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Effects of Soft X-rays on Urease and Catalase.

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Introduction. Many investigators have reported that X-rays had little or no effect on enzymes. Scott[1] concluded that enzymes are inactivated only when the dose of X-rays is enormous. On the other hand it is well known that cell division is inhibited by relatively small doses. Most of the work prior to 1937 was carried out on impure enzyme solutions and the results are inconsistent with a recent report by Dale[2] to the effect that polyphenol oxidase and carboxypeptidase are inactivated by hard X-rays in dilute solution of the pure enzymes.

The work described below was designed to answer the following questions: (1) To what extent is the susceptibility of enzymes to X-rays dependent upon the purity of the preparation? (2) Does the susceptibility vary with the concentration of the enzyme? The answer to these questions is necessary in order to evaluate the possible participation of enzyme destruction in the observed effects of X-rays on cells.

It can be said at the outset that the results show that crystalline preparations of urease and catalase are more easily inactivated than impure preparations of the same enzymes and that the susceptibility increases with dilution.

Procedure. Source of Radiation. The radiation was supplied by a gas X-ray tube having a copper target and window made of aluminum and cellophane. It was operated at 30 peak kv and 10 ma.[3] The shortest wavelength emitted was about 0.4 Ångström units, while the most intense part of the radiation was the K alpha line of copper which has a wavelength of 1.54 Ångströms. This region of the X-ray spectrum is of considerably longer wavelength than the one used in medical radiography and its energy is absorbed to a greater degree by matter. For this reason, one would expect that, having two sources each emitting the same energy, one in the long wavelength region and one in the short wavelength region, any

1 Scott, C. M., Medical Research Council, Great Britain, 1937, Special Series Report No. 223.

2 Dale, W. M., *Biochem. J.*, 1940, **34**, 1367.

3 Kersten, H., *Radiol.*, 1934, **23**, 60.

chemical or biological change due to X-rays would be more pronounced when caused by the long wavelength X-rays since more of the incident energy would remain behind in the substance irradiated. Thus it may happen that effects which are easily produced with long wavelength radiation may seem impossible of production with radiations used in medical radiography.

The enzyme solutions were irradiated in small glass cups placed in a special holder within a Dewar flask filled with crushed ice. The cup was covered with a cellophane window and the window exposed to the radiation at a distance of 3 cm from the focal spot of the target. The temperature within the irradiation chamber was 6-8°C as determined by a thermocouple arrangement. Five cubic centimeters of solution were irradiated at a time. In all cases the irradiated solutions were tested as soon as possible after irradiation and in each case the irradiated sample was measured against a control from the same dilution kept at the same temperature as the test sample.

Enzyme Preparation and Tests. Crude Urease. Ten grams of Arlco jack-bean meal were thoroughly mixed with 50 cc of distilled water in a beaker. The mixture was allowed to stand in the cold room at 4°C for 24 hours. The digest was then centrifuged from the debris at 4000 rpm in the cold. The supernatant fluid was then diluted to the desired concentration with distilled water. No stabilizers such as bisulfite were used. The solution was kept at 4°C when not in use. Fresh preparations were made daily.

Crystalline Urease. Samples of crystalline urease were prepared according to the method of Sumner[4] and recrystallized according to the method of Sumner and Hand.[5] Saturated suspensions in distilled water were stored at 4°C and diluted to a suitable concentration before use. The recrystallized samples of urease usually had an activity of 50,000-80,000 units per gram.

Method of Testing Urease Activity. Urease activity was measured by Sizer's method[6] of measurement of the rate of liberation of CO_2 from urea-phosphate solution.

Crude Catalase. The crude dioxane filtrates from beef liver and mother liquor immediately prior to dialysis for crystallization in the method of Sumner and Dounce[7] were used as "crude" catalase.

Crystalline Catalase. Crystalline catalase was prepared according to the method of Sumner and Dounce. The crystalline preparations usually had a Kat. f. = 17,000-37,000.

[4] Sumner, J. B., *J. Biol. Chem.*, 1926, **69**, 435.

[5] Sumner, J. B., and Hand, D. B., *J. Biol. Chem.*, 1928, **76**, 149.

[6] Sizer, I. W., *J. Gen. Physiol.*, 1939, **22**, 719.

[7] Sumner, J. B., and Dounce, A. L., *J. Biol. Chem.*, 1939, **127**, 439.

Method of Testing Catalase Activity. The test solution and all the reagents were cooled to 4°C and maintained at that temperature during the test. 0.5-1.0 cc of catalase solution was added to 35 cc 0.02 N H_2O_2 and 10 cc of M/15 phosphate buffer (pH = 6.8) and 5 cc distilled water. After a specified time 5 cc of 10 N H_2SO_4 were added and the residual peroxide was titrated with 0.02 N $KMnO_4$.

Results. Urease. Table I shows that irradiation for 20 minutes of urease solutions *below certain concentrations* resulted in inactivation, the percentage of which (1) increased with dilution for all crystalline preparations but (2) was relatively greater, *at the same concentrations,* for solutions made from highly active than from partially inactive material.

TABLE I.

Effect of Dilution of Crystalline Urease Solutions on Sensitivity to Soft X-ray Irradiation for 20 Minutes.

Activity before irradiation *Units per cc	Activity after irradiation Units per cc	% inactivation
†31	31	0
15.5	15.5	0
3.1	1.24	60
1.5	0.30	80
‡12	12	0
6.0	6.0	0
1.2	0.79	34
0.6	0.22	63

*1 urease unit = amount of enzyme which will liberate 1 mg ammonia N in 5 min. at 20°C.

†Prepared from crystals having 87,000 units per g.

‡Prepared from crystals having 28,000 units per g.

Urease solutions containing 1.5 units per cc were prepared from crude preparations and from crystals of high activity (87,000 units per gram). Eighty percent of the crystalline preparation was inactivated in 20 minutes whereas it required 70 minutes to produce the same degree of inactivation in the crude enzyme (Fig. 1). A similar difference in the reaction of crude and crystalline urease to oxidation and reduction reagents has been observed by Sizer and Tytell.[8] Crystalline urease responded to the oxidation and reduction reagents whereas the crude preparations were unaffected.

Catalase. The results show that catalase is less radiosensitive than urease to soft X-rays. Crude preparations did not show inactivation in either dilute or concentrated solution even after 8 hours' irradiation.

[8] Sizer, I. W., and Tytell, A. A., *J. Biol. Chem.*, 1941, **138**, 631.

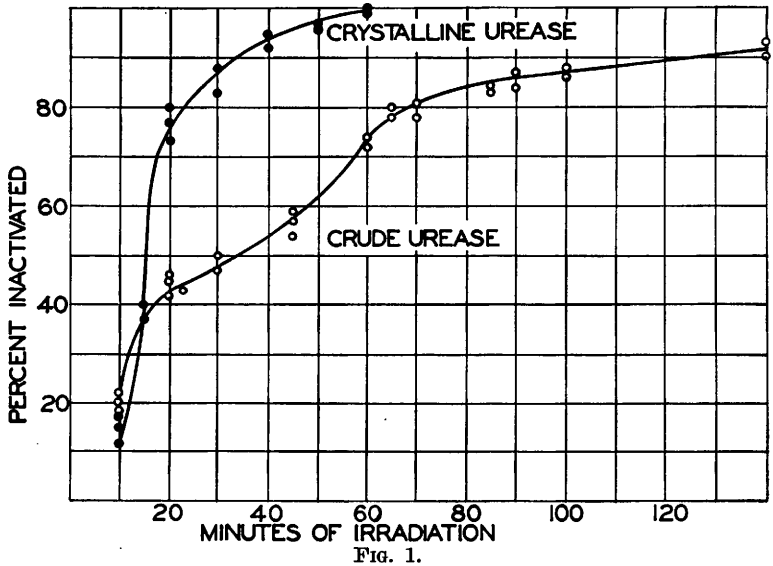

FIG. 1.

Time-inactivation curve for crude and crystalline urease solutions irradiated with soft X-rays.

Crystalline preparations were partially inactivated by large doses of radiation. Preparations made from crystals of high catalase activity were more easily inactivated than those made from crystals exhibiting lower activity (Table II). Sensitivity increased with dilution (Fig. 2). Except for the fact that catalase was less sensitive to X-rays, the results are similar to those obtained with urease.

Discussion. As a working hypothesis to explain the insensitivity of concentrated and impure enzyme solutions it may be reasonable to assume that the groups sensitive to X-rays are reversibly combined with other groups whereby they become stabilized. If the group-

TABLE II.

Sensitivity of Solutions of Crystalline Catalase Preparations of Varying Activities to Soft X-ray Irradiation for 4 Hours.

* Kat. f.	Dry solids of irradiated solutions, mg per cc	% inactivation
17,228	2.8†	0.0
27,337	3.1†	18.0
36,320	3.3†	23.0
36,320	0.66‡	52.5

*Kat. f. (Katalase fähigheit) is the ratio of k, catalytic activity expressed in terms of the monomolecular rate constant, to the total enzyme solids in reaction mixture. Kat. f. was always determined on the freshly prepared crystals and suitable dilutions made for test.

†These solutions were irradiated at a dilution of 1:25 by volume of the saturated preparation. Concentrated preparation in Fig. 2 was prepared in this manner.

‡This solution was prepared by diluting the 1:25 solution 5 times by volume, *i. e.*, final dilution 1:125.

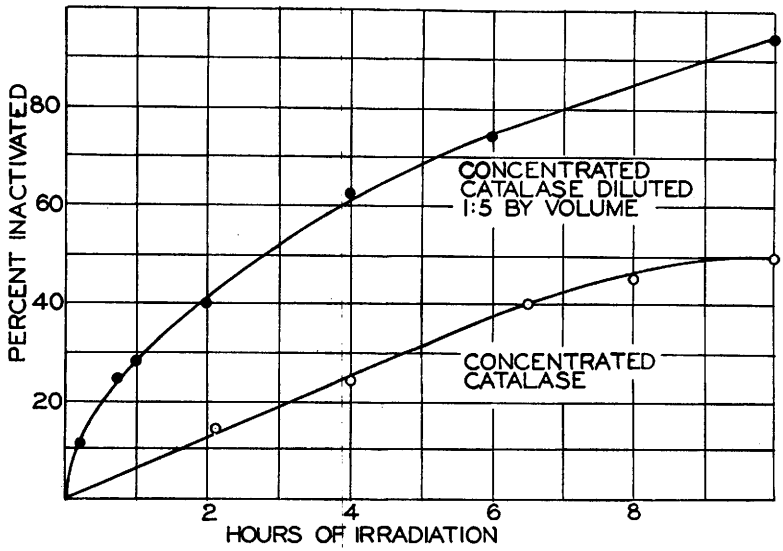

FIG. 2.

Effect of dilution on the time-inactivation curve of crystalline catalase solutions irradiated with soft X-rays.

ings on the enzyme are susceptible to X-rays only when dissociated, then this is consistent with the observed enhancement of sensitivity on dilution.

Estimation of the sensitivity of an enzyme within a cell to X-rays would then be contingent upon an understanding not only of the concentration but of the extent to which the enzyme is freely dissociated in the cell.

Summary. 1. The effects of soft X-rays on crude urease, crystalline urease, crude catalase, and crystalline catalase are described. 2. The sensitivity of the enzymes to the radiation is dependent upon purity and concentration. 3. Results are given which are in accordance with the observations of Dale on the effects of hard X-rays on polyphenol oxidase and carboxypolypeptidase.