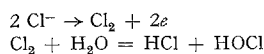


The mortality with platinum, an inert metal, was low and might be due to the toxicity of the salt (BaCl_2) or to HCl and HOCl formed as a result of reaction between chlorine and water molecules. The highest mortality with



BaCl_2 (Figure 3) might be due to the toxicity of barium because the control with BaCl_2 alone showed 18% mortality.

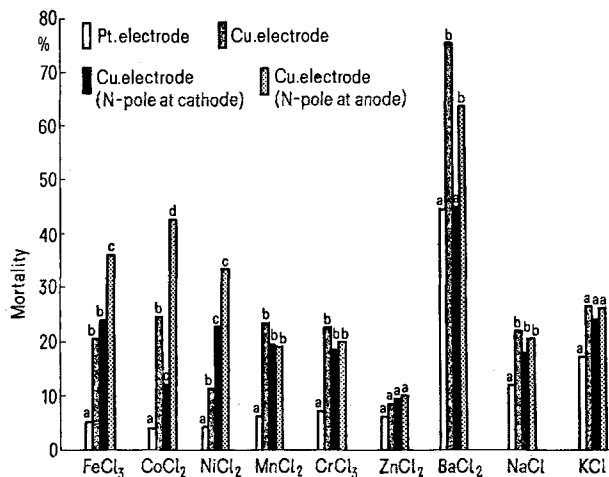


Fig. 3. Nematode mortality resulting from electrolysis of 9 salt solutions at $5 \times 10^{-3} \text{ g lit}^{-1}$ concentration for 2 h using Cu- and Pt-electrodes with and without magnetic field. Controls for each electrolyte excepting BaCl_2 : 1. without electric and magnetic field – no mortality in 2 h; 2. with magnetic field alone – no mortality in 2 h. 18% mortality in BaCl_2 in both 1 and 2. a, b, c, d, different letters indicate significant difference in mortality at 0.01 level within the graph for an electrolyte.

The average percentage mortality under crossed fields (E H) was the same as under E H.N⁺ and would therefore stand for both the situations in Figure 3. In solutions of ferro-magnetic salts there was a significant increase (at 0.05 level) in nematode mortality in both electric and magnetic fields (E H, E H.N⁺). In CoCl_2 there was a significant decrease (at 0.05 level) in mortality under E H.N⁻. No nematodes died in the control of 4.

The rate of chemical reaction is altered under the influence of magnetic fields⁹⁻¹². BHATNAGAR and MATHUR⁹ suggested that the altered rates in a number of chemical reactions were caused by a stirring effect of the magnetic field on the solution as well as an alignment of paramagnetic moments of the molecules involved. In the present experiment biomagnetic effects were observed only in case of ferro-magnetic salts and, as such, could be ascribed to the alignment of *para*-magnetic moments of the molecules which might have increased the velocity of electrochemical reactions.

⁹ S. S. BHATNAGAR and K. N. MATHUR, *Physical Principles and Applications of Magneto-Chemistry* (Macmillan and Co., London 1935), p. 327.

¹⁰ I. L. MULAY and L. N. MULAY, in *Biological Effects of Magnetic Fields* (Ed. M. F. BARNOOTHY; Plenum Press, New York 1964), p. 146.

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Degradation of Phenylalanine in the Presence of Hydrogen Peroxide

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Summary. UV-irradiation of phenylalanine by 253.7 nm light in the presence of hydrogen peroxide formed 5 ninhydrin reactive products and ammonia. Four of them were identified as aspartic acid, serine, alanine and lysine.

Stable and unstable substances which produce chemical effects have been shown to be formed by ultraviolet (UV) light. One such substance is hydrogen peroxide formed during UV-irradiation of nicotine in the presence of methylene blue². FERRARI and PASSERA³ have shown the formation of serine by UV-irradiation of aspartic acid. They suggested that the hydroxyl radical for its formation comes from hydrogen peroxide, produced by UV action on oxygen present in solutions.

We noticed different behaviour of hydrogen peroxide on UV-irradiation. Exposure to 253.7 nm light results in its disappearance and the same trend was noticed when irradiated in the presence of citrulline and arginine. However, in the presence of lysine, tyrosine and phenylalanine, the amount remained unchanged with varying radiation doses. In view of these observations, an investigation of the effect of 253.7 nm light on aqueous solutions of phenylalanine in the presence of hydrogen

peroxide was undertaken. Special emphasis has been laid on the separation and identification of amino acids thus formed.

A short wavelength UV-lamp Spectroline (manufactured by Black Light Eastern, Inc. USA, model R-51) having maximum emission at 253.7 nm and intensity $155 \mu\text{w}/\text{cm}^2$ at a distance of 18" was employed as radiation source. Aqueous solution of 2 mM phenylalanine were exposed to UV as previously reported⁴. To study the effects of hydrogen peroxide, equal amounts (v/v) were

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Formation of amino acids on UV-irradiation (28.28×10^7 ergs/cm²) of phenylalanine in the presence of hydrogen peroxide

Amino acid	Peak number (Fig. 1)	Amount (μ moles)
Aspartic acid	1	84
Serine	2	136
Alanine	3	159
Lysine	5	30

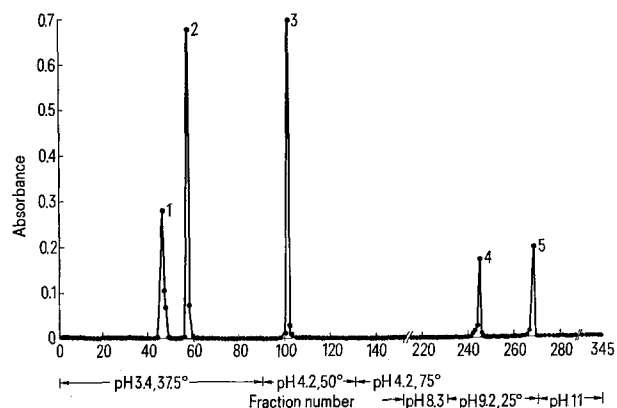


Fig. 1. Ion exchange chromatographic profile of UV-irradiated (28.28×10^7 ergs/cm²) phenylalanine in the presence of hydrogen peroxide.

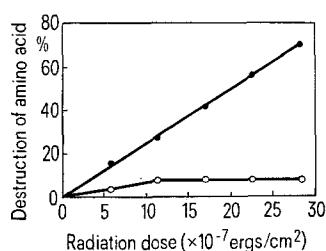


Fig. 2. Destruction of phenylalanine on UV-irradiation in the presence (●—●) and absence (○—○) of hydrogen peroxide.

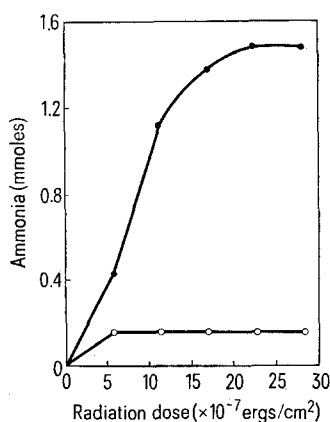


Fig. 3. Formation of ammonia from phenylalanine on UV-irradiation in the presence (●—●) and absence (○—○) of hydrogen peroxide.

added to the amino acid solution. The actual amount of hydrogen peroxide was estimated by iodometry⁵, its concentration was maintained at 1.87 mg/ml unless otherwise mentioned. Proper controls were included in each set of experiments. Descending paper chromatography on Whatman No. 1 paper was employed for separation of phenylalanine and its UV degradation product using *n*-butanol/acetic acid/water (60:15:25) as the developing solvent.

Ion exchange chromatography on Dowex 50W-X8 resin was the basic method used in the separation of amino acids essentially by the procedure of MOORE and STEIN⁶. The separation was carried out on a 0.9×100 cm jacketed column with the sequence of buffers and temperature as shown in Figure 1. In each of the 345 fractions, 2.0 ml were collected at the rate of 1.0 ml/6 min. The amount of amino acids was estimated by modified ninhydrin reagent⁷. To confirm the identities of the amino acids formed, dinitrophenol (DNP) derivatives were prepared essentially by SANGER's method⁸ and were separated by paper chromatography using pyridine/isoamyl alcohol/1.6 *N* ammonia (6:14:20) as the developing solvent. DNP derivatives of reference amino acids were prepared by the same procedure. The formation of ammonia was estimated by Nessler's reagent as previously reported⁹.

UV-irradiated phenylalanine in the presence of hydrogen peroxide (28.28×10^7 ergs/cm²) gave 5 ninhydrin reactive spots of *R_f* values 0.16, 0.25, 0.30, 0.42 and 0.52 besides phenylalanine (*R_f* 0.63). When the irradiated samples subjected to ion exchange chromatography, 5 distinct peaks were obtained (Figure 1). 4 peaks could be identified on the basis of elution volume as well as by preparing their DNP derivatives, running authentic DNP amino acids as standards. The products identified are aspartic acids, serine, alanine and lysine corresponding to peaks No. 1, 2, 3 and 5 respectively. Peak No. 4 was not identified. Alanine, serine and aspartic acid are formed in appreciable amounts, while lysine accounts for only a small fraction (Table). Estimation of phenylalanine by ninhydrin reagent revealed that the destruction of amino acid was several fold when irradiated in the presence of hydrogen peroxide (Figure 2). Irradiation without hydrogen peroxide was almost ineffective. Ammonia was the other product formed as a result of UV-irradiation of phenylalanine, its rate of formation increasing considerably when hydrogen peroxide was added (Figure 3).

The mechanism suspected to be involved in the photodegradation of citrulline and arginine in the presence of hydrogen peroxide seems to be different from the mechanism operating in the degradation of phenylalanine. In this case, the excited hydrogen peroxide molecule transfers a major portion of its absorbed energy to the substrate which may cleave a number of bonds, producing several radicals and thus forming a number of products. If any part of the energy is retained, this is not sufficient to photolyze the hydrogen peroxide molecule. This is the reason why during photolysis of phenylalanine, hydrogen peroxide was not consumed at all, irrespective to its amount and radiation dose. The formation of alanine and serine might be due to the participation of the alanine side chain, while lysine and aspartic acid seem to be products of ring rupture.

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