PRELIMINARY AND SHORT REPORT

STUDIES ON DOPA REACTION*

I. A Simple Technic for Dopa Reaction

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The influence of various chemicals on the activity of plant or mammalian tyrosinase has been studied extensively by manometric, colorimetric or chronometric methods (1–5). However, no comparable study has been made on the dopa reaction of the skin except for the recent report of Duijin (6). This may be due, at least in part, to the cumbersomeness encountered in handling many specimens at a time with classical dopa technic.

The present authors devised a simple method suitable for routine investigation on the influence of chemicals on dopa reaction.

MATERIALS AND METHOD

Skin specimens obtained by biopsy are fixed in 10% formalin (neutral formalin) for 30 minutes. After rinsing in tap water, they are cut at 25 μ in the freezing microtome and mounted on slides. A small amount of reaction mixture is dropped over the specimen and a cover glass is placed over it, and the edges of the cover slip are sealed with paraffin. The slide is kept at 37°C. and the course of reaction is observed at regular interval (usually 30 minutes) for three hours. In this way it is easy to prepare more than 10 specimens of different composition and observe them successively.

The composition of reaction mixture is:

0.2% solution of DL-dopa (Eastman)	1.5 ml.
1/15 M phosphate buffer pH 7.4	1.0 ml.
distilled water or aliquots of test substances	0.5 ml.

Preparations are made in duplicate for each experimental condition, the reaction intensity in two slides being always identical. Control slides contain distilled water in place of test substances.

The darkening of melanocytes after three hours is recorded. The difference in the intensity of reactions between control slides and the test slides is recorded as minus (no inhibition); one plus (slight inhibition), two plus (moderate inhibition), three plus (complete inhibition).

RESULTS

The reaction of melanocytes in control slides is of same degree of intensity with that of same specimen treated with conventional method, indicating that the small amount of oxygen contained in the reaction mixture is sufficient to cause full visualization of melanin deposit (figs. 1, 2). Moreover, there are some advantages over classical methods. First, the imbibition of the section with oxidation product of dopa is not encountered; second, the "unspecific" reaction of leukocytes does not occur.

DISCUSSION

Although the influences of ions and chemical compounds on the activity of tyrosinase have been investigated, it is difficult to apply the results obtained directly to the processes *in vivo*. For instance, cupric ion which causes increase in tyrosinase activity as measured

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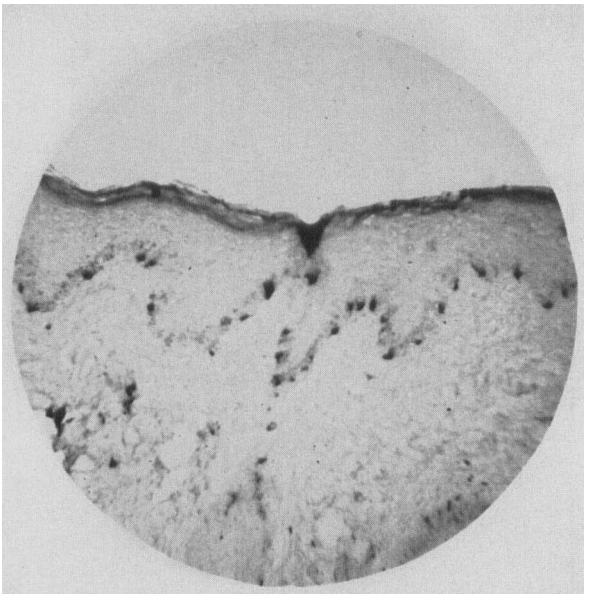

FIG. 1. Dopa reaction (conventional method). × 100

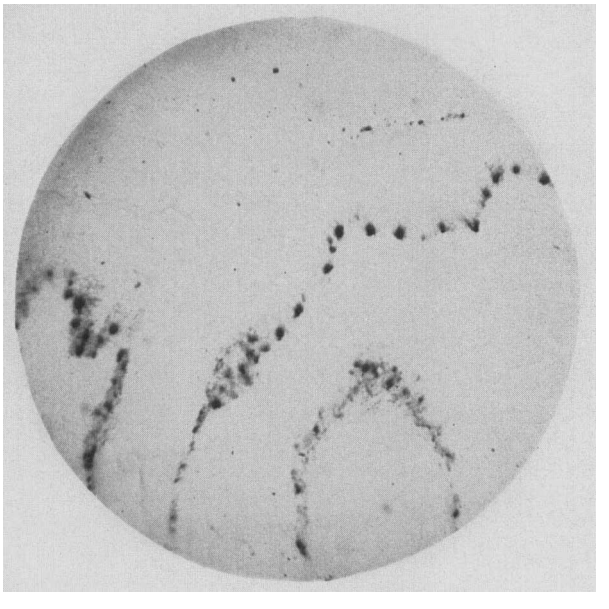

FIG. 2. Dopa reaction (presented method). × 100 No darkening of horny layer

by manometric method, tends to lower melanin output (7). So it is necessary to observe melanogenesis *in vivo* in the presence of test substance. In this respect dopa reaction of human skin may be interpreted as a semi-*in vivo* reaction. The presented dopa technic is simple and gives, though not strictly quantitatively, reproducible results and can be used

routinely as a screening test for the search of antipigmentary compounds. In the evaluation of results, however, it must be remembered that the dopa reaction is performed in the presence of unphysiologically large amount of the substrate and consequently competitive inhibitors may not be detected.

SUMMARY

A slide glass technic for dopa reaction is described.

This method is simple, gives reproducible results and has certain advantages over conventional methods.

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