PROTECTIVE ACTION IN IRRADIATION OF AMYLASE SOLUTIONS WITH ULTRAVIOLET LIGHT

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In another communication[1] the monomolecular character of the inactivation of amylase (pancreatin solution) with ultraviolet light has been demonstrated; *i.e.*,

$$(1) \qquad \frac{dQ}{dt} = -k \cdot Q \quad \text{or} \quad \frac{d \log Q}{dt} = -k,$$

where Q is the active enzyme concentration,[2-4] t is time in minutes, and k is a constant for given irradiation conditions. It was found,[5] however, that the addition of small amounts of dog serum to pancreatin solutions gave marked protection (a speed coefficient, k, of about 1/2 the value for simple pancreatin solutions being obtained by addition of 0.2 per cent of serum). The nature of this protection, whether the result of chemical combination with enzyme in a more stable form or of simple screening of the part of the solution more remote from the source of radiations, is of particular interest. Certain relations of chemical combination with the enzyme, trypsin, have been studied by Hussey and Northrop;[6] but whether similar results can be obtained in the case of amylase has not been established. In further investigation we have obtained evidence of a protective

[1] Thompson, W. R., and Hussey, R., *J. Gen. Physiol.*, 1931–32, **15**, 9.

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

[3] Wies, C. H., and McGarvey, S. M., *J. Gen. Physiol.*, 1932–33, **16**, 221.

[4] Thompson, W. R., McGarvey, S. M., and Wies, C. H., *J. Gen. Physiol.*, 1932–33, **16**, 229.

[5] Thompson, W. R., and Tennant, R., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 510.

[6] Hussey, R., and Northrop, J. H., *J. Gen. Physiol.*, 1922–23, **5**, 335.

effect in pancreatin solutions themselves, which is similar to that resulting from addition of serum. A simple competitive absorption theory seems to account satisfactorily for this effect, at least as a first approximation.

The method of irradiation was essentially the same as employed in former work[1,5] except that a different lamp was used. As before,[1] solutions were stirred during irradiations in a cylindrical quartz tube at 10°C. Radiations entering the tube were first filtered through 5 mm. of water at 10°C. as has been described previously.[1] The source was a mercury arc in quartz, 200 mm. below a quartz window in the bottom of the bath. Except in two instances the potential difference across the lamp was maintained at 75 volts, and the current was approximately 4 amperes. In this way several (5) irradiations with a 0.02 per cent pancreatin solution as used in the former work,[1,5] except that a standard content[7] of 3/10 of the amounts of phosphate buffers required for pH = 6.7 was adopted, gave a mean speed coefficient, $\bar{k} = 0.259$ with a.d. = 0.004. In contrast to this an irradiation under the same conditions except that the P.D. was 70 volts gave $\bar{k} = 0.172$ and one at 80 volts gave $\bar{k} = 0.302$. The amount of solution irradiated in these instances was 5.00 ml. as in all former work. The proposed method of investigation was to observe the corresponding values of $\bar{k}$ obtained in irradiation of 10 ml. quantities where a *screening* protective action would be made evident by a decreased mean speed coefficient. Three such observations gave a mean $k = 0.196$ with a.d. = 0.008.

The screening protection here is obvious, but in order to obtain even stronger evidence of such protection we contrasted similarly results in which 5 and 10 ml. of a solution of 0.07 per cent pancreatin were irradiated; obtaining, respectively, $\bar{k} = 0.156$ (a.d. = 0.002) and $\bar{k} = 0.0907$ (a.d. = 0.0007) for two instances of each.

In order to ascertain whether the reduced speeds of reaction evi-

[7] All amylase solutions were made to contain and diluted with solvent containing 30 ml. per 100 of M/15 standard phosphate buffer mixture for pH = 6.7, as was the case already adopted for the substrate. Subsequent to this work an improved viscosimetric method was developed. Thompson, W. R., Tennant, R., and Wies, C. H., *J. Biol. Chem.*, 1935, **108,** 85; Thompson, W. R., *J. Biol. Chem.*, 1935, **109,** 201.

dent in the second 5 ml. layer is due to a screening by the first 5 ml. or to a divergence of the rays, two irradiations were performed in which 5 ml. of the 0.02 per cent pancreatin plus 5 ml. of the solvent[7] were irradiated together. Thus all of the concentrations were approximately halved except those of the phosphate buffers, H^+ and OH^-. The mean k so obtained was 0.257 (a.d. = 0.018); and a similar result with the 0.07 per cent pancreatin gave $\bar{k} = 0.147$. These approximate the values obtained with 5 ml. of the undiluted solutions which have been given (0.259 and 0.156, respectively).

That there is a definite protective effect due to screening is obvious. Moreover, we may outline a simple theory of homogeneous absorption which gives close approximation with the observed results.

Accordingly, we assume that (1) holds in any volume element wherein the effective radiation intensity is constant; that the rays are parallel; and, therefore, that a coaxial disk volume element of the cylinder of thickness, ds, approaches the condition of equal radiation intensity throughout its volume as a limit as ds approaches zero. Furthermore, we assume that stirring is sufficient to maintain the concentration of active enzyme, Q, the same throughout the entire liquid mass at any instant of time. If k_s is the mean speed coefficient in the infinitesimal layer of thickness, ds, the bottom of which is at a distance, s, above that of the whole cylindrical liquid system whose total altitude is s_α and mean speed coefficient is K_α; then, obviously,

$$K_\alpha = \frac{\int_0^{s_\alpha} k_s \cdot ds}{s_\alpha}. \tag{2}$$

So far nothing definite has been assumed about the function, k_s; but we shall assume that k_s is proportional to effective radiation intensity and that this varies in accord with a law of equal fractional absorption in traversing a given thickness of solution, *i.e.*

$$k_s = k_0 \cdot e^{-\lambda s}, \tag{3}$$

where λ is the absorption coefficient for the solution. In actual observations where non-homogeneous radiation is involved a high relative absorption rate is generally found in the early layers, but (3) may approximately represent the facts, and the representation may be

close for large values of λs even if not throughout the whole range. In our ideal system we assume (3) to hold throughout. Then (2) and (3) give

$$K_\alpha = \frac{\int_0^{s_\alpha} k_0 \cdot e^{-\lambda s} \cdot ds}{s_\alpha} = \frac{k_0(1 - e^{-\lambda s_\alpha})}{\lambda \cdot s_\alpha}. \tag{4}$$

Now, as in the case of 5 and 10 ml. irradiations,

(5) if $s_2 = 2s_1$, then

$$\frac{2K_2}{K_1} = \frac{1 - e^{-2\lambda s_1}}{1 - e^{-\lambda s_1}} = 1 + e^{-\lambda s_1},$$

whence, in this case,

$$\lambda s_1 = \log_e \frac{K_1}{2K_2 - K_1}. \tag{6}$$

Now, let a *prime* refer to the 0.02 per cent and a *double prime* refer to the 0.07 per cent pancreatin solutions, and assume that $\lambda'' = 3.5\ \lambda'$. Then we may estimate $s_1\lambda'$ from the data on the 0.02 per cent solution by (6) and k_0 by substitution in (4). From these values we may calculate the corresponding values of K_1'' and K_2'' which are given in the third column of Table I. Similarly, we may estimate $s_1\lambda'$ and k_0 from the observed values for Solution 2 (0.07 per cent pancreatin) and calculate K_1' and K_2' as given in the fourth column. The observed values are entered in Column 1. The two sets of calculated values are obtained by regarding the data from one or the other of the two solutions (0.02 or 0.07 per cent) as perfect. A better result might be expected if we use instead a weighted mean of the two estimates of $s_1\lambda'$ and the mean value of k_0 obtained by substitution of this in (4). Thus we may give 3.5 times as much weight to the estimate of $s_1\lambda'$ from the data from the second solution as that from the first (roughly according to relative precision) and so obtain the values given in Column 2. These agree best with the observed values, and those by the extrapolation method in Column 3 worst as might be expected; but all indicate that the theory outlined above may serve at least as a first approximation.

We have made a further study of the protective action of dog serum using two additional dogs. In the former work it was merely demon-

strated that a protective action existed, but in this work we hoped to show something of the mode of action as in the case of the pancreatin solutions alone. Accordingly, 5 and 10 ml. portions of solutions of pancreatin (0.02 per cent) containing 0.2 per cent of serum were irradiated with results given in Table II; and from these the respec-

TABLE I

Effects of Competitive Absorption upon the Speed Coefficient in Inactivation of Amylase with Ultraviolet Light

	Observed	Calculated (assuming $\lambda'' = 3.5\lambda'$)		
		By weighted mean	From Solution 1	From Solution 2
k_0	.....	0.343	0.355	0.331
K_1'	0.259	0.263	0.259	0.264
K_2'	0.196	0.208	0.196	0.211
K_1''	0.156	0.152	0.138	0.156
K_2''	0.0907	0.087	0.076	0.0907
$\lambda' s_1$	.....	0.553	0.667	0.520

TABLE II

A Protective Action of Dog Serum (2 Parts per 1000) in Ultraviolet Irradiation of Pancreatin Solutions

No.	Dog No.	Pancreatin	Q_0	$\frac{Q}{Q_0}$	k	V
		per cent				*ml.*
18	2	0.02	10.07	0.627	0.0780	5
19	2	0.02	10.33	0.645	0.0489	10
20	3	0.02	9.61	0.452	0.132	5
21	3	0.02	9.98	0.462	0.0773	10
22	3	0.02	9.42	0.278	0.0711	10
23	3	0.02	10.12	0.305	0.119	5

tive values for λs_1 were 1.37 and 1.70 and for k_0 were 0.107 and 0.261. That some of the protection is due to screening is obvious in the table, but if the theory applied to the pancreatin solutions above held here also, then we should expect the same value for k_0, which is not the case. However, this does not mean that we must account for some of the protection upon some other basis than screening; but indicates at

least that if screening alone is responsible then the *screening* or *filtering* properties of the system are not uniform but greater in the lower layers. If all substances were in solution this is almost inconceivable. However, it was due to the accumulation of a sediment in these solutions that experiments with any given preparation could not be prolonged, particularly in the case of Dog 2; and it is here that the lowest value of k_0 was encountered. Furthermore, a slight drift toward increasing protection with time is apparent in the case of Dog 3. Accordingly, it is reasonable to assume that we had somewhat greater screening protection in the lower layers, possibly sufficient to account for the low k_0 observed. The protective action of the serum of these two dogs was roughly the same as in the instance previously reported.

It is obvious in (4) that $2K_2 - K_1$ is the mean speed coefficient for the *second layer* (upper half of the irradiated system), and that if we could measure this directly we should obtain more reliable results. Thus a system such that the two *layers* could not mix would be more advantageous, or we might use our 5 ml. system in the quartz tube as an instrument for measuring the effective intensity of radiations not absorbed by an interposed layer of solution of known thickness. The superiority of such a method over others such as the employment of a thermopile is obvious as is also the futility of dependence upon absorption spectra as a means of detecting the kind of radiations responsible for a given chemical reaction unless that reaction alone utilizes energy from this source.

CONCLUSION

Evidence has been presented which indicates that the protective action of dogs' sera in irradiation of pancreatin solutions with ultraviolet light is the result of a competitive absorption (screening action). A similar effect is found in simple pancreatin solutions for which we may account (at least to a first approximation) on the basis of assumed homogeneous absorption by a strong competitor in the solution for the radiations having inactivating power. These observations are of interest in connection with the theory of what has been called *negative catalysis*,[8, 9] especially in view of the marked effects of small quantities of the protecting substances.

[8] Taylor, H. S., *J. Phys. Chem.*, 1923, **27**, 322.

[9] Christiansen, J. A., *J. Phys. Chem.*, 1924, **28**, 145.