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KERATIN METABOLISM IN THE EPIDERMIS AND HAIR OF MICE WITH EXPERIMENTAL DIABETES

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The study of keratinization of the epidermis in diabetes is of great interest, because skin infections and nonspecific pruritis frequently occur in this disease, and the turgor of the skin is reduced. The study of the metabolism of epidermal keratin, a basic protein with a barrier function and also, possibly, facilitating the development of pathological changes in the skin, and also the study of the keratin metabolism of the hair may aid both research in the specific treatment of diabetes and the creation of a noninvasive test for its diagnosis.

In this investigation we studied the rate of biosynthesis and breakdown of prekeratin and keratin with the aid of ^{14}C-glycine. To characterize the process of keratinization in the epidermis, the content of –SH and –S–S– groups in prekeratin also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male mice in which diabetes was induced by a single intraperitoneal injection of streptozotocin, in a dose of 180 mg/kg body weight. Diabetes developed on the 2nd or 3rd day. The blood glucose level varied between 300 and 450 mg%. The animals were kept in this state for 1 month. The rate of formation and breakdown of keratin and prekeratin was estimated on the basis of incorporation of ^{14}C-glycine into

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TABLE 1. Content of −S−S− and −SH Groups in Prekeratin of Epidermis of Healthy and Diabetic Mice in μmoles/mg protein (M ± m)

Animals	SS+SH	SH	SS	SS/S
	In μmoles/mg protein (M ± m)			
Healthy	1,33±0,09	0,18±0,01	1,15±0,06	6,3
Diabetic	0,69±0,22	0,01±0,01	0,68±0,02	68,0 $p<0,001$

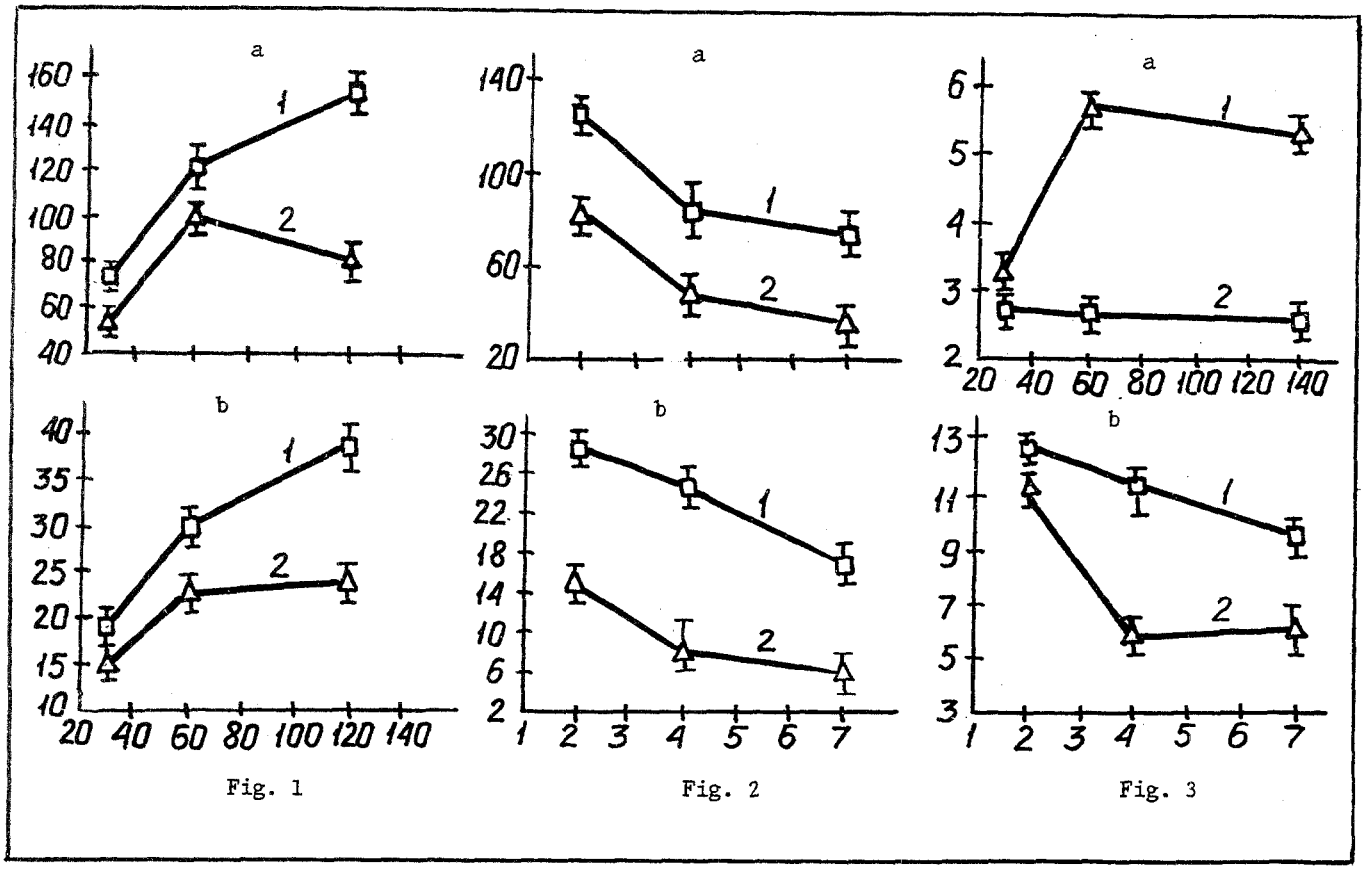

Fig. 1. Rate of biosynthesis of epidermal prekeratin (a) and keratin (b) in normal (1) and diabetic mice (2) as shown by incorporation of ^{14}C-glycine. Abscissa, time (in min); ordinate, specific activity (in cpm/mg·10^3).

Fig. 2. Rate of breakdown of epidermal prekeratin (a) and keratin (b) in normal (1) and diabetic mice (2) as shown by release of ^{14}C-glycine. Abscissa, time (in h); ordinate, specific activity (in cpm/mg·10^3).

Fig. 3. Rate of synthesis (a) and breakdown (b) of keratin in hair of normal (1) and diabetic mice (2) as shown by incorporation and release of ^{14}C-glycine. Abscissa, time: a (in min), b (in h); ordinate, specific activity (in cpm/mg·10^3).

these fractions at different time intervals after intraperitoneal injection of the labeled amino acid (2.5 μCi/g body weight) into normal and diabetic mice. The keratin fractions were extracted as in [1, 2]. After sacrifice of the animals, skin removed from the dorsal region was washed in physiological saline, incubated for 30 sec at 60°C, cooled on ice, and depilated, after which the epidermis was stripped off. The epidermis was homogenized in a solution of 25 mM Tris-HCl (pH 7.5), containing 1.5 mM KCl, 0.5% Triton X-100, 5 mM EDTA, and 1 mM EGTA. The homogenate was centrifuged for 15 min at 10,000*g* and the residue washed twice with the same solution, not containing Triton. The prekeratin fraction was extracted for 20 h at 4°C with 0.1 M sodium citrate solution (pH 2.6). The keratin

fraction was extracted for 20 h at 4°C by 9.5 M urea solution containing 25 mM Tris-HCl (pH 8.0). The method in [3] was used to determine −S−S− and −SH groups. To reduce −S−S− bonds a solution of prekeratin (50 μg/ml) was incubated for 10 min at room temperature with 0.1 mM dithiothreitol, and then dialyzed for 24 h against 0.01 M Tris-HCl solution (pH 8.0) containing 0.3 M KCl and 5 mM EDTA. To determine the content of −SH groups, prekeratin (50 μg/ml) and 50 μM M 5,5-dithio-bis-(2-nitrobenzoic acid) were added to the solution, after which optical density was measured at 412 nm. Dithiothreitol was used as the standard solution for calibration.

EXPERIMENTAL RESULTS

The experiments showed that the velocity of biosynthesis of prekeratin and keratin in the epidermis of diabetic animals was higher than in healthy animals, for incorporation of the labeled amino acid in both prekeratin and keratin fractions reached a maximum in the diabetic animals after 60 min, whereas in healthy mice incorporation of ^{14}C-glycine continued to rise (Fig. 1). The breakdown of prekeratin and keratin in the diabetic animals was similar in course to that of the healthy mice, as shown by the similar course of the curves of release of the label (Fig. 2). The study of keratinization in the epidermis by quantitative determination of −SH and −S−S− groups in prekeratin showed that the ratio of SS/SH groups rose in the prekeratin fraction of the diabetic mice by an order of magnitude (Table 1).

These results indicate that even in the stage of prekeratin formation more rigid structures than normally are formed in the epidermis.

The rate of formation of keratin of the hair, as shown by incorporation of ^{14}C-glycine, rose sharply above the normal level (Fig. 3). Meanwhile keratin breakdown in the hair took place more slowly (Fig. 3), as shown by the rate of release of the label after injection of ^{14}C-glycine.

The experimental data indicate that synthesis of epidermal keratin is intensified in diabetes and prekeratin with a more rigid structure than normal is formed. In the hair, in diabetes, biosynthesis and breakdown of keratin take place much faster. These results may form the basis of a noninvasive method for the diagnosis of diabetes.

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