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Cytochrome *b* and Bacterial Succinic Dehydrogenase

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Bach, Dixon & Zerfas (1946) pointed out that the dehydrogenases fall into distinct groups according to the intermediate hydrogen acceptors through which they react with oxygen. Succinic dehydrogenase and lactic dehydrogenase of yeast come into the category of 'cytochrome-reducing dehydrogenases' because they readily reduce cytochrome *c*, and do not depend on coenzyme I or II for the reduction of methylene blue (Mb). None of the enzymes of this group of 'cytochrome-reducing dehydrogenases' has been isolated. Bach *et al.* (1946) purified the lactic dehydrogenase of yeast to a considerable extent and found that a new cytochrome, *b*₂, was concentrated in the enzyme fractions. There was an apparent correlation between the strength of the absorption band and the enzyme activity. They presented evidence that cytochrome *b*₂ forms an essential part of the enzyme system, but were not able to determine whether cytochrome *b*₂ is the dehydrogenase itself or an essential intermediate carrier between lactate and methylene blue. They also pointed out that in the heart muscle, cytochrome *b* is intimately associated with succinic dehydrogenase as shown by Keilin & Hartree (1939),

and therefore it is possible that enzymes of this group are of the *b* type.

Ball, Anfinsen & Cooper (1947) state that the function of cytochrome *b* is unknown, and consider that it may be identical with that of succinic dehydrogenase.

In a study of the relationship between the iron enzymes of *Corynebacterium diphtheriae* and diphtheria toxin, Pappenheimer & Hendee (1947) obtained evidence that cytochrome *b* was directly concerned in the oxidation of succinate. They were unable to separate succinic dehydrogenase from cytochrome *b* by differential centrifugation. Pappenheimer & Hendee (1949) made a comparison between the diphtherial succinoxidase system and that of ox-heart muscle, and concluded that succin-dehydrogenase may well be identical with cytochrome *b* both in diphtherial extracts and in ox-heart preparations.

Slater (1949), working with heart-muscle and kidney tissue, found that cytochrome *b* was involved in the reduction of methylene blue by succinate. Slater was not able to decide whether cytochrome *b* is identical with succinic dehydrogenase or whether

cytochrome *b* is an enzyme additional to succinic dehydrogenase required for the reduction of methylene blue.

If cytochrome *b* is essential for succinic dehydrogenase activity it is obvious that one would not expect to find dehydrogenase activity without cytochrome *b*. The finding of such activity in the absence of the other would be proof of their non-identity.

A strictly anaerobic micrococcus, identified as *Veillonella gazogenes*, was isolated from the rumen of sheep (Johns, 1949). This micrococcus showed both succinic and lactic dehydrogenase activity, and as strictly anaerobic bacteria have not been reported as containing any of the cytochrome components (Stephenson, 1949) it was of interest to examine this organism for cytochrome bands and at the same time measure both lactic and succinic dehydrogenase activity.

METHODS

A very weakly catalase-positive strain of *V. gazogenes* was grown anaerobically in liquid media consisting of a general-purpose salt mixture (Stephenson, 1949) with 0.4% (w/v) Difco yeast extract and 1% (w/v) sodium lactate. Good growth was obtained in 16 hr. The culture was centrifuged down and washed twice in distilled water containing 0.01% (w/v) Na_2S .

Succinic and lactic dehydrogenase activity were determined by the Thunberg technique.

A portion of the bacterial suspension was placed in a wedge-shaped cell, frozen and cooled in liquid air according to the method of Keilin & Hartree (1949). It was examined with a direct vision hand spectroscope using a 250 W. projector lamp as a source of light.

RESULTS AND DISCUSSION

Keilin & Hartree (1949) have shown that freezing and cooling to very low temperature sharpens and intensifies the absorption bands of certain pigments, making possible the detection of cytochrome in cells where the pigment has not hitherto been seen. Examination of a suspension containing 34 mg. dry wt./ml. of bacteria with the addition of a little sodium dithionite revealed no absorption bands. When dilute sodium hydroxide, pyridine and hydro-sulphite were added to a bacterial suspension at air temperature, there was no sign of the bands of a pyridine haemochromogen.

Part of the same suspension was used for dehydrogenase determinations. The results are given in Table 1.

Table 1. *Succinic and lactic dehydrogenase activity of a washed suspension of Veillonella gazogenes*

(Thunberg tubes contained 1 ml. bacterial suspension (21 mg. dry wt./ml.), 1 ml. 0.0002M-methylene blue, 2 ml. 0.1M-phosphate buffer, pH 7. Tubes evacuated and filled with N_2 . Temp. 37°.)

	Contents of side arm (1.0 ml.)		
	0.1M-Sodium succinate	0.1M-Sodium lactate	Water
Reduction time (sec.)	30, 31	34, 30	No reduction in 2 hr.

Defining Q_{Mb} as the amount of methylene blue (in $\mu\text{l. H}_2$) reduced/mg. dry wt. of bacteria/hr. Q_{Mb} succinate = 25.6; Q_{Mb} lactate = 24.0.

A second experiment, similar to that shown in Table 1, was carried out with the addition of sodium malonate. The reduction of methylene blue in the presence of succinate was completely inhibited by 0.2M-malonate, and 50% inhibition was achieved by a concentration of 0.025M. Malonate is a competitive inhibitor of succinic dehydrogenase and this indicated that we were dealing with the reaction succinate + Mb $\rightarrow$ fumarate + Mb.H₂. Attempts to prepare an active cell-free solution of the succinic dehydrogenase were unsuccessful.

The evidence presented indicates that cytochrome *b* could not be detected in the intact cells of *Veillonella* even with the low-temperature technique, although the organism contained succinic and lactic dehydrogenases.

Bach *et al.* (1946) did not observe cytochrome *b*₂ in intact yeast. It was only after lactic dehydrogenase had been extensively purified that the two new absorption bands appeared. *Veillonella* may contain cytochrome *b*₂, but the methods used could not detect it. *Veillonella* strains vary considerably in their catalase content, and the one used in the present investigations showed only very slight activity when tested as a thick suspension. It was not present in sufficient concentration to show bands of pyridine haemochromogen at air temperature.

SUMMARY

Cytochrome bands could not be detected in an obligately anaerobic bacterium, *Veillonella gazogenes*, which contains succinic and lactic dehydrogenases.

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