**Acta physiol. scand. 1970. 78. 65—69**

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# Extraction of Dopa from the Integument of Pigmented Animals

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Received 13 March 1969

**Abstract**

CEGRELL, L., B. FALCK and A.-M. ROSENGREN. *Extraction of Dopa from the integument of pigmented animals.* Acta physiol. scand. 1970. *78.* 65—69.

The occurrence of high amounts of dopa has been demonstrated chemically in the piliary system of pigmented animals (guinea-pig, rabbit, and dog). Histochemically, a formaldehyde-induced fluorescence was recorded only in melanocytes of hair-bulbs. The spectral characterictics of these cells did not agree with those of the authentic dopa fluorophore. The discrepancy between the chemical and histochemical findings is discussed. Little or no dopa was found in non-pigmented skin of albino and multi-coloured guinea-pigs. This indicates that the integumentary dopa is related to the pigment formation.

It has long been well established that 3,4-dihydroxyphenylalanine (dopa) is an intermediary in the biosynthesis of catecholamines and melanin. Significant amounts of dopa, however, have not until recently been detected in mammalian tissues, and it was thought that it is not accumulated but rapidly metabolized. By means of the histofluorescence technique of Falck and Hillarp, a formaldehyde-induced fluorescence was demonstrated in human melanocytes and nevus cells (Falck *et al.* 1965, 1966a). On the basis of present knowledge regarding the synthesis of melanin, on the one hand, and the molecular structure required for a substance to form a fluorophore with formaldehyde, on the other, (Corrodi and Jonsson 1967) this cellular fluorescence was thought to reflect the presence of dopa (Falck *et al.* 1965). Recently, the presence of dopa in experimental melanoma (Takahashi and Fitzpatrick 1966) and in human malignant melanoma cells (Falck *et al.* 1966 a and b) has been demonstrated and dopa has been detected chemically in pooled samples of a great number of pigmented nevi (Cegrell, Falck, Jacobsson and Rosengren, unpublished results). Moreover, Bernheimer (1963) found dopa in cattle eyes, Ehinger and Rosengren (1967) could establish the presence of dopa in the ciliary epithelium and retinal pigment cells of albinotic embryos of some mammals and Cegrell *et al.* (1967) found dopa in skin of young pigmented mice. To obtain further evidence of a storage of

TABLE I. Dopa content in hair and shaved skin in different species. The figures express M±SEM or values of single determinations.

| Species | Extraction agent | Number of determinations | Dopa μg/g wet weight | |
|---|---|---|---|---|
| | | | Hair | Shaved skin |
| Pigmented guinea-pigs | boiling water | 7 | 2.1±0.44 | 0.29±0.10 |
| | perchloric acid | 7 | 0.90±0.16 | 0.10±0.007 |
| Albino guinea-pigs | boiling water | 2 | 0.09 and 0.14 | 0.03 and 0.03 |
| Multi-coloured guinea-pigs: | | | | |
| white parts | boiling water | 2 | 0.14 and 0.22 | 0.00 and 0.02 |
| coloured parts | boiling water | 2 | 3.4 and 5.3 | 0.27 |
| Pigmented guinea-pigs | ethanol | 2 | 0.55 and 0.97 | — |
| Pigmented young rabbits | boiling water | 4 | 8.5; 12; 23; 24 | 1.9 and 3.8 |
| | perchloric acid | 2 | 2.4 and 5.7 | 0.76 and 0.86 |
| Pigmented adult rabbits | boiling water | 1 | 1.3 | 0.05 |
| | perchloric acid | 1 | 0.46 | 0.02 |
| Pigmented dogs | perchloric acid | 3 | 4.6; 4.7; 5.6 | 0.63 and 1.0 |

dopa in the mammalian skin, the present study was undertaken. It disclosed that considerable amounts of dopa can be found in the integument of some species, but that high quantities were present in a quite unexpected location, *i.e.* the hairs.

## Material and methods

The material is composed of 13 young guinea-pigs, 4 young and 1 adult pigmented rabbits (grey and dark) and 3 young black dogs (4 weeks of age).

Two guinea-pigs had both multi-coloured and non-pigmented skin areas (below called multi-coloured animals), 9 were fully pigmented and 2 were albinos. They were killed by decapitation, and the hair of most of the body integument was removed with electric clippers. The hair from each of 7 pigmented animals was divided into two approximately equal samples as was the clipped skin (in the following called "shaved skin"). One sample of each tissue was extracted with boiling water for 5 min, and the other was extracted with perchloric acid. Four samples consisting a coloured and non-coloured hair and shaved skin, respectively, were collected from each of the multi-coloured animals. These samples were extracted with boiling water for five min. In two albino guinea-pigs, the body integument was divided into hair and shaved skin and extracted with boiling water. Finally, extraction with ethanol was performed on the hair from two pigmented animals.

The rabbits were killed by an intra-venous injection of air; the dogs, by bleeding in pentobarbital anesthesia. Samples of hair and shaved skin were obtained from suitable skin areas. The extraction was performed with either boiling water (5 min) or perchloric acid (see Table I).

The catechol derivatives were fluorimetrically determined according to Anton and Sayre (1964) and in some cases according to Ehrlén (1948). For further identification of the catechol derivatives, the $Al_2O_3$ eluates (guinea-pig) were chromatographed in three different systems: butanol-benzene-methanol-$H_2O$ (4 : 4 : 4 : 1), butanol-N HCl, and phenol-0.1 N HCl (9 : 1) (*cf.* Bertler *et al.* 1958). The catechol derivatives were visualized by spraying the papers with potassium ferrocyanide or by exposing the papers to dry formaldehyde gas (generated from paraformaldehyde) at 80° C for 1 hr.

Two samples of pigmented hair from two young rabbits were boiled in water (5 min) and the extracts obtained were incubated at 37° C for 30 min together with pyridoxal-5-phosphate

and an extract of rabbit kidney cortex (see Bertler and Rosengren 1959). The amount of dopamine formed under these conditions was determined according to Bertler *et al.* (1958).

At least two small tissue specimens from the shaved skin of each animal were taken for histochemical analyses according to the fluorescence method of Falck and Hillarp (for technical details see Falck and Owman 1965). The microspectrofluorimetric analyses were carried out with a modified Leitz' microspectrograph as reported by Björklund *et al.* (1968).

## Results and comments

The results of the chemical determinations according to the method of Anton and Sayre (1964) are summarized in Table I. Both the hair and the shaved skin from the pigmented animals contain significant amounts of a substance that, irrespective of the extraction procedure, behaved like dopa in these fluorimetric analyses. It also appeared that those samples of guinea-pig hair treated by the trihydroxyindole method of Ehrlén (1948) yielded a fluorescence typical for dopa and contained quantities of dopa that agreed with those found with the periodate method. Moreover, on the paper chromatograms a spot could be visualized with either $K_3Fe(CN)_6$ or exposure to formaldehyde gas. In the three systems used, this spot displayed the same Rf values, colour, and fluorescence as the spot of authentic dopa which was used in all the experiments as reference substance. Finally, in the two decarboxylation experiments with rabbit hair, a formation of dopamine occurred. The dopamine found after decarboxylation was of the same order of magnitude as that obtained in model experiments in which dopa was decarboxylated under as identical conditions as possible. The recovery in these studies is about 10—15 % and the amount of dopamine formed was 1.6 and 0.85 $\mu$g/g, which corresponded well to the amount of dopa in hair, 12 and 8.5 $\mu$g/g, respectively. Thus, it seems well established that dopa is present in the pilary system of pigmented animals.

The mild extraction procedures applied in this study can hardly release dopa from dopa-containing proteins, and with respect to melanin, it seems quite excluded that a hydrolysis can occur. It is thus reasonable to suppose that the demonstrated dopa exists in free form. Fitzpatrick (1965) found dopa in melanosomes, but only after hydrolysis of trichloracetic precipitates of the organelles with strong hydrochloric acid. He concluded that this dopa was present within the protein molecule.

It is evident from Table I that extraction with boiling water for 5 min gives a much higher yield of dopa than that obtained by perchloric acid, which is otherwise a more favourable agent for the extraction of dopa; thus, for example, more dopa can be extracted from an islet cell tumour of golden hamster with this acid than with boiling water (Cegrell, Falck and Rosengren, unpublished observations). The difference between perchloric acid and water at 100° C as extracting agents can possibly be explained by the fact that in the present case dopa had to be extracted from a keratinized tissue. Ethanol proved also to be an unfavourable extraction agent, which could be confirmed in recovery experiments on other tissues as well (dopa added to brain tissue).

The concentrations of dopa found in the pigmented hair are high; especially high values were recorded in young rabbits. In the shaved pigmented skin the values were

considerably lower. However, this obviously does not necessarily imply that the concentration of dopa is lower in the piliary system below the epidermal surface. In the albino guinea-pigs and in the non-pigmented integumental parts of the multicoloured guinea-pigs, comparatively slight amounts of dopa were found. This strongly indicates that the integumentary dopa has a relation to pigment formation.

In the fluorescence microscope, the hair showed an intense autofluorescence and the possible presence of any specific, *i.e.* formaldehyde-induced fluorescence could not be evaluated. In agreement with previous results from studies on guinea-pig skin (Olivecrona and Rorsman 1966) the hair matrix in the pigmented skin of the guinea-pigs and rabbits was found to contain melanocytes which displayed a specific granular green-yellow fluorescence. The number of fluorescent cells in the hair bulbs showed great intra- and inter-individual variations, and in some animals, including all the dogs, no fluorescence, but only cells heavily loaded with pigment, could be seen. In the pilary system of albino animals, neither pigment granules nor fluorescent granules were observed.

In the microspectrograph, the specifically fluorescent melanocytes showed excitation spectra with maxima at about 430 m$\mu$, whereas the maxima of the emission curves varied between 480- and 520 m$\mu$. Measured under the same conditions, the fluorophore of authentic dopa showed excitation/emission maxima at 410/480 m$\mu$. It seems unlikely that the difference in the emission spectra is caused by absorption in pigment granules since measured cells appeared colourless in transmitted light. Whether the formaldehyde-induced fluorescence reflects the presence of one or more fluorophore-forming substances with or without concomitant occurrence of dopa cannot at present be evaluated. The variations in emission maxima suggest the presence of a mixture of fluorophores in different proportions. Human malignant melanoma cells are known to contain at least two fluorogenic substances of which only dopa has so far been identified (Falck *et al.* 1966) and display spectral characteristics (Ehinger *et al.* 1968, Cegrell, Falck and Rosengren, unpublished observations) similar to those obtained from the melanocytes in this study.

The biological significance of dopa in the hair-shaft is obscure. It is tempting, however, to suggest that it is incorporated in connexion with uptake of pigment granules into the hair.

This work was supported by grants from the Swedish Cancer Society (Project No. 67—111) and Ollie and Elof Ericsson's Foundation and was carried out within a research organization sponsored by the Swedish Medical Research Council (Projects No. B69-14X-56-05C and No. B69-14X-712-04X).

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