Toxicological, Bacteriocidal and Fungicidal Properties of Fatty Acids and Some Derivatives

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ABSTRACT

The LD_{50} oral ingestion values for the common commercial fatty acids are in the range which are considered nontoxic; 24 hour primary skin irritation is considered positive for octanoic acid but negative for decanoic acid and upwards; 4 hour skin corrosivity is considered positive for decanoic acid and lower, negative for lauric acid and higher; eye irritation is considered positive for lauric acid and lower, negative for myristic acid and higher. Among the fatty acid derivatives that have well recognized bacteriocidal and fungicidal properties are undecanoic acid and its salts, sodium and zinc particularly, (athlete's foot fungus), fatty amine quaternary salts, (general bacteriocide properties), fatty amide derivatives, and sodium salts of common fatty acids. The highly purified monoglyceride prepared from high C-12 fatty acid has unprecedented activity and sanitizing properties.

INTRODUCTION

Lipids represent an important and controversial part of our diet. A quota of 20% to 40% of fat by weight in the diet improves the rate of growing animals. This is not entirely due to a high calorie intake but also to a greater efficiency of utilization of fat itself and other dietary constituents, particularly fat soluble nutrients. Besides their caloric value, lipids have several noncaloric roles. The pharmacological effect of lipids has been the subject of a recent review (1). One of the more exciting properties of lipids (fatty acids and derivatives) is their antimicrobial activity. The use of such material food and cosmetic preservative offers numerous advantages, the most important of these being the lack of toxicity to man and minimal adverse effect on the environment.

The present review collates information on the toxicology of fatty acids and esters of fatty acids. While most of these chemicals are Generally Regarded as Safe (GRAS), supporting data in the literature is sparse. Much of the lack of interest in the toxicology of these compounds is due to their long history of use as foods, and their general reputation for safety: the ultimate objective of this review is to form generalizations which will capsule the existing literature on the subject and make for an easier and better understanding of these substances as potential useful agents above and beyond their caloric value.

The term toxicity in this review will refer to mammalian lethality, while the term activity will be taken to mean antimicrobial activity.

A biological perspective of fatty acids and their esters can best be attained by quickly reviewing the digestion and absorption of fats. This will clarify the fate of fatty acids and contrast the metabolism of mono-, di- and triglycerides and esters of other polyols.

DIGESTION AND ABSORPTION OF FATS

Triglycerides, which are the main components of the food fats, are transformed into mono- and diglycerides during digestion. Since no significant amount of lipase is present in the mouth or stomach and no mechanics for emulsification exists, little or no fat digestion occurs in these organs. Moreover, the acid pH of both the mouth and stomach are not conducive to fat digestion.

The small intestine is the major site of fat digestion. In the intestinal lumen contents of man, Kayden et al. (2) identified two phases: an oil phase which contains mainly di- and triglycerides and some fatty acids, and a micellar phase which is a combination of bile salts, free fatty acids (FFA) and monoglycerides. From the micellar solution, monoglycerides and free fatty acids are absorbed through the intestinal wall (Fig. 1).

Pancreatic lipase, steapsin, is an α-lipase specifically attacking the ester linkages at the 1- and 3-positions in the triglycerides, leaving a monoglyceride with the fatty acid esterified at the glycerol carbon-2. This linkage may then be cleaved by an esterase to release the third fatty acid and glycerol.

Recent investigations (3) in which ^{14}C-labeled mono-, di-, and triolein was administered to rats with cannulated thoracic ducts indicated that there was digestive cleavage of all the fatty acids in the 1- and 3-positions. There was hydrolysis of only 22% of the fatty acids in the beta position, however. Thus, ca. 75% of the fatty acids of this particular dietary fat were split and absorbed as free fatty acids. The remainder were absorbed primarily as a β-monoglyceride.

Monoglycerides, along with bile salts, play an important role in stabilizing and further increasing the emulsification of fat in the small intestine. This further enhances the digestion of fats and other lipids solubilized in the micellar particle.

Thus, 50% to 75% of dietary fat is split by steapsin to free fatty acids, which are absorbed as such. A smaller amount is partially digested to and absorbed as β-monoglycerides, and still less as di- and triglycerides. Some estimates

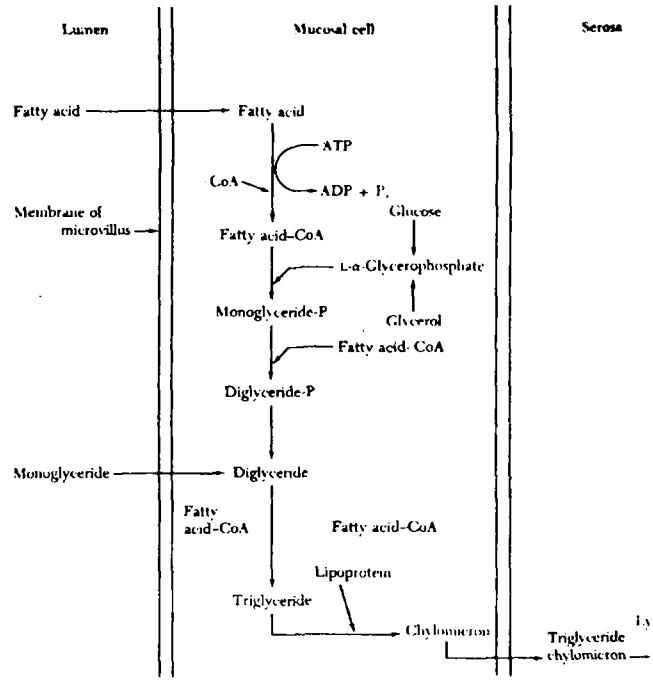

FIG. 1. Schematic representation of principal biochemical reactions during intestinal absorption of long chain fatty acids and monoglycerides. Adapted from Isselbacher, K.J., Fed. Proc. Sympos. 26:1420 (1967).

are that the final digestion and absorption of usual dietary fats are ca. 95% complete in normal individuals.

The review of the literature by Johnston (4) indicates that the monoglycerides and free fatty acids in the micellar solution are absorbed into the intestinal wall, leaving the bile salts within the lumen to form additional micelles. The monoglycerides and fatty acids migrate to the endoplasmic reticulum where resynthesis occurs. Fatty acids are activated enzymatically and resynthesized into triglycerides by the acylation of monoglycerides, the monoglycerides are esterified to triglycerides, and then the triglycerides are incorporated into chylomicrons for delivery to the intestinal lymphatics. Thus, diglycerides and triglycerides are digested through enzymatic action to monoglycerides, absorbed into the lumen of the intestinal wall, resynthesized into the triglycerides and delivered to the lymphatic system for final distribution to the extracellular fluids and to the fat depots.

Absorption of the digestion products of fats, primarily free fatty acids (70%) and β-monoglycerides (25%), occurs from the micelles in the microvil (brush border) of the epithelial cells of the small intestinal mucosa. Bile salt of the micelle apparently are not absorbed at this point but are redissolved in other emulsoid particles.

Current evidence (5,6) indicates that the products of fat digestion, free fatty acids and β-monoglyceride mainly, enter the microvilli and the apical pole of the absorptive mucosa epithelial cell by simple diffusion through the cell membrane. The short to medium chain (6 to 10 carbons) and unsaturated fatty acids are more readily absorbed than the long chain fatty acids (12 to 18 carbons). Also, the short chain fatty acids appear to enhance the absorption of fats in general, whereas long chain fatty acids tend to impair the process. Furthermore, the monoglycerides of the less well absorbed, long chain fatty acids (i.e., stearic) are better absorbed than the corresponding free fatty acids.

Differences in the rates of digestion and absorption of the individual fatty acids are reflected in the overall rates of digestion and absorption of the dietary fats from which they are derived.

Fats and oils with lower melting points (i.e., below 50 C.) are more rapidly and completely digested and absorbed than are those with higher melting points. Animal and vegetable triglycerides having similar melting points seem to be equally well digested and absorbed. Likewise, human milk fat is absorbed better than cow's milk fat because it contains a higher percentage of unsaturated fatty acids and more palmitate in the carbon-2 position and because milk has its own lipase activity.

The resynthesis of triglycerides from free fatty acids or monoglycerides in the mucosal cells entails the same reactions as those occurring in other cells. Briefly summarized, these are: (a) conversion of the free fatty acid to the fatty acid-CoA derivative by ATP and CoA; (b) conversion of the fatty acid-CoA derivative to monoglyceride phosphate in the presence of L-α-glycerophosphate; and (c) successive conversions, in the presence of two moles, of fatty acid-CoA to diglyceride phosphate, diglyceride, and finally triglyceride. Aggregates of triglycerides, plus small amounts of extraneous phospholipids, cholesterol, etc., are then "coated" with lipoprotein and secreted from the mucosal cell into the intracellular fluid, thence into the lacteals and lymphatics, and finally into the general circulation for metabolic disposal. These events are shown schematically in Figure 1.

Fatty acids play an important role as an energy source (7). Their oxidation is prominent in higher animals which can store large amounts of neutral fat as a fuel reserve. It is estimated that fatty acid oxidation provides at least half of the oxidative energy in the liver, kidneys, heart muscle and resting skeletal muscle. Fatty acids undergoing oxidation in tissues of higher animals came either from extracellular fluid or from endogenous intracellular lipids. Vertebrate blood contains considerable amounts of triacylglycols but very small amounts of free fatty acids. The major endogenous source of fatty acids for fuel is storage fat, in the form of fat droplets in the cytoplasm, which consists largely of triacylglycerol (7). Before the fatty acids can undergo activation and oxidation, the triacylglycerols first must undergo hydrolysis by intracellular lipases to yield free fatty acids and glycerols. Free fatty acids are therefore derived either from the blood stream in which they are transported bound to serum albumin, or from hydrolysis of intracellular lipids. They are first activated by esterification with CoA to form acyl CoA ester at the outer mitochondrial membrane. Then the fatty acyl group from CoA is carried across the inner membrane by the carrier molecule carnitine. Finally the fatty acyl group is transferred from carnitine to intramitochondrial CoA: through several enzymatic reactions, a two-carbon fragment of the fatty acid is removed as acetyl CoA and two pairs of hydrogen atoms are given to specific acceptors. A long chain fatty acid will go through several spirals. Thus, the 16-carbon palmitic acid undergoes a total of 7 such cycles, to yield 8 molecules of acetyl CoA and 14 pairs of hydrogen atoms. The acetyl CoA formed as a product of the fatty acid oxidation will then enter the tricarboxylic acid cycle, which is the final common pathway of oxidative catabolism of all fuel molecules in aerobic cells (7).

According to Florey (8), the glycerine derived from the hydrolysis of glycerides is converted directly into glucose, while the fatty acids are broken into 2-carbon units which contribute to the formation of citric acid. Thus, fats are fed into the pathways of glycolysis and the citric acid cycle.

In an experiment in which rats were given D-palmitic acid in free form or as mono- or triglyceride, Buensod and Favarger (9) found that monopalmitin is digested more completely than the free acid or triglyceride form, although it is digested more slowly than tripalmitin. Measurement of radioactivity 24 hr after oral administration of a mixture of mono- and diglycerides of ^{14}C-, 4-diacetyl tartaric acid (0.5-0.8 g/kg) by Lang and Schmidt (10) revealed that 26-31% of the activity was absorbed, 12-21% was oxidized to $^{14}CO_2$, 8-13% was excreted in the urine and 2% was retained in the carcass. According to Koppanyi and Dardin (11), TEM has a digestibility coefficient of roughly over 90%, it is absorbed from the small intestine, and the tartaric acid released during its metabolism is slowly excreted by the kidneys. Sourkes and Koppanyi (12) found that when 2.72 g of TEM per kg of body weight was administered *per oz* to dogs, 88% of the TEM was absorbed within 12 hr and less than 20% of the theoretically available tartaric acid was excreted in the urine during the same time. The digestibility coefficient of TEM in the adult rat was reported to be 95% (12).

Farber and Wynne (13) found that triacetin, when properly emulsified, caused inhibition of the hydrolysis of caseinogen by pancreatic proteinase. Zuckerman et al. (14) found that orally administered oleic acid (9 ml/day) stimulates the flow of liver bile in a cholecystectomized human subject. Administered with bile salts, oleic acid increases the choleretic effect of the bile salts beyond the additive effect of each of these stimulating agents (14). Pinter (15) reported that 25-50 g of glyceryl monolinoleate caused an increase in the serum triglyceride level of human subjects while similar quantities of glyceryl monostearate had little or no effect.

The foregoing discussion of the absorption of fatty acids and their resynthesis into triglycerides applies to long chain fatty acids. The lower molecular weight free fatty acids, representing less than 30% of the absorbed fat, are distributed, according to Frazer's partition theory, bound to plasma albumin mainly via the blood capillaries, into portal blood and thence directly to the liver for oxidation or

lengthening into long chain fatty acids (16).

This means that fatty acids and/or polyhydric alcohols are metabolized more or less independently of each other. This fact is very significant when the toxicity of fatty acid esters is considered. In general the oral toxicity depends on the effects of its individual lipid moieties.

THE TOXICOLOGY OF FATTY ACIDS
(see reference 19 for greater details)

Fatty acids as normal products found in animal and plants are not toxic to mammals in the usual sense of the word except at heroic dose levels. The most complete and recent report on this subject is a report prepared by Briggs et al. (7). The acids used in the study were a composite of materials obtained from 12 member companies of the Fatty Acid Producers Council. The upper levels for the saturated fatty acids (C_{10} to C_{18}) was 10.0 g/kg while octadecenoic ($C_{18:1}$) and octadecadienoic ($C_{18:2}$) were given at 21.5 ml/kg. Caprylic acid was given at the higher dosage.

Caprylic Acid

While no deaths occurred at the 10.0 ml/kg dose, all rats given 21.5 ml/kg of this fatty acid died within 2 hr after ingestion. The rats exhibited marked depression, coma absent rigating reflex, labored respiration and excessive salivation prior to death. The acute oral LD_{50} for the male albino rat is 14.7 ml/kg.

Gross necropsies performed on dead rats showed slight or moderate congestion of the lungs, adrenals and kidney. Gross necropsies performed on survivors at termination of the feeding studies (2 weeks) showed no significant gross pathology.

Capric Acid

Since death did not occur at the highest level tested, the oral LD_{50} for capric acid is greater than 10.0 gm/kg. While death did not occur at this high level, rats at the 4.64 and 10.0 mg/kg showed excessive salivation and diarrhea. Rats at highest level appeared to be depressed, had lower righting and placement reflexes, and had unkempt fur. After four days and all succeeding days rats exhibited normal behavior and appearance. Gross necropsies revealed no significant gross pathology.

Lauric Acid

The oral LD_{50} for lauric acid is greater than 10.0 g/kg since none of the animals died. The toxicity signs at higher levels and for a period of three days were similar to those detailed for capric acid. The exceptions were the oily unkempt fur and slight emaciation which were noted for the whole observation period. The average body weight gains were within normal limits for rats of the age, sex and strain used.

Myristic Acid

No mortalities occurred at any dosage level tested. Therefore, the acute oral LD_{50} is greater than 10.0 g/kg. Gross signs of toxicity could be seen on the first day. The unkempt fur was stained with diarrhea and a perosanguineous discharge from the nose and eyes. On the third postdosage day and for the remainder of the two weeks, the rats appeared grossly normal. The pattern of response of the other saturated fatty acids followed similar effects. The levels for oleic acid and linoleic acid (soya fatty acid) were similar except the highest dose tested was 21.5 ml/kg instead of 10 g/kg. As with the high dosage for saturated fats, toxic signs persist for 2-3 days after which the rats appeared normal.

Primary Skin Irritation

Caprylic acid. The fatty acid produced necrotic tissue at each intact and abraded site at the 24 hr reading. Very slight to moderate edema was observed. All intact and abraded sites were coriaceous at the 72 hr reading. The Primary Irritation Index (PII) was calculated to be *5.46.*

Capric acid. Following application of capric acid to intact and abraded skin sites of albino rabbits, the material produced necrosis or blanching in five or six intact sites and in all of the abraded sites at the 24 hr reading. Very slight edema was noted at one intact and two abraded skin sites at this reading. At the 72 hr reading coriaceous tissue was observed at all intact and abraded sites, and slight edema at three intact and abraded sites. The Primary Irritation Index was calculated to be 4.60.

Lauric acid. This material produced very slight erythema in five of six intact sites and three of six abraded sites, no irritation in one intact and one abraded site, and blanching in one abraded skin site at the 24 hr reading. No edema was present in either intact or abraded skin sites at this reading. No erythema was noted at any intact or four abraded sites at the 72 hr reading. However, coriaceous (leather like) tissue was observed at two abraded sites at this reading. Very slight or slight edema was observed at two abraded sites only at the 72 hr reading. The Primary Irritation Index was calculated to be 1.12.

The other saturated fatty acids (C_{14}, C_{16}, C_{18}) all had PII of O.O. No signs of irritation or corrosivity were observed. In contrast to the lack of irritation in the long chain (C_{12}) saturated fatty acids, oleic and Soya fatty acid (53% linoleic acid) were irritating.

Oleic acid. This acid produced very slight erythema in six intact and six abraded sites at the 24 hr reading only. No edema was observed at either reading. The Primary Irritation Index was calculated to be 0.50.

Soya fatty acid. This material produced spotty blanching at one intact and one abraded site, and very slight or well defined erythema at four intact and four abraded sites at the 24 hr reading. Very slight edema was noted at one intact and one abraded site at this reading. At the 72 hr reading coriaceous tissue and slight edema were noted at one intact and one abraded site. The Primary Irritation Index was calculated to be 1.64.

Patch Test for Corrosivity

No corrosive effects were noted at any site in any rabbit tested with lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid and soya fatty acids at any time during the study. Caprylic acid produced necrosis or spotted and/or entire blanching of each site at the 4 hr reading. At the 24 and 48 hr readings, entire or spotted coriaceousness was noted at each site. Capric acid produced blanching of one site and necrosis of one site at the 4 hr reading. The remaining sites exhibited no corrosive effects at the 4 hr reading. At the 24 and 48 hr readings, six sites exhibited coriaceousness while the remaining six sites exhibited no corrosive effects.

Acute Eye Application

Caprylic acid. This material produced corneal opacity and moderate or marked conjunctivitis in all rabbits, and iritis in three rabbits. In addition, blanching of the conjunctival tissues was noted in one rabbit. Irritative signs did not subside appreciably during the 72 hr observation period.

Capric acid. The fatty acid produced corneal opacity and moderate conjunctivitis in each rabbit, and iritis in four of six rabbits. There was no decrease in irritation during the observation period.

Lauric acid. This lipid produced corneal opacity and moderate conjunctivitis in all rabbits, and iritis in five of six rabbits. Irritative signs did not subside appreciably during the 72 hr observation period.

Myristic acid. The results following application of myristic acid to the eyes of albino rabbits were confined to

mild conjunctival erythema in three of six rabbits.

Palmitic acid. The material produced no signs of eye irritation in any rabbit.

Stearic acid. No signs of eye irritation were observed at any time during the study.

Oleic acid. The material produced mild conjunctivitis in five of six rabbits. No other irritative signs were observed and all except one rabbit showed no irritative signs at the 72 hr reading.

Soya fatty acid. This fatty acid mixture produced mild conjunctival erythema in four of the six eyes only. No other irritative signs were observed and all eyes were clear at the 72 hr reading.

Summary on Fatty Acid Toxicology

The acute oral toxicity and the primary skin and acute eye irritative potentials of the test materials were evaluated in accordance with the techniques specified in the Regulations for the Enforcement of the Federal Hazardous Substances Act (Revised, Federal Register, September 17, 1964). In addition, the corrosive potential of the fatty acids were evaluated in accordance with the procedure described in Section 173.240 under Title 49 of Code of Federal Regulations (Federal Register, February 12, 1973).

Caprylic acid and capric acid produced blanching, necrosis and coriaceousness. No corrosive effects were noted in any animals which were tested with lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid and soya fatty acids.

Based on these results, caprylic acid and capric acid are classified as corrosive as these terms are defined in the above cited Regulations, while the other fatty acids are not so classified. Capric, caprylic, and lauric acid as furnished were determined to be eye irritants. Also, two materials (caprylic acid and capric acid) proved to be corrosive under both FHSA and D.O.T. definitions.

A point to be made which was not discussed in the original work (19) is that the unsaturated acids were not tested for possible presence of peroxides. Consequently, the toxicity and irritability found for oleic and linoleic acids may be due to the presence of their peroxides which are known to be toxic. A discussion of the toxic effects of polyunsaturated fats has recently been published (8).

TOXICOLOGY AND SAFETY OF GLYCERIDES

The safety for the long continued use of mono-, di- and triglycerides was based earlier on the normal occurrence of such materials in food fats and oils; and on the similarity in chemical structure and metabolism to compounds found in man. Feeding studies carried out since 1941 tended to substanstiate the safety assessment of these compounds (21). The subject of glyceride safety has been reviewed recently with continued affirmation of their safety (22). The oral LD_{50} of the monooleate ester has been measured as 50 g/kg in rats and greater than 25 g/kg in mice (23-25).

Koppanyi and Dardin (11) reported that the mating of female rats fed a diet containing 10% TEM with male rats maintained on a 20% TEM diet resulted in the production of normal, healthy offspring which revealed no indication of damages attributable to the ingestion of TEM. Dietary levels of 0.5 and 5.0% oxystearin were found by Hodge et al. (11) to have no effect on the reproductive performance, nor the health of the offspring, of rats. In a test of reproduction by Ambrose et al. (26), at a dietary level of 10%, aceto-oleins AG-3 and AG-21, and acetostearin AG-26 supported reproduction and lactation through the test period of 3 generations, while acetostearins AG-194 and AG-31 failed to support reproduction beyond the first generation.

Solomides (27) reported that injections of glycerine produce a temporary immunity in rabbits followed by sensitization to re-injection.

Hine et al. (28) reported that no measurable irritation was produced by the application of glycerine to the skin and eyes of rabbits. Subcutaneous injections of mono- and diacetin were noted by Li et al. (29) to cause occasionally local irritation in mice and rats. In the rabbit eye, 50% monoacetin caused only a slight degree of irritation while diacetin and triacetin in similar concentrations caused marked congestion and moderate edema (29). The daily application, for 45 days, of a 30% acetoglyceride emulsion to the skin of albino guinea pigs was reported by Ambrose and Robbins (30) to result in no local irritation or systemic reactions.

In a study in which weanling male and female Holtzman rats were fed diets with or without 50% saturated, partially acetylated monoglycerides, Herting and Crain (31) noted the appearance of a foreign body-type reaction in the body fat occurring within 8 weeks of initiation of the test.

In the only reported feeding study using chicks, 10 one-day-old chicks were fed diets containing diacetyl tartaryl glycerol monostearate (TEM), glycerol lactopalmitate (GLP), or succinylated monoglyceride (SMG) at dosage levels of 570 mg/kg, 2.85 g/kg and 8.55 g/kg for a period of 90 days (32). TEM caused growth depression at all levels at 7 weeks, slight hyperemia of duodenum and ileum at the lower levels and moderate to severe hyperemia at the highest level (32). Gizzard erosion was observed at the high level, with only slight changes at the intermediate level (32). GLP caused growth depression at 90 days (1803). Liver weight was slightly increased, and there were instances of very slight hyperemia and gizzard erosion at the intermediate level at 90 days (32). SMG caused very slight growth retardation at 90 days (1803). Cecal size was increased at each level, slight hyperemia, and on instance of gizzard erosion, occurred at the intermediate level (32).

Wrestlind (33) found that injections of triacetin (LD_{50} = 1600 mg/kg) into the tail veins of mice produced almost immediate convulsions, failure of the righting reflexes and respiratory arrest. In some animals respiration did not return and death occurred in 1-3 min; other animals started to breathe spontaneously within a few minutes and survived. Li et al. (29) reported that subcutaneous injections of lethal doses of mono-, di-, and triacetin produced, in white mice, marked depression, weakness, prostration and, in some animals, labored respiration prior to death. Gross observations of animals poisoned by monoacetin revealed dilation of the heart and diffuse congestion of the lungs (29). In animals dying as a result of the toxic effects of di- and triacetin, hemorrhagic areas in the lungs frequently appeared (29). Preliminary studies of tissue sections showed some cloudy swelling of the convoluted tubules of the kidney, and in some cases the lumen was filled with casts (29). Hydropic degeneration and necrosis of the tubules was noted in some areas and the liver appeared to be congested (29).

For a period of 2 years, Fitzhugh et al. (34) maintained 24 rats on a basal diet with a 25% supplement of Myverol 18-00 (glyceryl monostearate). Compared to a similar number of control animals, growth and longevity of the test rats was normal, and detailed microscopic pathological examinations of all major organs and tissues revealed only a single change—an increase in the number of calcified renal tubular casts attributable to the treatment (34).

In a study by Mattson et al. (35) in which 12 groups of 10 weanling, male Sprague-Dawley rats were fed diets containing various pure mono-, di-, and triglycerides at a level of 25% for a period of 10 weeks, growth of all groups was normal and autopsies revealed no peculiarities. Ames et al. (36) reported that the feeding of monoglycerides derived from the fatty acids of cotton seed oil to rats for 3 generations disclosed no untoward effects attributable to the ingestion of the compounds. The only deleterious effect

noted by Braun and Shrewsburg (37) to result from the feeding of 8-24% monostearin or monolinolein in the diets of rats for 8 weeks was a somewhat slower growth of the monostearin-treated rats. On the basis of a feeding study in which rats were fed mono-, di-, and triglycerides (prepared from a mixture of partially hydrogenated soybean and cottonseed oils) at levels of 15 or 25% of the diet for 70 days, Harris and Sherman (38) stated that these compounds exhibit no differences in caloric efficiency nor produce any differences in body weight gain.

Groups of 5 male weanling albino rats were raised by Ambrose and Robbins (39) on diets containing 0, 0.25, 0.5, 1, 2, and 4% acetostearin for a period of 57 weeks. The authors suggested that the testicular hypoplasia and suppression of spermatogenesis, which was observed to varying degrees in all rats, may have been the result of insufficient vitamin E in the diet (39). For periods from 400 to 709 days, Ambrose et al. (40) maintained groups of 10 male and 10 female rats on diets containing acetostearin AG-194, AG-26, or AG-31 or aceto-olein AG-21 or AG-3 at dietary levels of 5, 10, and 20%. No significant differences in growth were noted between the control animals and those fed acetoglycerides except in the case of male rats on dietary levels of 20% acetostearins AG-194 and AG-31, and female rats receiving 20% AG-31 (40). Testes of rats receiving the three acetostearin diets were significantly smaller than those of the control rats or rats fed the two aceto-oleins (40). The livers of male rats fed 20% acetostearin AG-194 and of both male and female rats fed 20% acetostearin AG-31 were significantly larger than those of the control rats (40). Livers of female rats fed 20% of either aceto-olein were smaller than those of the control rats (40). Kidneys of female rats fed 20% acetostearin AG-31 were significantly larger than those of the controls. Acetostearins produced a foreign-body reaction in the fatty tissue of a number of the organs, presumably due to crystalline deposits of a high melting point fat (40). Acetostearins AG-194, AG-26, and AG-31 produced some scarring of kidney tissue, presumably due to deposited calcium (40).

In a study by Mattson et al. (41) in which rats were maintained for 8-12 weeks on diets containing 15-50% of various acetin fats, it was found that acetin fats derived from the usual edible triglycerides are nutritious materials.

Growth, organ weights, and mortality were normal for all groups of 8 young male rats which were maintained for 608 days on diets containing 10% glyceryl lactopalmitate with low monoglyceride content, 10% glyceryl lacto-oleate with high or low monoglyceride content, 10% polyglycerol lacto-oleate with high monoglyceride content, or 10% acetylated tartaric acid ester of glycerol monostearate (42).

Low growth rate, attributed to poor palatability of the test diets, was observed for groups of weanling rats which were given, for a period of 7 days, a basal diet containing 20% of either monoglyceride citrate or glyceryl lactopalmitate (43).

In a study in which groups of 50 male and 50 female weanling albino rats were given diets containing 0, 0.5, 5 or 15% oxystearin for 2 years, the only apparent effect was an unusual number of Leydig cell adenomas in the testes of the rats in the 15% oxystearin diet (44).

In a paired feeding study in which the effects of feeding a diet containing 20% triacetin for a period of 7 months were examined, McManus et al. (45) observed no discrepancies between the treated animals and the controls.

In a study by Orten and Dajani in which male weanling hamsters were maintained for 28 weeks on diets containing 5-15% glyceryl monostearate, the animals of the 45% group showed a slight weight loss while the 5% hamsters exhibited a higher weight gain then the controls; no consistent pathological changes were observed.

TOXICOLOGY AND SAFETY OF POLYOL ESTERS

While the metabolisms of fatty acids and triglycerides are well known, the biotransformation of ester of polyhydric alcohol is less known. What evidence is available continues to support their safety. In general these esters are cleared in the same manner as triglycerides so that one needs only to follow the effects of the fatty acid and polyhydric alcohol. This simple concept is supported by the following data.

Polysorbates

The chronic toxicity of polysorbate 80 (oleic acid ester) was followed in rats over a period of two years in one study (47) and for 3 generation reproduction in another (48). In neither ration was the emulsifier harmful at 2%.

Polysorbate 60 (stearate ester) was studied for two years in rat feeding experiments where the levels in the feed were 2, 5, 10 and 25%. While some tissue changes were noted in the liver, other microscopic changes were not seen in any other tissue (49).

In other studies (50-53) involving several polyoxyethylene sorbitan emulsifiers individually and as a mixture, it was determined that there was no cumulative toxicity over a two year period. There was no progressively changing response to the emulsifier over this time (4 generations). No evidence was shown to indicate that the emulsifiers had any carcinogenic potential.

The acute oral toxicity of the mono-oleate ester in rats is 50 g/kg and greater than 25 g/kg in mice (46-48). Doses of 30-40 g/kg gave only low incidence of death. Using emulsifiers labeled with radiocarbon in either the fatty acid or the polyol moiety (54), it was determined that the split fatty acid becomes indistinguishable from other fatty acids and that the polyol is very poorly absorbed. In man similar results were found (55).

Polyglycerols

The polyglycerol ester (PGE), decaglycerol deca-oleate, was fed to rats at levels of 2.5, 5.0 and 10% for 90 days (56). No adverse effects were found upon survival, organ weight or hematological values. There were no significant microscopic tissue changes which could be attributed to dietary treatment.

There was a decreased utilization of feed by males fed PGE at the 10% level. These data show that absorption of PGE was not complete. Studies dealing with the metabolian of tri-(G_3) and decaglycerol (G_{10}) and their corresponding oleic and eicosanoic acid were presented by Michael and Coots (56). The data showed that the ester bonds were hydrolyzed to a large extent prior to absorption. Oleic and eicosanoic acids were absorbed via the thoracic duct. The free or partially esterified polyglycerols were not well absorbed as the free fatty acids.

In vitro hydrolysis experiments indicated that the oleic ester bonds in the G_3 and G_{10} esters were cleaved as readily as is the same bond in triglycerides. Unlike glycerol, the polyglycerols were not retained appreciably in the carcass (25%). The study by Mitchell and Coots (57) supports the earlier conclusion of Babayan et al. (58) that the fatty acid moiety of the polyglycerol ester was absorbed and utilized as well as those of natural fats. The polyglycerol portion was not utilized but excreted nearly quantitatively (58).

Sucrose Esters

The two examples given above essentially establish the principle of nontoxicity for esters of polyols. It should be expected that the split products, fatty acid and sucrose, would not create problems. This is also true for sucrose esters but with an interesting point to be made. While the ester itself is nontoxic, it was first made by using dimethyl

TABLE I

Minimal Inhibitory Concentrations of Saturated Unsaturated Fatty Acids[a] (19)

	Pneumococci	Streptococcus group A	Streptococcus betahemolytic non-A	Candida	S. aureus
Caproic	NI	NI	NI	NI	NI
Caprilic	NI	NI	NI	NI	NI
Capric	1.45	1.45	2.9	2.9	2.9
Lauric	0.062	0.124	0.249	2.49	2.49
Myristic	0.218	0.547	2.18	4.37	4.37
Myristoleic	0.110	0.110	0.110	0.552	0.441
Palmitic	0.48	3.9	3.9	NI	NI
Palmitoleic	0.024	0.098	0.049	0.491	0.983
Stearic	NI	NI	NI	NI	NI
Oleic	NI	1.77	NI	NI	NI
Eladic	NI	NI	NI	NI	NI
Linoleic	0.044	0.089	0.089	0.455	NI
Linolenic	0.179	0.35	0.35	NI	1.79
Linolelaidic	NI	NI	NI	NI	NI
Arachidonic	NI	NI	NI	NI	NI

[a]Results are given in mM. NI=not inhibitory at the concentrations tested (1.0 mg/ml or 3 to 6.0 mM).

formamide (DMF) as a solvent. Under this circumstance sucrose esters made by the DMF process are not permitted to be added to foods on account of the toxicity caused by its DMF solvent. Consequently, the development of nontoxic manufacturing processes are a necessary part of the total picture and cannot be ignored. Indeed the filing of GRAS petitions in the U.S.A. included specific directions on the manufacturing process in order to obviate this problem.

Finally, as a note of caution, esters of polyols may have biological effects which have nothing to do with toxicity or irritation *per se* but may nevertheless cause clinical problems. The paper by Fisherman and Cohen (60) is a case in point. Oral challenge with Tween 80 gave positive results in 21 nonaspirin sensitive patients with intrinsic chronic rhinitis, nasal polyps and asthma. These studies suggest that the incidence of Tween 80 intolerance in patients with respiratory disease is about one half the incidence of aspirin intolerance and twice the incidence of iodide intolerance. Thus these nontoxic compounds can cause or precipitate clinical problems in patients with drug idiosyncrasies.

FATTY ACIDS AND DERIVATIVES AS ANTIMICROBIAL AGENTS

This subject has recently been reviewed by Kabara (61). The review emphasized the relationship between lipid structure and antimicrobial activity. That this group of chemicals should have biological activity is not surprising since the use of soaps (alkali salts of fatty acids) for cleansing goes back to antiquity. Soaps were first mentioned on a 4,000 year old clay tablet uncovered at Tello, Mesopotamia. Modern survey of literature after the turn of the century can be found in reports by Bayliss (62), Kodicek (63), and Nieman (64).

In subsequent years, the antifungal and bactericidal properties of fatty acids have been extensively investigated (65-67). Other reports point to virus inactivation by various soaps (68-70), as well as to the antitumor activity of fatty acids (71). In the past, certain structural generalizations were made concerning the activity of fatty acids on cells and microorganisms; however, owing to the impurity of the compounds used, the limited number of organisms tested in the same laboratory, or both, interpretation of structure-function relationships was misconstrued. Also, it must be kept in mind that considerable difficulty surrounds the testing of lipid materials because of their insolubility. Notwithstanding the above objections, a number of generalities have been made which will be useful.

Although in any homologous series fatty acids the bactericidal efficiency increases and the surface tension decreases with increasing chain length, there is no immediate cause and effect relationship. Thus, while all detergents which are highly bactericidal markedly lower the surface tension, the converse is not true. As an example, the nonionic surfactants are very surface active but have little effect on bacterial metabolism and generally are not bactericidal (72, 73). It has long been established (74) that surface active agents possess antibacterial activity to a greater or lesser extent depending on their type; the cationic agents are almost equally effective against both gram-positive and gram-negative organisms with maximum activity in alkaline solutions. In contrast, the anionic surfactants are selectively active against gram-positive organisms with maximum activity in acid solutions. Beside the diverse action of various surfactants, their antimicrobial activity can be altered by the environment in which their potency is measured.

The activity of the surface active agents is markedly reduced in the presence of proteins (i.e., the bactericidal concentration may be increased by a factor of up to 10 in the presence of serum). This is undoubtedly due to the formation of the detergent protein complex and the removal of the biologically active species from the solution (75). In a similar manner lipid materials can counteract the bactericidal effects of other surface active agents. Phospholipids prevent the inhibition of metabolism by soaps when added to the cell suspension before or with the detergent (73). This inactivation is possibly due to adsorption of the phospholipid onto the bacterial surface, and subsequently agents do not protect the cell if added after the detergent. The above are only a few of the variables which make the conversion of laboratory data to use conditions tenuous.

Abetting the failure of these lipids to have more industrial uses was the general philosophy prior to 1930 that bacteria could never be expected to respond to chemotherapy. After this time the discovery and potency of suphanilamides (76) penicillin (77) and other germicides (78) precluded the use of lipids as antimicrobial agents. The comparatively low antimicrobial effects of lipids needs, however, to be reexamined today in its light of safety.

While fatty acids and their derivatives may not represent the most active germicides to be designed, they ARE THE SAFEST.

As noted in prior publication, chain length is one of the more important variables relating chemical structure to antimicrobial activity (79). For anionic-saturated com-

TABLE II

The Antimicrobial Effect of Lauricidin Plus vs. Sorbic Acid

Organism/compounds	Sorbic acid	Lauribic
E. Coli	5,000	1,250
Klebsiella	5,000	2,500
PS. aeruginosa	10,000	5,000
Staph. aureus	5,000	62.5
Staph. epidermitidis	10,000	125
Strep. pyogenes	1,250	125
Strep. faecalis	10,000	250
Strep. mutans	5,000	250
S. cereviscae	10,000	125
C. albicans	10,000	1,250

pounds, the optimum length is 12 carbons (Table I). This statement is true for gram (+) organisms alone since compounds active against yeast are generally one or two carbons shorter in length. Fatty acids with six carbons or less, are active against gram (-) organisms.

While the esterification of a fatty acid to a monohydric alcohol leads to an inactive ester, esterification to a polyhydric alcohol forms an active biocide (80). Interestingly enough, the size of the polar group has little effect on the chain length optimum. Glycerol, as well as polyglycerol (tri-, bexa- and decaglycerol) derivatives seem to have lauric acid as the most important acyl fatty acid. The bulkier hydrophilic groups seem to impart a narrower spectrum of antimicrobial activity to the surfactant structure.

Indeed, polar groups direct action towards specific organisms while the hydrocarbon chain determines overall activity of the compound. This is noted in comparing surfactant activity against gram (-) strains. In these cases cationic agents are active against most organisms while anionic and nonionic materials (esters, amides and minimides) have narrower germicidal activities. Except where noted for fatty acids and their esters, amines, amides and aminimides reach optimal biocidal activity with chain lengths of C_{14}-C_{16} (81,82). In the case of aminimides chemical agents with rather diverse polar groups, all were active at a chain length of C_{16} (83).

All of these studies emphasize the priority of the hydrocarbon chain as compared to the polar group in determining surfactant biocidal activity against a given species. It is generally recognized that the antimicrobial property of an aliphatic surfactant is dependent on chain length. This relationship is complex since it varies in a nonlinear fashion and somewhat dependent on the specific class of organism tested. This nonspecific drug action is best understood in terms of Ferguson's principle (84). This principle is modified to include the statement that on ascending a homologous series, although the potency should increase, equipotent concentrations should require increasing thermodynamic concentrations, and beyond one particular member the series should become less active. More simply, the antimicrobial effect of an aliphatic surfactant becomes optimal at some specific chain length. This optimum length will vary depending upon the polar group and test organism used.

Whether or not isobranched fatty acids are more active is controversial. Recent studies indicate that branched chain acids, like those of the straight chain acids, are specific with regard to the test organism. There appears to be little overall difference in bactericidal effect which can be ascribed to branching (85).

With a given chain length, the position of the hydrophilic group(s) is an important variable in determining surface properties and biological activity. The kind, geometric isomer, and position of unsaturation can influence biological activity. In general, the acetylenic-containing fatty acids are more active than the thylenic members. In the ethylenic series, the *cis* form is more effective against microorganism than the *trans* form (86).

Whether unsaturation was important to biological activity was greatly dependent upon the length of alkyl chain. This fact has not been stressed in earlier reports. Unsaturated fatty acids with chain length of C_{12} or lower were generally less active than the saturated derivative. Unsaturated fatty acids with chain length of C_{14} to C_{18} were more active than the saturated compound. The unsaturation contributes the most biological activity to the longer chain fatty acid (Table I). Whether or not the position of unsaturation was important to biocidal activity follows this same trend, i.e., the position of unsaturation had no influence on $C_{11:1}$ fatty acids activity (87), some importance to $C_{12:1}$ derivatives and reached maximum effect in $C_{18:1}$ compounds (86). This was true whether unsaturation was ethylenic or acetylenic (86). In general the acetylenic derivatives were slightly more active than ethylenic isomers. In the $C_{12:1}$ series the most active isomer was the Δ^{10} $C_{12:1}$, while in the $C_{18:1}$ series the Δ^2, Δ^7 and Δ^8 were more inhibitory to group A Streptococcus than were the other $C_{18:1}$ acids.

The addition of a second ethylenic bond to $C_{18:1}$ further increases the biocidal activity (26). In contrast to mono-unsaturated fatty acids, the addition of a second double bond increases the activity of the fatty acid but without concern to specific positions of the second ethylenic bond. The addition of a third ethylenic bond, as in linolenic acid, made the fatty acid less active (86).

While the lipophilic (hydrocarbon) portion of the surfactant is important to biological function in general, the polar group also contributes to biocidal activity.

It was of interest to know what the chemical reduction of the carboxyl group to aldehyde and alcohol would do to the activity of the fatty acid. In general, the order of increasing antimicrobial activity was COOH, CHO, C-OH (79). While the reduction of the carboxyl group lead to a more active species, the oxidation of lauric acid to the diacarboxylic acid produced a less active compound. Wyss et al. (87) reported a similar finding. The esterification of the fatty acid with a mono-hydric alcohol produced an inactive ester. In contrast, esterification carried out with a polyhydric alcohol and yielding a monoester lead to biocidal compounds more active than the parent acid (81, 87). This distinction of monoester formation is extremely important since di- and tri-esters were much less active (80).

That esters of polyhydric alcohols would have antimicrobial activity was contrary to the present state of the art. Kabara's research results indicated that it was the monoester and not the di-, tri-, etc., which was the active species (79-83, 88).

While a number of mono-esters indicated activity, the most useful derivative was the monolaurin (Lauricidin). Fortunately this compound is now commercially available (Med-Chem Labs, Monroe, MI) and in high purity (>90%). The latter is important since other commercial samples proved to be inactive.

One of the biological limitations of Lauricidin is that its antimicrobial spectrum is limited to gram (+), yeasts, fungi and mold organism. However, other food grade materials such as tert-butylated anisole (BHA, the common antioxidant) and/or ethylendiaminetetracetic acid (EDTA) which have weak activity of their own are additive or even synergestic with Lauricidin against gram (-) organism. The high activity of these mixtures allows us to design antimicrobial systems specifically effective against certain microorganisms. Table II shows an example of increased activity of a Lauricidin proprietory mixture, Lauribie as compared to another well known fatty acid, sorbic acid.

This is only one example of a monoester, Lauruidin being useful as a food preservative. The reader is en-

couraged to read a new monograph printed by the Society (1). The monograph "Pharmacological Effects of Lipids" explains the multifacet areas for the use of fat derivatives for other than their caloric values.

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