of the association in all 12 patients tested. Withdrawal of the drug produced a rapid resolution of skin problems in all patients, and no recurrence was seen on instituting alternative beta-blockade therapy.

Zacharias (1972) reported that 16 out of 156 patients developed slowly progressive chronic skin reactions which appeared to be of a mixed eczematous and psoriatic nature. In our 21 patients 14 presented with a distinctive picture of a psoriasiform eruption which was most marked over bony prominences, invariably preceded by hyperkeratotic changes on the palms, soles, and sides of the fingers, and followed by an erythematous scaling, marginated rash involving other areas of the body. The full clinical picture tended to develop over several months. In only two of our patients was there a previous history of psoriasis, and we have no evidence that pre-existing psoriasis is aggravated by practolol. Despite the psoriasiform appearance of the eruption the histology never suggested true psoriasis. The slow evolution of this psoriasiform type of rash is in marked contrast to the acuteness of the lichenoid, L.E.-like, and erythrodermatous reactions.

Five of our patients (24%) had antinuclear factor in the serum whereas Raftery (1972) reported an incidence of 3%-4% in all patients on practolol. There were two patients with arthropathy who were positive for antinuclear factor, while six patients showed some histological features compatible with a diagnosis of lupus erythematosus. The one patient with polyserositis was negative for antinuclear factor and L.E. cell phenomenon were absent. In four patients with a positive response antinuclear factor persisted for two to three months after practolol had been stopped, which is in keeping with a diagnosis of drug-induced systemic lupus erythematosus (S.L.E.) (Bodman *et al.*, 1967; Alarcon-Segovia, 1969). In one patient the antinuclear factor persisted for 16 months. Antibodies to native DNA were not found in the patients who were positive for antinuclear factor, confirming the findings of Hughes (1971) that drug-induced S.L.E. can be distinguished from true S.L.E. by the absence of antibodies to native DNA.

Three of our patients had sore dry eyes as reported by Wright (1974), and tear secretion was found to be diminished in two on formal testing. The third patient developed bilateral corneal ulcers. In all three patients eye symptoms were still present at the time of writing despite drug withdrawal, and the ophthalmological problems may well prove to be the most serious aspect of this drug-induced syndrome. A strong case can be made for performing routine Schirmer's tear secretion tests at regular intervals during practolol therapy.

The mechanism whereby this drug reaction occurs has yet to be clarified. We have probably excluded immediate- and delayed-type allergies. It is possible that since practolol acts as a partial sympathomimetic agonist it will stimulate cyclic adenosine monophosphate activity. This in turn may have some direct effect on epidermal cells or, as Raftery and Denman (1973) suggested, impair the activity of T-lymphocyte populations, resulting in the production of strains of lymphocytes with autoimmune propensities. It is of interest that a histological picture closely resembling that seen in our patients has been reported in a patient with lichen planus associated with thymoma (Tan, 1974).

Further studies of cell dynamics in patients with practolol reactions are in progress.

We thank Dr. Graham Hughes, Hammersmith Hospital, for examining the sera for anti-DNA antibody, Mr. P. Drummond, ophthalmic department, Royal Victoria Infirmary, and Mr. D. Wilkins, Wellcome Laboratories for Research into Skin Diseases, for the immunofluorescent studies. We thank Professor Sam Shuster for his advice, and Dr. C. J. Stevenson for permission to reproduce (fig. 3).

Requests for reprints should be sent to Dr. R. H. Felix, University Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne.

References

Alarcon-Segovia, D. (1969). *Mayo Clinical Proceedings*, 44, 664.
Barrett, A. M. (1971). *Postgraduate Medical Journal*, January 1971 Supplement. No. 2, p.7
Bodman, S. F., Hoffman, J. J., and Condemi, J. J. (1967). *Arthritis and Rheumatism*, 10, 269.
George, C. F., Nagle, R. E., and Pentecost, B. L. (1970). *British Medical Journal*, 2, 402.
Felix, R. H., and Comaish, J. S. (1974). *Lancet*, 1, 1017.
Hughes, G. R. (1971). *Lancet*, 2, 861.
Raftery, E. B. (1972). In *New Perspectives in Beta-blockade International Symposium*, p. 239. Denmark. Ciba Laboratories.
Raftery, E. B. and Denman, A. M. (1973). *British Medical Journal*, 2, 452.
Rowland, M. G. M., and Stevenson, C. J. (1972). *Lancet*, 1, 1130.
Sandler, G., and Clayton, G. A. (1970). *British Medical Journal*, 2, 399.
Tan, R. S. H. (1974). *Proceedings of the Royal Society of Medicine*, 67, 196.
Wiseman, R. A. (1971). *Postgraduate Medical Journal* 47, supplement No. 2. p. 68.
Wright, P. (1972). *British Medical Journal*, 2, 560.
Zacharias, F. J. (1972). In *New Perspectives in Beta-blockade International Symposium*, p. 238. Denmark. Ciba Laboratories.

Haemoglobin Belfast 15 (A12) Tryptophan→ Arginine: A New Unstable Haemoglobin Variant

C. COTTON KENNEDY, GILLIAN BLUNDELL, P. A. LORKIN, A. LANG, H. LEHMANN

British Medical Journal, 1974, 4, 324-326

Summary

A new unstable haemoglobin, $\alpha_2\beta_2$15 Trp→Arg (Hb Belfast), with increased oxygen affinity has been found during the routine investigation of a long-stay psychiatric patient. It seemed to cause little haematological disorder. The reticulocytes synthesized normal and abnormal β-chains at the same rate but in the circulating blood Hb Belfast amounted to only 27·5% of the total haemoglobin.

Belfast City Hospital, Belfast BT9 7AB
C. COTTON KENNEDY, D.M., F.R.C.PATH., Consultant Clinical Pathologist, Department of Haematology
GILLIAN BLUNDELL, M.B., M.R.C.PATH., Senior Registrar, Department of Clinical Chemistry

M.R.C. Abnormal Haemoglobin Unit, University Department of Biochemistry, Addenbrooke's Hospital, Cambridge CB2 2QR
P. A. LORKIN, B.A., Member of Scientific Staff
A. LANG, PH.D., Member of Scientific Staff
H. LEHMANN, M.D., SC.D., F.R.S., Honorary Director

Introduction

About 40 unstable haemoglobins have now been described (Lehmann, 1974). A few were discovered by chance, and we report such a haemoglobin found in the course of routine investigation of a long-stay psychiatric patient.

Case Report

A 42-year-old Caucasian man had for the past 15 years been confined to hospital with paranoid schizophrenia. His history included appendi-

cectomy in 1952 and a myocardial infarct in 1972. He was a heavy smoker and had recently suffered an acute exacerbation of chronic bronchitis. A routine blood count performed on a model S Coulter counter gave the first indication of a possible haematological abnormality.

Physical examination showed a man of sallow complexion noted to become breathless on mild exertion. An enlarged, slightly tender mass thought to be the spleen was palpable on the left side of the abdomen. The liver was enlarged two to three fingerbreadths below the costal margin. Blood pressure was normal. He had never noticed dark urine and there was no history of blood loss. There was evidence of a persistent urinary infection, and a recent intravenous pyelogram had shown pronounced bilateral renal enlargement with distortion of the pelvis and calices consistent with polycystic disease.

He was the fifth-born child of unrelated Northern Irish and Scottish parents. There were no foreign antecedents. Polycystic renal disease had also been found in three sisters, two of whom had been given transplants.

Treatment included nitrazepam, chlorpromazine, and digoxin.

Haematological and Biochemical Studies.—The blood film showed marked hypochromia with microcytosis, polychromasia, and some target cells. Haemoglobin 13·7 g/100 ml; packed cell volume 44·5%; mean corpuscular volume 68 fl; red cells 6·51 × 10^6/mm^3; mean corpuscular haemoglobin concentration 30·6%; mean corpuscular haemoglobin 20·5 pg. The discriminant function (England and Fraser, 1973) was minus 10·4, suggesting thalassaemia trait. Over the previous two years the haemoglobin had varied from 10·6 to 13·6 g/100 ml and the mean corpuscular haemoglobin from 20·5 to 24·8 pg (normal range 27-32 pg). The reticulocyte count was 4%. Serum iron levels were 42 μg/100 ml with a total iron-binding capacity of 300 μg/ml. Oral and intramuscular iron therapy had no appreciable effect on these levels. Blood urea was in the range 44-50 mg/100 ml. Serum bilirubin, liver function, and haptoglobins were normal. No occult blood was detected in the faeces. Wassermann's reaction was negative. A test for unstable haemoglobins (Carrell and Kay, 1972) gave a positive result and after 48 hours of incubation at 37°C Heinz bodies were found in more than 60% of the red cells. Large numbers of inclusion bodies were shown after staining the red cells with brilliant cresyl blue. Electrophoresis of haemolysate on cellulose acetate (Bunn, 1973) showed, in addition to Hb A and Hb A_2, haemoglobin with a slightly slower mobility than Hb A.

Methods

Purification of the abnormal haemoglobin, isolation of the α-chains and β-chains, and identification of the amino-acid substitution were carried out as previously described (Beutler *et al.*, 1974).

Oxygen affinity measurements were made on the unfractionated haemolysate and on the chromatographically purified haemoglobin in 0·1 mol of potassium phosphate buffer per l. by the continuous recording method of Imai *et al.* (1970).

Biosynthetic studies were performed in vitro on reticulocyte-enriched preparations of red cells from peripheral blood (Lang *et al.*, 1972). Incubation of the reticulocytes was prolonged (two hours) at 37°C in the presence of 20 μCi of ^{14}C-leucine (270 mCi/mmol). Total ^{14}C-leucine (c.p.m.) incorporated into each globin chain isolated by ion-exchange chromatography was measured before dialysis of the pooled fractions against 0·5% formic acid at 4°C. Specific activities (^{14}C-leucine per optical density (O.D.) at 280 nm) were determined on the isolated globin chains after dialysis.

Results

Identification.—Ion-exchange chromatography of the total globin located the amino-acid substitution in the β-chain of the abnormal haemoglobin (fig. 1). The fingerprint of the aminoethylated β-chain differed from normal in two respects; three new tryptic peptides (Tp) (1, 2, and 3; fig. 2) were located and β Tp II, which normally stains intensely for tryptophan, though detectable was greatly reduced in intensity. This was probably tryptophan δ-15. The amino-acid composition of the three new peptides are shown in table I. Peptide 1 had the composition

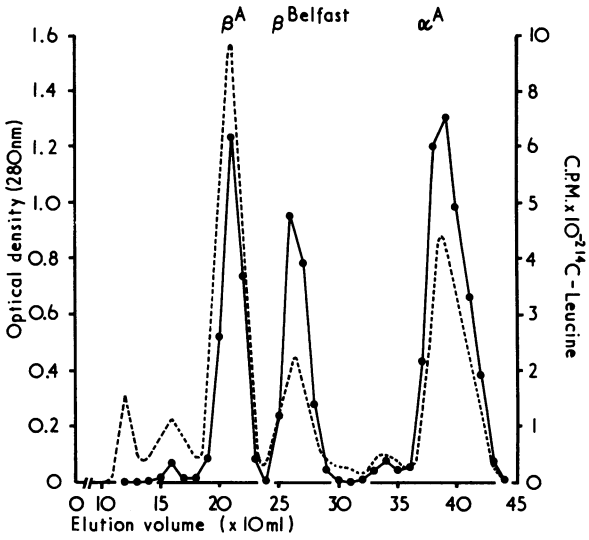

FIG. 1—Elution profile of ^{14}C-labelled total globin from CM 23 cellulose. Optical density at 280 nm (——). ^{14}C-leucine (c.p.m.) (●——●).

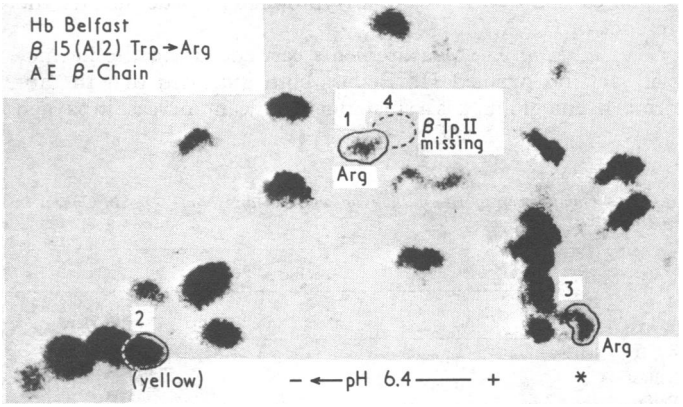

FIG. 2—Peptide-map fingerprint of aminoethylated (AE) β-chain of Hb Belfast. Electrophoresis was carried out at pH 6·4 for one hour at 2·5 kV. Ascending chromatography in isoamylalcohol: pyridine: water (6:6:7 v/v) 16 hours.

TABLE I—*Amino-acid Composition of New Peptides from βBelfast Chain*

	Molar Ratios		
Peptide:	1 (β9-15)	2 (β16-17)	3 (β16-30)
Asp			2·0
Thr	0·9		
Ser	0·7		
Glu			2·2
Gly*		0·7	3·6
Ala	2·0		1·0
Val	1·0		3·0
Leu	1·1		1·1
Lys		1·0	1·0
Arg	0·9		0·7

*N terminal glycine can be extensively destroyed by ninhydrin.

expected for the sequence β9-15 of β Tp II with β15 tryptophan replaced by a residue of arginine (fig. 3). The compositions of peptides 2 and 3 correspond to the sequences β16-17 and β16-30. All three peptides would result from tryptic hydrolysis at the new susceptible bond introduced by the mutation β15 Trp→Arg (fig. 3). The existence of peptide 3 (β16-30) suggests that the introduction of arginine at residue β15 must greatly reduce the rate of tryptic hydrolysis at the neighbouring Lys-Val (β17-18) bond. The mutation β15 (A12) Trp→ Arg has not been described before, and the haemoglobin has been called Hb Belfast.

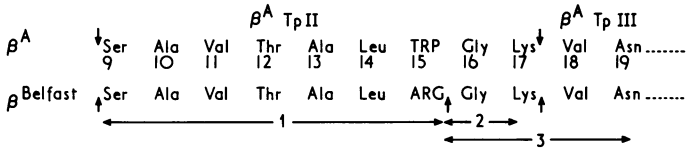

FIG. 3—Amino-acid sequence of new peptides (1, 2, and 3) from β-chain of Hb Belfast. Vertical arrows indicate position of hydrolysis by trypsin.

In-vitro Biosynthesis.—The incorporation of ^{14}C-leucine into the separated globin chains αA, βA, and ^{14}Belfast is shown in fig. 1. Comparison of the total synthesis of each chain as estimated from the total c.p.m. (table II) showed balanced synthesis of α-chain and non-α-chains in reticulocytes and equal synthesis of both βA and βBelfast chains. The δ-chain coeluting with the βBelfast chain does not contribute to the incorporation of ^{14}C-leucine since there is no detectable synthesis of δ-chains in reticulocytes (Rieder and Weatherall, 1965). G100 Sephadex gel filtration of the haemolysate gave no indication that the pool of free α-chains was greater than normal. Therefore, any reduction in the amount of Hb Belfast must occur after its synthesis due to instability of the unstable Hb Belfast. The haemolysates contained Hb A 69%, Hb Belfast 27·5%, and Hb A_2 3·5%. When the proportion of Hb Belfast was estimated from fig. 1 allowance was made for the reduction in the extinction coefficient at 280 nm caused by the loss of a tryptophan residue and for the presence of Hb A_2.

Oxygen Affinity.—Measurements carried out on the haemolysate and on purified Hb Belfast both indicated that the abnormal haemoglobin has a small but significant increase in oxygen affinity (fig. 4). The allosteric interaction, as measured by n, the exponent of the Hill equation, was normal in the case of the purified haemoglobin but somewhat diminished in haemolysates at an oxygen saturation of less than 50%. This apparent reduction of n in haemolysates is probably the result of interaction between two haemoglobins of different oxygen affinities. The Bohr effect of the haemolysate was normal.

TABLE II—*Comparison of Biosynthesis of α-globin and Non-α-globin Chains in Reticulocytes*

	Specific Activities		
	Total c.p.m.	c.p.m./O.D. 280 nm	^{14}C (c.p.m.)/μmol of ^{12}C-leu*
$\alpha^A/\beta^B + \beta^{Belfast}$	0·97		
$\beta^{Belfast}/\beta^A$	0·95		
$\beta^{Belfast} + \delta/\beta^A$		2·10	
$\beta^{Belfast}/\beta^A$			2·49

*$\beta^{Belfast}$ + δ-chain, isolated by CM cellulose chromatography, was fingerprinted (see Methods) to obtain abnormal peptides 1 and 3 (fig. 2). Specific activity ^{14}C-leucine (c.p.m.) per μmol of ^{12}C-leucine) was determined as described by Lang *et al.* (1972).

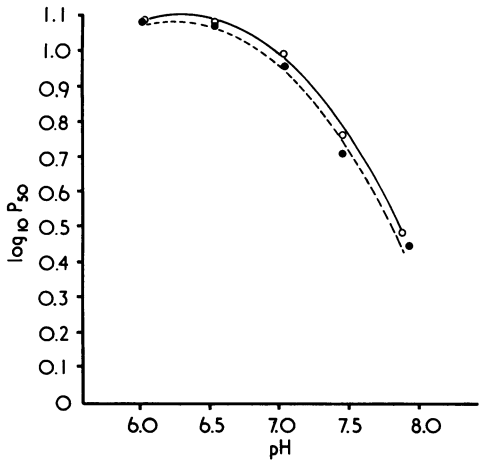

FIG. 4—Oxygen affinity (P_{50}) of haemolysate containing Hb Belfast. o—o Normal control ●—● patient.

Discussion

The substitution in Hb Belfast has occurred at position β15 in the sequential nomenclature and A12 in the helical nomenclature. A12 is an internal hydrophobic residue in all known globins, usually tryptophan but occasionally phenylalanine (Dayhoff, 1972). The introduction at this position of a hydrophilic arginine would be expected to destabilize the globin structure. The unstable haemoglobin was found in the course of a routine investigation and not because of any complaint such as an anaemia. The abnormal haemoglobin has a high oxygen affinity, yet no erythraemia was found and the packed cell volume was normal. This could well have been due to additional iron deficiency which may have been a compensatory factor and protected the patient, who had already suffered once from a myocardial infarct, from the consequences of a high cell volume. Interestingly it was possible to show that in the reticulocytes the unstable haemoglobin was produced at the same rate as the normal adult haemoglobin. In the circulating blood, however, the proportion of Hb A was 69% and that of Hb Belfast only 27·5%. Thus extensive destruction must occur after the haemoglobin has been synthesized.

Another point of interest is that the patient had polycystic kidneys, which were looked for and discovered only after it was found that there were several instances of this disorder in his family. His mother was stated to have died of uraemia due to a bilateral renal disorder, and one of her brothers had died from "poisoned kidneys." One of the patient's sisters died in 1972 after kidney transplantation. Another sister also had polycystic renal disease, and yet another had a kidney transplant operation in Belfast City Hospital in 1970, and one of her daughters was suffering from Still's disease and polycystic kidneys. Thus it was possible to find in three generations no fewer than seven instances of what probably was in all cases polycystic renal disease. The investigations continue but up to the time of writing no member of the patient's large family had been found to have an abnormal haemoglobin.

The blood of a brother living in New Zealand has now been examined by Dr. R. W. Carrell, who found that it contained an unstable haemoglobin. The brother's renal status is not yet known.

We thank Dr. G. A. Kernohan and Dr. J. F. Perry, Downshire Hospital, Downpatrick, for allowing us to study patients under their care.

Requests for reprints should be sent to Dr. C. Cotton Kennedy.

References

Beutler, E., Lang, A., and Lehmann, H. (1974). *Blood*, **43**, 527.
Bunn, H. F. (1973). *American Journal of Medical Technology*, **39**, 379.
Carrell, R. W., and Kay, R. (1972). *British Journal of Haematology*, **23**, 615.
Dayhoff, M. O. (editor) (1972). In *Atlas of Protein and Structure*, vol. 5. Silver Spring, Maryland, National Biomedical Research Foundation.
England, J. M., and Fraser, P. M. (1973). *Lancet*, **1**, 449.
Imai, K., *et al.* (1970). *Biochimica et Biophysica Acta*, **200**, 189.
Lang, A., White, J. M., and Lehmann, H. (1972). *Nature New Biology*, **240**, 268.
Lehmann, H. (1974). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 92.
Rieder, R. F., and Weatherall, D. J. (1965). *Journal of Clinical Investigation*, **44**, 42.