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Inhibitory Action of Human Epidermis on Melanin Formation.

STEPHEN ROTHMAN, HELEN F. KRYSA, AND ADELAIDE M. SMILJANIC.
From the Section of Dermatology, Department of Medicine, University of Chicago.

Geneticists discovered an inhibitory factor of pigmentation in crude aqueous extracts of guinea pigs' skin.[1] In this laboratory it was found that such a factor also occurs in human epidermis, inhibiting melanin formation in the tyrosine-tryosinase system and preventing the oxidation of *l*-dihydroxyphenylalanin ("dopa") in the air.

Method. Large strips of skin were removed from the abdominal region of white adult cadavers 24 to 48 hours after death. In a few instances fresh skin from female breasts or from amputated limbs were worked up immediately after surgery, but there was no substantial difference between results obtained with fresh and with cadaver skin.

The epidermis was removed according to the method of Baumberger *et al.*[2] by placing the skin on a slide warming table at 56°C with the corium side down. After 3 minutes of warming the epidermis could be easily pulled off. The thin small pieces of epidermis were weighed and sufficient water was added to make a suspension of 100 mg of epidermis per 1.0 cc of water. The suspension was shaken a few times and then placed in the icebox in a stoppered bottle for 24 hours. Next day a turbid or opalescent liquid was decanted and centrifuged. By subsequent ultracentrifugation most of the turbidity could be eliminated without decreasing the activity of the extract.

The extract was added in decreasing concentrations to measured amounts of solutions of tyrosine, tyrosinase and buffer. The pH was 7.4. The test tubes were covered with a cloth and left at room temperature. Results were read in a "Lumetron" colorimeter with blue (420) filter after 24 and 48 hours. The colorimeter was calibrated for measuring melanin in μg per 100 cc aqueous suspension.

Results. Table I shows the inhibition of melanin formation, increasing with the amount of epidermal extract added. Similarly prepared extracts of the corium were ineffective. Table II demonstrates that iodoacetamide, a specific poison for sulfhydryl groups, interferes with the inhibitory action of epidermal extracts. Iodoacetamide alone in similar concentration did not modify melanin formation in the tyrosine-tyrosinase system.

Discussion. Quantitative duplication of these experiments has not been possible, apparently because the epidermis loses varying amounts of water during its separation and weighing. The presence of sulfhydryl compounds in epidermal cells was demonstrated many years ago by histochemical application of the nitroprusside color reaction.[3] Also, it has been known that these compounds inhibit melanin formation.[4] The interference with the inhibitory action of epidermal extracts by iodoacetamide indicates that the inhibition is due to the presence of water-extracta-

TABLE I.

Inhibitory Effect of Epidermal Extract on Melanin Formation.

Each tube contains 9.9 cc of 0.05% tyrosine solution, 0.1 cc of tyrosinase solution (1000 Adams-Nelson units/cc),* 1.0 cc pH 7.4 phosphate buffer, and water sufficient to make a total volume of 12.0 cc.

Tube No.	Epidermal extract, 100 mg/cc cc	Melanin, μg/10 cc after 48 hrs
1	none	178
2	0.05	153
3	0.1	75
4	0.2	40

*Courtesy of the Upjohn Co.

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[3] Giroud, A., and Bulliard, H., *La keratinisation de l'epiderme et des phaneres*, G. Doin, Paris, 1930.

[4] Figge, F. H. J., and Allen, E., *Endocrinology*, 1941, **29**, 262; Rothman, S., *J. Investigative Dermatology*, 1942, **5**, 67.

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TABLE II.
Interference of Iodoacetamide with Inhibitory Effect of Epidermal Extract.
Tyrosine, tyrosinase, and buffer same as in Table I. Total volume 13 cc.

Tube No.	Epidermal extract 100 mg/cc cc	Iodoacetamide 24 mg/cc cc	Melanin μg after 24 hrs	48 hrs
1	1.0	0	100	158
2	1.0	1	240	460
3	0.5	0	138	230
4	0.5	1	270	460

ble sulfhydryl compounds in the epidermis. In preliminary experiments the —SH content of our epidermal extracts measured by the ferricyanide method[5] was found to be in the range of 0.0004 millimol of —SH per cc. The —SH content could be substantially depressed by ultraviolet irradiation of excised pieces of skin.

These findings lead to the hypothesis that in melanoblasts both substrate and active enzyme are present but no reaction takes place between them because of the inhibitory action of sulfhydryl compounds. Pigmentogenic stimuli such as sunshine, X-rays, heat and inflammatory cutaneous diseases of unknown origin act by oxidizing or otherwise destroying these compounds, whereupon the enzyme freely acts on the substrate. In addition to postinflammatory pigmentations, hyperpigmentations of endocrine origin may have the same mechanism as suggested by *in vitro* observations of Figge and Allen.[4]

Summary. Aqueous extracts of human epidermis contain substances inhibiting melanin formation in the tyrosine-tyrosinase system. Iodoacetamide interferes with this inhibition. A new hypothesis of the mechanism of postinflammatory pigmentation is presented.

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Significance of Histamine in Trypsin Shock as Determined by Benadryl.*

J. A. WELLS, H. C. MORRIS, AND CARL A. DRAGSTEDT.
From the Department of Pharmacology, Northwestern University Medical School, Chicago, Ill.

The following observations have been interpreted as supporting the hypothesis that trypsin owes its toxicity to the action of histamine which is liberated from tissues by this enzyme. Trypsin, like histamine, causes contraction of isolated segments of ileum and uterus from a number of animals.[1-4] This action, like that of histamine, is not inhibited by atropine but is abolished by arginine.[3-6]

Trypsin, like substances known to liberate histamine, when added to rabbit's blood results in a shift of histamine from cells into plasma.[7,8] Rabbits injected intravenously with trypsin show a leucopenia, a fall in total blood histamine, a fall in carotid and a rise in pulmonary arterial pressures,[3,5,9,10]

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