THE EFFECT OF RADIATIONS FROM A MERCURY ARC IN QUARTZ ON ENZYMES

II. The Effect of Ultra-Violet Radiation on Amylase in Solution

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In an earlier report[1] we have given the results of experiments which are concerned with the effects of irradiation of solutions of pepsin with ultra-violet light, wherein these results were compared with similar effects of irradiation with radiations from radon and its radioactive products in dynamic equilibrium with it, wherein also were included studies with other enzymes, namely, trypsin and invertase. Under fixed conditions of irradiation, it was shown that inactivation of the enzyme took place in each instance studied and that the relation between the enzyme concentration, Q, after irradiation and that before irradiation, Q_o, could be approximated closely in all cases by the relation,

$$Q = Q_o \cdot e^{-k \cdot W} \tag{1}$$

where W is a variable proportional to the radiant energy liberated by the source of radiation during the irradiation interval, and k is a positive constant (dependent in each case upon the enzyme system used and upon the conditions of irradiation aside from those which determine the power of the source and period of irradiation). Where the power of the source is constant (or approximately so) then the time, t, of irradiation may be substituted for W in (1) in the general sense there employed, though it should be borne in mind that if a fixed energy unit system for W in a given case has been adopted, as, for example, in the case of the β-ray experiments previously reported,[2]

[1] Hussey, R., and Thompson, W. R., *J. Gen. Physiol.*, 1925–26, **9**, 217.

[2] Hussey, R., and Thompson, W. R., *J. Gen. Physiol.*, 1922–23, **6**, 7.

then a change to a proportional variable in place of W should be accompanied by a change in the value of k in inverse proportion. In the case of ultra-violet irradiation of pepsin, wherein the power of the source (a mercury arc in quartz) might be assumed, if not constant, at least to fluctuate so that t is approximately proportional to the energy liberated under the existing conditions of irradiation, we have shown a satisfactory fit of the results obtained to the relation

$$Q = Q_o \cdot e^{-k \cdot t}; \tag{2}$$

or, in differential form,

$$\frac{dQ}{dt} = -k \cdot Q \quad \text{or} \quad \frac{d \log Q}{dt} = -k, \tag{3}$$

which obviously implies a linear relation between the logarithm of the enzyme concentration and the duration of irradiation under such conditions; or, in general, with the variable W.

Recently, we have been concerned in this laboratory with the estimation of active amylase concentration by means of a viscosimetric method described in another communication[3] a modification of which is suggested in another report[4] from this laboratory by Wies and McGarvey. By means of this modified method we have studied the effects of radiations from a mercury arc in quartz upon amylase solutions.

EXPERIMENTAL PROCEDURES

The solutions were prepared from pancreatin in 0.85 per cent saline as previously described,[3,4] and the irradiation system was essentially the same as that previously employed in the experiments[1] with pepsin mentioned above. Enzyme was irradiated in the same flat bottomed cylindrical quartz tube (about 25 mm. inside diameter, 1 mm. in thickness, and 36 mm. long) placed vertically above a quartz window (approximately 3 mm. thick and 25 mm. in diameter) in the bottom of a thermoregulated water bath at 10.0 ±.15°C., the water of which was freshly distilled (being replaced at least once every 3 days). The same mechanical stirring device was employed to agitate the enzyme solution during irradiation for which the same mercury arc was employed, tilted at a fixed angle

[3] Thompson, W. R., Johnson, C. E., and Hussey, R., *J. Gen. Physiol.*, 1931–32, **15**, 1.

[4] Wies, C. H., and McGarvey, S. M., unpublished.

of 30° to the horizontal, and in a position about 19.0 cm. vertically beneath the quartz window of the bath. The amount of enzyme solution irradiated in the present experiments was 5 ml. A control portion of the same enzyme solution was kept in the same bath in a light-screened container.

The results of a number of such irradiations are given in Table I. Successive estimations upon the control solution showed that the rate of spontaneous inactivation was negligible with respect to the rate of the radiochemical change. Accordingly, Q_o is taken in each instance as the concentration of amylase in the control solution at the end of the irradiation interval. Precise estimates of the rate of spontaneous inactivation of amylase under the control conditions are not available, but it is estimated as about 10 per cent per day; and this is obviously

TABLE I

t (min.)	Q_o	Q	$\frac{Q}{Q_o}$	k' (min.)$^{-1}$	$k' - k$
1.03	10.45	8.60	0.823	0.189	−0.049
2.00	10.85	6.52	0.601	0.255	+0.017
4.00	9.66	3.85	0.399	0.230	−0.008
6.00	12.64	3.14	0.248	0.232	−0.006
9.00	12.11	1.38	0.114	0.241	+0.003

Taking the approximation, $k = 0.238$ min.$^{-1}$

negligible in the present experiments with respect to a radiochemical change of about 50 per cent in 3 minutes as observed (approximately 3000 times as great). In Table I will be found the corresponding values of t, Q_o, Q, and $\frac{Q}{Q_o}$ for each irradiation, together with k'—defined as the value of k calculated in each such instance from the formula of (2). The value of k obtained by fitting the curve given by

$$\log \frac{Q}{Q_o} - k \cdot t = 0 \tag{4}$$

to the observed points, $(\log \frac{Q}{Q_o}, t)$, by the method of least squares was found to be 0.2376 min.$^{-1}$. The differences between 0.238 and the observed values of k' are given in the same table, where it may be

seen that they decrease in *absolute* value with increase in t, as might be expected.

It may be noted, furthermore, that inactivation has been extended as far as 88 per cent change, approximately. In order to estimate Q in such cases of great change, a flexibility of the viscosimetric method previously described was utilized by replacement of the usual addition of 5 ml. of enzyme to 25 ml. of substrate solution (3 per cent starch substrate) by the addition instead of first x ml. of 0.85 per cent saline and then y ml. of enzyme solution (where $x + y = 5$) to the above amount of substrate. Q is calculated from the resulting value of T (the time in hours for 15.8 per cent change in viscosity as described[4]) for the given digestion curves by the formula

$$Q = \frac{5}{y \cdot T}. \tag{5}$$

DISCUSSION

In the earlier work[1] upon the effects of ultra-violet radiation upon pepsin in solution it was observed in two successive experiences that, although the relation (2) held, it was necessary to introduce different constants for k in each instance. This was supposed to be due to a decrease in the intensity of radiation incident to the irradiated solution. Care was taken in the present experiments as to elimination of and prevention of accumulation of impurities in the water which might induce such decrease in intensity of radiation. The consistent results obtained indicate that the required condition of sensibly constant ratio between the time of irradiation, t, and the energy increment was realized. However, in subsequent work temporary deviations were noted which may be due to variation in the potential difference of the lamp electrodes. Further work in this connection is in progress.

Direct comparison of sensitivity of pepsin and amylase solutions is made impossible in these results due to the lack of definite information as to radiation intensities, but it seems evident that amylase solutions are much more sensitive than are pepsin solutions, perhaps more than 50 times as sensitive.

Further work involving different aspects of the radiochemical

inactivation of amylase is in progress in this laboratory, one of the immediate results of which is a demonstration that sensibly complete protection (within the limits of tolerance of the present work) is given by interposition of a No. 1 Crookes Glass filter (1.7 mm. thick) between the quartz window and the enzyme solution.

SUMMARY

Amylase in solution is inactivated by the radiations from a mercury arc in quartz, in a manner similar to that previously reported for pepsin. The reaction was followed to a point where more than 88 per cent change had taken place, the course being that of monomolecular radiochemical change. Apparently, this reaction is due to the influence of ultra-violet radiation alone.