The inconsistent results obtained with prolonged exposure in milk indicated that some factor other than the DDT was also toxic to the fish. This was substantiated by the fact that on occasions when mortality in the controls was low, the kill obtained in treated samples was proportional to the concentration of DDT. Bacteriostatic and surface-active agents, aeration, and homogenization were of no benefit in producing consistent results.

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Tyrosinase in Human Skin: Demonstration of Its Presence and of Its Role in Human Melanin Formation

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The mechanism of formation of melanin in human skin has been the subject of extensive investigation and vigorous controversy for the past 50 years (*14*). In 1895, Bourquelot and Bertrand (*5*) found in the mushroom an enzyme called tyrosinase, which catalyzed oxidation of the amino acid tyrosine to melanin. A few years later, tyrosinase was demonstrated in many plant, insect, fungus, and marine animal tissues. At this time, it was assumed that tyrosinase was also present in mammalian skin and that this enzyme catalyzed the oxidation of tyrosine to melanin. Although there was some experimental evidence to support this view, skepticism concerning the presence of tyrosinase in mammalian skin arose when reports demonstrating tyrosinase in rabbitskin could not be confirmed.

Bloch and his co-workers (*4*) found that on incubation of sections of human skin in a solution containing dihydroxyphenyl-L-alanine (dopa), under appropriate conditions, black granules were deposited in the melanoblasts of the basal layer of the skin. This process was shown to involve the enzymatic oxidation of dopa to melanin. The investigators (*4*) found that incubation of skin with compounds other than dopa, for example tyrosine and epinephrine, did not result in formation of melanin in the melanoblasts. Therefore, Bloch concluded that the melanoblasts in human skin contained a specific "dopa oxidase" which could catalyze the oxidation of dopa only to melanin.

[1] Abridgment portion of a thesis submitted by Dr. Fitzpatrick to the faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Master of Science in dermatology and syphilology.

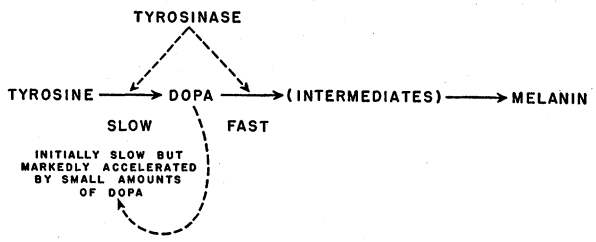

FIG. 1. Enzymatic oxidation of tyrosine to melanin by mammalian tyrosinase.

Several years later, Raper (*17*) showed that dopa was the first product formed in the enzymatic oxidation of tyrosine to melanin by tyrosinase of plant and insect sources. He also demonstrated that plant and insect tyrosinases could catalyze the oxidation of dopa, as well as of tyrosine, to melanin; that is, plant and insect tyrosinase possesses tyrosinase and dopa-oxidase activities.

In the past 8 years, it has been demonstrated that extracts from melanomas of mice (*9, 11, 15*), horses (*7*), and human beings(*10*) display both tyrosinase and dopa-oxidase activities. Furthermore, it has been shown (*15*) that, under certain conditions, a true distinction cannot be made between tyrosinase and dopa-oxidase activities in mammalian tissue. It was recommended (*15*) that the term *tyrosinase* be used for this enzymatic activity, instead of the separate terms *tyrosinase* and *dopa oxidase.* Dopa was also found to be a catalyst in the enzymatic oxidation of tyrosine (*15*). This concept is diagramed in Fig. 1. If the view represented in Fig. 1 is correct, as opposed to Bloch's hypothesis, it should be possible, under appropriate conditions, to demonstrate tyrosinase activity in human skin. The experimental results demonstrating the presence of tyrosinase activity in human skin form the basis of this report.

It has been reported (*3*) that under certain stimuli—for example, ultraviolet or roentgen rays—the human dendritic melanoblasts enlarge, become branched, and give a more pronounced dopa-oxidase reaction than when unirradiated skin is used. For this reason we performed experiments in which we used nonpigmented skin of normal individuals, which had been irradiated for 8 days with erythema doses of ultraviolet radiant energy obtained from a quartz mercury vapor lamp.[2] The results presented in this paper represent a study of the skin (normal before irradiation) of 30 human volunteers.

On the 8th day, a specimen for biopsy was taken, by means of a punch, from the irradiated site and was fixed immediately in 10% solution of formalin for 1 hr at 5° C. Slices of the fixed tissue, 1–2 mm thick, were placed in 25 ml of 0.005 M l-tyrosine made up in 0.1 M phosphate buffer at pH 6.8[3] where they remained for 24 hr at 5° C. The slices of tissue were then immersed in fresh tyrosine-phosphate buffer again, and were left in

[2] Alpine Sun Lamp, Luxor Model, Hanovia Chemical and Mfg. Co., Newark, N. J.

[3] This value is the optimal pH for the mammalian tyrosine-tyrosinase reaction (*15*). There was no change in the intensity of the reaction between pH 6.8 and 7.38.

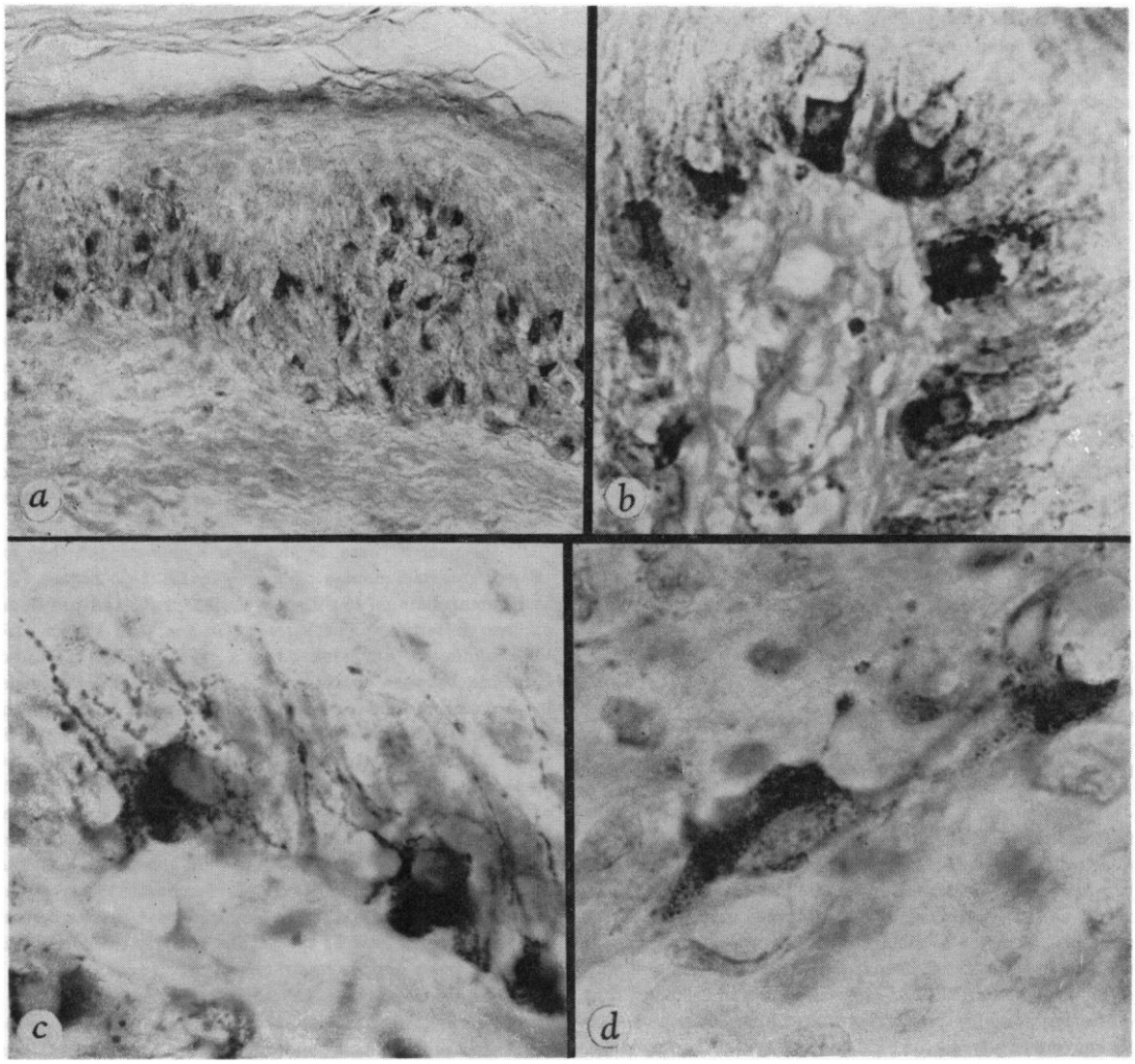

FIG. 2. Pigmented dendritic melanoblasts in human skin which has been exposed *in vivo* to ultraviolet radiant energy and incubated in tyrosine phosphate buffer. (Paraffin sections. Magnifications: *a*, × 375; *b*, × 700; *c*, × 1,025; *d*, × 1,450.)

an incubator at 37° C for another 24 hr. For one control, slices of irradiated skin were treated identically as has been described, except that phosphate buffer (without tyrosine) was used. For a second control, slices of unirradiated skin (from the same donor) were treated exactly as the first control. Gross examination of the slices of skin at this time revealed darkening of the epidermis in the slices incubated in tyrosine but no change in color in the control slices. To prepare the tissues for histologic examination, further fixation was carried out by immersing the slices of tissue in Bouin's solution (picroformal) for 24 hr; then they were dehydrated, cleared in toluene, imbedded in paraffin, sectioned at 15 μ, and counterstained with borax carmine.

Fig. 2 illustrates pigmented melanoblasts lying deeply in the basal cell layer at the epidermal-dermal junction. The outline of the melanoblast, with its dendritic processes, is delineated by the presence of fine, densely packed, brown granules which are deposited in the cytoplasm of the cell body and its dendrites. The cytoplasmic distribution of melanin granules is in agreement with results obtained by differential centrifugation of extracts from the Harding-Passey mouse melanoma, which show that tyrosinase is associated with particles in the cytoplasm of the pigment-producing cells (*15*).

In control sections the basal cells contained preformed melanin, but there were no dendritic melanoblasts containing tyrosinase activity at the epidermal-dermal junction. Evidence of melanin formation was not observed in the basal cells of the epidermis.[4] Whenever melanin was

[4] The absence of melanin formation in the basal cells of the epidermis is contrary to the impression held by others and formerly by one of the authors (H. M.).

found in the basal cells of the tissue that had been incubated in tyrosine, an equal amount of melanin was found in the basal cells of the control that had been incubated in buffer alone. A similar distribution of melanin formation was seen in sections from the same specimens when dopa-phosphate buffer was used in place of tyrosine-phosphate buffer.

Heating the tissue to 100° C for 10 min completely abolished the ability of the melanoblasts to convert tyrosine to melanin.

When the irradiated slices of skin were incubated with 0.01 M sodium diethyl-dithiocarbamate for 6 hr prior to incubation with tyrosine, the reaction was completely inhibited. Sodium diethyl-dithiocarbamate has been shown (*16*) to inhibit the oxidation of tyrosine catalyzed by mouse melanoma tyrosinase by combining with copper, which is necessary for enzymatic activity.

The deposition of granules of melanin in the cytoplasm of the cell body and dendritic processes of melanoblasts after incubation of human skin with tyrosine solutions indicates the presence of tyrosinase activity in these cells. The oxidation of dopa to melanin is also catalyzed by the melanoblasts, since incubation of sections of human skin with dopa results in deposition of pigment in these cells. Bloch's statement that the melanoblast contained only a specific "dopa oxidase" is apparently not supported by these findings. The data support the view that tyrosine can act as a precursor of melanin in human skin.

Human epidermal tyrosinase apparently exists in an inactive or partially inhibited state in normal unirradiated skin. Under the described experimental conditions irradiation of the skin with ultraviolet radiant energy, and possibly other types of radiation (such as roentgen rays, which are known to produce clinical pigmentation), is required before human melanoblasts can convert tyrosine to melanin. The mechanism by which ultraviolet radiant energy activates the enzymatic reaction is unknown. At least two factors appear to be involved. First, trace amounts of dopa, which are known (*15*) to accelerate greatly the tyrosine-tyrosinase reaction, may be formed in the melanoblast or surrounding cells. The dopa may be formed by direct photochemical oxidation of tyrosine present in the tissues and then catalyze the enzymatic oxidation of tyrosine to melanin. The conversion of tyrosine to dopa by ultraviolet radiant energy in the absence of the enzyme has been demonstrated (*2, 6, 18*). Second, ultraviolet radiant energy may decrease the concentration of normally occurring sulfhydryl groups in the epidermis. Rothman, Flesch, and others (*8, 19*) have shown that inhibition of the tyrosine-tyrosinase reaction *in vitro* by extracts of human epidermis is attributable to the presence of sulfhydryl groups. This inhibitory action was absent after irradiation of the epidermal extracts with ultraviolet radiant energy. Sulfhydryl groups inhibit tyrosinase by combining with the copper which is required for enzyme activity.

The inhibition of the human tyrosinase reaction by sodium diethyl-dithiocarbamate suggests that this enzyme, like tyrosinase in plants (*12, 13*), insects (*1*), and in mouse melanomas (*16*), requires copper for its activity.

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Zinc Precipitation of Plasmin

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The work of Vallée (*1*) on the various zinc concentrations in certain tissues has given significance to the study of the effects of low concentrations of zinc on blood proteins. During a study of plasmin activity in ulcer exudates, I noted that the activity was lower in those ulcers treated with zinc oxide. This observation suggested studying the effect of zinc on plasmin *in vitro.*

Plasmin,[1] assaying 1 unit per mg, can be completely precipitated and inactivated by small quantities of zinc. A method of obtaining this inactivation is given here.

One ml of a solution containing 30 mg of plasmin (30 units) was added to a tube containing 198 μg of $ZnSO_4 \cdot 7H_2O$ in 1 ml of distilled water. This represents 45 μg of ionized zinc, assuming it is all present in the ionized form. A precipitate formed that was removed from the solution by centrifugation. The supernatant was then tested for plasmin activity against gelatin in a viscometer. No activity was detected over a period of 4 hr. Varying degrees of precipitation were noted with 22.5 μg, 11.25 μg, 5.6 μg, and 2.8 μg of zinc. At 1.6 μg no precipitation was noted. Testing of the supernatants after sedimentation of the precipitates showed a loss of activity inversely propor-

[1] Supplied by Eugene Loomis, of Parke, Davis & Company Research Laboratory.