THE EFFECT OF RADIOACTIVE RADIATIONS AND X-RAYS ON ENZYMES.

IV. The Effect of Radiations from Radium Emanation on Solutions of Invertase.

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In our studies[1,2] concerned with the effect of the radiations (beta and gamma) from radium emanation, in equilibrium with its radioactive products, on enzymes in solution we have observed that trypsin and pepsin are inactivated. It has been possible to follow the course of these radiochemical reactions quantitatively; and, under the conditions of experiment employed by us, we have found that the change in the logarithm of the concentration of active enzyme is a linear function of the product of two variables, namely, the average activity or power of the radioactive source, P_a, expressed in terms of the unit curie-power,[3] and the time of exposure, t, expressed in hours. This product has the dimensions of energy and represents a single variable quantity which we designate by W and express in terms of the energy unit curie-power hour; *i.e.*, $P_a t \equiv W$. The relation between the chemical change observed and the variable W is simply expressed by the equation

$$\log Q - \log Q_0 = -kW \tag{1}$$

where Q_0 represents the initial concentration of active enzyme, and Q the concentration of active enzyme after an irradiation for a given increment of energy, W. The concentration terms are expressed in arbitrary units. The logarithms are to the base e.

[1] Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1922–23, v, 647.

[2] Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1923–24, vi, 1.

[3] Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1923–24, vi, 7.

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In order to determine whether the mode of behavior described in the case of trypsin and pepsin when irradiated with the radiations mentioned is a fact of wider application we have thought it desirable to extend our observations by similar experiments with another enzyme. The selection of a third system was influenced by a desire to employ some other type of enzyme than a proteolytic one, but one for which precise methods were available for determining its activity. It has been our good fortune to obtain from Professor J. M. Nelson a preparation of invertase for the purpose of our experiments.

Experimental Results.

The invertase preparation employed in our experiments is designated as Normal Invertase 8. For details regarding its preparation and general characteristics the reader is referred to papers by Professor Nelson and his coworkers.[4–7] The concentration of active invertase, Q, was determined from the relation $Qt = f_{(p)}$, where t is the time required for a given percentage inversion of sucrose, p.[8] Preliminary observations were made in which the inversion of two different preparations of sucrose was followed. One of these was obtained from the U. S. Bureau of Standards where it was prepared in accordance with the technique described by Bates and Jackson. The other preparation was Merck's Blue Label sucrose. No significant difference was observed in the form of the inversion curve[9] and consequently since

[4] Nelson, J. M., and Hitchcock, D. I., *J. Am. Chem. Soc.*, 1921, xliii, 2632.

[5] Nelson, J. M., and Vosburgh, W. C., *J. Am. Chem. Soc.*, 1917, xxxix, 790.

[6] Vosburgh, W. C., *J. Am. Chem. Soc.*, 1921, xliii, 219, 1693.

[7] Nelson, J. M., and Born, S., *J. Am. Chem. Soc.*, 1914, xxxvi, 393.

[8] In order that the symbols employed throughout this series of papers be consistent we have employed Q to represent the enzyme concentration instead of n as used by Nelson and Hitchcock.[4] One unit concentration of invertase ($Q = 1$) was arbitrarily taken as the concentration of a solution half the strength of the stock solution. 5 cc. of such a solution in 100 cc. of sucrose reaction mixture gave an average value for n of 1.78×10^{-2}. Accordingly the n value of the original solution (50 per cent of stock) must have been 3.56×10^{-1}, which is equal to one arbitrary Q unit.

[9] It is of interest to note that after the destruction of part of the invertase the form of the sucrose inversion curve was not altered although the rate of inversion was reduced.

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Merck's preparation is more readily available it has been used throughout the experiments to be reported in this paper.

In all of our experiments the composition of the reaction mixture was as follows:

Sucrose concentration..........	10 gm. per 100 cc.
Buffer (acetate) concentration....	0.01 molar
Invertase concentration.........	5.00 cc. of invertase dilution (50 per cent by volume) per 100 cc.

In each experiment a control test was run simultaneously with each test for determining the effect of irradiation on the invertase. The progress of the sucrose inversion was followed in all essential details in the manner fully described by Nelson and his coworkers. For these details the reader is referred to the papers already mentioned by these investigators.

The procedure followed in irradiating the samples of invertase was similar to that described by us for pepsin.[2] The radium emanation was contained in a small glass bulb and the amounts employed varied between 100 and 500 millicuries.

EXPERIMENTAL RESULTS.

Several experiments were made in which 8.12 cc. of a 50 per cent dilution of the stock invertase were irradiated in a spherical glass bulb as previously described for the irradiation of pepsin in solution, the value of W being varied. The results obtained from such an experiment are shown in Table I, where it will be observed that the value of the mean reaction speed coefficient is sensibly constant, as is required to satisfy the conditions stated in equation (1). Thus it is evident that the principles established for the radiochemical inactivation of trypsin and pepsin apply equally well to invertase.

So far in our experiments with trypsin and pepsin[1,2] we have reported only the results obtained where the volume of enzyme solution irradiated was constant. In this paper we present in addition the results of experiments wherein this volume was varied. If, as we have assumed to be the case in our previous experiments,[1] the layer of fluid around the emanation bulb is of sufficient thickness to absorb practically all of the beta radiation, and the speed of diffusion is great

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enough to maintain uniform concentration of enzyme throughout the liquid system, and in addition that the effect of the gamma radiation is negligible; it would be expected that the rate of change in the logarithm of Q with respect to W would vary inversely with the volume, *i.e.*

$$-\frac{d \log Q}{dW} = \frac{K}{V} = k, \text{ whence } K = kV \tag{2}$$

TABLE I.

Curie-power hours. (W)	$\frac{Q}{Q_0}$	$k = \frac{1}{W} \log_e \frac{Q_0}{Q}$ $k \times 10^2$
3.845	0.821	5.01
8.014	0.684	4.75
10.000	0.615	4.87
12.000	0.075	4.61
		Mean $k = 4.81 \times 10^{-2}$ a.d. $= \pm .16$

TABLE II.

Effect of Variation of the Volume of Enzyme Solution Irradiated.

Volume. (V)	Curie-power hours. (W)	$\frac{Q}{Q_0}$	$k = \frac{1}{W} \log_e \frac{Q_0}{Q}$ $k \times 10^2$	(kV) $K \times 10$
cc.				
4.59	9.000	0.544	6.77	3.11
8.12	9.000	0.678	4.33	3.51
11.88	9.000	0.771	2.90	3.45
18.34	9.000	0.832	2.05	3.76
			Mean $K = 3.46 \times 10^{-1}$ a.d. $= \pm .18$	

where V is the volume of solution irradiated, and K is a constant. In Tables II and III the results of two such experiments are given,[10]

[10] For these experiments spherical glass bulbs were made. The volume of the bulb was determined by making a pipette to deliver a volume of water sufficient to just fill the container when the emanation bulb was placed in position. The pipette was then calibrated by weight with distilled water.

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where the values obtained for K are shown. The agreement obtained between these values appears to be significant.

In the experiments described above the average power of the radioactive source lay between 100 and 550 millicurie-powers.

TABLE III.

Effect of Variation of the Volume of Enzyme Solution Irradiated.

Volume. (V)	Curie-power hours. (W)	$\frac{Q}{Q_0}$	$k = \frac{1}{W} \log_e \frac{Q_0}{Q}$ $k \times 10^2$	(kV) $K \times 10$
cc.				
4.59	5.089	0.708	6.82	3.13
8.12	9.000	0.653	4.74	3.85
11.88	13.17	0.681	2.93	3.48
18.34	20.34	0.650	2.11	3.87
			Mean $K = 3.58 \times 10^{-1}$	
			a.d. $= \pm .28$	

CONCLUSION.

The radiochemical inactivation of invertase by beta radiation from the radioactive products in equilibrium with radium emanation can be explained quantitatively on the same basis as that of trypsin and pepsin previously reported; namely, the rate of change in the logarithm of the concentration of the active enzyme with respect to the variable, W, is constant, under the conditions of irradiation described, when the volume of solution exposed is constant. When, within the limits stated in this paper, this volume (V) is varied, the rate of radiochemical change is inversely proportional to V; *i.e.*,

$$-\frac{d \log Q}{dW} = k = \frac{K}{V}$$

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