EFFECT OF TEMPERATURE ON CATALASE ACTIVITY

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A search of the literature for an enzyme already quite well known which might be involved directly or indirectly in photosynthetic reactions suggested catalase. Catalase occurs in chloroplasts (Neish, Krossing), its activity in light grown corn seedlings is greater in green ones than in albino or in yellow ones (Eyster, 1950) and it is heat sensitive at much below the boiling point of water and at a temperature which it was thought might possibly explain the maximum temperature for photosynthesis.

Leaves exposed for one minute at 55° C. and above lost the ability both to form chlorophyll and to transform sugar into starch (Eyster, 1949). It was therefore of interest to study the thermal stability of catalase, and to determine the critical temperature at and above which destruction of catalase in corn seedling leaves occurs.

METHODS AND MATERIALS

Green and etiolated corn seedlings were grown and were subjected to a series of temperatures by means of a water bath. The temperatures employed were 50°C, 55°C, 60°C and 65°C, respectively. These temperatures were rigidly maintained within 1°C. The seedlings, about 14 days old, were cut off at the base, inverted and submerged in the water of the water bath for periods of 1 minute, 5 minutes, 10 minutes and 30 minutes, respectively.

Catalase activity was determined by the "catalase tube" method of Knott (1925) who measured the oxygen evolved from H_2O_2 at a constant temperature and with constant shaking for a 5-minute interval on one gram of macerated tissue. The temperature of the water bath was 27.5°C ± 0.5, and the catalase tube was shaken at a constant frequency of 140 revolutions per minute. The motor driven apparatus was improved by replacing the leather belt with chain and gears. This eliminated all variations due to slippage, and standardized the shaking considerably. Present too, were an ammeter and a voltmeter to measure electric D.C. current feeding into the motor. A rheostat was installed to regulate the current and thus have the motor maintain a constant speed of 140 r.p.m.

The sample was prepared by taking 1 gm. of umblemished fresh leaves. These were cut with scissors, disregarding size and shape, and placed in small mortar. To this was added 1 gm. U.S.P. precipitated $CaCO_3$, approximately 1 gm. reagent grade sea sand 60–120 mesh, and 1–2 ml. distilled water, depending upon dryness of sample. The mixture was ground to a fine paste (for about 7 minutes) using large pestle. This was washed with distilled H_2O into a small bottle. The total volume of water added was 25 ml. This was found to be a most convenient amount for rinsing mortar and pestle.

After shaking the mixture, 2 ml. portion was quickly withdrawn by use of a large orifice pipette and transferred to one arm of catalase tube. Five per cent hydrogen peroxide was used to which some $CaCO_3$ had been added and shaken. This mixture was kept stoppered and standing in ice water to keep the H_2O_2 at constant concentration by reducing decomposition to a minimum. 2 ml. of this was withdrawn by pipette and placed into the other arm of catalase tube. To get checks the ends did not need to be the same for sample and for H_2O_2 respectively. The charged catalase tube was carefully secured (level and in line with rod) to stopper on shaft and allowed to sit in the bath exactly 3 minutes to come to temperature of bath. Meanwhile water level in gas burette was adjusted and air exit

clamped. It was made certain that the catalase tube would fit well up on the stopper. The motor and stopwatch were started simultaneously. The gas burette reading was taken immediately at end of 5 minutes by adjusting the water level during the run.

RESULTS AND DISCUSSION

The catalase measurements are given in Tables I and II. Table III is a summary of Tables I and II. A study of the data in these tables will reveal that 55°C was the temperature at and above which destruction of catalase occurred.

TABLE I

CATALASE MEASUREMENTS ON ETIOLATED CORN SEEDLINGS SUBJECTED TO TEMPERATURES BETWEEN 50°C AND 65°C FOR PERIODS VARYING FROM ONE MIN. TO THIRTY MIN.
Catalase value is expressed as ml. of O_2 in a 5-minute interval.

	CATALASE ACTIVITY AFTER:				
Temperature	1 min.	5 min.	10 min.	30 min.	Controls
50° C.	9.20	9.22	9.10	8.80	9.22
	9.30	9.15	9.00	8.80	9.20
	9.20	9.20	9.10	8.80	9.28
Average	9.23*	9.19*	9.07*	8.80*	9.23*
55° C.	8.70	8.42	8.20	1.95	8.28
	8.60	8.42	8.18	1.98	8.30
	8.60	8.40	8.22	2.02	8.28
Average	8.63*	8.41*	8.20*	1.98**	8.29**
60° C.	7.10	0.80			
	7.08	0.72			
	7.02	0.75			
Average	7.07**	0.76**			
65° C.	0.60				
	0.60				
	0.60				
Average	0.60**				

*Denotes comparability with proper control.

A pretreatment of seedlings at 55°C for one minute brought about some destruction. At 50° C there was no significant destruction except for pretreatment of green seedlings for 30 minutes. A comparison of the temperature effects on etiolated and green seedlings of approximately same age showed the catalase in green ones to be slightly the more heat-labile or destructible.

Why is it that 55°C is the critical temperature above which chloroplast catalase is destroyed as well as above which chloroplasts lose their ability both to form chlorophyll and to transform sugar into starch? There are at least two plausible explanations. Either (1) all of the chloroplast reactions are intimately tied up so that a disturbance to one affects the others also, or (2) the various chloroplast reactions are accomplished by independent proteinaceous enzymes which are denatured by much the same conditions.

Lantz (1927) reported that drying of corn seedlings at a temperature of 56°C reduced their catalase content, and that a continuous temperature of 42°C during

germination and growth decreased markedly the catalase content of corn seedlings. In the potato tuber, Appleman (1910) found that the catalase was completely destroyed at 50°C. The point of total destruction of catalase for most of the cases reported, however, ranges from 65 to 80°C (Miller, 1938).

It is well known that low temperature interfers with the utilization of nitrogen compounds and accounts for chlorosis in corn germinating on cool spring days. To determine whether catalase formation may also be markedly reduced by low temperature during early growth of corn seedlings, the thermostat of the greenhouse was adjusted to maintain a temperature of 10°C. This was done just as the green seedlings were pushing through the surface of the soil. After 10 days

TABLE II

CATALASE MEASUREMENTS ON GREEN CORN SEEDLINGS SUBJECTED TO TEMPERATURES BETWEEN 50°C AND 60°C FOR PERIODS VARYING FROM ONE MINUTE TO THIRTY MINUTES.

Catalase value is expressed as ml. of O_2 in a 5-minute interval.

Temperature	CATALASE ACTIVITY AFTER:				
	1 min.	5 min.	10 min.	30 min.	Controls
50° C.	8.80	8.75	9.85	7.95	8.60
	8.72	8.68	9.80	8.00	8.52
	8.75	8.52	9.72	7.90	8.45
	....	8.58	....	....	....
Average	8.76*	8.63*	9.79**	7.95**	8.52*
55° C.	9.52	7.55	4.30	1.02	10.20
	9.35	7.70	4.40	1.12	10.12
	9.28	7.72	4.30	1.05	10.20
Average	9.38**	7.66***	4.33***	1.06****	10.17**
60° C.	2.90	....	....	....	10.02
	2.90	....	....	....	10.25
	2.88	....	....	....	10.02
Average	2.89****	....	....	....	10.02***
					.10
					8.92
					8.88
					8.72
					8.84****

* Denotes comparability with proper control.

time four pots of these corn seedlings were removed to a place indoors where the temperature was 25°C and where there was constant artificial illumination (15 ft. candles). At this time the corn seedlings were 5–6 cm. tall and were quite yellow in appearance. The chlorophyll content remained quite low in ones remaining at 10°C, but the ones removed to 25°C became quite green in about one day. The catalase activity (Table IV) was found to be quite high in the chlorotic ones grown at the low temperature, but did increase somewhat in the ones placed at 25°C. These results are in general agreement with Lantz (1927) who found that there was a gradual accumulation of catalase in corn germinating and growing at 10° C, so that in 30 days the catalase content was nearly equal to that at 20 to 30°C.

While there is a remarkably close correlation in the way physical factors and chemical compounds affect catalase activity and photosynthesis, there is still no proof that the two are directly connected by a functional relationship.

TABLE III

SUMMARY OF EFFECT OF TEMPERATURE TREATMENTS ON CATALASE ACTIVITY, BASED ON CONTROL = 100

Etiolated				
Temperature	CATALASE ACTIVITY AFTER:			
	1 min.	5 min.	10 min.	30 min.
50° C.	100	100	99	95
55° C.	93.5	91	89	24
60° C.	85	9		
65° C.	7			
Green				
Temperature	1 min.	5 min.	10 min.	30 min.
50° C.	103	101	96	78
55° C.	92	76	43	12
60° C.	33			

TABLE IV

EFFECT OF TEMPERATURE DURING GROWING PERIOD ON CATALASE ACTIVITY

Catalase value is expressed as milliliters of O_2 in a 5-minute interval.

AGE OF SEEDLINGS	CATALASE ACTIVITY	
	Greenhouse 10° C.	In Doors 25° C.
Days		
14	9.25 (2)	.....
15	9.12 (2)	11.68 (2)
16	8.44 (2)	10.27 (2)
17	9.57 (2)	10.94 (2)

SUMMARY

1. Catalase in leaves of corn seedlings was destroyed by temperatures at or above 55°C.

2. A comparison of the temperature effects on etiolated and green seedlings of approximately the same age showed the catalase in green ones to be slightly the more heat-labile or destructible.

3. There seems to be a common thermal effect on catalase, starch synthesis enzyme, and chlorophyll synthesis enzyme. 55°C is the critical temperature above which all three enzymes are destroyed. A plausible explanation for this enzymatic destruction is that these enzymes are proteinaceous and are subject to thermal denaturation, as for example, egg albumin.

4. The catalase activity was found to be quite high in chlorotic corn seedlings grown in greenhouse at 10°C, but did increase somewhat in ones placed at 25°C.

5. There may be no direct functional connection between catalase activity and photosynthesis.

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