Inhibition of DOPA Oxidase by Borate

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In studies on the terminal oxidase system in leaf homogenates of higher plants, MacVicar and Burris (2) observed that the oxidation of dihydroxyphenyl-L-alanine (DOPA) was significantly inhibited by boric acid. They suggested that this inhibitory effect might be the result of the formation of a complex between the boric acid and the adjacent hydroxyl groups of the amino acid, thus effectively reducing the substrate concentration. Recently, Klein (1) has presented data in which this inhibitory effect was not observed in leaf preparations of tomato. In view of these conflicting results, it was deemed desirable to re-investigate the effect of borate on DOPA oxidase under the different conditions existing at the Oklahoma Station. The results of this investigation are summarized here.

Materials and Methods

Tomato (*Lycopersicon esculentum*, Mill), soybean (*Glycine max.*, Murr), and sweet potato (*Ipomoea Batatas*, Lam.) were employed in this study. Plants were grown in soil culture, with added fertilizer, in the greenhouse during the winter and early spring of 1950-51. Fully developed but still vigorous leaves were collected and the petioles and midribs excised. The resulting leaf blade tissues were ground in a mortar with sand with the addition of M/10 phosphate buffer at *p*H 6.0. The resulting mixture was expressed through muslin to remove tissue residue and the expressed material centrifuged at about 400 g. to remove starch granules, gross particles, and debris. The majority of the chloroplasts remained in suspension.

The Warburg constant-volume respirometer was used to measure oxygen consumption. Each flask contained the following: 1 ml. of leaf tissue extract; 1 ml. of M/50 DOPA (dihydroxyphenyl-D,L-alanine) in *p*H 6.0 phosphate buffer; 1 ml. of either *p*H 6.0 phosphate buffer or boric acid dissolved in phosphate buffer, so as to give a final concentration as indicated; and 0.15 ml. of 10 per cent KOH to act as a CO_2 absorbent. The oxygen consumption was measured for a period of 60 minutes after equilibration. The bath temperature was 37°C., flasks were shaken 120 strokes per minute, and the gas phase was air. Nitrogen was determined by a semi-micro Kjeldahl procedure (2).

Results and Discussion

The results obtained are presented in Table I in terms of microliters of O_2 consumed/mg. N/hour ($Q_{O_2}(N)$). Examination of these data shows that, as in the previous observations of MacVicar and Burris (2), boric

TABLE I

Effect of Boric Acid on Oxidation of DOPA by Leaf Tissue Homogenates

TRIAL NO.	PLANT MATERIAL	OXYGEN CONSUMED-MICROLITERS/MG. N/HOUR				
		CONTROL	0.001M	0.01M	0.1M	SATURATED
1	Tomato	179	161	155	153	122
2	Tomato	207	178	135	128	117
3	Soy bean	112	98	99	81	57
4	Soy bean	132	116	110	69	52
5	Sweet potato	154	154	151	69	48
6	Sweet potato	141	141	132	102	79

acid in relatively high concentrations was definitely inhibitory. Thus, 0.1M borate inhibited tomato 14 and 38 per cent, soybean 28 and 48 per cent, and sweet potatoes 55 and 28 percent in duplicate trials. Definite inhibition was observed at 0.01M concentrations only in tomato and soybean tissue preparations. Lower concentrations gave either no inhibition or such small decrease as to be inconclusive without more extended investigation. The previous studies of MacVicar and Burris (2) can be compared with these only in the case of soybean. $Q_{O_2}(N)$ values observed by them for this plant gave results closely paralleling those obtained in this study, although the percentage inhibition found here was slightly less at all concentrations.

Several explanations may be advanced to explain the divergence in results obtained by us and by Klein (1). Several minor differences in technique were apparent, the most significant of which was the lower *p*H used by him (5.3) compared to 6.0 by both MacVicar and Burris (2) and this study. Further it was observed that different plant species varied in their sensitivity to added borate. Change in the degree of inhibition has been observed from time to time in the same species. These facts suggest that some other variable aside from boron concentration may be involved in the inhibition. The state of nutrition of the plant with respect to boron may also be of some importance. Considerable difference between the boron supply in soil culture and in sand culture is to be expected. Finally, it might be pointed out that all of Klein's (1) studies were made using 0.01M H_3BO_3, a concentration which in one of the two experiments using tomato gave only slight depression in oxygen consumption and was ineffective in the case of sweet potato preparations. It would appear, therefore, that the differences between the initial observations of MacVicar and Burris (2) and those of Klein (1) may be accountable on the basis of differences in technique and the relatively low concentrations of inhibitor used by the latter worker.

SUMMARY

Oxidation of dihydroxyphenyl-D, L-alanine by leaf tissue preparations of tomato, soybean, and sweet potato was found to be decreased by the addition of borate in relatively high concentrations.

LITERATURE CITED

1. KLEIN, R. M. 1951. The relation of gas exchange and tyrosinase activity of tomato tissues to the level of boron nutrition of the plants. Arch. Biochem. *30*: 207-214.

2. MACVICAR, R. and R. H. BURRIS. 1948. The relation of boron to certain plant oxidases. Arch. Biochem. *17*: 31-39.