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SHORT REPORTS

Dopa and 5-S-Cysteinyldopa in the Serum of Albino, Black, and Red Guinea Pigs

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Abstract. Dopa and 5-S-cysteinyldopa levels in serum of albino, red, and black guinea-pigs were quantified. The levels of both amino acids were lower in the albino than in the pigmented animals. It is suggested that the serum levels of dopa and 5-S-cysteinyldopa in pigmented animals reflect tyrosine and dopa oxidation activity in the melanocytes.

Melanins are difficult to define chemically because of their insolubility. In the guinea pig, two percursors of melanin—dopa and 5-S-cysteinyldopa—can be extracted from pigmented skin and hairs. Black and red skin contain similar amounts of dopa, but 5-S-cysteinyldopa is present in definitely larger quantities in red skin than in black skin (4).

Recently developed methods for the detection of dopa and 5-S-cysteinyldopa make it possible to quantify these amino acids in serum.

The aim of the present study was to investigate if the serum levels of dopa and 5-S-cysteinyldopa were dependent on the colour of the animal.

MATERIAL AND METHODS

Inbred guinea pigs of uniform colour were studied, 6 white, 6 black, and 6 red animals. The animals were killed by a blow on the neck and the blood was collected from the jugular vessels. 3–4 cm^2 skin with hair from each animal was used for analysis. Skin was extracted with 0.4 M perchloric acid and serum proteins were precipitated by addition of 4 M perchloric acid to a final concentration of 0.4 M. Catecholic amino acids were adsorbed onto Al_2O_3 and determination of dopa and 5-S-cysteinyldopa was performed with HPLC and electrochemical detection (2).

RESULTS AND COMMENTS

The quantitites of dopa and 5-S-cysteinyldopa in the skin and hair and in the serum are given in Table 1. White skin contained small amounts of both amino acids, and the serum concentrations were also lower in white than in coloured animals. Black skin contained somewhat more dopa than did red skin, but red skin showed in contrast much higher levels of 5-S-cysteinyldopa. The black animals had higher serum levels of dopa than the red ones. The red animals showed much higher serum levels of 5-S-cysteinyldopa than the black ones.

Since serum concentrations of both cathecholic amino acids were lower in the albino animals than in the pigmented ones it can be concluded that a large amount of circulating dopa in pigmented animals has its origin in the melanocytes.

It is known that injected dopa also accumulates in white hairs (3) and keratin has a tendency to bind aromatic compounds (1). It is therefore conceivable that the small concentrations of catecholic amino acids found in the white skin may be due to the binding of such compounds formed elsewhere in the body. It seems more probable, however, that both dopa and 5-S-cysteinyldopa in the white guinea pigs originate in melanocytes having some tyrosinase activity but not producing pigment.

Table 1. *Dopa and 5-S-cysteinyldopa in unshaved skin and serum*

	Dopa		5-S-cysteinyldopa	
Colour	Skin (ng/g)	Serum (ng/ml)	Skin (ng/g)	Serum (ng/ml)
White				
Mean	12	0.4	1.5	0.4
Range	9–14	0.3–0.4	0.3–2.4	0.4–0.5
Black				
Mean	240	1.9	460	1.5
Range	150–330	1.0–2.9	280–640	1.0–2.0
Red				
Mean	130	0.7	3 600	11
Range	20–230	0.5–0.8	920–6 600	6.5–18

The finding that dopa and 5-S-cysteinyldopa levels in the serum reflect the pigmentation of guinea pigs will certainly facilitate studies on pigment metabolism.

5-S-cysteinyldopa has now for many years been considered a specific melanocyte amino acid. Dopa is formed in many organs where it is a precursor of catecholamines. The present findings on dopa make it likely that the serum levels of this amino acid mainly reflect melanocyte metabolism—at least in pigmented animals.

Since dopa is rapidly metabolized in the body, investigations into dopa metabolites such as dopamine may also prove helpful in the study of pigment metabolism.

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REFERENCES

1. Breuer, M. M.: Binding of aromatic compounds by hair. Nature Phys Sci *229:* 185, 1971.
2. Hansson, C., Edholm, L.-E., Agrup, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: The quantitative determination of 5-S-cysteinyldopa and dopa in normal serum and in serum from patients with malignant melanoma by means of high-pressure liquid chromatography. Clin Chim Acta *88:* 419, 1978.
3. Harrison, W. H., Gray, R. M. & Solomon, L. M.: Incorporation of L-dopa, L-α-methyldopa and DL-isoproterenol into guinea pig hair. Acta Dermatovener (Stockholm) *54:* 259, 1974.
4. Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Determination of 5-S-cysteinyldopa in melanomas with a fluorimetric method. Yale J Biol Med *46:* 516, 1973.

C3b Receptor in Normal Human Oral Mucosa

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Abstract. The presence of a receptor for C3b in normal human oral mucosa was studied using C3b produced by treating normal human serum with cobra venom factor. Cryostat sections of normal human oral mucosa were incubated with C3b, followed by a direct immunofluorescence technique using monospecific goat-antihuman C3. The histologic localization of C3b fluorescence was determined by fixing cryostat sections with glutaraldehyde and staining with hematoxylin and eosin. The endothelial cells of the capillaries and smaller venules in the oral mucosa showed staining by anti-C3. C3b did not bind to the intercellular substance nor to the basement membrane zone of normal human oral mucosa. Normal human serum treated with EDTA was negative, thus indicating that native C3 did not bind to the receptor. The endothelial C3b receptor of the oral mucosa may have an important function in the localization of immune complexes that occur in systemic diseases, as well as in diseases restricted to the oral mucosa.

Key words: C3b receptor; Oral mucosa; Endothelial cell

Direct immunofluorescence of oral lesions is of great diagnostic value in bullous and ulcerative diseases involving the oral cavity (4, 5). Findings of immunoglobulins and complement components have been interpreted as evidence of immunologic pathogenesis mediated either by autoimmune antibodies or by immune complexes (1, 2, 6).

Thyresson et al. (7) reported the presence of an endothelial C3b receptor in normal human skin. This endothelial C3b receptor was located in the capillaries of the papillary dermis and dermis and on the endothelial cells that line the lumina of arterioles and venules. C3b receptors were not found in the intercellular substance or in the basement membrane zone of normal human epidermis.

In an effort to further investigate normal human tissue for the presence of a similar C3b receptor, normal human oral mucosa was examined.

METHODS

Tissue. Normal human oral mucosa was obtained from healthy patients undergoing elective dental surgical procedures. The mucosal specimens were immediately snap-frozen in liquid nitrogen and stored at −70°C until used. Serial cryostat sections 4 μm thick were cut fresh each working day. No air drying or fixatives were used.

Preparation of C3b. Fluid-phase C3b was prepared accordnig to the method of Thyresson et al. (7). Normal human serum was obtained from whole blood of healthy donors and was allowed to clot at room temperature for 60 min, after which the serum was separated by centrifugation, aliquoted in 0.1 ml volumes, and stored at −70°C, until use. All sera had normal levels of total complement and C3. To produce C3b, a mixture of cobra venom factor (1 : 2) (Cordis Laboratory, Miami, FL) and normal human serum (1 : 2) was allowed to react for 30 min at 37°C. The