PRESENCE OF CYTOCHROMES IN THE REITER TREPONEME

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Recently, some of the heme proteins of some anaerobic bacteria have been definitely established (1). Although cytochrome c has been identified in *Leptospira icterohaemorrhagiae* by FULTON and SPOONER (2), no cytochrome system has been found in anaerobic spirochetes. The present communication concerns the presence of cytochromes in the Reiter treponeme, a strictly anaerobic spirochete.

Reiter treponemes were grown in KAWATA's thioglycolate medium (3) at 37° for 5 days. Cells were harvested by centrifugation at 2,000 × g for 15 min, washed 5 times by resuspension in 0.1 M phosphate buffer (pH 7.4) containing 0.002 M MgSO₄ and subjected to sonic vibration for 10 min at about 4°, using a Kubota 10-kc sonic oscillator. The sonicated suspension was freed of unbroken cells by centrifugation at 2,000 × g for 15 min and then spun down at 104,000 × g for 1 hr at 0°. The resulting particulate fraction was resuspended in the above phosphate buffer and divided into two aliquots. One aliquot was reduced with a few grains of Na₂S₂O₄ and another was oxidized by bubbling oxygen. Protein of the samples was determined according to LOWRY et al. (4). Reduced minus oxidized difference spectra were run in a Shimadzu recording spectrophotometer Model MPS-50.

As shown in Fig. 1, the particulate fraction gave a difference spectrum consisting of visible peaks at 630, 562, and 429 mμ. The peaks at 630 and 562 mμ seem to be quite similar to α-bands of a₃-and b-types of cytochromes, respectively, and a shoulder at 530 mμ may be characteristic of a β-band of the cytochrome b. The high peak with its maximum at 429 mμ, probably corresponding to a γ-band of the cytochrome b, is visible in the Soret region. A marked trough with its maximum at 460 mμ may be attributed to flavoprotein. Thus, the Reiter treponeme appears to contain the a₃- and b-types

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of cytochromes, but the nature of the cytochromes and the electron transport pathways in this organism must await further study. As in bacteria, the cytochromes seem to be associated with membranous components, probably the cytoplasmic membrane of the organism, because only the Soret region could be seen in the supernatant following centrifugation at 10,400 × g.

REFERENCES