Relative Toxicity of Three Homologous Series of Nonionic Surfactants in the Planarian

WITOLD SASKI*, MARIELLA MANNELLI, MARCO F. SAETTONE, and FRANCESCO BOTTARI

Abstract □ An investigation on the toxicity of three homologous series of nonionic surfactants was carried out using planarian *Dugesia lugubris* (O. Schmidt). The surfactants used in this study were polyoxyethylene stearic acid esters, polyoxyethylene sorbitan fatty acid partial esters, and polyoxyethylene alkyl ethers. The effect of surfactant concentration (C) on time of death (T) of planarian can be described by a regression equation, where $1/T$ is linearly related to C. Analysis of variance of $1/T$ showed that each regression was highly significant and that deviations from linearity were not significant. The behavior of the polyoxyethylene stearic acid esters and polysorbates was in good agreement with the Levy–Gucinski theory, which describes the effect of toxic agents on goldfish. No agreement with the same theory was shown by surfactants of the polyoxyethylene alkyl ethers, whose regression lines, in the C range 0.05–1.0%, failed to intersect the origin. Statistical differences in relative toxicity between the members of each series were assessed. In the polyoxyethylene stearic acid esters, a linear relationship between the length of the polyoxyethylene chain and lethal time was detected. Relative toxicity in the polysorbates decreases in the order of the 20, 40, 60, and 80 esters, thereby depending on the nature of the esterifying acid (lauric, palmitic, stearic, and oleic, respectively). No clear-cut pattern was found with the polyoxyethylene alkyl ethers; differences in relative toxicity between the ethers of the series were not statistically significant. The present study is indicative of the utility of the planarian in biopharmaceutical research.

Keyphrases □ Surfactants, nonionic—relative toxicity in planarian □ Toxicity, nonionic surfactants—polyoxyethylene chain-length effect □ Biologic membrane permeation—surfactants □ Surface tension, pH effects—surfactant absorption, toxicity

The part surface-active agents play in the passage of drugs across biological membranes continues to be of great interest in biopharmaceutical research (1–4). The use of surfactants to prepare micellar solutions of pharmaceutical materials may, in some cases, result in an increased absorption and biological activity, although cases are known in which solubilization affects drug absorption adversely or not at all (5). Effects on the absorbing membrane, interaction with the drug, and modification of the physical properties of the dosage form have been considered among the possible mechanisms by which surfactants may modify drug absorption (6).

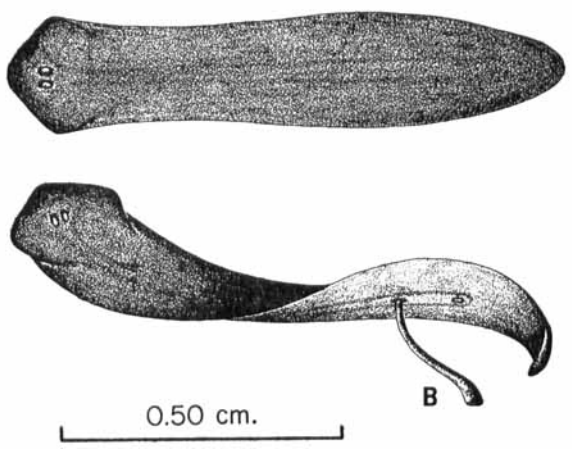

Figure 1—*Planarian* Dugesia lugubris. *Key: A, head; and B, pharynx.*

The effect of surface-active agents on biological membranes has been investigated using different approaches. Unicellular organisms such as bacteria, used in earlier studies, were later found unsuitable owing to the presence of enzymes and other vital cell constituents located in the cell membrane and, therefore, accessible to surface-active agents in the medium. Experimental systems using small animals or isolated intestines were recently employed (3, 7). The use of goldfish (*Carassius auratus*) for studies of factors influencing biological membrane permeation has been extensively illustrated in a recent series of papers (8–10). This animal has been used to investigate the influence of polysorbate 80 (1, 12) and of bile salts (4, 11) on pentobarbital and on 4-aminoantipyrine absorption.

The present authors set out to develop an experimental system involving an aquatic animal other than fish whereby studies on the kinetics of the transfer of drugs, both in the presence and in the absence of added surfactant, could be carried out satisfactorily. A platyhelminth, the planarian, was selected. The planarian, widely used in genetic, physiologic, and behavioral research (13), apparently has never been employed in pharmacology[1].

In the present paper, the effect on planarian of three homologous series of nonionic surfactants, which might be used as drug solubilizers, was investigated. The purposes of this study were: (*a*) to learn whether these agents exert any toxic action of their own, and (*b*) to assess on a statistical basis any functional relationship existing between toxic activity (*i.e.*, lethal time of planarian) and surfactant concentration. Indeed, nonionic surfactants may possess a pharmacologic activity of their own, due to their chemical structure or physical characteristics and independent of their surface-tension depressing activity (14). An investigation in this direction was considered to be an essential prerequisite to further studies dealing with membrane permeation by solubilized drugs.

[1] A possible objection to the use of planarian might be its low position in the biological scale. However, other low life forms have been successfully employed in pharmacologic research. Worms of the genera *Lumbricus* and *Tubifex*, for example, have been found useful in studies concerning evaluation of local anesthetic activity and standardization of chrysarobin, respectively; *cf.*, B. P. Block, D. J. Potts, and R. S. H. Finney, *J. Pharm. Pharmacol., Suppl.*, **16**, 85T(1964); and P. Boymond and P. Amacker, *Pharm. Acta Helv.*, **39**, 223(1964).

Table I—Effect of Surfactant Concentration on Time of Death of Planarian

Surface-Active Agent	Concentration, % w/v: 0.05	0.10	0.30	0.50	0.75	1.00
	Average Time of Death, hr.[a] (*CV*%)[b]					
Polyoxyethylene 20 stearate	19.60(12.0)	8.60(12.1)	3.32(13.4)	2.04(15.2)	1.34(3.8)	1.04(4.9)
Polyoxyethylene 30 stearate	48.43(11.8)	31.08(20.2)	10.14(6.5)	6.11(13.5)	4.18(20.3)	3.27(22.1)
Polyoxyl 40 stearate	—	40.75(48.1)	16.32(38.8)	8.90(28.9)	5.48(14.6)	3.75(12.1)
Polyoxyethylene 50 stearate	—	52.67(26.3)	17.94(13.7)	10.86(4.8)	7.65(15.3)	5.64(18.4)
Polyoxyethylene 100 stearate	—	—	51.45(14.7)	37.97(20.8)	24.81(14.2)	21.55(13.5)
Polysorbate 20	3.34(13.6)	1.86(36.0)	0.62(4.9)	0.37(2.9)	0.27(4.4)	0.20(7.3)
Polysorbate 40	—	8.96(27.5)	2.06(24.3)	0.98(27.8)	0.68(25.0)	0.47(16.7)
Polysorbate 60	27.68(22.8)	—	4.62(18.7)	2.74(18.2)	1.54(16.3)	1.21(17.3)
Polysorbate 80	9.85[c](4.5)	3.60[d](9.0)	3.16[e](15.2)	2.30[f](17.9)	1.82[g](7.8)	1.37[h](17.8)
Polyoxyethylene 23 lauryl ether	6.20(10.9)	5.32(5.7)	3.88(6.3)	2.74(10.3)	2.02(21.0)	1.74(6.4)
Polyoxyethylene 20 cetyl ether	1.13(24.8)	1.03(11.6)	0.72(11.4)	0.57(21.3)	0.47(17.5)	0.37(11.5)
Polyoxyethylene 20 stearyl ether	1.43(12.8)	1.16(12.4)	0.72(16.6)	0.58(22.9)	0.51(29.0)	0.43(31.4)
Polyoxyethylene 20 oleyl ether	1.54(6.3)	1.08(11.9)	0.82(15.8)	0.63(13.7)	0.48(10.9)	0.37(16.0)

[a] Average of 12 animals. [b] *CV*% = standard deviation/average × 100. Concentration, % w/v: [c] 1.00. [d] 2.00. [e] 2.50. [f] 3.00. [g] 4.00. [h] 5.00.

EXPERIMENTAL

The specimens of diploid type of *Dugesia lugubris* (O. Schmidt) (Fig. 1) were collected from an artificial pond[2]. The animals averaged 1.0 ± 0.2 cm. in length and 9.0 ± 1.0 mg. in weight.

Water and Food—All animals were kept in tap water brought into the laboratory directly from the mains *via* iron plumbing. It should be remembered that copper or brass tubing may cause cupric poisoning (15). Distilled water was used for the solutions in the experiments. Preliminary tests showed that planarians could survive for a long time in distilled water and that no significant differences in lethal times could be detected when the surfactant solutions were made with pond, tap, or distilled water. Animals were fed on beef liver cut into small pieces once a week, the water being changed and waste products removed the following day. All experiments were carried out on animals fasted for at least 10 days.

Nonionic Surface-Active Agents—The following compounds were used—*viz.*, a homologous series of polyoxyethylene stearic acid esters: polyoxyethylene 20 stearate, polyoxyethylene 30 stearate, polyoxyl 40 stearate, polyoxyethylene 50 stearate, and polyoxyethylene 100 stearate[3]; a homologous series of polyoxyethylene sorbitan fatty acid partial esters: polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80 USP[3]; and a homologous series of polyoxyethylene alkyl ethers: polyoxyethylene 23 lauryl ether, polyoxyethylene 20 cetyl ether, polyoxyethylene 20 stearyl ether, and polyoxyethylene 20 oleyl ether[3]. All compounds were commercially available materials, and no attempt at further purification was made.

Determination of pH of Immersion Fluids—The pH of the solutions was determined by means of a Photovolt 111 pH meter.

Determination of Surface Tension of Immersion Fluids—The ring detachment method, employing the du Nöuy tensiometer[4], was used. Three successive measurements were taken for each of the concentrations; after application of the usual corrections (17), the average was calculated.

Determination of Time of Death of Planarian—Three animals were placed in a beaker containing 20 ml. of a solution of the surfactant of varying concentrations: 0.05, 0.10, 0.30, 0.50, 0.75, and 1.0% w/v in distilled water at 20 ± 1°. From the moment of immersion, the planarians were observed with the help of a magnifying glass, and the time elapsed between immersion of the animals into the solutions and death was recorded. To eliminate bias in time-of-death determination, each beaker was marked with a code number by an individual not involved in the study, the code being broken only after completion of all determinations. When immersed, the planarians contract and curl at regular intervals and then distend again and swell. Their motility gradually decreases. The planarians eventually remain distended and become covered with a whitish substance. The pharynx is extruded. This precedes the moment of death (*T*) which corresponds to the state of perfect immobility and is followed, after 2–3 min., by a complete disintegration of the planarian which confirms the accuracy of the time of death recorded[5].

RESULTS AND DISCUSSION

The Levy–Gucinski theory (8), developed to describe the absorption of drugs by goldfish, states: $1/T = DA/L \cdot C$, where T is time of death, L is the lethal dose, D is the apparent absorption-rate constant, A is the surface area of the absorbing membrane, and C is the drug concentration in the aqueous medium. Provided a number of assumptions are fulfilled, a plot of reciprocal time of death *versus* concentration of drug in the medium should give a straight line intersecting the origin and having a slope equal to DA/L. Levy and coworkers (8, 9) also demonstrated that the CT value (product of drug concentration and time of death), used frequently as an index of relative toxicity of drugs, is a function not only of the inherent toxicity but also of the rate of absorption of a compound.

The effect on planarian *Dugesia lugubris* of the 13 nonionic surfactants in aqueous solution of concentration ranging from 0.05 to 1.0% (1.0–5.0% for polysorbate 80) are presented in Table I. Four separate experiments were carried out with each of the agents, using three animals for each solution concentration in any one of the experiments performed. The time of death of planarian was found to vary inversely with the concentration of surfactant in solution. The regression equations calculated for each compound are shown in Table II. To test the statistical significance of the $1/T$ *versus* C slopes and the linearity of the regressions, the data obtained with individual animals were submitted to an ANOVA test, with the aid of an Olivetti Programma 101 computer. The ANOVA showed that all regressions were highly significant ($p \ll 0.0005$) and that deviations from linearity were not significant. Only the regression line for polysorbate 20 showed some deviation from linearity, since the F value for nonlinear terms was 3.106, which is significant at the 5% level.

The following statistical tests were also performed. Within each series, the significance of difference between slopes was calculated using a test analogous to the t-test between two sample means. The results of this analysis are reported in Table III.

Using the Student t test at each concentration, significance of difference between reciprocals of death times at each surfactant

[2] In the botanical garden of the Department of Botany, the University of Pisa. For a comprehensive treatise on anatomy and physiology of planarian, *cf. Reference 16.*

[3] Trademarked as Myrj 49, 51, 52, 53, and 59; Tween 20, 40, 60, and 80; and Brij 35, 58, 78, and 98, respectively, by Atlas Chemical Industries, Wilmington, Del.

[4] Cenco Co., Chicago, Ill.

[5] When planarians are immersed in solutions containing pharmacologically active compounds of different types (barbiturates, chlorphenesin, *etc.*), the death pattern is essentially the same, but disintegration occurs 1–2 hr. after pharynx extrusion. Disintegration invariably follows death of a planarian. The rapid disintegration in the presence of surfactants is probably due to the wetting properties of these compounds.

Table II—Regression Equations Describing the Effect of Surfactant Concentration (C) on Time of Death of Planarian (T)

Surface-Active Agent	Regression Equation, $1/T = bC + a$
Polyoxyethylene 20 stearate	$1/T = 0.9540C + 0.0179$
Polyoxyethylene 30 stearate	$1/T = 0.3183C + 0.0045$
Polyoxyl 40 stearate	$1/T = 0.2659C - 0.0061$
Polyoxyethylene 50 stearate	$1/T = 0.1786C + 0.0029$
Polyoxyethylene 100 stearate	$1/T = 0.0406C + 0.0078$
Polysorbate 20	$1/T = 4.7932C + 0.1443$
Polysorbate 40	$1/T = 2.3039C - 0.1289$
Polysorbate 60	$1/T = 0.8771C - 0.0271$
Polysorbate 80	$1/T = 0.1571C - 0.0522$
Polyoxyethylene 23 lauryl ether	$1/T = 0.4234C + 0.1406$
Polyoxyethylene 20 cetyl ether	$1/T = 1.8780C + 0.8350$
Polyoxyethylene 20 stearyl ether	$1/T = 1.8548C + 0.7374$
Polyoxyethylene 20 oleyl ether	$1/T = 2.0700C + 0.6191$

concentration was determined by comparing the mean death times of any two members of a series until all the members of all the concentrations were tested. The results are listed in Table IV.

Polyoxyethylene Stearate Series—A plot of reciprocal time of death of planarian *versus* concentration of the five members of the homologous series of polyoxyethylene stearate series is presented in Fig. 2. A linear relationship is observed in each case and, as predicted by the Levy–Gucinski theory, the regression lines extrapolate to origin. The intercepts are not significantly different from zero at the 95% probability level. Differences between slopes of the polyoxyethylene stearic acid ester series are all significant or highly significant. Differences between reciprocals of death times at the lower concentrations of polyoxyl 40 stearate *versus* polyoxyethylene 50 stearate are not significant. The differences at the concentrations of 0.1 and 1.0% of polyoxyethylene 30 stearate *versus* polyoxyl 40 stearate are also not significant. Reciprocals of time for all remaining members of the series as compared with each other are significantly different at each concentration.

One further observation is apparent. The slopes of the lines for the individual members of the series decline as the ethylene oxide mole ratio R (*i.e.*, the number of moles of ethylene oxide added per mole of stearic acid) increases, the sequence being polyoxyethylene 20 stearate, polyoxyethylene 30 stearate, polyoxyl 40 stearate, polyoxyethylene 50 stearate, and polyoxyethylene 100 stearate.

This is further evidenced by a plot of the R values for polyoxyethylene stearate solutions of identical concentration *versus* time of death of planarian (Fig. 3). The points lie in straight lines, with intercept at $R \sim 19$. It must be remembered that, in a $1/T$ *versus* C plot, the slope is related to absorption rate and toxicity. In this case, a separate evaluation of each effect was not possible; hence, the decrease in slope is only indicative of a decrease in relative toxicity of the surface-active agents.

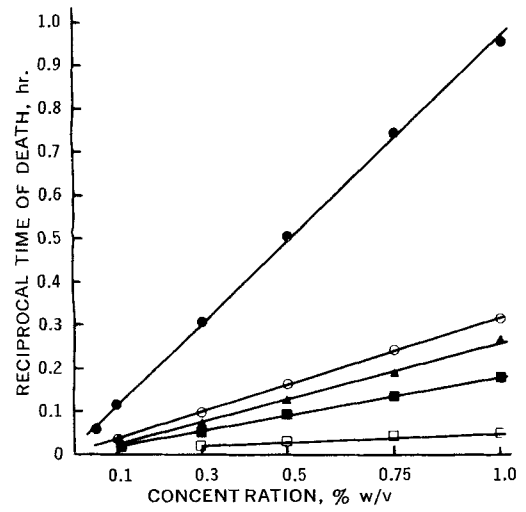

Figure 2—*Reciprocal time of death of planarian as a function of surfactant concentration at 20°. Key: ●, polyoxyethylene 20 stearate; ○, polyoxyethylene 30 stearate; ▲, polyoxyl 40 stearate; ■, polyoxyethylene 50 stearate; and □, polyoxyethylene 100 stearate.*

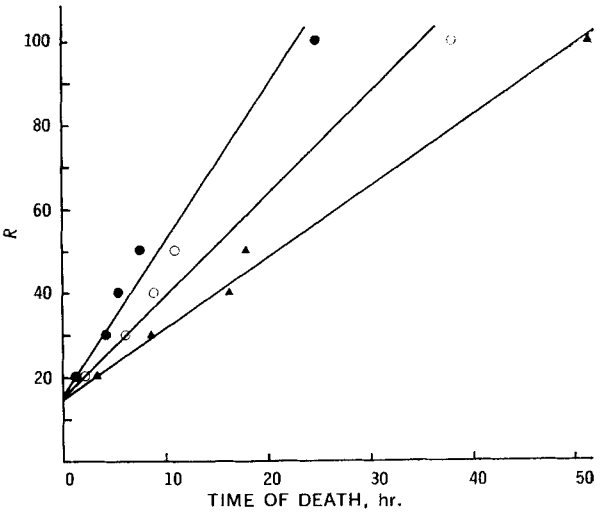

Figure 3—*Time of death of planarian as a function of ethylene oxide mole ratio* (R) *of polyoxyethylene stearates. Concentration of solutions, key: ▲, 0.30%; ○, 0.50%; and ●, 0.75%.*

The linear relationship existing between ethylene oxide mole ratio of surfactants and time of death of planarian appears rather interesting and deserves further investigation with other surfactants having a fixed hydrophobic moiety and different R values.

Since the polyoxyethylene moiety is an important segment of the polyoxyethylene stearates, the acute toxicity of polyethylene glycols is pertinent. Their acute oral toxicity (LD_{50} on a gram per kilogram basis) in the rat was reported to be decreasing with increasing molecular weight (18). Reduced absorption of polyethylene glycols from the gastrointestinal tract as the molecular weight was increased was also reported (18). The present findings are consistent with this report.

Products of surfactant concentration and time of death of planarian, *i.e.*, CT values based on actual experimental data, are presented in Table V. The CT values are relatively constant for each compound of the polyoxyethylene stearate series over the wide range of concentrations.

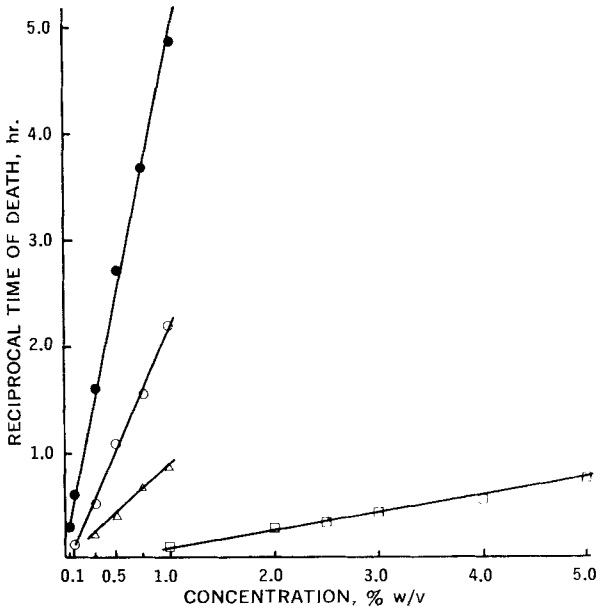

Figure 4—*Reciprocal time of death of planarian as a function of surfactant concentration at 20°. Key: ●, polysorbate 20; ○, polysorbate 40; △, polysorbate 60; and □, polysorbate 80.*

Table III—Significance of Difference between Slopes

Slopes	$F^{a,b}$	Student's t	Degrees of Freedom	Significance[b]
Polyoxyethylene 20 stearate *versus* polyoxyethylene 30 stearate	S	45.4	87	S
Polyoxyethylene 30 stearate *versus* polyoxyl 40 stearate	S	4.04	75	S
Polyoxyl 40 stearate *versus* polyoxyethylene 50 stearate	S	7.08	63	S
Polyoxyethylene 50 stearate *versus* polyoxyethylene 100 stearate	n.s.	34.1	104	S
Polysorbate 80 *versus* polysorbate 40	S	21.5	114	S
Polysorbate 80 *versus* polysorbate 60	S	37.8	85	S
Polysorbate 40 *versus* polysorbate 60	S	20.4	89	S
Polysorbate 40 *versus* polysorbate 80	S	34.8	59	S
Polyoxyethylene 23 lauryl ether *versus* polyoxyethylene 20 cetyl ether	S	28.2	74	S
Polyoxyethylene 23 lauryl ether *versus* polyoxyethylene 20 stearyl ether	S	10.5	70	S
Polyoxyethylene 23 lauryl ether *versus* polyoxyethylene 20 oleyl ether	S	19.8	70	S
Polyoxyethylene 20 cetyl ether *versus* polyoxyethylene 20 stearyl ether	S	0.167	98	n.s. ($p > 0.7$)
Polyoxyethylene 20 cetyl ether *versus* polyoxyethylene 20 oleyl ether	S	1.98	111	n.s. ($p > 0.05$)
Polyoxyethylene 20 stearyl ether *versus* polyoxyethylene 20 oleyl ether	S	1.36	116	n.s. ($p > 0.1$)

[a] F = variance ratio, s_1^2/s_2^2. [b] S = significant, $p < 0.001$. n.s. = not significant. Differences between slopes not indicated in the table were highly significant.

Polysorbate Series—Figure 4 is a plot of reciprocal time of death of planarian *versus* concentration of surfactants of the polysorbate series. All regression lines are highly significant; all four extrapolate virtually to origin, and each slope (*i.e.*, the action exerted on planarian by each surfactant) differs significantly from the others.

The toxicity of one of the surface-active agents, polysorbate 80, was so low that it was necessary to employ much higher concentrations (within 1.0–5.0% range) to obtain the end-point.

In this series, it can be seen that, the hydrophilic moiety of the surfactants being equal, relative toxicity depends largely on the nature of the hydrophobic portion, *i.e.*, the esterifying acid. The decreasing order of toxicity on planarian was polysorbate 20,

Table IV—Significance of Difference between Reciprocals of Death Times at Each Surfactant Concentration

	Concentration, %	Student's t	Significance
Polyoxyethylene Stearic Acid Ester Series			
Polyoxyl 40 stearate *versus* polyoxyethylene 50 stearate	0.1	2.26	n.s. ($0.05 > p > 0.02$)
	0.3	1.62	n.s. ($p > 0.1$)
	0.5	2.58	n.s. ($0.02 > p > 0.01$)
	0.75	4.91	S ($p < 0.001$)
	1.0	6.99	S ($p < 0.001$)
Polyoxyethylene 30 stearate *versus* polyoxyl 40 stearate	0.1	0.38	n.s. ($p > 0.1$)
	0.3	5.25	S ($p < 0.001$)
	0.5	3.93	S ($p < 0.001$)
	0.75	3.63	S ($0.001 < p < 0.002$)
	1.0	2.28	n.s. ($0.05 > p > 0.02$)
Polyoxyethylene 50 stearate *versus* polyoxyethylene 100 stearate; polyoxyethylene 30 stearate *versus* polyoxyethylene 50 stearate; polyoxyethylene 30 stearate *versus* polyoxyethylene 100 stearate; polyoxyethylene 20 stearate *versus* all others: all reciprocals of times are significantly different at each concentration			
Polysorbate Series			
Polysorbate 20 *versus* polysorbate 40; polysorbate 20 *versus* polysorbate 60; polysorbate 40 *versus* polysorbate 60: all reciprocals of times are significantly different at each concentration.			
Polyoxyethylene Alkyl Ether Series			
Polyoxyethylene 20 stearyl ether *versus* polyoxyethylene 20 oleyl ether	0.05	1.88	n.s. ($0.1 > p > 0.05$)
	0.1	1.27	n.s. ($p > 0.1$)
	0.3	1.90	n.s. ($0.1 > p > 0.05$)
	0.5	1.27	n.s. ($p > 0.1$)
	0.75	0.13	n.s. ($p > 0.1$)
	1.0	1.05	n.s. ($p > 0.1$)
Polyoxyethylene 20 cetyl ether *versus* polyoxyethylene 20 oleyl ether	0.05	3.00	S ($p < 0.01$)
	0.1	1.1	n.s. ($p > 0.1$)
	0.3	1.73	n.s. ($0.1 > p > 0.05$)
	0.3	1.46	n.s. ($p > 0.1$)
	0.75	0.66	n.s. ($p > 0.1$)
	1.0	0.11	n.s. ($p > 0.1$)
Polyoxyethylene 20 cetyl ether *versus* polyoxyethylene 20 stearyl ether	0.05	2.39	n.s. ($0.05 > p > 0.02$)
	0.1	2.32	n.s. ($0.05 > p > 0.02$)
	0.3	0.46	n.s. ($p > 0.1$)
	0.5	0.07	n.s. ($p > 0.1$)
	0.75	0.49	n.s. ($p > 0.1$)
	1.0	1.02	n.s. ($p > 0.1$)
Polyoxyethylene 23 lauryl ether *versus* all others: all reciprocals of times are significantly different at each concentration.			

Table V—Experimentally Determined *CT* Values in Grams per Deciliter × Hours for the Homologous Series of Polyoxyethylene Stearates

Surface-Active Agent	Concentration, % w/v 0.30	0.50	0.75	1.0	Average
Polyoxyethylene 20 stearate	0.99	1.02	1.00	1.04	1.0
Polyoxyethylene 30 stearate	3.04	3.05	3.13	3.27	3.1
Polyoxyl 40 stearate	4.89	4.45	4.11	3.75	4.3
Polyoxyethylene 50 stearate	5.38	5.43	5.74	5.64	5.5
Polyoxyethylene 100 stearate	15.40	18.98	18.60	21.55	18.6

Table VI—Effect of Low Concentrations of Polyoxyethylene Alkyl Ethers on Time of Death of Planarian

Surface-Active Agent	Concentration, % w/v 0.01	0.02	0.03	0.04
	Average Time of Death, hr.[a]			
Polyoxyethylene 23 lauryl ether	19.30(10.2)	11.68(77.7)	10.30(8.1)	6.75(10.0)
Polyoxyethylene 20 cetyl ether	3.08(19.5)	2.08(14.9)	1.35(11.6)	1.18(14.0)
Polyoxyethylene 20 stearyl ether	3.75(33.4)	2.21(12.2)	1.57(20.2)	1.35(18.2)
Polyoxyethylene 20 oleyl ether	5.15(9.1)	2.73(12.7)	1.80(10.0)	1.60(12.5)

[a] Average of 12 animals; *CV*% in parentheses.

polysorbate 40, polysorbate 60, and polysorbate 80, respectively. The acute oral toxicity (LD_{50} on a gram per kilogram basis) in the fasted rat was reported to be 34.7, 34.2, 33.8, and 38.0, respectively (18). The differences in relative toxicity between the individual members of the polysorbate series in the planarian are by far more pronounced. However, both in higher animals and in planarian, esterification of polyoxyethylene 20 sorbitan with oleic acid results in a significantly lower order of toxicity.

Polyoxyethylene Alkyl Ether Series—The results obtained for this series in the same concentration range (0.05–1.0%) as the other surfactants were somewhat different in that they did not seem to fit adequately the Levy–Gucinski theory. Indeed, although a linear relationship between reciprocal time of death of planarian and surfactant concentration was observed, all four lines extrapolated to the ordinate at a point (average intercept ± *SD*, 0.583 ± 0.31) which was significantly different from zero. The results are plotted in Fig. 5; one surfactant (polyoxyethylene 20 stearyl ether) is omitted for the greater clarity of the graph. As may be seen in Table III, the slopes of polyoxyethylene 20 cetyl ether, polyoxyethylene 20 stearyl ether, and polyoxyethylene 20 oleyl ether are significantly different from the slope of polyoxyethylene 23 lauryl ether but do not significantly differ from each other. In other words, the effect on planarian of the three former agents is practically identical, although they correspond to nominally different structures.

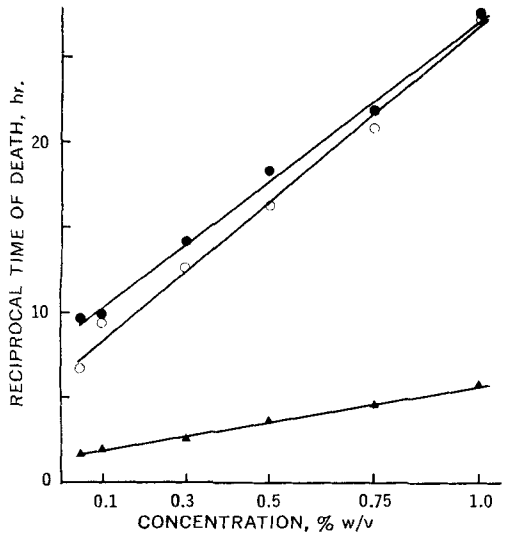

Figure 5—*Reciprocal time of death of planarian as a function of surfactant concentration at 20°. Key: ▲, polyoxyethylene 23 lauryl ether; ●, polyoxyethylene 20 cetyl ether; and ○, polyoxyethylene 20 oleyl ether.*

To gather additional data, the compounds were tested at concentrations ranging from 0.01 to 0.04%. The results are reported in Table VI and plotted in Fig. 6. It can be seen that the points lie on a curve ideally connecting the origin with the straight lines obtained in the experiments at higher concentrations of the surfactants.

These data indicate that polyoxyethylene 23 lauryl ether, the agent possessing the highest degree of hydrophilicity, is the least toxic of the series. This is in agreement with the acute toxicity data in terms of LD_{50} in grams per kilogram in fasted male rat (18).

Compounds of polyoxyethylene stearate and polysorbate series show a linear relationship between $1/T$ and C, and all lines have the origin as common intercept. This implies *absorption* of a toxic agent, whose concentration increases linearly with increasing overall surfactant concentration[6].

The concentration of these surfactants were well above their respective critical micelle concentrations (CMC's)[7]; therefore, the solutions contained micellar aggregates in equilibrium with surfactant monomers. Ultrafiltration studies of nonionic surfactant solutions have demonstrated that, above the CMC, monomers coexist with micelles, and the concentration of both species increases linearly with increasing overall surfactant concentration (19). Therefore, either species may be responsible for the toxic action on the planarian, although penetration of large micellar aggregates through a biological membrane seems less likely.

The behavior of the compounds of the polyoxyethylene alkyl ether series seems to indicate a much higher activity of the toxic species at low surfactant concentrations. A gradual decrease in activity occurs in the concentration range 0.01–0.05%; then a linear relationship between activity and overall surfactant concentration is observed. The effect results in a downward inflection of the $1/T$ *versus* C curve; hence, the linear portion of the curve extrapolates to the ordinate and not to the origin. Perhaps this phenomenon could be ascribed to the presence of toxic impurities undergoing gradual entrapment in surfactant micelles, *i.e.*, decreasing in activity, above the CMC. Indeed, GC analysis of commercial samples of polyoxyethylene alkyl ether surfactants has shown the presence of

[6] As an alternative hypothesis, it might be speculated that surface-active agents alter membrane permeability, with a loss of some essential planarian component, such as electrolyte.

[7] CMC's for Tween 20, 40, 60, and 80 are 0.0060, 0.0029, 0.0027, and 0.0013 g./dl., respectively; for Myrj 51, 52, 53, and 59, 0.0081, 0.011, 0.014, and 0.016 g./dl., respectively (*cf.*, P. Becher, in "Nonionic Surfactants," M. J. Schick, Ed., Marcel Dekker, New York, N. Y., 1967, p. 478). The CMC of Myrj 49, not reported in the literature, was found to be 0.015 g./dl. by the surface-tension method.

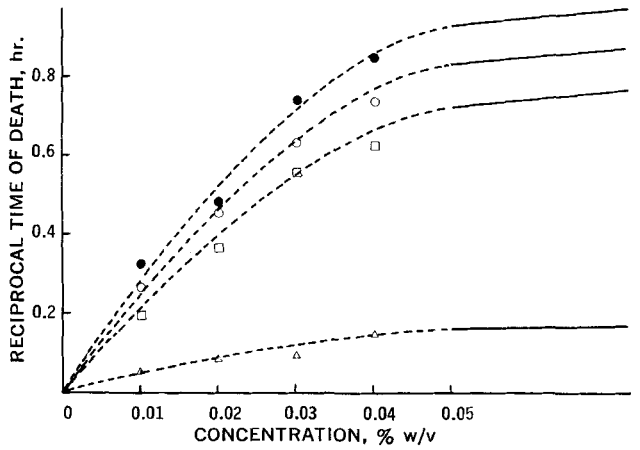

Figure 6—*Reciprocal time of death of planarian as a function of surfactant concentration at 20°. Key: △, polyoxyethylene 23 lauryl ether; ●, polyoxyethylene 20 cetyl ether; ○, polyoxyethylene 20 stearyl ether; and □, polyoxyethylene 20 oleyl ether.*

substantial amounts of alcohols and polyethylene glycols (20). From further work, now in progress, additional data may be obtained which might help clarify this question. The CMC's of the four surfactants, not reported in the literature, were determined by the surface-tension method. They were 0.012, 0.012, 0.010, and 0.018 g./dl. for polyoxyethylene 23 lauryl ether, polyoxyethylene 20 cetyl ether, polyoxyethylene 20 stearyl ether, and polyoxyethylene 20 oleyl ether.

Surface tension of the immersion solutions used throughout this study seems not to have influenced the results. The concentrations of the surface-active agents (except for compounds of the polyoxyethylene alkyl ether series at the 0.01% concentration) were above, or well above, their CMC's. Correspondingly, the variation in surface tension over the wide ranges in concentration was very slight (*cf.*, Table VII). The same can be said for pH of the solutions. Planarians have been reported to be rather indifferent to the pH of their environment, enduring without evident effect wide ranges in this factor (15). To verify this point, the survival time of planarians in solutions of pH ranging from 2.2 to 9.0 was recorded (Table VIII). All animals survived more than 7 days in the pH range 4–7. The pH of surfactant solutions at 1.0% concentration ranged from 4.5 to 7.0 (*cf.*, Table VII).

Table VII—Surface Tension and pH of Surfactant Solutions at 20°

Surface-Active Agent	Concentration, % w/v	Surface Tension, dynes/cm.	pH
Polyoxyethylene 20 stearate	0.05	41.4	
	1.0	38.0	6.3
Polyoxyethylene 30 stearate	0.05	43.0	
	1.0	38.3	5.1
Polyoxyl 40 stearate	0.05	42.7	
	1.0	40.0	4.9
Polyoxyethylene 50 stearate	0.05	44.6	
	1.0	43.4	4.5
Polyoxyethylene 100 stearate	0.05	48.1	
	1.0	47.2	5.0
Polysorbate 20	0.05	34.0	
	1.0	33.3	6.8
Polysorbate 40	0.05	38.8	
	1.0	37.0	6.6
Polysorbate 60	0.05	42.3	
	1.0	38.6	6.7
Polysorbate 80	1.0	40.8	6.7
	5.0	35.8	6.5
Polyoxyethylene 23 lauryl ether	0.05	40.7	
	1.0	40.0	6.5
Polyoxyethylene 20 cetyl ether	0.05	38.9	
	1.0	38.7	6.9
Polyoxyethylene 20 stearyl ether	0.05	40.6	
	1.0	40.3	7.0
Polyoxyethylene 20 oleyl ether	0.05	38.5	
	1.0	38.0	6.1

Table VIII—Survival Time of Planarian in Solutions of Different pH at 20°

pH of Solution[a]	Survival Time[b]
2.2	4.0 min.
3.0	1.0 hr.
4.3–7.5	7 days
8.0	30 hr.
9.0	3.0 hr.

[a] Solutions in the pH range 2.2–5.0 were prepared from 0.01 *N* HCl and 0.01 *M* dibasic sodium citrate; in the pH range 6.0–9.0 from 0.05 *M* tris(hydroxymethyl)aminomethane (Tris) and 0.05 *N* hydrochloric acid. [b] Average of six animals.

In conclusion, the data presented in this preliminary paper point out the utility of the planarian as an experimental animal. No direct evidence is presented concerning the nature of the toxic species (with the possible exception of the polyoxyethylene alkyl ethers at lower concentrations), although it is speculated that the agents in their monomeric state might be responsible for the toxic action.

Data on relative toxicity of surfactants obtained in this study are in satisfactory agreement with data in the literature obtained with higher animals. Planarians proved to be very sensitive to variations in type, structure, and concentration of nonionic surfactants and might prove a useful tool for investigations on these compounds. Further studies on the utility of the planarian in this and other areas of biopharmaceutical research are in progress.

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