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A new variant of abnormal methaemoglobin: Hb M_{Madon}

The ability of haemoglobin to bind reversibly molecular oxygen depends on the specific native structure of this haemoprotein. It is therefore to be expected that many different structural alterations of the haemoglobin molecule, of denaturational as well as genetic origin, result in a loss of the respiratory properties of haemoglobin and lead to spontaneous oxidation of the haemoglobin iron (formation of methaemoglobin) in presence of oxygen. About ten genetic variants of haemoglobin possessing abnormal α or β chains^{1,2} and occurring in methaemoglobin form (known as M haemoglobins) are known at present. A variant with different spectrochemical properties than those of the known Hb M types is described below.

The haemoglobin under study has been found in four cyanotic but otherwise symptomless subjects from three generations of a Polish family. The absorption spectrum of destomatized haemolysate treated with $K_3Fe(CN)_6$ showed in these cases an elevation characteristic for Hb M instead of a minimum at 600 m μ ; addition of cyanide resulted in a normal cyanmethaemoglobin spectrum (Fig. 1). When the

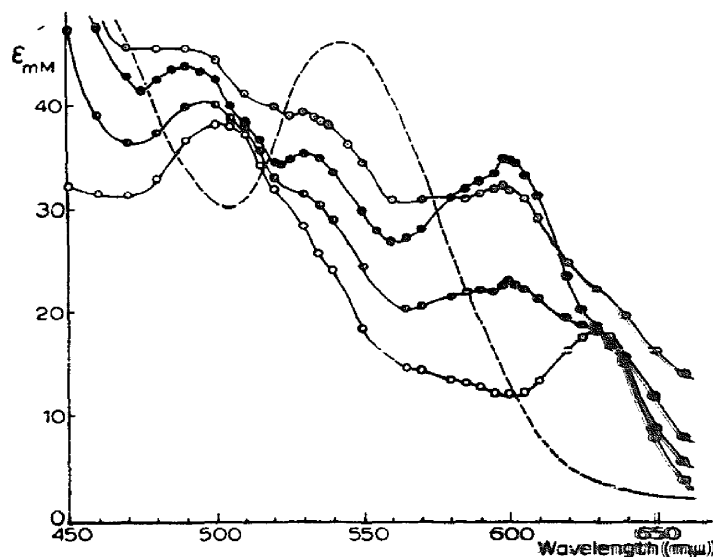


Fig. 1. Absorption spectra (0.0167 M phosphate buffer, pH 6.6) of methaemoglobins: O, normal; ●, patient; ●, calculated abnormal; ⊙, abnormal isolated from starch gel; —, cyanmethaemoglobin (identical for patient and normal control).

reaction of methaemoglobin ($1.6 \cdot 10^{-5}$ M) with cyanide ($1.5 \cdot 10^{-2}$ M) was followed spectrophotometrically at 600 or 630 $m\mu$ in 0.0167 M phosphate buffer (pH 6.6) and at room temperature³, the rate of conversion of methaemoglobin to cyanmethaemoglobin was found to be much slower than that of normal methaemoglobin A. The half time of this reaction was about 5 sec for normal and about 45 sec for abnormal haemoglobin (Fig. 2). The amount of the abnormal pigment was calculated from the

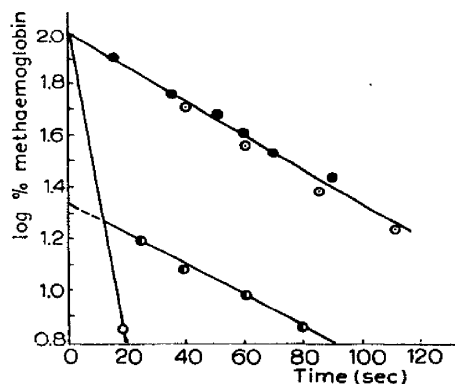


Fig. 2. The reaction of methaemoglobin with cyanide, measured at 630 $m\mu$ for normal (○) and patient (●) haemolysate and at 600 $m\mu$ for patient haemolysate (●) and for abnormal haemoglobin isolated from starch gel (⊙).

extrapolation of the curve obtained at 630 $m\mu$ to zero time to be 45 %. The theoretical absorption spectrum of abnormal methaemoglobin was constructed from the spectra of normal and abnormal methaemoglobin and from the known amount of the abnormal pigment in the patient's blood. The millimolar extinction coefficients were calculated assuming that of cyanmethaemoglobin to be 46.0 at 540 $m\mu$ (ref. 4) for both haemoglobins. The maxima of abnormal methaemoglobin are located at 598, 530 and 490 $m\mu$ and minima at 560, 522 and 475 $m\mu$ (Fig. 1).



Fig. 3. Starch-gel electrophoresis (pH 7.0) of patient (left) and normal (right) methaemoglobins.

Electrophoresis on starch gel⁵ in a 0.03 M phosphate buffer (pH 7.0) of the ferricyanide-treated haemolysate of the patient's red cells indicated the presence of two components (Fig. 3), the slower grey green and the faster (which was normal methaemoglobin A) brown. The absorption spectrum of the slower component eluted from the gel (shown in Fig. 1) was not identical with the calculated spectrum of abnormal haemoglobin. However, alterations of a similar character were observed in the absorption spectrum of normal methaemoglobin when prepared from starch gel under the same conditions.

Four M haemoglobins possess similar spectral properties to the one described above, *viz.* Hb M_{Saskatoon}^{6,7}, Hb M_{Leipzig I}^{1,3}, Hb M_{Kurume}⁸ and Hb M_{Chicago}^{1,9,10}. The Saskatoon and Kurume types react with cyanide rapidly and the Leipzig I and Chicago types react slowly. In this respect, the two latter resemble the variant described in this paper. The half times of the reaction with cyanide for Leipzig I and Chicago haemoglobins amount to about 30 sec (refs. 1, 10), ours being about 45 sec. The absorption spectra of these haemoglobins, although very similar, seem not to be identical. The respective absorption bands (for acid methaemoglobin) are located at 500, 540 and 602 m μ for Leipzig I (ref. 3), at 494 and 598 m μ for Chicago^{9,10} and at 490, 530 and 598 m μ for the haemoglobin under study. A clearly visible inflexion at 590 m μ is present in our haemoglobin and absent in the Leipzig I variant. The pigment described above can therefore be assumed to be a new variant of haemoglobin M. The name of Hb M_{Radom} is proposed provisionally for this haemoglobin since the affected family lives in Radom, Poland. A full account of this study including family and laboratory clinical data will be published elsewhere.

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