THE ROLE OF BACTERIA IN THE FORMATION OF FREE FATTY ACIDS ON THE HUMAN SKIN SURFACE*

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Nicolaides and Foster (1) and Nicolaides and Wells (2) presented data supporting the view that free fatty acids on the skin surface are secondary split products of glycerides. The evidence supporting this view is as follows: 1. incubation of human skin tissue with radioactive acetate yields radioactive glycerides but no radioactive free fatty acid (3, 4); 2. diglycerides and monoglycerides were isolated and identified in human skin surface fats (1)—these compounds are in all probability partial hydrolytic products of triglycerides; 3. sebum which is not emptied through ducts and follicles onto the surface, as present in steatocystomas, does not contain free fatty acids (2); 4. tripalmitin is lipolized on the human skin surface (2); 5. with histochemical methods the presence of a non-specific esterase can be demonstrated at the mouth of sebaceous ducts (2).

On the basis of these findings one may assume that lipolysis of sebum starts after the sebaceous cells have fallen apart and the amorphous lipid mass has reached the duct. However, the question arises whether tissue lipases of cellular origin alone are responsible for the lipolysis or whether bacteria which are present in the hair follicle and on the skin surface also contribute to the splitting of fat. It was observed that if human hair is stored the free fatty acid content of hair fat increases and that of glycerides decreases with the duration of storage (6). This finding can be best explained with bacterial activity because if one stores hair in a fat solvent which does not permit bacterial proliferation the free fatty acid content of hair fat does not increase with storage. The data of Herrmann *et al.* on seasonal and regional differences in free fatty acid content of the surface (5) indirectly supports the assumption that, at least in part, the free fatty acids on the skin surface are produced by bacterial activity.

In the present work we endeavored to assess the significance of bacteria in the lipolytic activity of the skin surface.

MATERIALS AND METHODS

Healthy adult white males, 25 to 38 years old, volunteered for these experiments.

Carboxyl-C^{14}-labelled tripalmitin was dissolved in petroleum ether, and spread from a dropper over a trapezoidal field of the back from both scapular areas converging down to the first lumbar vertebra. The total radioactivity of the material deposited on the skin varied from 70,000 to 390,000 counts. After evaporation of the solvent the back was covered with a clean towel and held in place with adhesive tape strips.

Twenty-four hours later the towel was removed and the back was wiped with fat-free ether-soaked cotton swabs. After wiping the skin of the back it was monitored with a Geiger counter for residual activity. The count was two to three times background which was low enough to indicate quantitative removal. Monitor count after the subject had a shower was of background intensity. The ether solution was extracted in a Soxleth extractor. The extracted fat was weighed. Its total amount varied from 33 to 95 milligrams. The radioactivity of a weighed aliquot of the total fat was counted. Another weighed aliquot was dissolved in petroleum ether and adsorbed on a Grade 5 alumina chromatographic column to separate the liberated free fatty acids from the rest of the material. After elution of other fatty materials with petroleum ether, ether and methanol, the free fatty acids were eluted with 20% hydrochloric acid in methanol. The free fatty acid fraction was extracted with petroleum ether in a separating funnel. After evaporation of the solvent the total free fatty acids were weighed and radioactivity of aliquots was counted.

The radioactive tripalmitin underwent some

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hydrolysis after being on the skin surface for 24 hours. The percentage hydrolysis was expressed as the ratio of activity of the free fatty acid fraction to the activity of total fat.

Sterilization of the skin on the back was done on the same individuals one week after the first experiment. The back was sprayed with an achromycin spray which was left on for 24 hours. Subsequently, the whole procedure of the first experiment was repeated with application of radioactive tripalmitin and wiping 24 hours later. In the experiments on sterile skin, however, the protective sterile towel was sprayed with achromycin.

In some of the experiments on non-sterilized skin the back was cleansed with ether prior to the application of radioactive material.

Prior to the tripalmitin application material was taken with swabs from the skin of the back, and hairs were removed from the back for bacteriological cultures in all experiments on non-sterile and sterilized skin. Material was planted on blood agar plates and into thioglycollate broth for anaerobic cultures. Number of colonies on the blood agar plates was counted after 48 hours incubation. Turbidity of the broth cultures was estimated visually 48 hours after incubation and graded from 0 to ++++.

RESULTS

Ten experiments were carried out on eight persons. In experiment number seven sterilization was also carried out with Neomycin spray. Seven days later the experiment was repeated on the same person after Achromycin sterilization (experiment number 8). Experiment 6 was not completed and only the bacteriological data are given.

Table I shows that the method used for sterilization was highly successful in degerming the skin and hairs. In eight out of ten experiments no bacterial growth was obtained from skin swabs and from hair 24 hours after the application of the antibiotic spray either on blood agar or on thioglycollate broth. The two experiments in which the sterilization was incomplete were done on the same person at different times. It is possible that the resident flora of this person's skin contained Achromycin-resistant strains.

The radioactivity data are presented in Table II. There we have listed counts per minute in the total fat and in the free fatty acid fractions as obtained from non-sterile and from sterilized skin in the same individual. The degree of lipolysis was calculated for each experiment in percentages, and the ratio of hydrolysis by sterilized skin to hydrolysis by non-sterile skin was recorded.

In all experiments the hydrolysis was found to be less on the sterilized than on the non-sterilized skin surface. However, the effect of

TABLE I

Effect of locally applied antibiotics on the surface flora

No. of Exp.	Subject	Age	Non-Sterile				Sterile (Achromycin)			
			Blood agar skin plate		Thioglycollate broth[3]		Blood agar skin plate		Thioglycollate broth[3]	
			Swab[1]	Hairs[2]	Swab	Hair	Swab[1]	Hair[2]	Swab	Hair
1	A. G.	30	7	3/15	++++	++++	0	0/11	0	0
2	R. R.	29	28	28/8	++++	++++	2	0/17	0	0
3	R. P.	30	5	2/5	++++	+	0	0/7	0	0
4	B. P.	36	442+	29/6	+++	+++	0	0/8	0	0
5	E. B.	30	313	15/16	++++	++++	0	0/20	0	0
6	J. L.	38	400+	185/18	++++	++++	7	2/19	+	+
7	K. H.	26	36	16/14	++	0	0*	0/17	0	0
8	K. H.	26					0	0/18	0	0
9	I. C.	25	7	0/10	+++	+++	1	0/27	0	0
10	J. L.	38	166	203/18	++++	++++	4	16/24	++	+++

[1] Number of colonies in forty-eight hours.

[2] Number of colonies/number of hairs.

[3] Degree of turbidity after forty-eight hours.

* Neomycin.

TABLE II

Lipolytic activity of the skin surface before and after sterilization

No. of Exp.	Subject	Age	Non-Sterile			Sterile (Achromycin)			
			Total fat[1]	FFA[2]	% hydrolysis	Total fat[1]	FFA[2]	% hydrolysis	Ratio[3]
1	A. G.	30	117,000	11,100	9.5	192,000	6,850	3.5	0.37
2	R. R.	29	166,000	9,700	5.8	217,000	4,590	2.1	0.36
3	R. P.	30	72,100	7,020	9.7	202,000	12,700	6.3	0.65
4	B. P.	36	164,000	13,000	7.9	241,000	7,170	3.0	0.38
5	E. B.	30	219,000	3,040	1.4	104,000	1,010	1	0.7
6	J. L.	38	experiment not completed						
7	K. H.	26	155,000	5,540	3.6	269,000*	5,370	2.0	0.56
8	K. H.	26				389,000	10,800	2.8	0.78
9	I. C.	25	144,000	38,000	25.6	104,000	16,800	16.2	0.63
10	J. L.	38	116,000	13,800	11.9	113,000	5,600	5.0	0.42

[1] Average counts per minute.
[2] Free Fatty Acid average count per minute.
[3] Ratio of % hydrolysis antibiotic treated to non-sterile.
* Neomycin.

sterilization in suppressing the fat-splitting ability of the skin surface varied greatly in intensity. In three experiments (numbers 1, 3 and 4) sterilization suppressed lipolysis by about two thirds, so that only one third of the original activity remained. In two experiments (numbers 7 and 10) about one half of the fat-splitting potency remained after sterilization. In the remaining four experiments (numbers 3, 5, 8 and 9) only one third to one fourth of the original fat-splitting ability was lost by sterilization.

COMMENT

The fact that sterilization of the skin surface suppresses its lipolytic activity makes it probable that surface bacteria participate in the formation of free fatty acids of the surface. Considering the fungistatic and bacteriostatic effects of some of these free fatty acids one may regard bacterial origin of free fatty acids as a new example of ecological equilibrium in the bacterial flora: some bacterial species produce substances which keep other (bacterial and fungal) species in check.

On the other hand, the results presented clearly indicate that sterilization of the skin surface does not completely abolish its lipolytic activity. Whether this non-bacterial lipolysis is due to lipolytic tissue esterases of cellular origin in the mouth of sebaceous glands or elsewhere or whether it is due to microorganisms which do not grow on culture media for bacteria, e.g., *Demodex folliculorum* or *Pityrosporon* is to be investigated.

The experiments indicate the possibility that individual differences in the bacterial flora of the skin surface substantially influence the chemical composition of this surface. Such differences may play a role in susceptibility and resistance to infections and also in such constitutional anomalies as seborrheic dermatitis.

SUMMARY

1. Human skin surface and hair can be rather easily sterilized by local application of antibiotic preparations.

2. The lipolytic activity of the skin surface is diminished after sterilization of the skin.

3. It is concluded that bacteria participate in the production of free fatty acids on the skin surface.

4. In addition to bacterial activity, the presence of lipolytic esterases of cellular origin is assumed because by sterilization of the surface its lipolytic activity is never completely abolished.

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CORRECTION

Brian Potter, M.D., author of "Histochemical Demonstration of Free Amino Groups in Cutaneous Proteins" (J. Invest. Dermat., 33: 245, 1959), reports that the statement "the granular cell myoblastoma is stained strongly by the ninhydrin-Shiff technic" is in error. Further work has shown that the granuloma cell myoblastoma does not stain differentially. The author wishes to report further that false positive reactions of this sort have been seen only in counterstained preparations. The histochemical validity of the method is not affected.