HAEMATOLOGY

Nature of Haemoglobin $M_{Oldenburg}$

In 1962, Tönz, Simon and Hasselfeld[1] described a German family with forty-six cyanotic members. This cyanosis was found to be due to an abnormal haemoglobin. This abnormal haemoglobin was characterized as an Hb M type on the basis of electrophoresis on starch block at *p*H 7·0 and on the absorption spectrum of the haemolysate. The abnormal haemoglobin was called Hb $M_{Oldenburg}$.

Cyanosis was present from birth, so an α-chain abnormality was postulated. The purpose of this communication is to describe the more detailed chemical investigation to find the amino-acid replacement in this Hb $M_{Oldenburg}$.

Haemolysate was prepared in the usual manner from the blood of one of the Hb $M_{Oldenburg}$ carriers. The Hb M fraction was separated on starch block electrophoresis at *p*H 7·0 (0·025 M $Na_2HPO_4.2H_2O$ + 0·025 M $NaH_2PO_4.2H_2O$) after converting the haemoglobin into the oxidized form[2]. The purified Hb M was digested with trypsin and the peptides were separated with high-voltage electrophoresis at *p*H 6·4 (water–pyridine 9 : 1, acidified with acetic acid). In Fig. 1 the pattern is given in comparison with that of a Hb A_1 digest. In the case of Hb M, peptide αT 8·9 is missing. No new peptide could be detected. Because peptide αT 9 has the same amino-acid composition as αT 8·9, with the exception of the N-terminal lysine, which is split off, differences in the amino-acid composition must occur in both peptides. We therefore compared the Hb M peptide αT 9 with the normal Hb A_1 αT 9 peptide. αT 9 usually occurs in the neutral zone after electrophoresis at *p*H 6·4. To separate this peptide, the neutral zone was eluted, dried and again electrophoresed at *p*H 3·6 (water–pyridine–acetic acid–*n*-butanol 7·6 : 1 : 7 : 2). Instead of the normal pattern, a new peptide appeared in the case of Hb $M_{Oldenburg}$ with sulphur, histidine and tyrosine staining reactions. A second sulphur-positive peptide could also be detected almost on the site of the normal peptide αT 9 (see Fig. 2). This suggests an amino-acid replacement, which acidified the peptides αT 9 and αT 8·9. On account of this more acid character, peptide αT 8·9 shifts to the neutral zone at *p*H 6·4 on electrophoresis, and both peptides αT 8·9 and αT 9 will run less to the negative side after electrophoresis at *p*H 3·6.

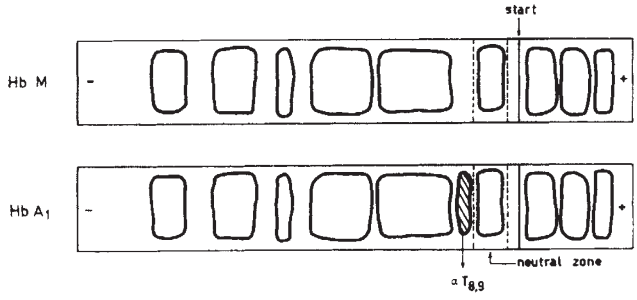

Fig. 1. Electrophoresis of the digest of Hb M and Hb A_1 at *p*H 6·4

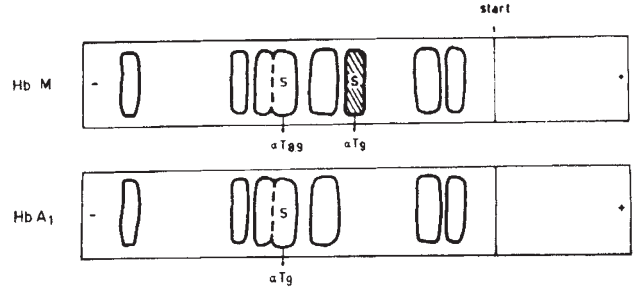

Fig. 2. Electrophoresis of the neutral zone at *p*H 3·6

The new peptide was extracted, hydrolysed and analysed. The amino-acid composition was that of the normal αT 9 peptide, except that one histidine unit was missing and a half-unit of tyrosine appeared in addition. Because of the positive tyrosine staining of the peptide and the fact that tyrosine breaks down on acid hydrolysis, it was assumed that a histidine group had been replaced by tyrosine. Which of the three histidines was replaced was not clear. It is reasonable to assume that it is histidine 87, because this histidine is connected to the iron atom of the haem group. This would explain the abnormal absorption spectrum of the haemolysate and the biochemical behaviour (lack of reduction of the methaemoglobin with sodium sulphate[1]). Such a replacement has already been described for Hb M_{Iwata} and Hb $M_{Kankakee}$ by Shibata *et al.*[3] and Jones *et al.*[4]. For this reason, Hb $M_{Oldenburg}$ is probably the same as these abnormal haemoglobins.

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IMMUNOLOGY

Heterogeneity of Heavy (γ) Chain Preparations from Human γG-Immunoglobulins

In recent publications, Cohen[1] and Cohen and Porter[2] have presented evidence that light chain preparations from partially reduced and iodoacetamide-alkylated γ-globulins of several species are heterogeneous. In glycine-urea starch-gel electrophoresis, *p*H 7–8, some eight to ten electrophoretically distinct bands were separated. Under similar electrophoretic conditions the heavy (γ) chains migrated as a diffuse band showing approximately the same spread as the original γ-globulin. In contrast to the light chains, the heavy chains did not give a band pattern.

We have prepared heavy and light chains of γ-globulins essentially according to the method of Fleischman, Pain and Porter[3]. The experimental conditions for reduction of the γ-globulin in neutral aqueous solution were similar to those described, but the chains were dissociated by dialysis against 0·1 M formic acid immediately after alkylation. Iodoacetic acid or iodoacetamide were used as alkylating agents. The chains from either reduced and dissociated products or reduced, alkylated and dissociated products were separated on a 'Sephadex *G*-200, *A* 50' (Pharmacia *AB*, Uppsala, Sweden) column in 0·1 M formic acid[4]. The separation of γ- and light chains from reduced but non-alkylated material was carried out in 0·1 M formic acid containing 1 ml. mercaptoethanol/l.

Urea starch-gel electrophoresis was performed in horizontal trays in formate buffer[5] and in glycine buffer[2]. When non-alkylated material was subjected to electrophoresis mercaptoethanol was incorporated into the gel.

The normal human γG globulins (7*S*) used in our experiments were either (1) Cohn-fraction II (supplied by Kabi *AB*, Stockholm, Sweden), further purified by chromatography on DEAE-'Sephadex', or (2) preparations