An unstable haemoglobin, Hb Tacoma β 30 (B12) arg→ser, detected at birth by the demonstration of red cell inclusions

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SUMMARY Incomplete expression of human haemoglobin β -chains at birth may lead to difficulty in the early demonstration of an inherited β -chain variant.

In this case, the rare unstable variant, Hb Tacoma β 30 (B12) arg→ser, although not present in cord blood in sufficient amounts to be easily detected by routine electrophoretic techniques, was readily shown to be present by the striking inclusions provoked by prolonged incubation of the neonatal red cells with new methylene blue.

Certain unstable haemoglobins, for example, Hb Hammersmith (Dacie *et al.*, 1967) and Hb Southampton/Casper (Hyde *et al.*, 1972; Koler *et al.*, 1973), produce severe continuous haemolysis starting early in life, while others, for example, Hb Zürich (Muller and Kingma, 1961), may be associated with significant haemolysis only when the carrier is given certain drugs. Yet other unstable variants, for example, Hb Sogn (Monn *et al.*, 1968) and Hb Toulouse (Rosa *et al.*, 1969), are not clinically important and their carriers remain well.

Many α - and β -chain unstable variants have been described after the introduction of the heat-instability test (Dacie *et al.*, 1964) and later the isopropanol precipitation test (Carrell and Kay, 1972).

Although abnormal α -chains must be present at birth in significant amounts, it appears rare for the diagnosis of unstable haemoglobinopathy to be made in a neonate. It is only recently, with the reporting of the first γ -chain labile mutant, Hb F Poole (Lee-Potter *et al.*, 1975), that an unstable haemoglobin has been implicated as a possible cause of haemolysis in the new-born, yet the early detection of carriers is obviously important if the susceptibility of the molecule to oxidative degradation is considered.

In February 1977, an abnormal haemoglobin was found when haemoglobin electrophoresis was carried out on the blood from a 34-year-old pregnant woman, because of her obscure ethnic background.

This was later shown to be Hb Tacoma β 30 (B12) arg→ser (Baur and Motulsky, 1965; Brimhall *et al.*, 1969). Hb Tacoma has not yet been shown to be associated with any clinical abnormality, but, because of the importance of diagnosing unstable variants as early in life as possible, it was decided to determine whether Hb Tacoma could be detected in the cord blood should the child be a carrier.

Material and methods

Routine haematological investigations used established techniques (Dacie and Lewis, 1975).

HAEMOGLOBIN ELECTROPHORESIS

Haemoglobin electrophoresis was carried out using cellulose acetate (Marengo-Rowe, 1965) modified to use tris buffer, pH 8.5 (Schneider and Schmidt, 1975), and agar gel (Marder and Conley, 1959). Globin chains were separated, using 2-mercapto-ethanol/urea, and then subjected to electrophoresis on cellulose acetate membrane (Schneider and Schmidt, 1975).

HAEMOGLOBIN QUANTITATION

Haemoglobins A₂ and F were quantitated using the methods of Marengo-Rowe (1965) and Pembrey *et al.* (1972), respectively.

STABILITY TESTS

Haemoglobin was checked for stability using the heat-instability test (Dacie *et al.*, 1964) and the isopropanol precipitation test (Carrell and Kay, 1972).

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These tests were performed with and without the prior addition of potassium cyanide (Brosious *et al.*, 1976).

INDUCEMENT OF RED CELL INCLUSION BODIES

One volume of whole blood was added to one volume of 1% new methylene blue or 1% brilliant cresyl blue in 0.015M NaCl and incubated at 37°C for one to four hours.

Where applicable, normal bloods were included as negative controls, and samples of Hb Southampton and Hb Köln (Carrell *et al.*, 1966) were used as unstable controls.

Results

Results of routine haematological investigations on the patient (PJ) and the cord sample were within normal limits (Table), and no significant abnormalities were detected in the blood films.

Table Haematological data from patient and child's cord sample

	Hb (g/dl)	RBC $\times 10^{12}/l$	Hct	MCV(f)	HbA ₁ %	HbF%
PJ	11.8	4.31	0.361	83	2.8	0.3
Cord sample	16.0	4.33	0.463	108	<1.0	—

Electrophoresis of blood from PJ for one and a half hours on cellulose acetate resulted in the major haemoglobin component being wider than normal. If electrophoresis was continued for up to four hours, separation into two bands occurred, the abnormal haemoglobin being slightly anodal to Hb A. This abnormal haemoglobin did not separate from Hb A on agar gel. Electrophoresis of the separated globin chains clearly demonstrated the presence of an abnormal β -chain.

Cellulose acetate and agar gel electrophoresis of the cord sample showed it to be mainly Hb F with small amounts of Hb A. Globin chain electrophoresis was not carried out because of the similar mobility of the aberrant chain in PJ to normal γ -chains.

Both the heat-instability and the isopropanol precipitation tests were weakly positive for PJ. The prior addition of potassium cyanide (KCN) completely inhibited the precipitation of the abnormal haemoglobin. The isopropanol test was weakly positive with the cord sample but this was not a significant result due to the presence of large amounts of Hb F.

When using redox dyes to induce the formation of inclusion bodies within the red cells, it was found

that different batches of brilliant cresyl blue gave inconsistent results. New methylene blue (NMB), obtained from Clin-Tech (Westminster Industrial Estate, London), was reliable.

Incubation with NMB for one to four hours resulted in all red cells from PJ showing inclusions similar to those found with Hb H (Fig. 1). Prior addition of KCN inhibited the formation of these inclusions. Incubation of the cord blood with NMB for four hours resulted in many red cells showing faint (and occasional red cells showing more prominent) inclusions similar to those found with PJ (Fig. 2). Normal cord red cells do not form inclusions in this time (Fig. 3).

Fingerprinting and aminoacid analysis, carried out by Professor H. Lehmann (MRC Abnormal Haemoglobin Unit, University Department of Biochemistry, Addenbrooke's Hospital, Cambridge), identified the abnormal haemoglobin carried by PJ as Hb Tacoma $\beta 30$ (B12) arg $\rightarrow$ ser. Haematological investigation of her English husband and 2-year-old son revealed no abnormality.

Discussion

Incomplete expression of the β -chains of human haemoglobin at birth often leads to difficulty in establishing whether a β -chain variant has been inherited. This difficulty is magnified when the variant haemoglobin is unstable. Many labile variants have the same electrophoretic mobility as Hb A (White and Dacie, 1971), and Hb F is itself inherently unstable and may therefore interfere with instability tests (Carrell, 1975; Lee-Potter *et al.*, 1975). In addition, the amount of unstable haemoglobin present in the adult carrier is often below the 40-45% usual for stable β -chain variants (White and Dacie, 1971), so that correspondingly lower values may be expected at birth. Hb Tacoma, however, is present in the adult to approximately 40% (Baur and Motulsky, 1965), and this may have facilitated its detection in the cord sample.

Haemoglobin A₂ levels are often slightly raised in heterozygotes for β -chain unstable variants, and were found to be so in both the previous reports of Hb Tacoma. In Hb Zürich, this may be due to loss of the unstable haemoglobin from the red cells (Rieder *et al.*, 1965). PJ, however, has a normal amount of Hb A₂.

Substitutions, involving the α_1 - β_1 contact area, may produce an unstable molecule, for example, Hb Tacoma and Hb Philly (Rieder *et al.*, 1969). Carriers of Hb Philly have a mild to moderate unstable haemoglobin disease, while as yet no clinical symptoms have been attributed to the presence of Hb Tacoma. It remains to be seen whether pharma-

Fig. 1 'Golf-ball' inclusions provoked by incubating red cells from PJ with new methylene blue for four hours. ($\times 2100$)

Fig. 2 Similar inclusions provoked under the same conditions in the child's cord blood. ($\times 2100$)

Fig. 3 Normal cord blood incubated under the same conditions as in Figs 1 and 2 shows two cells containing reticulofilamentous material and one cell with a superimposed granular artefact. No 'golf-ball' inclusions are present. ($\times 2100$)

colological or infective stress may lead to a haemolytic episode, and it seems prudent to exercise caution with known oxidant drugs, particularly in view of the dramatic reaction of Hb Tacoma with redox dyes *in vitro*.

Brosious *et al.* (1976) have suggested that KCN should be added to haemolysates before the isopropanol test is performed in order to eliminate some of the 'frequently observed false-positive results'. KCN reduces the instability of Hb Gun Hill (Rieder, 1970), Hb Köln, haem-depleted Hb A (Jacob and Winterhalter, 1970), and Hb Southampton. If added to Hb Tacoma, it renders the isopropanol test negative, and, as was found with Hb Philly (Rieder 1970), inclusion body production with redox dyes is also inhibited. Structural changes in some unstable haemoglobins may produce greater flexibility about the haem group, hence accelerating haemichrome formation and causing precipitation of the molecule (Winterbourn and Carrell, 1974).

KCN may, by binding with haem groups and consolidating interchain contacts, inhibit such haemichrome formation, thereby masking mild instability. It seems likely that the effect of KCN

varies according to the nature of the instability, and we would suggest that it is better to control stability testing carefully with known normal bloods and that KCN should not be added to the haemolysates.

The rather neglected technique of prolonged incubation with redox dyes may be a useful adjunct to haemoglobin electrophoresis when an early answer concerning the inheritance of unstable haemoglobin is required. The production of the characteristic 'golf-ball' appearance, originally described in Hb H disease (Gouttas *et al.*, 1955), is extremely striking with Hb Tacoma.

Hb Tacoma has been found only twice before: in an American family of European extraction (Baur and Motulsky, 1965) and in a Russian family (Idelson *et al.*, 1974).

PJ is from the county of Rautalampi, in the heart of Finland between Jyväskylä and Kuopio. All her grandparents come from this closely knit farming community, and there is no known connection with Russia, although Finland was an autonomous region within the Russian Empire until 1917.

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