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GENESIS OF FREE FATTY ACIDS*

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Fatty acids, those ubiquitous compounds in the biologic economy, form the building blocks of various lipids and lipid complexes. They were first described by Unna and Golodetz (1909) in a free or nonesterified state in skin fat and later by Mackenna et al. (1950) and Ricketts et al. (1951). However, there are no free fatty acids in freshly collected sebum (Nicolaides and Wells, 1957; Wheatley, 1951, 1957; Kellum, 1967).

Currently, free fatty acids are believed to play an important role in comedogenesis and in the formation of inflammatory lesions. Therefore, considerable experimental effort has been made to explain the origin of these substances. The experimental evidence accumulated by these studies has led to the current hypothesis that the free fatty acids of skin surface lipid are derived from sebum triglycerides through the lipolytic action of microbial lipases. These data will be summarized here.

TRIGLYCERIDE ORIGIN OF FREE FATTY ACIDS

Several investigators have indicated that free fatty acids are formed through the hydrolysis of sebum triglycerides in the follicular canal. Nicolaides and Wells (1957) found little or no free fatty acids in lipids recovered from steatocystoma, where sebum is not exposed to the follicle or the skin surface; otherwise the lipids were similar to those recovered from the skin surface. Also, the lipids of ovarian dermoid cysts contain no free fatty acids (Wheatley, 1951, 1957).

Kellum (1967) found triglycerides, but not monoglycerides, diglycerides, or free fatty acids, in human sebaceous glands and concluded that free fatty acids first arise after sebum is excreted from the sebaceous gland.

Although triglycerides, wax esters, and cholesterol esters all can yield free fatty acids when hydrolyzed, Nicolaides and co-workers (1956, 1957) determined that the free fatty acids in sebum are probably derived from the hydrolysis of sebum triglycerides. In a series of experiments, these investigators demonstrated that the total percentage of free fatty acids and triglycerides was fairly constant in a given individual and that the fatty acids increased as the triglycerides decreased. They identified the intermediate products of triglyceride hydrolysis, mono- and diglycerides, in skin surface lipid and demonstrated that the ratio of free to esterified fatty acids increased with time in free-flowing sebum. They also found that a pure triglyceride applied to the skin surface was rapidly hydrolyzed to the corresponding free fatty acid. After an unsuccessful attempt to find free wax alcohols in skin surface lipid, Nicolaides concluded that waxes are unlikely sources of free fatty acids. Cholesterol esters can be only minor sources for free fatty acids, since very little cholesterol or its esters are found in sebum.

BACTERIAL LIPOLYSIS

In recent years the theory that free fatty acids are formed from sebum triglycerides has led investigators to examine the mechanism by which this hydrolysis occurs. The presence of nonspecific esterases in the sebaceous duct and along the wall of the hair follicle (Montagna, 1955; Findlay, 1955; Nicolaides and Wells, 1957) suggested that these tissues furnished lipases since such enzymes are not uncommon in the ducts of epithelial glands.

Strauss and Mescon (1959) collected masses of comedones from several subjects and incubated them for 24 hr in an olive oil emulsion. Using suitable controls, they demonstrated significant hydrolysis of the oil as measured by an increase in the titratable acidity of the substrate. They speculated that *Corynebacterium acnes* (*C. acnes*), which was found in large amounts in the comedones, was the most likely source of the lipolytic enzyme. Scheimann et al. (1960) demonstrated a significant decrease in the hydrolysis of ^{14}C-labeled tripalmitin when it was applied to skin previously treated with topical antibiotics. This suggested an important role for the cutaneous bacteria in the lipolytic process. Later, Freinkel et al. (1965) and Strauss and Pochi (1966) observed that the administration of tetracycline and other broad-spectrum antibiotics resulted in a marked decrease in the titratable acidity of skin surface lipid. When the free fatty acids were measured directly, they too were decreased.

Thus, there was tentative evidence that bacteria played a role in the genesis of free fatty acids in skin. More recently, studies have focused on the specific role of the cutaneous microflora in the formation of these fatty acids. In a series of experiments, lipolytic activity has been demonstrated in vitro with isolates of *C. acnes*, staphylococci, and *Pityrosporum ovale* (*P. ovale*). Reisner et al. (1968) first studied the ability of *C. acnes* to hydrolyze various triglycerides by incubating fresh cultures of the organism with broth containing one of a series of saturated and unsaturated triglycerides. After solvent extraction, the free fatty acids were identified by thin-layer chromatography. Free fatty acids were liberated during

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incubation with *C. acnes* but not during control incubations without the organism; this indicated that *C. acnes* possessed lipolytic activity. In similar experiments, Kellum et al. (1970) examined isolates of *C. acnes* from patients with and without acne vulgaris and studied their ability to hydrolyze trilaurin, tripalmitin, and triolein in vitro. The hydrolysis products were determined by solvent extraction and thin-layer chromatogaphy. A significantly greater number of lipolytic isolates was found from patients with acne than from patients without acne. Using a dye-indicator lipase-screening media, Smith and Willet (1968) found only limited lipolytic activity in *C. acnes* isolates. Only tributyrin was hydrolyzed by *C. acnes*, but the value of such screening media for the critical analysis of lipases is questionable. Other workers have demonstrated that two distinct types of *C. acnes* inhabit sebaceous follicles and that the lipolytic activity of the two groups is demonstrably different (Whiteside and Voss, 1972).

Staphylococci have long been known to possess lipolytic activity, a subject of considerable interest to the dairy industry. With regard to the cutaneous cocci, Freinkel and Shen (1969) demonstrated the lipolytic activity of coagulase-negative staphylococci by incubating culture supernatants with an olive oil emulsion and measuring the titratable acidity of the reaction product. In numerous strains of straphylococci, Holt (1971) and Smith and Willet (1968) observed lipase activity on screening media; this indicates that staphylococci possess lipases that can hydrolyze various triglycerides.

Experimental evidence also suggests that *P. ovale* can hydrolyze triglycerides in vitro (Weary, 1970). When triolein, tripalmitin, trimyristin, and tristearin were incubated in culture media, the corresponding free fatty acids were liberated from all but tristearin.

Using a partially purified lipase preparation, Troller and Bozeman (1970) observed that staphyloccal lipase produced significant hydrolysis of tributyrin, tricaprin, and triolein, but not of triglycerides of intermediate chain lengths (C_6, C_8, C_{12}, C_{14}, and C_{16}). We have prepared purified staphylococcal lipase preparations by batch culture of the organism and purification of the culture supernate. After centrifugation at relatively low speed, the supernatant fraction was either concentrated by ultrafiltration or its protein was precipitated by ammonium sulfate or 50% ethanol. Column chromatography achieved an even better purification; that is, a 200- to 300-fold increase in specific activity over that of the culture supernatant. After column chromatography at least two peaks of activity and several bands of protein were shown to be present by disc gel electrophoresis. Biogel 300 produced further purification; more recently, we have investigated the possibility of obtaining highly purified enzyme preparations by affinity chromatography. Our experiments indicate considerable variation in the lipolytic activity of cutaneous cocci (Shalita, 1972). When isolates of *Staphylococcus aureus* were compared with group II *Staphylococcus albus*, the former was considerably more active against various triglyceride chain lengths whereas the latter hydrolyzed short-chain triglycerides preferentially.

The enzyme purification procedures are of practical significance. They are necessary to define the substrates of these lipases and should prove useful in isolating other lytic enzymes elaborated by these microorganisms. Furthermore, accurate characterization of these enzymes will make it possible to design inhibitors that may be valuable in the treatment of acne. It must be emphasized, however, that ultimately the data from these in vitro experiments must be correlated with those from in vivo studies. Assays performed even on crude enzyme preparations demonstrate considerably more activity than that present in the follicle. Thus, the unusually high concentrations of an inhibitor required in vitro can be lowered considerably for in vivo situations.

EFFECTS OF ANTIBIOTICS

While these direct studies of the lipolytic activity of the various cutaneous bacteria were being carried out, another approach to the problem of the relative importance of each species in the genesis of fatty acids was being studied cooperatively by investigators at the University of Pennsylvania and Boston University. These studies were initiated in a group of 12 volunteers who were treated with topical 1% neomycin for 4 weeks (Marples et al., 1970). Pre- and posttreatment samples were analyzed to determine the composition of the skin surface lipid and the microbiologic composition of scalp scrubbings. With treatment, the density of the aerobic flora decreased dramatically but there was no significant alteration in the density of *C. acnes*. Thin-layer chromatography and photodensitometry of the charred thin-layer chromtography (TLC) spots indicated little or no change in the percentage of free fatty acids before and after treatment.

In a second study, volunteers treated with 600 mgm of demethylchlortetracycline (DMCT) daily showed a marked reduction in the *C. acnes* population, but there was no effect on the yeasts or the aerobic organisms because of the emergence of resistant organisms (Marples et al., 1971). A significant decrease in the percentage of free fatty acids was observed in this study.

The investigators then began another series of experiments to clarify the role of lipid-dependent yeasts in the formation of free fatty acids (Marples et al., 1972). Although the density of Pityrosporum species was reduced by the topical application of amphotericin B, no appreciable change in fatty acids was observed when subjects were treated only with this antimicrobial agent. The addition of systemic DMCT to this regimen reduced not only

the *C. acnes* density but the percentage of free fatty acids on the skin surface as well. The decrease, however, was greater when DMCT was used with amphotericin B as compared to DMCT treatment alone, an indication that yeasts may play a role when the *C. acnes* population is reduced.

Other antibiotics that affect both the bacterial flora and the surface lipid composition have also been demonstrated. In a study of the effects of various antibiotics on the bacterial population of the skin, Marples and Kligman (1971) observed a significant reduction in *C. acnes* in subjects treated with clindamycin. On the basis of this study, clindamycin might be expected to affect the composition of skin surface lipid. Studies by Cunliffe et al. (1972) and in our own laboratories (Shalita et al., 1973) have demonstrated that patients receiving clindamycin therapy did indeed show a marked decrease in the percentage of free fatty acids on the skin surface.

The results of these experiments furnish substantial evidence that the free fatty acids in the surface lipids of human skin originate from the hydrolysis of sebum triglycerides. The demonstration of lipolytic activity in cutaneous bacteria and the experiments of Marples et al. (1970, 1971, 1972) indicate that the action of the cutaneous microflora could easily account for most if not all of the free fatty acids normally present. Some of the free fatty acids could be derived from bacterial lipid synthesis. However, studies on the lipid composition of these organisms indicate that their predominant fatty acid is a 15-carbon branched chain acid that is found only in trace amounts in sebum (Freinkel, 1971).

In the numerous discussions of the genesis of free fatty acids in sebum, there has been much speculation but little critical investigation about the role of tissue lipases. Conceivably, lysosomal lipases could be liberated from follicular cells as a secondary event after trauma to the follicular wall by the formation of fatty acids during the bacterial lipolysis of sebum.

Increased concentrations of fatty acids, accompanied by an exacerbation of acne but no change in sebum production, have been reported in patients under emotional stress (Kraus, 1970). The role, if any, of the pituitary–adrenal axis in the control of sebum composition is poorly understood; but serum fatty acids may be increased during periods of stress. Again, the role of systemic lipid metabolism in the control of sebum composition is not clear. Total caloric deprivation affects sebum composition in man (Pochi et al., 1970), but, in general, sebaceous glands seem to possess their own homeostatic mechanism which allows for little change in lipid composition when essential precursors and the appropriate cell replicating stimuli are provided§.

§ Wheatley, V. R. (1972). Personal communication.

THE ROLE OF FREE FATTY ACIDS IN THE PATHOGENESIS OF ACNE

In regard to the pathogenesis of acne, current thinking assigns a central role to free fatty acids. Many investigators now believe that free fatty acids, liberated from sebum triglycerides through the action of microbial lipases, induce changes in the follicular epithelium; these changes eventually result in comedo formation. At some point in the process, these free fatty acids penetrate through the follicular wall into the surrounding dermis and thus cause an inflammatory reaction. Whether or not the degree and type of inflammation are related to the free fatty acids is not known.

This theory, developed from a series of experiments in several laboratories, demonstrates that free fatty acids are both irritating and comedogenic. Strauss and Pochi (1965) first showed that intracutaneous injections of skin surface lipid, particularly the free fatty acid portion, could incite an intense inflammatory reaction similar to that of acne. The injection of the free fatty acid fraction produced a reaction equal to that of whole skin surface lipid, but the injection of this lipid without free fatty acids resulted in the formation of only a small papule.

Later, Kellum (1968) found that a series of pure, even chain length, free fatty acids, particularly the C_8–C_{14} chain lengths, applied to human skin under occlusive patches produced primary irritant reactions. Similar findings have been observed with odd chain length, free fatty acids¶. Lorincz et al. (1968), Kligman and Katz (1968), and Kanaar (1971) have demonstrated that the repeated application of skin surface lipid to the rabbit ear almost uniformly results in comedo formation. Again the free fatty acid fraction appears to be the most comedogenic portion of this lipid (Kligman et al., 1970).

Lately there has been disagreement about the relative properties of the various free fatty acids both as primary irritants and as comedogens. From a pragmatic point of view, the shorter chain fatty acids are obviously more irritating; for example, a drop of acetic acid burns the skin whereas a drop of capric or lauric acid does not. On the other hand, equal volumes or weights of molecules of different chain lengths contain unequal numbers of molecules of the given compound. Thus the only valid comparison of the properties of a given compound is an equimolar one. To understand the situation as it applies in vivo, however, only quantitative analysis of the free fatty acids of different chain lengths in sebum can yield definitive data.

The clinical corollary of the fatty acid theory of the pathogenesis of acne is the fact that drugs which lower skin surface fatty acids are effective in the treatment of the disease. Thus the tetracyclines, erythromycin and clindamycin, which re-

¶ Stillman, M., Shalita, A., and Maibach, H.: Unpublished data.

duce the free fatty acid fraction of sebum, are beneficial in acne therapy. However, these drugs also affect the density of *C. acnes*; thus, this organism may play a more direct role than merely hydrolyzing sebum triglyceride. Recent experiments in our laboratories indicate that for optimal clinical improvement, both *C. acnes* density and free fatty acids must be decreased, since a topical agent which lowers the fatty acid composition of skin surface lipid was less effective than antibiotics in treating acne, even when comparable sebum composition changes were produced.

SUMMARY

According to our current state of knowledge, free fatty acids are formed in sebaceous follicles primarily through hydrolysis of sebum triglycerides by microbial lipases. Because of their comedogenic and irritant qualities, these free fatty acids are probably responsible for many of the alterations in the follicle that result in acne lesions.

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