FATTY ACIDS, DETERGENTS AND OTHER SURFACTANTS

CARCINOGENICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS. OTHER BIOLOGICAL PROPERTIES. METABOLISM. ENVIRONMENTAL SIGNIFICANCE.

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5.2.2.9 Fatty Acids, Detergents and Other Surfactants

5.2.2.9.1 Introduction.

A number of epidemiologic and laboratory studies have associated increased incidence of gastric carcinomas with the consumption of heated fats (1-3; see also Section 5.1.1.3.2 in Vol. IIA). However, subsequent studies (4, 5) failed to provide any convincing evidence for a causal relationship. The role of dietary fat in the induction of cancer has been critically reviewed by Arffmann (6) and Cooper (7), and was the subject of a workshop in 1981 sponsored by the U.S. National Cancer Institute (8).

Already during the early studies on skin carcinogenesis it was established that certain lipophilic-hydrophilic substances, used as vehicles for carcinogen administration, can considerably modify the effect of carcinogens (9). For instance, many polar-nonpolar compounds, in the group of non-ionic detergents (e.g., Tweens and Spans) were shown to be potent promotors of skin tumorigenesis (9, 10). In connection with these findings, some long-chain fatty acids, which are present in the molecules of certain Tweens and Spans, were themselves found to have carcinogenic, cocarcinogenic, and/or tumorigenesis-promoting properties (11-15). These studies generated concern about the possible carcinogenicity of certain lipids. Because of the increasing growth of the synthetic detergent industry and the widespread application of many of its products (see Section 5.2.2.9.5), the possible carcinogenicity of lipophilic-hydrophilic (surface active) agents has become a focus of concern. The toxicological properties and carcinogenic activities of this interesting group of compounds have been extensively reviewed (16-20).

5.2.2.9.2 Physical and Chemical Properties. Biological Effects.

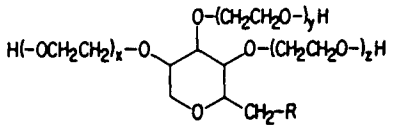
5.2.2.9.2.1 PHYSICAL AND CHEMICAL PROPERTIES.

Fatty acids are saturated or unsaturated straight-chain monocarboxylic acids, usually with an even number of carbons. Short-chain fatty acids are miscible with water; fatty acids with 10 or more carbon atoms are, on the other hand, virtually insoluble in water, but readily soluble in nonpolar solvents. The boiling points and melting points of fatty acids increase with the chain length. Esterification of the carboxyl group and reaction of the double bond(s) (if any) are the most important chemical reactions of fatty acids. The common fatty acids (around 18 carbon atoms) exist mostly in the form of esters as fats, oils, or waxes in animals and plants. Oxidation of the double bond(s) in unsaturated fatty acids yields unstable hydroperoxides which further break down to keto and hydroxy-keto acids.

In order to understand the biological activities of detergents and surface active agents ("surfactants") in general, their physico-chemical properties must be considered. As shown in Tables I and III, all these agents contain characteristic water-insoluble (hydrophobic, lipophilic, or non-polar) groups such as alkyl-, alkylaryl-, or other more complex hydrocarbon moieties and water-soluble (hydrophilic, lipophobic, or polar) moieties such as $-(CH_2-CH_2O)_n-$, $-OH$, $-SO_3^-$, $-COO^-$, or $-\overset{+}{N}R_3$ in their molecules. For a detailed discussion of the molecular mechanism of action of surface active agents, see Sections 3.3.2.1 and 3.3.2.2 in Volume I.

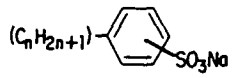
In water/oil or water/air systems, surfactants tend to form oriented monolayers at interfaces; the hydrophilic portion extends into the aqueous phase whereas the lipophilic part of the molecule is directed toward the lipid

TABLE I Structural Formulas of Surfactants Tested for Carcinogenic Activity

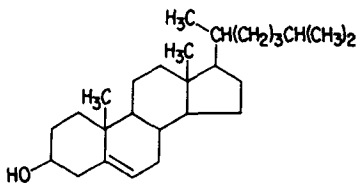


Tweens and Spans

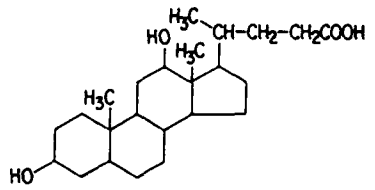
R = fatty acids
Tweens – x,y,z = 5 to 100
Spans – x,y,z = 0



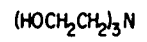
Alkylbenzene sulfonates



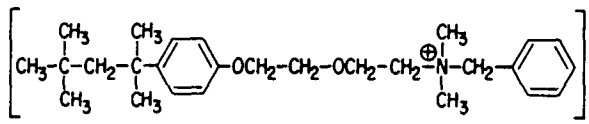
Cholesterol



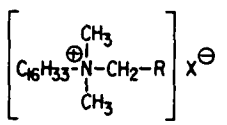
Deoxycholic acid



Triethanolamine



Benzethonium chloride



Cetyldimethylbenzylammonium chloride
(R = C_6H_5; X = Cl)
Cetyltrimethylammonium bromide
(R = H; X = Br)

Table II. Acute Toxicity of Some Fatty Acids, Detergents and Other Surfactants

Compound	Species and Route	LD_{50} (mg/kg)	Reference
A. Fatty Acids			
Lauric acid	Rat, oral	12,000	(21)
	Mouse, i.v.	131	(22)
Palmitic acid	Mouse, i.v.	57	(22)
Stearic acid	Mouse, i.v.	23	(22)
Oleic acid, sodium salt	Mouse, i.v.	152	(21)
B. Detergents and Other Surfactants			
(1) Anionics			
Alkylbenzene sulfonates	Rat, oral	2,200	(23)
	Mouse, oral	4,600	(23)
Deoxycholic acid	Mouse, i.p.	130	(24)
(2) Cationics			
Triethanolamine	Rat, oral	8,680	(25)
	Mouse, i.p.	1,450	(21)
Benzethonium chloride	Rat, oral	665	(26)
	Rat, s.c.	119	(27)
	Mouse, oral	485	(26)
Cetyldimethylbenzyl-ammonium chloride	Rat, oral	234	(28)
Cetyltrimethylammonium bromide	Rat, oral	410	(21)
	Mouse, i.p.	106	(29)
(3) Nonionics			
Tween 20	Rat, oral	> 30,000	(30)
	Rat, i.p.	3,500	(30)
	Mouse, oral	> 30,000	(30)
	Mouse, i.p.	2,400	(30)
Tween 40	Rat, i.v.	1,580	(21)
Tween 60	Rat, oral	> 20,000	(31)
	Rat, i.v.	1,220	(21)
Tween 80	Rat, i.v.	1,790	(31)
Span 20	Rat, oral	> 20 (ml)	(31)
Span 40	Rat, oral	> 10,000	(31)
Span 60	Rat, oral	> 30,000	(31)
Span 80	Rat, oral	> 10 (ml)	(31)

Table III. Carcinogenicity of Fatty Acids and Derivatives in the Mouse

Compound	Structure	Strain	Principal Organ Affected and Route	Reference
A. Saturated	$[CH_3(CH_2)_n-\overset{O}{\overset{\|\|}{C}}-R]$			
Lauric acid	n=10, R=OH	Albino	No significant effect, topical	(11)
		Swiss-Webster	No significant effect, s.c.	(12)
Isopropyl myristate	n=12, R=$OCH(CH_3)_2$	Swiss	No significant effect, topical	(32, 33)
Palmitic acid	n=14, R=OH	Swiss-Webster	No significant effect, s.c.	(12)
Stearic acid	n=16, R=OH	BALB/c, ICR/Ha, Swiss-Webster	No significant effect, s.c.	(12, 13)
2-, 9-, or 10-Hydroxystearic acid	n=16, R=OH (hydroxy group at C-2, C-9, or C-10)	BALB/c	No significant effect, s.c.	(12)
Methyl stearate	n=16, R=OCH_3	ICR/Ha	Local sarcoma, s.c	(13)
		Swiss-Webster	No significant effect, s.c.	(12)
12-Hydroxy-stearic acid	n=16, R=OH (hydroxy group at C-12)	Swiss-Webster	Local sarcoma, s.c.	(12)
Methyl 12-hydroxy-stearate	n=16, R=OCH_3 (hydroxy group at C-12)	Swiss-Webster	Local sarcoma, s.c.	(12)
4-Keto-stearic acid	n=16, R=OH (keto group at C-4)	BALB/c	Local sarcoma[a], s.c.	(12)
Stearohydroxamic acid	n=16, R=NHOH	BALB/c	Local sarcoma[a], s.c.	(12)

Table III. Carcinogenicity of Fatty Acids and Derivatives in the Mouse (continued)

Compound	Structure	Strain	Principal Organ Affected and Route	Reference
B. Unsaturated	$[CH_3(CH_2)_n-R-\overset{\overset{O}{\|\|}}{C}-O-R']$			
Oleic acid	n=7, $R=-CH=CH(CH_2)_7-$, $R'=H$	Swiss-Webster	No significant effect, s.c.	(12)
Methyl oleate	n=7, $R=-CH=CH(CH_2)_7-$, $R'=CH_3$	ST/a	Skin, lymphoid tissue, topical	(14)
Methyl 12-oxo-trans-10-octadecenoate	n=5, $R=-\overset{\overset{O}{\|\|}}{C}-CH=CH-(CH_2)_8-$, $R'=CH_3$	ST/a ST/a	Skin, lymphoid tissue, topical No significant effect, oral	(14, 15)
Methyl 12-hydroxy-10-octadecanoate	n=5, $R=-\underset{\underset{OH}{\|}}{CH}-CH=CH-(CH_2)_8-$, $R'=CH_3$	ST/a	Skin, topical	(14)
Methyl 13-hydroxy-9,11-octadecadienoate	n=4, $R=-\underset{\underset{OH}{\|}}{CH}-CH=CH-CH=CH-(CH_2)_7-$, $R'=CH_3$	ST/a	Skin, topical	(14)

[a]Marginal activity

phase or the air. This molecular orientation brings about a lowering of interfacial or surface tension. Another characteristic of surfactants is the formation of micelles in aqueous solution when their concentration in the solution exceeds the "critical micelle concentration" (CMC). Owing to these properties, solutions of surfactants exhibit, to various degrees, the following functional activities: detergency, emulsifying, wetting, solubilizing, foaming, and dispersing, which are closely related to the balance between their hydrophilic and lipophilic properties. For more details on the physico-chemical properties of surfactants the reader is referred to the following publications (34-37, 38a).

On the basis of their ionizing properties, surfactants may be classified into anionic, cationic, and non-ionic types. Alkylbenzene sulfonates are typical anionic surfactants because the electrically charged group of these molecules, the hydrophilic sulfonate moiety, is anionic. Benzethonium chloride, cetyldimethylbenzylammonium chloride, and cetyltrimethylammonium bromide are cationic surfactants since the charged moiety of these compounds, the hydrophilic quaternary ammonium ion, is cationic. The Spans and Tweens, which do not ionize, are non-ionic surfactants. Chemically, the Spans are fatty acid esters of the cyclic sorbitol anhydride, sorbitan. The fatty acid moieties of Span 20, Span 40, Span 60, and Span 80 are lauric acid, palmitic acid, stearic acid, and oleic acid, respectively. Addition of polyoxyethylene chains of variable length to the three vicinal tertiary hydroxyl groups of Spans results in the respective Tweens (Tween 20, Tween 40, Tween 60, and Tween 80). The Tweens commonly used in carcinogenicity studies contain a total of about 5-100 ethyleneoxide units. The Spans are lipophilic, due to the fatty acid moieties. The Tweens are hydrophilic and their hydrosolubility increases with the number of the hydrophilic ethyleneoxide units.

The emulsifying properties of surfactants is determined by the balance of the size and strength of the hydrophilic and lipophilic groups in the molecule, the "hydrophile-lipophile balance" (HLB). A strongly lipophilic emulsifier has a low HLB, usually < 10; a highly hydrophilic emulsifier has a HLB value usually >10. The HLB values may be determined experimentally or may be calculated. The HLB values of most polyol fatty acid esters can be calculated with the formula:

$$HLB = 20\ (1 - \frac{S}{A})$$

where "S" is the saponification number of the ester and "A" the acid number of the acid. For example, the HLB values for Tween 20, Tween 40, and Tween 60 are 16.7, 15.6, and 14.9, respectively (10, 35).

5.2.2.9.2.2 BIOLOGICAL EFFECTS OTHER THAN CARCINOGENIC

Toxicity. Despite little direct evidence for significant toxic effects of oxidized fats or fatty acids to humans, heated fats and oxidation products of certain fatty acids have been reported to retard growth and induce pathological lesions, anorexia, diarrhea, and death in animals (7; cited in ref. 38b). Administration of methyl linoleate derivatives intragastrically to mice caused atrophy of the spleen, dilatation of the intestine, histological changes in the lymphatic system, and leukopenia (39). Severe inflammatory reaction with leukocyte infiltration and necrosis were observed following subcutaneous injection of methyl-12-oxo-trans-10-octadecenoate (38b). Among other lipophilic acids, linoleic acid and palmitic acid were shown to be potent inhibitors of HeLa cell replication (40). Linoleic acid was also reported to be highly toxic to lung (41) and cultured liver cells of rats

(42). Oro and Wretlind (22) have determined the LD_{50} values for saturated fatty acids from C_2 to C_{18} by intravenous injection into mice. They found that stearic acid (C_{18}) was the most toxic, with LD_{50} of 23 mg/kg body weight, which was ten times lower than that of its unsaturated analog, oleic acid. Toxicity decreased with the number of carbon atoms in the molecule, reaching the lowest point for caproic acid (C_6): LD_{50} = 1,725 mg/kg. With further decrease in the number of carbon atoms, toxicity increased; the LD_{50} for acetic acid (C_2) was found to be 525 mg/kg.

In general, most surfactants are relatively innocuous. The oral LD_{50} values for anionic surfactants in various laboratory animals range between 500 and 5,000 mg/kg; cationic surfactants are slightly more toxic and nonionics are somewhat less (17) (Table II).

Irritation of tissues, mucous membranes, and skin is among the most common local effects of surfactants. The degree of irritation depends largely on their surface active properties which in turn depend on their physico-chemical characteristics. When tested in animals and humans, many anionic and nonionic surfactants which are used as household detergents do not induce skin or eye irritation, or sensitization, even at high concentrations (23, 42). However, exposure of the skin of mice to certain anionic and cationic surfactants (e.g., cetyltrimethylammonium bromide) at a concentration of 10% results in cellular damage and necrosis. Following multiple applications of 1-2.5% of the agents, acanthosis and hyperkeratosis were observed (44). Toxic myocardial changes and damage in the mucous membrane of the gastrointestinal tract have also been reported in animals receiving high doses of anionic surfactants (20). Alkylbenzene sulfonates with alkyl chains of 10-12 carbon atoms are more irritating and toxic than those with shorter or longer alkyl chains; linear and branched chain compounds have similar acute oral toxicities (20). Upon

parenteral administration, certain anionic and cationic detergents are hemolytic, due primarily to their capability to solubilize and elute phospholipids from the cell membrane. The concentration required for hemolysis is related to the content of phospholipids in the membrane of the erythrocytes (18).

Mutagenicity. There is a scarcity of information on the mutagenic activity of fatty acids and detergents. A 1980 study showed that neither 12-hydroxystearic acid nor the oxidized fractions of deep frying fats are mutagenic in different Salmonella typhimurium tester strains with or without metabolic activation (45). Linoleic acid, oleic acid, and methyl oleate were tested for mutagenicity in the U.S. National Toxicology Program. All three compounds were negative in the Ames test (46). Tween 60 and Tween 80 did not induce chromosomal aberrations either in vitro in Chinese hamster cells or in vivo in mouse bone marrow cells (47, 48). They were also nonmutagenic in the Ames test and in the silkworm oocyte system (47). Consistent with these findings, Tween 60, Span 60, alkylbenzene sulfonates, cetyltrimethylammonium chloride, dicetyldimethylammonium chloride, and cetyldimethylbenzylammonium chloride (49), as well as benzethonium chloride and cholesterol (50) gave negative results when tested for mutagenicity in strains TA100 and TA98 of S. typhimurium in the presence and absence of S-9 activating mix. Tween 80 was also not mutagenic in the dominant-lethal test in mice (51) or in the sex-linked recessive lethal test in Drosophila melanogaster (52). The mutagenic properties of anionic surfactants have recently been reviewed by Oba (19). Absence of mutagenicity was reported for alkylbenzene sulfonates or other anionics assayed in various bacterial and mammalian systems. Tween 60 but not Tween 80 was found mutagenic in strains H-17 and M-45 of Bacillus subtilis and strain WP-2 of Escherichia coli (47). Moreover, synergism between Tween 60 and ethyleneimine in inducing chlorophyll mutation in barley plants was

reported (53). Various mutagenic effects have also been described in cluster beans treated with triethanolamine (54). Hoshino and Tanooka (55), however, found triethanolamine itself non-mutagenic to Bacillus subtilis; mutagenic effect to the bacteria was only observed after reacting with sodium nitrite under acidic conditions or when the mixture was heated.

Teratogenicity. Studies on chemical teratogenesis during the past few decades have indicated that teratogenic effects may result from a number of different mechanisms. Freese et al. (40) have noted a high correlation between inhibition of mammalian cell growth and reported teratogenicity of certain lipophilic organic acids; it is interesting to note that palmitic and linoleic acid are inhibitors of HeLa cell replication (see above under "Toxicity"). It remains to be tested if exposure to unusually high levels of these fatty acids is potentially teratogenic. Linoleic acid was positive in an in vitro cell surface recognition assay system for potential teratogens (56). An oxidized linoleic acid sample (which contained about 25% linoleic acid hydroperoxide), but not purified linoleic acid, induced an elevated incidence of malformations in the offspring of treated rats (57). The teratogenic activity of other fatty acids is still unknown.

A considerable number of studies on the teratogenic potential of detergents and other surfactants (anionics in particular) have been carried out in various animal species by oral, dermal, and subcutaneous administration. The results show no conclusive evidence of teratogenicity. A Japanese group headed by Mikami (cited in ref. 19) reported that linear alkylbenzene sulfonates and other commercial detergent formulations based on anionic surfactants caused increased incidence of malformations in rats and mice. However, their data were considered to be inadequate and incomplete, and other investigators failed to confirm their results in other strains of animals. Studies by

Mikami's group as well as others on the teratogenic activity of anionic surfactants have been critically reviewed (19, 58). Similarly, there is no significant evidence fo the teratogenicity of cationic surfactants. Except for a general increase in the incidence of variations of cervical vertebral arches in the offspring of mice treated with dicetyldimethylammonium chloride (50 or 200 mg/kg body weight), no significant change in the rate of malformations was observed (59). In rats, a high dose (35.6 mg/kg/day) of benzethonium chloride produced delayed ossification; however, the effect was not regarded to be related to teratogenicity, but to the decrease of fetal growth secondary to maternal toxicity (60). However, when pregnant mice were administered cetyltrimethylammonium bromide at an intraperitoneal dose of 10.5 or 35.0 mg/kg (10 or 33% of the LD_{50}), a higher incidence of malformed fetuses was found (29). At the time of this writing, the evidence is conflicting regarding the teratogenicity of the typical non-ionic surfactants, the Tweens. Verrett et al. (61) found Tween 60 and Tween 80 not teratogenic in developing chick embryo. However, in recent studies, Kocher-Becker et al. (62) observed that Tween 20, which was previously regarded to be an inert vehicle in teratogenicity assays, produced malformations in mice strikingly similar to those produced by thalidomide. Pregnant mice were given a single intraperitoneal injection of Tween 20 on day 9 of gestation; the doses that caused thalidomide-like malformations were 1.0, 1.7, 2.5, and 3.3 mg/kg body weight.

5.2.2.9.3 Carcinogenicity and Its Structure-Activity Relationships.

5.2.2.9.3.1 FATTY ACIDS.

A short-term bioassay for preliminary screening of possible carcinogenicity of a series of fatty acids was developed by Arffmann (63) [based on injection of the fatty acids into the tail of the newt (Triton cristatus)]. Epidermal hyperplasia and downgrowths into the dermis, suggestive of carcinogenic activity, was observed with certain derivatives of methyl oleate and methyl linoleate (64). The most active compound was methyl 12-oxo-trans-10-octadecenoate, which is derived from methyl oleate and has an oxo group in the α-position relative to the double bond. Moderate activity was found with derivatives of both methyl oleate and methyl linoleate with an α-hydroxy group, namely, methyl 12-hydroxy-10-octadecenoate and methyl 13-hydroxy-9,11-octadecadienoate. The corresponding derivatives having a hydroxy group in the β-position were inactive. On the basis of the chemical structures, it appears that conjugation of double bond(s) with an oxygen-containing group in the α-position may be important for the carcinogenic activity of fatty acid derivatives.

In studies by application of methyl oleate and two oxo- and hydroxy-derivatives of its 10-octadecenoate isomer (20% v/v in acetone) to the skin (3 times weekly for 1 year) of ST/a mice all three compounds exhibited low level of complete carcinogenic activity, but were potent promotors of papillomas, lymphomas, and malignant skin tumors induced by 7, 12-dimethylbenz[a]anthracene (14, 64, 65). In another experiment, the incidence and number of forestomach papillomas initiated by 4-nitroquinoline-N-oxide were also increased in ST/a mice given methyl-12-oxo-trans-10-octadecenoate (15 mg/day) in the diet for 300 days; when administered orally alone, however, methyl-12-oxo-

trans-10-octadecenoate did not induce tumors in the mice (15). Data yielded by these and other studies on the complete carcinogenicity of fatty acids and derivatives are given in Table III.

Swern et al. (12) investigated the carcinogenic activity of 11 fatty acids and their derivatives by repeated subcutaneous injection to female BALB/c or CFW (Swiss-Webster) mice. Lauric acid, palmitic acid, oleic acid, stearic acid, methyl stearate, and 2-, 9-, or 10-hydroxystearic acid were regarded by the authors as noncarcinogenic under the conditions of their experiment. Methyl 12-hydroxystearate and 12-hydroxystearic acid induced sarcomas in 8 of 27 and 9 of 28 mice, respectively, and were considered to be carcinogenic. Stearohydroxamic acid and 4-ketostearic acid, which elicited 3 and 2 sarcomas in 13 and 14 mice, respectively, were regarded to be marginally carcinogenic toward the subcutaneous tissue of mice. The absence of carcinogenic activity of stearic acid toward the subcutaneous tissue of mice was later confirmed by Van Duuren et al. (13); methyl stearate, however, was found to be weakly carcinogenic (13). Oleic and lauric acid did not induce tumors when applied to the skin of mice daily, 6 times a week for 31 weeks but displayed significant promoting effect in skin tumor induction (11). Interestingly, isopropyl myristate which was reported to cause various cutaneous lesions, did not significantly increase the tumor incidence in female Swiss mice (32, 33) or New Zealand rabbits (33) by repeated application to the skin for the life-span of the animals.

5.2.2.9.3.2 DETERGENTS AND OTHER SURFACTANTS.

Studies on the complete carcinogenicity of these compounds are summarized in Table IV. Their cocarcinogenic and tumorigenesis-promoting activities will be extensively discussed in Section 6, Vol. IV.

Table IV. Carcinogenicity of Detergents and Surfactants

	Compound[a]	Species and Strain	Principal Organ Affected and Route	Reference
A.	Non-ionic			
	Tween 60	Mouse, Swiss and unspecified	Skin, topical	(10, 66-68)
		Rat, Osborne-Mendel and Bethesda Black	Local sarcoma, s.c.	(69)
	Tween 80	Rat, Shell and Carworth Farms E	Local sarcoma, s.c.	(70)
	Cholesterol	Mouse, C57, MRC and CBA	Local sarcoma, s.c.	(71-73)
		Mouse, albino and Swiss	Bladder, implantation	(74-76)
B.	Anionic			
	Alkylbenzene sulfonates (ABS)	Rat, albino	No significant effect, oral	(77)
		Rat, Wistar	No significant effect, oral	(78)
		Rat, Moriyama	No significant effect, oral	(79)
		Mouse, Swiss	No significant effect, topical	(80)
		Mouse, C57	No significant effect, s.c.	(80)
		Rabbit, unspecified	No significant effect, topical	(80)

Table IV. Carcinogenicity of Detergents and Surfactants
(continued)

Compound[a]	Species and Strain	Principal Organ Affected and Route	Reference
B. Anionic (cont'd)			
Deoxycholic acid	Rat, unspecified	Local sarcoma, s.c.	(cited in ref. 16)
	Mouse, C3H	Local sarcoma, s.c.	(cited in ref. 16)
	Mouse, CF-1	No significant effect, topical	(81)
C. Cationic			
Triethanol-amine	Rat, albino	No significant effect[b], topical	(82)
	Mouse, ICR-JCL	Lymphoid tissue, mammary gland, lung, ovary, oral	(55)
	Mouse, CBA x C57	No significant effect[b], topical	(82)
Benzethonium chloride	Rat, Fischer 344	Local sarcoma, s.c.	(27)
Cetyldimethyl-benzylammonium chloride	Rat, Osborne-Mendel	No significant effect, oral	(83)
	Rat, albino	No significant effect, oral	(28)
Cetyltrimethyl-ammonium bromide	Rat, Sprague-Dawley	No significant effect, oral	(84)

[a]See Table I for structure

[b]The duration of the study was only 26 weeks

Anionics. Because of the evident structural relationship to alkylbenzenes, there has been concern about the possible carcinogenicity of mixtures of alkylbenzene sulfonates averaging twelve carbon atoms (ABS), which are among the strongest surfactants known and the major components of commercial detergent products. However, experiments to date failed to demonstrate any carcinogenic effect of these compounds. Paynter and Weir (77) conducted a 2-year toxicity study, in which 120 albino rats of both sexes were given dodecylbenzene sodium sulfonate at levels of 0, 200, 1,000, and 2,000 ppm in the diet and the authors found no pathological changes in various organs attributable to the intake of the test substance. Similar studies were performed by Tusing et al. (78) in 120 male and 120 female Wistar rats. ABS was administered either in the diet at levels of 0, 0.1% (1,000 ppm) and 0.5% (5,000 ppm) or in the drinking water (about 0.05%) for 104 weeks. Gross and microscopic examination of the tissues revealed no evidence of toxic changes resulting from the treatment with ABS by either route. Saffiotti et al. (80) tested ABS for possible carcinogenicity in the skin of mice and rabbits. Application of ABS twice weekly to the skin of Swiss mice or of rabbits for 110 weeks, at concentrations of 5 or 10%, induced no tumors; no carcinogenic activity was observed either by subcutaneous injection of ABS into C57 mice (80). Additional evidence for the absence of carcinogenic activity of ABS has been provided by other bioassays using rats and mice (cited in ref. 19).

In contrast to the lack of complete carcinogenic activity, there is evidence for the tumorigenesis-promoting properties of ABS. Takahashi (79) reported significantly higher incidence of gastric cancers in rats which received concurrent treatment of 4-nitroquinoline-N-oxide and ABS by gavage than in those given 4-nitroquinoline-N-oxide alone; no neoplasms of the glandular stomach or other organs were found in the control group of rats

administered only ABS. ABS also enhanced the incidence of N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric tumors in rats when administered simultaneously in the drinking water (85).

Deoxycholic acid, a bacterial metabolite of bile acid (cholic acid), has long been suspected to be a carcinogen, cocarcinogen, or promotor in the pathogenesis of human colon cancer (cited in ref. 86). While several investigators reported its sarcomatogenic activity following subcutaneous injection into rats and mice, others found it not carcinogenic (rev. in ref. 16). Painting of deoxycholic acid on the skin of mice induced no tumors (81). Several experiments seem to support the view that deoxycholic acid or its metabolites (e.g., 12-ketolithocholic acid) may act as a cocarcinogen or promotor in colon carcinogenesis (87-92). However, studies in the classic mouse skin system (93) or in the dimethylhydrazine-induced rat colon cancer model (86) showed no promoting activity of deoxycholic acid or related agents.

Cationics. The carcinogenic potential of triethanolamine has been investigated by Hoshino and Tanooka (55). Following feeding to male and female ICR-JCL mice 0.03 and 0.3% of triethanolamine in the diet, significantly higher incidences of tumors of the lymphoid tissues were found in the females. Moreover, malignant tumors of various other tissues were also produced in higher rates in both sexes of the experimentals than in the untreated controls. Most tumors appeared after at least 60 weeks of treatment. Kostrodymova et al. (82) conducted a 26-week study in which triethanolamine (13%) was administered epicutaneously to albino rats and CBA x C57 mice. However, because of the short duration of administration no conclusion can be made regarding the carcinogenicity or inactivity of this compound.

Benzethonium chloride, when administered subcutaneously at doses of 0.1, 0.3, 1.0, and 3.0 mg/kg body weight twice weekly for 52 weeks to 200 F344 rats, gave rise to 26 fibrosarcomas at the injection site. Only one injection site-related tumor was observed in the 200 controls (27). Several structurally-related cationics such as cetyltrimethylammonium chloride, dicetyldimethylammonium chloride, and cetyldimethylbenzylammonium chloride were reported negative in the in vitro transformation of hamster embryo cell bioassay (49). Oral administration of cetyldimethylbenzylammonium chloride (28, 83) or cetyltrimethylammonium bromide (84) to rats for 4 to 24 months produced no carcinogenic effects.

Nonionics. In a series of studies on the tumorigenesis-promoting effects of Span and Tween surfactants, Setälä (10) was the first to observe occasional papillomas on the skin of mice painted with Tween 60 alone (daily, 6 times a week for 52 weeks) without prior initiation by hydrocarbon carcinogens. Subsequently, the complete carcinogenic action of Tween 60 in mouse skin was observed by various investigators, reporting the induction of malignant squamous cell carcinomas as well as benign tumors (66-68). The compound was also shown to induce local fibrosarcomas of moderate malignancy in 5 of 30 rats receiving 1 ml (6%) weekly by subcutaneous injection for 73 weeks (69). Similarly, repeated subcutaneous injections of 2 ml (6%) Tween 80 twice weekly for 40 weeks induced local sarcomas in 11 of 17 rats (70). Since large doses are required to induce tumors, these compounds are regarded as only weakly carcinogenic. Some authors (66, 68) interpreted the effects as due to a physical phenomenon (the so-called "solid-state carcinogenesis") rather than to chemical action. No evidence of carcinogenicity was found with other Tween and Span surfactants (94, 95). This group of nonionic hydrophilic lipophilic substances are better known in the cancer literature for their tumorigenesis-promoting properties.

Indeed, as early as 1954, Setälä and coworkers (9) observed that Span 20 and Tween 60, when applied topically and repeatedly to the skin of mice enhanced the carcinogenic action of 7,12-dimethylbenz[a]anthracene. Subsequent studies on a series of Tween and Span surfactants established that these compounds represent a new type of physico-chemically defined promoting agents for the mouse skin (10, 96, 97). Evidence for the tumorigenesis-promoting action of various Tweens was also provided by other investigators (66-68, 98). The hydrophilic nature of as well as the fatty acid moiety in these compounds are key features for their promoting activity. The most potent promoting agents in the group are Tween 40, Tween 60, and Tween 80, which all possess high HLB values (15.6, 14.9, and 15.0, respectively) and a relatively long-chain fatty acid in their molecules. Compounds of the Span type, which lack the hydrophilic polyoxyethylene moieties and have, therefore, lower HLB values (1.8-8.6) are only weak promotors. The optimum HLB range for skin tumor promotion is 11.0-15.6 (10). In order to be effective in tumorigenesis promotion, it is also essential that they are applied in relatively large doses and with sufficient frequency (10). The difference between this group of compounds and croton oil lies mainly in the amount of substance necessary for tumorigenesis promotion (68). More recently, the cocarcinogenic effects of Span 20 and several Tweens in gastric carcinogenesis, by oral administration of N-methyl-N'-nitro-N-nitrosoguanidine or methylnitrosocyanimide, have also been shown in rats (85, 99, 100).

The carcinogenicity of cholesterol -- which may be regarded structurally as a strongly lipophilic nonionic surfactant -- has been a subject of controversy and has been critically discussed by several authors (6, 16, 101, 102). Bioassays of cholesterol in many laboratories failed to induce tumors in experimental animals (cited in ref. 6). An International Agency for

Research on Cancer working group (102) considered the tumorigenicity of cholesterol difficult to evaluate in a number of feeding experiments, because of the presence of other components and impurities in the diet. Positive carcinogenic effects were noted in several experiments involving subcutaneous injection (e.g., 71-73) or implantation (74-76) of cholesterol pellets into mice. However, the effects were variably interpreted (72, 102, 103) and the carcinogenic action of cholesterol is still unclear. On the other hand, the compound has been reported to exhibit cocarcinogenic activity with dimethylnitrosamine in the induction of bladder tumors in hamsters (104) as well as with N-hydroxy-acetylaminofluorene in the induction of liver tumors in mice (105). The carcinogenic activity of cholesterol has also been dicussed in Vol. IIA, Section 5.1.1.2.1.

5.2.2.9.4 Metabolism and Mechanism of Action.

In contrast to the well established β-oxidation pathway of normal fatty acids, there is a relative paucity of information regarding the metabolism of the carcinogenic fatty acid derivatives. The metabolism and tissue distribution studies on methyl 13-hydroxy-9,11-octadecadienoate in the rat (106, 107) are rather unique in this regard. On the other hand, several important reviews on the absorption, metabolic degradation, and excretion of various detergents and other surfactants are available (18, 31, 108). There is no evidence for bioactivation which might be responsible for the weak carcinogenic and promoting effects of these hydrophilic-lipophilic compounds toward the skin and subcutaneous tissue of rats and mice. Instead, there is overwhelming support for the hypothesis that the action of these polar-nonpolar substances is due primarily to their surfactant properties, whereby they structurally disorganize endoplasmic lipoprotein membranes and affect cellular

homeostasis (16). Interference with mitochondrial oxidative phosphorylation by 12-hydroxystearic acid and its methyl ester is believed to be the mechanism of carcinogenesis by these fatty acid derivatives (109), due possibly to disruption of membrane systems and denaturation of functional proteins. Many surfactants have been shown to alter the intestinal permeability of various nutrient substances (cited in ref. 84). "Solvent action" which facilitates the absorption and penetration of other carcinogens through the skin or gastrointestinal tract is believed to play a major role in tumorigenesis promotion or cocarcinogenesis (10, 11, 79, 85, 99, 110). The promoting activity of individual agents, on one hand, and the nature and intensity of histological changes in the skin (9) and stomach wall (111), on the other hand, have both been correlated with the hydrophile-lipophile balance (HLB value) of the compounds.

Interestingly, Rohrschneider and Boutwell (112) noted a structural similarity between phorbol esters, the active principles of croton oil, and methyl esters of polyunsaturated fatty acids. The authors speculated that there might be a receptor site in the cell membrane for natural cellular regulators which are structurally related to fatty acids -- prostaglandins, for instance. The effect of phorbol esters was interpreted as being mediated by interaction with the receptor site due to structural similarity with the natural cellular regulator. The weak carcinogenic and promoting activities of fatty acid derivatives observed may mimic the action of the phorbol esters. Like croton oil and other promoting agents, some fatty acids, unsaturated in particular, and their derivatives, have actually been found to induce mitosis and stimulate cell division when applied to the mouse skin (10, 113). Jirgensons (114) has recently studied, by circular dichroism, the effects of various promoting agents on the conformation of histones, which play an impor-

tant role in gene regulation: the order of conformation-modifying activity of phorbol esters and Tween 60 (polyoxyethylene sorbitan monostearate) was found to parallel their promoting activity.

Furthermore, there are observations that Tween and Span surfactants are inhibitors of DNA repair (115-117), and it has been suggested that the carcinogenic, cocarcinogenic, and tumorigenesis-promoting activities of these agents are related to their inhibition of DNA repair replication (115). However, other studies (116, 117) failed to demonstrate any specificity of these compounds in the inhibition of DNA synthesis. It is possible that the effects are due to the general action of these substances to denature proteins, including DNA repair enzymes, by virtue of their surfactant properties.

5.2.2.9.5 Environmental Significance.

Fatty acids are normal components of animal fats and plant oils. Unsaturated fatty acids readily undergo autooxidation to yield hydroperoxides and various oxygen-containing secondary products including epoxides and hydroxy compounds. (The carcinogenic action of peroxides and epoxides have been discussed in Sections 5.2.1.7.3 and 5.2.1.1.5 of Vol. IIIA). Altered fatty acids may also result from polymerization and other chemical reactions during heating.

Certain fatty acids occur as the lipophilic moiety of soaps and synthetic surfactants (e.g., Tweens and Spans), which are used to formulate a wide variety of household and industrial cleansing products. In addition, many of the surfactants are used as food additives as well as co-solvents, solubilizers, dispersants, and emulsifiers in various industries (37, 118).

Because of their relatively low cost and rapid rate of biodegradation, the anionics are the most widely used. An estimate in 1968 indicated that the production rate of ABS in the U.S. was then about 540 million lbs/yr (37). The cationics (e.g., benzethonium chloride, cetyldimethylbenzylammonium chloride) are used principally as disinfectants and germicides, rather than as detergents. Many of these are used in hospitals and restaurants, as well as in the pharmaceutical, cosmetics, and toiletries industries (119). Moreover, a considerable amount of triethanolamine, which may be converted to the carcinogenic nitrosamines is often present in cutting fluid formulations (e.g., 55).

The widespread applications of this group of chemicals, both in the home and in industry, make it almost certain that there is ubiquitous exposure of humans either through direct contact with the skin, mucous membranes, or indirectly by ingestion through food and water. The intake of detergents per person in the U.S. has been estimated to be 0.3-3.0 mg/day (17).

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Notes Added After Completion of Section 5.2.2.9

The promotion of experimental carcinogenesis by dietary unsaturated fatty acids has been repeatedly demonstrated in rodents. Sakaguchi *et al.* (1) reported that rats fed a diet containig 5% linoleic acid or 4.7% stearic acid had significantly higher incidence of colon tumors induced by azoxymethane. Lipid analysis showed that unsaturated fat diet markedly altered the phosphatide fatty acyl composition of colon mucosa and increased the level of arachidonic acid in the neutral lipid of colon tumors (1). The altered lipid composition of the mucosa can lead to changes in the fluidity and permeability of cell membranes. In line with these findings, recent research has shown that dietary unsaturated fatty acids, as many tumorigenesis-promoting chemicals, inhibit "metabolic cooperation" between Chinese V79 hamster cells (2, 3). Interestingly, no such activity was observed with *trans*-oleic (elaidic) acid and with saturated fatty acids, suggesting that the *cis*-double bond orientation may be essential for the inhibition of intercellular communication by fatty acids (3). The excess of arachidonic acid, the precursor of prostaglandins, in colon tumors has been suggested to play a role in the promotion of carcinogenesis by the unsaturated fat diet (1). Ip *et al.* (4) have demonstrated that at least part of the promoting effects of polyunsaturated fat on 7,12-dimethylbenz[a]anthracene-induced mammary tumorigenesis in rats is mediated through increased synthesis of prostaglandins, since the addition of indomethacin (an inhibitor of prostaglandin synthesis) to the diet completely abolished the stimulatory effect of linoleic acid on tumorigenesis. On the other hand, arachidonic acid and unsaturated fatty acids (but not saturated fatty acids) have been found to activate protein kinase C in human (5) and mouse (6) tissues to similar extent as the tumorigenesis-promotor phorbol esters. McPhail *et al.* (5) proposed that the release of arachidonic acid

could play the role of a second messenger in the regulation of cellular activities by the stimulation of protein kinase C. It is interesting that the potency of unsaturated C_{18} fatty acids in activating protein kinase C parallels the number of cis-double bonds, in that γ-linolenic acid > linoleic acid > oleic acid, containing three, two and one double bonds, respectively.

Triethanolamine has been selected for carcinogenesis bioassay by the U.S. National Toxicology Program; the study on a closely related compound diethanolamine, has been completed and the histopathology of animal tissues are being examined at the time of this writing (7). The toxicological properties of a large number of surfactants used in cosmetic formulations has been assessed by the Cosmetic Ingredient Review expert panel (8-11).

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