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This Week's Citation Classic

Clegg J B, Naoghton M A & Weatherall D J. Abnormal human haemoglobins: separation and characterization of the α and β chains by chroraatography, and the determination of two new variants, Hb Chesapeake and Hb J (Bangkok). *J. Mol. Biol.* **19**:91-108, 1966. [Depts. Biophys. and Med., Johns Hopkins Univ. Sch. Med., Baltimore, MD]

The paper described a chromatographic technique for the quantitative fractionation of the peptide chains of human hemoglobins. The high resolution allowed separation on a preparative scale of globins differing by only a single charged residue. [The *SCI*® indicates that this paper has been cited over 890 times since 1966.]

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"My first postdoctoral job in the US turned out to be a disaster which culminated after six months in a simultaneous resignation/firing ceremony and a hectic search for alternative employment. Eventually, relief came in the form of Howard Dintzis and Mike Naughton of the department of biophysics, Johns Hopkins University School of Medicine. I joined them in studying the biosynthesis of insulin by pulse-labelling, but nine months later the project was in the doldrums. There seemed to be no rhyme or reason for the labelling patterns of the peptides we had so laboriously isolated from our pancreas incubations. We subsequently discovered that our troubles had been caused by a fluorescent whitener in the blotting paper used to dry the papers for high voltage electrophoresis!

"By the way of a palliative, Naughton suggested that perhaps as a sideline I should turn back to protein work; in particular he had long been interested in hemoglobin and had tried unsuccessfully over some years, as had many others, to devise a way of easily fractionating human globin chains. It was clear from the amino acid compositions that the most likely pHs for optimum resolution by ion-exchange chromatography or electrophoresis would be in the neutral range, where the relative net charges on the globins were minimal –precisely the pHs where globin was virtually insoluble in aqueous solution. Here I was on home ground, for I had spent most of my PhD devising a method for fractionating the peptide chains of fibrin.[1] This too was insoluble, a complication that I had overcome by doing all the experiments in 8M urea. It seemed natural, then, to try on globin the method that had finally worked for fibrin, chromatography on CM-cellulose in urea.

"The first attempts were disappointing, with poor recoveries and what can only be described as a splurge instead of peaks, but we had ignored the free sulphydryl groups in globin; neutral pH in 8M urea would be ideal for forming mixed disulphides of any oxidised cysteines. A few mls of mercapthethanol in the next experiment produced a beautiful separation, and by the end of a week we had settled the conditions, essentially as they were subsequently published. Shortly afterward, David Weatherall came over from the department of medicine to try out the method on some of his radioactive thalassemic globin samples,[2] thus beginning a friendship and collaboration that has continued ever since.

"The method appeared at an appropriate time. Interest in human hemoglobin genetics, and the hereditary disorders like thalassemia, was rapidly gathering pace in the 1960s and there was a need for a good quantitative preparative technique, hence its appeal.

"This story seems to me to illustrate the absurdity of some of the pure-versus-applied science arguments. In recent years the method has been widely used for the antenatal diagnosis of the thalassemias.[3] It arose out of a personality clash and fluorescent blotting paper, and was paid for out of an insulin project. What politician would have ordained it that way?"

1. **Clegg J B & Balley K.** The separation and isolation of the peptide chains of fibrin. *Biochim. Biophys. Acta* **63**:525-7, 1962.
2. **Weatheralll D J, Clegg J B & Naughton M A.** Globin synthesis in thalassaemia: an in vitro study.*Nature* **208**:1061-5, 1965.
3. **Alter B P & Nathan D G.** Antenatal diagnosis of haematological disorders *Clin. Haematol.* **7**:195-216, 1978.