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The Effect of Ultraviolet Irradiation on Certain Amino Acids (M.D.F.R.L.) 07 274

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Quantitative studies were made of the destruction and conversion of amino acids in aqueous solution under ultraviolet irradiation, with and without the influence of inhibitors. These amino acids and also tryptophane were irradiated up to 180 min. Their relative stability are shown by the observation that at 90 min of irradiation 95% of the histidine, 34% of the tryptophane, 27% of the dopa, and 18% of the tyrosine were decomposed. For the same time period, 20% of the phenylalanine was converted into tyrosine and 1.5% of the tyrosine into dopa. Of the amino acids studied, cysteine and cystine exerted the greatest protective influence on a solution of histidine. Homocystine and methionine are less protective while alanine, and leucine exert only slight protection.

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~~Sciences, General (33)~~
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~~Optics (7)~~

Amino acids
Ultra violet radiation
$C_6H_5NCH_2$ $CH(NH_2)$ COOH

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MEDICAL DEPARTMENT

FIELD RESEARCH LABORATORY

Fort Knox, Kentucky

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15 June 1950

THE EFFECT OF ULTRAVIOLET IRRADIATION
ON CERTAIN AMINO ACIDS*

*Sub-project under Studies of Physiological and Psychological Problems of Military Personnel in Relation to Equipment, Environment and Military Tasks. Approved 31 May 1946. MDFRL Project No. 6-64-12-08-(4).

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THE EFFECT OF ULTRAVIOLET IRRADIATION ON CERTAIN AMINO ACIDS*

by

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from

Medical Department Field Research Laboratory
Fort Knox, Kentucky
15 June 1950

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Project No. 6-64-12-08
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15 June 1950

THE EFFECT OF ULTRAVIOLET IRRADIATION ON CERTAIN AMINO ACIDS

OBJECT

The amino acids phenylalanine, tyrosine, dihydroxyphenylalanine and histidine have been implicated in the process of skin pigmentation and erythema production. Although ultraviolet irradiation of these amino acids is known to lead to conversion and decomposition in aqueous solution, no quantitative data are available. A study of the degree of their conversion and of decomposition was therefore undertaken in order to find quantitative data and to use them as a means by which the effects of added accelerators and inhibitors could be judged.

RESULTS AND CONCLUSIONS

Quantitative studies were made of the destruction and conversion of amino acids in aqueous solution under ultraviolet irradiation, with and without the influence of inhibitors. These amino acids and also tryptophane were irradiated up to 180 minutes. Their relative stability is shown by the observation that at 90 minutes of irradiation 95 per cent of the histidine, 34 per cent of the tryptophane, 27 per cent of the dopa, and 18 per cent of the tyrosine were decomposed. For the same time period, 20 per cent of the phenylalanine was converted into tyrosine and 1.5 per cent of the tyrosine into dopa. Of the amino acids studied, cysteine and cystine exerted the greatest protective influence on a solution of histidine. Homocystine and methionine are less protective while alanine, and leucine exert only slight protection.

RECOMMENDATIONS

These measurements should be continued with monochromatic ultraviolet light and with exact determinations of the absorbed energy.

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THE EFFECT OF ULTRAVIOLET IRRADIATION ON CERTAIN AMINO ACIDS

I. INTRODUCTION

The amino acids, phenylalanine, tyrosine, dihydroxyphenylalanine and histidine have been implicated in the process of skin pigmentation and erythema production (1, 2). Although ultraviolet irradiation of these amino acids is known to lead to conversion and decomposition in aqueous solution, no quantitative data are available. A study of the degree of their conversion and of decomposition was therefore undertaken in order to find quantitative data and to use them as a means by which the effects of added accelerators and inhibitors could be judged (3).

II. EXPERIMENTAL

The above amino acids, and in addition tryptophane were investigated because they are known to be influenced by ultraviolet light. Solutions of these amino acids were buffered with citric acid-disodium phosphate to about pH 4 or 7, and with boric acid-KCl-NaOH to pH 9, and then irradiated with the unfiltered light of a Hanovia quartz mercury lamp at 30 cm distance, the radiation being incident from above without any absorber (except air) between the lamp and the solution. Test tubes, 1 inch in diameter and containing the solutions in layers from 0.5 to 2.5 cm, were kept at room temperature in running water. After 10 minutes to 3 hours irradiation, the respective amino acid solutions were analyzed for tyrosine (4, 5) or dopa (6) or trytophane (4) or histidine (7, 8). Concentrations were measured with a registering spectrophotometer.

To study the effect of inhibitors on the decomposition of histidine various amino acids were added to a histidine solution at pH 7 and 9. The higher pH was necessary for solubility of some of the amino acids; the histidine solution was chosen as the test substance because of its rapid decomposition with ultraviolet irradiation. The effect of accelerators was studied by addition of ferrous sulfate and ascorbic acid to phenylalanine and tyrosine solutions at pH 4 and 7 (5). However, the methods (4 and 5) were inapplicable because ferrous sulfate and ascorbic acid were found to interfere with the color reaction used for the determination of these amino acids.

III. RESULTS AND DISCUSSION

A. Destruction of Amino Acids. Table 1 shows the degree of destruction of the amino acids following irradiation. The degree of decomposition increased with the duration of irradiation. In descending order the degree of stability was tyrosine, dopa, tryptophane, and histidine. For example, in 90 minutes under the same conditions of irradiation, about 18 per cent of the tyrosine, 27 per cent of the dopa, 54 per cent of the tryptophane, and 95 per cent of the histidine were decomposed.

Under the influence of ultraviolet irradiation, histidine yields, among other products, histamine and imidazolacetaldehyde (9), which are similar in their pharmacological action. Since it is generally assumed that skin erythema formation is due to these substances, it seemed of interest study

the stability of histamine under ultraviolet irradiation. In a series of measurements, not recorded in the tables, it was found that this amine was decomposed even to a larger extent than histidine under the same experimental conditions. After 10 minutes of irradiation, 55.5 per cent was decomposed, and after 20 minutes, 80.5 per cent.

B. Conversion of Phenylalanine and Tyrosine. L. E. Arnow has reported that irradiated solutions of phenylalanine give a positive color reaction for phenol and also for dopa (10). As can be seen from Table 2, after 90 minutes irradiation 20 per cent of the phenylalanine was converted into products, presumably largely tyrosine and dopa, giving a positive test with the color reagents used (4, 5). The tests employed do not distinguish between monohydroxy and dihydroxyphenylalanine derivatives and do not permit any conclusion with respect to the position of the introduced hydroxyl groups. However, the product formed probably is largely tyrosine, since in separate experiments (not reported here) using Arnow's procedure (6), the amount of dopa present was found to be negligible. Since, after 180 minutes of irradiation only 14 per cent of the phenylalanine could be accounted for as hydroxylated phenylalanine derivatives, it seems probable that the extension from 90 to 180 minutes of irradiation under the given experimental conditions destroyed more of these substances than it produces.

The data in Table 2 show that the conversion of tyrosine to dopa was much less than the conversion of phenylalanine to tyrosine, only 2 per cent occurring after 180 minutes of irradiation. Since, with the same amount of irradiation, 29.5 per cent of the tyrosine was destroyed (Table 1), it is obvious that not all tyrosine acted upon by the radiant energy was converted to hydroxylated phenylalanine derivatives. Other reactions, no doubt, take place, such as the oxidation of the side chain, deamination, decarboxylation and others, leading to reaction products different from hydroxylated phenylalanine derivatives. Furthermore, some of the dopa formed under these conditions was converted to melanin-like derivatives. Similar reactions may occur with phenylalanine, although no data are available.

C. Inhibitors. Of the amino acids tested for their inhibitory effect on histidine decomposition, cysteine and cystine were most effective, homocysteine and methionine less protective, while alanine and leucine afforded only mild protection (Table 3). It can be seen that at the higher pH of 9 the destruction of histidine (experiments 5 vs. 1), as well as its protection by cysteine, was greater than at pH 7. (Experiment 6 vs. 2.)

Since cysteine contains a sulfhydryl group and l-cystine is partially transformed into cysteine by irradiation (9), whereas alanine and leucine do not contain this group, the results obtained are compatible with the idea of Rothman et al. (3) that the protective influence observed is exerted by the sulfhydryl group. It is possible that the protection afforded by homocystine and methionine also arises from the formation of sulfhydryl groups during their irradiation.

IV. SUMMARY AND CONCLUSIONS

Quantitative studies were made of the rate of destruction and conversion of certain amino acids in aqueous solution under ultraviolet irradiation, with and without the influence of inhibitors. The amino acids tested were

phenylalanine, tyrosine, histidine, dopa, and tryptophane, with the irradiation lasting up to 180 minutes. Their relative stability was shown by the observation that at 90 minutes of irradiation 95 per cent of the histidine, 54 per cent of the tryptophane, 27 per cent of the dopa, and 18 per cent of the tyrosine were decomposed. For the same time period, 20 per cent of the phenylalanine was converted into tyrosine and 1.5 per cent of the tyrosine into dopa. Of the amino acids tested, cysteine and cystine exerted the greatest protective influence on a solution of histidine. Homocystine and methionine are less protective while alanine and leucine exert only slight protection.

V. RECOMMENDATIONS

These measurements should be continued with monochromatic ultraviolet light and with exact determinations of the absorbed energy.

Thanks is expressed to the Biochemistry Branch of the Laboratory for its assistance in this project.

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TABLE 1

DESTRUCTION OF AMINO ACIDS IRRADIATED IN BUFFER SOLUTION OF pH 7.0

Each value is an average of 2 experiments made simultaneously.
Mean percentage difference between samples = 3 per cent.

Amino Acid Irradiated	Amount Irradiated	Time of Irradiation	Degree of Decomposition
	Mg.	Minutes	Per Cent
Tyrosine	0.25	0	0
Tyrosine	0.25	90	17.9
Tyrosine	0.25	180	29.5
Dopa	0.40	0	0
Dopa	0.40	45	21.0
Dopa	0.40	90	27.0
Dopa	0.40	180	44.7
Tryptophane	0.25	0	0
Tryptophane	0.25	90	33.6
Tryptophane	0.25	180	50.2
Histidine	0.20	0	0
Histidine	0.20	10	49.0
Histidine	0.20	20	54.6
Histidine	0.20	45	70.6
Histidine	0.20	90	95.4
Histidine	0.20	180	96.9

TABLE 2

DEGREE OF CONVERSION OF PHENYLALANINE AND OF TYROSINE TO HYDROXYLATED COMPOUNDS IRRADIATED IN BUFFER SOLUTION OF pH 7.0

Each value is an average of 2 experiments made simultaneously. Mean percentage difference between samples = 3 per cent.

Amino Acid Irradiated	Amount Irradiated	Time of Irradiation	Amino Acid Tested for	Degree of Conversion
	Mg.	Minutes		Per Cent
Phenylalanine	0.10	0	Tyrosine & Dopa	0
Phenylalanine	0.10	90	Tyrosine & Dopa	20.00
Phenylalanine	0.10	180	Tyrosine & Dopa	24.00
Tyrosine	0.80	0	Dopa	0
Tyrosine	0.80	45	Dopa	1.25
Tyrosine	0.80	90	Dopa	1150
Tyrosine	0.80	180	Dopa	[illegible]

TABLE 3

INHIBITING EFFECT OF VARIOUS AMINO ACIDS ON THE DECOMPOSITION OF HISTIDINE

0.20 mg. of histidine irradiated in buffer solution of pH 7.0 (experiments 1 to 4) and of pH 9.0 (experiments 5 to 18). Irradiation time 10 minutes. Number of experiments shown in parenthesis. Equimolecular amounts of inhibitor in experiments 2,3,4,6,9,13,17; experiments 7,10,14,18; and experiments 8,11,15.

Number of Experiment	Inhibiting Substance	Amount of Inhibitor	Degree of Composition
		Mg.	Per Cent
1	--	--	48.5 (12)
2	Cysteine HCl	10.0	3.5 (4)
3	dl-Alanine	6.0	37.5 (4)
4	l-(-)-Leucine	8.0	34.0 (4)
5	--	--	66.0 (6)
6	Cysteine HCl	10.0	0 (4)
7	Cysteine HCl	5.0	8.5 (4)
8	Cysteine HCl	2.5	20.5 (4)
9	l(-)-Cystine	15.0	0 (4)
10	l(-)-Cystine	7.5	6.5 (4)
11	l(-)-Cystine	3.75	25.5 (7)
12	l(-)-Cystine	1.875	20.0 (4)
13	dl-Homocystine	15.0	18.0 (4)
14	dl-Homocystine	7.5	22.5 (4)
15	dl-Homocystine	3.75	28.0 (4)
16	dl-Homocystine	1.875	36.0 (4)
17	dl-Methionine	10.0	19.5 (10)
18	dl-Methionine	5.0	24.5 (4)