

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 nor-epinephrine 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

Klaus Schocken

Army Medical Research Laboratory,  
Fort Knox, Kentucky

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<sup>2</sup>. The energy measurements, made with a calibrated thermopile, were accurate within  $\pm 1\%$ . 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<sub>4</sub>OH. 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

TABLE 1  
EFFECT OF ULTRAVIOLET RADIATION ON CYSTINE AND CYSTEINE\*  
(1 mg in 1.5 ml 0.1 N HCl)

I	II	III	IV	V	VI	VII
Amino acid irradiated (1 mg)	Time of irradiation (min)	Cystine and cysteine after irradiation (mg)	Cysteine after irradiation (mg)	Cystine after irradiation calculated by difference of columns III and IV	Amount destroyed (mg)	Degree of decomposition (%)
Cystine	30	0.821 ± 0.044†	0.283	0.538	0.179	17.9
"	60	0.702 ± 0.039	0.396	0.306	0.298	29.8
"	120	0.587 ± 0.043	0.449	0.138	0.413	41.3
Cysteine	30	1.090 ± 0.012	0.740	0.350	0	0
"	60	0.890 ± 0.047	0.591	0.299	0.110	11.0
"	120	0.560 ± 0.045	0.469	0.091	0.440	44.0

\* Each value is an average of five experiments made simultaneously.

† 95% fiducial limits for the mean of cystine and cysteine after irradiation.

the solution was slightly acid to litmus. After standing 48 hr in the refrigerator at 2° C the precipitate was filtered off, washed with ice-cold water, and recrystallized from boiling water. This procedure was repeated twice. Altogether 39.9 mg of a benzylated product was obtained.

The procedure used in the attempts to identify the irradiation product of methionine was in principle the same.

The results obtained on irradiation of cystine and cysteine with the Hanovia lamp are illustrated in Table 1. It can be seen that, with increasing exposures of cystine, increasing amounts of this substance were reduced to cysteine, and simultaneously increasing amounts of it were destroyed. On irradiation of cysteine, at first only oxidation to cystine took place without destruction; then, with increasing time of irradiation, increasing destruction occurred. During the irradiations the formation of hydrogen sulfide was noticeable by odor.

It is possible that in addition to the direct effect of the ultraviolet radiation some of the oxidation effects may also have been due to the diffusion into the solution of ozone generated in the vicinity of the solutions, and to the formation of H<sub>2</sub>O<sub>2</sub> in the solution.

Monochromatic irradiation (wavelength 225 mμ, spectral band width 10 mμ) of cystine (1 mg in 1.5 ml 0.1 N HCl) for 8 hr was found to result in approximately 16% reduction of S—S to SH in the range of energies from 0.33 to 2.50 J/mg. Similar experiments carried out with cysteine indicated a small but definite degree of oxidation of cysteine to cystine in the range of energies from 0.63 to 5.28 J/mg. No destruction of either cysteine or cystine could be detected, probably because the radiant energy was considerably less than that produced by the Hanovia lamp. The absorption of cysteine, cystine, and methionine increases with decreasing wavelength; the A-H6 mercury lamp possesses no measurable output below 225 mμ; it was established that 98% of the incident radiation was absorbed by the methionine solution,

97% by the cystine solution, and 78% by the cysteine solution at this wavelength.

After irradiation of homocystine a positive test for sulfhydryl (—SH) was obtained. From the irradiation mixture *S*-benzylhomocystine could be isolated. The mixed melting point with an authentic sample of *S*-benzylhomocystine gave no depression.

C<sub>11</sub>H<sub>15</sub>NO<sub>2</sub>S. Calcd: C 58.64, H 6.71, N 6.22, S 14.23  
Found:<sup>1</sup> C 57.95, H 7.00, N 6.78, S 13.35  
C 58.31, H 7.04, N 6.58, S 13.56

Although the sulfur content is too low and the nitrogen content too high to furnish unequivocal proof of the identity, there seems little doubt that the substance is *S*-benzylhomocystine.

The finding of the conversion of homocystine to homocysteine agrees with the observation that cystine is reduced to cysteine by ultraviolet irradiation (5). The precipitate I was identified as unchanged homocystine.

TABLE 2  
EFFECT OF ULTRAVIOLET RADIATION ON METHIONINE\*  
(1 mg in 1 ml 0.3 N HCl)

Time of irradiation (min)	Degree of decomposition (%)
30	30.0
60	40.5
120	76.0

\* Each value is an average of two experiments made simultaneously.

The results obtained on irradiation of methionine with the Hanovia lamp are illustrated in Table 2. The destruction increased with the incident energy.

Monochromatic irradiation (wavelength 225 mμ, spectral band width 10 mμ) of methionine (1 mg in 1 ml 0.3 N HCl) for 8 hr was found to result in approximately 10% destruction within the range of energies from 0.42 to 1.12 J.

It was found that a solution of methionine gave a

<sup>1</sup> The analyses were performed by Carl Tiedcke, of the Laboratory of Microchemistry, Teaneck, N. J.

positive color reaction with sodium nitroferrieyanide after irradiation for 1 hr with the Hanovia lamp but not before, and not if the radiation was first passed through an acetone filter which absorbs wavelengths shorter than 300 m $\mu$ . Because of this finding the isolation of homocysteine was attempted from an irradiated methionine solution. No *S*-benzylhomocysteine could be isolated; only unchanged methionine could be identified. It is possible that methyl mercaptan CH<sub>3</sub>SH was formed.

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## Comments and Communications

### Zoological Nomenclature

AS FROM March 1, 1953, the International Commission on Zoological Nomenclature will start to vote on the following cases involving the possible use of its plenary powers for the purposes specified in brackets against each entry. Full particulars of these cases were published on August 29, 1952, in the *Bulletin of Zoological Nomenclature*, those relating to cases (1) to (4) in Part 10, and those relating to cases (5) to (14) in Part 11 of Vol. 6. (1) *Sphinx* Linnaeus, 1758 (Class Insecta, Order Peidoptera) [designation of type species]; (2) Houttuyn (M. H.), 1787, *Animalium Musei Houttuinensi Index* [suppression]; (3) *Phalaena* Linnaeus, 1758 (Cl. Insecta, Ord. Lepidoptera) [suppression, and validation as of generic status of eight terms used by Linnaeus for groups thereof: *Bombyx*, *Noctua*, *Geometra*, *Tortrix*, *Pyrallis*, *Tinea*, *Alucita*, as from 1758, *Attacus*, as from 1767; and names of families based thereon; alternatively, for *Bombyx* and *Pyrallis* (as from Fabricius, 1775), designation of type species]; (4) *Episema* Oechsenheimer, 1816 (Cl. Insecta, Ord. Lepidoptera) [designation of type species, thereby also preserving *Diloba* Boisduval, 1840]; (5) *cydippe* Linnaeus, 1761, *Papilio*, and *adippe* Linnaeus, 1767, *Papilio* (Cl. Insecta, Ord. Lepidoptera) [suppression, and validation of *adippe* Denis & Schiffermüller, 1775, *Papilio*]; (6) *hispidus* Olivier, 1811, *Palaemon* (Cl. Crustacea, Ord. Decapoda) [validation]; (7) *Sicyonia* Milne Edwards, 1830 (Cl. Crustacea, Ord. Decapoda) [validation]; (8) *Hymenocera* Latreille, 1819 (Cl. Crustacea, Order Decapoda) [designation of type species]; (9) *Pyramidella* Lamarck, 1799 (Cl. Gastropoda, Subclass Prosobranchia) [validation, by suppression of *Plotia* Roeding, 1798]; (10) *Dasypeltis* Wagler, 1930 (Cl. Reptilia) [validation]; (11) *Trichopsylla* Kolenati, 1863 (Cl. Insecta, Ord. Siphonaptera) [suppression]; (12) *pungens* Waleknaer, 1802, *Pulex*, and *vespertilionis* Duges, 1832, *Pulex* (Cl. Insecta, Ord. Siphonaptera) [suppression]; (13) *simus* Linnaeus, 1767, *Coluber* (Cl. Reptilia) [determination of application]; (14) *Mellita* Agassiz, 1841 (Cl. Echinoidea) [validation].

Comments on the above cases should be sent to me as soon as possible.

FRANCIS HEMMING, *Secretary*

*International Commission on  
Zoological Nomenclature  
28 Park Village East, Regent's Park  
London, N.W. 1, England*

### Comparative Anatomy, Embryology, and Histology Synthesized

COMPARATIVE anatomy, embryology, and histology of vertebrates are usually separate semester courses in undergraduate schools—an arrangement that results in some duplication of material. For the past four years I have offered at Lawrence College a year course in vertebrate morphology, which attempts to combine the three courses into an integrated whole. After a preliminary study of testis and ovary histology and germ-cell formation, we investigate fertilization, cleavage, and germ-layer formation. Then the various body systems are studied in the following order: skeletal, muscular, nervous, endodermal, urinogenital, and circulatory. For each system the development and the microscopic and gross structure of adult morphology are considered comparatively.

The forms used are frog, chick, and pig embryos; shark, *Necturus*, alligator, and cat adults; and histological sections from the four adult forms. The approach varies with the system. In the skeletal and muscular systems, a brief histological and embryological survey is followed by much more extensive work on comparative anatomy. The embryology of the nervous system is given a great deal of time, with histology and anatomy somewhat less. The endodermal systems emphasize histology most, embryology next, and anatomy less. The embryology and histology of the urinogenital systems are considered together, with anatomy following. In the circulatory system a short study of histology is followed by study of the morphology in the frog embryo, shark, and *Necturus* adults, chick embryo, alligator adult, pig embryo, and cat adult.