P. ACNES AND THE CHEMISTRY OF SEBUM

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INTRODUCTION

Numerous investigators (reviewed in 1, 2, 3) have performed chemical analysis of sebum and skin surface fat in order to find a specific component, which could be related directly or indirectly to the patho-mechanism of acne. One of the striking findings was that sebum in comedones contain up to 40% free fatty acids. On this basis the well-known hypothesis was developed that sebaceous triglycerides are hydrolyzed by lipases, which have been excreted by *Propionibacterium acnes* (P. acnes). The resulting free fatty acids, especially those of medium carbon chain length were thought to diffuse through the follicular wall into the surrounding tissue and to provoke the typical inflammation, since these fatty acids were shown to cause inflammation on topical application (4—6). The sensitivity of *P. acnes* to tetracyclines and the simultaneous inhibition of lipases by these drugs underlined this hypothesis (7—13).

A closer look at the composition of human skin surface fat casts, however, some doubt upon this theory. The average composition as reported by different authours and reviewed by us (2, 3) shows about 16% free fatty acids. In recent investigations we observed, however, a pronounced penetration of free fatty acids into human skin (14). It is not clear why this obviously normal backdiffusion should cause inflammation in acne patients only. The increased amounts of free fatty acids in comedones are not likely to be responsible for this discrepancy, since they occur in non-acne persons too and since penetration does not increase proportionally to the given amount.

The argument that free fatty acids with medium chain length could be responsible for the inflammation is also not very likely to be correct. A more detailed analysis by Ramasastry, Downing, Pochi, and Strauss (15) revealed that free fatty acids in surface fat decreased from childhood to puberty though they *should* increase drastically at the age of onset of acne. Boughton and Wheatley analyzed the chain length of total free fatty acids of skin surface lipids. They showed that there are only small amounts of medium chain length fatty acids (less than 14 carbon atoms). Lipases, however, are not able to form these acids since they only split triglycerides. The subsequent Ω- or β-oxidation is bound to an intracellular multienzyme complex, i.e., cannot be performed by enzyme excretion but only within the cells of living organisms. The sebaceous gland of acne patients is not likely to form triglycerides much different than those of normal glands and these are what are eventually split by lipases.

One could, however, argue that *P. acnes* indeed forms these acids intracellularly and then excretes them. In order to investigate this problem we performed a series of experiments with cultures of *P. acnes*. The medium in which the organism was grown was then tested for inflammatory responses in acne patients and in volunteers without acne (16).

MATERIALS AND METHODS

From pustules and comedones of 5 acne patients five different strains of *P. acnes* were isolated and characterized* (17). The strains were precultured for 7 days on Brain Heart Infusion (BHI) Agar (Oxid comp.).

Triglycerides (containing natural fatty acids of C_{12}—C_{18} carbon chain length) were emulsified in BHI-dextrose bouillon to give a final concentration of 1% with an Ultra-Turrax-homogenizer (Jahnke and Kunkel comp.). The medium was sterilized and 15 ml were added to sterile test tubes. These were inoculated with more than 10^8 colony forming organisms (P. acnes) and incubated for 10 days at 37°C. Thereafter the media was sterilized by passage through a Seitzfilter EKS I. The filtrate was lyophilized and then redissolved to give a 50% aqueous solution for clinical testing. The following media were prepared:

1. Medium plus 1% triglycerides, inoculated with *P. acnes*
2. Medium, inoculated with *P. acnes*
3. Medium defatted, inoculated with *P. acnes*
4. Medium plus 1% triglycerides, not inoculated
5. Medium defatted, not inoculated

*These experiments were performed in collaboration with Professor Staib, Robert Koch Institute, Berlin.

In order to extract the inherent fat of BHI-medium the dry powder was suspended in chloroform + n-hexane 1:1, stirred for two hours at room temperature, filtered, washed with the solvent mixture, dried and used as described.

To investigate the heat stability of potential inflammatory products formed by *P. acnes* the sterile filtrates of all 5 strains grown on defatted media were combined. One-half of this material was stored at room temperature, the other part was boiled for 10 minutes under reflux. The same procedure was performed with defatted non-inoculated medium. Thus the following media were prepared and tested:

6. Media defatted, inoculated, sterilized by filtration, combination of media of 5 strains
7. Media defatted, inoculated as under 6, denatured by heat
8. Medium defatted, not inoculated, sterilized by filtration
9. Medium defatted, not inoculated, sterilized by filtration, denatured by heat.

Patch tests were performed on the upper back of 30 volunteers (22 females, 8 males) with normal skin and on normal-appearing sites of 20 acne patients. Patch tests were performed after cleaning the skin with petroleum ether and on cleaned sites which were stripped 6 times with adhesive tape. No reactions were observed to the stripping alone. One tenth of a ml of the test solution was dropped on patch test plasters and these were attached to the upper back skin. The medium of each strain was tested separately. The plasters were removed 48 hours later and the reaction was read after 20 min. according to the following criteria:

0 = no reaction
1 = follicular erythema
2 = confluent erythema with perifollicular irritation
3 = papules surrounded by erythema
4 = confluent papules (infiltrated erythema)
5 = pustules with erythema

Mean values were calculated and significance was calculated by means of the student-test.

RESULTS

After 9 days incubation the *P. acnes* colonies were surrounded by a clear halo in the otherwise turbid emulsion of fat in medium. The non-inoculated media remained turbid. This can be considered as proof of excretion of lipases by the organisms and the subsequent hydrolysis of fat in the medium around the colonies.

The readings of the patch tests can be summarized as follows:

1) Every reading of non-inoculated media remained negative.
2) Media of different strains showed no prominent differences; if one patient had a pronounced reaction against one strain, the reaction against the other strains was clearly positive too. The readings of the different strains were pooled.
3) The readings of acne patients were significantly higher in both the normal patch tests and after stripping ($p < 0.0005$).
4) The readings on patch tests with stripping were always considerably higher than for patch tests done without stripping (medium plus triglyceride $p < 0.0005$).
5) Acne patients as well as volunteers showed a positive reaction against culture media which was defatted before inoculation. In acne patients the readings were even higher though this was not significant. The more intense reaction of acne skin in comparison to normal skin was significant ($p < 0.0005$). Furthermore, readings on acne skin with defatted media were the same as with media plus triglycerides.
6) Normal volunteers showed stronger reactions to the stripping patch test with media plus triglycerides in comparison to normal media (the difference was not significant, $p = 0.025$), the reaction against defatted media, however, was equal to that with triglycerides.
7) Normal volunteers reacted against defatted and heatdenatured media too, the reading being about 50% of that against non-denatured media. Controls were negative.

DISCUSSION

P. acnes obviously produced noxious substances which could hardly consist of free fatty acids; and acne patients showed stronger inflammatory reactions upon the application of this material than non-acne volunteers. The nature, however, of the toxic agent, which might be an endotoxin excreted by living bacteria or those dying during the culture, or an allergen or metabolite different from fatty acids, remain unclear.

The results of the first test series could be interpreted to signify that lipases excreted by *P. acnes* had been applied to the skin, penetrated into the follicles, there split triglycerides to form free fatty acids and thereby caused the inflammation, though this was not very likely because of the lack of evidence of the pre-

sence of corresponding short chain fatty acids, as argued in the introduction. However, this possibility could be ruled out since lipase would not withstand boiling. We were interested in looking at the problem from a different point of view; specifically we were interested in whether and to what extent triglycerides are hydrolyzed on the skin surface. A quick breakdown of triglycerides could be anticipated because there was a large surface area relative to the amount of substrate. Humidity, skin temperature, and the frequently mentioned presence of nonspecific, esterases should also facilitate this process. Therefore radiolabelled triglyceride (tripalmitate) was applied to skin *in vitro* under the mentioned conditions. Twenty-four hours later the surface lipid was removed with ether and analyzed by thin-layer chromatography. The hydrolysis was only about 1% (18). If this in vitro result may be generalized it means that lipolysis occurs mainly in the follicle.

In 1976 and 1977 three different groups of investigators reconsidered the fatty acid hypothesis. Anderson, Cook and Smith (19) reported that by oral as well as by topical treatment with tetracyclines the severity of acne could be reduced. However, neither the amount of surface lipid nor that of any component, i.e., squalene + hydrocarbons, wax esters, sterol + diglycerides, triglycerides, and free fatty acids changed with improvement of acne. Thus, the severity of acne could be reduced by tetracyclines without concomitant quantitative changes in surface lipids.

Puhvel and Sakamoto (20) analyzed the surface lipids and the sebum content of follicles and of isolated comedones and injected the corresponding free fatty acids intracutaneously in volunteers. They observed no inflammation exceeding that of the injection trauma.

Weeks, McCarty, Black, and Fulton (21) purified and characterized bacterial lipases and used them for selection of potent lipase inhibitors. The most potent inhibitor was able to suppress fatty acid formation 40% within 12 hours. However, this compound showed no favourable effects in a five-week treatment period, although the free fatty acids were decreased dramatically in all patients.

These investigations suggest that the liberation of fatty acids by bacterial lipases is unrelated to the pathogenesis of acne and that bacteria do not depend on lipases and lipids for energy requirements. Thus the question is open again as to which agents are responsible for the inflammation in acne and how these agents are formed.

SUMMARY

For several years the sebum was believed to be responsible for inflammation in acne. Triglycerides were thought to be hydrolyzed by lipases which are excreted by *P. acnes*. The resulting fatty acids, especially those of medium chain length could cause inflammation by diffusing into the tissue surrounding the infected follicle.

However, several observations cast doubt on this hypothesis:

1. Diffusion of free fatty acids from the skin surface back into the skin appears to be a normal process.
2. The free fatty acid content of surface fat decreases from childhood to puberty instead of increasing at the age of onset of acne.
3. Surface fat contains only small amounts of medium chain length fatty acids. The formation of long chain acids by oxidation cannot be achieved by the action of lipases only.
4. Even if the fat had been extracted, culture media contain inflammatory agents after growth of *P. acnes* and sterile filtration. The toxic principle was 50% resistant to 10 min. boiling; therefore it could not be a lipase.
5. The severity of acne can be reduced by oral and topical tetracycline treatment without concomitant quantitative changes in surface lipids.
6. The intracutaneous injection of concentrations of free fatty acids found in surface fat, fat from follicles, and from comedones did not cause inflammation.
7. A potent lipase inhibitor was able to suppress free fatty acid formation but showed no favourable effect in acne.

Taken altogether these findings render the mentioned hypothesis highly unlikely. Further research should be focused on *P. acnes* toxins or allergens.

REFERENCES

1. Downing, D.T., Strauss, J.S.: Synthesis and composition of surface lipids of human skin.
   J Invest Dermatol 1974; 62:228-44.
2. Hermann, F. et al.: Biochemie der Haut.
   Thieme Verlag, Stuttgart, 1972.
3. Stüttgen, G., Schaefer, H.: Funktionelle Dermatologie.
   Springer Verlag, Berlin-Heidelberg-New York, 1975.
4. Kellum, R.E.: Acne vulgaris. Studies in pathogenesis: Relative irritancy of free fatty acids from C_2 to C_{16}.
   Arch Dermatol 1968; 97:722-26.
5. Kellum, R.E., Strangfeld, K., Ray, L.F.: Acne vulgaris. Studies in pathogenesis: Triglyceride hydrolysis by corynebacterium acnes in vitro.
   Arch Dermatol 1970; 101:41-48.
6. Reisner, R.M. et al.: Lipolytic activity of corynebacterium acnes.
   J Invest Dermatol 1968; 51:190-96.
7. Smith, E.L., Mortimer, P.R.: Tetracycline in acne vulgaris.
   Br J Dermatol 1967; 79:78-82.
8. Strauss, J.S., Pochi, P.E.: Effect of orally administered antibacterial agents on titratable acidity of human sebum.

J Invest Dermatol 1967; 47:577-81.
9. Kraus, St.J.: Reduction in skin surface free fatty acids with topical tetracycline.
J Invest Dermatol 1968; 51:431-34.
10. Faget, H., Landes, E.: Untersuchungen über die Wirkung der Tetracycline bei der Acne vulgaris.
Hautarzt 1968; 19:469-72.
11. Juhlin, L., Lidén, S.: A quantitative evaluation of the effect of oxytetracycline and doxycycline in acne vulgaris.
Br J Dermatol 1969; 81:154-58.
12. Bordácová, J., Výmola, F.: Die Empfindlichkeit von Corynebacterium Acnes gegen Antibiotica.
Arzneim Forsch 1969; 19:386-88.
13. Wereide, K.: Long-term treatment of rosacea with oral tetracycline.
Acta Derm Venereol (Stockh) 1969; 49:176-79.
14. Schalla, W., Schaefer, H.: unpublished results.
15. Ramasastry, P. et al.: Chemical composition of human surface lipids from birth to puberty.
J Invest Dermatol 1970; 54:139-44.
16. Hägele, W., Schaefer, H., Stüttgen, G.: Über die Bedeutung der Triglycerid-Spaltung durch Corynebacterium acnes für die Acne vulgaris.
Arch Dermatol Forsch 1973; 246:328-34.
17. Ray, L.F., Kellum, R.E.: Corynebacterium acnes from human skin. Identification by morphologic, cultural, biochemical, serological, and chromatographic methods.
Arch Dermatol 1970; 101:36-40.
18. Artuc, M.,
Thesis, Techn University Berlin, 1976.
19. Anderson, R.L., Cook, C.H., Smith, D.E.: The effect of oral and topical tetracyclines on acne severity and on surface lipid composition.
J Invest Dermatol 1976; 66:172-77.
20. Puhvel, M.S., Sakamoto, M.: A re-evaluation of fatty acids as inflammatory agents in acne.
J Invest Dermatol 1977; 68:93-97.
21. Weeks, J.D. et al.: The inability of a bacterial lipase inhibitor to control acne vulgaris.
J Invest Dermatol 1977; 69:236-43.

DISCUSSION

Cunliffe, Leeds: Dr. Schaefer, when I read the paper you presented today in the German literature a few years ago, we tried to repeat your experiments and technically we were not quite as successful as yourself in producing the reactions in the skin. Overall, however, our results were similar in that we could produce an inflammatory reaction in the complete absence of fat. We have not yet published these results and I do not know if we will, because in our control data there were quite a lot of positive results. This worried us, and I am interested to see that in your control studies you had no inflammation. Perhaps you will comment on that? The other point is that there was the rather considerable variation between subjects in the way that they responded.

Schaefer, Berlin: We did use the three different preparations. We did examine biopsy specimens but we did not mention it in the paper. These were typical follicular inflammatory lesions but not acne lesions. You cannot produce acne lesions within 24 hours. Concerning the controls, we applied control media in every patient. Maybe we had a somewhat stronger solution than you had but nevertheless we never saw any positive reaction in the controls.

Juhlin, Uppsala: I would like to return to Dr. Schaefer's studies and ask him what do the patch tests look like? You mentioned that they were perifollicular. Are they urticarial or are they more like delayed reactions? Could it be histamine since the reaction was not destroyed by boiling the material before injection?

Schaefer, Berlin: We had the impression that it is something like histamine since it was almost stable against boiling and because it was more of an immediate type follicular inflammation which disappeared within 2—3 days. It was not a stable lesion.

Cunliffe, Leeds: Dr. Schaefer, did you do any sephadex separations of these various fractions to see if any particular fraction of *P. acnes* and its products were more inflammatory than any other fraction?

Schaefer, Berlin: We did thin layer chromatography and we observed that there were no free fatty acids.

Cunliffe, Leeds: I am sorry, that is not what I meant. Once you put the preparation on the skin and found out that it could produce an inflammatory reaction, were you then able to seprate that fraction into higher and lower molecular weight material to determine what caused the inflammation?

Schaefer, Berlin: I have not. Brain-Heart media is a very complex medium, that would be exceedingly difficult to fractionate. We intended to try a more simple medium, but as you know *P. acnes* is a very sensitive strain to media changes. We did not succeed in growing it on other media.