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EFFECTS OF SELENITE SELENIUM ON RESPIRATION IN MAIZE ROOTS

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SUMMARY

One-centimeter root-tip segments submerged in phosphate buffer solutions (pH 4.5 to 5.5) were treated with Na_2SeO_3 concentrations ranging from 0.005 to 10.0 m*M*. Uptake of O_2 and CO_2 output were measured manometrically. Segments exposed aerobically to 1.0 and 10.0 m*M* selenite for several hours exhibited a considerable decrease in O_2 input, particularly at the higher concentration. In contrast, CO_2 output was greatly accelerated by selenite concentrations of 2.5 to 10.0 m*M*. Consequently respiratory quotient values were large for these high concentrations. Under conditions of marked O_2 deficiency, CO_2 output was appreciably decreased regardless of the presence or absence of selenite. Use of Se^{75} showed that the gas output with selenite did not include a volatile selenium compound. A striking parallel was indicated between concentration effects of selenite and published arsenite effects on aerobic fermentation. This was confirmed by including both selenite and arsenite treatments in the same experiments.

INTRODUCTION

Selenium, while not proved to be essential for the growth of higher plants, is known: (a) to induce increased growth in some species, particularly of the genus *Astragalus*, (b) to be tolerated by other species, and (c) to be injurious to still others [9]. The present study is concerned with the effects in maize-root tips of selenite selenium on the respiratory gas exchanges under varied Se concentrations and O_2 supply. A test was made with tracer selenium for possible evolution of volatile selenium compounds. Finally a comparison between selenite and arsenite effects has been included.

MATERIALS AND METHODS

One centimeter-tip segments from primary roots of sterilized[4], germinating maize grains, *Zea mays* Purdue hybrid WF 9×38−11, were used. Tip segments were employed because of their greater respiratory activity compared with older root portions [5]. Fifteen segments each were transferred to phosphate buffer (pH 4.5 to 5.5) in Warburg-respirometer flasks. Concentrations of Na_2SeO_3 ranged from 0.005 to 10.0 m*M*. The roots were in contact with the solutions for approximately three hours: one hour during setting up, plus one-half hour equilibration, plus 100 minutes respiratory rate measurement at 25°C. Oxygen-input and CO_2-output rates were measured by standard methods [10].

Study of selenite effects at deficient O_2 concentrations (ca. 0.4%) involved preventilation of the flasks containing the roots with commercial N_2 gas bubbled through pyrogallate solution. In the tracer experiments Se^{75} was added to the Na_2SeO_3 solution bathing the roots. A concentration of 5.0 m*M* $NaAsO_2$ was chosen for the selenite-arsenite experiments to compare selenite and arsenite effects. Some published arsenite results are included.

RESULTS AND DISCUSSION

Selenite concentration

Curves representing duplicate experiments show that at selenite concentrations of 1.0 and 10.0 *mM* O_2-input rates had decreased to approximately 90 and 70 per cent of the control average, respectively (Fig. 1A). Similar inhibitory action on O_2 input has been observed in yeast respiring glucose [3].

Results from three experiments measuring CO_2 output are plotted in Figure 1B. These curves show that the CO_2 output was considerably increased within the 2.5 to 10.0 m*M* selenite range. At 5.0 m*M* the average rate was 30 per cent above that of the control.

The effects of high selenite concentrations on the respiratory quotient (RQ) are shown in Figure 2. Curve I resembles those for CO_2 output, in general, and shows that the control RQ of approximately 0.9 was markedly increased to 1.6 for the highest selenite concentration. This marked increase was the result of both increased CO_2 output and the accompanying decreased O_2 input.

A plot (Curve II) of the logarithms of the respiratory quotients for the range 0.005 to 10.0 m*M* Na_2SeO_3 resulted in a curve made up of two straight lines. These lines intersect in the range of 0.25–

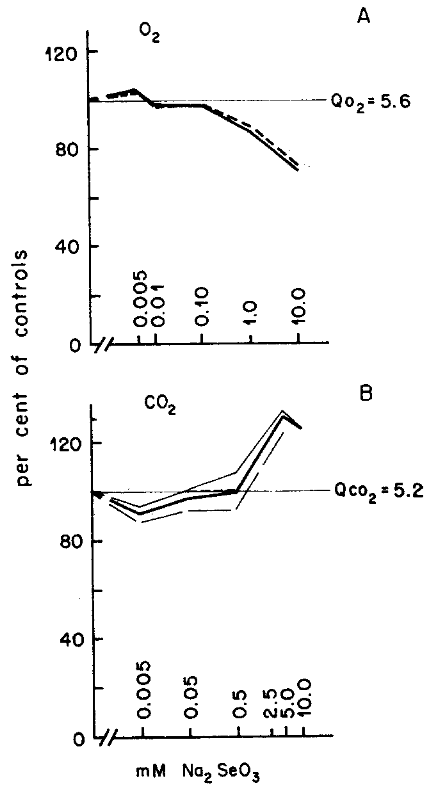

Fig. 1. Selenite-concentration effects on the respiratory gas exchange. Q-values are µl gas/h/mg dry wt. **A**. Effect on O_2 input. **B**. Effect on CO_2 output. *Heavy line* represents average values.

0.50 m*M* selenite concentration. This is the region in which O_2 input began to fall and CO_2 output to rise relative to their controls. It is here that the dual effects of selenite began to occur.

The final color of the 10.0 m*M* selenite-treated roots was light brown. Roots in 2.5 and 5.0 m*M* Na_2SeO_3 were light yellow; all others remained white. Discoloration in selenite-treated roots has been explained as deposition of elemental Se by reducing substances within the cells [8].

Selenite vs *oxygen supply*

Results from a study of selenite effects on submerged roots where the cultures were treated with air and with N_2 gas containing only

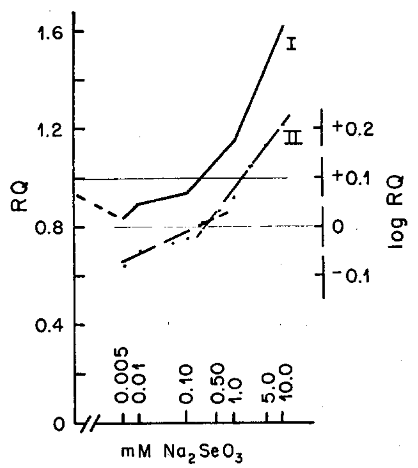

Fig. 2. Selenite-concentration effects on the respiratory quotient (CO_2/O_2). Curve-**I** data are from O_2- and CO_2-rate values of Fig. 1. Curve **II** is a logarithmic plot of curve-I values.

traces of O_2 are given in Figure 3. Again O_2 input under aerobic conditions was depressed and CO_2 output increased by high (5.0 m*M*) selenite concentration. In the N_2 treatment, however, CO_2 output was decreased to rates averaging 83 per cent for the minus-selenite and 82 per cent for the plus-selenite treatments. This latter rate, therefore, was not decreased by the Na_2SeO_3 but by the absence of O_2.

A somewhat parallel case in the literature may be cited [6]. One-centimeter three-day barley-root tips treated aerobically with 1.0 m*M* 2,2 dipyridyl solution had their O_2 input decreased 33 per cent. Under anaerobic conditions, however, the CO_2 output was not appreciably inhibited by the addition of dipyridyl. It was concluded that glycolysis was not materially inhibited by dipyridyl. A similar conclusion is suggested for the effect of selenite upon maize-root respiration as indicated by the lack of selenite inhibition of CO_2 output in N_2.

It can be seen from the CO_2 values obtained that the Fermentation/Respiration ratio (CO_2 in N_2/CO_2 in air) was approximately 0.8 in the absence of selenite. In its presence this ratio was 0.7. Both ratios suggest a Pasteur effect operating, that is a sparing action of O_2 on substrate consumption [7].

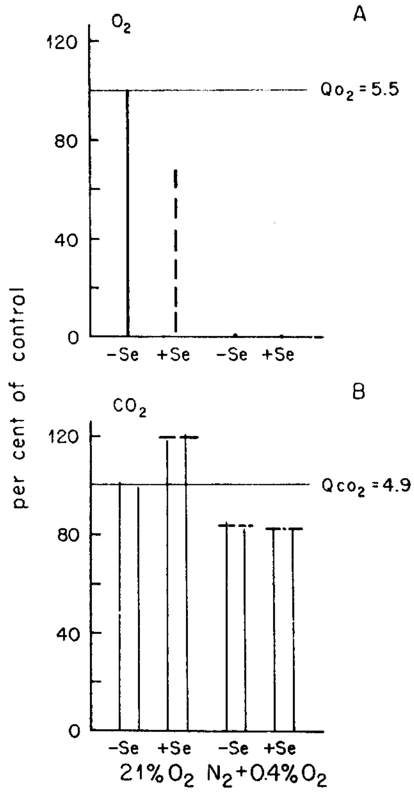

Fig. 3. Selenite-oxygen concentration effects on the respiratory gas exchange: (+) Se = 5.0 m*M* Na_2SeO_3. **A**. Effect on O_2 input. **B**. Effect on CO_2 output.

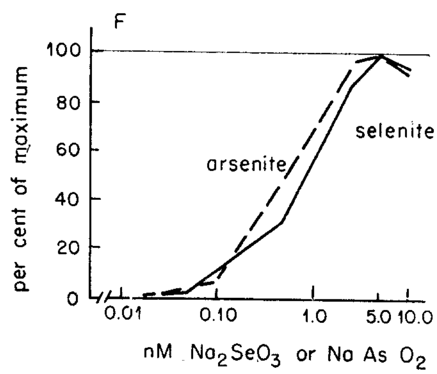

Fig. 4. Effects of selenite and arsenite concentrations (m*M*) on aerobic ferment ation (F), expressed as per cent of the maximum rates. Arsenite curve based on published data [1].

Tracer-selenium experiment

An experiment was conducted to determine if volatile selenium compounds were produced in the aerobic CO_2-output studies with selenite since it had been suggested that the increased gas output observed might be due to the decomposition of the added selenite. That this was not so was shown when minute quantities (0.0014 μg) of a Se^{75} compound added to 2.2 ml of 5.0 m*M* nonradioactive Na_2SeO_3 in the ambient solution gave no evidence of volatilization when the alkali-treated filter paper in the Warburg flasks was tested for radioactivity. Also no difference in respiratory rates was observed in the selenite-treated roots between those with and those without Se^{75}.

Selenite vs *arsenite*

The fact that 5 m*M* Na_2SeO_2 markedly depressed O_2 input and at the same time enhanced CO_2 output thereby increasing the RQ suggests a parallel effect on RQ obtained with $NaAsO_2$ applied to maize root tips [1]. The writer's RQ values for endogenous respiration reached a maximum of 1.6 for the 5.0 m*M* Na_2SeO_3 treatment compared with the 0.9 average control value. The published value referred to for sugar-fed root tips receiving arsenite was 2.3 compared with a control value of 1.08. Comparable aerobic fermentation curves, observed CO_2 output-(control RQ $\times$ observed O_2 input) for roots in the presence of selenite and of arsenite show marked similarity (Fig. 4). The arsenite effect was explained as inhibition of pyruvate and a-ketoglutarate oxidation through action on the SH group of lipoic acid. The result is an increased supply of pyruvate and a consequent promotion of aerobic fermentation. It is suggested that a similar action may explain the results with selenite also.

Results of experiments designed to compare directly the responses to selenite and arsenite are shown in figure 5. Both compounds depressed O_2-input rates but arsenite was much more effective than selenite (5A). When the two were combined the depressive effect was the same as with arsenite alone which shows that the effects of the two compounds were not additive.

In contrast to selenite, a depression in CO_2 output was obtained with arsenite (5B). As with O_2 input, the addition of selenite to ar-

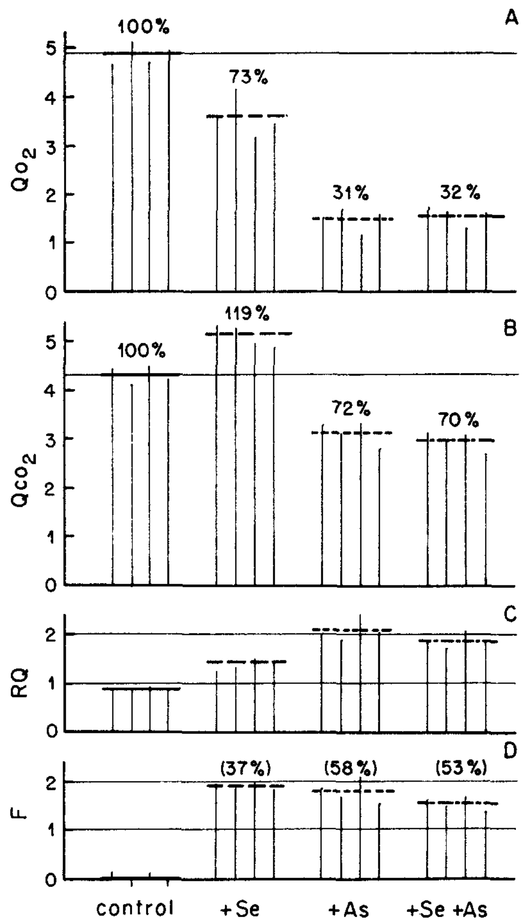

Fig. 5. Effects of 5.0 m*M* concentrations of Na_2SeO_3 and $NaAsO_2$ on respiration. **A**. Effect on O_2 input. **B**. Effect on CO_2 output. **C**. Effect on the respiratory quotient. **D**. Effect on aerobic fermentation. F-values are μl CO_2/h/mg dry wt. Per cent values here are based on total CO_2 output per treatment.

senite had no apparent effect over the action of arsenite alone. Increase in RQ values over the control was obtained with all three treatments, however, the increase with arsenite and arsenite plus selenite was roughly double that with selenite alone (5C).

Aerobic fermentation (F) was induced by both selenite and arsenite and to about the same magnitude in terms of μl CO_2/h/mg dry wt. (5D). However, when expressed as per cent of the total CO_2

output, the per cent values for the arsenite treatments were higher owing to the lack of stimulation in CO_2 output obtained with selenite.

CONCLUSIONS

The following conclusions are drawn from the results of these experiments. Maize-root tips had their O_2-input rates markedly depressed by relatively high (5 to 10 mM) concentrations of Na_2SeO_3. On the other hand the CO_2-output rates were considerably increased by these same high Na_2SeO_3 concentrations. As a result the respiratory quotient was nearly doubled relative to the controls by the highest selenite concentration. Volatile selenium compounds did not contribute to the observed CO_2-output measurements. In N_2-saturated solution selenite lost its stimulatory effect on CO_2-output rate. This indicates that the selenite-stimulatory effect was basically aerobic in character. Selenite treatments initiated and promoted aerobic fermentation. Parallel effects between selenite and arsenite were observed in depressing O_2 input and in promoting aerobic fermentation. However arsenite at 5 mM concentration depressed rather than stimulated CO_2 output. Respiratory quotients were increased with both compounds but to a greater extent with arsenite.

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