Respiratory Metabolism of Sugar Beets[1]

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Introduction

The sugar beet has an unusually favorable balance between photosynthesis and respiration. It far outranks all other temperate zone plants in its ability to photosynthesize and accumulate food on a per acre basis (3)[3]. In agricultural practice, huge quantities of sugar beets must be stored for extended periods. During storage the beets respire and, as a result large quantities of sugar are lost. If ways could be developed for decreasing this loss, it would be of obvious practical value. At present, the most practicable method of decreasing respiratory sugar losses is the forced-ventilation technique (1). The respiratory rate of the stored beets is decreased by forcing cool air through the beet piles. This reduces the temperature and thus decreases the respiratory rate and consequent sugar loss.

Another obvious approach to the control of beet respiration is the use of chemical inhibitors. For this purpose it is desirable to know something of the various enzymatic reactions which make up the over-all respiratory process in sugar beets. Although considerable research on respiration has been carried out with animal tissues and with certain plant tissues (2), little is known of the intermediary reactions in sugar beet respiration. The present report deals with some preliminary studies on the effects of a number of chemicals which are known to inhibit certain enzymes. It is hoped that basic studies of this type will provide a foundation for more detailed studies of the application of chemical inhibitors for the reduction of sugar beet respiration. In addition, such information will be useful in studies being carried out on the effects of ionizing radiations on sugar beet metabolism and storage.

Materials and Methods

Respiratory measurements were carried out with the standard manometric (Warburg) technique (7), using tissues from large (2.5 lb.) sugar beets of the variety SL 202. The beets were harvested in November and were stored in darkness in a root cellar at a temperature of 40° F. Most of the experiments were performed during the following March and April.

Conical vessels (16 ml.) with side arms and without center wells were used. Each vessel contained 25 slices of beet tissue (1.0 mm. thick x 8.5 mm. diameter, wet weight approximately 1.5 grams, dry weight approximately 0.25 grams) in a total of 4.0 ml. of solution. The final reaction solution was 0.4 *M* in sucrose, 0.05 *M* in potassium phosphate buffer of pH 6.8, and 0.04 *M* in potassium chloride unless otherwise specified. For pH values below 5.7, 0.05 *M* citrate buffer was used. Inhibitors were added to give the

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[3] Numbers in parentheses refer to literature cited.

final concentrations indicated. Carbon dioxide was absorbed by 0.2 ml. of 5 percent potassium hydroxide on a fluted filter paper in the side arm of the vessel.

A shaking rate of 110 oscillations per minute with an amplitude of 2.5 cm. was used routinely after preliminary tests. All respiratory measurements were made at 20° C. after preliminary tests indicated some reduction in the slope of the rate-temperature curve above 25° C. (see Figure 1).

Slices of sugar beet tissue 1.0 mm. in thickness were cut with a hand microtome at right angles to the vertical axis of the beet. Discs of tissue 8.5 mm. in diameter were cut from stacks of 10-15 slices by means of a sharp, stainless steel cork-borer. A simply constructed, adjustable holder kept the tissues in place while cutting the discs. Tissues from near the skin and core of the beet were not used. The discs were washed, with good agitation, for 15 minutes in running cold (13-16° C.) tap water, rinsed three times in distilled water, twice with 0.4 *M* sucrose containing 0.04 *M* KCl, and stored in sucrose-KCl at 1° to 2° C. until used. No discs were used that had been sliced for more than six days, except to study the respiratory rate in relation to time after cutting. There was a small progressive increase in respiratory rate of the cut discs during storage at 1-2° C. Bacterial contamination was usually obvious in discs stored for more than two weeks. Other studies showed that the respiratory rate of discs increased steadily with time during storage with aeration in either water or sucrose-KCl solution. Discs stored in running tap water behaved in the same way. The best technique found was to store the discs without aeration in sucrose-KCl solution at 1-2° C. The medium was changed and the discs were washed in distilled water immediately before use. There was little change in the respiration rate of discs handled in this manner over a period of one week. After the respiration measurements, the discs were washed in distilled water, blotted, and dried at 65-70° C. for about 15 hours. The discs were then weighed and the respiration rates in microliters (mm^3) oxygen per gram dry weight per hour calculated to 0° C., and 760 mm. pressure.

Experimental Results

Temperature had a large effect on the respiratory rate of sugar beet discs as shown in Figure 1. The rate vs. temperature curve is sigmoid in shape and shows a very definite reduction in slope above 25° C. Preliminary studies showed that discs 1-1.5 mm. in thickness exhibited a maximum rate of respiration as shown in Figure 2. Discs of this optimum thickness suspended in 4 ml. of solution respired at essentially the same rate as similar discs impaled on stainless steel wire so they did not touch each other, and measured in water-saturated air. This latter experiment indicated that oxygen availability was probably not limiting in the media in which the Warburg measurements were made.

Sugar beet roots are made up of alternate rings of vascular and parenchymatous tissues. Slices 1 mm thick were separated roughly into the two kinds of tissues and respiratory rates were determined for each tissue. The data in Table 1 show that on a fresh weight basis the vascular tissue had the higher respiratory rate. When compared on a dry weight basis, however, the

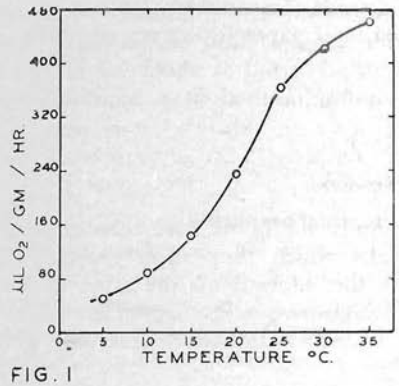

FIG. 1

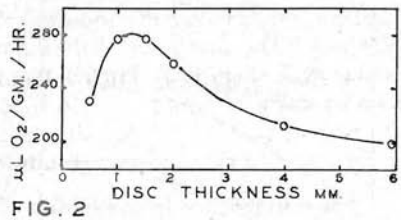

FIG. 2

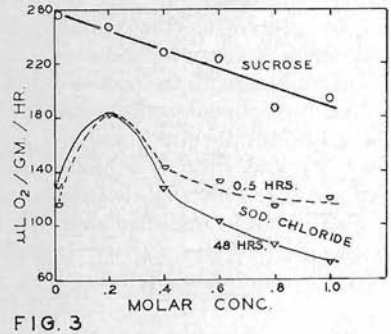

FIG. 3

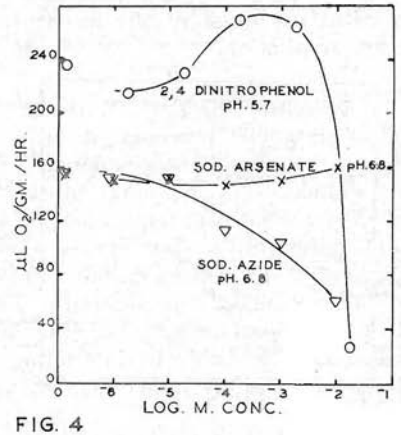

FIG. 4

Figures 1 to 4.—The effect of: 1, temperature; 2, disc thickness; 3, sucrose and sodium chloride concentration; and 4, reagents affecting phosphate transfers on the respiration rate of sugar beet root tissues.

parenchyma respired more rapidly due to the much greater percentage of dry matter in the vascular tissue. An increase in the sucrose concentration of the medium in which the tests were made caused a reduction in respiratory rate as shown in Figure 3. Previous studies with whole beets (5) and with 10-gram pieces of tissue (4) have shown that there is, in general, a positive correlation between the sucrose concentration in the tissue and respiratory rate.

Table 1.—Respiratory Rate of Vascular vs. Parenchyma Tissue at 25° C.

Tissue	ul 0_2/gm./hr.		Dry matter
	Fresh wt.	Dry wt.	Percent
Vascular	55.5	333.	16.7
Parenchyma	43.7	404.	10.8

Sugar beet tissues in the present studies showed a pronounced increase in respiratory rate as the sodium chloride concentration of the media was increased. The maximum "salt respiration" occurred at about 0.2 *M* NaCl as shown in Figure 3. Higher concentrations resulted in a decreased respiratory rate.

Inhibitor Studies

For convenience in presenting the results, inhibitors have been grouped according to the apparent mechanism by which they affect enzymes or enzyme systems. The system of grouping the inhibitors is the same as that used in the review paper of James (2) which may be examined for a more detailed discussion of the mechanisms by which inhibitors interfere with plant respiration.

Reagents Affecting Phosphate Transfer—Inhibitors in this category affect metabolic processes of many types by preventing the synthesis of energy-rich phosphorus compounds. Respiration may continue and even may be stimulated. Compounds of this type examined with respect to their effects on sugar beet respiration included 2,4-dinitrophenol, sodium arsenate, and sodium azide. The figures show that 2,4-dinitrophenol stimulated respiration at low concentrations (10^{-4} to 10^{-5} *M*) and strongly inhibited respiration at higher concentrations. These effects were only slightly influenced by pH. Sodium arsenate had little effect. Sodium azide inhibited sugar beet respiration, probably by interfering with metallic oxidase activity (as will be discussed later) rather than by any effect on phosphate transfer.

Thiol Reagents—Many enzymes involved in respiration are active only when surface sulfhydryl (-SH) groups are free. Inhibitors can act by combining with -SH groups thereby inactivating the enzyme involved. Figure 5 shows the effects of two inhibitors in this group, iodoacetamide and sodium arsenite, on sugar beet respiration. In these short-term experiments iodoacetamide inhibited only moderately. Sodium arsenite was more effective, giving 50 percent inhibition at 10^{-3} *M*. Since compounds of this type react slowly, longer periods of contact might result in greater inhibition.

Carbonyl Reagents—Some of the steps in the respiratory process can be inhibited by compounds such as hydroxylamine and sodium cyanide which react with essential carbonyl groups. As shown in Figure 6, both hydroxylamine and cyanide inhibit the respiration of sugar beet tissue. Since cyanide can also inhibit by other mechanisms as discussed below, it is difficult to speculate on the details of the mechanisms involved here.

Reagents Forming Fluorophosphate—Fluoride inhibits the enzyme enolase which catalyzes an essential step in the respiratory process, the conversion of phosphoglyceric acid to phosphopyruvic acid. Figure 7 shows that sugar beet respiration is inhibited by sodium fluoride. Inhibition was increased with decreasing pH, because of an increased permeability of the cells to the inhibitor.

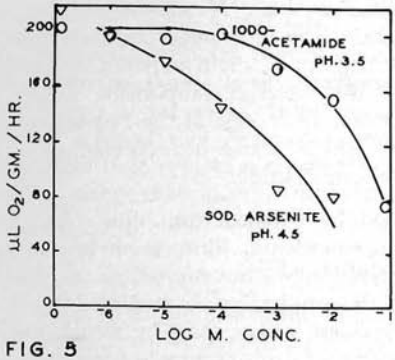

FIG. 5

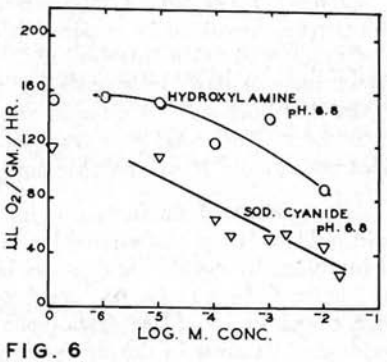

FIG. 6

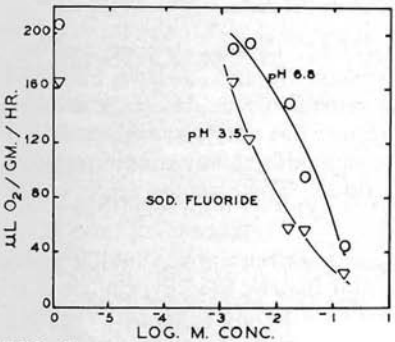

FIG. 7

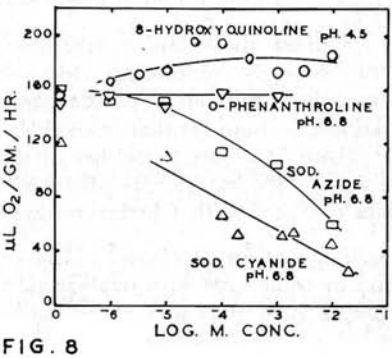

FIG. 8

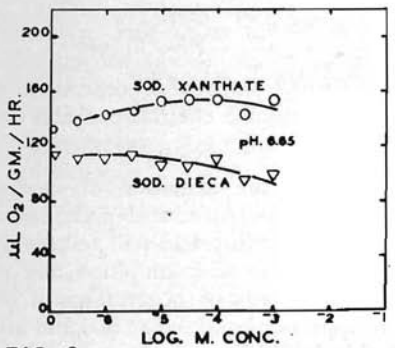

FIG. 9

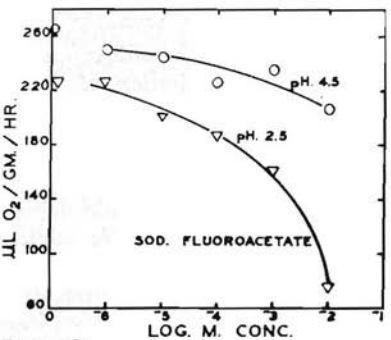

FIG. 10

Figures 5 to 10. The effect of chemical inhibitors on the respiration rate of sugar beet root tissues: 5, thiol reagents; 6, carbonyl reagents; 7, reagents forming fluorophosphates; 8 and 9, reagents forming complexes with transition metals; and 10, reagents for oxaloacetic acid.

Reagents Forming Complexes with Transition Metals—A number of the enzymes involved in respiration contain iron or copper. Inhibitors such as cyanide and azide interfere with the activity of these enzymes, especially cytochrome oxidase, by combining with the metallic component. Figure 8 shows that both sodium cyanide and sodium azide inhibit the respiration of sugar beet tissue. Azide is less effective which would be expected since it does not usually form as stable complexes as cyanide.

A number of enzymes are inhibited by 8-hydroxyquinoline. Ascorbic acid oxidase is especially sensitive to this compound. Since sugar beet tissue is inhibited by cyanide and azide, but not by 8-hydroxyquinoline, as shown in Figure 8, it may be presumed that the respiration is mediated by way of a cytochrome oxidase or polyphenoloxidase rather than by ascorbic acid oxidase. As shown in the same figure, sugar beet respiration is insensitive to o-phenanthroline which strongly chelates ferrous iron.

Sodium diethyldithiocarbamate (DIECA) chelates copper and as a result strongly inhibits ascorbic acid oxidase. It also chelates iron under some conditions but has little effect on cytochrome oxidase at low concentrations. Sodium xanthate also chelates copper but not as strongly as DIECA. As shown in Figure 9, neither of these compounds at low concentrations inhibits the respiratory rate of sugar beet tissue. These results agree with the data obtained with 8-hydroxyquinoline.

Reagent for Oxaloacetic Acid—Sodium fluoroacetate can inhibit respiration by combining with oxaloacetic acid and thereby blocking the citric acid cycle. Figure 10 shows that sodium fluoroacetate inhibits sugar beet respiration at low pH, probably because it penetrates into the cells more rapidly under such conditions.

Miscellaneous Inhibitors—Some phenols inhibit respiration, apparently by forming complexes with copper-containing proteins. Resorcinol was found to reduce the respiration of sugar beet tissue. Since a concentration of 8×10^{-2} *M* was required for a 50 percent decrease, the effect is probably not very specific.

Malonic acid competitively interferes with the oxidation of succinic acid by the enzyme succinic dehydrogenase in the citric acid cycle. Since the inhibition is competitive, addition of more succinic acid will reverse the effect. In the present work the picture of malonic acid inhibition and reversal was not too clear, probably due to slow rates of penetration of the compounds into the sugar beet tissue. It appears, though, that malonic acid does inhibit to some extent and that this inhibition can be reversed by succinate.

Maleic hydrazide had little or no effect on the respiration of sugar beet root tissue at pH 6.8 or 3.5. Previous studies (6) have also shown that it had little or no effect on the respiration of whole roots when applied as a pre-harvest foliar spray or when the roots were dipped in a solution of maleic hydrazide after harvest.

Summary

Preliminary studies have been made on the respiratory metabolism of sugar beet root tissues using the Warburg technique and respiratory inhibitors. Techniques of preparing, handling, and storing tissue discs were developed. On the basis of the effects of various types of inhibitors, it would appear that sugar beet root respiration involves cytochrome oxidase or polyphenol oxidase rather than ascorbic acid oxidase.

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