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This Week's Citation Classic®

Postgate J R. Cytochrome c_3 and desulphoviridin; pigments of the anaerobe *Desulphovibrio desulphuricans*. *J. Gen. Microbiol.* **14**:545-72, 1956.
[Chemical Research Laboratory, Teddington, England]

The presence of an autoxidizable c type cytochrome in *Desulfovibrio desulfuricans* was reported, together with its purification to apparent homogeneity, its characterization, and evidence for its role as an electron transport factor in the bacterium's reductive metabolism. Also, an enigmatic green pigment, desulfoviridin, was recognized, partially purified, and described as a porphyroprotein. [The *SCI*® indicates that this paper has been cited in over 175 publications since 1956.]

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In 1953 I was working at a government laboratory in Teddington, near London, on the basic physiology of the obligately anaerobic sulphate-reducing bacteria (*Desulfovibrio* species). June Lascelles, a colleague from Oxford University, had just obtained a new Hartridge Reversion Spectroscope that could measure the spectra of cytochromes and pigments in nitrate-reducing and photosynthetic bacteria as well as the wavelengths of their absorption peaks. She offered to look at a sulphate reducer. Our group was one of the few that could grow them without producing an optically impenetrable mass of FeS. I posted off to her the pooled residues of a Warburg experiment I had just completed on the so-called Hildenborough strain. June soon called to say that my cultures were contaminated. She had seen a perfectly clear cytochrome of the c type, and it was well known that anaerobes did not have cytochromes.

Research on *Desulfovibrio* had been dogged for decades by impure cultures; I was demoralized: how sloppy could I get? I set up new cultures of three different strains and purity checks on Hildenborough. A week later my morale was restored: Hildenborough was as pure as the driven snow and all three new strains had cytochromes. So I rushed to Oxford with a new batch of Hildenborough. The cells had a cytochrome α band at about 500 mμ and a β band at about 525 mμ, both reversibly discharged by shaking in air and stable to KCN. We saw another band, unaffected by aeration, at 630 mμ (cytochrome a_2?); D.D. Woods could see one around 590 mμ although June and I could not.

Could the dogma be wrong? In late 1953 I went to Cambridge to see D. Keilin, the godfather of cytochromes, duly armed with a dense suspension of Hildenborough and a crude cell-free extract. In one day I learned more about cytochromes than in the previous five years, mostly using an old brass micro-spectroscope with Keilin's associate, E.F. Hartree, making derivatives (with nitrite, CO, pyridine, and so on). Much of the essential characterization of the cytochrome was done that afternoon. Keilin was skeptical. He accepted that I had a cytochrome but doubted that I had an anaerobe; it took a second visit with more material to convince him.

The rest is history. The cytochrome was of a new type characteristic of the genus *Desulfovibrio*: relatively stable with high Fe content and low redox potential. The 630 band was not a cytochrome at all but a green protein, which I named desulfoviridin; over a decade later it proved to be a bisulfite reductase. Woods's 590 band was a decomposition product: desulfoviridin readily loses its prosthetic group, releasing a porphyrin.

During 1954 I learned that Ishimoto at the University of Tokyo had also found the cytochrome and the green protein in *Desulfovibrio*.[1] We both had preliminary publications in press, so credit was (and is) shared. Several published preliminary accounts of the more unusual properties of the new cytochrome, which I named c_3, enabled me to relax and take a good two years preparing the definitive paper cited here. Earlier work by Davenport and Hill[2] on cytochrome *f* was an invaluable guide to its content.

It is ironic that, over succeeding decades, several of the paper's details proved to be wrong. There were three, not one, cytochromes. My purification method was crude and damaging, yielding material at best half-denatured. I had the E_h right, but there were four Fe atoms (not two) per molecule of cytochrome c_3. I missed its role in sulfite reduction because my reductase preparations were poor. And desulfoviridin was not a porphyroprotein but a sirohaematin—though I score points for an inspired guess about the structure of its prosthetic group that, 20 years later, proved not far wrong. But the work was seminal because cytochrome c_3 led to revision of a dogma and made scientists think again about anaerobic oxidative metabolisms. I have recently surveyed this area in a book.[3]

1. **Ishimoto M & Koyama J.** Biochemical studies on sulfate-reducing bacteria. 6. Separation of hydrogenase and thiosulfate reductase and partial purification of cytochrome and green pigment. *J. Biochemistry* **44**:233-42, 1957.
2. **Davenport H E & Hill R.** The preparation and some properties of cytochrome *f*. *Proc. Roy. Soc. London Ser. B* **139**:327-33, 1952. (Cited 230 times since 1955.)
3. **Postgate J R.** *The sulphate-reducing bacteria*. Cambridge, England: Cambridge University Press, 1984. 208 p.

