BIOCHEMISTRY OF ACNE

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The topic to be discussed, namely the biochemistry of acne, overlaps other presentations in this monograph. Therefore, I will restrict my presentation and present data related to a few specific questions dealing with the relationship of the sebaceous glands and sebum to acne. There are numerous statements that acne is a disease of the sebaceous glands, and for this reason, acne is almost always classified as a disease of the sebaceous glands in dermatologic textbooks. It is not a disease of the sebaceous glands; rather it is a disease of the pilosebaceous follicle and in particular of the sebaceous follicle. There is little question that sebum does play a role in the pathogenesis of acne. In fact, even though some doubts have been raised in the last few years, acne can be tied to sebaceous gland development. In the newborn child, the sebaceous glands are well developed and acne may be present. The sebaceous glands undergo atrophy shortly after birth and neonatal acne tends to disappear, although in certain instances it may persist for several months. In these patients, the sebaceous glands probably remain enlarged, and studies of sebum composition done in our laboratories support this concept. As part of the early pubertal spectrum, the sebaceous glands enlarge again; this is when acne commonly starts.

There is other evidence to link the sebaceous glands and sebum with acne. Sebum is comedogenic in the rabbit ear model, as described by Kligman and his coworkers. True, the rabbit ear is not the human face, but we have observed the development of comedones when sebum has been injected into the scalp.

Many years ago we showed that sebum is an irritant, and in particular we were able to demonstrate that free fatty acids were more irritating than any other component of sebum. Recently, the concept that the fatty acids are the prime irritants in acne has come under question. In our studies relatively large amounts of sebum were injected into the skin. Puhvel and her co-workers have now found that when smaller amounts of sebum, considered by her to represent what is found in the follicle, are injected there is no visible evidence of inflammation. Her calculations have been made with data based on solvent extraction of isolated follicles. While she has determined the amount of lipid that is present at a static period of time, her studies do not take into consideration that lipid is being constantly formed. Therefore, she has probably not used sufficient material to duplicate the dynamics of lipid formation in the sebaceous follicle.

Be that as it may, when sufficient material is injected into the skin, whole sebum is a very strong irritant, as is its free fatty acid fraction. However, if the free fatty acids are removed, and the residual material is injected into the skin, a minimal amount of inflammation is produced. If *Propionibacterium acnes* is injected into a closed cyst containing keratinous material and lipid, but no bacterial organisms, inflammatory components are generated. A massive dermal inflammation response is induced with rupture of the cyst and liberation of all of the inflammatory products into the dermis. If heat-killed organisms are injected into a comparable cyst in the same individual or if living organisms are injected intradermally into the same individual, there also is very little inflammation. All of this supports the concept that sebum and its degenerative products are inflammatory. While the fatty acids may be the prime irritants, other bacterial exoenzymes or other products generated by these exoenzymes may be responsible for the inflammation.

The next question to be discussed is whether there is an increase in sebaceous gland activity in association with acne. Studies from our laboratories, as confirmed by Shuster and his co-workers, have shown that mean sebum production is increased in acne, as compared to a comparable age-matched group of subjects without acne. The mean difference between such groups is highly significant. However, the standard deviations are large and there is great overlap in the values. Therefore, there is no absolute value for sebum production which indicates that an individual does or does not have acne, and acne is not a disease that can be considered to be related only to the size and functional capacity of the sebaceous glands. However, in patients with acne, as the severity of the

grade increases, there is a significant increase in mean sebum production. Once again, however, the range of values within each group are large.

It is obviously important to know whether there are any specific changes in sebum composition in acne. The available literature on this subject is confusing, and most of the studies have not involved the complete analysis of the lipid film. If there is any consistency at all in the published data, it is that the squalene content is slightly increased, but this might only reflect an increase in sebum production. Kellum has reported an increase of a minor component of sebum with a gas-chromatographic retention value of 17.52. We have identified this particular component as Δ 5,8 octadecadienoic acid, a C-18 straight-chain fatty acid with double-bond unsaturation at the 5 and 8 position. By chromatographic techniques, we have found that this Δ 5,8 C-18 fatty acid, which Kellum felt was increased in acne, to be unchanged in amount in patients with varying degrees of acne. However, the percentage of Δ 9,12 octadecadienoic acid is decreased in patients with acne and the percentage decrease parallels the degree of acne. This finding had not been readily apparent since the two unsaturated fatty acids have similar retention times and are difficult to separate under normal circumstances. The differences in the concentration of Δ 9,12 octadecadienoic acid in the surface lipid samples from those with and without acne are highly significant and there is very little overlap. This is a most interesting finding, for the Δ 9,12 fatty acid is the essentially fatty acid, linoleic acid. While the pathogenesis of acne is complex, there is little doubt that the prime pathogenic factor is abnormal keratinization, and in the essential fatty acid-deficient animal, the prime cutaneous change is abnormal keratinization. The obvious question, therefore, is whether the decreased linoleic acid level in sebum could be the etiological factor causing the early keratinization alterations within the follicle. This is obviously a most attractive concept, for it makes it possible to link sebaceous gland function to the early changes in the follicular wall. We are actively exploring this possibility.

In summary then, while considerable work has been done, we are just beginning to understand the biochemistry of acne. The biological effects of sebaceous lipids have been relatively well characterized, but other substances which could be of great significance such as bacterial enzymes and their products are just beginning to be studied. Much more will be heard about these, I expect, in the near future.

DISCUSSION

Hagerman, Malmö: Lately the role of one of the other fatty acids in the pathomechanism of acne has been discussed. I want to mention one acid that appears to play a rather interesting role. My 25 years study of acne has shown that in at least 80 per cent of the patients, the disease will improve considerably with the use of a specially adapted elimination diet. A closer analysis has demonstrated that small or even minimal quantities of particular well-defined substances in certain foods can cause inflammatory changes (Table).

Eliciting acnegens (percent reacting of 117 selected patients with acne)

Lipids		Colours		Aromas		Halogens	
Chocolate	52	Citrus	31	Spices	52	Fish	18
Cheese	52	Tomato ketchup	17	Tomato ketchup	17	Pickled herring	10
Other dairy products and margarine	33	Amaranth	17	Pickled herring	10	Shellfish (mostly shrimps, colour?)	9
Eggs	8	Strawberries	10	Mustard	12	Iodized salt	2
Other "fatty" foods	65	Tomatoes	9	Pepper	12		
		Red apple peel	9	Onion	7		
		Grapes	9	Smoked meat	7		
		Currant juice	8				
		Hips	7				

Time does only allow a discussion of the vegetable oils also used in margarine and dressings. I have found that certain oils appeared to have an acne-provoking capacity, but others could be eaten with impunity by the same patients. Therefore, a special experiment was made on a very co-operative patient. When the acne was minimal on this particular diet, the patient tested one oil after another in a dose of 30 ml and noted the changed in acne. After testing eight different oils, including raw linseed oil, the results were compared with the respective composition of the oils as far as this was available. Only one component appeared to have any relation to the clinical flares and this was linolenic acid. The linolenic acid content of the tested oils is (according to the literature): corn oil 0.1%, sunflower oil 0.1%, cotton oil 0.2%, olive oil 0.4%, soy bean oil 7.5%, mustard oil 8%, rape oil 11%, and raw linseed oil about 60%. The estimated rate of skin flare followed the respective content of linolenic acid very closely.

Linolenic acid is an unsaturated C-18 carbonic acid with three double bonds. The correlation between the flares of acne and the content of linolenic acid is so obvious that there can be no doubt that this acid, or possibly a metabolite or an obligatory contamination is the offender in this particular case. Furthermore, the reaction pattern is in very good agreement with that observed during dietary experiments with many other patients during many years. They will for instance be able to eat corn oil and oleo margarine in which linolenic acid is not a component, but they will flare when they eat certain other margarines, oils or dressings in which more linolenic acid-rich oils (e.g. rape oil) are incorporated.

As with my other acnegens the reactions appear within 48 hours, which excludes the possiblity that the linolenic acid could have been excreted through the sebaceous glands. On the contrary, it must have reached the follicle from the blood supply. Raw linseed oil is not an irritant, but the free acid might well be an irritant after being metabolized, probably in the skin. Maybe the acne bacillus in addition to producing proteases, lipases, hyaluronidases and so on, could also possess a syntetase or a similar enzyme, capable of rearranging the linolenic acid molecule into agents with strong vasoactive tissue toxic or leucotactic properties similar to the metabolic conversions of prostaglandins from their precursors.

Plewig, Munich: I have a question for Dr. Strauss. When you treat your patients systemically with steroidal compounds, is it conceivable that you influence the epidermal lipid components as well? If you would study epidermal lipid in sebaceous gland-free areas, do they change in composition?

Strauss, Iowa: What steroidal compounds are you referring to?

Plewig, Munich: I mean the oral contraceptives such as chlormadinone or an anti-androgen such as cyproterone acetate.

Strauss, Iowa: The only one mentioned by you that we have had any experience with is chlormadinone, but we worked with this many years ago. Cyproterone is not available for use in the United States. So essentially our experience has been with estrogens. We have not detected any changes in sebum composition in lipid recovered from the skin surface. But the problem is very simple. It is very difficult to separate epidermal and sebaceous lipids. Under normal circumstances from the face, which is our collection site, somewhere on the order of 95—98 per cent of the total recovered lipid is of sebaceous origin. In order to be able to see a change in sebum composition with a reduction in production rate you probably have to produce reduction in sebum excretion on the order of 80—90 per cent. This is what we have observed with 13-cis-retinoic acid. We do have close to a 90 per cent reduction in sebum production with this drug. Under these circumstances we can observe chemical changes in the surface lipids. Separation of the epidermal lipid from sebaceous lipids is most difficult because there is so little epidermal lipid in the total lipid film. It is much easier to identify the sebaceous lipids because you can identify the wax esters or squalene which are present in significant amounts since cholesterol makes up only 1 or 2 per cent of mature sebum, it is very unlikely that there will be any measureable changes unless there is a great decrease in sebum production, as already mentioned.

Plewig, Munich: I want to ask Dr. Strauss how stable is lipid composition in any one individual person oir ethnic groups when you collect repeated samples?

Strauss, Iowa: I cannot give you any data on ethnic groups, but within a single individual, sebum composition is quite stable if you disregard triglyceride or cholesterol ester lipolysis. If the per cent composition is grouped as wax esters, squalene, combined choleste-

rol-cholesterol esters and combined triglycerides-diglycerides and free fatty acids as total triglyceride derived fatty acids, then the values are quite stable. Furthermore, the pure sebaceous components which are squalene and wax esters usually comprise approximately 40 per cent of the recoverable film. This is a fairly constant figure under normal circumstances.

Plewig, Munich: Would it make sense to study the effects of nutritional factors with this lipid system?

Strauss, Iowa: I can answer that partially. We have studied sebum composition in severe starvation in obese patients who were on total starvation for as long as 42 days and in a healthy group of individuals who were given less than 50 calories a day for 10 days. We have also determined surface lipid composition in a group of patients with pellagra in South Africa and in a group of very malnourished children in South America. In all of these groups we have observed changes in lipid synthesis which essentially consist of a marked decrease of all lipid synthesis except for squalene. Squalene synthesis remains unchanged so that there is a relative increase in squalene and a decrease in the other components of the surface lipid film. However, we have no data on individuals who are on less restrictive diets.

Some of these patients were obese patients that were on a totally acaloric diet. They were older patients who were being studied on a metabolic ward. The largest group of patients had pellagra and were admitted to a hospital in South Africa for a period of 7 days for refeeding. Interesting, just at the end of the 7th day, we did observe changes in sebum composition which reflected a return towards normal.

Shuster, Newcastle-upon-Tyne: I think I can partially answer Dr. Plewig's question about the epidermal contribution. You cannot, as Dr. Strauss says, measure it by lipid output, but you can measure it if you look at lipid synthesis, which you can do quite separately for the epidermis and the sebaceous glands. We have done this fairly extensively in the rat and to a lesser degree in man. There are rather different changes occurring in different situations, but in general the epidermal contribution is negligible.

Zachariae, Aarhus: Maybe you have given the answer before, but I want to know about the stability of the different components in sebum. I would like to ask how stable the composition is in the female during the menstrual cycle?

Strauss, Iowa: We have no data on sebum composition during the menstrual cycle.

Plewig, Munich: I would like to ask both Dr. Strauss and Dr. Schaefer what is known about the excretion of other metabolites or food products through the sebaceous glands? I mean things such as colours, dyes, tetracyclines, drugs and so forth?

Strauss, Iowa: Well, I can express my viewpoint. First of all, remember that all evidence that we have at present indicates that sebum is synthesized de novo in the sebaceous gland. There is no transfer of preformed free fatty acids or triglycerides or any other lipid components to sebum. The lipids are probably synthesized from acetate. There is no data on the excretion of other compounds through the sebaceous gland, but I frankly doubt that much material would pass through the glands. On the other hand, substances could probably pass through the follicular wall. There is evidence that tetracycline does get into the follicle, so that it is being excreted. My viewpoint, which I cannot back with data, is that the drug is probably passing through the follicular wall rather than going through the sebaceous gland.

Schaefer, Berlin: I agree with Dr. Strauss, exactly the same. I could not really imagine how this could happen because the process of accumulation of drugs within the sebaceous gland is quite different.

I would like to have Dr. Strauss's comment on the concept of unsaturated fatty acids. You and Dr. Hagerman seem to present exactly opposite points of view. You see a decrease in the fatty acid and he states that an increase is responsible for acne. I have to add that we have studied the penetration of a lot of fatty acids through skin and they penetrate freely in and out. Nevertheless, I absolutely doubt that an essential fatty acid like linoleic acid would be able to penetrate into the skin and cause inflammation. If you apply linoleic acid to the skin surface, it is somewhat proinflammatory, but only at very very high levels such as 10 per cent.

Strauss, Iowa: Dr. Hagerman and I are talking about two different fatty acids. The fatty acid that we have identified as being decreased is linoleic acid, while Dr. Hagerman is talking about linelenic acid. We are talking about a fatty acid which has two sites of unsatu-

ration; he is talking about a fatty acid with three sites of unsaturation. I have had no experience with linolenic acid. The two viewpoints are not dramatically opposite, since we are talking about different fatty acids.

Hagerman, Malmö: I was talking about the peroral exposure to the linolenic acid and not about what goes into the sebum. Therefore we are speaking on very different topics and it is not at all peculiar that we have different results.