

pentoses, did not appear unless both hexosediphosphate and glycolaldehyde were present as substrates, and then only after the formation of xylulose, it is most unlikely that it was the product of the oxidative decarboxylation of hexose compounds. The evidence suggests that the synthesis was the result of the coupling of dihydroxyacetone phosphate and glycolaldehyde to give xylulose-1-phosphate, and the successive conversion of this compound to a ribulose phosphate and thence to ribose phosphate.

Since liver tissue has been shown to contain a pentose-phosphate isomerase<sup>6</sup>, the synthesis of ribose in a system already giving rise to ribulose phosphate might be expected; the conversion is established as an essential step in the formation of ribose by the oxidative decarboxylation of hexose derivatives<sup>7</sup>. The mechanism of the formation of ribulose phosphate from xylulose phosphate, involving an inversion at carbon-3, is less readily explained, though it could be regarded as bearing some analogy to the conversion of glucose to galactose for which a specific waldenase enzyme system exists<sup>8</sup>, or to the interconversion of ribulose and arabinose, for which an enediol intermediate has been suggested<sup>2</sup>. It seems clear, however, from these experiments that, contrary to earlier views, ribose-like deoxy-ribose can be synthesized in animal tissues by a condensation of 2-carbon and 3-carbon compounds.

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### Prosthetic Group of Cytochrome $a_2$

CYTOCHROME  $a_2$ , which is found in certain bacteria, for example, *Aerobacter aerogenes* and *Azotobacter*, has particular interest as it may play the part of a cytochrome oxidase in these organisms. The main absorption band of this cytochrome in the visible part of the spectrum is in the red region: reduced, 630 m $\mu$ ; oxidized, 645 m $\mu$ ; carbon monoxide complex, 635 m $\mu$ . Spectroscopic similarities to some ferrophæophorbides<sup>1</sup> as well as to certain bile pigment iron complexes<sup>2</sup> have been observed.

We have now obtained hæmin  $a_2$  free from protohæmin. *A. aerogenes* was cultivated under conditions of vigorous aeration by which the cytochrome  $a_2$  content is increased<sup>3</sup>. The hæmins were extracted with acid-acetone and, after removal of phospholipids, the green hæmin  $a_2$  was separated from protohæmin by precipitation of the latter by benzene.

The acid hæmatin (in glacial acetic acid) has a principal maximum of absorption at 603–5 m $\mu$  and a smaller maximum, of one-fifth the height, at 750 m $\mu$ . The alkaline hæmatin shows a maximum

at 662–64 m $\mu$ , the hæm at 618 m $\mu$ , and the pyridine hæmochromogen at 613–15 m $\mu$ . Cyanide forms a compound with hæmatin  $a_2$  in alkaline solution, causing the disappearance of the band at 662 m $\mu$ ; on reduction with dithionite, a band appears at 618 m $\mu$ . Carbon monoxide also reacts with hæm  $a_2$ ; the band has the same position as that of the hæm, but is of greater intensity.

By removal of the iron from the hæmin by means of the ferrous acetate-hydrochloric acid or the ferrous formate method a chlorin-like pigment is obtained. Re-introduction of the iron restores the spectrum of hæmatin  $a_2$ . In neutral solvents this chlorin has two principal maxima of absorption. In dioxane they are 654 m $\mu$  and 503 m $\mu$ . Three much weaker bands are found at 597, 572 and 533 m $\mu$ . The Soret band has a maximum at 405 m $\mu$ . In 10 per cent hydrochloric acid a maximum is shown at 630 m $\mu$ . The position of these maxima differs from those of the chlorins, phæophorbides or rhodins, for which data are recorded in the literature.

The 'HCl number' of the free chlorin is close to that of protoporphyrin; but the  $R_F$  value of the methyl ester of the chlorin when run on paper chromatograms using the kerosene-chloroform or kerosene-propanol solvents of Chu *et al.*<sup>4</sup> is markedly less than that of protoporphyrin ester.

Lipoid material remains even in the purest preparations. It is possible that, like porphyrin  $a$ <sup>5</sup>, the prosthetic group of cytochrome  $a_2$  possesses a lipophilic side-chain.

When the chlorin and the hæmatin were treated with hydroxylamine by the methods of Lemberg and Falk<sup>6</sup>, there was no shift in the position of the band at 652 m $\mu$  of the chlorin in pyridine or the band at 615 m $\mu$  of the pyridine hæmochromogen. Thus it is unlikely that the side-chains of the prosthetic group possess a formyl or carbonyl group.

The evidence for assuming that this hæmin and chlorin were derived from cytochrome  $a_2$  is as follows. The intensity of the acid hæmatin band at 603 m $\mu$ , or of the chlorin band at 652 m $\mu$  in acetone, was proportional to that of the cytochrome  $a_2$  band. *Azotobacter vinelandii*, *Escherichia coli* and *Proteus vulgaris*, all known to contain cytochrome  $a_2$ , yielded this hæmin and chlorin. When the bacteria were grown anaerobically, neither cytochrome  $a_2$ , hæmin  $a_2$ , nor chlorin was obtained. Some organisms such as *Bacillus mycoides*, *Bacillus subtilis* and *Pseudomonas aeruginosa* developed relatively small amounts of cytochrome  $a_2$  when grown in liquid medium with vigorous aeration, and correspondingly gave small amounts of this hæmin and chlorin.

We therefore conclude that the isolated hæm and chlorin are the prosthetic group and iron-free prosthetic group, respectively, of cytochrome  $a_2$ .

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