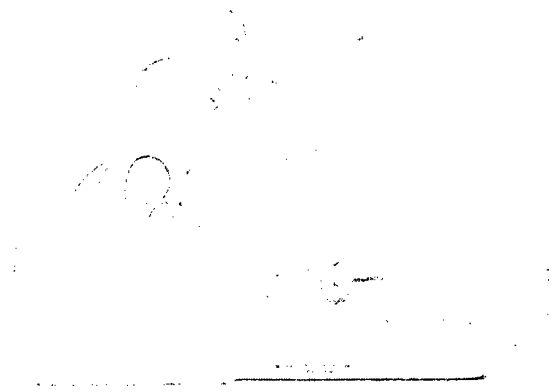

FIG. 1. Vacuum microsublimation apparatus in operation. The vapor trap in the vacuum line, consisting of a vacuum flask partially filled with glass beads, aids in preventing turbulence in the vacuum hood.

bottom test tubes, 15 mm in diameter. These may be cut down to any height, depending on the capacity desired (10 mm in height is equivalent to about 1 ml in capacity). Commercially available 5-ml beakers also may be used.

The biological fluid, tissue extract, or solution is concentrated by evaporation to 1 or 2 ml and then transferred to a microbeaker. The beaker is placed on the heating block of the melting point apparatus and heat applied. The ground-glass lip of the vacuum hood is then placed over the beaker on the block. Vacuum is applied to assist in drying the residue.

When the residue is completely dry the vacuum hood is removed, and the microbeaker is encased in aluminum foil. This prevents sublimation from occurring on the walls by keeping the wall temperature the same as that on the bottom. A microscope cover slip is now placed over the mouth of the microbeaker, and the vacuum hood is placed over this assembly on the heating block. The hood is pressed down on the block and vacuum slowly applied through a screw-type pinch clamp in the vacuum line (Fig. 1). Caution must be exercised in applying the vacuum to prevent turbulence, which may displace the cover slip. The temperature of the block is gradually raised by means of a voltage regulator until crystals of sublimation are seen forming on the cover slip. This temperature is maintained for a few moments to ensure quantitative separation. The vacuum is slowly released by means of the pinch clamp, and the vacuum hood is removed. The microbeaker is removed from the block and the cover slip inverted and transferred to the block. Another cover slip is placed on top, and the melting point of the sublimate determined. For sharper definition in determining the melting point the heating block may be placed on the stage of a dissecting microscope and the crystals thus magnified during the melting point determination (Fig. 2).

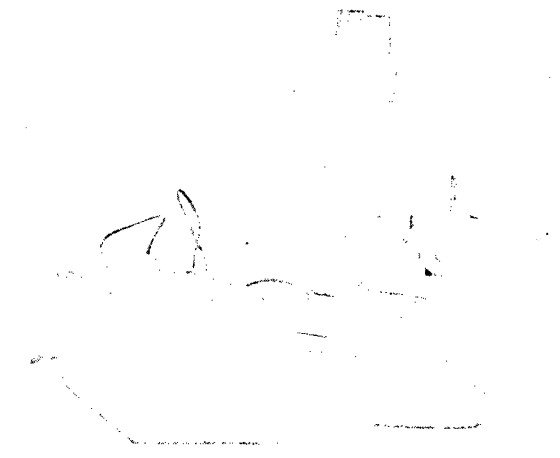

FIG. 2. Micromelting point apparatus with heating block mounted on stage of dissecting microscope. Contrast between crystals and background is enhanced by coating the heating block surface with a black, heat-stable substance such as ceramic ink.

By employing this apparatus the author has been successful in quantitatively recovering 4 μg Amytal from 2.5 ml of whole blood.

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The Effect of Carbon Monoxide on Respiration in the Sweet Potato, *Ipomoea batatas* Lan.[1]

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Since the addition of certain *ortho*-diphenolic substances (catechol, chlorogenic acid, etc.) to thin slices of roots of sweet potatoes increases the rate of oxygen consumption and carbon dioxide evolution, it has been suggested that a polyphenol oxidase is the terminal oxidase in sweet potato respiration (*1, 2*). In line with this, Walter (*3*) reported that sweet potato homogenates showed considerable phenol oxidase activity, but little or no cytochrome oxidase activity. This has been interpreted as further evidence for the possible operation of the phenol oxidase as a terminal oxidase, since the apparently insignificant amounts of cytochrome oxidase that were found could hardly play a major respiratory role in this tissue (*4*).

Recently, however, the author was able to demonstrate a very active cytochrome oxidase in sweet potatoes by a spectrophotometric method (*5*). It was also shown that crude sweet potato homogenates are not

[1] The author wishes to thank Albert W. Frenkel, of the University of Minnesota, for his helpful comments.

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suitable for the manometric estimation of cytochrome oxidase by the technique used by Walter (*3*), because the high phenol oxidase activity of these preparations masks other oxidative activity in the same system. Distinct cytochrome oxidase activity could be demonstrated manometrically if the homogenates were dialyzed thoroughly, or if the particulate cellular fraction (containing the cytochrome oxidase activity) was separated from the phenol oxidase by high-speed centrifugation.

Therefore, since a cytochrome oxidase is also present in sweet potatoes, and since the elicitation of increased gas exchange on the addition of phenolic substances does not seem to be a very critical basis for determining the nature of a terminal oxidase in living cells, it was of interest to re-examine the role played by phenol oxidase in sweet potato respiration. For this purpose, the effect of carbon monoxide on respiration was studied, as it had been shown earlier (*6*) that the carbon monoxide inhibition of sweet potato phenol oxidase activity was insensitive to light, whereas the carbon monoxide inhibition of sweet potato cytochrome oxidase was easily eliminated by light. The respiration of thin disks (7 mm diam, 0.5 mm thick) of root tissue in 0.05 *M* potassium phosphate buffer (pH 7.1) was measured at 25° C by standard manometric techniques (*7*) in various ratios of carbon monoxide or nitrogen to oxygen. The system was irradiated with light of about 300 ft-c incident on the manometer vessels.

In Table 1 are given values for oxygen consumption by sweet potato disks in several gas mixtures and in darkness and light. Respiration was strongly inhibited at the higher ratios of carbon monoxide to oxygen. It is evident that the inhibitions were completely eliminated by illumination except for a small amount at the highest CO/O_2 ratio. For comparison, the results with purified preparations of both sweet potato cytochrome oxidase and phenol oxidase under the same conditions are also presented.

Several varieties of sweet potatoes were examined, but no essential differences in results were noted that were due to difference in variety. Inasmuch as the carbon monoxide inhibition of respiration was so sensitive to light, and the carbon monoxide inhibition of phenol oxidase activity was completely insensitive, it would seem that sweet potato phenol oxidase is precluded from consideration as a terminal oxidase of respiration under the conditions used here. Conversely, the participation of the cytochrome oxidase in respiration, although not entirely proved, is rendered likely by the similar reaction of respiration and of cytochrome oxidase activity to carbon monoxide inhibition and to illumination.

TABLE 1

EFFECT OF CARBON MONOXIDE ON OXYGEN CONSUMPTION BY SWEET POTATO SLICES, BY PURIFIED PHENOL OXIDASE, AND PURIFIED CYTOCHROME OXIDASE FROM SWEET POTATO, AND THE EFFECT OF LIGHT ON THE OBSERVED INHIBITION

Experimental material	Gas ratio (in the liquid phase)	Percentage inhibition of oxygen consumption in a given ratio of CO/O_2 as compared with a control in N_2/O_2 of the same ratio	
		In the dark	In the light
Slices	3.1	25	0
"	6.8	46	0
"	14.4	67	12
Phenol oxidase	6.8	80	81
Cytochrome oxidase	6.8	68	0

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The Dependence of the Secondary Sex Ratio in Humans on the Age of the Father[1]

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That older mothers produce fewer male offspring than younger mothers is well known. This decrease in sex ratio with increasing age has given rise to a number of conjectures, some of which are based on embryological or gynecological considerations (*1–3*). From the genetic point of view, one might be inclined to wonder if possibly this decrease in sex ratio is, in fact, a function of the age of the father, with the above relationship being a simple consequence of the correlation between ages of spouses.

A simple statistical test has been made of data bearing on this point. From the analysis described below, it appears that this decrease in sex ratio is actually linearly related to the age of the father and is independent of the age of the mother.

The appropriate statistics giving the sex of each child at birth, with the ages of the mother and of the father, are given in the yearbooks of the U. S. Bureau of Vital Statistics for the years 1947, 1948, and 1949. The figures for the three years for the whites have been combined, including only those births for which the ages of both parents were known.

A multiple linear regression has been calculated for these data. Only those aspects of the calculations bearing on the general argument and the tests of significance will be presented here.

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