Haemoglobin Rahere (β82 Lys-Thr) : a new high affinity haemoglobin associated with decreased 2, 3-diphosphoglycerate binding and relative polycythaemia

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Summary

A new haemoglobin with increased oxygen affinity, β82 (EF6) lysine→threonine (Hb Rahere), was found during the investigation of a patient who was found to have a raised haemoglobin concentration after a routine blood count. The substitution affects one of the 2, 3-diphosphoglycerate binding sites, resulting in an increased affinity for oxygen, but both the haem-haem interaction and the alkaline Bohr effect are normal in the haemolysate. This variant had the same mobility as haemoglobin A on electrophoresis at alkaline pH but was detected by measuring the whole blood oxygen affinity; it could be separated from haemoglobin A, however, by electrophoresis in agar at acid pH. The raised haemoglobin concentration was mainly due to a reduction in plasma volume (a relative polycythaemia) and was associated with a persistently raised white blood count. This case emphasises the need to measure the oxygen affinity of haemoglobin in all patients with absolute or relative polycythaemia when some obvious cause is not evident.

Case Report

A 35-year-old Englishman presented to his doctor in 1973 with giddiness and deafness in one ear. These symptoms were ascribed to vestibular neuritis, but a routine blood count showed raised haemoglobin (20 g/dl). He was referred to St Bartholomew's Hospital for further investigations. His giddiness had by then disappeared. He had been investigated for hypertension two years earlier but the raised blood pressure was a transient finding. He smoked 20 cigarettes a day but exercise tolerance was normal and in his schooldays he had held the record for the 100-yard sprint. There was no history of renal or respiratory disease and there was no family history suggestive of polycythaemia. His father had died in an accident aged 55, and his mother was alive and well and aged 66. He was married with three young children.

Examination showed him to be a nervous and tense man weighing 83 kg; he was slightly depressed and obsessed that there was something seriously wrong with him. Blood pressure was 160/90 mm Hg and the heart sounds were normal. The liver was palpable 2-cm below the costal margin, but there was no splenomegaly, no lymph nodes were palpable, and there was no renal bruit.

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The laboratory tests carried out by standard methods[1] gave the following results: haemoglobin 19·0 g/dl; packed cell volume 57%; red blood count 6×10^{12}/l (6 000 000/mm³); mean cell volume 95 fl (95 μm³); mean cell haemoglobin 32 pg; mean cell haemoglobin concentration 33 g/dl; reticulocytes 0·4%; white blood count $13·5\times10^{9}$/l (13 500/mm³), with a neutrophil leucocytosis; platelets 275×10^{9}/l (275 000/mm³). All these results were the mean values of 18 determinations over one and a half years. Blood volume studies performed on two occasions using ^{51}Cr-labelled red cells and ^{131}I-labelled albumin showed a red cell mass of 35 ml/kg (normal range 30 ± 5 ml/kg), and a plasma volume of 32·5 ml/kg (normal range 45 ± 5 ml/kg); these indicated a relative polycythaemia, the raised haemoglobin level being mainly secondary to a reduction in plasma volume. Other findings included a leucocyte alkaline phosphatase score of 80 IU/l, serum B_{12} 225 ng/l, serum folate 5·9 μg/l, red cell folate 304 μg/l, serum iron 23·3 μmol/l (130 μg/100 ml), and TIBC 73·0 μmol/l (408 μg/100 ml). There was thus no evidence of any myeloproliferative disorder or deficiency state which could have held down the red cell mass. Bone marrow examination was refused. Intravenous pyelogram showed a small radio-opaque calculus at the lower pole of the left kidney, but no cause was found for this.

The oxygen dissociation curve of the patient's whole blood measured by tonometry at pH 7·4 and 37°C (fig 1) was shifted to the left of that of a normal control, which indicated a raised oxygen affinity.

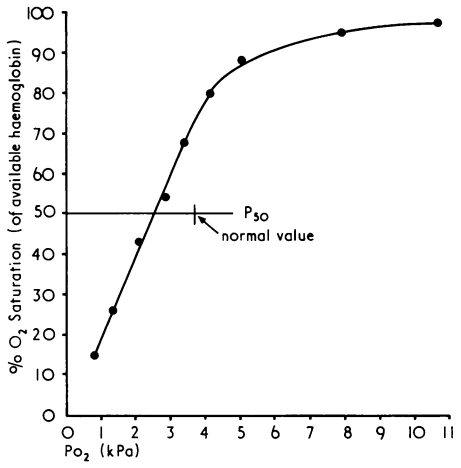

FIG 1—Patient's whole blood oxygen dissociation curve at 37°C, pH 7·4, (4·5% carboxyhaemoglobin). Curve is shifted to left.
Conversion: SI to traditional units—Po_2: 1 kPa ≈ 7·5 mm Hg.

The P_{50} (partial pressure of oxygen at which the haemoglobin is 50% saturated with oxygen) was 2·4 kPa (18 mm Hg) on two separate occasions (normal $3·6\pm0·2$ kPa ($27\pm1·5$ mm Hg)). Arterial blood analysis was normal and showed a pH of 7·45, oxygen pressure (Po_2) of 10·7 kPa (80 mm Hg), carbon dioxide pressure (Pco_2) of 5·1 kPa (38 mm Hg), and an oxygen saturation of 97%. The carboxy haemoglobin level was 4·5%. The 2, 3-diphosphoglycerate (2,3-DPG) level, measured by a modification of the method of Lowry *et al*,[2] was 8·5 μmol/g haemoglobin (2·3 mg/g), which was at the lower end of the normal range of 8·2-17·4 μmol/g-haemoglobin (2·2-4·6 mg/g). The oxygen affinity of the haemolysate was also increased when measured by the method of Imai[3] at 20°C in 0·1-M potassium phosphate at five different pH values (fig 2). The P_{50} was about 83% of the value of a normal haemolysate but the Hill coefficient, n, and the alkaline Bohr effect were normal.

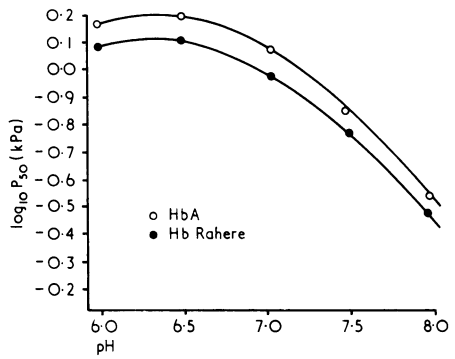

FIG 2—Oxygen affinity data for haemolysates from patient (●) and normal control (○) measured in 0·1-M potassium phosphate buffers at 20°C showing variation of log P_{50} with pH. P_{50} is partial pressure of oxygen at which haemoglobin is 50% oxygenated (for whole blood see fig 1).
Conversion: SI to traditional units—P_{50}: 1 kPa ≈ 7·5 mm Hg.

IDENTIFICATION OF THE ABNORMAL HAEMOGLOBIN

Electrophoresis on paper (pH 8·9) and cellulose acetate (pH 8·6)[4] showed only the normal bands with the mobilities of Hb A and Hb A_2. The Hb A_2 and Hb F (alkali-resistant haemoglobin) concentrations were in the normal ranges (2·8% and 1·0% respectively). Agar gel electrophoresis at pH 6·0 and electrofocusing, however, both showed two major bands of about equal intensity. In agar the bands ran in the positions of Hb A and Hb F, but on electrofocusing the new band focused on the anodal side of Hb A with a lower isoelectric point and well separated from Hb F. The new variant (Hb Rahere) was also eluted after Hb A on chromatography on DEAE Sephadex in TRIS-HCl buffers[5] and constituted 48% of the total haemoglobin. A sample of globin prepared from the whole haemolysate was separated by chromatography on CM cellulose[6] in 8-M urea-phosphate buffer, pH 6·7, into three main fractions corresponding to normal α^A-chains, normal β^A-chains, and abnormal β-chains, which were less positively charged at pH 6·7. In the fingerprint of the aminoethylated (AE) abnormal β-chains (fig 3) two tryptic peptides normally present in the $AE\beta^A$-chains—βTpIX (β67-82) and AEβTpX (β83-95)—were replaced by a single new peptide. Amino-acid analysis showed that this was a new composite peptide AEβTpIX-X (β67-95) in which the lysine at position 82 was replaced by threonine (fig 4), thereby preventing tryptic hydrolysis at this position.

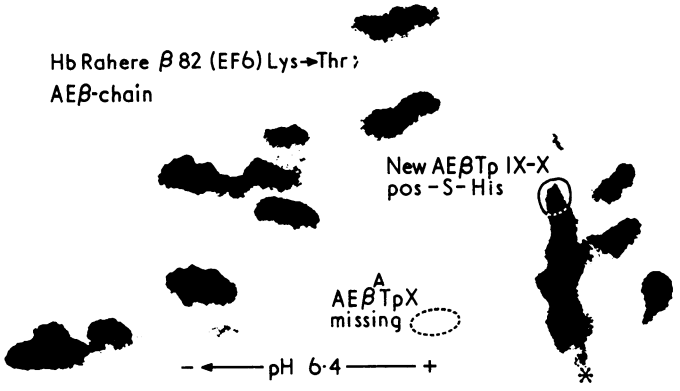

FIG 3—Fingerprint of tryptic digest of isolated AEβ-chain of Hb Rahere; electrophoresis in pyridine-acetic acid buffer pH 6·4 in horizontal direction; ascending chromatography in isoamyl alcohol-pyridine-water (6:6:7 by vol) in vertical direction. -S- = positive reaction for "divalent sulphur," indicating the presence of S-β aminoethylcysteine or methionine. New peptide AEβTpIX-X replaces two missing peptides β^ATpIX and $AE\beta^A$TpX; it occupies about the same position on the fingerprint as β^ATpIX.

OXYGENATION PROPERTIES OF ISOLATED HB RAHERE

The oxygen affinity of haemoglobin is regulated in vivo by intracellular concentrations of organic phosphates, the most important of which are 2,3-DPG in mammals and inositol hexaphosphate (IHP) in birds and reptiles.[7] They combine specifically with the deoxy-form, thereby stabilizing it and lowering the oxygen affinity. The structure of complexes of human deoxyhaemoglobin A with 2,3-DPG and IHP has been investigated by x-ray crystallography,[8 9] and the binding site has been identified as a cavity formed between the two partner β-chains and lined with positively charged groups that bind the negatively charged phosphate molecule by electrostatic attraction. Hb Rahere is one of several variants with amino-acid substitutions in the DPG binding site (see table). The replacement of lysine by threonine results in the loss of two positively charged groups, one from each chain, which should cause a weakening in the binding of 2,3-DPG. This supposition was confirmed by the measurement of the oxygen affinity of purified Hb Rahere in the presence and absence of 2-mM 2,3-DPG and 2-mM IHP (fig 5) "Stripped"—that is, phosphate-free—Hb A has a very high oxygen affinity; in the presence of the organic phosphates there is a large shift to the right of the dissociation curves, representing an increase in P_{50} and a decrease of affinity. Although purified stripped Hb Rahere had nearly the same P_{50} as stripped Hb A, the shifts to the right in the presence of 2,3-DPG or IHP were much smaller (fig 5), indicating that it was less responsive to the regulatory effect of the organic phosphates. These findings explain why the oxygen affinity of blood containing Hb Rahere is higher than that of normal blood. Similarly, fetal blood has a higher affinity than adult blood because the replacement of positively charged histidine β143 by the neutral serine γ143 also weakens the binding of 2,3-DPG.

	+						−				+		−			
Val	Leu	Gly	Ala	Phe	Ser	Asp	Gly	Leu	Ala	His	Leu	Asp	Asn	Leu	Thr	...
67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	
							−		+	+		−	+ −			
... Gly	Thr	Phe	Ala	Thr	Leu	Ser	Glu	Leu	His	AECys		Asp	Lys			
83	84	85	86	87	88	89	90	91	92	93		94	95			

FIG 4—Amino-acid sequence of new peptide βTpIX-X (67-95) of Hb Rahere. + and − = electric charges at pH 6·4.

*Haemoglobin variants with substitutions in the 2,3-DPG binding site**

Name of variant	Substitution	Oxygen affinity	Erythrocytosis	Reference
Hb AIc	β1 (NA1) blocked	Increased	−	10
Hb Tokuchi	β2 (NA2) His→Tyr	?	?	11
Hb Deer Lodge	β2 (NA2) His→Arg	?	−	12
Hb Rahere	β82 (EF6) Lys→Thr	Increased	+	This paper
Hb Little Rock	β143 (H21) His→Gln	Increased	+	13
Hb Syracuse	β143 (H21) His→Pro	Increased	+	14
Hb Abruzzo	β143 (H21) His→Arg	Increased	−	15, 16

*Hb Shepherd's Bush β74 (E18) Gly→Asp also shows a reduced binding of 2,3-DPG,[31] which would explain the raised oxygen affinity of this variant.[32]

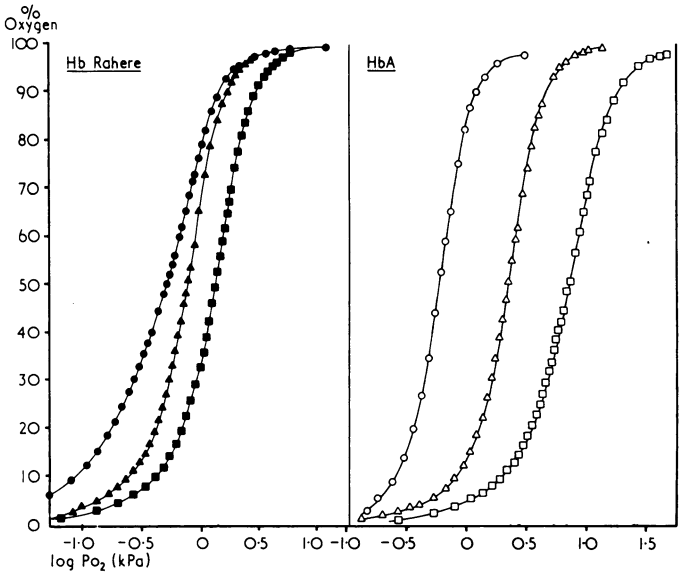

FIG 5—Oxygen dissociation curves for purified Hb Rahere (closed symbols) and Hb A (open symbols), stripped (●, ○) and in presence of 2-mM 2,3-DPG (▲, △) and 2-mM IHP (■, □). Note that shift to right caused by organic phosphates is smaller in Hb Rahere than in Hb A.

Discussion

Familial polycythaemia was first described[17] in 1914, but it was not until 1966 that Charache *et al*[18] showed that it could result from the inheritance of a haemoglobin variant (Hb Chesapeake) with a high oxygen affinity. Subsequently 15 more high affinity variants associated with "polycythaemia", as judged by a haemoglobin concentration above the normal range, have been described (summarised in refs 4, 19, and 20). In those cases in which the red cell count was reported it was usually raised but in others the white cell and platelet counts were nearly always normal, and so some would prefer the term "erythrocytosis" to "polycythaemia." Blood volume studies were carried out in only a few cases. In some patients the red cell mass was unequivocally raised, indicating an absolute polycythaemia, but in others was near the upper limit of normal, suggesting the possibility of a relative polycythaemia or haemoconcentration. The white cell and platelet counts are often normal, but in many cases these data were not recorded. Nevertheless, a raised white count was found in at least four families from the 16 variants so far reported: in association with Hb Ypsilanti[21] (1 out of 14 cases); in the American family with Hb Malmö[22 23] (3 out of 16 cases), though not in the Swedish family[24]; in Hb Heathrow[25] (3 out of 5 cases); and in our case, in which only the propositus has been tested. No haemoglobin, red cell, or packed cell volume values were reported for Hb J Capetown,[26] and in Hb Hirose[27] they were normal. In a patient with Hb Abruzzo[15 16] the haemoglobin concentration. was normal (14 g/dl), though the red cell count was raised ($7{\cdot}5 \times 10^{12}$/l (7 500 000/mm^3)) with hypochromic erythrocytes. This case was complicated, however, by the possible presence of β-thalassaemia, which may have prevented the development of a high haemoglobin concentration.

Some high-affinity haemoglobins are also unstable and these are associated with a haemolytic anaemia of the Heinz-body type rather than polycythaemia, but the haemoglobin level is often slightly higher than would otherwise be expected for the degree of haemolysis.[4] In our case peripheral blood studies showed raised haemoglobin and red cell and white cell counts. Blood volume studies, measuring the red cell mass and plasma volumes independently, showed that the red cell mass was only at the upper limit of normal but that there was a relatively greater reduction in the plasma volume. This would suggest that relative polycythaemia was the predominant cause of the high haemoglobin concentration and erythrocytosis. Nevertheless, there is such controversy over the ways used to express blood volume results[28 29] that it is difficult to state categorically that there was not an element of absolute polycythaemia contributing to the high haemoglobin concentration and red count in our case.

The polycythaemia associated with the stable high-affinity haemoglobins is assumed to develop in compensation for a reduction in oxygen delivery to the tissues. One might expect the polycythaemia to be of the absolute type with a raised red cell mass as in other cases of secondary polycythaemia. Possibly, however, tissue oxygenation could be maintained equally well by either an absolute or a relative polycythaemia since what matters to the tissue is the haemoglobin concentration in the local blood vessels not the whole body red cell mass. Possibly in certain circumstances a reduction in the plasma volume could represent a compensatory mechanism when oxygen delivery to the tissues is impaired. At present we have no satisfactory explanation for the raised white count, nor is one available for the other cases in which it was reported to be raised.

The mild polycythaemia in the patient might be explained by the following considerations. In blood containing equal amounts of Hb A and Hb Rahere a relatively large proportion of the haemoglobin may exist as hybrid tetramers of the form $\alpha_2^A \beta^A \beta^R$, in which one of the lysine β82 residues is still present. Crystallographic studies indicate that the carboxyl group of 2,3-DPG is bound more closely to one of the lysine β82 residues than to its partner in the opposite β-chain. Consequently the hybrid tetramers might bind 2,3-DPG nearly as well as Hb A and the main contribution to the raised affinity would come from the $\alpha_2\beta_2^R$ tetramers. Assuming a binominal distribution, there would be 25% of the latter form, 50% of hybrid tetramers, and 25% $\alpha_2\beta_2^A$.

In Hb Rahere blood the P_{50} is reduced to 2·4 kPa (18 mm Hg) (66% of the normal). Blood gas analysis showed a carboxyhaemoglobin level of 4·5%, however, which was not unexpected in a smoker. The presence of some carboxyhaemoglobin increases the average affinity of the haemoglobin molecule for oxygen[30] and therefore reduces the P_{50}; 4·5% carboxyhaemoglobin will reduce the P_{50} by about 0·2 kPa (1·5 mm Hg). Allowing for this, the molecular substitution and consequent reduction in 2,3-DPG binding accounts for a 1-kPa (7·5-mm Hg) (or 27%) fall in P_{50}.

This patient's initial haematological findings resembled polycythaemia rubra vera, the erythrocytosis being associated with a persistently raised white count and a serum urate at the upper limit of normal. This emphasises the need to consider haemoglobin variants with high oxygen affinity in all cases of polycythaemia, relative or absolute. The best screening test is the P_{50}, but if this is unavailable haemoglobin electrophoresis at alkaline and acid pH will detect some of the variants. The prognosis in such variants is likely to be good with a normal life span, but follow-up should be maintained to monitor the packed cell volume and the patient's general health. Treatment will be required only if the packed cell volume becomes unduly high, predisposing to cardiovascular complications. If this occurs venesection would be the treatment of choice.[22 23 25]

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