References

1. CHASMAR, R. P. *Nature,* Lond., 1948, **161**, 281.
2. GENZEL, L. and MUSER, H. *Zeits. f. Physik,* 1950, **127**, 194.
3. JAMES, W. *Science,* 1949, **110**, 254.
4. MOSS, T. S. *Proc. phys. Soc., Lond.,* 1949, **62**, 741.
5. RITTNER, E. S. Unpublished reports to the Air Materiel Command.
6. SIMPSON, O. Unpublished dissertation.
7. SOSNOWSKI, L., STARKIEWICZ, J., and SIMPSON, O. *Nature,* Lond., 1947, **159**, 818.
8. SOSNOWSKI, L., SOOLE, B. W., and STARKIEWICZ, J. *Nature,* Lond., 1947, **160**, 471.
9. VON HIPPEL, A. *et al. J. Chem. Phys.,* 1946, **14**, 355.
10. VON HIPPEL, A. and RITTNER, E. S. *J. Chem. Phys.,* 1946, **14**, 370.

Technical Papers

Hemolytic Activity of Some Nonionic Surface-active Agents

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For a long time it has been known that surface-active agents, as exemplified by the soaps, possess marked hemolytic activity. Recent studies using synthetic anionic surface-active agents have confirmed these observations and demonstrated that the cytolytic efficiency of a homologous series of these compounds is dependent upon their carbon chain length. In discussion of these observations, emphasis has been placed upon correlation of the cytolytic power of these compounds to their surface activity (*3*) and upon complex formation, based upon electrostatic interaction, between the anionic surface-active agents and lipid, lipoprotein, and protein components of the red blood cell ultrastructure (*6*).

It is desirable to report upon the hemolytic activity of certain nonionic surface-active compounds at this time, both because of its pertinence to any consideration of the mechanism of these cytolytic effects and because of the practical importance of having available surface-active agents with desirable physical properties but largely devoid of such deleterious biological effects as hemolysis and toxicity.

This report will be confined primarily to a series of nonionic polymeric surface-active agents[1] based upon alkyl phenols made water-soluble by interaction with an alkylene oxide.[2] The alkyl-substituted phenol represents the hydrophobic portion of the molecule, while the ether-alcohol groups are the hydrophilic portion. The hemolysis studies utilized a 5% suspension of washed sheep red cells made up in 0.16M NaCl + 0.015M phosphate, buffered at pH 7.4. Hemolysis was studied by mixing 0.2 ml of the 5% sheep red cell suspension with 4.5 ml of buffered NaCl to which the desired amount of the surface-active agent had been added. This mixture is translucent, due to the scattering of light by the intact erythrocytes, but upon hemolysis it becomes transparent. The time required to achieve hemolysis of 75% of the cells has been used as an end point and was determined by visual comparison with a standard (*4*). Twofold dilutions of the surface-active agents were used and the time for hemolysis was plotted against the concentration of these compounds. From these plots the concentration per ml of surface active agent necessary for an hemolysis time of 100 min was estimated and used as a basis of comparison. Surface tensions at 25° C were determined with the duNouy tensiometer, previously standardized against H_2O and benzene.

It will be seen (Table 1) that two of the nonionic compounds, Tritons WR-1352 and A-20, are distinguished from the other surface-active agents listed in being nonhemolytic. This is evident at concentration levels approximately 1,000-fold greater than is necessary to produce hemolysis with the other surface-active compounds. These two compounds, which in high concentration are nonhemolytic to sheep cells, evidence this innocuousness at concentration levels at which they depress the surface tension of H_2O from a control value of 72 to 33 or 42 dynes per cm. In contrast, the other nonionic and ionic surface-active agents in Table 1 are hemolytic at concentration levels which depress the surface tension of their aqueous solutions to 37 dynes per cm for the least hemolytic and to 68 dynes per cm for the most hemolytic compound. Thus, alteration of the physical properties of the solution as exemplified by measurements of surface tension can in no way suffice to explain the presence or lack of hemolytic activity of surface-active agents. Both anionic and cationic surface-active agents have been shown to form saltlike, stoichiometric complexes with proteins and to cause their denaturation (*2, 7*). Nonionic compounds, on the other hand, do not possess strong polar groups and are thus unable to enter into electrostatic interaction with proteins, as is evident from studies of electrophoresis (*5*), precipitation (*1, 2*), and alteration of biological activity (*1, 2*). This means that the hemolytic activity of those nonionic compounds which evidence this property must be based upon nonelectro-

[1] The Triton compounds, as listed in Table 1, were made available through the courtesy of the Rohm and Haas Company.

[2] Bock, L. H. and Rainey, J. L. 1948 U. S. Patent 2,454,541.

static interactions with cellular components involving van der Waals' forces or hydrogen bonding. The degree of polymerization may be an important factor in determining the hemolytic activity of these nonionic surface-active agents with the most highly polymerized compounds evidencing the least hemolytic activity. However, an insufficient range of compounds was available to establish this point.

TABLE 1

HEMOLYTIC ACTIVITY OF SELECTED SURFACE-ACTIVE AGENTS

Surface-active agent	Concentration (μg/ml) for 100-min hemolysis time	Surface tension (dynes/cm) at that concentration
Nonionic compounds		
Triton WR-1352	>10,000	(42)*
Triton A-20	>10,000	(33)
Triton M-3619	33	37
Triton N-100	30	40
Triton X-155	10	51
Triton WR-1363	10	40
Triton WR-1360	6	40
Triton WR-1364	4	57
Anionic compounds		
Dioctyl sodium sulfosuccinate (Aerosol OT)	20	52
Sodium lauryl sulfate (Duponol WA)	17	60
3,9-Diethyltridecanol-6 sodium sulfate (Tergitol 7)	5	57
Cationic compounds		
Cetyl dimethyl benzyl ammonium chloride	9	57
Cetyl pyridinium chloride (Ceepryn)	4	67
Cetyl trimethyl ammonium bromide (CTAB)	4	68

* Values in parentheses were measured at a concentration of 10,000 μg/ml.

It is of interest to record, in addition, that the two compounds observed to have such low hemolytic activity also displayed a low toxicity. Upon intraperitoneal injection into mice, both Tritons WR-1352 and A-20 had an LD_{50} (10-day observation period) of more than 2,500 mg per kg body wt. No higher concentrations were used, due to the viscosity of these compounds at this level. This is in contrast with the LD_{50}'s of the other compounds listed in Table 1, which ranged from 1 to 50 mg per kg for the cationic, 70 to 100 for the anionic, and 100 to 300 for the other nonionic compounds.

In summary, attention has been called to two nonionic surface-active agents capable of altering the physical properties of solutions but largely devoid of such deleterious biological effects as hemolytic activity and toxicity.

References

1. GLASSMAN, H. N. *Bact. Rev.*, 1948, **12**, 105.
2. ———. *Ann. N. Y. Acad. Sci.*, 1950, in press.
3. HÖBER, R. and HÖBER, J. *J. gen. Physiol.*, 1942, **25**, 705.
4. JACOBS, M. H. *Biol. Bull.*, 1930, **58**, 104.
5. LUNDGREN, H. P. *Textile res. J.*, 1945, **15**, 335.
6. PONDER, E. *J. gen. Physiol.*, 1946, **30**, 15.
7. PUTNAM, F. W. *Adv. protein Chem.*, 1948, **4**, 79.

The Inhibitory Effect of Three Antihistaminic Compounds on the Growth of Fungi Pathogenic for Man

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Recent observation in this laboratory revealed that several cases of *tinea pedis* (athlete's foot) responded dramatically to applications of a cream containing 2% pyribenzamine. This observation suggested that the antihistamines should be assayed for inhibitory activity against the pathogenic fungi. The following studies were conducted to determine whether the effect noted was solely against allergic manifestations incited by the etiologic agent (*4*) or whether such compounds possessed fungistatic or fungicidal properties as well.

Three crystalline antihistaminic compounds selected for investigation were pyribenzamine hydrochloride (Ciba),[1] antistine hydrochloride (Ciba),[2] and di-phenyl-pyraline (Nopco).[3] Final concentrations of 0.1, 0.25, 0.5, 0.75, and 1.0 mg/ml of the freely soluble drugs were prepared in Mycophil broth (Baltimore Biological Laboratory). These were dispensed to tubes in 5.0-ml quantities and autoclaved at 15 lb for 15 min. After sterilization the pH of the control broths as well as those containing the varying concentrations of di-phenyl-pyraline and antistine was 6.65; those containing pyribenzamine were pH 6.3.

The dermatophytic species tested included four strains of *Microsporum*, eight of *Trichophyton* and seven of *Epidermophyton floccosum*. Single strains of *Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum*, and *Blastomyces dermatitidis*, causative agents of systemic mycoses, were also employed in the study. With the exception of three organisms (*T. ferrugineum, C. albicans*, and *C. neoformans*) all strains had been isolated from human lesions within the previous six months.

The test media containing the antihistamines and the control media without antihistamine compounds were inoculated with ½-ml amounts of organism suspension prepared according to the method outlined in a previous publication from this laboratory (*1*). All cultures were incubated at 28° C and examined for comparative growth at three-day intervals for a period of two weeks.

[1] N,N-dimethyl-N'-benzyl-N'-(α-pyridyl)-ethylenediamine monohydrochloride.

[2] 2N-benzyl-N-phenyl-aminomethyl-imidazoline hydrochloride.

[3] 1-Methyl-piperidyl-4-benzhydryl ether.