CLXXXI. IRRADIATION OF *DOLICHOS* TYROSINASE.

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Irradiation of enzyme solutions has been studied by several investigators, and excepting in a few isolated instances inactivation has been observed to follow this treatment. The interesting observation made by Narayanamurti and Ramaswami [1929] that tyrosinase from the field bean *Dolichos lab lab* is activated by exposure to the rays from a quartz mercury arc led us to undertake the present study.

Experimental.

Enzyme preparation. The enzyme was prepared by extracting 200 g. of the finely ground meal of the ungerminated seed of *Dolichos lab lab* with 600 cc. of distilled water in presence of toluene for three days at room temperature. After filtering through paper pulp the extract (containing a large quantity of protein) was dialysed under slight pressure in collodion bags against flowing distilled water for a week when all the proteins were precipitated and the solution was then centrifuged to remove suspended matter, the resulting brown liquid being clear. The enzyme thus prepared is very stable in aqueous solution for many weeks at 0°. As source of ultra-violet rays two lamps have been used in this investigation. A 110 v. D.C. atmospheric type lamp was used in the earlier experiments. A Hanovia "Homesun" 220 v. vacuum type arc was used in the later experiments. Both gave accelerations. The distance of the silica flask containing the enzyme solution was 15 cm. from the lamp and irradiation was done in presence of air. The experimental arrangement was very similar to that described by Narayanamurti and Ramaswami [1929] and the activity was estimated by the method of Raper and Wormall [1923].

Irradiation of dialysed extracts. The effect of exposing the dialysed extracts as such to the rays was first investigated. The results taken from the previous paper by Narayanamurti and Ramaswami [1929] are given in Table I.

Fresh experiments were conducted with two different extracts: (*A*) 3 or 4 months old, dialysed for over a month and colourless, (*B*) fresh and dialysed for 10 days. The effect of having a water-jacket of silica to absorb the heat rays

was also tried. In all cases acceleration was observed. The results are given in Table II.

Table I.

Time of exposure in minutes	Activity (cc. thiosulphate required)	Increase
	Fresh extract.	
0	4·4	—
5	8·0	3·6
15	8·9	4·5
30	9·2	4·8
90	9·4	5·0
	Old extract.	
0	7·1	—
5	8·7	1·6
30	10·9	3·8
60	10·1	3·0
180	9·8	2·7

Table II. *The reaction mixture was composed of 10 cc. of Walpole's acetate buffer of* p_H *6·5, 40 cc. of 0·05 % tyrosine solution, 5 cc. of water, 5 cc. of enzyme extract, and 10 cc. of toluene. The temperature was 30°. In this and following tables activity is expressed as cc. of thiosulphate required.*

Time of exposure in min.	Old extract				Fresh extract	
			Water jacketed			
	Activity	Increase	Activity	Increase	Activity	Increase
0	1·5	—	—	—	2·2	—
5	4·2	2·7	2·4	0·9	4·3	2·1
15	2·8	1·3	2·2	0·7	5·8	2·6

It is clear that the enzyme is activated whether fresh or old, water-jacketed or not. However, in the case of old extracts the increase in activity diminishes on continued exposure as observed in earlier experiments.

Influence of the container. The next point to be investigated was the influence of the container. The enzyme solution contained in silica and glass flasks of similar size and shape was irradiated with a Hanovia "Homesun," 220 v. D.C. 1·0 amp. normal current vacuum type lamp at a distance of 15 cm. The lamp was started 5 minutes prior to irradiation. The results are recorded in Table III.

Table III. *The reaction mixture contained 50 cc. of 0·06 % tyrosine solution, 5 cc. of water, 10 cc. of enzyme extract and 10 cc. of toluene. The temperature was 30° and the duration of reaction 3 hours.*

Time of exposure in min.	Activity		Increase in activity	
	Glass	Silica	Glass	Silica
0	1·9	1·9	—	—
5	2·0	4·5	0·1	2·6
15	3·5	5·0	1·6	3·1

It is seen that in the case of glass acceleration is still caused but to a much smaller extent than in the case of silica.

Effect of addition of irradiated water. The influence of irradiated water was tried (Table IV).

Table IV. *The reaction mixture was composed of 50 cc. of 0·06 % tyrosine solution, 5 cc. of water and 10 cc. of enzyme extract, 10 cc. of toluene being added. Temperature was 30° and duration of reaction 3 hours.*

Time of exposure of water in min.	Activity	Increase
0	1·9	—
15	2·6	0·7

Addition of irradiated water causes less marked acceleration than irradiation of the reaction mixture in glass vessels.

Effect of period of exposure. It was shown in the older experiments that activation depended on the time of exposure and that the increase in activity diminished with longer exposure in the case of old solutions. Some new experiments with old solutions are given in Table V.

Table V. *The reaction mixture contained 50 cc. of 0·06 % tyrosine solution, 10 cc. of enzyme extract and 10 cc. of toluene. Temperature was 31° and duration 4 hours.*

Time of exposure in min.	Activity	Increase
0	1·0	—
5	1·6	0·6
10	3·4	2·4
20	1·2	0·2
30	0·8	−0·2
60	3·6	2·6
75	4·2	3·2
90	3·4	2·4

Effect of keeping the irradiated enzyme on its activity. The next point to be investigated was the effect on its activity of keeping the irradiated enzyme. A quantity of the enzyme was irradiated with the help of a Hanovia "Homesun" at a distance of 15 cm. for 15 minutes and the activity of the enzyme was determined immediately and after several periods of exposure. The results are given in Table VI.

Table VI. *The reaction mixture was composed of 20 cc. of 0·06 % tyrosine solution, 10 cc. of enzyme solution and 5 cc. of toluene. The temperature of the reaction was 35° and duration 3 hours.*

Description of enzyme	Activity	Increase
Unexposed	6·9	—
Immediately after exposure	9·9	3·0
5 minutes after exposure	9·7	2·8
15 minutes after exposure	8·9	2·0
30 minutes after exposure	8·6	1·7
60 minutes after exposure	8·2	1·3

It is clear that the activation decreases on keeping. Whether this inactivation is due to destruction of an activator produced by irradiation or to increased lability of the activated enzyme only future experiments can decide.

Mechanism of the activation process. In a previous paper it was suggested that the activation might be related to the electro-kinetic potential of the enzyme particle. It was shown that the optimum p_H of the enzyme lies near its isoelectric point. It was therefore suggested that on exposure to ultraviolet light the cataphoretic mobility of the enzyme particle might be reduced. To test this assumption samples of the enzyme (old extract) exposed to two different periods of irradiation were subjected to cataphoresis and after 18 hours the liquids in the two arms of the apparatus were examined for their activities. The results are given below.

Table VII.

Time of exposure in min.	Activity	Increase in activity
0	1·0	—
5	1·6	0·6
30	0·8	−0·2

Description of the enzyme	Activity
Positive pole—unexposed	2·2
Negative pole—unexposed	4·8
Positive pole—5 minutes' exposure	4·4
Negative pole—5 minutes' exposure	5·0
Positive pole—30 minutes' exposure	3·8
Negative pole—30 minutes' exposure	6·8

It is clear from the results recorded that the enzyme is most active when it is least charged. On longer exposure the positive charge on the enzyme particle is increased thus causing a slight diminution in activity.

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REFERENCES.

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