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Effects of High-voltage Cathode Rays on Aqueous Solutions of Histidine Monohydrochloride

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It has been reported by Proctor & Bhatia (1950) that irradiation of haddock fillets by cathode rays caused no significant loss of amino-acids in the protein. The investigation reported was limited to ten amino-acids, namely, arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and cystine. The observations indicated that these amino-acids either were not affected in the concentrations in which they were present in the fish protein, or that they were protected from the action of cathode rays (electrons) when present in native environments.

Although there are reports in the literature relating to the irradiation of histidine by ultraviolet rays and by X-rays, no reference to the effect of cathode rays is known. Ellinger (1928) reported that irradiation of histidine hydrochloride with ultraviolet radiation led to the formation of a pharmacologically active product with histamine-like action. Further irradiation at 2970 and 3020 Å. resulted in a loss in pharmacological activity. This work was confirmed by Holtz (1933, 1934), who isolated crystalline histamine picrate. Investigations conducted by Szendrő (1931) indicated that imidazole acetaldehyde is also formed from histidine. Splitting off of ammonia from a number of amino-acids by light of the quartz mercury lamp has been measured by Lieben & Molnar (1931) and by Lieben & Urban (1931, 1934). It was indicated that the rate of decomposition of histidine by irradiation is independent of pH. Dale, Davies & Gilbert (1949) reported that 10.6 µg. NH₃/ml. were liberated from 0.13M solution of histidine monohydrochloride after exposure to an X-ray dose of 166,000 r.

The present report describes experiments conducted to determine the chemical and physical effects of cathode rays on histidine, and presents evidence regarding the mode of action of cathode rays on histidine.

EXPERIMENTAL

Source of cathode rays. A pressure-insulated, electrostatic generator of the Van de Graaff type, operating at 3,000,000 V. and capable of producing a continuous supply of monoenergetic electrons, was used for the production of the cathode rays (Robinson, 1947; Trump & Cloud, 1943; Trump & Van de Graaff, 1948). The dosages of cathode rays are expressed in terms of röntgen-equivalent-physical (r.e.p.) (Evans, 1947).

Irradiation. Samples (10 ml. each) of the amino-acid solution were irradiated in stainless-steel dishes 5.08 cm. in diameter and 0.8 cm. in depth. The depth of the liquid when the sample amounted to 10 ml. was 0.5 cm. This is well within the penetration range of electrons at the voltages used. The dishes were uncovered during irradiation and all irradiations were made at room temperature. After irradiation, the samples were transferred to glass bottles.

Estimation of histidine. Determinations of histidine were made microbiologically, *Leuconostoc mesenteroides* P-60 being used as the test organism. The basal medium 'D' developed by Dunn, Shankman, Camien, Frankl & Rockland (1944) was used.

For chemical analysis of histidine, a colorimetric method (Macpherson, 1942; Block & Bolling, 1945) based on a modification of Pauly's (1904, 1915) reaction was used. The red colour was measured in an Evelyn colorimeter having a 515 mµ. filter. A standard curve of histidine monohydrochloride monohydrates, covering a range of 0–100 µg., was prepared.

Estimation of deamination. For the observations on deamination, 3 ml. samples of histidine solution, prepared in 0.1N-HCl, were sealed into 5 ml. glass ampoules, which were subjected to the cathode-ray beam. After irradiation, the

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tip of the ampoule was broken and the liquid was withdrawn for NH_3 analysis. NH_3 was determined by the micro-diffusion method (acidimetric) as described by Conway (1947). The test fluid (1 ml.) was put in the central chamber of the standard unit. NH_3 was absorbed in 0.001 N-HCl. Non-irradiated histidine solution was taken as the control in the blank unit.

Ultraviolet absorption. The ultraviolet absorption spectra of the irradiated and the non-irradiated amino-acid solutions were determined with a Beckman quartz spectrophotometer model DU.

RESULTS

Disappearance of histidine on irradiation. A yellow pigmentation was produced in histidine solutions upon irradiation. The intensity of the colour increased progressively with dosage at a given concentration and also with concentration at a given dosage.

Four aqueous solutions of histidine containing 100, 250, 500 and 1000 μg . of the amino-acid per ml. of solution were irradiated. The results on the amounts of unchanged amino-acid as determined microbiologically are shown in Table 1.

Two irradiations (I and II) were conducted on two different days, and the amounts of unchanged amino-acid in each sample were measured both

chemically and microbiologically. The results are shown in Table 2. Comparisons of the data presented in Tables 1 and 2 indicate the degree of variation that may be encountered in this type of irradiation and the subsequent evaluation of the results.

Production of ammonia. The yields of ammonia obtained on irradiation of solutions of histidine monohydrochloride containing 100 μg ./ml. at four levels of 100,000, 250,000, 500,000 and 1,000,000 r.e.p. are presented in Table 3.

Ultraviolet absorption spectra. Fig. 1 shows the ultraviolet absorption spectra of histidine monohydrochloride solutions (100 μg ./ml.) irradiated at different dosages. The increased absorption by the irradiated solutions indicates that the molecule of histidine is affected by irradiation.

DISCUSSION

Inspection of Table 1 shows that histidine monohydrochloride was decomposed upon irradiation with cathode rays. The difference in the amino-acid content before and after irradiation has been considered as representing the amount decomposed on irradiation. The decomposition of amino-acid over

Table 1. *Effect of high-voltage cathode rays on aqueous solutions of L-histidine hydrochloride hydrate*

Dosage (r.e.p.)	Percentage retention of amino-acid in solutions with concentrations per μg . per ml. of			
	100 (pH 4.71)	250 (pH 4.48)	500 (pH 4.34)	1000 (pH 4.18)
Control (non-irradiated)	100.0	100.0	100.0	100.0
100,000	44.5	52.4	66.8	80.7?
250,000	17.5	35.9	61.3	62.7?
500,000	6.0	29.2	53.2	64.3
1,000,000	0.0	12.4	28.2	43.1

Table 2. *Effect of high-voltage cathode rays on aqueous solutions of L-histidine monohydrochloride hydrate (100 μg ./ml.)*

Dosage (r.e.p.)	Percentage retention of histidine as determined					
	Chemically			Microbiologically		
	Run I	Run II	Average	Run I	Run II	Average
100,000	58.8	60.3	59.6	48.4	51.3	49.9
250,000	38.9	31.5	35.2	21.1	20.2	20.7
500,000	20.0	12.3	16.2	8.0	7.2	7.6
1,000,000	7.0	1.3	4.2	0.0	0.0	0.0

Table 3. *Deamination of histidine monohydrochloride (100 μg ./ml. in 0.1 N-HCl) by irradiation with high-voltage cathode rays*

Dosage (r.e.p.)	Ammonia N (μg ./ml.)		NH_3 (μg ./ml.)
	Replicates	Average	
100,000	3.00, 2.82, 3.60, 3.48	3.22	3.90
250,000	5.31, 5.25, 5.70, 5.70	5.49	6.66
500,000	7.35, 7.32, 7.38	7.35	8.92
1,000,000	9.36, 9.46, 9.06, 9.12	9.25	11.23

the range of concentrations studied was related exponentially to the dosage. This was found to be a first-order reaction. With an equal dosage of irradiation, the dilute solution was relatively more affected than the more concentrated solutions. This observation demonstrates the 'dilution phenomenon' in radiochemical reactions.

Fig. 1. Ultraviolet absorption spectra of aqueous solutions of L-histidine hydrochloride hydrate (100 µg./ml.). Cathode-ray dosage: curve A, control (non-irradiated); curve B, 100,000 r.e.p.; curve C, 250,000 r.e.p.; curve D, 500,000 r.e.p.; curve E, 1,000,000 r.e.p.

Dale, Gray & Meredith (1949) have stated that if the action of ionizing radiation is indirect, one would expect the ratio of inactivation dose D (energy required to reduce the amount of solute to 37% of the initial value) to concentration (C), which they defined as the specific inactivation dose (D/C), to be independent of concentration over a wide range. Table 4 gives the specific inactivation doses of histidine at four concentrations, as calculated from the data in Table 1. The calculations were made from the following equation

$$n = n_0 e^{-D/D_0},$$

where n_0 is the initial concentration of the solute, n is the concentration of the solute that survives a dose D , and D_0 is the dose required to reduce the solute to 37% of its initial concentration ($1/e$). The values in Table 4 show that the specific inactivation dose (D/C) is almost independent of concentration within the range of concentrations studied. The above observations demonstrate that the action of high-voltage cathode rays on aqueous solutions of histidine is indirect.

When the concentration of the solute is low, it is possible that an appreciable proportion of the total number of active radicals may recombine with one another rather than react with solute molecules. This situation will not arise in concentrated solutions and the activated radicals will have a greater chance to react with the solute molecules before they can recombine to form water. It is seen from Table 4

Table 4. *Decomposition of histidine by high-voltage cathode rays*

Concentration of solution, C (g./ml.)	Inactivation dose, D (r.e.p.)	Specific inactivation dose, D/C (r.e.p./g./ml.)
1.0×10^{-4}	1.34×10^5	1.34×10^9
2.5×10^{-4}	3.21×10^5	1.29×10^9
5.0×10^{-4}	5.69×10^5	1.14×10^9
10.0×10^{-4}	11.60×10^5	1.16×10^9

that the specific inactivation dose does show a trend towards increasing values with decreasing concentration of solute, indicating that there may be some loss of radicals either by recombination or in other ways when the more dilute solutions are irradiated.

Ionic yield of the decomposition of histidine. From the data shown in Table 1, the ionic yields over a range of concentrations have been calculated, and the values are presented in Table 5. In the calculation of ionic yields, 1 r.e.p. is considered to produce 1.6×10^{12} ion-pairs/g. of tissue (Dainton, 1948).

Table 5. *Ionic yields of the decomposition of histidine monohydrochloride by high-voltage cathode rays*

Concentration of solution (mg./ml.)	Dosage (r.e.p. $\times 10^5$)	Ionic yield M/N
0.1	1.0	1.00
	2.5	0.59
	5.0	0.34
0.25	1.0	2.14
	2.5	1.16
	5.0	0.64
	10.0	0.39
0.50	1.0	3.00
	2.5	1.40
	5.0	0.84
	10.0	0.64
1.0	1.0	3.40
	2.5	2.62
	5.0	1.29
	10.0	1.02

The overall ionic yields of histidine ranged from 0.34 to 3.40. At a given dosage the ionic yield increased with concentration. This observation is typical of radiochemical reactions, to which the indirect action theory may be applied and in which the amount of solute remaining unchanged is exponentially related to the dose. Lea (1947) considers that activated water is formed at the rate of 1 mol.

(one effective radical pair) per ion-pair and that reaction yields smaller than this are due to the proportion of collisions between reactant molecules and activated water leading to deactivation without reaction.

The data given in Table 5 demonstrate the high ionic yields in concentrated solutions. In view of the mechanism suggested by Miller (1948) and by Dainton (1948), the high ionic yields of the decomposition of histidine monohydrochloride can be explained by assuming the formation of several pairs of active radicals per ion-pair.

Evidence of rupture of the imidazole ring of histidine. In the colorimetric method used for the determination of histidine, colour develops on treating histidine with *p*-diazobenzenesulphonic acid. Pauly proposed the following structure for the coloured complex:

If the ring is broken, histidine cannot be estimated by this method.

Inspection of Table 2 shows that the residual amino-acid, as measured colorimetrically, diminished progressively with increasing dosage of irradiation. A comparison of these values with those obtained microbiologically shows that the microbiological values are lower than the chemical values in every case. This suggests that the microbiological assay measures the change both in the ring part of the molecule and the side chain containing the α -amino group.

Table 3 shows the yields of ammonia liberated from histidine solutions containing 100 $\mu\text{g./ml.}$ Upon irradiation with a dose of 1,000,000 r.e.p., 11.23 $\mu\text{g.}$ of $\text{NH}_3/\text{ml.}$ were obtained. Microbiological assay showed in every case that no amino-acid was retained at this dosage of irradiation. If only the α -amino group were split off, 100 $\mu\text{g.}$ of histidine monohydrochloride hydrate (mol.wt. = 209.64) would give rise to 8.11 $\mu\text{g.}$ of ammonia, whereas 11.23 $\mu\text{g.}$ of $\text{NH}_3/\text{ml.}$ were obtained. This signifies that ammonia nitrogen is formed from the nitrogen atoms present both in the ring and in the side chain. This is further evidence that the imidazole ring of histidine is broken upon irradiation with cathode rays.

SUMMARY

1. Aqueous solutions of histidine monohydrochloride containing 100, 250, 500 and 1000 $\mu\text{g./ml.}$ were irradiated with high-voltage cathode rays at four levels, namely, 100,000, 250,000, 500,000 and 1,000,000 r.e.p. Histidine was decomposed at all concentrations, and the decomposition was related exponentially to the dosage of irradiation.

2. The ionic yields ranged from 0.34 to 3.4 over the range of concentrations studied.

3. Data are presented to show that the action of high-voltage cathode rays on histidine is indirect.

4. It is concluded that cathode rays decompose histidine through deamination of the α -amino group and the fission of the imidazole ring.

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