EFFECTS OF CERTAIN INORGANIC IONS ON CORN LEAF CATALASE

H. C. EYSTER
The Charles F. Kettering Foundation, Yellow Springs, Ohio

With the technical assistance of THOMAS E. BROWN

Smirnow and Alissowa (1924) reported the relative activity of catalase extracted in salt solutions from wheat seeds as compared with water extracts and found that with M/20 sodium salts the reduction in catalase activity was $NO_3 > Cl > B_2O_4 > SO_4$, and that with M/20 chloride salts the reduction was Mn > Ca > Mg > NH_4 > K > Li > Na. These results do not give a direct measure of the effect of various salts on catalase inhibition. Instead, the results seem to be a measure of the effect of the salt on extractability of catalase in addition to the salt's inhibiting influence on the activity of the extracted catalase. They measured the catalase activity by adding 5 ml 1% H_2O_2 to the seed extract, letting it stand 30 minutes, adding 3 ml 10% H_2SO_4 and then measuring the undecomposed H_2O_2 by titrating with 0.1 N $KMnO_4$.

Charmandarjan and Tjutjunnikowa (1930) likewise reported on the relative activity of catalase extracted from germinating barley seeds. They worked with water extracts to which various salt solutions were added and with salt solution extracts to which water was added. Water extracts to which an equal volume of M/20 sodium salt solution was added showed following inhibition sequence $NO_3 > Cl > SO_4$, and Zn > Mn > Ca > Mg > NH_4 > Na for water extracts to which an equal volume of M/20 chloride salt solution was added. Catalase measurements by these investigators were made by titrating excess H_2O_2 with 0.1 N $KMnO_4$.

Santesson (1923) examined the effects of several anions and gave the succession in the following way on a rising scale: $SO_4 < HPO_4 < F < B_4O_7 < CO_3 < Br < I < Cl < NO_3 < ClO_3$. Michaelis and Pechstein (1913) working with blood catalase found that, at high hydrogen ion concentrations, low anion concentrations were sufficient to provoke a certain inhibition, and high anion concentrations were necessary to produce the same inhibition at low hydrogen ion concentration, and that sulfate ions inhibit only to a relatively slight extent, whereas chloride and especially acetate and nitrate are strong inhibitors. Agner and Thoerell (1946) stated that much of the supposed pH effect was really due to the inhibition by anions which increased with decreasing pH.

Boas (1934) in experiments with potato tuber tissue reported greatest inhibition of catalase by SCN and least by SO_4. Using chloride salts, the order of inhibition from greatest to least was as follows: Ca > Mg > K > Li. Boas measured the amount of O_2 given off in a gas burette by cork borer made cylinders of potato tubers of equal weight placed in 30 ml 1% H_2O_2.

Favre (1911) studying the activity of catalase in blood gave data which showed that chlorides inhibited more than sulfates and that Mn and Mg cations were more inhibitory than Na and K. He had the excess H_2O_2 react with iodide to form iodine, and then measured the amount of iodine formed by titrating with $KMnO_4$.

Since catalase exists in chloroplasts associated with chlorophyll, there is a possibility that catalase may be involved in photosynthesis (Yakushiji, 1933). Only very limited studies have been made on the direct and immediate effects of solutes and catalyst poisons on leaf catalase. For this reason it was deemed desirable to investigate this problem further. On the basis of toxicity effect of various solutes and catalyst poisons it may eventually be possible to decide the

question whether catalase is concerned with photosynthesis. A subsequent paper will describe the effects of catalyst poisons.

METHODS AND MATERIALS

The method described previously (Eyster, 1950) was used for the quantitative determinations of catalase activity. A 1 g sample of fresh leaves from green corn seedlings, two to four weeks old, usually grown in the greenhouse was macerated and placed in a small flask. Distilled water was added to make the total volume of the suspended leaf tissue 25 ml. Usually two control measurements of the catalase were obtained by placing 2 ml of suspension in one arm of a catalase tube for each determination. This brought the total volume of the macerated corn leaf tissue down to 21 ml, and then to make the volume 20 ml,

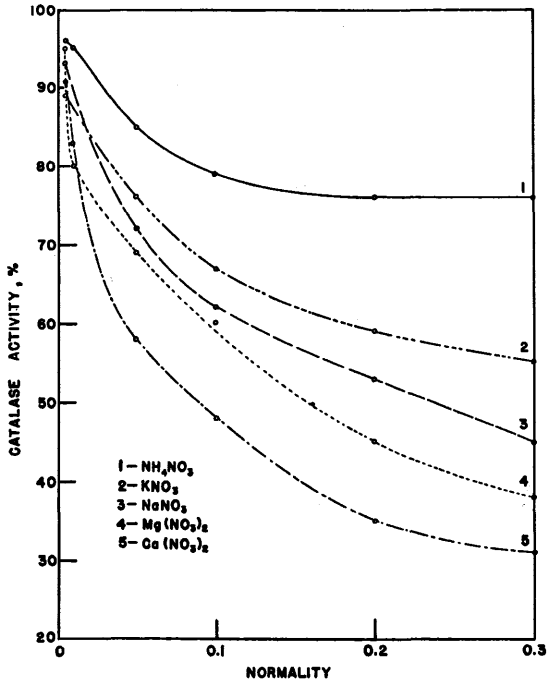

FIGURE 1. Varying effects of different nitrates on the activity of catalase in macerated corn leaves. Catalase activity is expressed in percent on the basis of 100% for the control measurement.

1 ml was drawn off and discarded. To test the influence of a chemical compound on catalase activity, a weighed amount of the compound was added to the 20 ml to make a definite known concentration. A magnetic stirrer aided in getting the compound quickly dissolved and thoroughly mixed. Measurement of catalase activity was resumed after the chemical compound was added and determinations were continued until two close measurements (within 0.2 ml) were obtained. The last two measurements were then averaged and compared with the average of the two control measurements on a percentage basis. The computations were done with the assumption that the control average represented 100%.

Landon (1934) states: "It is well known that the activity of most catalase preparations made from plant tissues declines more or less rapidly from the time of preparation." To determine what effect standing has on catalase activity corn

leaf preparations were tested immediately and after 4½ hours of standing. Measurements made during early stages of this study were exactly the same as measurements made near the end. Both showed a decline in catalase activity

TABLE 1

Solute effect on activity of catalase in macerated corn leaves. Catalase value is expressed as milliliters of O_2 in a 5-minute interval.

Solute	Concentration	Catalase Activity A Without Solute	Catalase Activity B With Solute	B/A
		ml.	ml.	%
NH_4NO_3	0.005 N	10.56	10.12	96
	0.01 N	7.61	7.26	95
	0.05 N	6.50	5.53	85
	0.1 N	11.13	8.77	79
	0.2 N	11.40	8.42	74
	0.3 N	8.86	6.54	74
KNO_3	0.01 N	9.72	8.36	86
	0.05 N	7.46	5.65	76
	0.05 N	11.06	8.37	76
	0.1 N	9.56	6.40	67
	0.2 N	9.16	5.32	58
	0.3 N	10.07	5.52	55
$NaNO_3$	0.001 N	10.96	10.80	99
	0.005 N	9.98	9.26	93
	0.01 N	9.86	8.22	83
	0.05 N	10.90	7.87	72
	0.1 N	9.18	5.70	62
	0.2 N	10.07	5.36	53
	0.3 N	11.20	5.00	45
$Mg(NO_3)_2.6H_2O$	0.005 N	9.10	8.67	95
	0.01 N	9.03	7.24	80
	0.05 N	9.93	6.88	69
	0.1 N	10.52	6.34	60
	0.2 N	11.61	5.26	45
	0.3 N	8.74	3.36	38
$Ca(NO_3)_2.4H_2O$	0.005 N	11.36	10.35	91
	0.01 N	8.48	7.06	83
	0.05 N	8.46	4.94	58
	0.1 N	9.85	4.68	48
	0.2 N	10.34	3.61	35
	0.3 N	10.92	3.41	31
$NaNO_2$	0.0001 M	11.62	10.70	92
	0.001 M	11.56	5.63	49
	0.01 M	12.00	1.66	14
	0.1 M	6.66	0.32	5
$CaCl_2.2H_2O$	0.1 N	11.12	9.72	87
NaCl	0.1 N	11.22	10.26	92
	0.3 N	10.28	8.78	85
	0.5 N	10.70	9.17	86
$Na_2HPO_4.7H_2O$	0.3 N	11.03	10.72	97
Na_2SO_4	0.1 N	5.74	5.90	103
$NaC_2H_3O_2.3H_2O$	0.3 M	8.00	7.80	98

to only 95% of what it was in the fresh preparations. Most of the experiments were completed in an hour and some may have required as much as three hours to attain an equilibrium, but none of the experiments required 4½ hours. It is assumed then that standing did not reduce the activity more than 5%, and that any reduction to 90% or less of its original activity might be significant.

RESULTS AND DISCUSSION

The solute effects on catalase activity are given in table 1. Nitrate ions had a marked effect and chloride ions had a slight effect, whereas sulfate and phosphate ions appeared to have no influence on catalase activity. The cation associated with the nitrate ion had a modifying influence in the order: $NH_4 < K < Na < Mg < Ca$ (fig. 1). The cation associated with chloride showed $Na < Ca$, and it is likely that the cation effect is comparatively the same regardless of the associated anion.

The nitrite ion had a far more depressing effect on catalase activity than the nitrate ion. Sodium nitrite toxicity began at about 0.0001 M as compared with sodium nitrate toxicity which began at about 0.001 M. An inhibiting effect of sodium nitrite on blood catalase was reported by Rotini and Snassel (1933).

SUMMARY

1. Nitrate ions had a marked effect and chloride ions had a slight effect, whereas, under the conditions of the experiments reported, sulfate and phosphate ions appeared to have no influence on catalase activity.

2. The cation associated with the nitrate anion had a modifying influence in the order: $NH_4 < K < Na < Mg < Ca$.

3. $NaNO_3$ was toxic in concentrations down to 0.001 M.

4. Nitrites were even more toxic than nitrates. $NaNO_2$ was toxic in concentrations down to 0.0001 M.

REFERENCES

Agner, K., and **H. Theorell.** 1946. On the mechanism of the catalase inhibition by anions. Arch. Biochem., 10: 321–338.

Boas, F. 1934. Über Ionenwirkungen, besonders über das Anionenphanomen. Planta, 22: 445–461.

Charmandarjan, M. O., und **A. B. Tjutjunnikowa.** 1930. Einfluss von Salzen auf die Tätigkeit der Malzkatalase. IV. Biochem. Zeitschr., 221: 273–283.

Charmandarjan, M. O., und **A. W. Tjutjunnikowa.** 1930. Einfluss von Salzen auf die Tätigkeit der Malzakatalase. V. Biochem. Zeitschr., 222: 272–283.

Eyster, H. C. 1950. Catalase activity in chloroplast pigment deficient types of corn. Plant Physiol., 25: 630–638.

Eyster, H. C. 1950. Effect of temperature on catalase activity. Ohio Jour. Sci., 50: 273–277.

Favre, W. 1911. Zur Frage von der hemmenden Wirkung anorganischer Salze auf die Katalaze. Biochem. Zeitschr., 33: 32–48.

Landon, R. H. 1934. The effect of certain chemicals on the catalase activity in plants. Am. J. Bot., 21: 583–591.

Michaelis, L., and **H. Pechstein.** 1913. Untersuchungen über die Katalase der Leber. Biochem. Zeitschr., 53: 320–355.

Rotini, O. T., and **F. Snassel.** 1933. Über die Giftwirkung von $NaNO_2$ auf die Katalase von Rinderblut. Fermentforschung, 13: 499–504.

Santesson, C. G. 1923. Über die Eintwirkung von Giften auf einen enzymatischen Prozess. Skand. Arch. Physiol., 44: 262–305.

Smirnow, A. J., and **S. P. Alissowa.** 1924. Zur Frage über die Rolle der Aschenbestandteile in den Pflanzen. I. Mitteilung: Das Einwirken von Neutralsalzen auf die Katalase. Biochem. Zeitschr., 149: 63–78.

Yakushiji, E. 1933. Über die Katalase und ihre Rolle im Reaktionsmechanismus der Photosynthese. Acta Phytochim. (Tokyo), 7: 93–116.