SURFACE ACTIVE AGENTS AND THEIR APPLICATION IN BACTERIOLOGY

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Surface active agents have a wide range of utility as manifested by their applications in detergency, solubilization, emulsification, capillary penetration, wetting, and spreading. Because of their growing industrial utilization these compounds have become commercially available on an increasing scale during the past twenty years with consequent opportunities for application to systems of biological interest. In interaction with such systems, these compounds have exhibited marked effectivness in low concentrations, with phenomena such as precipitation, complex formation and denaturation of proteins, cytolysis of cells, destruction of microörganisms, and inactivation of viruses as examples.

It is the purpose of this review to present the chemical structure and physical properties of surface active agents in relation to their biological activity. The effects of surface active compounds on proteins are discussed briefly. A more comprehensive discussion of the interactions of proteins and surface active agents may be found in a current review by Putnam (185).

Before starting on the main thread of development of this review it may be well to define a few of the terms which will find constant usage. This is an especial necessity because numerous terms have been utilized to characterize certain industrially important activities of these compounds, and, through widespread usage, have taken on meanings of a more generic nature than was originally intended.

Surface active agents may be defined as substances which alter the energy relationships at interfaces. Among the manifestations of these altered energy relationships is the lowering of surface or interfacial tension. More specifically,

the following types of important industrial usages of these compounds are recognized:

(*a*) Wetting Agents. Promotors of spreading of liquids on surfaces or of penetration of liquids into materials.

(*b*) Detergents. As in the cleansing of dirt from textiles.

(*c*) Emulsifying Agents. Aids in the dispersion of one phase within another, ordinarily immiscible, phase.

All the above types of compounds are surface active, but the utility of any compound as a wetting agent, detergent, or emulsifying agent is an expression of an aggregate of properties, including specific chemical configuration, and is inadequately expressed by any one simple measurement such as surface tension lowering. Thus, two compounds may display an equal ability to lower surface tension, with one being an efficient wetting agent, whereas the other may be quite deficient in this respect (for an excellent example see Wilkes and Wickert (242)).

For these reasons the term surface active agents has been chosen as the most general appellation of those compounds. Such terms as wetting agents, detergents, and emulsifying agents should be reserved for denoting specific functions of a given compound. It is well to keep in mind, however, that this has not been scrupulously observed in the literature and, especially in work of a biological background, the term detergent is often used synonymously with surface active agent.

Compounds displaying surface activity are characterized by an appropriate structural balance between one or more water-attracting groups and one or more water-repellent groups. Various synonyms have been used for the water-attracting groups. They have been known as hydrophilic or polar groups. Similarly, the water-repellent groups have been known as hydrophobic, non-polar, or hydrocarbon groups. For purposes of this review the terms *hydrophilic* and *hydrophobic* will be used.

CHEMICAL STRUCTURE AND PHYSICAL PROPERTIES OF SURFACE ACTIVE AGENTS

Classification of Surface Active Agents

The electrical charge on the hydrophilic portion of a surface active agent may serve as a convenient basis of classification of these compounds. Dependent upon the nature of this charge, or the absence of ionization, surface active agents have been classified as: Anionic, Cationic, Non-Ionic, or Amphoteric.

An *anionic* surface active agent is characterized by a structural balance between a hydrophobic residue (e.g., paraffinic chain, alkyl substituted benzene or naphthalene ring) and a negatively charged hydrophilic group (e.g., carboxyl, sulfate, sulfonate, or phosphate). In *cationic* surface active agents the same hydrophobic residues may be balanced with a *positively* charged hydrophilic group (e.g., quaternary ammonium, sulfonium, arsonium, phosphonium or iodonium). *Non-Ionic* surface active agents possess no ionized groups. The hydrophobic portion of a non-ionic surface active agent is balanced by such non-ionized hydrophilic groups as polymerized ethylene oxide or polyhydric alcohols. *Amphoteric* surface active agents are compounds of mixed cationic-

anionic structure. This latter type of compound is of no practical importance at the present time.

A brief outline has been compiled of the type structures of various commercially important surface active agents (adapted from Price (184)). It should be emphasized that this outline (see figures 1–6) is illustrative only and does not attempt to list all the types of chemical structures which might display surface activity. Paraffinic carbon chains are represented by rectangular strips in the figures.

A few commercially available examples of each type compound are listed below.

Paraffin Chain Salt Types (see fig. 1 and table 1)

Soaps: Sodium oleate

Alkyl Sulfonates: Fatty acid sulfonate ('arctic syntex A')

Alcohol Sulfates: Lauryl sulfate ('duponol WA')

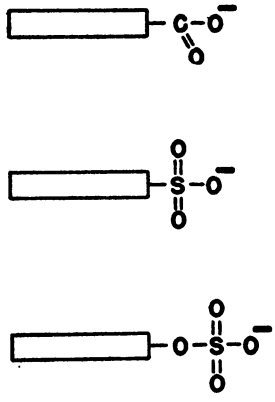

FIG. 1

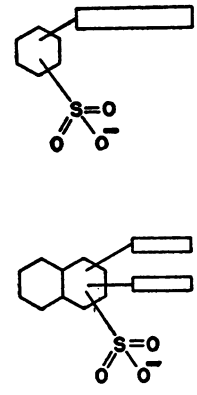

FIG. 2

FIG. 1. ANIONIC SURFACE ACTIVE AGENTS. Paraffin chain salt types. Top: soap; center: alkyl sulfonate; bottom: alcohol sulfate.

FIG. 2. ANIONIC SURFACE ACTIVE AGENTS. Alkyl aryl sulfonates. Top: alkyl benzene sulfonate; bottom: alkyl naphthalene sulfonate.

Alkyl Aryl Sulfonates (see fig. 2 and table 1)

Alkyl Benzene Sulfonates: Dodecyl-benzene sulfonate ('santomerse 3')

Alkyl Naphthalene Sulfonates: ('alkanol B')

Paraffin Chain Salts With Complex Hydrophilic Groups (see fig. 3 and table 1)

Amide of oleic acid and methyl taurine ('igepon T')

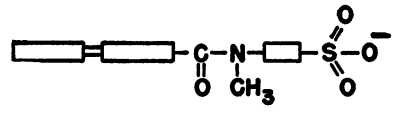

FIG. 3. ANIONIC SURFACE ACTIVE AGENTS

Paraffin chain salts with complex hydrophilic groups: amide of oleic acid and methyl taurine.

Compounds With Hydrophilic Groups Near Middle of Hydrocarbon Chain (see fig. 4 and table 1)

Dioctyl-sulfosuccinate ('aerosol OT')

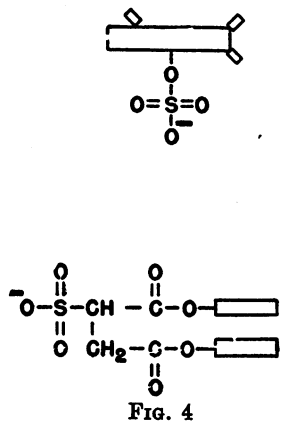

FIG. 4

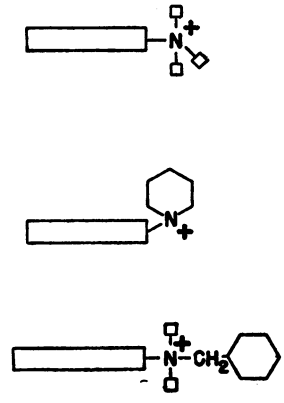

FIG. 5

FIG. 4. ANIONIC SURFACE ACTIVE AGENTS. Compounds with hydrophilic groups near middle of carbon chain. Top: sulfate of 3,9-diethyl-tridecanol-6; bottom: dialkyl sulfosuccinate.

FIG. 5. CATIONIC SURFACE ACTIVE AGENTS. Top: alkyl derivatives of aliphatic amines; center: alkyl derivatives of aromatic amines; bottom: alkyl-aryl derivatives of aliphatic amines.

Cationic Surface Active Agents (see fig. 5 and table 2)

Alkyl Derivatives of Aliphatic Amines: Cetyl-trimethylammonium (CTAB)

Alkyl Derivatives of Aromatic Amines: Cetyl-pyridinium ('ceepryn')

Alky-Aryl Derivatives of Aliphatic Amines: Cetyl-dimethylbenzylammonium

Non-Ionic Surface Active Agents (see fig. 6 and table 3)

Partial Esters of Polyhydric Alcohols with Fatty Acids: 'Polyethylene glycol 400 monolaurate'

Esters of Polyhydric Anhydrides and Fatty Acids: Sorbitan mono-oleate ('span 80')

Polyether Alcohols: Polymerized ethylene oxide condensate ('igepal CA')

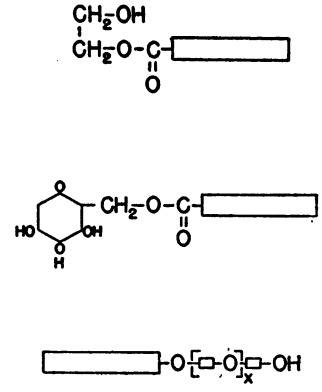

FIG. 6. NON-IONIC SURFACE ACTIVE AGENTS

Top: partial esters of polyhydric alcohols and fatty acids; center: esters of polyhydric anhydrides and fatty acids; bottom: polyether alcohols.

Selected List of Commercial Surface Active Agents and Their Properties

No attempt has been made to present a complete listing of commercially available surface active agents. Indeed, with the rapid appearance of new compounds, any attempt would soon be outdated. Instead, a list of 10 anionic, 10 cationic, and 6 non-ionic surface active agents, selected to illustrate a variety of structures, has been drawn up.[1]

TABLE 1

Characteristics of selected anionic surface active agents

TRADE NAME	MANUFACTURER	CHEMICAL DESCRIPTION	ACTIVE AGENT	SURFACE TENSION (DYNES/CM AT 25 C) AT THE FOLLOWING CONCENTRATIONS (IN PER CENT)					
				1.0	0.1	0.01	0.001	0.0001	0.00001
			%						
Aerosol OT	American Cyanamid	Dioctyl sodium sulfosuccinate	100	26.3	29.9	43.0	56.8	63.0	72.1
Alkanol B	du Pont	Alkyl-naphthalene sulfonate	?	31.8	44.1	61.3	70.6	71.5	71.6
Arctic Syntex A	Colgate	Fatty acid sulfonate	85	28.2	28.9	33.8	56.5	70.3	71.2
Aresklene 400	Monsanto	Dibutyl-phenylphenol sodium disulfonate	100	29.2	34.1	45.8	61.6	70.9	71.7
Duponol WA	du Pont	Sodium lauryl sulfate	?	28.7	32.0	46.2	64.3	71.5	71.8
Igepon T	General Dyestuff	Sodium salt of amide of oleic acid and methyl taurine	?	27.7	29.6	35.7	55.4	71.0	71.5
Santomerse 3	Monsanto	Dodecyl-benzene sulfonate	100	31.0	33.4	41.8	60.0	70.0	70.9
Sodium oleate	Eimer and Amend	Same as trade name	100	25.0	25.0	30.0	48.0	68.5	71.5
Tergitol 7	Carbide and Carbon	Sodium sulfate derivative of 3,9-diethyltridecanol-6	25	25.8	28.5	44.2	54.7	66.0	71.1
Victawet 58 B	Victor	Phosphorated capryl alcohol	70	22.8	24.9	36.0	58.9	70.5	72.0

Note: Surface tension of water at 25 C is 72.0 dynes/cm.

To make the listing more useful certain basic data have been obtained for each of the compounds (65). Trade names, manufacturers, chemical descriptions, percentage of active material,[2] and surface tension measurements at six concentration levels are listed for anionic compounds in table 1, for cationic compounds in table 2, and for non-ionic compounds in table 3.[3] It is interesting

[1] For an extensive listing of trade names, chemical descriptions, and manufacturers see (141).

[2] In those cases where it was impossible to ascertain the percentage of active material (indicated by ? in the tables), it was assumed that the commercial samples were 100% active.

[3] Surface tension was determined with the du Nouy tensiometer calibrated against water and benzene as standards.

to note that, despite marked differences in chemical configuration, similar ranges of surface activity may be obtained in all three classes of surface active agents.

TABLE 2

Characteristics of selected cationic surface active agents

TRADE NAME	MANUFACTURER	CHEMICAL DESCRIPTION	ACTIVE MATERIAL	SURFACE TENSION (DYNES/CM AT 25 C) AT THE FOLLOWING CONCENTRATIONS (IN PER CENT)					
				1.0	0.1	0.01	0.001	0.0001	0.00001
			%						
Ceepryn	Merrell	Cetyl-pyridinium chloride	?	38.5	40.7	51.2	67.0	69.8	71.3
Cetyl dimethyl-amine oxide	Onyx	Same as trade name	20	27.5	28.5	30.1	40.7	70.3	72.0
Cetyl dimethyl-benzyl ammonium chloride	Onyx	Same as trade name	25	30.9	31.1	34.4	56.2	69.6	70.7
CTAB	J. T. Baker	Cetyl-trimethyl ammonium bromide	?	33.9	35.3	46.5	65.9	70.5	71.3
Ethyl cetab	Rhodes	Cetyl-dimethyl-ethyl ammonium bromide	100	32.5	33.3	36.0	54.7	70.2	71.5
Ethyl decab	Rhodes	9-octadecenyl-dimethylethyl ammonium bromide	100	32.1	32.7	41.4	66.5	70.2	71.3
LPC	Hooker	Lauryl-pyridinium chloride	26	40.8	38.0	55.7	68.7	71.3	71.6
Octab	Rhodes	Octadecyl-dimethyl-benzyl ammonium chloride	100	32.9	34.5	37.0	59.9	68.5	71.2
Phemerol	Parke-Davis	*p*-tertiaryoctyl-phenoxyethoxyethyl-dimethylbenzyl ammonium chloride	100	36.4	36.2	52.0	62.5	69.0	72.0
Roccal	Winthrop	Alkyl-dimethyl-benzyl ammonium chloride	20	31.6	32.2	40.5	61.9	69.5	71.5

Physical Properties of Solutions of Surface Active Agents

Studies of the physical properties of solutions of surface active agents have revealed a number of anomalies, with the experimental facts being largely unchallenged but the underlying theory still nebulous and, to a large extent, highly controversial. The behavior of extremely dilute solutions of ionic surface active agents has been found to approximate that of an ordinary strong electrolyte (KCl) but, as the concentration increases, marked divergencies in such

physical properties as equivalent conductivity, ionic transference number, osmotic coefficient, and surface tension become apparent (136, 195).

As an illustration, the equivalent conductivity of a typical homologous series of cationic surface active agents, amine hydrochlorides, may be presented (196). Figure 7 represents the equivalent conductivities as a function of the square root of the concentration of an homologous series of amine hydrochlorides over the range C_8 to C_{18}. A portion of the same data has been plotted on an enlarged scale and the theoretical Onsager slope for each curve included in figure 8. Three ranges are demonstrated by these results. In the first range the equivalent conductivity falls as a linear function of the square root of the concentration

TABLE 3

Characteristics of selected non-ionic surface active agents

TRADE NAME	MANUFACTURER	CHEMICAL DESCRIPTION	ACTIVE MATERIAL	SURFACE TENSION (DYNES/CM AT 25 C) AT THE FOLLOWING CONCENTRATIONS (IN PER CENT)					
				1.0	0.1	0.01	0.001	0.0001	0.00001
			%						
Carbowax 1500 dioleate	Glyco Products	Oleic acid ester of a polymerized polyethylene glycol	?	32.0	33.7	36.3	37.4	63.0	71.8
Igepal CA	General Dyestuff	Polymerized ethylene oxide condensation	?	30.3	30.2	40.7	59.7	71.0	72.0
Polyethylene glycol 400 monolaurate	Glyco Products	Lauric acid ester of a polymerized polyethylene glycol	?	31.8	34.1	36.5	53.7	68.5	71.2
Span 80	Atlas Powder	Sorbitan mono-oleate	100	29.0	29.5	30.7	57.5	71.4	71.7
Tween 80	Atlas Powder	Sorbitan mono-oleate polyoxyalkylene derivative	100	40.4	40.2	44.6	51.5	70.3	72.0
Triton A-20	Rohm and Haas	Polyether alcohol	25	33.7	36.2	43.5	59.3	71.3	71.4

similar to the behavior observed in strong electrolytes. At a concentration which has been designated the "critical concentration" and which is dependent upon the length of the paraffin chain, the temperature, and the ionic environment, a sharp break from the behavior expected of strong electrolytes is observed. In the example illustrated, the "critical concentrations" are 0.04 M for the C_{10}, 0.013 M for the C_{12}, 0.004 M for the C_{14}, 0.0008 M for the C_{16}, and 0.0003 M for the C_{18}. A rapid fall in equivalent conductivity with increasing concentration characterizes this second range. In the third range the equivalent conductivity remains constant or rises. Results similar to these in character have been obtained in studies on anionic surface active agents (249).

The basis of explanation for these marked deviations from expected behavior has been the formation of aggregates, designated as micelles. McBain postu-

lates the existence of spherical ionic micelles and much more poorly conducting lamellar micelles. Both types of micelles are considered coexistent in solution, the relative amounts of each dependent upon the nature of the solution and the temperature (136). Hartley (74), on the other hand, believes that these effects can be explained by predicating only one type of colloidal particle, a spherical

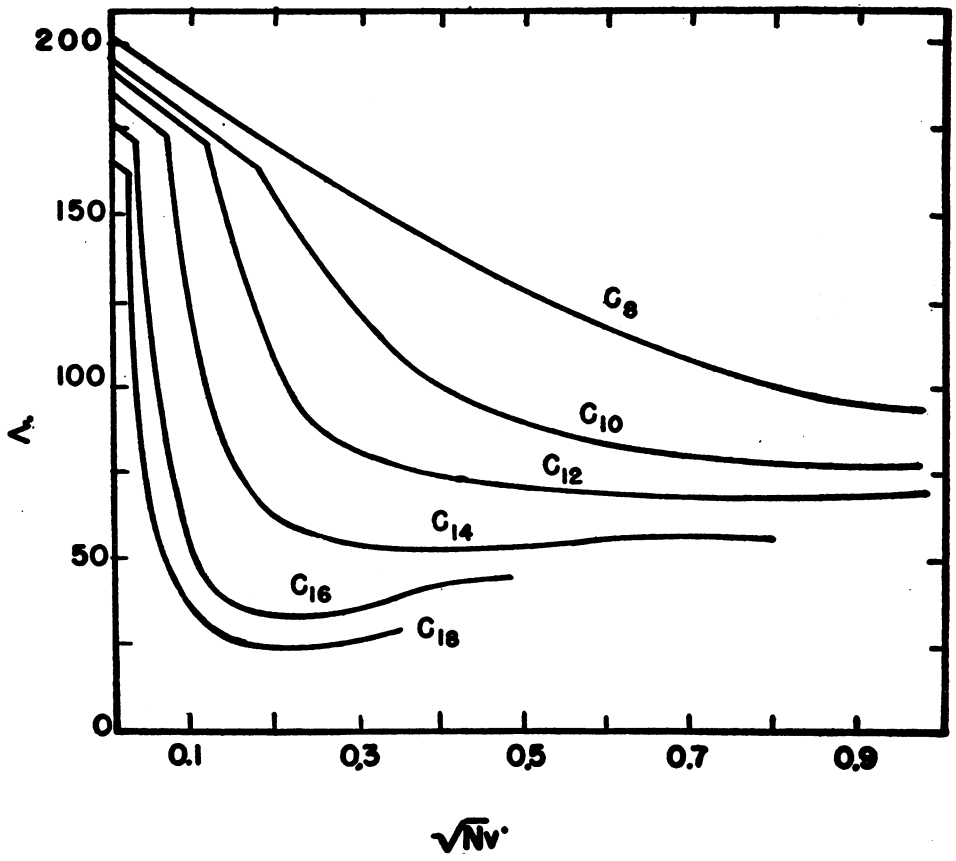

FIG. 7. EQUIVALENT CONDUCTANCES AT 60 C OF AN HOMOLOGOUS SERIES OF ALKYL AMINE SALT SOLUTIONS ($C_nH_{2n+1}NH_2 \cdot HCl$)

The number of carbon atoms in the alkyl portion of the molecule is indicated on each curve. Adapted from (196).

ionic micelle formed by the association of large ions. To this spherical micelle are attached a number of oppositely charged ions, so-called "gegen-ions". Anomalous conductivity effects are thought to be due to changes in the degree of ionization of the "gegen-ions". Although recent x-ray studies indicate the presence of both spherical and lamellar particles in solutions of colloidal electrolytes, adherents of both of the above views on micellar structure may still be found.

While this discussion has concerned itself with the ionized surface active agents,

it can be demonstrated by cryoscopic measurements that their non-ionic counterparts are also characterized by micelle formation, a critical concentration, and expansion of micellar structure with dilution (67).

Structure of Surface Active Agents and its Relation to Function

a. Anionic Surface Active Agents

Chain Length. For the anionic surface active agents with long paraffinic chains in the hydrophobic portion of their molecules, it has been found that, other things being equal, the length of the carbon chain has an important influence upon the degree of surface activity of the compound. Surface and interfacial tension measurements have demonstrated increasing surface activity as chain length is increased over the range C_6 to C_{16}, with a maximum being reached

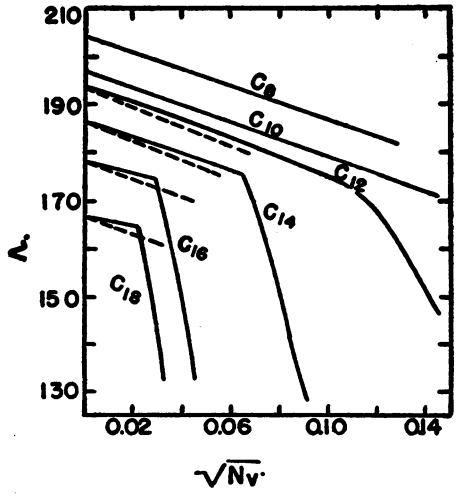

FIG. 8. ENLARGED PLOT OF PORTION OF DATA IN FIGURE 7
The broken lines indicate the Onsager values for each salt: Adapted from (196).

at C_{14} or C_{16}. Above C_{16} surface activity decreases. This importance of carbon chain length in determining surface activity has been demonstrated with soaps (213), alcohols (34, 39, 181, 207), and aliphatic acids (216).

Position of the Hydrophilic Group. With a given chain length, the positions of the hydrophilic groups are important variables in the determination of the surface active properties of the resultant molecule. Dreger and co-workers (39) prepared an isomeric series of sodium *sec*-pentadecanol sulfates in which the sulfate appeared successively on C_2, C_4, C_6, and C_8. The surface activity was found to have increased in the same order. This increased surface activity of compounds with hydrophilic groups near the center of the carbon chain has been the basis for the development of the 'tergitol' (242) and 'aerosol' series (31) of surface active agents.

When these compounds were tested for detergency and wetting ability, it was found that, other things being equal, the nearer the hydrophilic group is to the

end of a straight-chain alcohol the better the detergency but the poorer the wetting ability and vice versa. These results emphasize the limited applicability of measurements of surface activity at model interfaces such as the air-water interface (surface tension) or the hydrocarbon-water interface (interfacial tension).

As one's attention is transferred from straight chain to aromatic compounds the possibilities for alteration in substituent hydrophilic groupings increases markedly. Unfortunately, there has been a dearth of careful, systematic investigation of the relation of structure to function of these compounds.

Electrolytes and Surface Activity. It is well known that colloidal micelles are stabilized by an electric double layer and that the presence of added electrolytes within the solution markedly affects this stability. Those colloids that are *negatively* charged have their charge diminished by the presence of *cations* of an added electrolyte. If the concentration of the added electrolyte is high enough, the electrical charges of the micellar surfaces are diminished to the extent that their stability is destroyed, and they flocculate. In general, the flocculating power of an ion follows the Schulze-Hardy rule in that the precipitating power of an electrolyte depends upon the *valence* of the ion, the charge of which is opposite to that on the colloidal particle.

The fact that surface active agents tend to form colloidal micelles at low and somewhat indeterminate concentrations has already been commented upon. It is to be expected, therefore, that the nature and amount of the electrolytes present will have an effect upon the properties of solutions of a given surface active agent. This expectation has been found to be experimentally true. The hydrophilic portion of the anionic type of surface active agent is negatively charged. Therefore, these compounds should be sensitive to the nature and amount of the cationic component of the electrolytes present in the solution. Progressive and considerable lowering of the critical concentration and minimal interfacial tension (water-xylene) of dodecyl sulfate solutions has been demonstrated by the addition of NaCl (182). The influence of the added salts on the interfacial tension was demonstrated to be due solely to the added cation since all the sodium salts of non-complex anions gave results similar to those given by sodium chloride. In accordance with the predictions of the Schulze-Hardy rule, cited above, there is a considerable valency effect in the concentration of added electrolyte necessary to produce a given change in surface or interfacial tension. With calcium chloride, effects equivalent to those obtained with sodium chloride, require only 1/40 to 1/200 the concentration. Similar results were obtained by studying the effect of electrolytes upon the surface tension of tridecane-7-sulfate solutions (39). The valency effect of the cations was well illustrated in this work by measurement of the molar ratio of $NaCl:CaCl_2:AlCl_3$ necessary to reduce the surface tension a given amount (53 to 27.4 dynes/cm). The values were found to be 54:6:1.

b. Cationic Surface Active Agents

In the case of the cationic surface active agents which, since the publication of Domagk (38), have found widespread use because of their bactericidal powers,

little published work exists on the relation of structure to function in terms of chemical and physical properties. The bulk of the recent literature on this subject is confined to biological activity as the main criterion of function.

While earlier sporadic publications may be found, it seems certain that the papers of Jacobs and co-workers were the first to investigate extensively the bactericidal properties of quaternary salts and the relation of their structure to function (89, 90, 91). The general discussion of chemotherapy by Jacobs (89) emphasizes bactericidal power, specificity, compatibility with tissue components, and resistance to metabolic alteration as viewpoints for evaluation of chemical compounds as potential chemotherapeutic agents. The type of compounds studied were the quaternary salts of hexamethylenetetramine coupled with benzyl halides. While the routine testing was performed on *Eberthella typhosa*, enough tests were made on other organisms to demonstrate a variability in the bactericidal powers of various compounds dependent upon the test organism used (90). In the benzyl hexamethylenetetrammonium salts, introduction of the methyl, chlorine, bromine, iodine, cyano, and nitro groups into the benzene nucleus notably enhanced the bactericidal powers of these compounds. Substitution in an ortho position almost invariably was more effective than substitution in the meta or para position. These classical studies in chemotherapy evidently failed to strike a responsive chord since practically no further work on quaternary salts as bactericides can be found prior to that of Domagk (38) to which reference has already been made.

Chain Length. Systematic studies of the structure of quaternary ammonium compounds and their bactericidal functions have uniformly emphasized the importance of carbon chain length in determining activity. Considering the simple aliphatic quaternary ammonium compounds of the general type $RN^+(CH_3)_3$, where R is a straight chain alkyl group, it has been found that as the chain length was increased from C_6 to C_{18} the bactericidal activity increased substantially, reaching a maximum at C_{16} (84, 208). Similar conclusions of dependence of bactericidal activity of cationic surface active agents upon their carbon chain length have been derived from studies of alkyl pyridinium chlorides (111, 210), alkyl-dimethyl-sulfonium iodides (118), dialkyl-methyl-benzyl-ammonium chlorides (122), dialkyl-benz-triazolium bromides (124), azinium salts (238), sulfamyltetrazolium salts (93), alkyl-triethyl phosphonium and arsonium salts (94), alkyl-dimethyl-benzyl-phosphonium and arsonium salts (94), alkyl-colaminoformylmethyl-pyridinium chlorides (49), alkyl-phenoxy-ethoxyethyl-dimethyl-benzyl-ammonium chlorides (197), imidazolium and imidazolinium salts (211), alkyl-benzyl-ammonium chlorides, alkyl-dimethyl-ethyl-ammonium bromides, alkyl-dimethyl-allyl-ammonium bromides, and alkyl-trimethyl-ammonium bromides (232).

It might be well, at this juncture, to emphasize the fact that species differences may play a large part in the results obtained in testing chemical compounds for bactericidal activity. Not only are the minimal bactericidal concentrations quantitatively different for different species of bacteria but qualitative differences may well show up in studies of effects of alteration of chemical structure upon activity. As an example of the latter point, when the bactericidal activ-

ities of the alkyl-trimethyl-ammonium bromides were studied over the range C_6 to C_{18} against *Staphyloccus aureus* and *Eberthella typhosa* it was found that with the C_6, C_8, and C_{12} compounds *E. typhosa* was killed at higher dilutions than was *S. aureus* whereas with the C_{14}, C_{16}, and C_{18} compounds the reverse was true (208). As another example the experience of Kuhn and Westphal with dialkyl-benztriazolium salts may be mentioned (124). In this structure (see figure 9) when one compares the bactericidal activity of the following two compounds:

(A) where $R_1 = C_8H_{17}$ and (B) where $R_1 = C_{12}H_{25}$
$R_2 = C_8H_{17}$ $R_2 = C_2H_5$

the ratio of activities (A/B) is found to be 8/1 for *Staphylococcus aureus* but 1/1 for *Salmonella paratyphi* B.

Nature of Anion. In considering the effects of the structure of various portions of the molecules of cationic surface active agents upon their properties several investigations have been made of the effect of alteration of the anionic component of quaternary ammonium salts. Hauser and Niles (76) studied the

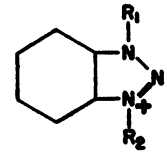

FIG. 9. DIALKYL BENZTRIAZOLIUM ION

surface tension of cetyl-pyridinium (I) chloride, bromide, and iodide and of cetyl-trimethyl-ammonium (II) chloride, bromide, and iodide. In the case of both cationic groups (I) and (II) the presence of the anionic groups enabled them to lower the surface tension of water in the order I>Br>Cl. Furthermore, the magnitude of surface tension lowering is the same for each pair of halide salts, i.e., (I) chloride and (II) chloride. This suggests that the surface tension is not dependent only upon the cation but that the nature of the anion determines its final degree.

In contrast to the physical properties of the quaternary ammonium salts, which we have seen are markedly affected by the nature of the anion, the bactericidal properties seem quite independent of the identity of the anion. It has been shown for cetyl-trimethyl-ammonium salts that the chloride, bromide, iodide, nitrate, sulfate, methosulfate, acetate, benzoate, cyanide, hydrocinnamate, and fluosilicate gave no significant differences in bactericidal power (208). The phosphate, laurate, and salicylate gave a lower activity. Similarly with cyclic compounds it was shown for the cetyl-pyridinium salts, that the nitrate, sulfate, methosulfate, bromide, and chloride have approximately identical activities (210). With acetoxy and carbethoxy derivatives of aliphatic quaternary ammonium salts the chlorides, bromides, iodides, and nitrates again were not significantly different in bactericidal activity (209).

Nature of Central Atom. Quaternary salts containing central atoms other than N can be formed. A series of alkyl-dimethyl-sulfonium iodides (substitution of S for N) have been prepared and their bactericidal effects against *Staphylococcus aureus* and *Escherichia coli* compared with the corresponding ammonium salts (118). The sulfonium compounds have been found to be qualitatively the same but quantitatively about one-third as active as the corresponding ammonium compounds. This work was later extended to a comparative study of quaternary ammonium, phosphonium, and arsonium compounds (94). Corresponding alkyl-triethyl ammonium, phosphonium, and arsonium iodides were compared over the range of C_8 to C_{16}. Similarly, alkyl-dimethyl-benzyl ammonium, phosphonium, and arsonium compounds were compared over the same carbon chain range. The criterion of activity used was the inhibition of glycolysis of lactic acid bacteria (for method, see Jerchel (93)). For both series of compounds the relative order of activity was As>P>N.

Electrolytes and Surface Activity. The comments, previously made, of the effects of added electrolyte upon the properties of anionic surface active agents should be applicable to their cationic counterparts (keeping in mind, of course, their difference in electrical sign). This has been confirmed by the demonstrated effectiveness of added electrolyte in increasing the efficiency of cetyl-trimethyl-ammonium bromide both from the standpoint of surface tension lowering and bactericidal effect (81).

Cationic Surface Active Agents of Complex Structure. Many cationic surface active agents of complex structure have been investigated as potential bactericides. Departure from the relatively simple aliphatic quaternary ammonium salts (e.g., cetyl-trimethyl-ammonium bromide) or the simple aromatic quaternary salts (e.g., cetyl-pyridinium chloride) has not, as yet, been too fruitful.

Kuhn and co-workers have investigated the quaternary salts of aminophenol ethers (119), of hydroxyquinoline ethers (123), alkyl-triazolium compounds (124), alkyl-tetrazolium compounds (120, 121).[4] The compound of outstanding activity reported by these workers was the ethobromide of *n*-dodecylbenzotriazole. Dilutions of 1:615,000 were claimed to be enough to kill *Staphylococcus aureus* as compared with 1:1,200 for lauryl-dimethyl-benzyl-ammonium bromide which was used as a standard (124). These results, however, have failed to be confirmed (197, 231, 239). Varied syntheses of complex quaternary ammonium salts have been reported from the laboratory of Niederl but physical and biological data on these compounds are meager.

c. Non-Ionic Surface Active Agents

Comprehensive reviews of methods of preparation, properties, and applications of non-ionic surface active agents have recently been published (66). Little information was found, however, which would relate the structure of these compounds and their activity.

[4] A brief summation of this work is presented by Westphal and Jerchel (239).

INTERACTION BETWEEN PROTEINS AND SURFACE ACTIVE AGENTS

Studies of the interaction of surface active agents and proteins are of importance in providing a potential theoretical basis applicable to the interaction of surface active agents with other, more complex, biological systems. It has been found that these interactions may result in precipitation, complex formation, and denaturation.

Precipitation. Protein and surface active agents, upon interaction, may precipitate from solution. This precipitation is dependent upon such factors as the length of the paraffinic carbon chain of the surface active agent, the pH, the mass ratio of surface active agent to protein, temperature, and ionic strength (187).

The relative effectiveness of compounds in precipitating crystalline lactalbumin and egg albumin has been determined for several homologous series, such as sulfate esters of primary alcohols, sulfonates of diesters of succinic acid, and sulfonates of benzene ring compounds (144). Under constant conditions of temperature, acidity, and concentration, sulfates and sulfonates containing less than 8 carbon atoms in their paraffinic chain were ineffective precipitating agents. Increasing the length of the chain above 8 carbon atoms enhanced the precipitating effectiveness with each additional carbon until a maximum amount of precipitate per mole of reagent was reached. Further lengthening of the carbon chain was without effect. It may be well to point out that compounds with paraffin chains of 8 carbons or less do not have a marked tendency to form micelles (e.g., see figure 7). This increased efficiency of the higher molecular weight sulfates and sulfonates as protein precipitants is similar, in outline, to the increased affinities of these same anions for wool proteins as demonstrated in extensive titration data (220, 221).

With surface active agents of carbon chain length appropriate for effective precipitation, it has been reported that cationic surface active agents precipitate protein only at pH values alkaline to the isoelectric point of the protein (117). Conversely, anionic surface active agents have been found to precipitate protein only on the acid side of the isoelectric point of the protein (167, 187). Indeed, it has been suggested that rough estimations of the isoelectric point of a protein may be made by noting the lowest pH at which the protein is precipitated by a cationic surface active agent[5] (92).

With other factors kept constant, the mass ratio of surface active agent to protein has been found to be a decisive factor in precipitation (187). In their studies of the dodecyl sulfate-horse serum albumin system, Putnam and Neurath (187), keeping the protein concentration constant and varying the concentration of surface active agent, found that they could distinguish three separate regions. In the first region, that of protein excess, the weight ratio of surface active agent

[5] While this paper confines its discussion to estimation of the isoelectric point by determination of the most acid pH at which a cationic surface active agent will cause precipitation, there is no reason why the converse situation, determination of the most alkaline pH at which an anionic surface active agent will cause precipitation, could not be used equally well.

to protein is less than 0.2 and the protein is incompletely precipitated. In the second region, the equivalence zone, the weight ratio of surface active agent to protein falls between 0.2 and 0.4 and complete precipitation of the protein is accomplished. In the third region, that of surface active agent excess, the weight ratio of surface active agent to protein exceeds 0.4 and any precipitate initially formed may be partly or completely dispersed on shaking. This effect of high concentrations of surface active agents has been generally observed and, indeed, can prevent the precipitation of denatured protein at the isoelectric point or the precipitation of protein denatured by trichloracetic or tungstic acid (5).

Complex Formation. While the interaction of proteins and surface active agents to form precipitates has been shown to be limited to regions in which these compounds bear charges of opposite sign, it should not be inferred that there is no interaction when both are similarly charged. Studies of such physical properties as electrophoresis, viscosity, and diffusion have demonstrated that interaction and complex formation may occur when both reactants bear the same net charge (e.g., an anionic surface active agent forming a complex with a protein on the alkaline side of its isoelectric point) (135, 160, 186, 188, 189). Electrophoretic studies (135) of the interaction of crystalline egg albumin and an anionic compound, alkylbenzene sulfonate, at pH 6.5 have demonstrated that up to a weight ratio of surface active agent to protein of 0.3 a complex is formed, the composition of which remains constant at 1 part by weight of surface active agent to 3 parts by weight of protein, and any excess of protein migrates as a separate boundary. When the weight ratio of surface active agent to protein exceeds 0.3 the electrophoretic mobility is proportional to the composition but intermediate between the mobilities characteristic of the protein and surface active agent. Similarly, the interaction at pH 6.8 of crystalline horse serum albumin and the anionic compound, dodecyl sulfate, has resulted in the formation of two discrete complexes, the electrophoretic mobilities of which are intermediate between those for the dodecyl sulfate and the horse serum albumin (189). The first complex corresponds to a weight ratio of 0.22 grams sodium dodecyl sulfate per gram of albumin while the second complex corresponds to a weight ratio of 0.45. Any excess surface active agent migrates as an independent boundary. Viscosity and diffusion studies of the same system confirm these results (160).

Reference to our discussion of precipitation will show that the minimum and maximum weight ratios of surface active agent to protein for complete precipitation of horse serum albumin by sodium dodecyl sulfate are identical with the weight ratios required for the formation of the two complexes observed electrophoretically.

Denaturation. We have seen, thus far, that interaction of surface active agent with protein may, with appropriate mass ratios of reactants, result in the formation of discrete stoichiometric complexes. In the pH range in which the components are oppositely charged precipitation may occur. Complex formation is, however, independent of pH over a wide region.

In addition to precipitation and complex formation, surface active agents have a powerful denaturing effect upon proteins (5). Hemoglobin and egg albumin,

at their isoelectric points, were found to be denatured by surface active agents with the denatured isoelectric protein kept in solution.[6] The most striking observation in these experiments was the relatively minute amount of surface active agent necessary to accomplish denaturation. For example, in the denaturation of beef methemoglobin in neutral solution, 0.0008 M duponol PC[7] denatured rapidly, in contrast to 8 M urea which took a considerable amount of time.

Egg albumin, a protein upon which many previous studies of denaturation have been reported, demonstrates the same proportion of sulfhydryl groups liberated by duponol PC as by guanidine hydrochloride (6). Expressed as cysteine, the sulfhydryl groups liberated in egg albumin denatured by either of the above compounds is 1.24 per cent which may be compared with the figure of 1.41 per cent reported by Hess and Sullivan (80) as the total cysteine content of egg albumin. The maximum proportion of sulfhydryl groups liberated by the surface active agent from egg albumin, 1.24 per cent, exceeds that liberated by urea, 0.96 per cent (154), or heat coagulation, 0.58 per cent (155). The latter two methods evidently fail to liberate the total number of sulfhydryl groups in the protein.

While the liberation of certain reactive groups, which are initially inert, is a widely studied manifestation of protein denaturation, there are other chemical, physical, and biological differences between native and denatured protein which have been demonstrated as a result of interaction with surface active agents. Solutions of globular proteins possess a higher viscosity in the presence of neutral denaturants, thought to be due to an unfolding and consequent greater asymmetry of the protein molecule (159). Both cationic and anionic surface active agents produce a large increase in the relative viscosity of serum albumin which is independent of pH but varies with the mass ratio of surface active agent to protein (160). In agreement with the methemoglobin experiments cited previously, these viscosity studies demonstrate that surface active agents produce changes comparable to those produced by other neutral denaturants but at much lower concentrations. Thus, similar alterations in the intrinsic viscosity of serum albumin are brought about by 8 M urea and 0.17 M sodium dodecyl sulfate, by 8 M guanidine hydrochloride and 0.28 M sodium dodecyl sulfate (160). Some of the other manifestations of protein denaturation which have been observed as a result of the presence of surface active agents include: a less ready crystallizing tendency of the protein after removal of the denaturant (187), alteration of x-ray diagram, indicating change from corpuscular to fibrous arrangement (166), alteration of molecular weight (153, 215), and loss of specific biological activity (13, 14, 215). Serum proteins regenerated after denaturation by urea or guanidine have been shown to retain the immunological specificity characteristic of the native protein, although possessing a decreased antigenic activity (50, 150). However, similar studies with proteins denatured by surface active agents are lacking.

[6] This solubilization of the denatured protein is valuable in enabling titration of the liberated sulfhydryl groups in homogeneous media.

[7] A mixture of the C_{10} to C_{18} alcohol sodium sulfates.

It will be noted that the discussion of the interaction of surface active agents and proteins has, thus far, been limited to the ionized agents. This is natural since the phenomena which we have reviewed have been primarily the consequences of electrostatic interaction. However, it should not be forgotten that another type of surface active agent remains: the non-ionic. Relatively little data exist in the literature concerning the interaction between this type of compound and proteins. No evidence could be found by electrophoresis (134) for any interaction or complex formation between either native or heat denatured egg albumin and a polyether alcohol. These results are consonant with the hypothesis of strong polar groups in surface active agents being requisite to interaction with proteins. This lack of interaction of non-ionic surface active agents and proteins has important consequences since it allows their coexistence in solution with maintenance of altered physical characteristics such as lowering of surface tension, solubilization, etc., but divorced from such biologically deleterious effects as denaturation.

INTERACTION BETWEEN SURFACE ACTIVE AGENTS AND ISOLATED BIOLOGICAL SYSTEMS

Enzymes

Studies of the interaction of surface active agents and enzymes are meager. In common with other proteins, such enzymes as pepsin, urease, yellow ferment, and catalase are precipitable only when their electrical charge is opposite in sign to that of the surface active agent (117, 187). The activity of urease and phosphatase has been found to be only slightly inhibited by alphasol Ma[8] (148), but, since the enzyme concentrations were not given and only a single concentration of surface active agent was used, evaluation of these results is difficult. Freeman *et al.* (55) have demonstrated that complete inhibition of enzymatic activity of amylase, lipase, and pepsin could be effected by an anionic surface active agent (an alkyl-aryl sulfonate, not further identified) while partial inhibition was obtained with trypsin and phosphatase. In agreement with the low concentrations of surface active agent previously shown to be sufficient for denaturation, those complete inhibitions require solutions of only 0.01 M or less surface active agent.

The activity of crystalline trypsin can be diminished by interaction with soaps, and complete inhibition is achieved in the presence of sufficient soap (169). Soaps of the same paraffin chain length (C_{18}) but differing in the number of double bonds were equal in their ability to inhibit tryptic activity. Partial reversibility of this inhibition occurred when the soap was precipitated from the soap-enzyme-substrate mixture by addition of calcium chloride. It can be calculated from the data presented in this paper that the mass ratio of soap to protein necessary for complete inhibition approximates 2.5.

In what may well be a process analogous to the dissociation of conjugate proteins, anionic surface active agents can activate protyrosinase to produce active tyrosinase (3, 24). When an homologous series of alkyl sulfates was tested,

[8] Dihexyl sodium sulfosuccinate.

optimal activating efficiency was found in the 12 to 16 carbon range. Attempts to reverse this activation by removal of the surface active agent from the field of action have not been successful (25). Cationic surface active compounds, however, were not effective as protyrosinase activators (4).

The absence of interaction between non-ionic surface active agents and protein has been mentioned previously. Advantage has been taken of this situation to formulate a method for the determination of lipase activity (7). Utilizing as a substrate the non-ionic compound, tween 20,[9] which is completely soluble in water, lipase activity can be determined in a one-phase system instead of the two-phase emulsion previously required.

Toxins

Bacterial exotoxins have properties which have, for a long time, caused them to be identified with proteins. This hypothesis has been greatly strengthened by the isolation, in recent years, of several toxins as essentially pure, highly active proteins (diphtheria (47, 168), tetanus (176), botulinus, type A (1, 126), and botulinus, type B (125)). It is reasonable to expect, therefore, that these toxins upon interaction with ionic surface active agents, will be subject to alterations similar to those exhibited by other proteins. Like enzymes, toxins furnish a potent biological activity for measurement in addition to the physical properties susceptible to measurement in other proteins.

Tetanus toxin has been inactivated by bile salts and soap (131, 132, 234) and by the cationic compound zephiran[10] (158). Similarly, diphtheria toxin may be inactivated by soaps (17, 19, 132) and the salts of fatty acids (205). The lower members of the fatty acid series produced little inactivation, but, beginning with the acid containing 8 carbon atoms in the paraffinic chain, a destruction of toxic, flocculating, and immunizing properties is produced by solutions as dilute as 0.01 M. The lecithinase of *Clostridium welchii*, which is probably identical with the specific alpha toxin, is readily inactivated by sodium dodecyl sulfate (142).

While it has been claimed that tetanus and diphtheria toxins detoxified by sodium ricinoleate are excellent antigens (130), contrary results have also been obtained (205).

Erythrocytes

Surface active agents, in contact with biological cells, frequently cause cytolysis. This has been long recognized in conjunction with red blood cells where the hemolytic effect of such natural surface active compounds as the bile salts and saponins have been the subject of many investigations (179). Since the introduction of synthetic surface active agents, several observations of their hemolytic properties have been recorded (5, 21, 83, 94, 211). The hemolytic capacity of an homologous series of anionic compounds has been found to vary with chain length (83, 180), with optimal efficiency, in the sulfated alcohols, at 14 carbon atoms,

[9] A polyoxyalkylene derivative of sorbitan monolaurate.

[10] A mixture of the C_8 to C_{18} dimethyl benzyl ammonium chlorides.

where a 1:100,000 dilution is lytic within a few minutes. Plasma and its protein and lipid components are capable of inhibiting these hemolytic effects.

At sub-lytic concentrations, surface active agents still produce changes as evidenced by alteration of erythrocyte shape from discoidal to spheroidal. These disk to sphere transformations may be produced at surface active agent concentrations only 1/10 of that necessary for hemolysis. For the most efficient alcohol sulfates, calculation of erythrocyte surface area per molecule of lysin shows that sphering may be accomplished even though there are not sufficient molecules of lysin in the system to cover the erythrocyte surfaces with a continuous monolayer (180).

Within a restricted number of homologous anionic compounds, a relationship between surface activity and hemolytic activity is found (83). When the scope of the chemical structures tested is broadened, however, this relationship no longer holds true. Ionic compounds with like surface activity are found to have widely different hemolytic activities and certain non-ionic compounds are found to be non-hemolytic at concentrations which demonstrate surface tension properties equivalent to those of anionic and cationic compounds which are intensely hemolytic (65). This lack of hemolytic activity by non-ionic compounds lends support to the view of Ponder (180) that surface active agents form complexes with lipid, lipoprotein, and protein components of the erythrocyte ultrastructure as a stage in the hemolytic process. That this interaction of surface active agents and erythrocytes may operate to alter the normal ionic permeability of the cellular membrane to produce a so-called "colloid osmotic hemolysis" has recently been indicated (240, 241).

Many of the above mentioned experimental observations on the interaction of erythrocytes and surface active agents and the theoretical inferences as to their mechanism will find analogies in the coming discussion of the interaction of bacteria with similar compounds.

Bacterial Growth

Alteration of surface tension may produce marked effects on the growth of microörganisms. The bulk of the studies that were completed before the advent of modern synthetic surface active agents utilized such substances as soaps, bile salts, and saponin as surface tension depressants (for literature of this early work see 56, 133).

It is well to recognize that culture media ordinarily have surface tension values lower than that of water, due to their protein content. Thus Marshall (149), investigating a number of routinely used culture media, found their surface tensions in the order of 45 to 54 dynes/cm as compared with 72 for water.

Reducing the surface tension of the medium below 45 dynes/cm may well change the character of growth of microörganisms. In media of low surface tension, *Bacillus subtilis* and *Mycobacterium tuberculosis* may cease pellicle formation and grow submerged and dispersed throughout the bulk of the liquid, and some anaerobes, particularly *Clostridium tetani*, have been reported to grow aerobically (128, 129). It is difficult to ascribe this last effect to surface activity

and, as a matter of fact, doubt exists as to the accuracy of the observation (64). In addition to alteration of the character of growth, culture media of low surface tension may depress or prevent growth. Pneumococci[11] and streptococci have been shown to grow poorly in media, the surface tension of which was lower than 50 dynes/cm (9, 127, 178) while 46 dynes/cm has been found as a limiting surface tension for the growth of *Bacillus anthracis* (127). Growth of *Mycobacterium tuberculosis*, human type, was inhibited at surface tensions of 42 dynes/cm while the avian type and *Mycobacterium phlei* were inhibited at surface tensions below 30 dynes/cm (2). Bacteria flourishing in the gastrointestinal tract seem to be resistant to the deleterious action of surface active compounds in their culture media (127, 218, 245). The following order of sensitivity of growth to depression of surface tension has been determined by Wolf (245). The list commences with the most sensitive.

Corynebacterium diphtheriae
Clostridium welchii
Erysipelothrix murisepticus
Bacillus subtilis
Clostridium sporogenes
Bacillus proteus
Eberthella typhosa
Pseudomonas pyocyaneus
Escherichia coli
Salmonella paratyphosus A
Salmonella paratyphosus B

It is well to consider these early experiments on the relation of surface tension to bacterial growth with a good deal of reserve. Most of the observed effects are probably more ascribable to specific effects of the surface active agent used than to alteration of surface tension *per se*. As was seen in our discussions of the interaction of surface active agents with proteins, or with erythrocytes, the ionic nature of the surface active compound is an important factor in the results obtained. Non-ionic compounds do not interact with proteins even though they possess surface tension depressant properties similar to the ionic compounds which, under similar circumstances, may cause precipitation, complex formation and denaturation. With the erythrocyte, too, some non-ionic compounds were non-hemolytic despite surface tension alterations which, in the case of the ionic compounds, were far above the minimum necessary for complete hemolysis. That similar factors are of importance in determining the results of the interaction of surface active agents and bacterial cells may be concluded from the more recent work, which shows that, dependent upon the nature of the surface active agent used, a whole gamut of results may be obtained from bactericidal activity at the one extreme to facilitation of submerged, disperse growth at the other.

Studies of the effects of a variety of cationic and anionic surface active agents on the respiration and glycolysis of both gram positive and gram negative micro-

[11] Synthetic surface active agents will cause pneumococci to lyse (18) and may be used instead of bile salts in the differentiation of pneumococci from streptococci (73).

organisms have demonstrated the superior effectiveness, at physiological pH, of the cationic compounds as inhibitors[12] of bacterial metabolism (10). While the cationic compounds are equally effective against both gram positive and gram negative organisms, the anionic compounds show a selective activity against the gram positive organisms. A similar selectivity is illustrated in the effects of anionic compounds on the lactic dehydrogenase and cytochrome systems of suspensions of *Staphylococcus aureus* and *Escherichia coli* (163). As one departs from physiological pH the cationic compounds become more active in the alkaline range, and the anionic in the acid range. In common with the previously discussed effects of chain length on the activity of surface active agents, maximal effectiveness for inhibition of bacterial metabolism has been demonstrated in the C_{12} to C_{16} range.[13]

An outstanding current application of surface active agents in bacteriology has been the discovery of the effect of tween 80, a non-ionic, fatty acid ester type coupound[14], in promotion of submerged and diffuse growth of tubercle bacilli (44). Although virulent tubercle bacilli are among the least fastidious of pathogenic microörganisms in their metabolic requirements, *in vitro* growth of these organisms has necessitated relatively large inocula and long periods of incubation with all the disadvantages inherent in the resultant heterogeneity of the cellular population.

Utilizing a modified Kirchner medium Dubos and Davis have demonstrated that addition of tween 80, up to an optimal level of 0.1 per cent, greatly enhanced the rate and abundance of growth of an avian (Kirchberg) strain. Corresponding experiments with a human strain (H37RV) are more difficult to assess from the standpoint of the amount of growth due to the granular masses of the control cells as compared with the isolated cells and microscopic loose clumps prevailing in the culture grown in the presence of tween 80. This ability to facilitate submerged and diffuse growth of mycobacteria in liquid media seems resident in the surface active properties of the substance since non-surface active oleic acid esters are not effective.

While tween 80 promotes the diffuse submerged growth of tubercle bacilli, early experiments required relatively large inocula. This inhibitory effect against small inocula has been found to be due to small amounts of unesterified oleic acid[15] present either in the original commercial tween 80 or formed as a result of hydrolytic action of biological material on this product. Chemical

[12] At concentrations below those effective in causing inhibition of bacterial metabolism an actual stimulation of metabolism may, sometimes, be found (10). This stimulatory effect has been found much more frequently with anionic than with cationic surface active compounds.

[13] Growth retardation and inhibition may, however, occur at concentrations of surface active agent which have little or no effect on cellular energy production (201).

[14] A polyoxyalkylene derivative of sorbitan monooleate.

[15] Although free long chain fatty acids may be inhibitory to many microörganisms, the same compounds esterified or formed into complexes with native serum albumin may have these toxic effects minimized or eradicated and the resultant atoxic compounds may actually enhance the growth of certain bacteria (43).

extraction of this unesterified fatty acid (35) or its removal from the field of action by complex formation with such substances as serum albumin[16] allows successful growth from truly minimal inocula of 2 or 3 cells (36, 37).

Tubercle bacilli grown diffusely in submerged liquid culture in the presence of tween 80 retain their characteristic morphology and staining (45). This has been true even in cultures maintained for over a year with repeated transfers in liquid media containing this product. Return of these cultures at any time to standard media (e.g., egg yolk media) causes reversion to a granular type of growth. In addition to retention of characteristic morphology and staining, pathogenic mycobacteria grown in the presence of tween 80 are extremely virulent for mice, guinea pigs, and chick embryos and are able to elicit the production of agglutinins for the homologous cultures. It is well to caution, however, that tween 80 in conjunction with the tubercle bacilli may act as a haptene, thus serving as a potential source of confusion in immunological analysis.

Successful applications, using both fluid and solid media incorporating this surface active agent, have been made to studies of the pathogenesis of experimental tuberculosis in mice (174), the morphological characteristics correlated with the virulence of mamalian tubercle bacilli (151), the rapid cultivation of tubercle bacilli from pathological material (54), the demonstration of naturally occurring streptomycin resistant variants of the tubercle bacillus (233), and the revelation of the antibiotic activity of subtilin against *Mycobacterium tuberculosis* (247).

Stimulated by the obvious advantages of having water-soluble non-toxic solutions of oleic acid in the form of tween 80, Williams *et al.* (243) have made use of this compound in studying the metabolism of lactic acid bacteria.

Bacteriostatic and Bactericidal Activity

Current interest in the application of surface active agents, other than soaps, as bactericides stems from the work of Domagk (38) although scattered previous indications of the bactericidal possibilities of these compounds is available in the literature (75, 89, 90, 91). We have already discussed the role of such factors in chemical structure as alkyl chain length, nature of the anion, nature of the central atom, and biological specificity in determination of the bactericidal properties of quaternary ammonium salts.

Since it has been widely observed that in any homologous series of surface active compounds the bactericidal efficiency and the surface depressant properties increased with increasing alkyl chain length, it has been natural to consider the possible relation between surface activity and bactericidal activity. While all surface active compounds which are efficient bactericides have been found to possess a marked ability to reduce surface tension, the converse is not necessarily true. Extensive experimental evidence of this may be found in surface tension determinations on a series of salts of high molecular weight aliphatic acids which had been previously studied for their bactericidal effects on *Mycobacterium*

[16] Even purified serum albumin may contain some lipase which may be inactivated by heating at 56 C.

leprae and other acid-fast bacteria (216, 217). Similar conclusions have been reached from a study of various commercial surface active compounds (59). Further confirmation may be inferred from the demonstration that non-ionic surface active agents, although effective as depressants of surface tension have little or no effect on bacterial metabolism and are not bactericidal (12, 85).

In our discussion of the inhibitory effects of surface active agents on bacterial metabolism we saw that cationic compounds, at physiological pH, are more effective than anionic (10). In addition, cationic compounds possess equal effectiveness against both gram positive and gram negative organisms while the anionic compounds show a selective activity against gram positive organisms. In general, the results of studies of inhibition of bacterial metabolism and bactericidal activity of surface active agents parallel each other, although quantitative correlation has not always been obtained (11)[17]. With respect to the selective activity of anionic and cationic surface active compounds, similar conclusions may be drawn from studies of their bactericidal activity[18] as were obtained from studies of their inhibition of bacterial metabolism (11, 23, 33).

In these comparative studies of the effects of surface active agents on bacterial metabolism and viability, some interesting observations were made on a non-quaternary type of cationic compound (10, 11). These compounds are alkyl esters of amino acids (e.g., the lauryl ester of alpha-amino isobutyric acid). When they possess an optimal number of carbon atoms in the amino acid they are effective in inhibiting bacterial metabolism but quite ineffective as bactericides. No explanation of this discrepancy is apparent. Unfortunately, there seems to be no other published work on the biological activity of these compounds to which these results can be compared.

As one departs from a neutral pH, the cationic compounds tend to become more efficient bactericides in more alkaline solutions and less efficient in acid solutions with the reverse situation holding true for the anionic compounds (48, 59, 60, 61, 84, 190). Cetyl pyridinium chloride, however, seems to maintain an even level of bactericidal activity over the pH range of 2 to 10 (190) with no explanation apparent for its individuality in this respect. This dependence of the bactericidal efficiency of surface active agents on pH is analogous to the relationship obtaining when inhibition of bacterial metabolism is used as a criterion of activity (10) and has many points of similarity to the effects of pH on the bactericidal activity of acidic and basic dyes (219). This pH dependence also means that the relative bactericidal superiority evidenced by cationic compounds when compared, at neutral pH, with anionic compounds may not only change quantitatively as the pH is altered but, under appropriate conditions, anionic compounds may display a marked superiority over cationic. Table 4

[17] This may be largely due to the methods involved. In studying inhibition of bacterial metabolism one measures the actual per cent inhibition as compared with the control, whereas in bactericidal studies the presence or absence of viable cells is measured without any intermediate possibilities.

[18] However, in the bacteriostatic range a greater activity against gram positive bacteria may be shown by cationic compounds (107).

with figures selected from data of Gershenfeld and Milanick (59) illustrates this by a comparison between aerosol OT (an anionic compound) and triton K-12 (a cationic compound).

In common with other chemical bactericides, the efficiency of surface active agents is affected by the presence of organic matter. Whereas, under standardized experimental conditions, cetyl trimethyl ammonium bromide displays a phenol coefficient of 1200 in the absence of added organic matter, this figure decreases to 380, 225, 150, and 62 respectively in the presence of 2, 5, 10, and 20 per cent added horse serum (84). Further illustration of the effect of the presence of protein material upon the bactericidal efficiency of cationic compounds against a variety of microörganisms may be found in table 5. When one keeps in mind the complexes formed by the interaction of proteins and surface actve agents this inhibition of bactericidal activity by the presence of added protein is not at all surprising. In addition to proteins, the lipids display

TABLE 4

Bactericidal activity of an anionic and cationic surface active compound at varying pH

Adapted from (59)

COMPOUND	MAXIMAL DILUTIONS KILLING STAPHYLOCOCCUS AUREUS IN 10 MINUTES BUT NOT IN 5, AT 37 C					
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
Aerosol OT	1:37,500	1:32,500	1:5,000	<1:100	—	—
Triton K-12	<1:100	1:150	1:400	1:900*	1:10,000	1:20,000

* This test performed at pH 7.2.

a marked ability to counteract the bactericidal effects of surface active compounds (58). Phospholipids added before or simultaneously with surface active agents decrease or prevent the inhibitory effects on bacterial metabolism displayed by surface active compounds (12). Similar protection by phospholipids is evidenced when the bactericidal activities of surface active compounds are studied. Since the phospholipids possess a characteristic polar-nonpolar structure, they are surface active. Although they do not by themselves alter bacterial metabolism it is conceivable that, by adsorption to the bacterial surface, they alter its nature. Indirectly, this view is supported by such evidence as the retention of the protective effects of phospholipids by bacteria which have been exposed to these compounds and then washed. Then too, phospholipids added subsequent to the surface active agent are without protective effect (12). Ordinary fats, such as butterfat, (possibly due to their phospholipid content), have been demonstrated to protect bacteria against the bactericidal activity of surface active agents (194).

Methods of Evaulation. While there is little doubt that certain surface active agents, as represented by the quaternary ammonium and pyridinium salts, are highly efficient bactericides, concern has been expressed as to the validity and

significance of some of the high phenol coefficients reported in the literature. This concern has been engendered both by thoughts of the general inapplicability of the phenol coefficient test to determination of the bactericidal efficiency of non-phenolic compounds which may be totally unlike phenol in chemical and bactericidal properties (27, 28, 100, 198) and, more specifically, by reason of

TABLE 5

Germicidal activity of cetyl pyridinium chloride aqueous solution from Quisno and Fotor (190)

ORGANISM	NO. STRAINS TESTED	AVERAGE CRITICAL KILLING DILUTION IN TERMS OF ACTIVE INGREDIENTS AT 37 C*	
		No serum	10 % bovine serum
Staphylococcus aureus	5	1:83,000	1:12,500
Staphylococcus albus	1	1:73,000	1:12,000
Streptococcus viridans	1	1:42,500	1:12,000
Streptococcus hemolyticus	2	1:127,500	1:17,000
Neisseria catarrhalis	2	1:84,000	1:13,000
Diplococcus pneumoniae I	1	1:95,000	1:14,000
Diplococcus pneumoniae III	1		1:20,000
Pseudomonas aeruginosa	2	1:5,800	<1:1,000
Klebsiella pneumoniae	2	1:49,000	1:5,500
Corynebacterium diphtheriae	1	1:64,000	1:14,000
Mycobacterium phlei	1	1:1,500	1:1,000
Eberthella typhosa	5	1:48,000	1:3,000
Escherichia coli	2	1:66,000	<1:1,000
Proteus vulgaris	2	1:34,000	1:2,000
Shigella dysenteriae	1	1:60,000	1:5,000
Shigella paradysenteriae (Flexner)	2	1:52,000	1:3,500
Shigella paradysenteriae (Hiss)	1	1:49,000	1:2,000
Shigella sonne	2	1:68,000	1:6,500
Lactobacillus acidophilus	1		1:16,500
Brucella abortus	1		1:19,500
Trichomonas vaginalis	1		1:3,000†
Candida albicans	1	1:37,000	1:3,500
Cryptococcus neoformans	1	1:61,000	1:6,000
Trichophyton mentagrophytes	1	1:36,000	1:3,000
Microsporum canis	1	1:34,000	1:5,000

* The critical killing dilution is defined as the highest dilution of germicide which will kill in 10 minutes but not in 5.

† Twenty-five per cent human serum.

the individualistic properties of cationic surface active agents (22, 103, 104, 199). Factors in the Food and Drug Administration method of testing germicides (203) against which criticisms have been leveled include the following features of especial interest in testing surface active agents:

(*a*) Inconstancy of the culture medium.

(*b*) Failure to distinguish between bacteriostatic and bactericidal action.

(*c*) Lack of uniformity in size of transfer in preparing subcultures.

(*d*) Presence of organic matter.

Variability of the peptone component of the medium used in determination of the phenol coefficient may have serious consequences in the results obtained (26, 190, 200, 248). Beyond the obvious metabolic importance of the identity and reproducibility of the peptone content, it is important to consider the variable amounts of phospholipids possible in peptones prepared from natural materials. Phospholipids, as previously stated, are capable of interfering with bactericidal activities of cationic surface active agents (12). Recognition of this problem has caused synthetic and semi-synthetic media to be recommended (102, 246).

Distinction between bactericidal and bacteriostatic effects represents another problem which the cationic surface active agents share with other bactericidal compounds of high efficiency, such as the heavy metal salts. Methods based both on dilution and the presence of specific antagonists have been devised to meet this problem. Subculturing of organisms, which have been subjected to the action of the experimental bactericide, into large volumes of culture media or even retransferring a loopful of the first subculture into a second tube of culture medium has been suggested to minimize, by dilution, the effect of any surface active agent carried over (103, 212). Neutralization of any surface active agent carried over in subculturing may be accomplished by incorporation of lecithin (193) or sodium stearate (183) into the subculture medium. A semi-solid broth containing lecithin has also been suggested (8) which allows discrete colony growth and detection of partial inhibition.

Since the presence of surface active agents in the medium alters the surface tension, the standard loopful used in subculturing will contain varying volumes of media (and therefore varying numbers of organisms). A volumetric transfer has been recommended (183, 226).

It is universally agreed that the presence of organic matter lowers the efficiency of bactericides. It is important to keep in mind, however, that, due to such factors as the concentration, reactivity, and adsorbability of the bactericide, the presence of a given amount of organic matter may affect the efficiency of chemically different types of bactericides to varying degrees. The cationic surface active agents are used in low molar concentration so that removal of a given number of molecules from the field of action will have a proportionately greater effect upon them than on many bactericides of lower efficiency (101). The presence of phospholipids (12) and fats (194) inhibits the bactericidal activity of cationic surface active agents, in addition to proteins forming complexes with them. The high degree of surface activity of these compounds while advantageous from the standpoint of facilitating tissue penetration is disadvantageous in increasing the tendency of surface active compounds to be adsorbed out of solution. The effect of the presence of 10 per cent bovine serum on the average critical killing dilution of cetyl pyridinium chloride on a variety of microörganisms is illustrated in table 5.

An interfering factor of obvious importance in testing bactericides, but one difficult to evaluate quantitatively, is the tendency for organisms exposed to quaternary ammonium compounds to agglomerate and to adhere to the walls

of the containing vessel (40, 103, 105, 140, 191). This agglomeration and adhesion leads to obvious difficulties in obtaining uniform exposure or representative sampling of organisms and is a contributing factor to the inconsistencies universally reported in the testing of quaternary ammonium compounds by the phenol coefficient method.

Modifications of the phenol coefficient test which have been recommended for use with surface active agents include the use-dilution method, where actual concentrations recommended for sanitary use are tested (146, 199), a glass slide technique utilizing a 99.9 per cent endpoint (95), a combination of the phenol coefficient test with plate count and swab count (30), a semi-micro adaptation of the phenol coefficient test in which the entire volume rather than an aliquot of the bacterial suspension-bactericide solution is cultured (103), and a filter paper transfer technique (103, 104). Agar-plate or cylinder-plate methods of testing bactericidal activity, while widely used with chemotherapeutic agents, have not been found applicable to quaternary ammonium compounds (173, 192), possibly due to the inability of ionic aggregates to pass through the agar network (173) or due to physical adsorption of the cationic compounds on the agar (192).

An attempt has been made to avoid the difficulties and inconsistencies of the *in vitro* methods of evaluation by resort to *in vivo* testing. The developing chick embryo has been used by inoculation of the chorioallantoic membrane with *Staphylococcus aureus*, treatment of the infected membrane over a period of five days with the bactericide under test, followed by culture of the membrane for estimation of the remaining organisms (68, 69). Another method utilizes a virulent strain of *Salmonella typhimurium* as the test organism and follows the procedure of the phenol coefficient test with the modification of subculturing the treated organisms by inoculation into mice rather than culture broth. Endpoints were determined by recovery of organisms from the heart blood of the infected mice (98). The technique of Nungester and Kempf (161), where the tail of a mouse is immersed in the bacterial culture-bactericide solution and then a portion of the tail transplanted into the abdominal cavity of the mouse, has also been applied (175). These extensive efforts in the methodology of evaluation of the bactericidal efficiency of the cationic surface active agents have yet to yield a method which is unequivocal and universally accepted by workers in the field.

If it is proposed to utilize bactericides in contact with animals tissues it is, of course, important to have a measure of the relative toxicity of the bactericide to pathogenic bacteria and to tissue. A number of workers have measured toxicity indices (i.e., ratio of bacterial toxicity to tissue toxicity), where the toxicity to bacteria has usually been determined by the phenol coefficient test and the toxicity to tissue by the effect of the bactericide upon the phagocytosis of artifically opsonized staphylococci (82, 236, 237), or by the effect on the chick embryo (63, 244). In general, the toxicity index has been found to be lower for bactericides of the surface active quaternary ammonium type than for compounds dependent upon chlorine, mercury, permanganate, alcohol, or formaldehyde for their action (235).

Microbicidal Activity. The activities of several commercial quaternary ammonium salts against a variety of bacteria and fungi have been reported in the literature (e.g., 46, 96, 175, 190). As an illustration[19] of the bactericidal and fungicidal spectrum of these compounds the activity of cetyl pyridinium chloride has been chosen as an example and tabulated (see table 5).

In addition to their utility as bactericides and fungicides, quaternary ammonium salts have been found useful as cysticides. Several cationic surface active agents have demonstrated efficiency in the destruction of water borne cysts of *Entamoeba histolytica* while anionic and non-ionic compounds were found of lesser utility (51).

Synergistic Activity. Surface active agents have also attracted interest in recent years because of their possibilities in potentiating or synergizing the activity of bactericides and fungicides of different chemical nature. Addition of anionic surface active compounds to phenol derivatives has, in several studies, increased their bactericidal efficiency 50 to 100 per cent (61, 164, 165, 227). Evidence has been obtained that this enhancement of bactericidal properties is primarily due to a synergistic action between the surface active compound and the undissociated phenols (164). Ordinary soaps, which are commonly used to emulsify phenolic bactericides of limited aqueous solubility, will actually tend to decrease the bactericidal efficiency of these phenolic compounds due to the alkalinity the soaps produce upon hydrolysis and the consequent decrease in the proportion of undissociated phenol present. Ordinary soaps are not usable in acid solutions due to the limited solubility of their anionic fatty acids. The synthetic anionic surface active agents, on the other hand, are soluble in acid solution and mixtures of phenolic bactericides and surface active agents can be formulated with the phenols present in the undissociated form. This allows a maximal bactericidal effect of the phenolic compound *per se* plus maximal synergistic effect of the added surface active agent. It has also been indicated that some synergistic effects can be demonstrated between surface active agents and mercurials, permanganate, and hexylresorcinol[20] (53, 61).

While discussing the synergistic effects of surface active agents used in conjunction with bactericides, it may be well to caution against the formulation of mixtures of anionic surface active agents and quaternary ammonium salts. Since the quaternary ammonium salts are cationic compounds they will combine electrostatically with the anionic surface active agents and may even precipitate out of solution, with a loss of bactericidal efficiency rather than the desired increase.

Theory of Antibacterial Activity. Certain speculations as to the probable mechanism of the high degree of antibacterial activity of surface active agents have been made. Perhaps the first of the series of processes involved in the interaction of surface active agents and bacteria is a reversible adsorption or

[19] Quaternary ammonium compounds, however, are not very effective in killing spores (41).

[20] This synergistic effect was not demonstrable when pH changes were not carefully controlled (62).

combination of ions of these compounds with bacteria. Evidence, by analogy, for this view may be obtained by reference to the demonstrated ability of bacteria to adsorb hydrogen ions and other cations from solution and to enter into ion exchange (137, 138). Further evidence is obtained from the dependence of the bactericidal activity of surface active agents on pH which operates to increase the activity of the surface active compound when the production of oppositely charged ions in the bacterial cell is favored (231). More directly applicable is the demonstration that the inhibitory action on bacteria of cationic surface active agents may be delayed by prior treatment of the bacteria with anionic surface active agents (42). Indeed, within certain time limits, this inhibitory action may be reversed by subsequent introduction of an anionic compound (231); in addition, there is reported a protective action on bacteria of the presence of a cationic compound, which is bactericidally relatively ineffective, in conjunction with an effective cationic compound. Presumably, this protective effect operates through shifting the adsorption equilibrium of toxic cation and bacteria through competition for reactive sites.

Recent work, however, has cast some doubt on the validity of these observations (107). Anionic compounds added after the exposure of bacteria to cationic compounds were shown not to be able to reverse the bactericidal effect. Repetition of the demonstration of competitive action of bactericidally ineffective versus effective cationic compounds was not attempted. While there seems to be little doubt of the ability of bacteria to act as ion exchangers and to adsorb ions of surface active compounds from solution, the role of this mechanism in the bactericidal activity of these compounds has yet to be clearly delineated experimentally.

One of the observations in studies of the effects of surface active agents on bacterial metabolism has been an upper limit to the percentage inhibition obtained (80 to 95 per cent) regardless of increase in concentration of surface active agent (10, 11). These data have been interpreted as evidence against the hypothesis that an enzyme of key importance exists which is very sensitive to surface active agents, since it might be expected that increasing the amount of such agent would give an excess capable of totally blocking metabolic activity (85).[21] As an alternative consideration, the possibility of disruption of some cellular membrane component with a consequent increase in permeability is suggested—a mechanism reminiscent of that associated with erythrocyte hemolysis. With this mechanism, intracellular constituents such as enzymes, ions, coenzymes, and metabolic intermediates would be released to the surrounding medium by the lytic action of surface active agents. This dilution of the intracellular contents would reduce to a low level the metabolic activity observed with no further effect to be expected upon addition of more surface active agent until the concentration was high enough to interfere by denaturation and inac-

[21] However, the possibility of alternate metabolic pathways should be considered e.g., metabolic reactions involving amino acids in which dehydrogenation reactions take place through the intermediary functions of various hydrogen acceptors, without the utilization of oxygen (206).

tivation of the enzymes present. In experimental support of this lysis mechanism of the function of surface active agents, chemical analyses have been made of the trichloracetic acid soluble phosphorus and nitrogen compounds released in the washings of staphylococci in the presence of various anionic, cationic, and non-ionic surface active agents (85). Whenever the nature and concentration of the surface active agent are adequate to be bactericidal, a leakage of nitrogen and phosphorus compounds from the cells is observed (85, 86). Confirmation of this lytic mechanism is found in studies of the release of specific amino acids from bacterial cells (57). Determinations of the release of lysine from *Streptococcus faecalis* showed that surface active agents (tyrocidin, CTAB, and aerosol OT) liberate lysine proportionate to the concentration of surface active agent used up to a level that is sufficient to liberate the total lysine of the bacterial cells. This lytic effect is quantitatively similar to the bactericidal effect of the same compounds. Non-surface active bactericides (penicillin,[22] acriflavin, sulfa drugs do not exhibit this lytic effect).

The two theoretical considerations presented above have emphasized the role of the bacterial surface in determining the results of interaction of surface active agents and bacteria. Within the bacterial cell there exist many compounds essential to metabolism and growth which are susceptible to interaction with surface active agents and consequent alteration of their specific metabolic activity. The ability of surface active agents, at concentrations of the same order of magnitude as were bactericidal, to precipitate proteins and, in the case of conjugated proteins, to cause dissociation, has impressed several workers (12, 117) as a possible mechanism of their antibacterial activity. This view has been challenged (85) on the basis of the quantitative differences in mass ratio of protein to surface active agent required for denaturation of ordinary proteins as compared with bactericidal effects. In view of the meagerness and roughness of the data thus far available, it would seem premature to disregard denaturation in theorizing on the mechanism of the antibacterial activity of surface active agents.

Interaction with Viruses

To the extent that virus activity may be considered resident in a protein structure, it might be expected that the conclusions drawn from studies of the interaction of proteins and surface active agents would be applicable to the interaction of viruses and surface active agents. In a limited way, this has been demonstrated with plant viruses (172). The bulk of the work on interactions of viruses and surface active agents, however, suffers both from the nature of the virus preparations and the mode of attack. Virus preparations, especially animal viruses prepared from tissue homogenates and chick embryo fluids, and protein-containing dilution fluids may carry over large amounts of non-virus compounds (proteins and phospholipids) which would greatly alter at least the quantitative aspects of the interaction of surface active agent and virus. In

[22] Compare, however, the recent revelation of a high degree of surface activity for the sodium salt of penicillin (77).

addition, most studies have been planned solely on the basis of the demonstration of ability or inability of the surface active agent to inactivate virus activity.

Studies of the purified plant viruses of tobacco mosaic, tomato bushy stunt, and potato "X" in the presence of the anionic compound, dodecyl sulfate, have demonstrated virus inactivation with a concomitant splitting of the nucleic acid from the protein (13, 14, 16, 215). These viruses varied in susceptibility to the action of the dodecyl sulfate, with potato "X" being most susceptible and tomato bushy stunt least susceptible. Within a rather narrow range of experimental conditions (pH, temperature, and dodecyl sulfate concentration), inactivation of tobacco mosaic virus without loss of serological activity can be demonstrated (16) and under these conditions anisotropy of flow is retained. With increased concentrations of dodecyl sulfate, however, more severe molecular changes are produced leading not only to loss of infectivity, but to abolition of serological activity and anisotropy of flow as well. Similar results are possible with the tomato bushy stunt virus although, in general, it is easier to demonstrate destruction of infectivity without loss of serological activity with the tomato bushy stunt virus than with the tobacco mosaic virus. Many aspects of the interaction of dodecyl sulfate and these plant viruses are similar to the effects of urea, as a denaturant, upon the same viruses (15).

In the interaction of cationic compounds with tobacco mosaic virus, aggregation paralleling inactivation has been demonstrated (172). The efficiency of cationic compounds in causing virus aggregation was proportional to the length of their alkyl chain, and concentrations necessary for precipitation of the virus were similar to those necessary for precipitation of such simpler proteins as egg albumin and lactalbumin.

Paralleling the investigations of inactivation of toxins by soap and bile salts, and the attempted use of these inactivated products as vaccines, many investigators have studied the effect of these same compounds on animal viruses with similar ends in view (20, 32, 79, 139, 177). Perhaps the investigations of Klein *et al.* (106) bring out most of the relevant points, other than immunogenic capacity, evident from interactions of animal viruses and surface active agents reported, thus far, in the literature. When anionic, cationic, and non-ionic surface active agents were allowed to interact with animal viruses and bacteriophages, the ability of a surface active compound of a given homologous series to inactivate was found to be proportional to alkyl chain length; a non-ionic surface active compound had no inactivating effect; and various viruses and phages differed from each other in susceptibility to inactivation. One of them, the gamma phage active against *Escherichia coli* B was highly resistant to all surface active agents used. Indeed, on the basis of these results, cationic surface active compounds have been recommended for the isolation of coli phage from sewage (97).

This difference in susceptibility of various viruses and phages to inactivation by surface active agents and the seeming inability of inactivation of some of them has been observed elsewhere (29, 214). Thus, Burnet and Lush (29) utilizing sodium desoxycholate, saponin, and sodium dodecyl sulfate as surface

active compounds found the following order of resistance to inactivation. The list is given in order of increasing resistance: herpes, louping ill, influenza, Sabin's B virus, pseudorabies, myxomatosis, fowl-pox, vaccinia, ectromelia, psittacosis. Psittacosis virus was found to be practically unaffected by the highest concentration of inactivating agents used. Three dysentery phages, two Salmonella phages, and two phages active against *Staphylococcus aureus* also showed no inactivation by dodecyl sulfate.

Although high dilutions of surface active agents may fail completely to inactivate the influenza virus (116) and on this basis have been recommended for freeing throat washings, egg fluid, or ground mouse lung from adventitious bacteria (115), many reports of the inactivation of influenza virus by higher concentrations of both anionic and cationic surface active agents exist (29, 108, 110, 214, 222, 223). In the case of the influenza and also the lymphocytic choriomeningitis virus, attempts to recover active virus by removal of the surface active agent were unsuccessful (224). The PR 8 strain of influenza virus, type A, rendered non-infectious by treatment with sodium oleate has been found to retain its immunogenic capacity unaltered (222, 223). Although the antigenic capacity of only this one strain was tested after inactivation by sodium oleate, various other strains of both type A and B influenza virus were shown to be inactivated by the same procedures. Inactivation of virus by surface active agents with retention of antigenic capacity has also been reported with the neurotropic strain of the yellow fever virus (52), but, on the other hand, immunization experiments with lymphocytic choriomeningitis virus inactivated with surface active agents were unsuccessful (224). Although there are early reports of the antigenic capacity of sodium ricinoleate inactivated poliomyelitis virus (112, 143), later work has not confirmed this (113, 162).

APPLICATION OF SURFACE ACTIVE AGENTS TO PROBLEMS OF SANITATION

The surface active agents of primary interest in application to problems of sanitation are the quaternary ammonium compounds which, we have seen, have outstanding bactericidal properties. A more concrete idea of the extent of current use of these compounds may be obtained from the latest government statistics (229) showing 1945 production in the United States to exceed 3 million pounds.

Quaternary ammonium compounds have several advantages for consideration as bactericides (191). At effective concentrations they are virtually odorless, tasteless, have low oral toxicity, and are relatively non-irritating to the skin. By virtue of their surface activity they penetrate and wet surfaces. As chemical compounds they are stable, non-corrosive, and neutral. The lack of taste and odor makes them especially desirable in the sanitation of food establishments.

In contrast to these advantages, it is well to keep in mind that the bactericidal efficiency of quaternary ammonium compounds is more markedly diminished by decrease in temperature or presence of organic matter than is true for phenolic compounds. Quaternary surface active compounds are cationic and as such have certain incompatibilities, among which might be mentioned soap

and anionic surface active compounds in general, sodium hexametaphosphate, sodium metasilicate, and acidic dyes. It is especially important to keep these incompatibilities in mind when compounding formulations designed to have high detergent as well as bactericidal properties. Since the bactericidal efficiency of most quaternary compounds is not constant over a wide pH range it is important also to evaluate the compounds at the pH actually to be encountered in particular sanitary applications.

Because of the considerable price differential between the quaternary ammonium and phenolic compounds, accurate evaluation of their relative bactericidal efficiency is most important in arriving at a decision as to their comparative merits in any practical situation. Unfortunately, as we have seen in our discussion of methods of evaluation of these compounds as bactericides, no single test method gives an unequivocal answer to this problem. Indeed, it has been concluded (199) that the only reliable basis for selection of a bactericide lies in actual determination of its utility under the conditions of ultimate use.

Some of the current sanitary applications of quaternary ammonium compounds will be briefly reviewed to give an idea of the scope of their application. In eating establishments they have been recommended for sanitizing dishes, glasses, and utensils (114, 145, 147). Detergency of these bactericidal dishwashing formulations may be increased by the addition of appropriate non-ionic surface active compounds. It should be kept in mind that if the alkalinity of the detergent is sufficiently high to saponify fat the resulting soap will inactivate the cationic quaternary ammonium compound with resultant loss in bactericidal activity (70). The dairy industry finds quaternaries useful in the sanitation of milk cans, dairy machinery (72, 156, 157), cow udders, and milkers' hands (87, 204). In food processing industries these compounds, besides being useful in general environmental and machinery sanitation, have found use in washing dirty eggs, resulting in a reduction of the bacteria count of dried egg pulp (170, 171). An interesting attempt to make fish-packing ice bactericidal by addition of quaternary ammonium compounds has not, as yet, proved successful (225). Many medical uses have been suggested for these compounds. Antisepsis of the skin of the patient and hands of the operator (71, 78, 152) and surgical instruments (88) have been reported. This antisepsis of the skin may be more apparent than real since cationic surface active compounds applied to the hands have been shown to deposit a non-perceptible film which retains viable bacteria underneath it (152). This film has a low bactericidal power on the inner surface whereas the outer surface is strongly bactericidal. Recent interest in combatting air-borne infections is reflected in the use of quaternary ammonium compounds impregnated into blankets or other cotton or woolen textiles (202, 228) to impart an efficient bactericidal activity. The possible use of quaternary ammonium compounds as components of aerosol bomb type bactericides has also been suggested (109, 230).

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