ON CYANMETHÆMOGLOBIN AND PHOTOMETHÆMOGLOBIN.

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FOUR years ago a derivative of methæmoglobin was described by Bock[1] and named by him 'photomethæmoglobin'. This substance, which has a red colour and characteristic spectrum, somewhat resembling that of hæmoglobin, but with the dark band broader and less defined, is obtained by exposing very dilute solutions of methæmoglobin to bright daylight. Bock prepared it by the action of light on a solution of methæmoglobin crystals which had been purified by crystallisation. He also succeeded in crystallising it, and described fully its spectrophotometric properties. It is reducible to hæmoglobin, though with difficulty, by reducing agents such as hyposulphite, ammonium sulphide, or bacteria, and its spectrum is the same in acid and alkaline solution.

Having recently had occasion to prepare 'cyanmethæmoglobin' I was at once struck by its resemblance to photomethæmoglobin, and after careful comparison of the spectra and behaviour to reducing agents of the two substances, could detect no difference between them. Cyanmethæmoglobin was discovered by Kobert[2] in 1891. He was led to the discovery by an investigation of the nature of the patches of red colour which occur on the mucous membrane of the stomach and elsewhere in the bodies of persons who have died of hydrocyanic acid poisoning. He found that when hydrocyanic acid or a cyanide is added to methæmoglobin solution the latter becomes red, and gives a spectrum like that of reduced hæmoglobin. He showed also that this change of colour and spectrum is an exceedingly delicate test for hydrocyanic acid.

Bock's photomethæmoglobin was obtained from methæmoglobin prepared by the action of ferricyanide on oxyhæmoglobin; and it seemed possible that, in spite of the care taken in repeatedly crystallising the

[1] *Skand. Archiv für Physiologie*, VI, p. 299. 1895.

[2] *Maly's Jahresbericht*, 1891, p. 443.

methæmoglobin, some of the ferricyanide was still present, and was decomposed, with liberation of hydrocyanic acid, by the light. To test this supposition I first endeavoured to prepare photomethæmoglobin from methæmoglobin prepared in another way, namely by the action of iodine on blood. The result was entirely negative, in spite of prolonged exposure of dilute solutions to bright sunlight. I then exposed to the light very dilute solutions of ferricyanide, without hæmoglobin, afterwards adding methæmoglobin in the dark. The result was that the photomethæmoglobin spectrum was at once obtained. Moreover the exposed solution smelt distinctly of hydrocyanic acid, and showed a precipitate of ferric hydrate, so that the ferricyanide had been decomposed by the light. The solution exposed required to be exceedingly dilute. A solution strongly coloured by ferricyanide was not appreciably decomposed.

It thus seems clear that the formation of photomethæmoglobin is not directly due to the action of light on methæmoglobin, but to the action on methæmoglobin of hydrocyanic acid liberated in consequence of decomposition of ferricyanide by the action of light. 'Photomethæmoglobin' is thus in reality identical with 'Cyanmethæmoglobin.'

Bock regarded photomethæmoglobin as a modification of methæmoglobin containing just as much oxygen as methæmoglobin, but in a more firmly combined form. The fact that reducing agents reconvert photomethæmoglobin or cyanmethæmoglobin to hæmoglobin certainly indicates that they contain more oxygen than hæmoglobin; and Bock showed that the change from methæmoglobin to photomethæmoglobin is not accompanied by any absorption of oxygen. In order to ascertain definitely whether any oxygen is driven out from methæmoglobin by the action on it of hydrocyanic acid I made use of the apparatus employed in the ferricyanide method of determining the oxygen of oxyhæmoglobin[1]. At the end of an oxygen determination with fresh blood the bottle was opened and the small tube taken out, filled with a dilute solution of potassium cyanide, replaced, and the bottle closed. As soon as the reading of the burette was constant this tube was upset in the usual way, so that the cyanide acted on the methæmoglobin. It was found that there was not the slightest evolution of gas although the solution now gave the spectrum of cyanmethæmoglobin. It is therefore clear that the cyanide does not displace oxygen from methæmoglobin in the same way as nitric oxide

[1] This *Journal*, XXII. p. 303. 1898.

does. Cyanmethæmoglobin is doubtless a cyanogen compound of some kind with hæmoglobin, but not a compound in the formation of which oxygen is displaced. Bock's conclusion that there is just as much oxygen in 'photomethæmoglobin' as in methæmoglobin would thus seem to be correct.

If blood solutions to which ferricyanide has been added be allowed to stand for two or three days cyanmethæmoglobin is formed, in consequence, perhaps, of putrefactive changes leading to decomposition of the ferricyanide. Amyl nitrite, when added in excess to dilute blood solutions, may also give the cyanmethæmoglobin spectrum, on account, doubtless, of the presence of traces of hydrocyanic acid in the reagent.

Besides cyanmethæmoglobin the substance known as cyanhæmatin has been described by Hoppe-Seyler, and I made a few experiments to ascertain whether this body is identical with cyanmethæmoglobin, as has been recently stated by Szigeti[1]. I cannot, however, confirm his conclusions. The spectra and tints of cyanmethæmoglobin and cyanhæmatin are, it is true, very similar. The spectra are not, however, quite identical, the absorption band of cyanhæmatin being somewhat narrower and less diffuse. To obtain the cyanhæmatin spectrum a considerable excess of cyanide must be added to an alkaline solution of hæmatin, so that hæmatin is not a good reagent for the detection of hydrocyanic acid. The two substances can readily be distinguished by the action of ammonium sulphide. This at first causes no sensible alteration in the cyanmethæmoglobin, which is only very gradually reduced to hæmoglobin on warming and allowing the solution to stand. The spectrum and colour of the cyanhæmatin solution, on the other hand, are at once altered, a new and very striking spectrum appearing, and the solution becoming pinkish red. The latter spectrum is two-banded, the bands being very similar as regards their position and relative breadths to those of oxyhæmoglobin. They are, however, slightly nearer the violet end of the spectrum, and are not quite so well separated as those of oxyhæmoglobin. This spectrum is doubtless that of a reduction product of cyanhæmatin, but is not that of hæmochromogen, as asserted by Szigeti: nor has exposure to a vacuum any effect on the spectra of either cyanhæmatin or cyanmethæmoglobin.

[1] *Maly's Jahresbericht*, 1893, p. 620.