David C. Rees

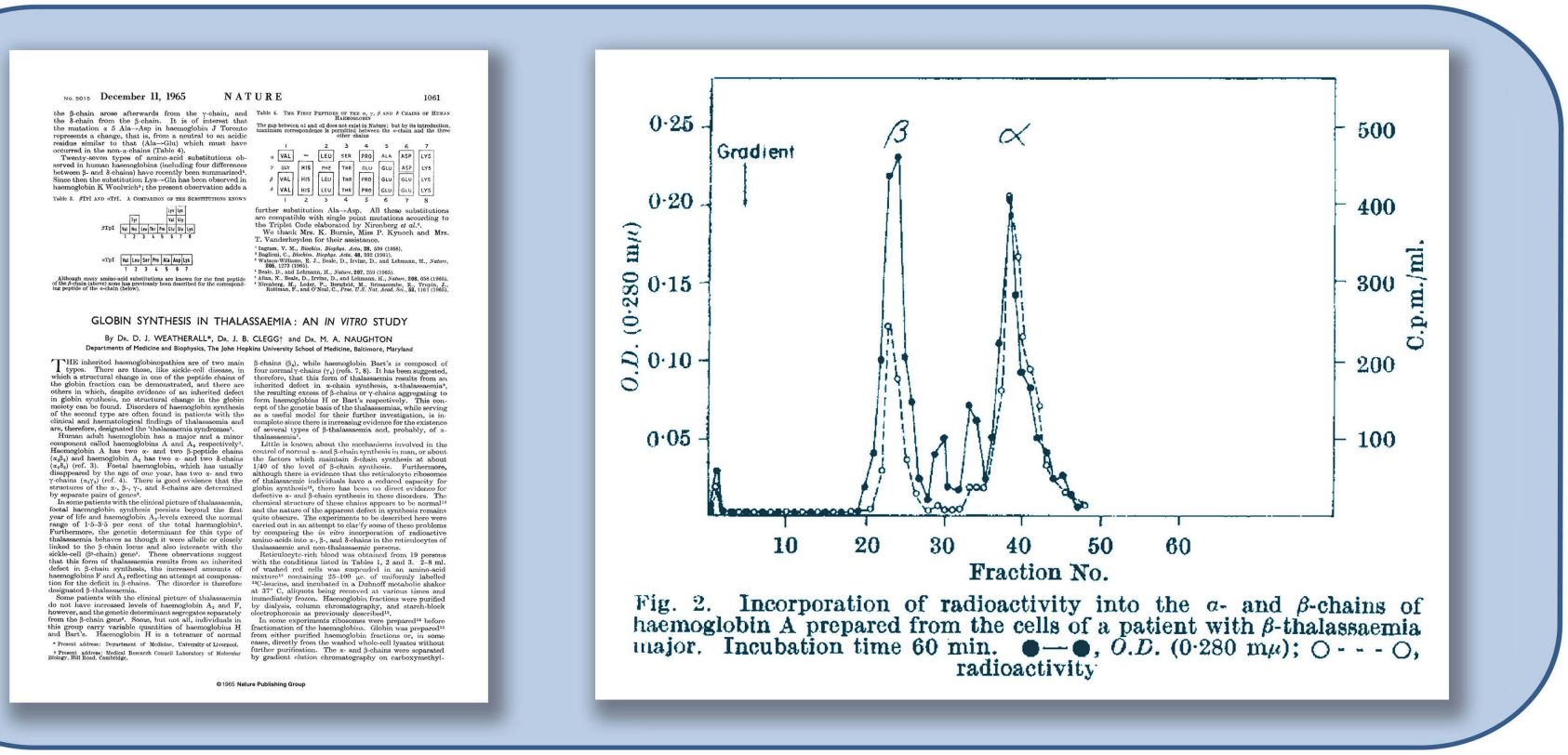
The beginnings of molecular medicine

David C. Rees
Department of Haematological Medicine, King's College Hospital and King's College London, UK
E-mail: david.rees@kcl.ac.uk

https://doi.org/10.3324/haematol.2022.281711

TITLE	Globin synthesis in thalassaemia: an in vitro study.
AUTHORS	Weatherall DJ, Clegg JB, Naughton MA.
JOURNAL	Nature. 1965;208(5015):1061-1065. DOI: 10.1038/2081061a0 PMID: 5870556

Thalassemia was first described in the 1920s by Thomas Cooley and Pearl Lee in Detroit. They described four children with massive hepatosplenomegaly, anemia, jaundice and marked bony abnormalities, including enlargement of cranial and facial bones.[1] It initially seemed more likely that this was a disease of bones than blood, as more clinical descriptions gradually emerged from the Mediterranean and Asia, where this seemed to be a common problem. In parallel with advances in genetics, red cell biology, and hemoglobin analysis, it became apparent that thalassemia was inherited in an autosomal recessive manner, with mild red cell abnormalities typically present in both parents. Protein sequencing had shown that some hemoglobinopathies, most notably sickle cell disease, were caused by structural abnormalities of the globin chains, whereas others had no detectable globin abnormalities, and were referred to as thalassemia syndromes. It was suspected that thalassemia was caused by quantitative defects of globin chain synthesis, but there was no direct evidence of this until Weatherall *et al.* published their landmark paper.[2] The authors were able to assess the relative rates of globin chain synthesis by incubating reticulocytes with radiolabeled leucine, and measuring the rate of leucine incorporation over different time periods. In this way, they showed that the rates of β and α globin synthesis were matched in normal reticulocytes, whereas there were quantitative deficits of β and α globin in the respective types of thalassemia. This discovery was the beginning of the molecular understanding of thalassemia, and led to an exponential increase in the under-



No. 5015 December 11, 1965 NATURE 1061

the β-chain arose afterwards from the γ-chain, and the δ-chain from the β-chain. It is of interest that the mutation α δ Ala→Asp in haemoglobin J Toronto represents a change, that is, from a neutral to an acidic residue similar to that (Ala→Glu) which must have occurred in the non-α-chains (Table 4).

Twenty-seven types of amino-acid substitutions observed in human haemoglobins (including four differences between β- and δ-chains) have recently been summarized[3]. Since then the substitution Lys→Gln has been observed in haemoglobin R Woolwich[4]; the present observation adds a further substitution Ala→Asp. All these substitutions are compatible with single point mutations according to the Triplet Code elaborated by Nirenberg *et al.*[5].

We thank Mrs. K. Burnie, Miss P. Kynoch and Mrs. T. Vanderheyden for their assistance.

GLOBIN SYNTHESIS IN THALASSAEMIA: AN *IN VITRO* STUDY

By Dr. D. J. WEATHERALL*, Dr. J. B. CLEGG† and Dr. M. A. NAUGHTON
Departments of Medicine and Biophysics, The John Hopkins University School of Medicine, Baltimore, Maryland

THE inherited haemoglobinopathies are of two main types. There are those, like sickle-cell disease, in which a structural change in one of the peptide chains of the globin fraction can be demonstrated, and there are others in which, despite evidence of an inherited defect in globin synthesis, no structural change in the globin moiety can be found. Disorders of haemoglobin synthesis of the second type are often found in patients with the clinical and haematological findings of thalassaemia and are, therefore, designated the 'thalassaemia syndromes'.

Human adult haemoglobin has a major and a minor component called haemoglobins A and A_2 respectively[1]. Haemoglobin A has two α- and two β-peptide chains ($\alpha_2\beta_2$) and haemoglobin A_2 has two α- and two δ-chains ($\alpha_2\delta_2$) (ref. 3). Foetal haemoglobin, which has usually disappeared by the age of one year, has two α- and two γ-chains ($\alpha_2\gamma_2$) (ref. 4). There is good evidence that the structures of the α-, β-, γ-, and δ-chains are determined by separate pairs of genes[5].

In some patients with the clinical picture of thalassaemia, foetal haemoglobin synthesis persists beyond the first year of life and haemoglobin A_2 levels exceed the normal range of 1·5–3·5 per cent of the total haemoglobin[6]. Furthermore, the genetic determinant for this type of thalassaemia behaves as though it were allelic or closely linked to the β-chain locus and also interacts with the sickle-cell (β^S-chain) gene[7]. These observations suggest that this form of thalassaemia results from an inherited defect in β-chain synthesis, the increased amounts of haemoglobins F and A_2 reflecting an attempt at compensation for the deficit in β-chains. The disorder is therefore designated β-thalassaemia.

Some patients with the clinical picture of thalassaemia do not have increased levels of haemoglobin A_2 and F, however, and the genetic determinant segregates separately from the β-chain gene[8]. Some, but not all, individuals in this group carry variable quantities of haemoglobins H and Bart's. Haemoglobin H is a tetramer of normal β-chains (β_4), while haemoglobin Bart's is composed of four normal γ-chains (γ_4) (refs. 7, 8). It has been suggested, therefore, that this form of thalassaemia results from an inherited defect in α-chain synthesis, α-thalassaemia[9], the resulting excess of β-chains or γ-chains aggregating to form haemoglobins H or Bart's respectively. This concept of the genetic basis of the thalassaemias, while serving as a useful model for their further investigation, is incomplete since there is increasing evidence for the existence of several types of β-thalassaemia and, probably, of α-thalassaemia[7].

Little is known about the mechanisms involved in the control of normal α- and β-chain synthesis in man, or about the factors which maintain δ-chain synthesis at about 1/40 of the level of β-chain synthesis. Furthermore, although there is evidence that the reticulocyte ribosomes of thalassaemic individuals have a reduced capacity for globin synthesis[10], there has been no direct evidence for defective α- and β-chain synthesis in these disorders. The chemical structure of these chains appears to be normal[11] and the nature of the apparent defect in synthesis remains quite obscure. The experiments to be described here were carried out in an attempt to clarify some of these problems by comparing the *in vitro* incorporation of radioactive amino-acids into α-, β-, and δ-chains in the reticulocytes of thalassaemic and non-thalassaemic persons.

Reticulocyte-rich blood was obtained from 19 persons with the conditions listed in Tables 1, 2 and 3. 2–8 ml. of washed red cells was suspended in an amino-acid mixture[12] containing 25–100 μc. of uniformly labelled ^{14}C-leucine, and incubated in a Dubnoff metabolic shaker at 37° C, aliquots being removed at various times and immediately frozen. Haemoglobin fractions were purified by dialysis, column chromatography, and starch-block electrophoresis as previously described[13].

In some experiments ribosomes were prepared[14] before fractionation of the haemoglobins. Globin was prepared[15] from either purified haemoglobin fractions or, in some cases, directly from the washed whole-cell lysates without further purification. The α- and β-chains were separated by gradient elution chromatography on carboxymethyl-

Table 4. THE FIRST PEPTIDES OF THE α, γ, β AND δ CHAINS OF HUMAN HAEMOGLOBIN

	1	2	3	4	5	6	7
α	VAL	–	LEU	SER	PRO	ALA	ASP	LYS
γ	GLY	HIS	PHE	THR	GLU	GLU	ASP	LYS
β	VAL	HIS	LEU	THR	PRO	GLU	GLU	LYS
δ	VAL	HIS	LEU	THR	PRO	GLU	GLU	LYS
	1	2	3	4	5	6	7	8

Although many amino-acid substitutions are known for the first peptide of the β-chain (above) none has previously been described for the corresponding peptide of the α-chain (below).

	1	2	3	4	5	6	7
αT1	Val	Leu	Ser	Pro	Ala	Asp	Lys

Fig. 2. Incorporation of radioactivity into the α- and β-chains of haemoglobin A prepared from the cells of a patient with β-thalassaemia major. Incubation time 60 min. ●—●, *O.D.* (0·280 mμ); ○ - - - ○, radioactivity

Figure 1. Rates of globin synthesis in reticulocytes from a patient with β-thalassemia major. Incorporation of radioactivity into globin chains after reticulocytes from a patient with β-thalassemia major had been incubated for 60 minutes with ^{14}C-leucine. The solid line shows the amount of total protein, which is approximately the same for both β and α globin chains. The dotted line shows the rate of synthesis of new globin chains over the 60 minutes of the incubation, with significantly less synthesis of β globin compared to α globin.

standing of its pathophysiology, including an appreciation of ineffective erythropoiesis, and that the cellular pathology resulted from an excess of the unaffected globin chain rather than a deficiency of the protein from the mutated gene. This technique of globin chain synthesis was refined slightly over the years, but remained essentially the same. It was central to many of the discoveries concerning transcriptional and translational control of the globin genes, which in turn acted as paradigms for molecular biology in general. In particular, globin chain synthesis was used to identify the significance of many variants identified in the globin genes, and was only really replaced when quantitation of messenger RNA became routine in the 1990s. Similarly, it was used in the routine diagnosis of hemoglobinopathies until the 1980s, and in particular was used in the early prenatal diagnosis of hemoglobinopathies from fetal blood samples.[3]

This landmark paper arose primarily from the interaction between a very astute hematologist, David Weatherall, who had identified an important question, and a very skilled protein chemist, John Clegg, who knew how to answer it. Together they went on to write 200 papers together, including more than 20 published in *Nature*. This interaction was one of the driving forces behind the development of molecular medicine, with the establishment of the Institute of Molecular Medicine (later the Weatherall Institute of Molecular Medicine) in Oxford, and the spawning of numerous clinical and laboratory scientists across the world.

Disclosures

No conflicts of interest to disclose.

References

1. Cooley TB, Lee P. A series of cases of splenomegaly in children with anemia and peculiar bone changes. Trans Am Pediatr Soc. 1925;37:29-30.
2. Weatherall DJ, Clegg JB, Naughton MA. Globin synthesis in thalassaemia: an in vitro study. Nature. 1965;208(5015):1061-1065.
3. Fairweather DV, Modell B, Berdoukas V, et al. Antenatal diagnosis of thalassaemia major. Br Med J. 1978;1(6109):350-353.