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BIOLOGICAL EFFECTS OF SURFACTANTS, III HYDRA AS A HIGHLY SENSITIVE ASSAY ANIMAL

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ABSTRACT

Effects on hydra of five homologous series of surfactants representing nonionic, amphoteric and anionic classes were examined. When concentrations which had no effects were increased tenfold the animal disintegrated within 24 *h. Lethal concentrations always coincided with a surface tension of* 49 ± 4 *dynes/cm. This is probably the level which disrupts the cell membranes. Because the deleterious effects are very rapid and obvious, hydra is a useful organism for monitoring the potential toxicity of detergents in freshwater environments.*

INTRODUCTION

A large proportion of the surfactants in commercial use (i.e. detergents, wetting agents, spreaders) end up in surface or ground water. There they may have profound biological effects. In plants, surfactants may inhibit growth (see Parr & Norman, 1965 for a review), drastically alter the cellular ultrastructure (Healey *et al.*, 1971) and on occasion enhance growth (Stone, 1960; Ernst *et al.*, 1971). These effects are probably mediated by the adsorption of the surfactants to the cell wall of the plants (Fujita & Koga, 1966).

Little is known about surfactant properties which are responsible for biological effects. Therefore, one of us (R.E.) has synthesised a series of surfactants varying systematically with respect to charge, structure, molecular weight and hydrophilic-lipophilic balance. We have used these compounds to investigate the effects of surfactant characteristics on several biological systems.

Hydra is one of the organisms employed in these investigations. This fresh water coelenterate is a useful test organism for two reasons. First, it is ubiquitous throughout freshwater habitats in the United States and other parts of the world. Secondly, it has no protective outer layers of any consequence so that the epithelial cells of the ectoderm are in direct contact with the surrounding aqueous

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environment. Therefore, hydra provides a very sensitive system for studies of surfactant effects on living tissue.

MATERIAL AND METHODS

Culture methods

Hydra attenuata was used in all experiments. The stock culture was maintained in 'M' solution consisting of 1×10^{-3} M $CaCl_2$, 1×10^{-3} M $NaHCO_3$, 1×10^{-4} M KCl and 1×10^{-4} M $MgCl_2$ in distilled water (Lenhoff & Brown, 1970) at $20° \pm 1$ °C. They were fed 3–8 larvae of the brine shrimp *Artemia salina* daily and washed 6–8 h after feeding.

Assay procedure

Ten control animals were maintained in 10 ml 'M' solution at $20° \pm 1$ °C. They were fed heavily (8–15 larvae) three times per week and washed 6–8 h after feeding. The animals were removed and the dishes thoroughly cleaned once a week. Under these conditions a single hydra produced 10–12 buds in three weeks, which is a typical growth rate. Experimental animals were treated in the same manner except that surfactant dissolved in 'M' solution at a specific concentration was added to the culture medium. Each time the animals were washed they were placed in culture solution containing fresh surfactant.

The number of buds produced between two feedings was measured by counting those that had detached from the adult since the last feeding. Subsequently the buds were removed and the animals fed. Each non-lethal surfactant concentration was assayed in duplicate and the cumulative number of buds produced by the ten animals in each dish over a period of three weeks determined. Lethal concentrations in most cases led to disintegration of the animals one day after exposure to the surfactant. In two cases the animals disintegrated within 10 days after the start of treatment.

SURFACTANTS

Synthesis and purification

A total of 24 surfactants belonging to three major groups were synthesised (see Appendix). These consisted of 14 fatty alcohol ethoxylates, 6 sulphobetaines and 4 sodium alkyl sulphates. All compounds were repurified following synthesis to remove reaction by-products or unreacted starting materials (see Appendix, Tables A1, A2, A3).

Surface tension measurements

Determinations of surface tension (ST) for each of the several concentrations of every surfactant were made using the du Nouy surface tension (or ring) method (Schwartz & Perry, 1949) with a Fisher Model 20 Tensiomat.

RESULTS

Effect of surfactants on budding rate and survival

Ten of the twelve fatty alcohol ethoxylates (nonionics) were toxic to hydra at 2×10^{-2} mM and all of these surfactants had a lethal effect at 2×10^{-1} mM within a period of 24 h (Table 1).

At concentrations of 2×10^{-3} mM or below, the entire range of nonionics

TABLE 1

EFFECT OF SURFACTANTS ON THE RATE OF BUDDING IN *Hydra attenuata*

			Budding rate[b] (*Number of buds produced*/10 *animals*/3 *weeks*) *Surfactant concentrations* (*mM*)					
Surfactant[a]	*n*	*MW*	2	2.10^{-1}	2.10^{-2}	2.10^{-3}	2.10^{-4}	2.10^{-5}
Fatty alcohol ethoxylates								
Tetradecyl	5·16	441	○	○	○	+ + +	+ + +	+ + +
Tetradecyl	7·11	533	○	○	○	+ + +	+ + +	+ + +
Tetradecyl	10·70	685	○	○	○	+ + +	+ + +	+ + +
Tetradecyl	18·14	1012	○	○	○	+ + +	+ + +	+ + +
Decyl	5·07	381	○	○	○	+ + +	+ + +	+ + +
Dodecyl	6·30	464	○	○	○	+ + +	+ + +	+ + +
Tetradecyl	7·11	533	○	○	○	+ + +	+ + +	+ + +
Hexadecyl	8·18	602	○	○	○	+ + +	+ + +	+ + +
Octadecyl	9·32	680	○	○	○	+ + +	+ + +	+ + +
Decyl	6·70	454	○	○	+ + +	+ + +	+ + +	+ + +
Dodecyl	7·18	502	○	○	○	+ + +	+ + +	+ + +
Tetradecyl	7·11	533	○	○	○	+ + +	+ + +	+ + +
Hexadecyl	7·00	550	○	○	○	+ + +	+ + +	+ + +
Octadecyl	7·00	578	○	○	+ + +	+ + +	+ + +	+ + +
Sulphobetaines								
Octyl		286	+ +	+ + +	+ + +	–	–	–
Decyl		312	+	+ + +	+ + +	–	–	–
Dodecyl		338	○	+	+ + +	–	–	–
Tetradecyl		370	○	○	±	+ + +	+ + +	+ + +
Hexadecyl		399	○	○	○	+ + +	+ + +	+ + +
Octadecyl		423	○	○	○	+ + +	+ + +	+ + +
Sodium alkyl sulphates								
Decyl		260	○	○	+ + +	–	–	–
Dodecyl		288	○	±	+ + +	–	–	–
Tetradecyl		316	○	+ + +	+ + +	–	–	–
Hexadecyl		344	+ + +	+ + +	+ + +	–	–	–

[a] Surfactants within a class are identified by the n-alkyl group (R). The fatty alcohol ethoxylates are also characterised by the number of ethylene oxide units (n). The general formulae for the three classes are: Fatty alcohol ethoxylate: $R—O—(CH_2CH_2O)_n—H$; Sulphobetaines:

$$R—\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{\overset{|}{\underset{|}{N^+}}}}—CH_2CH_2CH_2—SO_2O^-$$

Sodium alkyl sulphates; $R—O—SO_2O^-Na^+$

[b] Budding rates measured relative to controls: + + + : 100–120 buds (controls); + + : 90–100 buds; + : 70–90 buds; ± : 20 buds, lethal to adults within 10 days; ○ : lethal to adults within 24 h; – : not tested.

evaluated had no effect on these animals and budding rates were the same as those of the controls (Fig. 1, Table 1).

The response of hydra to sulphobetaines showed a direct relation to their molecular weight. Toxicity increased in line with increasing chain length of the lipophile (Table 1). A cumulative suppression on budding rate was further observed with these detergents at concentrations between their lethal and non-toxic levels (Fig. 2). Octyl sulphobetaine at 2 mM and dodecyl sulphobetaine at 2×10^{-1} mM induced the budding rate to fall off after about two weeks of exposure. The

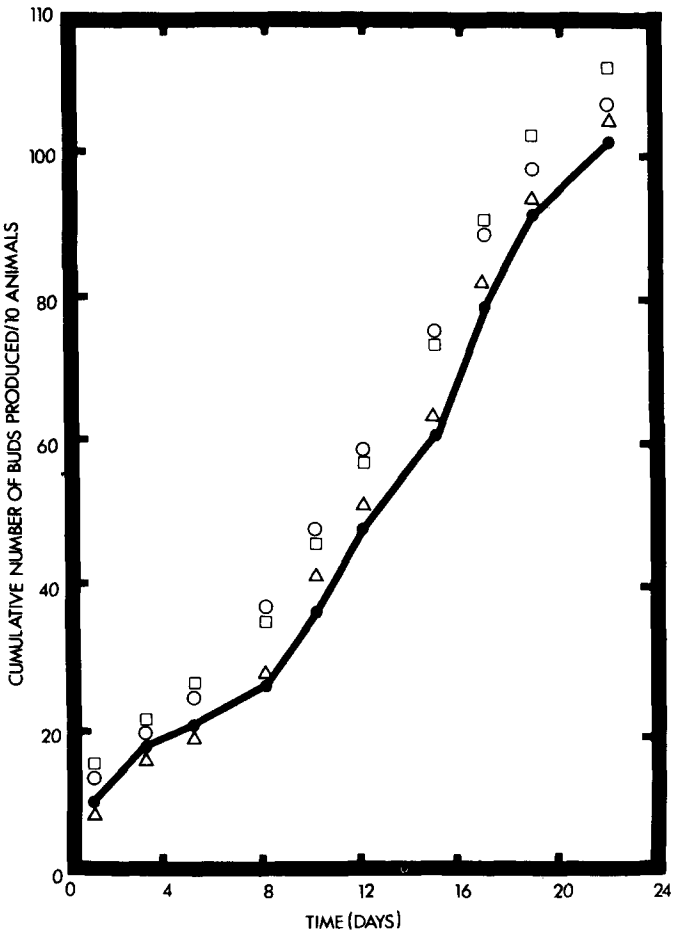

Fig. 1. Effect of three non-lethal concentrations of tetradecyl-polyethoxyethanol (7·11 moles adduct of ethylene oxide) on the budding rate. Explanation of symbols: ○; 2×10^{-3} mM, (1·05 ppm), □; 2×10^{-4} mM, (0·10 ppm), △; 2×10^{-5} mM, (0·01 ppm), ●; control. Each point represents the average of two measurements.

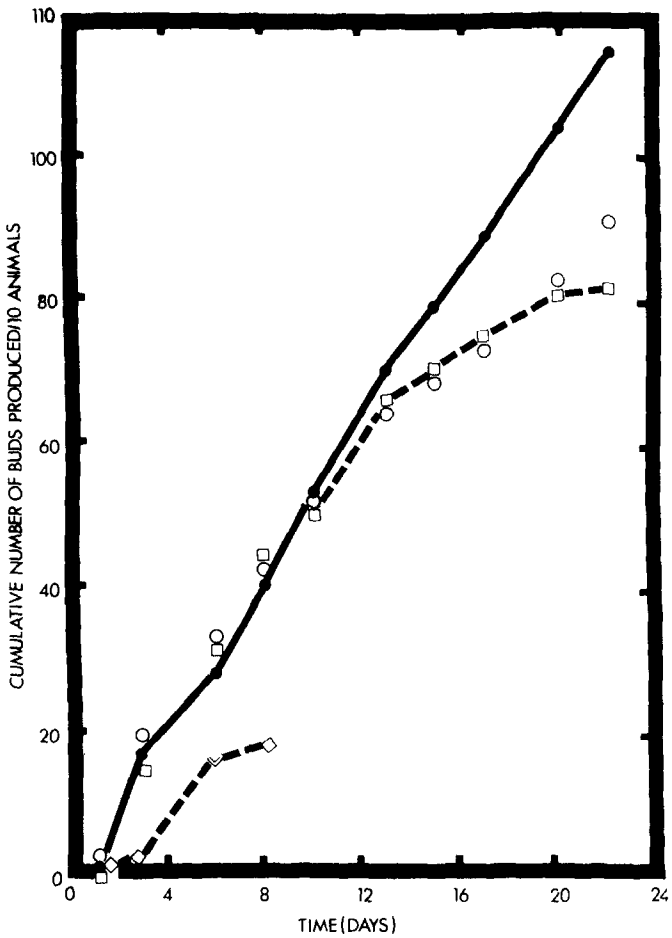

Fig. 2. Cumulative effects of three surfactants on the budding rate. Explanation of symbols: ●; control, ○; 2 mM 3-(octyldimethylammonio)-1-propanesulphonate, (536·2 ppm), □; 2×10^{-1} mM 3-(dodecyldimethylammonio)-1-propanesulphonate, (67·5 ppm), ◇; 2×10^{-2} mM 3-(tetradecyldimethylammonio)-1-propanesulphonate, (7·4 ppm). Each point represents the average of two measurements.

tetradecyl derivative caused budding rates to decline severely within the first week (Fig. 2).

A molecular weight-dependent effect was also exhibited by the anionic sodium alkyl sulphates. However, in this case the relation was the opposite of that of the sulphobetaines: the toxicity decreased with increasing molecular weight (Table 1).

Of the animals subjected to a lethal concentration (2·0 mM), one member from each class was examined with phase microscopy. Within 10–15 min of exposure the

animals were highly contracted. The ectodermal epithelium was either distorted or already disrupted in the case of the anionic alkyl sulphates. By 24 h all animals in all three classes were reduced to cellular debris.

Effects of surfactants on the surface tension of the culture medium

The surfactant concentration employed made possible observation of effects above and below the critical micelle level. This is reflected in a decreasing surface tension with increasing molarity up to the critical micelle concentration (CMC)

TABLE 2
EFFECT OF SURFACTANTS ON THE SURFACE TENSION OF THE CULTURE SOLUTION

			Surface tension (dynes/cm)					
			Surfactant concentration (mM)					
Surfactant	*n*	*MW*	2	2.10^{-1}	2.10^{-2}	2.10^{-3}	2.10^{-4}	2.10^{-5}
Fatty alcohol ethoxylates								
Tetradecyl	5·16	441	**42·0**	**40·2**	**43·0**	51·0	55·0	62·0
Tetradecyl	7·11	533	**33·0**	**33·7**	**37·5**	54·1	57·5	60·5
Tetradecyl	10·70	685	**38·1**	**37·6**	**47·1**	50·7	58·5	64·4
Tetradecyl	18·14	1012	**41·1**	**42·0**	**50·0**	60·0	64·1	69·3
Decyl	5·07	381	**38·7**	**41·0**	**48·8**	53·9	58·5	70·5
Dodecyl	6·30	464	**29·6**	**31·8**	**47·2**	57·2	65·3	71·2
Tetradecyl	7·11	533	**33·0**	**33·7**	**37·5**	54·1	57·5	60·5
Hexadecyl	8·18	602	**38·0**	**39·0**	**43·5**	53·0	58·5	61·0
Octadecyl	9·32	680	**43·0**	**44·5**	**47·0**	55·5	62·0	68·0
Decyl	6·70	545	**31·0**	**42·0**	54·2	62·9	67·8	70·0
Dodecyl	7·18	502	**31·0**	**32·0**	**41·5**	57·0	62·3	71·0
Tetradecyl	7·11	533	**33·0**	**33·7**	**37·5**	54·1	57·5	60·5
Hexadecyl	7·00	550	**39·0**	**41·0**	**43·0**	52·2	61·0	64·0
Octadecyl	7·00	578	**41·0**	**46·5**	49·0	53·0	61·0	71·4
Sulphobetaines								
Octyl		286	50·2	63·3	64·0	—	—	—
Decyl		312	55·0	65·7	70·8	—	—	—
Dodecyl		335	**41·0**	47·8	70·0	—	—	—
Tetradecyl		370	**36·0**	**42·0**	47·0[a]	64·1	65·2	71·5
Hexadecyl		399	**38·0**	**39·5**	**42·7**	58·0	60·0	70·0
Octadecyl		423	**38·4**	**37·5**	**43·9**	48·0	61·2	62·0
Sodium alkyl sulphates								
Decyl		260	**31·0**	**51·4**	71·4	73·0	72·0	72·0
Dodecyl		288	**44·0**	52·0[a]	60·0	66·0	72·0	72·0
Tetradecyl		316	**46·0**	57·7	60·5	64·3	71·7	73·0
Hexadecyl		344	50·0	69·0	65·0	68·0	71·0	72·0

Surfactants used are the same as those in Table 1. Values in bold type are lethal concentrations.
[a] These values were lethal within 10 days.

range where surface tension levels off (Vold & Vold, 1964). The data reveal a very striking correlation: of more interest, surfactant concentrations which reduced the surface tension to or below 49 $\pm$ 4 dynes/cm were also the lethal levels (values in bold type in Table 2).

DISCUSSION

The response of hydra to the three classes of surfactant tested differed. However, one striking correlation was observed for all three ionic series: concentrations of the surfactants yielding surface tension values below 49 ± 4 dynes/cm coincided with levels lethal to hydra.

Effects of nonionics

A group of twelve fatty alcohol ethoxylates varying in size of the alkyl group and the number of ethylene oxide units were used to examine the effects of hydrophilic-lipophilic properties and balance.

Our results show that the hydrophilic-lipophilic balance had surprisingly little effect on toxicity as in all but two cases the lethal level was 2×10^{-2} mM. This concentration is at the threshold or above the CMC, which for alcohol ethoxylates lies between 2×10^{-2} and 2×10^{-1} mM (Becher, 1967).

The surface tension values drop with increasing detergent concentration and level off as micelles form. This occurs within the same range shown above (Table 2). In addition, detergents above the CMC have solubilisation effects on water insoluble organics such as membrane lipids (McBain, 1950).

Effect of zwitterionics (sulphobetaines)

The six sulphobetaines employed by us covered a range of 8–18 carbon atoms in the lipophilic chain. Toxicity to hydra increased with the increase in lipophilic properties of these detergents and correlates well with the logarithmically decreasing CMC of sulphobetaines which accompanies increasing alkyl chain length (Herrmann, 1966). It is also in line with the lower surface tension values of sulphobetaines having higher alkyl radicals (Table 2). Similarly, solubilisation efficiency and extraction of proteins from 3T6 mouse fibroblasts by sulphobetaines improves with increasing alkyl chain length (A. Gonenne & R. Ernst, pers. comm.).

With hydra, lethal concentrations are above the CMC. The sulphobetaines are known to be 'mild' in their effects on biological materials (Allen & Humphries, 1975), so that a considerable concentration of micelles may be necessary for any observable effect on these animals.

Effect of anionics

Alkyl radicals of sodium alkyl sulphates employed by us ranged from 10–16 carbon atoms. Here, toxicity decreased with increasing lipophilic chain length. This, however, was undoubtedly due to reduced water solubility and resulting loss of surfactant activity at the assay temperature.

The temperature at which ionic surfactants and water become homogeneous is known as the Krafft point (Krafft & Wiglow, 1895; Hutchinson & Shinoda, 1967). In this connection, solubilities of sodium alkyl sulphates decrease sharply below their

Krafft points with increasing alkyl chain length. These are about 35 °C for sodium tetradecyl sulphate and 45 °C for sodium hexadecyl sulphate (Osipow, 1962). The reduced solubility of these surfactants at the assay temperatures would account for their lower toxicity to hydra (Table 1) and their higher surface tensions (Table 2).

In the laboratory, hydra fed heavily every day will double their tissue mass in 3–4 days (David & Campbell, 1972). Instead of increasing in size, the excess tissue is removed by budding in the form of new individuals. Therefore, the budding rate is a direct measure of the growth rate of the tissue mass and this was the parameter measured in these studies.

Budding rates are known to be sensitive to environmental conditions. Under normal circumstances they are proportional to the daily feeding level (Bode, pers. observation; J. Otto & R. D. Campbell, pers. comm.). However, if the culture dishes are not kept clean, the budding rate will decrease despite heavy feeding due to the presence of accumulated detritus and slime exuded by the animals (Bode, pers. observation). Similarly, any surfactants that interfere with growth in hydra would be expected to decrease the budding rate. Surfactants clearly have disruptive effects on cell membranes. Observations on a member from each class subjected to 2·0 mM concentrations indicated that the cell membranes were lysed either rapidly in anionics or more slowly for the nonionics and amphoterics. Other information indicates that the sodium alkyl sulphates and the sulphobetaines are very effective in disrupting membranes. For example, 3 mM sodium dodecyl sulphate (SDS) has been used to dissolve the tentacles of hydra for purposes of isolating nematocysts (Bode & Flick, 1976). In general, SDS and sulphobetaines have been used to solubilise proteins from membranes (Steck & Fox, 1972; Allen & Humphries, 1975). Thus, the surface tension value of 49 $\pm$ 4 dynes/cm may reflect the concentration where the lytic effect of surfactants on hydra membranes is lethal.

Most reports on the biological effects of surfactants are based on experiments involving concentrations which are higher than those found in ground and surface water. Because hydra is very sensitive to chemicals in the environment, due to the absence of a sufficiently protective outer layer, we have been able to employ detergent concentrations which are in line with pollution levels. Therefore our findings are more representative of the situation in the environment.

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REFERENCES

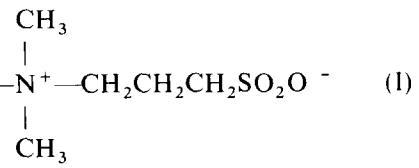
ALLEN, J. C. & HUMPHRIES, C. (1975). Isoelectric focusing of biological membranes. In *Isoelectric focusing*, ed. by J. P. Arbuthnott & J. A. Becky, 347–53. London, Butterworth and Company.

BECHER, P. (1967). Micelle formation in aqueous and nonaqueous solutions. In *Nonionic surfactants*, ed. by M. J. Schick, 478–513. New York, Marcel Dekker.
BODE, J. R. & FLICK, K. M. (1976). Distribution and dynamics of nematocyte populations in *Hydra attenuata*. *J. Cell Sci.*, **21**, 15–34.
DAVID, C. N. & CAMPBELL, R. D. (1972). Cell cycle kinetics and development in *Hydra attenuata*. I. Epithelial cells. *J. Cell Sci.*, **11**, 557–68.
EPTON, S. R. (1948). A new method for the rapid titrimetric analysis of sodium alkyl sulfates and related compounds. *Trans. Faraday Soc.*, **44**, 226–30.
ERNST, R. (1966). *Process for producing acyclic surfactant sulfobetaines*. US Patent 3,280,179.
ERNST, R., ARDITTI, J. & HEALEY, P. L. (1971). Biological effects of surfactants I. Influence on growth of orchid seedlings. *New Phytol.*, **70**, 457–75.
FUJITA, T. & KOGA, S. (1966). The binding of a cationic detergent by yeast cells in relation to its germicidal action. *J. gen. appl. Microbiol.*, **12**, 229–37.
HEALEY, P. L., ERNST, R. & ARDITTI, J. (1971). Biological effects of surfactants II. Influence of the ultrastructure of orchid seedlings. *New Phytol.*, **70**, 477–84.
HERRMANN, K. W. (1966). Micellar properties of some zwitterionic surfactants. *J. Colloid & Interface Sci.*, **22**, 352–9.
HUTCHINSON, E. & SHINODA, K. (1967). An outline of the solvent properties of surfactants. In *Solvent properties of surfactant solutions*, ed. by K. Shinoda, 12–13. New York, Marcel Dekker.
KÖNIG, H. (1972). Zur Analyse der Sulfobetaine. *Z. Analyt. Chem.*, **259**, 191–4.
KRAFFT, F. & WIGLOW, H. (1895). Über das Verhalten der fettsauren Alkalien und der Seifen in Gegenwart von Wasser. III. Die Seifen als Krystalloide. *Ber.*, **28**(3), 2566–73.
LENHOFF, H. M. & BROWN, R. (1970). Mass culture of hydra; an improved method and its application to other aquatic invertebrates. *Lab. Animals*, **4**, 139–54.
MCBAIN, J. W. (1950). *Colloid science*. Boston, D. C. Heath & Company.
MOORE, C. D. & BELL, M. (1956). Non-ionic surface-active agents and their utilization in cosmetic preparations. *Soap Perfum. Cosm.*, **29**, 893–6.
OSIPOW, L. I. (1962). Theory and industrial applications. In *Surface chemistry*, 189–91. New York, Reinhold Publishing Company.
PARR, J. R. & NORMAN, A. G. (1965). Considerations in the use of surfactants in plant systems: A Review. *Bot. Gaz.*, **126**, 86–96.
SCHWARTZ, A. A. & PERRY, J. W. (1949). *Surface active agents. Their chemistry and technology*, 1, 268–72. New York, Interscience Publishing Company.
STECK, T. L. & FOX, C. F. (1972). Membrane proteins. In *Membrane molecular biology*, ed. by C. F. Fox & A. D. Keith, 40, 44–50. Stanford, Conn., Sinauer Associates Inc.
STONE, B. B. (1960). Growth promotion in pea stem sections. I. Stimulation of auxin and gibberellin action by alkyl lipids. *Pl. Physiol., Lancaster*, **35**, 262–9.
VOLD, J. M. & VOLD, R. D. (1964). *Colloid chemistry*. New York, Reinhold Publishing Company.

APPENDIX

Preparation of surfactants

Sulphobetaines. 3-(alkyldimethylammonio)-1-propanesulphonate



(I)

R = n-octyl, n-decyl, n-dodecyl, n-tetradecyl, n-hexadecyl, or n-octadecyl. These surfactants were prepared from corresponding, fractionally distilled, tertiary alkyldimethylamines as described previously (Ernst, 1966). Upon reaction the solutions were refluxed for one hour to hydrolyse any residual traces of

propanesulton precursor (monitored by infrared spectophotometric procedures for the disappearance of the typical sultone absorption at 10·3 μ and 11·2 μ). The solutions were then diluted with 50% aqueous ethanol to give 10% solids, and 250 ml portions were extracted repeatedly with 100 ml petroleum ether, to remove fatty matter present as impurities. Extracted solutions were agitated with 100 g mixed bed ion exchange resin (Amberlite MB 1, Rohm and Haas Company), filtered and dried.

Sulphobetaines, although having strong anionic and cationic radicals, act as 'ionic neutral' zwitterion compounds and pass through cation and anion exchange resins (König, 1972), but ionic impurities are removed. After this the dried, crystalline solids (20–25 g) were stirred into boiling acetone, and the dispersion filtered upon cooling. The yield was recrystallised from acetone once more, the crystals dried at 60 °C and percent nitrogen determined by the Kjeldahl method. Molecular weights were calculated on the basis of Kjeldahl nitrogen (Table A-1).

TABLE A-1
3-(ALKYLDIMETHYLAMMONIO)-1-PROPANESULPHONATE SURFACTANTS

n-Alkyl group	*Molecular weight*	
	Theory	*Calculated*
Octyl-	279·4	268·1
Decyl-	307·4	309·8
Dodecyl-	335·6	337·5
Tetradecyl-	363·6	370·2
Hexadecyl-	391·7	398·8
Octadecyl-	419·7	422·6

Purity was assayed by thin layer chromatography.

Silica gel G plates are spotted with 50–250 μg of the surfactant and are developed with chloroform-methanol 9:1 v/v. Visualising is by spraying first with 0·8% ninhydrin in ethanol, followed by heating for 10 min at 120 °C, indicating the presence of residual precursor amines, and over-spraying with Dragendorff's reagent to stain the sulphobetaines. Thin layer chromatograms of purified sulphobetaines contain only a single bright yellow spot near the origin.

Anionics. Sodium alkyl sulphate

$$R—O—SO_2O—Na^+ \qquad (II)$$

R = n-decyl, n-dodecyl, n-tetradecyl, n-hexadecyl, or n-octadecyl, were prepared from fractionally distilled, higher alcohols by sulphation with chlorosulphonic acid, followed by neutralisation with NaOH. For purification, each surfactant was dissolved in 50% aqueous alcohol to yield a solution containing 10% solids. Unreacted fatty alcohol was removed by repeated extraction of 250 ml of this solution with 100 ml hexane. The extracted solution was dried and the solids re-dissolved in boiling anhydrous ethanol, filtered hot on a Buchner filter, and the

filtrate evaporated to dryness. Crystalline solids were then re-dissolved in anhydrous isopropanol, the solution re-filtered and filtrate dried to constant weight. Activity was determined by methylene blue-cationic titration (Epton, 1948), using 0·004 M Hyamine 1622 (Rohm and Haas Company) as the titrant (Table A-2). Molecular weights were calculated (Table A-2).

TABLE A-2
SODIUM ALKYL SULPHATE SURFACTANTS

n-Alkyl group	*Molecular weight, theory*	*Methylene blue activity, %*
Decyl-	260·2	98·9
Dodecyl-	288·4	99·9
Tetradecyl-	316·4	99·3
Hexadecyl-	344·5	100·3
Octadecyl-	372·6	98·7

Nonionics. Ethoxylated higher alkanols

$$R—O—(CH_2CH_2O)_nH \qquad (III)$$

These nonionics, prepared from fractionated normal alcohols (C_{10}–C_{18}) and ethylene oxide, were supplied by the Textilana Corporation. Purification and determination of equivalent molecular weight were carried out as previously reported (Ernst *et al.*, 1971). Ethylene oxide units (CH_2CH_2O) were determined as the difference between the equivalent molecular weight and the molecular weight of the alkanol (alcohol) precursor.

TABLE A-3
ETHOXYLATED HIGHER ALKANOLS

n-Alkanol group Name	*Polyethylene glycol group (ethylene oxide units, n) and HLB numbers*											
	n	*HLB*	*n*	*HLB*[c]	*n*[a]	*HLB*	*n*	*HLB*	*n*	*HLB*	*n*	*HLB*
Decanol			5·07	50·7	6·7	67·3						
Dodecanol			6·3	52·3	7·18	60·0						
Tetradecanol[b]	5·16	36·9	7·11	51·0	7·11	51·0	10·7	76·5	18·14	129·5	40·35	288·3
Hexadecanol			8·18	51·1	7·0	44·0						
Octadecanol			9·32	51·7	7·01	38·9						

[a] Constant hydrophile, target = 7·0 ± 0·3.
[b] Constant lipophile.
[c] Constant HLB number, target = 50·0 − 52·0.

Hydrophilic-lipophilic balance (HLB numbers) were calculated by the equation of Moore & Bell (1956). Three series of nonionics were prepared and purified (Table A-3) having:

(a) constant hydrophile and increasing lipophile;
(b) constant lipophile and increasing hydrophile; and
(c) hydrophile and lipophile balanced to give constant HLB numbers.