Antimicrobial lipids: Role in innate immunity and potential use in prevention and treatment of infections.

Halldor Thormar[1]*, Hilmar Hilmarsson[1] and **Gudmundur Bergsson[2]**

[1]Faculty of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland
[2]Department of Immunology, Landspitali, The National University Hospital of Iceland, Reykjavik, Iceland
* Address correspondence to this author at the Faculty of Life and Environmental Sciences, University of Iceland, Sturlugata 7, 108 Reykjavik, Iceland; Tel: 354-525-4602; Fax: 354-525-4069; E-mail: halldort@hi.is.

The antibacterial activities of lipids have been known for more than a century. With the advent of antibiotics in the 1940s and 50s the interest in fatty acids and other lipids as antibacterial agents diminished but in the 1970s there was a renewed interest in antimicrobial lipids, particularly milk lipids. Fatty acids and monoglycerides released by hydrolysis of milk fats were found to inactivate enveloped viruses and the stomach contents of neonates became microbicidal one hour after feeding of breast milk. Fatty acids and monoglycerides inactivate all enveloped viruses which have been studied. They kill Gram positive and Gram negative bacteria, but the susceptibility of bacteria varies. Long-chain unsaturated fatty acids and medium-chain saturated fatty acids and monoglycerides thereof are most active. A potential use of these lipids in prevention and treatment of skin infections, sexually transmitted infections, respiratory infections, oral infections and foodborne infections is discussed.

Keywords: Antimicrobial lipids; germicidal soaps; microbicides; innate immunity; skin infections; sexually transmitted infections; mucosal membranes; foodborne infections.

1. Introduction

In 1881, Robert Koch [1] wrote a report on disinfection, particularly on the effect of various chemical compounds on the anthrax bacillus, which he a few years earlier had shown to be the cause of anthrax. The anthrax bacillus thus became the first bacterium conclusively shown to be the cause of a disease. In his report on disinfection, Koch showed that a 1:5000 dilution of potassium soap had an inhibiting effect and a 1:1000 dilution (0.1%) completely stopped the growth of anthrax bacillus in nutrient broth. Koch concluded that the fatty acid moiety of the soap caused the inhibition, because free potassium had much less effect. This was the first time lipids were shown to have an antimicrobial action and suggested that ordinary soaps, which are potassium or sodium salts of fatty acids, could be used as disinfectants as well as for cleaning, a function of soaps that had been known for thousands of years. In the late 19th and early 20th century, numerous studies were carried out on the antimicrobial effect of various types of soap on a variety of pathogenic bacteria, which at this time caused serious illnesses in the Western world, but are now a problem only in developing countries. The main aim was to test the potential of germicidal soaps as general disinfectants. These early studies were reviewed by Reithoffer in 1896 [2], Serafini in 1898 [3] and Konrádi in 1902 [4]. According to these authors, sodium soaps killed cholera bacteria (*Vibrio cholerae*) in culture medium within minutes to hours, whereas it took longer to kill typhoid and anthrax bacilli, and *Staphylococcus pyogenes aureus* isolated from pus survived for at least 24 hours.

As shown by Reichenbach [5], there was a distinct difference in the germicidal activity of soaps depending on their fatty acid contents. Following his work, studies increasingly focused on the fatty acids which are commonly used in soaps and their derivatives. Walker [6-9] studied the germicidal properties of chemically pure soaps, particularly from a hygienic point of view, emphasizing the importance of knowing which soaps are germicidal against different pathogens. He concluded that pathogenic bacteria could be placed into two groups. One group which included pneumococci, gonococci, meningococci, streptococci and diphtheria bacilli were highly susceptible to sodium and potassium salts of both saturated and unsaturated fatty acids and were readily killed by any ordinary soap. In the other group were enteric bacteria, that is typhoid, paratyphoid, dysentery and coli bacilli, which showed a moderate susceptibility to salts of saturated fatty acids and were only killed by soaps which contained these salts, such as coconut oil soap.

Despite promising reports on the germicidal action of soaps they did not reach a widespread use as disinfectants. Walker [9] discussed the apparent lack of interest in soaps as antiseptics and disinfectants, in spite of their documented activity against a number of pathogenic bacteria such as streptococci, pneumococci, meningococci, gonococci, diptheria bacilli, *B. influenzae* and *Spirochaeta pallida*, the causative agent of syphilis. Common soaps were known to kill these bacteria in a couple of minutes, in a much higher dilution than used in normal handwashing. This indicated that thorough washing with soap destroyed the above bacteria on the hands. Therefore, soaps compared favorably with many newly synthesized chemical disinfectants and, when properly used for cleaning of the hands or for dishwashing, could play an important role in preventing the spread of disease. Still, soaps were hardly mentioned as disinfectants in standard textbooks on surgery and bacteriology. Probably, the reason for this was their low activity against many common pathogens such as staphylococci and enterobacteria.

The discovery over one hundred years ago that common natural soaps kill many types of pathogenic bacteria raised hopes that these alkaline salts of fatty acids might be used as disinfectants or even for prevention and treatment of infectious diseases. The germicidal actions of soaps were therefore actively studied for more than half a century, because few resources were available at this time to fight bacterial infections. These studies may have helped improve hygienic practices, but did not contribute much to prevention or treatment of infections or infectious diseases. The interest in the germicidal action of fatty acids and other lipids decreased with the advent of antibiotics around the middle of the 20th century, which led to a breakthrough in the therapy and cure of many previously fatal infectious diseases. In the1970s there was a renewed interest in antimicrobial lipids, largely due to the pioneering work of Kabara and his associates and of Welsh and May on antiviral lipids in human milk. The most studied antimicrobial lipids are summarized in Table 1.

The early studies on the antibacterial activity of lipids, particularly the germicidal action of soaps, have recently been reviewed [10, 11].

Table 1 Antimicrobial lipids.

Fatty acids	Monoglycerides	Carbon atoms:double bonds
Caprylic	Monocaprylin	8:0
Capric	Monocaprin	10:0
Undecylenic	–	11:1
Lauric	Monolaurin	12:0
Myristic	Monomyristin	14:0
Palmitoleic	Monopalmitolein	16:1Δ9
Sapienic	–	16:1Δ6
Oleic	Monoolein	18:1
Linoleic	–	18:2
Linolenic	–	18:3
Arachidonic	–	20:4

2. Antimicrobial lipids in milk

The antimicrobial activity of milk lipids has been known for more than half a century. Sabin and Fieldsteel reported in 1962 [12] that human milk reduced the infectivity of several viruses, such as herpes simplex virus and poliovirus. They demonstrated that the anti-herpes virus activity was associated with the cream fraction of the milk, whereas the anti-poliovirus activity was not. These findings were confirmed by several workers who found antiviral activity against several enveloped viruses in milk fat. Electron microscopy of negatively stained virus particles showed that the lipid envelope was damaged, making the particles permeable and degrading their structure and infectivity. However, the causative agent was not identified and it was even suggested that the RNAase present in human milk might play a role.

2.1. Antimicrobial activity of human milk is associated with free fatty acids and monoglycerides.

In a later study, Welsh et al. [13] showed that the antiviral activity of human milk was associated with free fatty acids and monoglycerides, released by hydrolysis of triglycerides by milk lipase. Free oleic and linoleic acids in the milk were particularly active. In a series of studies on the antimicrobial activity of human milk, Isaacs and coworkers [14-18] confirmed and extended these earlier studies. They found that fresh human milk did not inactivate enveloped viruses but became highly antiviral after storage in a refrigerator at 4^{o}C for 2 to 5 days. A separation into cream and supernatant fractions before storage showed that a factor in the supernatant was required for the appearance of antiviral activity in the cream fraction, most likely a lipase. This was supported by the finding that milk with low levels of lipoprotein lipase did not become antiviral upon storage, in contrast to milk with high levels of this enzyme. It was concluded that the appearance of antiviral activity in human milk upon storage is caused by hydrolysis of triglycerides to free fatty acids by lipoprotein lipase in the milk. This was confirmed by addition of a specific lipase inhibitor.

2.2. Antimicrobial fatty acids are released in the gastrointestinal tract by hydrolysis of milk fat.

To answer the question of whether human milk became antimicrobial in the suckling infant, samples were obtained from low birth weight neonates fed human milk through a nasogastric tube [14, 16-18]. The milk fed to the infants had no antimicrobial activity. At 1 and 3 hours after feeding, samples of the stomach contents were removed by aspiration and tested for antimicrobial activities. All of the 1 hour samples were highly active against the enveloped viruses tested, whereas the 3 hour samples had lower or no activities. The variability of antimicrobial activity at 3 hours probably

reflects various rates of digestion by the infants, since emptying of the stomach may be complete around that time [14]. The antiviral activity of the stomach contents was located in the lipid fraction which contained monoglycerides and free fatty acids [17]. Most likely, their release in the stomach is due to a rapid hydrolysis of milk triglycerides by salivary and gastric lipases, and not by lipoprotein lipase in the milk which is not stable in the gastric environment [16]. In addition to enveloped viruses, the enteric bacteria *E. coli* and *Salmonella enteritidis* were killed by stomach contents from milk-fed infants [17,18].

Milk lipids may have a protective function against infectious agents in the gastrointestinal tract, as first reviewed by Kabara in 1980 [19]. A study on the effect of low fat consumption on acute gastrointestinal illness in children over 1 year of age showed that those fed only low fat milk were 5 times more likely to develop intestinal infections than children fed whole milk [20]. The effect of milk lipids on intestinal infections has been studied experimentally in rats. The studies showed that feeding with high milk fat diets reduced intestinal colonization of *Listeria monocytogenes* in orally infected rats, but not of *Salmonella enteritidis* which is less susceptible to the antibacterial activity of lipids at intestinal pH [21]. In contrast, as previously described, enterobacteria are killed by milk lipids in the acid environment of the stomach [18]. Fatty acids and monoglycerides released by hydrolysis of milk fat by digestive lipases are present not only in the stomach but also at other sites in the digestive tract. Their action, in concert with bile salt-stimulated lipase, results in a complete digestion of triglycerides in the intestinal tract, with free glycerol and fatty acids as the final products [22]. Although most of the lipids are absorbed, a small amount is excreted in the stools of newborn infants [19]. Antibacterial lipids may therefore be present in most parts of the gastrointestinal tract of neonates and thus contribute to the prevention of infections.

In addition to intestinal infections caused by bacteria, a human enteric coronavirus, which is an enveloped virus, can cause necrotizing enterocolitis in infants [14, 23]. It is therefore possible that antiviral lipids could protect against this infection.

The antimicrobial action of milk lipids and the role of breast milk in innate immunity have recently been reviewed [24, 25].

3. Antimicrobial skin lipids

3.1. The self-disinfecting power of the skin.

The human skin has an extraordinary power to rid its surface of harmful bacteria and to maintain its endogenous bacterial flora. This ability to destroy exogenous microbes has been referred to as the self-disinfecting power of the skin. The question of the nature of the disinfection was addressed in some early work, but the first study to show that skin lipids are antibacterial and may play a role in the self-disinfection of human skin was done by Burtenshaw in 1942 [26]. His experiments showed that ether extracts of skin scrapings were highly active against streptococci but less active against staphylococci. Fractionation of the ether extracts and testing of the various fractions against streptococci demonstrated that all of the bactericidal activity was in the fraction containing fatty acids. Further studies of this fraction showed that it contained oleic acid and other long-chain fatty acids. In nearly all experiments, the disinfecting extracts and fractions thereof were far more bactericidal at an acid than at a more alkaline pH, leading to the conclusion that the less dissociated fatty acid molecules are more active than their salts**.**

These studies were confirmed and extended by Ricketts et al. [27] who studied self-disinfection (self-sterilization) of the skin of young adult volunteers. They showed that if lipids were removed from the skin of one forearm by aceton extraction, the rate of self-sterilization was greatly reduced, compared with the other untreated forearm. When the acetone extracts were replaced on the skin, reduction was restored. An analysis of the skin lipids removed by acetone showed that about 40% consisted of free fatty acids, about half of them unsaturated, with oleic acid the most abundant. The fatty acid fractions from the skin extracts were tested for bactericidal activities against *Streptococcus pyogenes* and *Staphylococcus aureus* as well as against bacteria which are normally present in skin microflora. Low concentrations of the unsaturated fatty acid fraction killed *S. pyogenes* in 10 minutes, whereas saturated fatty acids were much less active. *S. aureus* was less susceptible than *S. pyogenes* to the bactericidal effect of the unsaturated fatty acid fraction, and the normal microflora was not affected. This study showed that the removal of surface lipids reduced the self-sterilizing power of the skin against *S. pyogenes* and to a lesser degree against *S. aureus* and the sensitivity of these bacteria to fatty acids ran parallel with their rate of disappearance from normal skin. It was concluded that the unsaturated fatty acids are a major factor in self-sterilization of *S. pyogenes,* and to a lesser degree *S. aureus*, and that natural skin lipids therefore play an important role in elimination of these pathogenic organisms from the skin.

3.2. Antimicrobial fatty acids derived from triglycerides in the sebum.

Later studies showed that the antimicrobial fatty acids on the skin surface are largely derived from triglycerides in the sebum, which is a complex mixture of lipids secreted from the sebaceous glands into the hair follicles [28, 29]. During the passage from the sebacious glands through the follicular canal to the skin surface, the sebum triglycerides are hydrolysed into free fatty acids and glycerol, to some extent by a lipase of the bacterium *Propionibacterium acnes* [30].

Triglycerides produced by the sebacious glands of humans are unique in that they contain fatty acids not found in other tissues, for example sapienic acid which is an unusual palmitoleic acid isomer C16:1Δ6 [28]. The free fatty acids of human sebum and their antibacterial spectrum were studied by Wille and Kydonieus [31]. Both saturated and monounsaturated fatty acids were equally active against *S. aureus*. Of the saturated fatty acids, lauric acid, a minor component of skin lipids, was the most active. The predominant unsaturated fatty acid, sapienic acid, was active against streptococci but not against several Gram negative bacteria. An unusual oleic acid isomer C18:1Δ8 was the next most abundant unsaturated acid. This study demonstrated that fatty acids, unique to human sebum, are strongly antibacterial and may to a great extent account for the self-disinfecting activity of the skin surface.

Staphylococci normally reside on the skin as harmless commensals. However, under certain conditions, *S. aureus* can cause a variety of skin infections, such as pimples, boils, abscesses and cellulitis. Bactericidal fatty acids may play a role in preventing that colonization of *S. aureus* leads to skin infection. Thus, a deficit in free sapienic acid is associated with increased susceptibility to *S. aureus* colonization in the skin of atopic dermatitis patients. Fatty acids may therefore be a part of the innate defense mechanisms in the skin [32-34].

3.3. Stratum corneum as a source of antimicrobial lipids in the skin.

The fact that the skin in some regions of the body, for example the abdomen, is devoid of sebacious glands without being particularly prone to infections led to studies of the stratum corneum as another source of antimicrobial lipids in the skin [35]. The lipid composition of stratum corneum is different from that of sebum, mainly in that it contains breakdown products of phospholipids and sphingolipids, derived from membrane bilayers of epidermal keratinocytes. In addition to free fatty acids, released by phospholipase-mediated hydrolysis of the phospholipids [36], the stratum corneum contains free sphingosines which are long-chain unsaturated amino alcohols, normally a component of sphingolipids in cell membranes [37]. Sphingosines were shown to be bactericidal against staphylococci and streptococci [38] and topical application of sphingosines to the skin of volunteers caused a 3 $\log_{10}$ reduction in viable *S. aureus* [39]. Thus, simple sphingolipids may be of importance as antimicrobial agents in the skin. This is supported by a study which showed an association between reduced levels of sphingosine in the stratum corneum of patients with atopic dermatitis and increased colonization by *S. aureus* [40]. Together with the sebacious glands, the stratum corneum may therefore play a role in the antimicrobial defense of the skin.

3.4. A possible role of antimicrobial lipids in the innate immunity of the skin.

As outlined above, several studies show that antimicrobial lipids contribute to the host defense against pathogenic microorganisms in skin and mucosa. The question of whether their role is merely incidental or if lipids have a biological mission as protectors against pathogens has not been unequivocally answered. Recent studies support the notion that lipids have a specific function in the innate immune response, possibly in connection with pathogen recognition receptors and antimicrobial peptides [41, 42]. Thus, lipids may be a factor in the complex multifunctional innate immune system.

Antimicrobial lipids and their role in defense mechanisms in the skin have been reviewed recently in detail [25, 43].

4. Potential use of antimicrobial lipids in prevention of sexually transmitted infections.

4.1. Use of microbicides as preventive measures against sexually transmitted pathogens.

In the 1990s there was a considerable interest in the application of microbicides as a preventive measure against sexual transmission of human immunodeficiency virus (HIV) and other sexually transmitted pathogens. A number of compounds with microbicidal activities were tested *in vitro* and in clinical trials with various results [44, 45]. Some of them, like the non-ionic surfactant nonoxynol-9 (N-9), were too toxic for the vaginal and cervical mucosa to be routinely used by women as a microbicide [46].

4.2. Microbicidal lipids kill sexually transmitted bacteria and viruses.

Several studies done in the late 1990s and early 2000s showed that microbicidal lipids are highly active against common sexually transmitted viruses and bacteria [47]. Bergsson et al. [48] studied the action of free fatty acids and monoglycerides on *Chlamydia trachomatis*, a Gram negative bacterium which is a common cause of genital infection in humans. After incubation at 37^{o}C for 10 min, 10 mM lauric acid, capric acid and monoglyceride of capric acid, monocaprin, caused a greater than 4 $\log_{10}$ reduction in viable bacteria. When further compared at lower concentrations and shorter exposure times, monocaprin at a concentration of 5 mM was the most active causing a greater than 5 $\log_{10}$ reduction in 5 min. In a similar study of *Neisseria gonorrhoeae*, monocaprin was the most active of the lipids tested *in vitro*, reducing the viable counts by 6 $\log_{10}$ or greater in 1 min at a concentration of 0.6 mM [49]. In addition to these two important sexually transmitted bacteria, the effect of microbicidal lipids on herpes simplex virus type 1 (HSV-1) was also studied. Monocaprin at a concentration of 5 mM reduced the virus titer by greater than 5.5 $\log_{10}$ in 1 min [50].

4.3. Hydrogels containing monocaprin as the active ingredient kill sexually transmitted pathogens including human immunodeficiency virus (HIV) in human semen.

To prevent infection by a sexually transmitted pathogen it is important that it be killed rapidly and in large numbers on the genital mucosa before it has time to infect cells of the mucosal membranes. Second, the microbicidal agent must be solubilized in a pharmaceutically acceptable formulation which can be easily applied at the entry sites of the pathogen. Third, the formulation must not cause harmful side effects in the concentrations used. A number of hydrogels have been formulated which contain monocaprin as the microbicidal ingredient, propylene glycol as the solvent and carbopol and sodium carboxymethyl cellulose as gel forming ingredients with good adhesion to mucosal membranes [50]. Their pharmaceutical properties have been studied in detail [51]. Three hydrogels were tested *in vitro* for microbicidal activities against *C. trachomatis, N. gonorrhoeae*, HSV-2, and human immunodeficiency virus type 1 (HIV-1) [52]. All of them were highly active against these 4 pathogens, reducing their viable numbers by 4-5.5 $\log_{10}$ in 1 min, except for *C. trachomatis* where 2.5 minutes were needed to kill the bacteria. The results for one of the hydrogels containing 10 mM monocaprin are shown in Table 2. The bacteria and HSV-2 were suspended in culture medium before being mixed with the hydrogel. HIV-1, on the other hand, was suspended in fresh human semen to mimick the way by which the virus is introduced into the genital tract of women. When mixed with fresh semen, the hydrogels caused a 10,000-fold or greater reduction in the number of viable leucocytes in the semen in 1 min [52]. The killing of these cells is important because they may be carriers of HIV-1 in the semen of infected individuals [53] and sexual transmission of the virus can occur through cell to cell contact between infected leucocytes and cells of the genital mucosa [54]. The rapid killing by the microbicidal hydrogels of both free HIV-1 and leucocytes in semen is essential because cell to cell transmission and infection by cell free virus appear to take place in a short time, probably within a few minutes.

Table 2 Microbicidal activities of a hydrogel formulation containing 10 mM monocaprin mixed with suspensions of virus/bacteria for a given time.

Virus/bacterium	Time (min)	Reduction in titer/viable counts (log10)
HSV-2	1	≥ 5.5[1]
HIV-1	1	4.1[2]
N. gonorrhoeae	1	≥ 5.4[3]
C. trachomatis	2,5	5.0[3]

[1] HSV-2 suspension in cell culture fluid mixed with hydrogel
[2] HIV-1 suspension in human semen mixed with hydrogel. Titers of HIV-1 suspended in cell culture fluid were reduced by $\geq 4,8$ log10 after mixture with hydrogel for 1 min.
[3] Bacteria suspended in culture medium mixed with hydrogel.

4.4. Low toxicity of hydrogels containing microbicidal monocaprin.

The toxicity of microbicidal hydrogels was tested in the vaginal mucosa of rabbits by a standard irritation test. No abnormalities were observed either by macroscopic or microscopic examination [52]. The low toxicity was confirmed in a later study in which no irritation or toxicity was observed following application of the hydrogels to the vaginal mucosa of NMRI mice or to scarified skin of hairless mice [55]. A small phase 1 clinical trial, in which about 50 young women participated, showed that the hydrogels were easy to apply intravaginally and were well tolerated [56].They caused only a short burning sensation or itching in about half of the women and did not adversely affect the normal bacterial flora, such as the number of vaginal lactobacilli. The microbicidal hydrogels thus seem to have low toxicity at the site of application. Evidence for absence of systemic toxicity of monocaprin comes from a number of clinical studies of humans, including infants, who received large quantities of triglycerides containing medium-chain fatty acids, either by feeding or by intravenous administration [57]. The triglycerides are hydrolysed by lipases in the digestive tract. The free medium-chain fatty acids are directly absorbed by the intestinal mucosa and transported via the portal vein to the liver where they are rapidly oxidized and used as a source of energy. Therefore, they do not accumulate in the fat tissues of the body. These studies have recently been reviewed [47].

Despite the microbicidal activity of monocaprin hydrogels against HIV-1 and other sexually transmitted agents *in vitro*, their apparent low toxicity and easy application, there has been little interest in studying their safety and efficacy in clinical trials. One reason for this is the fact that monocaprin, like other microbicidal lipids, is cytocidal to cells directly exposed to the lipid. Cytotoxicity is therefore observed when monocaprin is tested in cell monolayers by the standard method used to assay antiviral activities of compounds which inhibit HIV-1 replication, but are not microbicides. Mucous membranes are normally covered with a mucus layer which protects the membrane from a direct contact with the environment. Microbicides are intended to kill pathogens in the mucus layer before they reach the cells of the membrane and cause infection. Therefore, microbicides need not come in a direct contact with mucosal cells.

That mucosal membranes are protected by the mucus layer is indicated by the fact that the stomach of infants contains microbicidal lipids in high concentrations shortly after feeding, without a harmful effect on the cells of the gastric musosa [14]. Microbicidal lipids should therefore be well suited to prevent transmission of pathogens to mucosal membranes.

5. Therapeutic use of antimicrobial lipids in the pre-antibiotic era.

5.1. Treatment of leprosy.

There are several reports on therapeutic use of fatty acids in the pre-antibiotic era [10]. Thus, chaulmoogra oil, made from the seeds of a tree common in Southeast Asia, was used to treat leprosy. Because of the reputation of this oil in treatment of leprosy, Walker and Sweeney [58] tested its activity *in vitro* against a number of acid-fast bacteria. The total fatty acids of chaulmoogra oil were bactericidal to a dilution of 1:100,000 against the bacilli of rat and human leprosy and 3 tubercle bacilli. In order to identify the active ingredients of the oil, chemically distinct fatty acids were isolated and found to consist mostly of the cyclic 18-and 16-carbon fatty acids chaulmoogric and hydnocarpic acids. They were tested against the rat leprosy bacillus and found to account for the bactericidal activity of the total fatty acids. In contrast to the high activity of chaulmoogric acid against acid-fast bacteria it was inactive against 8 different nonacid-fast bacteria. The authors concluded that their results provided a scientific basis for the use of chaulmoogra oil and its products in the treatment of leprosy.They hypothesized that the bactericidal action was due to an aberration in lipid metabolism of acid-fast bacteria, causing accumulation of chaulmoogric acid molecules in their fatty capsules, with a toxic effect.

As reviewed by Stanley and his coworkers [59], chaulmoogra oil was used for centuries to treat leprosy. Rubbing of lesions with the oil or taking it by mouth seemed to give some relief to lepers. Subcutaneous or intramuscular injection was somewhat more effective than oral administration, but had the disadvantage of causing severe pain. Treatment with ethyl esters or sodium salts of the fatty acids of chaulmoogra oil was more successful, so that a small percentage of advanced cases and a much larger percentage of incipient leprosy cases became negative for the bacteria and could be discharged as cured. Stanley and coworkers [59] studied the chemistry of the active principles of chaulmoogra oil, chaulmoogric and hydnocarpic acids, and concluded that the ring structure of the molecules as well as the number of carbon atoms were largely responsible for the bactericidal action. The use of sulfones to treat leprosy in the 1940s put an end to experiments with chaulmoogra oil treatments.

5.2. Activity against tuberculosis.

In the late 1940s, Hänel and Piller [60] reported clinical improvement in patients with pulmonary tuberculosis treated by mouth with an emulsion of unsaturated fatty acids from cod liver oil, which had been shown to be highly active in preventing growth of the tubercle bacillus *in vitro*. These clinical trials suggested that treatment of patients with concentrated emulsions of unsaturated fatty acids from fish oil might be beneficial. The authors reasoned that unsaturated fatty acids may play a role in the natural defense of the body against the tubercle bacillus and that therapy with high doses of the fatty acids would therefore be helpful. Native populations in tropical areas have used fats against mycobacterial infections, especially leprosy and tuberculosis. In Nigeria palmkernel oil has been used and turtle oils have been used by Mexican Indians. Notably, turtle oils are rich in 10-, 12-, and 14-carbon saturated fatty acids which have been shown to be strongly bacteriostatic for *M. tuberculosis* and other mycobacteria *in vitro* [61].

5.3. Benefits of microbicidal therapy.

A therapeutic application of antimicrobial lipids was suggested more recently by Kabara [62] who emphasized the low toxicity of lipids derived from natural sources. Despite these considerations, pharmaceutical formulations containing antimicrobial lipids have only been tested in limited clinical trials and not yet been approved as prophylactic or therapeutic drugs. Possibly, the great success of chemotherapy, using synthetic antibiotics, has discouraged researchers and the pharmaceutical industry in making serious efforts to develop drugs which contain simple natural compounds with antimicrobial activities. However, this may now be changing with the increasing problem of bacterial and viral resistance to antibiotic and antiviral drugs. Besides specific synthetic drugs, there may be a place for less specific microbicidal compounds, such as lipids, which kill the pathogens on contact, thus launching a two-pronged attack on the invading pathogen, with antibiotics and microbicides acting in concert. Direct killing, in addition to growth inhibition, might delay the development of antibiotic-resistant strains. Therefore, in the last decade several studies have been done to test the preventive and therapeutic efficacy of microbicidal lipids in experimental animals and in small clinical trials.

6. Potential use of antimicrobial lipids in prevention and treatment of infections.

Several pathogenic bacteria, viruses and fungi are susceptible to the microbicidal action of lipids, which might therefore be used to prevent or treat infections caused by these pathogens. Pharmaceutical formulations have been developed as vehicles for microbicidal lipids to prevent transmission of pathogens to mucosal membranes or to treat mucosal or skin infections caused by a pathogenic microorganism. In the formulation of dosage forms with lipids as the active ingredient it is important to determine which lipids have the highest microbicidal activity against various pathogens and are therefore the most desirable as active ingredients. As described earlier, monocaprin proved to be the most active lipid against several sexually transmitted pathogens, leading to the development of monocaprin hydrogels which could be applied to the mucosal membranes most exposed to these pathogens [50]. Monocaprin maintains full microbicidal activity in hydrogels and they have low toxic effect on skin and mucosal membranes [52, 55, 56]. It is suggested that such hydrogels could be used to prevent or to counteract infections in mucosal membranes, for example sexually transmitted infections by HSV, HIV-1, *C. trachomatis* and *N. gonhorreae*, or to treat skin and mucosal infections by HSV.

6.1. The activity against oral herpes of hydrogels containing monocaprin and doxycyclin.

A randomized, double blind, placebo-controlled clinical trial was carried out to investigate the antiviral and wound-healing effect of a hydrogel containing either monocaprin or a combination of monocaprin and doxycycline against oral herpes [63]. The rationale for the combination was that while monocaprin efficiently inactivates HSV-1, doxycycline inhibits matrix metalloproteinases that contribute to the breakdown of tissue in ulcers. Application of the hydrogel containing a combination of monocaprin and doxycycline, 5 times a day for 5 days, resulted in a significant reduction of the healing time and pain, compared with placebo and monocaprin alone. The latter had some effect, though not reaching significance. More clinical studies along these lines are desirable, because hydrogels containing monocaprin with or without doxycycline might prove effective in treatment of cold sores.

6.2. Intranasal delivery of formulations containing virucidal lipids for treatment of respiratory syncytial virus infection in rats.

Respiratory syncytial virus (RSV) is a common cause of serious lower respiratory tract infection in infants and elderly people. There is no effective treatment for RSV infection and new prophylactic or therapeutic compounds for general use against RSV and other respiratory viruses would be desireable. A study of the virucidal activity of fatty acids against RSV and parainfluenza type 2 virus showed that lauric acid and monocaprin were the most active [64]. A pharmaceutical formulation was designed which contained lauric acid and monocaprin as the active ingredients, propylene glycol as the solvent, polysorbates as surfactants and Carbopol 974P as a mucoadhesive polymer [65].The formulation was active against RSV *in vitro*, causing a reduction in the virus titer of 4.5 $\log_{10}$ or greater upon contact for 1 min. When applied to the nasal mucosa of rats immediately before inoculation of RSV and then daily for 4 days, the formulation significantly reduced the viral load, compared to controls receiving saline ($P<0.01$). It did not cause irritation in the mucosal membranes or any other ill effects in intranasally treated rats.The formulation may have a potential as nasal spray to suppress or ameliorate respiratory infections by RSV and other enveloped viruses.

Several studies have addressed the question of whether lipids, particularly free fatty acids, may serve in the natural defense of the skin and mucosal membranes against pathogens. These studies have recently been reviewed [25, 66]. Antimicrobial lipids are found in bronchoalveolar lavage of many animals, including humans, and it has been suggested that they play a role in extracellular clearance of microbes in the respiratory tract. The idea of applying free fatty acids, or other microbicidal lipids which inactivate RSV and parainfluenza virus, to respiratory mucosa may therefore not be far-fetched, since they might enhance a natural defense by lipids in the mucosa.

6.3. Topical treatment of skin infections by fatty acids and monoglycerides.

Recent studies indicate that natural defense mechanisms can be exploited to combat drug-resistant pathogens [67, 68, 70, 71]. A unique palmitoleic acid isomer C16:1Δ6, sapienic acid, which is the predominant unsaturated fatty acid in human sebum, is active against *S. aureus* and streptococci [31] and is considered to play a role in the innate immune mechanisms of the skin. Clarke et al. [67] showed that sapienic acid was effective in treating systemic and skin infections by *S. aureus* in a mouse model. In a group of mice with systemic infection, injection of purified sapienic acid led to a significantly reduced bacterial load and completely prevented death, compared with 4 deaths in10 untreated controls. In mice with atopic dermatitis, topical application of sapienic acid caused a highly significant reduction in the number of viable *S. aureus* in the skin. In another study, oleic acid native to the skin inhibited the growth of MRSA at a dose which was not cytotoxic to human sebocytes. It was also antibacterial in the skin of mice infected with a strain of MRSA. The results suggested a therapeutic approach against MRSA by boosting the bacteriocidal activities of a native fatty acid [68].

Lauric acid shows strong bactericidal activities against many Gram positive bacteria such as streptococci and staphylococci [69]. It has recently been shown that lauric acid is strongly bactericidal against the Gram positive

bacterium *Propionibacterium acnes* which causes acne vulgaris, a chronic follicular inflammation in the skin [70]. In this study, intradermal injection and epicutaneous application of lauric acid effectively decreased *P. acnes* colonization of mouse ears, relieving swelling and granulomatous inflammation. Since lauric acid was not cytotoxic to human sebocytes, the results suggested that it could be used for treatment of acne. In a further work [71], lauric acid was incorporated into liposomes for topical delivery. In this formulation, the activity against *P. acnes* was well maintained, supporting the notion that a liposome formulation containing lauric acid could be used for treatment of acne vulgaris and other *P. acnes* associated diseases.

In another study of a potential therapeutic effect of lipids on infections of skin and mucosa, combinations of monocaprin with organic acids showed antibacterial activities *in vitro* against a number of isolates of *S. aureus*, both methicillin-susceptible and resistant [72]. Experiments in a murine model of MRSA nasopharyngeal colonization showed that monolaurin ointment was more effective in reducing the bacterial colonization than the antibiotic mupirocin which is used as a topical treatment for this type of skin infections. The authors concluded that monolaurin formulations have the potential to prevent and reduce *S. aureus* colonization of the nose and skin in humans and that a clinical evaluation is warranted [72].

6.4. Activity of fatty acids and monoglycerides against *Helicobacter pylori*.

Based on reports in the literature that diets rich in polyunsaturated fatty acids lower the incidence of peptic ulcer in humans and the apparent importance of *Helicobacter pylori* infection in peptic ulceration, Thompson et al. hypothesized that polyunsaturated fatty acids may protect against peptic ulcer by inhibiting the growth of *H. pylori* [73]. To test this hypothesis, they did an *in vitro* study of the antibacterial effect of a series of C18 and C20 unsaturated fatty acids. All of these common dietary fatty acids were strongly bactericidal against *H. pylori* by disrupting the bacterial cell membrane. It was estimated that after digestion of a fatty meal, the concentration of free fatty acids in the stomach would be well within the range shown to be bactericidal against *H. pylori*. It was therefore suggested that treatment with dietary polyunsaturated fatty acids might protect against peptic ulcer by bactericidal action. However, a small trial on the efficacy of polyunsaturated fatty acid treatment of patients with ulcer did not show a significant change in the colonization of the stomach by *H. pylori* nor in inflammation characteristic of *H. pylori* gastritis [74].

In addition to polyunsaturated fatty acids, medium-chain monoglycerides and lauric acid are bactericidal against *H. pylori* in vitro [75, 76]. Further studies are needed to establish whether monoglycerides, particularly monocaprin and monolaurin, may be useful in prevention of or protection against gastric colonization by *H. pylori* in humans. A treatment of either established infection or full blown stomach ulcers by bactericidal lipids is less likely to be successful, due to the inaccessibility of the bacteria underneath the mucus layer.

6.5. Treatment of *Candida*-associated denture stomatitis by monocaprin.

It has been suggested that antimicrobial lipids could be used in the prevention of dental caries or for treatment of infections in the oral mucosa [77]. When monocaprin was tested *in vitro* against a number of microorganisms commonly found in the oral cavity, *Candida albicans* was found to be the most sensitive but *Streptococcus mutans* also showed considerable sensitivity. This suggested that monocaprin might be used as a topical agent against *Candida* contamination of dentures. A study of denture disinfection in 32 patients attending a geriatric day care centre showed a significant, but short-term, reduction in *Candida* counts on the fitting surface of full dentures [77]. However, the time of monocaprin treatment of the dentures was short and no direct treatment of the oral mucosa itself was possible. It was concluded that a suitable vehicle for retaining monocaprin on the denture fitting-surface was needed. An *in vitro* study in which monocaprin was incorporated into a denture adhesive showed a slow release of monocaprin and a microbicidal effect on oral strains of *Candida* [78]. This indicated that incorporation of monocaprin into denture adhesives may have a potential as a means to prevent or inihibit denture stomatitis. Further studies confirmed that monocaprin added to a denture adhesive gave the material an anti-candidal activity without reducing its adhesive properties [79]. It was concluded that a denture adhesive containing monocaprin is suitable for a clinical trial to assess its efficacy in treating denture stomatitis.

6.6. Concluding remarks.

As outlined above, experiments to treat bacterial and viral infections in skin and mucosa with pharmaceutical formulations containing microbicidal lipids have given promising results. However, sufficient clinical trials have not yet been carried out to warrant their acceptance as drugs for prevention or treatment of bacterial and viral infections in animals or humans. On the other hand, pharmaceutical dosage forms containing salts of fatty acids, particularly the C11 unsaturated undecylenic acid, have been commercially available for many years as fungicidal drugs. Topical treatments with creams, ointments and powders containing either undecylenic acid or zink undecylenate were found to be safe and effective and comparable to a common fungicidal cream. Desinex cream and a number of other preparations containing undecylenic acid as the active ingredient are on the market as over-the-counter antifungals [80].

Antimicrobial lipids and their potential use in prevention and treatment of infections have been recently reviewed in detail [81, 82].

7. Antimicrobial lipids in prevention of foodborne bacterial infections.

7.1. Bacteria causing foodborne infections in humans.

Most of the pathogenic bacteria which cause foodborne infections in humans originate in farm animals, where they colonize the intestinal tract, usually without causing clinical illness. The most common intestinal bacterial pathogens in food animals are *Campylobacter jejuni*, *Salmonella enteritidis*, *Listeria monocytogenes, Escherichia coli* strain O157 and other Vero-toxin producing strains, and *Yersinia enterocolitica* [83].

Fig. 1 *Campylobacter jejuni*, the most common foodborne bacterial pathogen. Electron micrograph of a negatively stained bacterium. Bar: 0.5 µm. (Bergsson, G. and Arnfinnson, J. 2001, unpublished).

Campylobacteriosis is the most common foodborne bacterial infection in humans. Intestinal *C. jejuni* colonization of broiler chickens is the major source of the human infection since it causes contamination of carcasses in the slaughterhouse. Cross-contamination of bacteria during food preparation in the kitchen can result in human exposure to these foodborne pathogens, due to transfer of bacteria from poultry carcasses via cutting boards or other unwashed surfaces to salad vegetables and ready-to-eat foods [84]. Salmonellosis is the second most common foodborne bacterial infection and is most frequently caused by consumption of undercooked chicken and pork. Human listeriosis is much less common than campylobacteriosis and salmonellosis but is a more serious infection often requiring hospitalization, sometimes with fatal outcome [83]. The most important sources are raw food, for example cheese, raw milk and ready-to-eat meat and fish products [85]. Infections of humans with toxin-producing *E. coli s*trains are most often caused by consumption of undercooked ground beef, soiled vegetables or contaminated water, but the occurrence is low. The same is true for infections by *Yersinia* which are caused by consumption of undercooked pork [86].

7.2. Activity of lipids against foodborne bacteria.

In a study of the bactericidal action of lipids on foodborne bacteria [87], fatty acids and monoglycerides were tested *in vitro* against *C. jejuni*. Capric acid and its monoglyceride, monocaprin, reduced the viable bacterial counts by almost 7 $\log_{10}$ in 10 min, whereas the other fatty acids and monoglycerides had no or very little activity. A monocaprin-in-water emulsion was prepared which was stable at room temperature for at least 1 year and remained strongly bactericidal against *Campylobacter*. Monocaprin emulsions acidified by addition of citrate-lactic buffer at pH 4.1-5.0 killed clinical isolates of *Salmonella* spp. and *E. coli* strain O157, reducing the viable counts by greater than 6.7 $\log_{10}$ in 10 min at room temperature [87]. A recent unpublished study shows that monocaprin emulsions are active against *Listeria*. All of the most important foodborne bacteria are therefore killed by monocaprin emulsions, either at neutral pH or below pH 5.

7.3. Use of monocaprin to control the transmission of foodborne bacteria to humans.

There are several ways to control the transmission of foodborne pathogens such as *Campylobacter* from animals to humans [11]. One way is to prevent or reduce intestinal colonization of pathogenic bacteria in farm animals. Another way is to reduce surface contamination on fresh or processed food products by treatment with bactericidal compounds. The third way is sanitization or disinfection in kitchens and other food preparing and food processing facilities to prevent cross-contamination. A number of studies have been carried out to determine whether monocaprin emulsions can be used to reduce *Campylobacter* contamination of poultry at various stages from farm to fork.

7.3.1. Control of intestinal colonization by *Campylobacter* in broiler chickens.

Monocaprin at a concentration of 0.12% reduced the *Campylobacter* counts by 4 $\log_{10}$ in contaminated chicken feed and reduced the counts in drinking water to undetectable levels, without any adverse effects on the health or growth rate

of chickens [87, 88]. This suggests a possible use of monocaprin emulsions to prevent infection of broiler chickens by contaminated drinking water and feeds.

Further experiments were done to determine whether or not an already established intestinal *Campylobacter* colonization in broiler chickens could be reduced by adding monocaprin emulsions to their drinking water and feed [88]. Notably, there was a significant 1.5-2.2 $\log_{10}$ reduction in viable *Campylobacter* counts in cloacal swabs of naturally infected broilers by 0.24% monocaprin added to water and feed for 2-3 days before slaughter. This may be useful as a means to reduce *Campylobacter* colonization immediately before slaughter, thus decreasing the likelihood of carcass contamination in the slaughterhouse.

7.3.2. Reduction of Campylobacter contamination on chicken carcasses by immersion in monocaprin emulsions.

Reduction of the number of viable *Campylobacter* on poultry carcasses in the slaughterhouse makes the spread of the bacteria to humans less likely. Thus, a risk assessment study has estimated that a 2 $\log_{10}$ reduction in the number of viable *Campylobacter* on chicken meat will lead to a 25- to 30-fold reduction in the number of human cases of campylobacteriosis [89]. Because of the strong bactericidal activity of monocaprin against *Campylobacter*, experiments were done to test whether the number of viable *Campylobacter* on poultry carcasses could be reduced by immersion into monocaprin emulsions [90]. Immersion of naturally contaminated chicken legs in 0.5% monocaprin emulsions for 1 min at 20^{o}C caused a greater than 2 $\log_{10}$ reduction in the number of *Campylobacter* and a 0.25% emulsion caused a significant reduction in the contamination of chicken legs treated for 5 min at 6^{o}C. Emulsions were also tested on contaminated duck legs and found to be more active than on chicken legs tested under the same conditions.

The lower efficacy of bactericidal compounds on chicken skin, compared with their activity in water, is probably due to the firm attachment of bacteria to the skin [91]. However, under favorable conditions, treating of poultry carcasses or parts thereof with monocaprin emulsions is an effective and economical method to reduce *Campylobacter* contamination and thus make the meat safe for human consumption.

7.3.3. Use of monocaprin emulsions as kitchen sanitizers to prevent cross-contamination of foodborne bacteria.

Cross-contamination of pathogens from soiled surfaces in the kitchen, such as cutting boards, to ready-to-eat foods is a risk factor in human exposure to these pathogens, and can cause transmissions of foodborne bacteria [92]. It is therefore important to promptly clean and sanitize contaminated surfaces. The effect of monocaprin emulsions as kitchen sanitizers was tested on cutting boards and other food contact surfaces commonly found in the kitchen. Three foodborne bacterial species were tested in these experiments, namely *C. jejuni, E. coli* and *S. enteritidis*. Plastic (polypropylene) cutting board surfaces soiled with chicken meat juice containing 6 $\log_{10}$ cfu of *Campylobacter* per ml were washed for 2 min with a 0.24% monocaprin emulsion. After washing, bacteria were not detectable in swabs from the plastic surfaces, the reduction in viable *Campylobacter* counts being greater than 4 $\log_{10}$ [93]. Similarly, plastic cutting boards soiled with *Salmonella*-spiked chicken meat juice were sanitized by washing with a 0.5% monocaprin emulsion at pH 4.1, the viable bacterial counts reduced by 5.5 $\log_{10}$ [94]. Washing with monocaprin emulsions therefore proved to be an effective method for sanitization of polypropylene cutting boards soiled with chicken meat juice heavily contaminated with *Campylobacter* or *Salmonella*.

To simulate routine cleaning procedures in a kitchen, laminated plastic kitchen counters soiled with *S. enteritidis* or *E. coli* in diluted nutrient broth, either fresh or dried on the surface, were washed with a 0.5% monocaprin emulsion at pH 4.1. *Salmonella* was more easily killed than *E. coli*, the viable cell counts in swabs reduced from about 8 $\log_{10}$ to undetectable levels, both on a wet surface and in dry spots. *E. coli* was less easily killed on wet surfaces than in dry spots. However, the viable cell counts on swabs were reduced by about 6 $\log_{10}$ or more in both cases.

Killing of *E. coli* by washing with a 0.5% monocaprin emulsion was tested on various surfaces common in kitchens. The emulsions were most effective against *E. coli* on wet surfaces of glass and stainless steel, a little less effective on laminated plastic and tiles and least effective on plastic cutting boards. On dry surfaces, the viable *E. coli* counts were reduced to undetectable levels on all 5 surfaces.

These experiments show that cleaning with monocaprin emulsions kills the foodborne bacteria *C. jejuni, S. enteritidis* and *E. coli* on hard surfaces, particularly in dry spots. This might be a highly effective way of sanitization or even disinfection of hard surfaces in kitchens and in other food preparing and food processing facilities and may help in preventing transmission of foodborne bacteria to humans.

The potential use of microbicidal lipids as sanitizers in the food industry has recently been reviewed [11, 95].

8. The nature of the microbicidal action of lipids.

8.1. Virucidal action.

A number of studies have been done to elucidate the virucidal and bactericidal action of fatty acids. Viruses containing a lipid envelope are inactivated and several studies have shown that the inactivation is caused by disintegration of the viral enevelope [15, 96, 97]. This was demonstrated by an electron microscope study of vesicular stomatitis virus particles exposed to linoleic acid for 30 min [15]. At a concentration of 0.5 mg/ml of linoleic acid the viral envelope was partly disrupted and at 1 mg/ml it was completely disintegrated (Fig. 2).

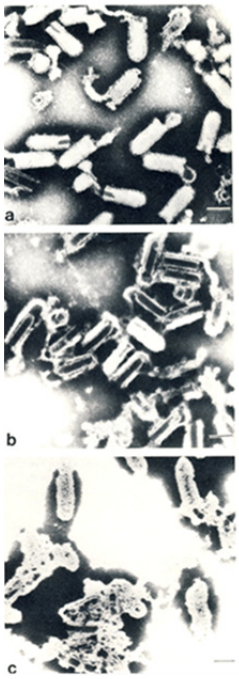

Figure 2. Negative staining of vesicular stomatitis virus particles showing the effect of exposure to linoleic acid. The virus was incubated for 30 min in culture medium (a), in 0.5 mg/ml of linoleic acid (b), and in 1 mg/ml of linoleic acid (c). a. Normal intact particles covered with spikes. b. The viral envelope is partly disrupted, allowing penetration of stain into particles. c. Virus particles in various stages of disintegration. Bar: 100 nm. (Reprinted from [15] with kind permission from the American Society for Microbiology).

8.2. Bactericidal action.

A mechanism similar to the one which inactivates viruses seems to cause the killing of bacteria by fatty acids and monoglycerides. The viability of the Gram negative bacterium*, C. trachomatis*, is irreversibly lost after treatment with monocaprin [48]. The negatively stained elementary bodies of *C. trachomatis* appeared deformed and partly disintegrated after 10 min, suggesting that monocaprin kills the bacteria by disruption of the cellular membrane (Fig. 3). A study of the effect of monocaprin on the structure of group B streptococci, using a 2-color fluorescent bacterial viability kit, showed that monocaprin caused damage to the cell membrane which became permeable to propidium iodide [69]. Examination of thin sections of group B streptococci by transmission electron microscopy confirmed this finding, since after treatment with monocaprin the plasma membrane was no longer visible, indicating a disintegration of the membrane. This suggests that the lipids can penetrate the cell wall of Gram positive bacteria and thus reach the cell membrane where it causes a partial solubilization of the membrane, leading to its disintegration and to cell death. The Gram negative bacterium *H. pylori* is rapidly killed by fatty acids and monoglycerides at neutral pH [75, 76] and electron micrographs showed that polyunsaturated fatty acids disrupt the cell membrane leading to cell lysis [73]. This study also demonstrated that the bactericidal fatty acids are incorported into the bacterial membranes.

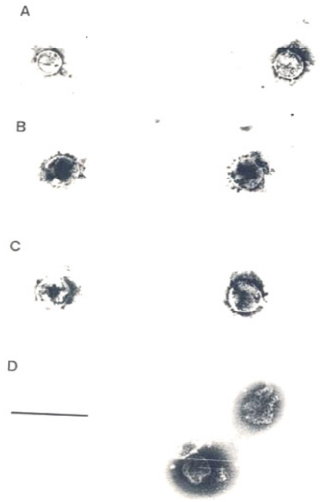

Figure 3. Electron micrographs of negatively stained elementary bodies of *Chlamydia trachomatis*. Untreated (A), treated with 10 mM monocaprin for 1 min (B), 5 min (C) and 10 min (D). After treatment for 10 min the cells appear deformed and partially disintegrated. Bar: 1 μm. (Reprinted from [48] with kind permission from the American Society for Microbiology).

The differential susceptibility of bacterial species to the action of lipids is notable, as exampled by *Campylobacter* and *H. pylori* and the enterobacteria *E. coli* and *Salmonella* which are only killed at low pH [87, 94]. The differential susceptibility of these bacterial species to the action of lipids is most likely due to differences in the structure of the outer membrane which in the enterobacteria can act as a barrier and protect the inner cell membrane. Increased acidity may cause a weakening of the permeability barrier in the outer membrane, allowing penetration of the lipid through the membrane and the cell wall. The bactericidal lipid thus gains access to the cell membrane, leading to its disintegration and to cell death [76, 82, 95].

9. Conclusion.

The antimicrobial activities of lipids, particularly of free fatty acids and monoglycerides, have been studied for decades. The activity profiles for many viruses and bacteria have been determined showing that long-chain unsaturated fatty acids and medium-chain saturated fatty acids and monoglycerides thereof are generally most active. Notably, bacteria vary greatly in their susceptibility to the bactericidal action of lipids, but many common pathogenic bacteria are rapidly killed by fatty acids and monoglycerides. Fatty acids, derived from triglycerides in sebum and from phospholipids of epidermal keratinocytes, are bactericidal against pathogens in the skin and may be a part of the innate immune system. There is considerable experimental evidence that the natural defense can be boosted by application of microbicidal lipids. More clinical trials are needed to test the safety and efficacy of lipids in prevention and treatment of infections in the skin and mucosal membranes.

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