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

V. THE INFLUENCE OF VARIATION OF THE THICKNESS OF THE ABSORB- ING LAYER OF SOLUTIONS OF PEPSIN UPON THE RATE OF RADIOCHEMICAL INACTIVATION OF THE ENZYME.

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Some time ago we commenced an investigation of the effect of the radiations from the radioactive products in equilibrium with radium emanation upon enzymes in solution. In our first experiments we irradiated dilute solutions of trypsin and found that definite inactivation resulted. We succeeded in following the course of this radiochemical change and found that the rate of change in the logarithm of the concentration of active enzyme was proportional to the power of the radioactive source. The experimental arrangement employed in these experiments had many practical disadvantages and in addition it was not suitable to the study of the efficiency of power utilization in the irradiated system. A satisfactory method of procedure was found in the use of a spherical glass flask to contain the solution to be irradiated with the source of radiation in a smaller spherical glass bulb at its center. Under these conditions of irradiation the course of inactivation of trypsin¹ was followed with results in agreement with those found in the original experiments.² Our observations were then extended to a study of the radiochemical inactivation of pepsin³ and invertase⁴ where we observed results that were in agreement with those found in the case of trypsin.

¹ Unpublished experiments.

² Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1922-23, v, 647.

³ Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1923-24, vi, 1.

⁴ Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1925-26, ix, 211.

With these facts established we were in a position to study other important aspects of this type of radiochemical change. Our attention was directed to the consideration of the effect of varying the thickness of the liquid layer upon the value of the mean speed coefficient (k).

$$\frac{dQ}{dt} = -kQP \quad (1); \quad \text{or} \quad \frac{d \log Q}{dt} = -kP \quad (2)$$

where Q is the concentration of active enzyme (expressed in arbitrary units) at the time, t (expressed in hours from the start of the irradiation), and P is the power of the source at that same instant. The relation between these variables may be variously expressed as was indicated in a previous communication.⁵ A convenient form of expression for the purpose of computing the value of k is as follows:

$$k = \frac{1}{W} \log_e \frac{Q_0}{Q}$$

where $W = \int_0^t P dt$ and Q_0 is the initial concentration of active enzyme.

As has been shown by our previous observations, when the volume of the irradiated system (in the spherical arrangement described above) is constant, the value of the speed coefficient is also constant. As this relation holds for any of the enzymes studied we are at liberty to choose any one of them for the present purpose. Our first attempt was made with invertase as a matter of convenience. We found that over a certain range of variation the product of the values of the speed coefficient obtained and the volume of solution irradiated was approximately a constant.⁴ Unfortunately, the determination of speed coefficients at small volume could not be made satisfactorily with the invertase preparation then available. The results obtained indicated that the effect of the gamma radiation was negligible with respect to that of the beta radiation; but, however, from these results no definite information could be obtained in regard to what might be considered the lower limit of liquid layer for sensibly complete absorption of beta radiation by the radiosensitive system. Since this phase of our

⁵ Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1923-24, vi, 7.

study is of considerable importance to our investigation as a whole we have employed pepsin in an attempt to gain this information and to confirm the results obtained with invertase. It is the purpose of this communication to present the results obtained.

Experimental Procedure.

The pepsin solution was prepared by dissolving 3.125 gm. of granular pepsin in distilled water containing 2.50 cc. of 0.1 M HCl, diluting to 250 cc. in a volumetric flask with distilled water, filtering, and add-

TABLE I.

Pepsin solution irradiated. Volume (V).	Thickness of layer (d).	Curie-power hours. (W)	Units of active pepsin. (Q)	$k = \frac{1}{W} \log_e \frac{Q_0}{Q}$	$K = kV$
cc.	cm.		$Q_0 = 2.913 \pm 0.015$		
18.34	1.419	23.22	1.934 ± 0.023	0.0177	0.324
11.88	1.199	18.03	1.886 ± 0.012	0.0289	0.343
8.12	1.031	10.29	1.947 ± 0.017	0.0392	0.319
4.59	0.816	5.811	2.024 ± 0.017	0.0627	0.288
2.83	0.661	3.572	1.985 ± 0.036	0.108	0.304
1.26	0.454	1.600	2.262 ± 0.027	0.158	0.199
1.26	0.454	1.600	2.207 ± 0.029	0.174	0.218

The precision measure of Q is the a. d. The values of Q given are in each case the mean of eight determinations with the exception of the two results for the volume 1.26 cc. where the value given is the mean of four determinations. The value of Q_0 is the mean of 40 determinations made at different times distributed over the course of the experiment. The power of the radioactive source in these experiments lay between 550 and 50 curie-powers.

ing a crystal of Merck's Reagent Thymol to the filtrate. The pH of this solution was found to be 4.4 by colorimetric measurement. The stock (control) solution was kept in a pyrex flask (painted with asphaltum on the outside) in a thermostat at 10.0°C. Irradiations were performed in another thermostat regulated to $10.00 \pm 0.03^\circ\text{C}$. That the same form of curve is obtained for the radiochemical inactivation of pepsin at 10°C. as had been found at 0°C. was shown by a short series of irradiations, the results of which are given in another communication.⁶

⁶ Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1925-26, ix, 315.

The volumes of the spherical irradiation flasks used in the experiments were such that when the small radium emanation bulb (previously described⁴) was in position at their center they would contain 18.34, 11.88, 8.12, 4.59, 2.83, and 1.26 cc. respectively. Pipettes were made to deliver these volumes of solution, and calibrated gravimetrically. Obviously, when solution was placed in these flasks and the radium emanation bulb inserted in the center, the solution assumed the form of a spherical shell. The thickness (d) of this spherical shell has been previously referred to as the thickness of the liquid layer. It may be computed for any given irradiation flask from a knowledge of the outside diameter ($2a$) of the radium emanation bulb (in this case 0.432 cm.) and the volume of the spherical shell (V). The calculated values of d for the flasks mentioned above were 1.419, 1.199, 1.031, 0.816, 0.661, and 0.454 cm. respectively. The results of a series of irradiations with these flasks of pepsin solution at 10°C. is given in Table I where the values of the speed coefficient (k) are given and in addition the product of this value and the volume (V) of solution irradiated ($K = kV$).

The method of determining the active pepsin concentration was the same as we have employed in our previous experiments.³

DISCUSSION.

As has been indicated in the paper previously referred to,⁴ when the thickness of the liquid layer is sufficient to absorb practically all of the beta radiation, and the speed of diffusion is great enough to maintain uniform concentration of enzyme throughout the liquid system, and in addition the effect of the gamma radiation is negligible; it would be expected that the mean speed coefficient of the reaction would vary inversely with the volume (V), *i.e.*

$$-\frac{d \log Q}{P dt} = -\frac{d \log Q}{dW} = k = \frac{K}{V} \quad (5)$$

where K is constant over this range.

It will be observed in Table I that the values of the product kV are not significantly different when the thickness of liquid layer (d) lies between 0.661 and 1.419 cm.; *i.e.*, the differences are not significant

with respect to the precision measure. The region explored with invertase lay within this range (0.816 to 1.419 cm.) where satisfactory agreement was obtained between the values of K . Our present results, therefore, serve as a confirmation of those obtained with invertase. However, the values of kV obtained where $d = 0.454$ cm. do differ significantly from the others. If we assume that in this case the speed of diffusion was not significantly changed, and it seems reasonable to make this assumption, it follows that the fraction of the available energy absorbed by the system was decreased. Indeed, it may be remarked that a suggestion of a drift appears in the data in the direction of decreasing values for K with decreasing values of d as the value of d approaches 0.454 cm.; but this drift (it appears in the observations on invertase also⁴) cannot be considered as significant without the evidence of additional data, although it is in keeping with what might be expected, and approximately with what was anticipated at the beginning of these studies. It is suggested, furthermore, that, given proper experimental conditions, K may be employed as a measure of the absorbing power⁷ of an irradiated system.

CONCLUSION.

Evidence is presented which indicates: (1) that the effect of gamma radiation is negligible with respect to that of beta radiation upon pepsin in dilute solution under the conditions employed in the experiments made; (2) approximately the thickness of fluid layer which may be regarded as necessary and sufficient to practically completely absorb the available beta radiation; (3) that the mean reaction speed coefficient in radiochemical inactivation of pepsin varies inversely with the volume of solution irradiated if the thickness of the fluid layer satisfies the sufficient condition stated in (2), and beyond this as far as has been studied.

⁷ Duff, A. W., A text-book of physics, Philadelphia, 4th edition, 1916, 641.