A Sensitive Micro-Spectrophotometric Method for Succinate Analysis

ASSAY of succinic acid by estimation of reduction of a redox dye in a succinic dehydrogenase and cyanide – poisoned system has been reported by Lardy[1] for methylene blue, and by Vishniac and Ochoa[2] for 2,6-dichloroindophenol. The disadvantage to the use of these dyes is that they are non-enzymatically reoxidized directly by oxygen and there is uncertainty concerning the end-point of the reduction of the dye and thus about the amount of succinate oxidized.

In our experiments air was displaced with argon in the solutions used and in the ground-glass stoppered Beckman cells employed. The cells were charged in an argon atmosphere in an open-top rectangular jar.

A 20 per cent succinoxidase[3] suspension which was quite stable in ice for several hours was prepared with 0·1 *M* phosphate buffer (*p*H 7·4) and homogenized in ice (using a low-speed drill press) for 7 min. with a 'Teflon' pestle glass homogenizer. This homogenization time of 7 min. was found to be the best of many intervals tested in order that sedimentation effects of tissue particles in the Beckman cells be minimized.

Each Beckman cell, including the 'blank' cell, contained 0·1 ml. 20 per cent tissue suspension (0·67 per cent after dilution in cell), 0·3 ml. 0·1 *M* potassium phosphate buffer (*p*H 7·4), 0·1 ml. 0·05 *M* potassium cyanide, 0·5 ml. 0·01 per cent, 2,6-dichloroindophenol dihydrated sodium salt, and 1·8 ml. glass-distilled water. It was found that the 0·67 per cent tissue suspension used in the Beckman cell media was preferable to the 2 per cent suspension used by Umbreit[3] for manometric analyses, because of both optical considerations and difficulties incurred with reduction of the dye by endogenous substrate in the succinoxidase preparation. Beckman readings showed that the endogenous reactions went to completion in about 6 min. At this point a further addition of 0·2 ml. of water was made to the 'blank' cell, and 0·2 ml. of 5×10^{-4} *M* sodium succinate solution (or any unknown solution) was added to all other cells. The 'blank' cell was adjusted to a high enough scale reading so that negative readings need not be taken on the other cells.

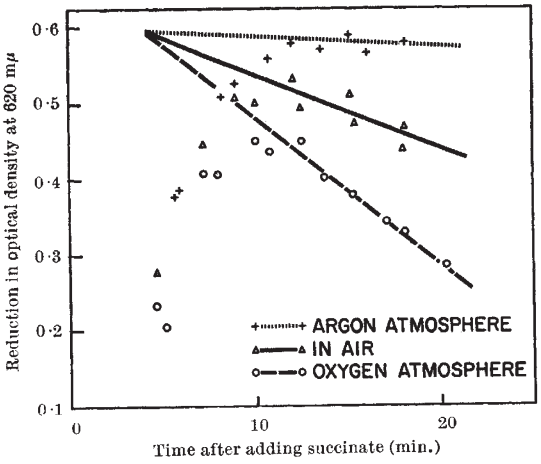

Fig. 1. Decreases in density relative to the 'blank' cell during the stated periods of incubation in systems varying from 0 to 100 per cent saturation with oxygen. The straight lines coming to a common point represent extrapolation of the slopes of the three curves

The relative effects of atmospheres of argon, air and oxygen on changes in optical density after addition of the stated amount of sodium succinate in each system are compared in Fig. 1. The Beckman density readings refer to relative decreases in density from the 'blank' cell. It is clear from these comparisons that by substituting argon for oxygen, reversal of the reduction of the dye through direct re-oxidation is avoided, and a more valid measurement of succinate content thus achieved. By extrapolating the slopes of the three curves in Fig. 1, and observing a common point of intersection, one again may see the desirability of working in the anaerobic system described in order to achieve maximum effects. The sensitivity of the argon system is such that each 0·01 Beckman density unit is proportional to 0·00167 μmole of succinate. Accuracy to the nearest 0·01 μmole of succinate may thus be achieved.

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Formation of Carbon by Decomposition of Carbon Suboxide at 750-780° C.

THE recent communication by Deitz and Prosen[1], which suggests that carbon suboxide is the means of transport of carbon in the system carbon monoxide–graphite at 450° C., prompts us to record some observations on the decomposition of carbon suboxide.

During attempts to prepare carbon suboxide by the pyrolysis of diacetyl tartaric anhydride[2] at 750–780° C., the formation of lustrous flakes was observed in the hot part of the reactor. This material was probably formed by polymerization of carbon suboxide, followed by decomposition of the polymer[3].

These lustrous flakes (0·5 per cent w/w of the diacetyl tartaric anhydride initially present) were shown by chemical and spectrochemical analyses to be essentially carbon, with about 1 per cent of hydrogen and of oxygen; trace impurities (silicon, aluminium, iron and magnesium) were present. A Debye–Scherrer X-ray diffractogram showed a strong 002 band and weak 100 and 110 bands. The optical reflectance measured in cedar wood oil was 18 per cent, a value higher than that (about 11 per cent[4]) reported for natural graphite.

It is noteworthy that instead of a soot, a hard graphite-like material was deposited at these low temperatures.

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