Dermatitic Effect of Nonionic Surfactants I

Gross, Microscopic, and Metabolic Changes in Rabbit Skin Treated with Nonionic Surface-Active Agents

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Nonionic surface-active agents were applied to rabbit skin daily in an attempt to determine the physiological properties and irritative potential of some selected surfactants. Three methods of evaluation were used: gross examination, microscopical examination, and measurement of the respiratory metabolic activity of the treated skin. The gross and microscopical evaluations of these tests both indicated that the greatest irritation was produced by the polyoxyethylene ethers. These substances produced gross inflammation in even short periods of time (3 days) in the weakest dilutions (1 per cent) in which they were tested. Polysorbates caused more irritation than sorbitans. The metabolic measurements indicated a two, three, and fourfold increase in the oxygen consumption of the inflamed, treated skin sample, depending on the length of the treatment and the type of agent used.

IN THE PAST 20 years there has been a considerable increase in the utilization of synthetic surfactants as household and industrial cleansing products, and as solubilizers and emulsifiers in both pharmaceutical and cosmetic preparations. Consequently, because of this frequency of contact among the general population, the effects on the human skin of these surfactants become of extreme importance.

At present a literature survey presents a wide variety of results. In 1963, Treon (1) conducted standard patch tests with a large number of nonionic surfactants, including those that were used in the present experiment. He reported no irritation with human skin and only mild irritation on rabbit skin in the cases of sorbitan trioleate,[1] polysorbate 80,[2] and polyoxyethylene esters 52, 56, and 72.[3] The other nonionic surfactants caused no irritation in rabbit skin. The same year Choman (2) published observations on the effects of aqueous nonionic surfactants on excised calf and human skin, in which he indicated that irritation or cellular structural alteration did not occur.

Contrary to these reports there are several studies claiming carcinogenic effects of some of the same nonionic surfactants in *in vivo* mouse skin experiments (3–9).

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[1] Marketed as Span 85 by Atlas Chemical Industries, Inc., Wilmington, Del.
[2] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.
[3] Marketed as Brij 52, 56, and 72 by Atlas Chemical Industries, Inc., Wilmington, Del.

This presentation will consider the gross anatomic, histological, and metabolic changes of rabbit skin caused by the daily application of selected nonionic surface-active agents.

EXPERIMENTAL

Three main groups of nonionic surface-active agents were selected for this study: (*a*) partial esters of sorbitan fatty acids, sorbitan series; (*b*) partial esters of polyoxyethylene sorbitan fatty acids, polysorbate series; and (*c*) polyoxyethylene ethers.[3]

A detailed list of these surfactants is in Table I. These surfactants were used both in undiluted and in diluted forms, using distilled water, hydrophilic

TABLE I.—NAME AND CHEMICAL COMPOSITION OF SURFACTANTS USED

Partial Esters of Sorbitan Fatty Acids (Sorbitan Series)
Sorbitan monolaurate
Sorbitan monostearate
Sorbitan monooleate
Sorbitan trioleate
Partial Esters of Polyoxyethylene Sorbitan Fatty Acids (Polysorbate)
Polyoxyethylene (20) sorbitan monolaurate (Polysorbate 20)
Polyoxyethylene (20) sorbitan monostearate (Polysorbate 60)
Polyoxyethylene (20) sorbitan monooleate (Polysorbate 80)
Polyoxyethylene (20) sorbitan trioleate (Polysorbate 85)
Polyoxyethylene Ethers
Polyoxyethylene (4) lauryl ether
Polyoxyethylene (2) cetyl ether
Polyoxyethylene (10) cetyl ether
Polyoxyethylene (2) stearyl ether
Polyoxyethylene (2) oleyl ether
Polyoxyethylene (10) oleyl ether[a]

[a] Marketed as Brij 96 by Atlas Chemical Industries, Inc., Wilmington, Del.

TABLE II.—GROSS OBSERVATIONS AFTER 3 DAYS OF APPLICATION[a]

Substance	60%				10%				5%				1%
	100%	in Water	in H.O.[b]	in H.P.[c]	in Water	in H.O.	in H.P.	in Pet.[d]	in Water	in H.O.	in H.P.	in Pet.	in Pet.
Sorbitan monolaurate	±			+			0	+					0
Sorbitan monostearate				0			0	0					0
Sorbitan monooleate	+			0			0	0					
Sorbitan trioleate	+							+					+
Polysorbate 20	+				0	+		+					+
Polysorbate 60	+				+	+		+		+			+
Polysorbate 80	+				+	+			0	+			
Polysorbate 85	+				+	+		±		+			+
Polyoxyethylene ether 30	+++	++	+++		+	++		++					+
Polyoxyethylene ether 52	++	++		++	+			++			+	+	+
Polyoxyethylene ether 56	++	++	++		+	+		++	+	+		+	+
Polyoxyethylene ether 72		±			+		++	++	+		++		+
Polyoxyethylene ether 92	+++	++	+++					++					±
Polyoxyethylene ether 96								++		±		±	±
H.O.	0												
H.P.	0												
Pet.	0												

[a] 0, no visible change; +, erythema, edema; ++, thickening; +++, hyperkeratinization; ++++, formation of fissures and open lesions; ± to ++++, underline signifies more intense irritation. [b] H.O., hydrophilic ointment U.S.P. [c] H.P., hydrophilic petrolatum U.S.P. [d] Pet., petrolatum U.S.P.

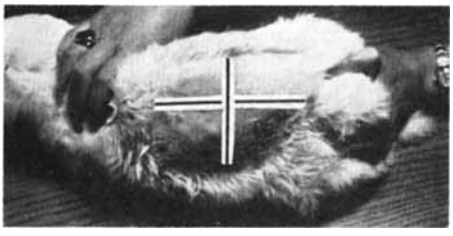

Fig. 1.—There is erythema and thickening of the lower left quadrant of the shaved area (polyoxyethylene ether 56, 5% in hydrophilic ointment) and crusting with induration of the lower right quadrant (polyoxyethylene ether 30, 10% in hydrophilic ointment). The upper right quadrant is the control area. One week of application.

ointment U.S.P., hydrophilic petrolatum U.S.P., and white petrolatum U.S.P. as diluents. The diluents were also applied alone as controls.

Increasing concentrations from 1–100% of the above surfactants were prepared and applied to the trunks of New Zealand white rabbits. Each trunk, clipped free of hair by an electric clipper (Oster model A2), provided 8 test areas. On each rabbit, one area was untreated and one area was treated with only an ointment base, to act as control sites. Fifty rabbits were used in this investigation, providing 400 separate areas. About 0.3 Gm. of each different preparation was evenly applied and then gently rubbed in for 3 sec. with a hard rubber spatula to the appropriate area, once a day. For the evaluation of histological changes biopsy specimens were taken from representative sites after application for 10 and 30 days and at the completion of the experiment. After each biopsy, that area was discontinued for use as an experimental site. With forceps, the skin was elevated, and a full thickness biopsy was taken by scissors. The specimens were kept in a 10% formol–saline solution until routine histological slides were prepared, stained with hematoxylin and eosin, and examined microscopically.

The oxygen consumptions of the treated and control skin samples were determined by the direct Warburg method (10). The animals were killed by fracturing the neck. The test areas were washed with water quickly to remove the remainder of the substances previously applied, and the samples were taken with the aid of the Castroviejo keratotome (11) set to cut 0.2 mm. thickness of skin. The skin slices were cut to small pieces with cold scissors and were immediately weighed on an analytical balance (referred to as wet weight), then transferred to Warburg flasks containing 3.0 ml. of Kreb's Ringer phosphate–glucose solution (10) and 0.2 ml. of 20% KOH solution in the center well. The measurement was carried out at 37°, after a 30-min. equilibration period. Room air was used as the gas phase. At the end of the measurement the samples were removed from the flasks, rinsed in distilled water, and placed in preweighed crucibles. Dry weights of the samples were obtained by drying overnight in an oven at 105°. The oxygen consumption was calculated in microliters per milligram of skin (dry) per hour: this value is the QO_2.

RESULTS AND DISCUSSION

The samples were evaluated by three methods: (*a*) direct inspection, (*b*) histological examination, and (*c*) measurement of the respiratory metabolic activity of the treated skin.

Gross observations were made and recorded daily, but representative tables are made up for intervals of 3 days and 10 days after starting applications. After 3 days of application, the first gross changes, namely erythema and edema, resulting in a hard induration began to appear where certain of the polyoxyethylene ethers were being applied, even in a dilution of 10%. Irritation was also observed, but to a lesser degree (Table II), where 100% concentrations of polysorbates were applied. At the sites of application of 10% concentrations of polysorbates, only a slight erythema was noticeable. With the exception of sorbitan monolaurate[4] and sorbitan trioleate, no changes were seen at the sorbitan areas. As the treatment continued, a de-

[4] Marketed as Span 20 by Atlas Chemical Industries, Inc., Wilmington, Del.

TABLE III.—GROSS OBSERVATIONS AFTER 10 DAYS OF APPLICATION[a]

Substance	100%	60%			10%			5%			1%
	in Water	in H.O.	in H.P.	in Water	in H.O.	in H.P.	in Pet.	in Water	in H.O.	in H.P.	in Pet.	in Pet
Sorbitan monolaurate	+		++			+++	+|+|+|+|+|+|+|+					++++++++
Sorbitan monostearate			+++									
Sorbitan monooleate	++											
Sorbitan trioleate	+++											
Polysorbate 20	++++			+++	++							
Polysorbate 60	++++			+++	+++							
Polysorbate 80	++++			+++	+++			0	+++			
Polysorbate 85	+++	++		+++	+++		++					
Polyoxyethylene ether 30	+|+|+	++		++	+		++					
Polyoxyethylene ether 52	+|+|+	++	+	+++	++		+++	+++		++	+|+	+++
Polyoxyethylene ether 56	+|+|+	++	+	+++	++		+++	+++		++	+|+	+++
Polyoxyethylene ether 72	+|+		+	++		+	++			+	+	+
Polyoxyethylene ether 92	+|+		+	++		+	++			+	+	++
Polyoxyethylene ether 96	+|+		+	++		+	++			+	+	++
H.O.	0								+++			
H.P.	+											
Pet.	+											

[a] See footnotes under Table II.

Fig. 2.—Control skin, plain hydrophilic ointment application only (−).

gree of irritation became apparent in most treated areas. The skin treated with polyoxyethylene ethers of all concentrations showed scaling and thickening, with marked induration and fissuring. A type of crust formation, shown microscopically to consist of necrotic and sloughing epidermis, often developed after 1 week of treatment with various concentrations of polyoxyethylene ether, (as illustrated by Fig. 1). Area 1 (polyoxyethylene ether 56, 5%) and area 3 (polyoxyethylene ether 30,[5] 10%) showed considerable irritation. Area 4, the untreated skin, showed no sign of irritation. The inflamed necrotic and irreversibly damaged epidermis sloughed off after 2 or 3 weeks of polyoxyethylene ethers application, but reepithelialization of the surface followed rapidly.

Table II illustrates the gross evaluations after 3 days of treatment. It is evident from this table that the surfactants belonging to the polyoxyethylene ether series produced irritation even after 3 days of application, and in a dilution of 1%. Table III records observations made after 10 days of application, where even the areas treated with surfactants belonging to the sorbitan series begin to show some irritation. An interesting observation was the increased rapidity of hair growth, chiefly in areas of application of the polysorbates. New skin was formed satisfactorily from remaining epidermal appendages in almost all the areas which had sloughed off after treatment with the polyoxyethylene ether-type of surfactants.

The microscopic appearances in the treated areas paralleled the degree of gross irritation observed. Figures 2, 3, 4, and 5 illustrate the degrees of inflammation and irritation after 10 days of application. The microscopic changes which were observed are illustrated by Tables IV and V.

The respiratory metabolic activity of selected samples of surfactant-treated skin was studied using oxygen consumption measured by the Warburg method. It has been reported (12–14) that in *in vitro* tests the surfactants influence enzymatic reactions, damaging the function of the respiratory chain. The authors found that the surfactant-treated skin consumed 2, 3, or 4 times as much oxy-

[5] Marketed as Brij 30 by Atlas Chemical Industries, Inc., Wilmington, Del.

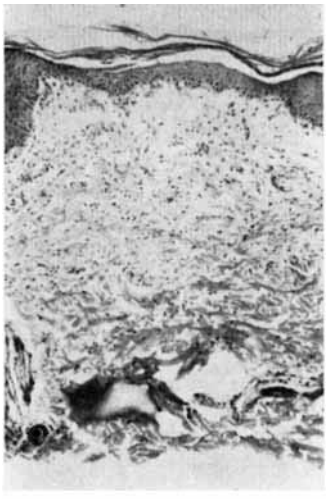

Fig. 3.—Polysorbate 80 in 100% concentration; there is irregular acanthosis of the epidermis and edema and inflammation in the dermis (2+).

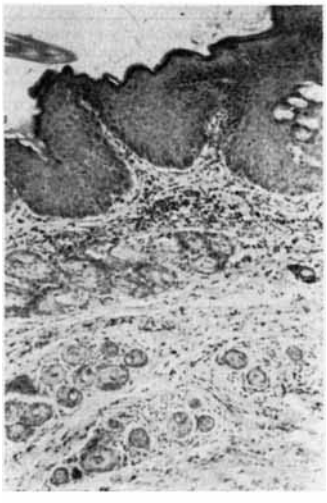

Fig. 4.—Polyoxyethylene ether 52 in 5% concentration. There is acanthosis and hyperplasia of the epidermis, together with some edema and inflammation of the dermis (3+).

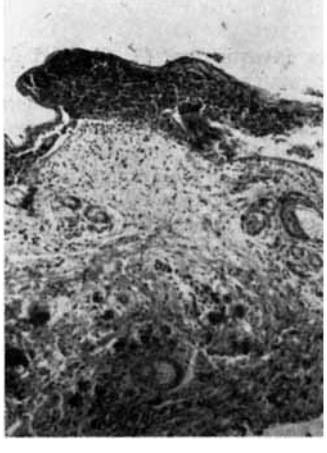

Fig. 5.—Polyoxyethylene ether 30 in 60% concentration; the epidermis is destroyed. It is replaced by eosinophilic staining material filled with many polymorphonuclear leucocytes. These cells extend to the dermis and in some instances dip down into the sebaceous glands and hair follicles. There are scattered polymorphonuclear leucocytes and indication of edema in the dermis (4+).

gen as the control skin, depending on the length of time of the treatment and on the type of agent used. The results are illustrated by Figs. 6 and 7.

Figure 6 represents the QO_2 values of the control and treated skin samples obtained from 13 rabbits at a period of 3- to 15 days (1 rabbit a day) after starting application. Figure 7 illustrates the QO_2 values of skin samples taken from the second group of rabbits, 15 in number, within the period of 30–81 days after starting application. During this period the samples were taken 30, 31, 40, 42, 43, 44, 45, 50, 51, 52, 55, 58, 77, 78, and 81 days after starting the application.

A specific explanation for the increased respiration rate due to the topical application of surfactants has not yet been found. The surfactants can directly influence enzymatic reactions as reported (12–14). On the other hand, the permeability changes of cell membranes induced by the surfactants could also be the source of the altered respiratory activity. The effect of these surfactants on the cell membranes, and in a smaller scale, on the membrane of the mitochondrion is very probable, due to their hydrophilic and lipophilic properties. By these properties they might be able to disturb the balance of the cell system, *e.g.*, they can weaken the hydrophobic bond, which is a key to the structure and function of the membrane of the mitochondrion, consequently causing alteration in the oxidative metabolism.

One of the difficulties in measuring skin respiration is to provide samples containing mainly the epidermis and relatively small amounts of dermis. The oxygen consumption of the epidermis, which is ordinarily actively proliferating, is significantly higher than that of the dermis. Usually, skin respiratory results refer to milligrams of dry skin or milligrams of tissue nitrogen. While the dry weight is a rather poor standard, due to the possible variations in amounts of epidermis, dermis, or physiologically inert constituents present, no other standard has been shown to be superior. Tissue nitrogen varies directly with the dry weight and is, in addition, more time consuming. With the aid of the Castroviejo keratotome samples were obtained containing relatively uniform structural components of the skin. The definite pattern in the authors' results, and the reproducibility of repeated measurements during these investigations, indicate that with this technique, the dry weight (imperfect as it may be) is the most practicable standard of reference.

A number of rabbits, of which the gross and histological skin-change data were presented above, were being treated with surfactants for several months in a long-term study to ascertain whether any carcinogenic effect can be observed arising from application of the above surfactants, as has been reported in mouse skin experiments (3–9). The authors found no gross or microscopic signs of carcinogenic effect of these agents in 3 rabbits which were treated with sorbitans and polysorbates for a period of 5 months and of 22 rabbits which were treated for more than 2 months.

The authors' results are in conflict with previous reports regarding the irritative potential of these substances.

In these reports (1, 2), no irritation was reported caused by sorbitan monolaurate, sorbitan mono-

TABLE IV.—MICROSCOPIC OBSERVATIONS AFTER 10 DAYS OF APPLICATION

		60%		10%		5%	
	100%	in Water	in Ointment Base	in Water	in Ointment Base	in Water	in Ointment Base
Sorbitan monolaurate	+[a]				0		0
Sorbitan monostearate							
Sorbitan monooleate	+				0		0
Sorbitan trioleate	+						
Polysorbate 20	+			+	+	0	
Polysorbate 60	+			+	+	+	
Polysorbate 80	++			+	+	+	
Polysorbate 85	++			+	+	+	
Polyoxyethylene ether 30	++++ N[b]	++++ N	++++ N	++	++ H[c]		
Polyoxyethylene ether 52		+++ N		++			+++ H
Polyoxyethylene ether 56		+++ N		+++	++ H	++	++
Polyoxyethylene ether 72		+++		++	++	++	++ H
Polyoxyethylene ether 92	++++ N	++++ N	++++ N				
H.O.	0						
H.P.	0						
Untreated skin	0						

[a] + to ++++, inflammation, from minimally increased numbers of inflammatory cells in the dermis, chiefly perivascular to marked degrees of polymorphonuclear and round cell infiltrate throughout the depth of the dermis. [b] Necrosis, a complete destruction of the normal cellular structure of the epidermis, replaced by an eosinophilic amorphous mass thoroughly infiltrated by polymorphonuclear leucocytes. This process involved hair follicular epidermis and sebaceous glands in severe cases. It was followed in all instances, when the rabbit lived, by re-epithelialization from the remaining appendages. [c] Acanthosis, an irregular acanthosis of the normally flat, thin, and regular epidermis.

TABLE V.—MICROSCOPIC OBSERVATION AFTER 1 MONTH OF APPLICATION[a]

		60%		10%		5%	
	100%	in Water	in Ointment Base	in Water	in Ointment Base	in Water	in Ointment Base
Sorbitan monolaurate	+				+		+
Sorbitan monostearate							
Sorbitan monooleate	+++				+		
Sorbitan trioleate	++ H						
Polysorbate 20	++			+	+		
Polysorbate 60	++ H			++++ N		++	
Polysorbate 80	+++ N H			+++	++	++	
Polysorbate 85	+++ H					++	
Polyoxyethylene ether 30	++++ N H	++++ N	+++ N		++		
Polyoxyethylene ether 52		+++					++
Polyoxyethylene ether 56	++ N	++ N		+++ H	+++ H	+++	+++ H
Polyoxyethylene ether 72	+++	+++ H			++ H	++	++
Polyoxyethylene ether 92	++++ N H	+++ H	++++ N H				
H.O.	+						
H.P.	+						
Untreated skin	0						

[a] See footnotes under Table IV.

stearate,[6] sorbitan monooleate,[7] polysorbate 20,[8] polysorbate 60,[9] polysorbate 80; and polyoxyethylene ethers 30 and 92;[10] and only mild irritation caused by sorbitan trioleate, polysorbate 85,[11] and polyoxyethylene ethers 52 and 72. The difference in the degree of irritation, which in our case was extreme, and in some instances, resulted in complete destruction of the epidermis, may be due to the different methods by which the surfactants were applied. The above investigators (1, 2) used *in vitro* tests and/or patch tests. The authors' method was an attempt to reproduce the frequent application of dermatologic and of cosmetic preparations by the general public. It is not always advisable to apply the results of animal experiments to humans and the fact that these evidences of irritation have been produced in rabbits must be kept in mind in assessing their significance.

The closed patch test applied for 48 hr. is less desirable as a measure of capacity of a primary irritant than a daily application of the test material for a period of at least 10 days.

Studies have not proved that synthetic detergents are entirely responsible for the reported increase in primary irritant dermatitis and "housewife's dermatitis" cases (15–18). Evidence has been presented that surface-active agents cause denaturation of keratin (19) and produce harmful

[6] Marketed as Span 60 by Atlas Chemical Industries, Inc., Wilmington, Del.
[7] Marketed as Span 80 by Atlas Chemical Industries, Inc., Wilmington, Del.
[8] Marketed as Tween 20 by Atlas Chemical Industries, Inc., Wilmington, Del.
[9] Marketed as Tween 60 by Atlas Chemical Industries, Inc., Wilmington, Del.
[10] Marketed as Brij 92 by Atlas Chemical Industries, Inc., Wilmington, Del.
[11] Marketed as Tween 85 by Atlas Chemical Industries, Inc., Wilmington, Del.

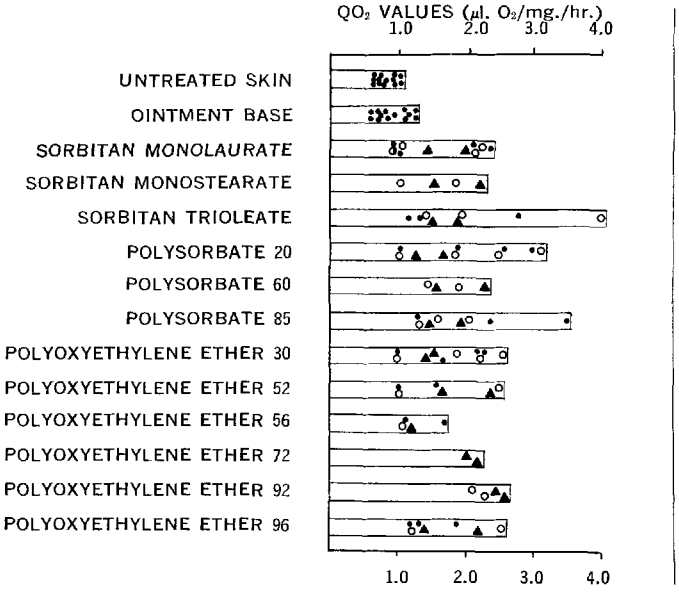

Fig. 6.—The oxygen consumption of control and treated skin samples taken from 3–13 days after starting application. Key: ●, control, ointment base alone, or 100% surfactant; ○, 10% surfactant in ointment base; ▲, 1% surfactant in ointment base.

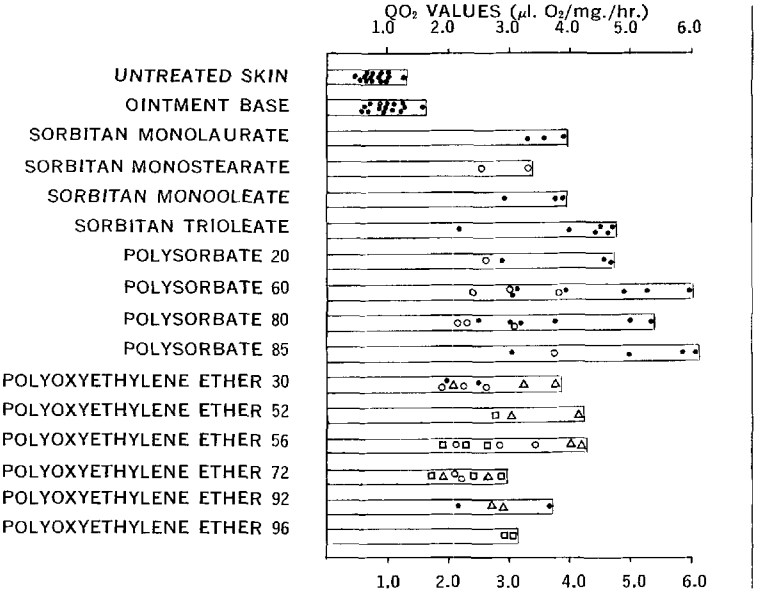

Fig. 7.—The oxygen consumption of control and treated skin samples taken from 30–81 days after starting application. Key: ●, control, ointment base alone, or 100% surfactant; Δ, 60% surfactant in ointment base; ○, 10% surfactant in ointment base; □, 5% surfactant in ointment base.

effects on the horny layer as defatting agents by removal of the lipids and other substances (20–23). The surface-active agents are usually present in cosmetic and dermatological preparations in concentrations varying from 1–10%. Considering the above studies (15–23) and these results, the possibility arises that, if the increased number of cases of hand dermatitis in females cannot be explained by the increased use of synthetic household detergents then the surfactants present in hand lotions and other cosmetic or dermatological preparations could possibly be one factor in some of the cases of hand dermatitis in the female population. There are, as yet, only animal tests to prove that a number of polyoxyethylene ether surfactants, a common component of hand lotions, have a distinct potential to irritate the skin. Certain other surfactants, namely the polysorbates and sorbitans, also have undesirable influences on the skin, if used in daily applications for a longer period.

REFERENCES

(1) Treon, J. F., *Proc. Sci. Sect. Toilet Goods Assoc.*, **40**, 40 (1963).
(2) Choman, R. R., *J. Invest. Dermatol.*, **40**, 177(1963).
(3) Setälä, K., Setälä, H., and Holsti, P., *Science*, **120**, 1075(1954).
(4) Setälä, H., *Acta Pathol. Microbiol. Scand. Suppl.*, **115**, 1(1956).

(5) Shubik, P., Della Porta, G., and Spencer, K., *Acta Unio. Intern. Contra Cancrum*, **15**, 232(1959).
(6) Merenmies, L., *Acta Pathol. Microbiol. Scand. Suppl.*, **130**, 1(1959).
(7) Della Porta, G., Terracini, B., Dammert, K., and Shubik, P., *J. Natl. Cancer Inst.*, **25**, 573(1960).
(8) Della Porta, G., Shubik, P., Dammert, K., and Terracini, B., *ibid.*, **25**, 607(1960).
(9) Shubik, P. A., *Acta Unio. Intern. Contra Cancrum*, **17**, 22(1961).
(10) Umbreit, W. W., Burris, R. H., and Stauffer, J. F., "Manometric Techniques," 4th ed., Burgess Publishing Co., Minneapolis, Minn., 1964.
(11) Blank, H., Rosenberg, E. W., and Sarkany, I., *J. Invest. Dermatol.*, **34**, 307(1961).
(12) Wilmsmann, H., and Marks, A., *Fette, Seifen, Anstrichmittel*, **61**, 965(1959).
(13) Wilmsmann, H., *Am. Perfumer Cosmet.*, **78**, 21(1963).
(14) Polgár, L., *Acta Physiol. Acad. Sci. Hung.*, **21**, 313(1962).
(15) Jambor, J. J., and Suskind, R. R., *J. Invest. Dermatol.*, **24**, 379(1955).
(16) Jambor, J. J., *ibid.*, **24**, 387(1955).
(17) Ferguson, E. H., and Rothman, S., *Arch. Dermatol.*, **80**, 300(1959).
(18) Suskind, R. R., Meister, M. M., Scheen, S. R., and Rebello, D. J. A., *ibid.*, **88**, 117(1963).
(19) Van Scott, E. J., and Lyon, J. B., *J. Invest. Dermatol.*, **21**, 199(1953).
(20) Blank, I. H., *ibid.*, **25**, 391(1955).
(21) Szakall, A., *Arch. Klin. Exptl. Dermatol.*, **201**, 311(1955).
(22) Paschound, J. M., and Schmidli, B., *Dermatologica*, **110**, 323(1955).
(23) Flesch, P., and Esoda, E. C. J., *Nature (London)*, **178**, 211(1956).

Occurrence of Amanita Toxins in American Collections of Deadly Amanitas

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Chromatographic examination of *Amanita phalloides* carpophores collected in the Pacific Northwest revealed the presence of relatively large amounts (1.5–1.9 mg./Gm. dry wt.) of β-amanitin but lesser concentrations (trace to 0.93 mg./Gm.) of α-amanitin. It was concluded that these represent a distinct chemical race of *A. phalloides*, apparently restricted in its occurrence to the states of Washington, Oregon, and California. Other species investigated included *Amanita bisporigera*, the most toxic yet found in the United States, containing 2.25–5.0 mg. of α- and β-amanitins per Gm. *Amanita verna* specimens contained variable amounts of the two toxins (0 to 1.7 mg./Gm.), but very small quantities (0 to <0.1 mg./Gm.) of α-amanitin only were detected in samples of *Amanita virosa*.

CONFUSION ABOUNDS in the literature pertaining to the identity and toxicity of *Amanita* species. In the United States this is particularly true of *Amanita phalloides* (Fr.) Secr. and related species (so-called deadly amanitas); in fact, all literature prior to the last decade or two must be carefully evaluated to determine, if possible, the identity of the mushroom which was actually studied under a particular designation.

Until 1918, all species of deadly amanitas occurring in the U. S. were generally referred to *A. phalloides*, which was considered to represent a single polymorphic species. For example, Murrill (1), referring to *A. phalloides* in 1916, wrote of "The variety of colors assumed by this species—white, yellow, green, gray, brown, blackish . . ." In 1918, Atkinson (2) recognized that the most common *Amanita* species in the eastern U. S., usually interpreted as a dark brown form of *A. phalloides*, was actually a different species. He subsequently described it and assigned the name *Amanita brunnescens* Atk. The various color forms were gradually sorted out with the passing years, the white forms being identified as *Amanita verna* (Fr.) Vitt. s. Boud., *Amanita virosa* Secr., or *Amanita bisporigera* Atk., the yellow or green as *Amanita citrina* S. F. Gray, and the blackish or gray as *Amanita porphyria* (Fr.) Secr. Finally, none remained which could actually be designated *A. phalloides*.

Changes in nomenclature are generally accepted with reluctance; thus, *A. phalloides* is still frequently referred to in the popular press and even in scientific writings. As late as 1955, the term "brown *A. phalloides*" was used to designate *A. brunnescens* (3). Disregarding this use of antiquated nomenclature, mycologists began to assume that authentic *A. phalloides* did not occur in the U. S. (4).

In 1958, Smith (5) reported that the species did occur rarely in California, but details were not presented. A year later, specimens were found in Ashland, Ore., which greatly resembled *A. phalloides*, and analysis of them revealed the presence of β-amanitin (6) as well as a smaller amount of α-amanitin (7). A fatal case of mushroom poisoning with symptoms identical to those

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