RESPIRATION OF SACCHAROMYCES CEREVISIAE IN THE PRESENCE OF SELENIUM AND ARSENIC[1]

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Moxon and his coworkers (1, 2, 3) have demonstrated that small amounts of sodium arsenite are effective in preventing the symptoms of selenium poisoning in rats. Similar results have been observed in farm animals (4, 5). Pengra and Berry, however, were unable to demonstrate any such antagonism by yeast growth studies. It was the purpose of the work reported here to determine whether or not the inhibition of yeast respiration by inorganic selenium could be prevented by arsenic.

EXPERIMENTAL

The respiration studies were carried out by measuring oxygen uptake of resting yeast cells **(Saccharomyces cerevisiae)** by the usual Warburg technique. One ml. of a distilled water suspension of yeast (ca 30 mg. wet weight of cells), 1 ml. of 0.15 M phosphate buffer pH 6.5 containing M/6 glucose, and either water, sodium selenite or selenate solution, and/or sodium arsenite solution to give a total volume of 3 ml. were added to each reaction vessel. The flasks were shaken for 15 minutes before closing the stopcocks, and then readings were taken every 10 minutes. The temperature of the water bath was 31° C.

The yeast used was an isolate from Fleischmann's baker's yeast which was carried on molasses agar (4 per cent Brer Rabbit molasses, 0.12 per cent $NH_4H_2PO_4$ and 2 per cent Difco Bacto agar). A transfer of cells was made from this agar to a molasses broth (4 per cent molasses and 0.12 per cent $NH_4H_2PO_4$). After 48 hours incubation at 31° C, one drop of the culture was used to inoculate more of the same medium, which was then incubated in a similar manner. After 48 hours the cells were harvested by centrifugation, resuspended in a volume of sterile water equal to the volume of the original medium and again centrifuged. After decanting, a similar volume of sterile 0.85 per cent saline was add-

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ed and the cell suspension was incubated at 31° C for 18 hours and then shaken on a wrist action shaker for one hour at room temperature. The cells were again harvested by centrifugation and after resuspending in sterile distilled water (1 ml. for each 5 ml. of original medium) were ready for use. The incubation in saline had been found necessary to reduce the high rate of oxygen uptake when no substrate was present.

RESULTS

Figure 1 illustrates the relative toxicities of selenium as sodium selenite and sodium selenate to yeast under the conditions of these experiments. For both salts the substrate contained 400 parts per million of selenium (0.005 M). Since the selenite reduced oxygen uptake more than did the selenate, it was decided that it should be used in studies with arsenic.

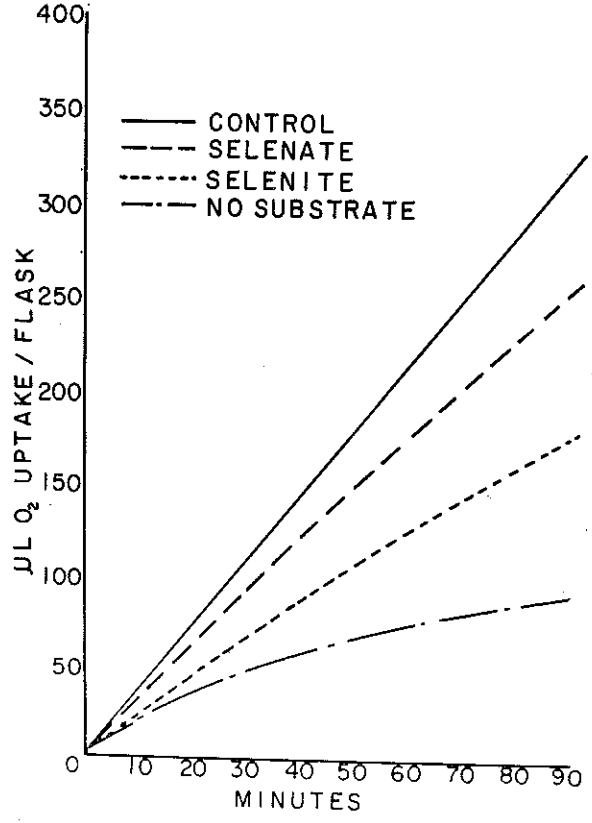

FIGURE 1. OXYGEN UPTAKE OF SACCHARIMYCES CEREVISIAE IN THE PRESENCE OF SELENITE AND SELENATE

Sodium arsenite is known to prevent the toxicity of sodium selenite in rats (3) and it was therefore the form of arsenic used here. In preliminary studies, the arsenite was found to be about as effective in reducing oxygen uptake as was the selenite. Therefore the amount used along with the selenite (80 parts per million of arsenic or about 0.001 M) was reduced to about one-fifth of the molar level of selenium, at which concentration its inhibitory effect on oxygen uptake was small. Figure 2 illustrates that the arsenite did not prevent the selenite toxicity, but rather it added to it.

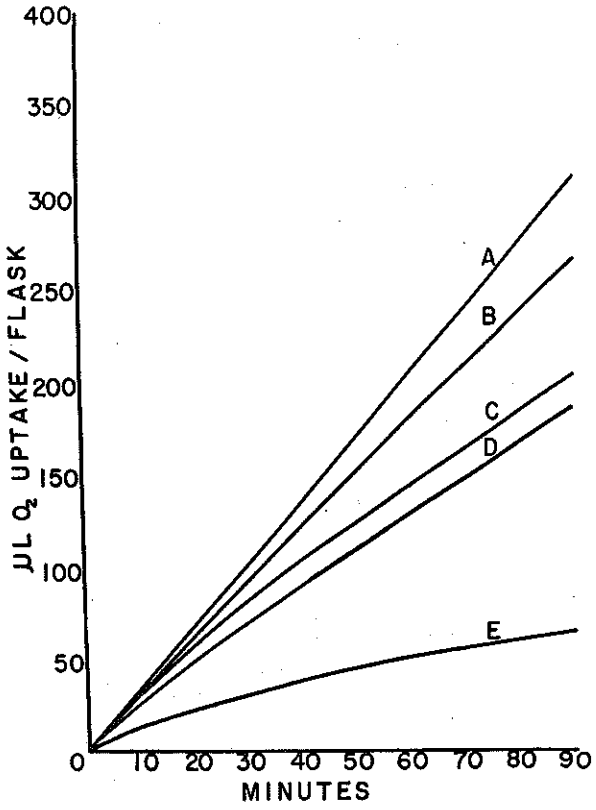

FIGURE 2. EFFECT OF ARSENITE ON OXYGEN UPTAKE WITH AND WITHOUT SELENIUM. A, NO SELENIUM OR ARSENIC; B, ARSENITE (80 p.p.m. As); C, SELENITE (400 p.p.m. Se); D, SELENITE PLUS ARSENITE; E, NO SUBSTRATE.

DISCUSSION

As in previous work (6) the selenium-arsenic antagonism observed in animals could not be demonstrated in yeast. Lardy and Moxon (7) were able to demonstrate some effect of arsenite on selenite inhibition of carbon dioxide production by baker's yeast in unbuffered glucose medium. They also found both selenate and selenite to be more inhibitory to carbon dioxide production than they were in these studies to oxygen uptake. After this work had been completed, studies in this laboratory showed that in the absence of added phosphate, selenium becomes much more inhibitory to the yeast glucose metabolism system. In view of the findings of Lardy and Moxon, this work should be repeated with substrates containing no added phosphates.

SUMMARY

Using a phosphate buffered glucose medium, no antagonism between arsenite and selenite could be demonstrated by **Saccharomyces cerevisiae** oxygen uptake measurements.

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