HISTIDASE ACTIVITY OF THE SKIN IN RELATION TO THE STATE OF MELANOGENESIS

Kh. Kurbanov, V. A. Burobin, and T. T. Berezov

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The histidase reaction of the skin in relation to the state of melanogenesis was studied in animals (18 rabbits) and patients with vitiligo. The histidase activity and the content of urocanic acid were determined in skin biopsy material in order to study the reaction. The histidase activity of the skin of black rabbits was found to be twice as high as that of white rabbits. In the depigmented areas of the skin of patients with vitiligo the histidase reaction was depressed by comparison with areas of the skin with normal pigmentation in the same patients and with the skin of control persons. The dependence of the histidase reaction of the skin on the state of melanogenesis is dependent, it is suggested, on the inhibitory action of cysteine on the histidase and tyrosinase activity of the skin.
KEY WORDS: skin; melanogenesis; histidase; urocanic acid.

The protective function of the epidermis against light and other forms of radiation is explained by the fact that it contains not only keratins, but also nucleic acids, carotenes, and porphyrins, chiefly melanins [1, 10]. The melanins possess high electron-acceptor activity, ability to undergo reversible oxidation and reduction, and a high concentration of stable free radicals; the latter are responsible for protection against extremal conditions that may be created as a result of disturbance of oxidation and reduction through the action of ionizing radiation [2, 3, 13].

The epidermis of mammals also contains a high concentration of urocanic acid (UA) [16], which increases after irradiation with ultraviolet rays or after exposure to the sun [5, 11]. Its concentration in the surface layers of the skin is controlled by the enzyme histidase, which actively deaminates L-histidine into trans-urocanic acid in the epidermis. UA can absorb a considerable proportion of the ultraviolet radiation falling on the skin surface [6, 14]. Under the influence of ultraviolet radiation trans-UA is converted into the cis-isomer; the trans–cis isomerization of UA, according to some workers, is the most effective mechanism of utilization of the energy of ultraviolet radiation and because of this fact it is an important physiological mechanism for the protection of the deep layers of the skin against the harmful action of irradiation [2, 7, 8].

The object of the present investigation was to study the histidase reaction of the skin in animals with different state of melanogenesis and in patients with a disturbance of melanin synthesis.

EXPERIMENTAL METHOD

The histidase activity and UA concentration in the skin were studied in nine white and nine black rabbits of the same age, weighing 2.45-3.20 kg and kept on the standard laboratory diet. Skin samples (200-300 mg) were taken from the same area (the dorsum) after decapitation. The hair and subcutaneous cellular tissue were removed from the skin.

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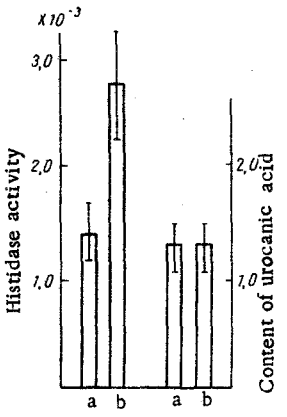

Fig. 1

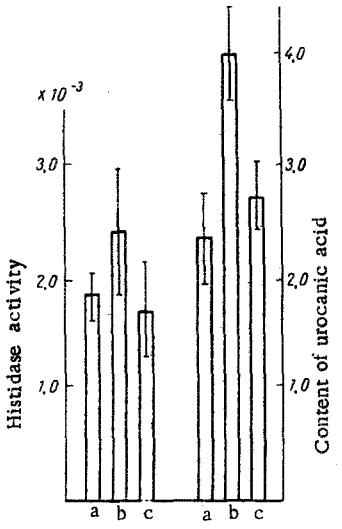

Fig. 2

Fig. 1. Histidase activity and content of urocanic acid in the skin of white (a) and black (b) rabbits. Ordinate: on the left, histidase activity in μmoles UA/mg protein/min; on the right, UA concentration in μmoles/g skin.

Fig. 2. Histidase activity and concentration of urocanic acid in human skin: a) pieces of skin of healthy persons; b) pieces of skin with normal pigmentation in patients with vitiligo; c) pieces of depigmented areas of skin from patients with vitiligo. Remainder of legend as in Fig. 1.

The histidase activity of the skin and the UA concentration also were investigated in biopsy skin samples (100-120 mg) from 18 patients (13 men and five women) with vitiligo, aged 19-53 years. The duration of the disease varied from 2 to 21 years. The affected areas of the skin accounted for 15-20% of the total body surface. As a control the histidase reaction of the skin was investigated in nine healthy persons (seven men and two women) aged 17-49 years. Skin biopsy on the patients and healthy persons was performed on approximately the same areas of skin (chiefly from the abdominal wall and the back). Biopsy of healthy and affected skin of the same patient with vitiligo was performed on symmetrical areas. Histidase activity and the UA concentration were determined by the method of Whitfield and Shepherd [15] in the modification of Mardashev et al. [2].

Pieces of skin (100-150 mg) were minced with scissors and homogenized with powdered glass and distilled water (1:100). The homogenate was centrifuged and the UA concentration determined in the residue. The residue was extracted with 0.05 M Na pyrophosphate buffer, pH 8.2 (1:50). The incubation mixture contained 1 ml skin extract, 0.1 ml 0.05 M reduced glutathione, pH 8.2, 0.2 ml 0.1 M L-histidine, pH 8.2, 4 mg albumin, and water to 3 ml. The samples were incubated for 2 h at 37°C; the reaction was stopped by the addition of 1 ml 20% TCA, the samples centrifuged, and the optical density of the experimental samples was measured relative to the controls (without histidine) on a type SF-4 spectrophotometer at 267 nm.

The UA content in the samples was calculated from a calibration curve. The histidase activity was expressed in micromoles UA formed during incubation for 1 min per mg extractable protein determined by Lowry's method. The concentration of UA in the skin was determined from the distance between the extinctions of the residue of an acidified (2 N HCl) aqueous extract of skin before and after reduction of the UA with powdered metallic zinc for 60 min at 37°C into imidazolylpropionic acid at 267 nm. The quantity of UA was expressed in micromoles per gram of skin.

EXPERIMENTAL RESULTS AND DISCUSSION

Anglin et al. [6], who investigated the histidase activity in the skin of 30 guinea pigs, found a mean value of $6.03 \cdot 10^{-3}$ unit. Other workers [7] found that the histidase activity in the guinea pig epidermis is the same as in man and in noninbred mice. The present experiments showed that the histidase activity in

rabbit skin varies from $(1.37 \pm 0.24) \cdot 10^{-3}$ to $(2.63 \pm 0.47) \cdot 10^{-3}$ unit, whereas in human skin it is $(1.84 \pm 0.2) \cdot 10^{-3}$ unit. The histidase activity in the skin of black rabbits was almost twice as high as in white rabbits. Meanwhile, there was no difference between the skin of black and white rabbits as regards the content of UA (Fig. 1).

In the patients with vitiligo the histidase activity in the depigmented areas of the skin was lower than in the areas of their normal skin and in the skin of healthy subjects (Fig. 2). A much higher histidase activity was observed in the unaffected areas of skin of the patients with vitiligo. This correlated with the UA concentration. Considering that a marked increase in pigmentation is found in the unaffected areas of skin, especially at the borders of the depigmented areas, a general correlation was noted between the degree of pigmentation and the level of histidase activity of the skin.

The degree of pigmentation of the skin is linked with the level of sulfhydryl (SH) groups in the skin. Glutathione reductase activity and the concentration of reduced glutathione in white skin are known to be higher than in black [12]. The content of SH groups in skin affected with vitiligo is greater than in skin with normal pigmentation [9]. The histidase activity of the skin is also linked with the content of compounds containing SH groups. Reduced glutathione increases the histidase activity in the skin [6, 15]; its activity was highest when added to the incubation medium at the rate of 4.1 mmole to 10-20 mg skin tissue. The content of reduced glutathione in normal skin was 1.30-1.99 nmole/mg fresh skin tissue, much lower than the level of reduced glutathione required to activate the skin histidase. Meanwhile, other sources of SH groups than glutathione are cysteine and homocysteine. The content of free cysteine in the skin is much higher than that of reduced glutathione, especially in the stratum corneum: 22.3 nmole/mg skin tissue [4]. L-cysteine is known to reduce the histidase activity sharply in the skin and liver [6].

The low histidase activity in the skin in which melanogenesis is depressed is thus evidently connected to some degree with the inhibitory action of SH groups most probably supplied by cysteine. The hypothesis [12] that the increase in pigmentation caused by ultraviolet irradiation or exposure to sunlight is connected with the oxidation of SH groups under the influence of these factors is evidently correct. If this is so, the mechanism of potentiation of the histidase reaction of the skin is similar in nature to the mechanism of pigment formation.

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