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Autoradiographic Investigation on Benzoyl Peroxide Treated Skin

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Abstract. In vitro autoradiography was used to study the effect of a 5% benzoyl peroxide preparation, or its gel base alone. After three weeks' local application, there was no change in the labelling index of epidermis or follicular infundibulum, while there was a significant reduction in the labelling index of the sebaceous glands. Treatment with the base alone brought about no change.

Key words: Benzoyl peroxide; In vitro autoradiography; Sebostatic effect; Acne

In recent years, preparations containing benzoyl peroxide (BP) have been increasingly used in the treatment of acne (2, 6, 7, 11). Clinically, inflammatory lesions show a rapid response, while long-standing comedos respond slowly.

Current opinions on the mode of action of BP have reflected the finding that after 14 days' treatment with a 10% preparation, there is a decrease in the microbial flora similar to that found with systemic tetracycline administration (3), and also the demonstration of a significant fall in the percentage content of free fatty acids in the skin surface lipids, these serving as a parameter of the presence of *Propionibacterium acnes*. These observations have led to the view that BP works principally through a bacteriostatic action similar to that of many other peroxides (9), perhaps based on the

conversion of bacterial amino acids to pantothenic acid.

Treatment with BP, however, is also followed by significant desquamation, and a diminution in the oily appearance of affected skin. It was believed that the benzoic acid produced by benzoyl peroxide in contact with organic tissues might have a keratolytic action similar to that of a salicylic acid, leading to a loosening of the epidermis, thus giving the skin its "drier" appearance (3).

The present study was intended to clarify this mechanism further, using autoradiographic investigations of skin treated with BP.

MATERIAL AND METHODS

A commercially obtainable 5% BP preparation (PanOxyl 5, Stiefel Laboratories, Offenbach/Main), or its base alone, was used.

The gel base was composed of colloidal magnesium aluminium silicate, hydroxypropyl methylcellulose, polyoxyethylene lauryl ether, citric acid, absolute alcohol and distilled water.

Nine male subjects were studied (8 test subjects and 1 control); their mean age was 30 years. Before and after a 3 weeks' course of treatment with the 5% BP preparation, or the base alone, punch biopsies were taken under local anaesthesia from the skin of the side of the cheek, in an area free from inflammatory changes.

Biopsy material was incubated for one hour at 37°C in Trowell's T8 tissue culture medium containing 20 μ Ci/ml H³[thymidine] (Radiochemical Centre, Amersham), fixed for 2 hours in 3.5% glutaraldehyde and post-fixed with osmium tetroxide (Palade). After dehydration in ethanol, it was embedded in Epon 812. Semi-thin sections were prepared using the Reichert OMU 2 Ultramicrotome, laid on slides and covered with Kodak NTB 2-Emulsion (dilution 1:1.5). After 4 weeks' exposure they were developed in Kodak 19B, fixed with Fixon Ultra Rapid and stained with Toluidine blue (pH 5).

A count was made of labelled cells in the basal layer of the epidermis, the follicular infundibulum and the sebaceous glands, and divided by the total number of basal cells in the corresponding area. At least 1000 basal cells were counted in each area. The value obtained was denoted the labelling index.

Statistical evaluation

The differences in labelling index of single sections before and after the 3 week course of treatment were estimated. Statistical evaluation was carried out using Student's *t*-test.

RESULTS

The epidermis and follicular infundibulum showed no significant change in labelling index before and after the 3 week treatment. The germinative zone of

Table 1. *Labelling index in skin in treatment with benzoyl peroxide 5 %*

I: before treatment, II: after treatment (3 weeks)

Case no.	Epidermis		Follicles		Sebaceous gland ^a	
	I	II	I	II	I	II
1	15.3	12.5	12.2	9.4	7.1	3.7
2	8.9	10.6	11.1	8.2	9.4	5.7
3	16.3	16.7	10.6	10.0	7.2	5.9
4	9.2	10.7	6.6	5.3	7.5	5.5
5	9.6	7.1	7.4	6.4	11.6	6.5
6	6.8	6.9	6.6	8.5	9.9	6.2
7	9.0	13.3	6.8	8.2	12.1	6.6
8	9.2	6.7	4.7	7.0	8.1	4.3
Sum	84.3	84.5	66.0	63.0	72.9	44.4
Average	10.54	10.5	8.25	7.9	9.11	5.55
Control	11.83	13.35	5.55	6.67	8.9	9.22

	^a Before:	After:
$\bar{x}$	9.11	5.55
	72.9	44.4
n_2	4.3	1.9
Range	7.1–12.1	3.7–6.6
	$p < 0.001$	

the sebaceous glands, however, showed a significant fall in proliferative activity ($p < 0.001$), and this reached 45 % in some cases (Table I). The control subject, treated with base alone, showed no reduction in labelling index in these sebaceous glands (pretreatment value 8.9 % post-treatment 9.12 %).

DISCUSSION

Seborrhoea is an essential part of the pathogenesis of acne; it arises from a genetically determined increased sensitivity of the sebaceous glands to the androgenic stimulation arising at puberty. All severe cases of acne show seborrhoea (1, 11, 13); the degree of severity of the acne is proportional to the size of the sebaceous glands and the proliferative activity of the germinative cells (9).

Studies on the mode of action of Vitamin-A acid, which has for several years been found to be extremely effective in the treatment of acne, have shown that it exerts its therapeutic effect on the comedo. In the epidermis, it stimulates mitotic activity, inhibits keratin production, and leads to parakeratosis and thinning of the epidermis (5); it also stimulates proliferation of keratinocytes in the epithelium of the follicular infundibulum, thus preventing the formation of coherent horny lamellae—the raw material of the comedo. No effect on proliferative activity in the sebaceous glands has been observed.

The present study demonstrates that BP has a totally different mode of action.

As reported earlier (8) the autoradiographic studies showed no change in the labelling index of the epidermis or follicular infundibulum; any effect on existing comedos must therefore be secondary, due to the keratolytic effect of benzoic acid. There was, however, a significant reduction in the labelling index of the germinative zone of the sebaceous glands after 3 weeks' treatment with the 5 % BP preparation. This finding is in agreement with the clinical observations of defatting of the skin. The therapeutic efficacy of BP in acne vulgaris can therefore be attributed not only to its bacteriostatic effect but also to sebostatic qualities.

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An Ultrastructural Study of the Epidermis in Hyperkeratosis Lenticularis Perstans

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Abstract. A 60-year-old Japanese man contracted hyperkeratosis lenticularis perstans (Flegel). Biopsy specimens of the keratotic papules on the dorsa of the feet were studied with electron microscopy. An increase in the number of membrane coating granules, keratohyalin granules and tonofilaments was the most prominent feature of the keratinocytes underlying the keratotic papule. However, in the area just above the cone-shaped dermal infiltration, membrane coating granules were absent and keratohyalin granules and tonofilaments were poorly developed.

Key words: Electron microscopy; Membrane coating granules; Keratohyalin granules

Hyperkeratosis lenticularis perstans (HLP) is a rare dermatosis characterized by inflammatory keratotic papules involving the extremities. Since the first

report by Flegel (3), several reports have confirmed HLP as a distinct entity. In a previous paper (8), we described the ultrastructure of the infiltrating cells of HLP in which were present intracytoplasmic inclusions.

In this report, we present electron microscopical findings of the keratinocytes in HLP.

MATERIAL AND METHODS

A 60-year-old Japanese man was seen primarily because of increasing numbers of keratotic papules (Fig. 1) on the legs and forearms of 2 years' duration. The eruptions first appeared over the anterior aspects of the lower legs and dorsa of the feet.

Biopsy specimens were obtained from well-developed keratotic papules on the dorsa of the feet. All tissue was cut into two. One-half was processed for routine microscopy and sections were stained with hematoxylin-eosin. The other half was divided into small pieces for electron microscopy. They were fixed in cold 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 2 hours, rinsed overnight in several changes of the same buffer containing 10% sucrose at 4°C, post-fixed in cold 1% osmium tetroxide for 2 hours, dehydrated in graded



Fig. 1. Typical lesions of HLP on the dorsum of the foot.