cytoplasmic staining was homogeneous except in the zona reticularis, where red and purple droplets stood out against the red cytoplasmic background, perhaps because of solution of the dye in fat droplets. Cell nuclei and the capsule of the gland were unstained, and cells of the adrenal medulla stained very lightly.

Sulfatase activity was not demonstrable in rat and mouse collagen, cartilage, bone, and blood cells.

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The Destruction of Epinephrine by the DOPA-Oxidase System of Ocular Tissue[1]

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The principal enzyme systems responsible for the inactivation of epinephrine in the body are generally considered to be monoamine oxidase and cytochrome oxidase (*1–3*); in addition, epinephrine is conjugated in the liver. In seeking a possible enzymatic basis for the observation that mydriatics are more effective in light than in dark eyes (*4*), assays were conducted on homogenates of the irides and ciliary bodies of pigmented and albino rabbits for enzymes which might be involved in the destruction of the cholinergic or adrenergic mediators. No significant differences were found between the two groups in respect to monoamine oxidase (*5*), cytochrome oxidase, succinic dehydrogenase (*6*), cholinesterase (*7*), or reducing substances (*8*), employing the specific substrates called for by the methods listed. Dihydroxyphenylalanine (DOPA) was oxidized by the pigmented but not by the albino homogenates; DOPA-oxidase has previously been demonstrated in the pigment granules of steer irides (*9*). It appeared that this enzyme was responsible for

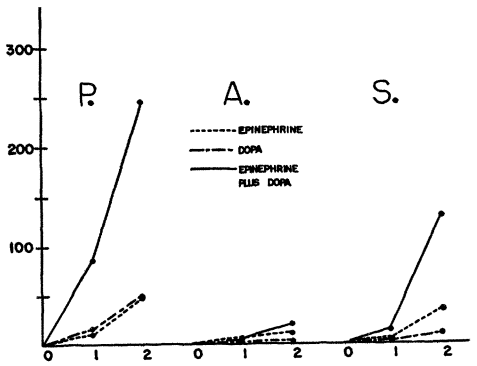

FIG. 1. Rates of oxygen uptake of homogenates of pigmented (*P.*) and albino (*A.*) rabbit iris-ciliary bodies, and spontaneous oxygen uptake (*S.*), in the presence of epinephrine (2.75 mg), D-L-DOPA (2.0 mg), and epinephrine plus DOPA. Each flask contained 60–75 mg wet wt tissue in 0.133 *M* phosphate buffer (pH 7.0), 38° C, total fluid volume 3.0 ml. Points represent averages of two to four determinations. Ordinates: μl O_2/100 mg wet wt tissue; abscissae: time in hours.

the small but consistent difference in O_2-uptake observed when epinephrine was used as substrate, since preparations of melanomas have been reported to oxidize epinephrine, although at rates considerably below those for DOPA (*10, 11*). When a mixture of epinephrine plus DOPA was used as substrate, the O_2-uptake by the pigmented homogenates was considerably greater than the sum of the values with the two substances alone (Fig. 1). When the concentrations of the two substrates were varied, the rate was found to be dependent upon the concentration of each. Bio-assays of trichloracetic acid filtrates of the reaction mixtures by the dog's blood pressure method showed that significant amounts of epinephrine were inactivated during the reaction. A lower degree of augmentation of O_2-uptake was recorded with the vessels in which autoxidation was followed, but none occurred with the albino homogenates. Recent evidence has indicated that mammalian "DOPA-oxidase" and "tyrosinase" are identical (*12*). The addition of 0.001 *M* α-naphthothiourea (ANTU), a relatively selective inhibitor of plant tyrosinase (*13*), reduced the O_2-uptake by 50% during the first hour in vessels containing the combined substrate and pigmented homogenate, but did not affect the rate of autoxidation. No significant degree of deamination or decarboxylation occurred during the enzymatic or spontaneous oxidation. When mixtures of DOPA and norepinephrine were used as substrate, the augmentation of O_2-uptake was absent.

The development of a reddish-orange color prior to a fine black precipitation in the autoxidation vessels and in the supernatant solutions with pigmented homogenates, and the absence of deamination or decarboxylation, indicate that both substrates followed the same path of oxidation to and beyond indole-quinones as they have been shown to follow with plant tyrosinase (*14, 15*). The basis of the greatly augmented O_2-uptake with the combined substrates remains to be determined. The quinone and indole-quinone forms of epinephrine and DOPA constitute redox systems; the

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quinone forms are highly unstable (*16*). It is possible that the oxidant of one form of either compound may promote directly the oxidation of some stage of the other. Another possibility is that liberation of hydrogen peroxide with the oxidation of the leuko-form of either indole-quinone may oxidize either or both substrates (*15*). The absence of acceleration with norepinephrine is in keeping with this concept, since it is known to be oxidized to an indole-quinone with much less ease than epinephrine (*17*). Martin *et al.* (*18*) have reported that DOPA and several phenolic compounds accelerate the destruction of epinephrine by mushroom tyrosinase, and Lerner *et al.* (*19*) have more recently found a similar accelerating action of DOPA on the oxidation of epinephrine and similar compounds by a tyrosinase prepared from mouse melanoma.

Experiments are now in progress to determine the extent to which the DOPA-DOPA-oxidase system may participate in the destruction of epinephrine in pigmented irides *in vivo*. The results of these studies and a detailed account of the present findings will be reported elsewhere.

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The Effect of Ultraviolet Radiation on Sulfhydryl and Disulfide Containing Amino Acids

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In previous studies quantitative measurements were made on the influence of ultraviolet irradiation on the rate of hydroxylation and of destruction of those amino acids that have been implicated in the process of skin pigmentation and erythema production (*1*). It was found that these effects were inhibited by the presence of sulfhydryl containing amino acids. The results obtained were compatible with the hypothesis of Rothman and his associates that pigmentogenic stimuli such as ultraviolet radiation cause pigmentation by oxidizing or destroying inhibitory sulfhydryl compounds, thus enabling an enzyme to act on the pigment precursor (*2*).

It thus became of interest to study the effect of ultraviolet irradiation on the possible reduction of the disulfide (—S—S—) linkage of cystine and homocystine to —SH and possible oxidation of the sulfhydryl (—SH) group of cysteine to —S—S—. Methionine was also studied.

Solutions of either cystine or cysteine in 0.1 *N* HCl and of methionine in 0.3 *N* HCl were irradiated in test tubes 1 in. in diameter mounted in running water. The height of the solutions in the test tubes varied between 0.5 and 2.5 cm. The unfiltered radiation of a Hanovia quartz mercury lamp was incident from above at 3 cm distance without any absorber (except air) between the lamp and the solution. After 30 min to 2 hr of irradiation the respective amino acid solutions were analyzed colorimetrically for cysteine and cystine (*3*) or methionine (*4*) with a recording spectrophotometer.

For irradiations with approximately monochromatic radiation, the Double Monochromator Model 300 DUV of the Farrand Optical Company was used in conjunction with the 1000-w type A-H6 G-E mercury lamp. In this case, the solutions were contained in a quartz vessel with a cross-section area of 1 cm^2. The energy measurements, made with a calibrated thermopile, were accurate within ±1%. The total energy incident on the solution was absorbed, except for small reflection losses amounting to about 4%. The reflection losses, which occur on the interfaces of the quartz cell, were not subtracted from the energies as they were measured.

In those experiments in which the isolation of homocysteine was attempted, solutions of homocystine and methionine in 0.1 *N* HCl were irradiated with the Hanovia lamp, as described above, in layers of 4 cm for 2½ hr. The procedure used in the identification of homocysteine from homocystine was as follows: The dry residues of 4 irradiations of 400 mg homocystine each in 200 ml of 0.1 *N* HCl were dissolved in 12 ml water, filtered, and neutralized with NaOH. A precipitate (I) formed at pH 5–6, which was filtered, washed with water, and then dissolved in 1 *N* HCl and reprecipitated by neutralization with NH_4OH. This procedure was repeated twice. Then the precipitate was filtered, washed with water, and dried in the desiccator *in vacuo*. To the filtrate of precipitate I, 600 mg NaOH was added, followed by 0.5 ml benzylchloride, and the mixture was shaken for 100 min in a small separatory funnel. The solution was extracted with 5 ml ether four times to remove the uncombined benzylchloride. The alkaline solution was freed from ether *in vacuo* and filtered; acetic acid was added until