological membranes, these changes probably indicate changes in epidermal membrane structure. If, as is generally believed, membrane lipids constitute a barrier to water diffusion (7), the proportions of individual membrane lipids or their total amount can influence water and ion transport through membranes.

The effects of surfactants on skin permeability have been studied by several investigators (8–13), and the present experiments were designed to supplement existing data of surfactant-induced changes in biological membranes (2, 4–6). There is no reliable, direct method for studying changes in the structure or permeability of isolated biological membranes; however, if the skin is viewed as a biological membrane system, changes in its permeability can be deduced from similar changes in membranes of isolated skin samples.

Although the degree of permeability varies greatly with different substances (14), the rate of water desorption could provide a useful parameter of the skin's barrier function. Most methods (15–23) for measuring water diffusion or desorption of excised skin are elaborate, require special laboratory devices, and lend themselves to error (*e.g.*, leakage of water vapor, lack of properly controlled experimental conditions, and the possibility of artifacts). We devised a simple, reliable technique using an analytical electric microbalance to record the weight changes of excised skin samples kept in a constant-temperature and constant-humidity chamber.

EXPERIMENTAL

The procedure for the treatment of rabbit skin was described previously (2). Briefly, the trunk of each rabbit (N = 14) was clipped free of hair and divided into four areas. With the first group of rabbits (N = 4), one area was untreated; the other three areas were treated daily with petrolatum USP alone or with 10% polysorbate 85[1] or 10% polyoxyethylene ether[2]. Thereafter (N = 10), the untreated area was omitted and treatment with 10% sorbitan trioleate[3] in petrolatum was substituted. Throughout the study, the area treated with petrolatum alone was regarded as the control. The areas for the specific ointment preparations were selected randomly to compensate for possible variations in response to treatment due to different areas of the body.

At the end of the 4-day treatment, one of the surfactant preparations was applied to the untreated and control areas. It was left there for 15 min.; then the test areas were washed with cotton soaked in ether to remove surface lipids as well as the ointment.

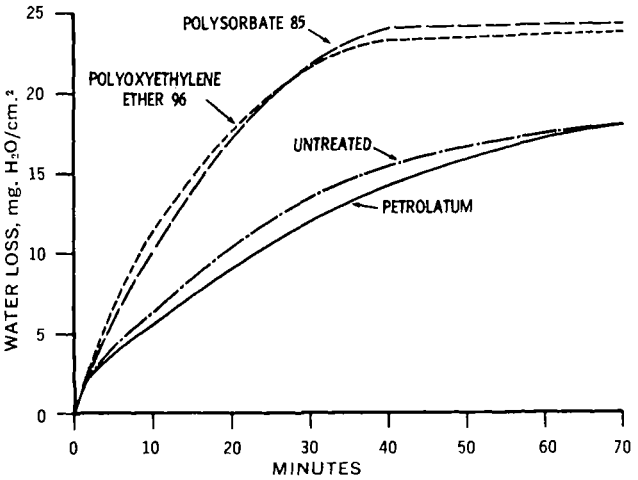

Figure 1—*Water loss from four samples of skin, subjected to different treatments, from one rabbit.*

[1] Tween 85, Atlas Chemical Industries, Inc., Wilmington, Del.
[2] Brij 96, Atlas Chemical Industries, Inc., Wilmington, Del.
[3] Span 85; Atlas Chemical Industries, Inc., Wilmington, Del.

Table I—Diffusional Water Loss from Petrolatum- and Surfactant-Treated Rabbit Skin

Minutes	Water Vapor (mg. water/cm.²/10 min.), Mean ± SE			
	Control: Petrolatum (N = 14)	Polysorbate 85 (N = 14)	Polyoxyethylene Ether 96 (N = 9)[a]	Sorbitan Trioleate (N = 7)[a]
0–10	6.7 ± 0.29	9.2 ± 0.47	9.2 ± 0.63	8.9 ± 0.69
>10–20	5.0 ± 0.20	6.9 ± 0.32	6.2 ± 0.58	6.5 ± 0.46
>20–30	3.4 ± 0.24	4.4 ± 0.33	3.5 ± 0.49	3.4 ± 0.59
>30–40	2.3 ± 0.22	1.7 ± 0.20	1.0 ± 0.17	1.4 ± 0.34
>40–50	1.3 ± 0.16	0.6 ± 0.09	0.3 ± 0.06	0.6 ± 0.17
>50–60	0.8 ± 0.10	n.d.[b]	n.d.	n.d.
>60–70	0.5 ± 0.08	n.d.	n.d.	n.d.

[a] Specimens from the other rabbits were either untreated or were damaged during removal. [b] Not detectable.

The rabbits were anesthetized with ether. Skin samples (about 2–3 cm.² × 0.4 mm. thick) were cut with a Castroviejo keratotome (24) and were placed immediately on a cooled glass plate; a piece measuring 1.0 cm.² was excised with a scalpel. Each piece was placed flat on a wire screen suspended vertically in a chamber[4] in which the temperature was maintained at 37° and relative humidity at 36%. The wire holding the sample was connected, through a small hole drilled in the top of the chamber, to an electronic microbalance[5] connected to a recorder[6]. The chart speed was set at 5 mm./min., and a full-scale response of 40 mg. was employed.

RESULTS AND DISCUSSION

Figure 1 was constructed from the recorded graph of water loss from four skin samples from one rabbit in the first group; the curve rose more sharply initially (during the first 20 min.), but leveled off sooner, for the surfactant-treated samples than for the untreated and petrolatum-treated samples. In later groups of rabbits in which the untreated area was omitted, the pattern observed with every rabbit tested was similar, the rate of water loss being greater from surfactant-treated than from control samples.

As shown in Table I, at 50 min. the diffusion was almost complete from the surfactant-treated samples but was still considerable from the control samples; thereafter, until 70 min. (the end of the experiment), the difference was even more marked. The changes in rate of water loss, calculated from individual data and based on control values (petrolatum only) as reference, were as follows. During the first 10 min., the rate of loss from samples treated with polyoxyethylene ether 96 was 47.3% greater than from the control samples. Treatment with polysorbate 85 and sorbitan trioleate preparations also considerably increased the rate of water loss; during the second 10-min. period, the increase was 40.7 and 35.2%, respectively. The relative effects of these selected surfactants were similar during earlier investigations (1, 2, 4–6), in which the morphological and biochemical changes were greater with the polyoxyethylene ether-type surfactants than with other types.

There was no great difference in rates of water loss in the first 2 min. (Fig. 1), during which period mainly the surface moisture evaporated, evidently uninfluenced by membrane permeability. After 2 min., however, when the water molecules from deeper layers of the tissue had to diffuse, the rate of evaporation was limited by the rate of diffusion, the latter being governed by both the water concentration and the degree of permeability of barrier membranes.

There was no significant difference in the water content of samples obtained from areas treated with petrolatum, polyoxyethylene ether 96, and sorbitan trioleate (Table II). Samples treated with polysorbate 85 contained about 30% more water than did those treated with petrolatum, but after 30 min. the rate of loss was lower from the former and desorption was virtually complete earlier, despite their initially higher water concentration. This indicates that surfactant-induced changes in permeability played the major role in the control of water desorption.

The "crossover" effect at 30–40 min. (Table I) confirms that the differences in rate of water loss were due to permeability changes,

Table II—Water Content of Petrolatum- and Surfactant-Treated Rabbit Skin

	Water Vapor (mg. water/mg. Skin[a]), Mean ± SE
Petrolatum (N = 14)	1.65 ± 0.08
Polysorbate 85 (N = 14)	2.15 ± 0.08
Polyoxyethylene ether 96 (N = 9)	1.74 ± 0.09
Sorbitan trioleate (N = 7)	1.85 ± 0.14

[a] Water content is expressed as mg. water/1 mg. dried weighed skin: $\left(\frac{\text{milligrams wet weighed skin} - \text{milligrams dry weighed skin}}{\text{milligrams dry weighed skin}}\right)$.

since the present experiments were designed to eliminate or control most other factors that could influence the rate of removal of water from excised skin. Thus, the humidity and temperature of the environment and the size and surface area of the samples were standardized and kept constant, and the apparatus and techniques ensured that the accuracy of weight loss measurement was achieved with maximal sensitivity and readability.

In addition, factors that may influence the water-binding capacity of petrolatum- and surfactant-treated skin other than by affecting barrier membranes were taken into account. These include the interaction of water molecules with the absorbed surfactant, diffusibility changes due to treatment-induced changes in the structure of the skin surface, and changes in osmosis or in collagen hydration that might relate to the amount of surfactant absorbed. Therefore, a surfactant preparation was applied to both the untreated and the petrolatum-treated areas 15 min. before the samples were taken to provide some surfactant in the control samples without inducing measurable biochemical changes (2). If water desorption were influenced mainly by the *presence* of the surfactant, there would be no difference in the rate of water desorption; if any difference occurred, it should be due mainly to surfactant-induced *effects* on biological membranes in samples treated with surfactant for the longer period.

It could be argued that the amount of surfactant in control samples (exposed for 15 min.) differed from the amount in treated samples (exposed for 4 days). However, findings in earlier studies (2, 4–6) of surfactant-induced effects on the content, composition, and biosynthesis rate of phospholipids indicated that differences in rates of water loss probably relate mainly to the effect of surfactant on biological membranes. If, as was concluded previously (4–6), the tested surfactants affect the epidermal barrier membranes, the damaged or regenerating membranes are less of a barrier to water diffusion than are the intact membranes in petrolatum-treated samples. In fact, the present results confirm this conclusion. Possible mechanisms of a surfactant–membrane interaction were described previously (2, 6).

[4] Model VP-100 RAT-1, Blue M. Electric Co.
[5] Cahn.
[6] Coleman 165.

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Substituted Hippuramides as CNS Depressants

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Abstract □ A series of substituted hippuramides related to tricetamide was prepared, and a preliminary pharmacological evaluation was performed.

Keyphrases □ Hippuramides, substituted—synthesis and preliminary pharmacological testing □ CNS depressants, potential—synthesis and preliminary pharmacological testing of substituted hippuramides

Tricetamide (trimethoxybenzamide of glycine diethylamide), reported in 1960 by Kusserow and Drapir (1), was investigated pharmacologically and reported by Cronheim *et al.* (2, 3) to possess interesting sedative properties. Röhnert (4) also reported the synthesis of a series of *N*-substituted 3,4,5-trimethoxybenzoylglycine diethylamides. It was of interest to prepare a series of ring-substituted benzoylglycine dimethyl- and diethylamides in order to compare them to tricetamide with regard to their ability to potentiate pentobarbital sleeping time.

EXPERIMENTAL[1]

General Procedures—Two successful routes of synthesis were used. In general, the best yields were obtained by condensing the desired benzoyl chloride with glycine dimethyl- or diethylamide (Method A) (4). The synthesis of *N*-[(diethylcarbamoyl)methyl]-2,5-dimethylbenzamide is presented as an example.

In some cases it was necessary to use the mixed anhydride of the substituted hippuric acid formed from reaction with ethyl chloroformate. Dimethyl- or diethylamine was then added to the anhydride formed to give the desired amide (Method B) (5). The synthesis of *N*-[(dimethylcarbamoyl)methyl]-2,4-dichlorobenzamide is presented as an example.

Method A: N-[(*Diethylcarbamoyl)methyl*]-*2,5-dimethylbenzamide*—A solution of 9.1 g. (0.054 mole) of 2,5-dimethylbenzoyl chloride in 70 ml. anhydrous ether and a solution of 8 g. (0.062 mole) of glycine diethylamide in 100 ml. of 8% $NaHCO_3$ solution were added slowly dropwise into a beaker at such a rate that twice the volume of aqueous solution was added per volume of ethereal solution. After the addition was complete, the mixture was stirred vigorously for another hour. The layers were allowed to separate. The ether layer was removed and dried overnight using anhydrous sodium sulfate. The solvent was removed and then the residue was recrystallized once from ethyl acetate and then twice from alcohol and water. A yield of 76% of theory based on the acid chloride was obtained.

Method B: N-[(*Dimethylcarbamoyl)methyl*]-*2,4-dichlorobenzamide*—A solution of 4.6 g. (0.022 mole) of 2,4-dichlorohippuric acid in 50 ml. of acetone and 5 ml. of 25% trimethylamine (0.034 mole) in water was cooled to about −10°.

A solution of 2 ml. (0.025 mole) of ethyl chloroformate in 10 ml. of acetone was added with stirring at such a rate that the temperature was maintained at −10°. The mixture was allowed to stand 15 min.; then 10 ml. of 25% dimethylamine (0.056 mole) in water was added with stirring, keeping the temperature near −10°. The mixture was stirred for another hour, the ice and salt bath was removed, and the mixture was allowed to stand overnight at room temperature. The solvent was then removed in a flash evaporator. The oily residue was taken up in ether, and the ether solution was washed with water and then dried overnight using anhydrous sodium sulfate. On removal of the ether, a semicrystalline mass resulted. This was recrystallized several times from ethyl acetate. The yield was 1.8 g. or 39% of theory.

[1] All melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by F. B. Strauss Microanalytical Laboratory, Oxford, England. IR spectra for all compounds were recorded on a Beckman IR-8 spectrometer and were found to be in agreement with the assigned structures. No attempt was made to optimize the yields. The method of preparation, yields, melting points, recrystallization solvents, molecular formulas, and analyses are shown in Table I.