

Hemoglobin Indianapolis (beta 112[G14] arginine). An unstable beta-chain variant producing the phenotype of severe beta-thalassemia.

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Research Article

Hemoglobin (Hb) Indianapolis is an extremely labile beta-chain variant, present in such small amounts that it was undetectable by usual techniques. Clinically, it produces the phenotype of severe beta-thalassemia. Biosynthetic studies showed a beta:alpha ratio of 0.5 in reticulocytes and about 1.0 in marrow after a 1-h incubation. These results, similar to those seen in typical heterozygous beta-thalassemia, suggested that betaIndianapolis was destroyed so rapidly that its net synthesis was essentially zero. To examine the kinetics of globin synthesis, reticulocyte incubations of 1.25--20 min were performed with [3H]leucine. The betaIndianapolis:beta A ratio at 1.25 min was 0.80 suggesting that beta Indianapolis was synthesized at a near normal rate. At 20 min, this ratio was 0.46 reflecting rapid turnover of beta Indianapolis. The erythrocyte ghosts from these incubations contained only betaIndianapolis and alpha-chains, and the proportion of betaIndianapolis decreased with time, indicating loss of betaIndianapolis. Pulse-chase studies showed little change in beta A:alpha ratio and decreasing betaIndianapolis:alpha and betaIndianapolis:beta A with time. The half-life of betaIndianapolis in the soluble hemoglobin was approximately equal to 7 min. There was also rapid loss of beta Indianapolis from the erythrocyte membrane. From these results, it may be inferred that betaIndianapolis is rapidly precipitated from the soluble cell phase to the membrane, where it is catabolized. Heterozygotes for beta 0-thalassemia usually have minimal hematologic abnormalities, [...]

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Hemoglobin Indianapolis ($\beta^{112}[\text{G14}]$ Arginine)

AN UNSTABLE β -CHAIN VARIANT PRODUCING THE PHENOTYPE OF SEVERE β -THALASSEMIA

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ABSTRACT Hemoglobin (Hb) Indianapolis is an extremely labile β -chain variant, present in such small amounts that it was undetectable by usual techniques. Clinically, it produces the phenotype of severe β -thalassemia. Biosynthetic studies showed a β : α ratio of 0.5 in reticulocytes and about 1.0 in marrow after a 1-h incubation. These results, similar to those seen in typical heterozygous β -thalassemia, suggested that $\beta^{\text{Indianapolis}}$ was destroyed so rapidly that its net synthesis was essentially zero. To examine the kinetics of globin synthesis, reticulocyte incubations of 1.25–20 min were performed with [^3H]leucine. The $\beta^{\text{Indianapolis}}:\beta^{\text{A}}$ ratio at 1.25 min was 0.80 suggesting that $\beta^{\text{Indianapolis}}$ was synthesized at a near normal rate. At 20 min, this ratio was 0.46 reflecting rapid turnover of $\beta^{\text{Indianapolis}}$. The erythrocyte ghosts from these incubations contained only $\beta^{\text{Indianapolis}}$ and α -chains, and the proportion of $\beta^{\text{Indianapolis}}$ decreased with time, indicating loss of $\beta^{\text{Indianapolis}}$. Pulse-chase studies showed little change in $\beta^{\text{A}}:\alpha$ ratio and decreasing $\beta^{\text{Indianapolis}}:\alpha$ and $\beta^{\text{Indianapolis}}:\beta^{\text{A}}$ with time. The half-life of $\beta^{\text{Indianapolis}}$ in the soluble hemoglobin was ≈ 7 min. There was also rapid loss of $\beta^{\text{Indianapolis}}$ from the erythrocyte membrane. From these results, it may

be inferred that $\beta^{\text{Indianapolis}}$ is rapidly precipitated from the soluble cell phase to the membrane, where it is catabolized. Heterozygotes for β^0 -thalassemia usually have minimal hematologic abnormalities, whereas heterozygotes with $\beta^{\text{Indianapolis}}$, having a similar net content of β -chain, have severe disease. The extremely rapid precipitation and catabolism of $\beta^{\text{Indianapolis}}$ and the resulting excess of α -chains, both causing membrane damage, may be responsible for the severe clinical manifestations associated with this variant. It seems likely that other, similar disturbances in the primary sequence of globin polypeptide chains may produce clinical findings similar to those seen with hemoglobin Indianapolis and thus produce the phenotype of severe β -thalassemia.

INTRODUCTION

Thalassemia syndromes share as a common feature unequal production of the globin subunits of the hemoglobin tetramer (1–3). Most often, there are decreased amounts of the specific globin messenger RNA which serves as a template for the synthesis of the affected globin chain (4–6). The messenger RNA deficit results from either globin gene deletions (7–10) or transcriptional or post-transcriptional impairment of the expression of globin genes (11–13). Analysis of the hemoglobins synthesized in patients with thalassemia has not shown abnormalities in the primary structure of the globin chains (1–3). Thus, with the exception of the thalassemia-like conditions associated with $\delta\beta$ -chains of Lepore hemoglobins (1–3) or the elongated α -chains

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of Hb Constant Spring (14) and similar termination codon mutants (15, 16), the globin produced in thalassemia is normal. The clinically important thalassemias may be divided into those in which there is impaired synthesis of α -globin chains or β -globin chains. The β -thalassemias, in which there is a deficit in the production of β -chains relative to α -chains, may be associated with either the complete failure to synthesize β -chain (β^0)¹ or a suboptimal production of β -chain (β^+) directed by the β -thalassemia allele (1–3). There is a great deal of clinical heterogeneity in both the heterozygous and homozygous β -thalassemias.

In this study, we report clinical, hematologic, and biosynthetic findings from two individuals with the phenotype of severe β -thalassemia. The unique aspect of these patients was the presence of a β -chain variant that was synthesized at a near normal rate but was rapidly and virtually totally catabolized. The degree of destruction was such that no variant β -chain protein was detectable by electrophoretic or chromatographic methods, and its amino acid substitution could only be determined by a novel technique of structural analysis employing isotopically labeled globin. We have called this most unstable of all hemoglobins yet described Hb Indianapolis (β 112[G14] arginine). It is possible that similar types of globin chain mutants are responsible for some of the clinical syndromes that resemble severe β -thalassemia.

Case report

A 9-yr-old Caucasian female, of northern European ancestry, was noted to suffer from fatigue at 6 mo of age. She was found to be anemic with a hemoglobin level of 4 g/dl and a reticulocyte count of 10.6%. She had scleral icterus and hepatosplenomegaly. At age 2, the spleen reached to 2 cm above the iliac crest. From the age of 3 on, she received monthly transfusions with packed erythrocytes. The peripheral blood film showed hypochromia, microcytosis, anisocytosis, poikilocytosis, basophilic stippling, polychromatophilia, and nucleated erythrocytes. The bone marrow, aspirated from the posterior iliac crest, was hyperplastic with marked normoblastic hyperplasia. Heinz bodies were found after splenectomy at age 9.

The father of the proband, age 34, had a history almost identical to that of his daughter. He was also splenectomized at age 9. He received transfusions with 2 U of packed erythrocytes every 6 wk until age 24 when they were discontinued because of hemosiderosis, manifested by cardiomyopathy, skin discoloration, and increased hepatic iron stores.

¹ Abbreviations used in this paper: β^0 , failure to synthesize β -chain; β^{ind} , $\beta^{indianapolis}$; NKM, 140 mM NaCl, 5 mM KCl, and 1.5 mM MgCl₂; PCV, packed cell volume.

METHODS

Standard hematologic techniques were used. Blood counts and erythrocyte indices were done with a Coulter electronic cell counter (Coulter Electronics Inc., Hialeah, Fla.). Erythrocyte survival studies were performed following the International Committee for the Standardization of Hematology panel recommendations (17), and ferrokinetics were done according to the method of Cavill et al. (18). Hemoglobin electrophoresis was carried out on cellulose acetate membranes, starch gel, polyacrylamide gel, and citrate agar gel (19). Isoelectric focusing of hemoglobin was done by the method of Drysdale et al. (20).

Preparation of reticulocytes for incubation. Heparinized blood from the proband, various members of her family, and normal controls was collected by venipuncture and immediately placed on ice. For the preparation of blood for the initial incubations, enough plasma was removed by centrifugation at 2,500 g to attain a packed cell volume (PCV) of 50%. All further procedures, unless otherwise stated, were performed at 4°C. For incubation in medium, the plasma was removed by centrifugation at 2,500 g for 5 min. The packed erythrocytes were washed four times by centrifugation at 2,500 g for 5 min in 140 mM NaCl, 5 mM KCl, and 1.5 mM MgCl₂ (NKM).

Preparation of bone marrow for incubation. Bone marrow samples were obtained by iliac crest aspiration. The bone marrow cells were collected in heparin and placed on ice. A single cell suspension of bone marrow cells was prepared by repeated passage of the cells through a 20-gauge needle. The plasma and fat were removed by centrifugation at 1,000 g for 10 min, and the packed cells were washed four times with NKM as described above.

Incubation of reticulocytes and bone marrow cells. Initial incubations of peripheral blood reticulocytes were carried out by addition of 200 μ Ci of L[4,5-³H]leucine (40–50 mCi/mmol; Amersham Corp., Arlington Heights, Ill.) to 5 ml of whole blood with the PCV adjusted to 50% as described above. The whole blood was then allowed to incubate for 1 h at 37°C in a metabolic shaker. Incubation of peripheral blood reticulocytes for kinetic studies and of bone marrow cells was carried out according to Boyer et al. (21), with L-leucine omitted from the incubation medium. The washed, packed erythrocytes and the incubation medium were mixed to give a final volume of 1 ml and a PCV of 40%. All samples were incubated at 37°C in a metabolic shaker under room air with the exception of one sample which was incubated at 22°C for 1 h. The samples were allowed to renew metabolites in the incubator for 5 min before the addition of [³H]leucine. Incubations were continued for the desired time and terminated by washing the cells three times in ice-cold NKM.

Hemolysis and preparation of erythrocyte ghosts. The erythrocytes were lysed with 2 ml of 1.5 mM MgCl₂ and rapidly returned to isotonicity by the addition of 0.5 ml of a buffer containing 0.06 M Tris, 1.5 mM MgCl₂, and 0.3 M KCl, pH 7.4. Stroma-free hemolysates were prepared by centrifugation at 30,000 g for 20 min. The stromal pellet was washed three times at 4°C according to the procedure of Dodge et al. (22) with 20 mosmol phosphate buffer, pH 6.0. Supernates and stroma were stored at –70° until needed.

Pulse-chase incubations. Washed erythrocytes and incubation mixture (5 ml) from the proband and her father were incubated with [³H]leucine for 5 min as described above. After this time, 1.0 ml of the mixture was removed, lysed, and treated as described. The remaining 4 ml of mixture was washed three times in ice-cold NKM and resuspended in a medium containing a 10-fold excess of L[¹²C]leucine and incubated at 37°C. 1-ml aliquots were removed at 2.5-, 5-, 10-, and 20-min intervals.

Hemoglobin chain separation. The lysates were converted

into globin by precipitation in 2% HCl in acetone (vol/vol). Hemoglobin chain separation was carried out on columns of CM-52 (Whatman Inc., Clifton, N. J.) by a modification of the procedure of Clegg et al. (23). Instead of the linear gradient proposed by Clegg et al., an exponential gradient, as reported elsewhere (24), was employed, or a nonlinear gradient was produced with an Ultragrad (LKB Instruments, Inc., Rockville, Md.). The 4.2-ml fractions were placed into 15 ml of aqueous counting scintillant (Amersham Corp.) and counted in a liquid scintillation spectrometer. The washed erythrocyte ghosts were dissolved in Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) and NaOH with carrier globin added as described (25).

Structural studies. The structure of $\beta^{\text{Indianapolis}}$ was solved by using an isotopic and chromatographic technique described elsewhere (24). HLA typing of the proband, parents, paternal grandparents, siblings, and a paternal uncle was done using standard methods (26).

RESULTS

Hematologic and electrophoretic results. The pre-splenectomy hematologic findings on the proband and the postsplenectomy findings on her father, as well as the hematologic findings on immediate family members, are presented in Table I. The patients were severely anemic with microcytosis, marked degrees of reticulocytosis, and many nucleated erythrocytes in the blood. The unusually high reticulocyte count of the father is likely the result of counting of some cells with precipitated hemoglobin as reticulocytes. Blood films also showed anisocytosis, poikilocytosis, and basophilic stippling. Other family members were normal.

Hemoglobin electrophoresis of the hemolysates of the proband and father, using cellulose acetate membranes, starch, polyacrylamide and agar gels, showed only HbA, A₂, and F. Isoelectric focusing of the hemolysate of the father did not show a variant hemoglobin band. The HbA₂ levels of the proband and father were 2.6 and 2.5%, respectively, whereas the HbF levels were 12 and 10%. Hemoglobin electrophoresis of the mother and two siblings was normal. Blood counts and

hemoglobin electrophoresis of the paternal grandparents, three sisters, and two brothers of the father were normal.

An unstable hemoglobin could not be demonstrated by heat precipitation or isopropanol incubation of the hemolysate of the proband and father.

Erythrokinetics. The results of erythrokinetic studies done on the father are given in Table II. There is accelerated disappearance of ⁵⁹Fe from the plasma, a marked increase in plasma iron turnover, and decreased ⁵⁹Fe incorporation into erythrocytes. The ⁵¹Cr half-life is shortened. All these findings indicated a high degree of ineffective erythropoiesis typical of severe β -thalassemia (27, 28).

Globin chain synthesis. An imbalance in globin chain synthesis was present in peripheral blood reticulocytes of the proband and her father (Table III). The α :non- α ratio in the nucleated erythrocytes of the marrow of both patients was near unity (Table III). Results similar to this are typical of heterozygous β -thalassemia (29, 30). The α : β ratios in reticulocytes of the proband's paternal grandparents and of her siblings were unity (data not shown).

A chromatograph of globin, obtained from a peripheral blood incubation, is shown in Fig. 1. A consistent peak of radioactivity, which eluted in the position occupied by the β -chain of sickle hemoglobin but without corresponding absorbance at 280 nm, was present on multiple studies of blood and marrow. This peak was abolished by heating the hemolysate to 50°C before chromatography and did not appear in control samples. Subsequent structural studies described elsewhere (24) identified this peak as $\beta^{\text{Indianapolis}}$ (β^{ind} , $\beta_{112}[\text{G14}]$ arg). When the peripheral blood was incubated at 22°C, there was a 30% increase in the counts recovered from β^{ind} .

Kinetic studies. Time-course incubation studies are presented in Table IV. These studies revealed that the β^{A} : α ratio remained essentially constant with time,

TABLE I
Hematologic Findings in Patients with Hb Indianapolis and in Unaffected Family Members

	Age	Hb	PCV	RBC*	MCV†	MCH‡	MCHC*	Corrected WBC¶	nRBC**	Reticulocytes
	yr	g/dl		10 ⁹ /liter	f	pg	g/dl	10 ⁹ /liter	per 100 WBC	%
Proband	9	4.7	16	2.54	75	26.2	29.4	7.1	42	14
Father	34	5.7	18	2.77	72	22.7	31.4	8.6	133	45
Mother	32	12.5	37	4.42	82	28.1	33.8	11.3	—	1.5
Brother	12	12.0	36	4.95	81	28.0	33.3	9.1	—	1.0
Sister	14	12.0	36	5.01	82	29.0	33.3	7.7	—	1.0

* Erythrocytes.

† Mean corpuscular volume.

‡ Mean corpuscular hemoglobin.

§ Mean corpuscular hemoglobin concentration.

¶ Leukocyte count.

** Nucleated erythrocytes.

TABLE II
⁵¹Cr and ⁵⁹Fe Erythrokinetic Studies in Hb Indianapolis

	⁵¹ Cr t _{1/2}	Plasma Fe	⁵⁹ Fe t _{1/2}	PITR*	EITR†	Erythrocyte Fe Use
	d	μg/dl	min	mg/kg/d	mg/kg/d	% at day 14
Father	14	207	40	2.74	0.96	38
Normal	28–32	80–150	60–100	0.46–0.78	0.43–0.72	>80

* Plasma iron turnover rate.

† Erythrocyte iron turnover rate.

whereas the β^{Ind}:α and β^{Ind}:β^A decreased with increasing time. The β^{Ind}/β^A ratio of 0.8 at 1.25 min of incubation of the proband suggests that β^{Ind} was synthesized at a normal or near normal rate. Also of interest are the findings in the erythrocyte ghosts (Table IV). There were only β^{Ind} and α-chain counts in the stroma, with no evidence of β^A radioactivity. Furthermore, the proportion of β^{Ind} chains in the stroma decreased with time, suggesting loss of these chains.

Pulse-chain studies. The pulse-chain studies (Table V) demonstrated little change in the β^A:α ratio and decreasing β^{Ind}:α and β^{Ind}:β^A ratios with time, suggesting loss of β^{Ind} chains rather than a rapid decline in their synthesis. As in the kinetic studies, there was a decrease in the proportion of β^{Ind} in the stroma with time.

HLA typing and erythrocyte antigens. The results of HLA tissue typing are presented in Table VI. False paternity can be excluded for both the proband and her father; thus, as would be expected, there is no linkage of the gene for Hb Indianapolis with the major histocompatibility locus. Studies of ABO, Rh, MNS, P, Kell, Lutheran, Duffy, and Kidd blood groups in the father and paternal grandparents again gave no indication of false paternity.

DISCUSSION

With the exception of the thalassemia-like conditions associated with the δβ-fusion gene products of Lepore

TABLE III
 Globin Chain Biosynthesis Studies in Reticulocytes and Bone Marrow in Hb Indianapolis*

	Peripheral blood		Bone marrow	
	β ^A :α	β ^A + β ^{Ind} :α	β ^A :α	β ^A + β ^{Ind} :α
Proband	0.38	0.51	1.14	1.30
Father	0.40	0.52	0.83	1.00
Normal ± SE (n = 27)	0.98 ± 0.02	—	1.01 ± 0.10	—

* Results are reported as the ratio of the total number of counts incorporated into the α- and non-α-chains separated by CM-cellulose column chromatography after 1 h incubation at 37°C.

hemoglobins (1–3) and the elongated α-chains of Hb Constant Spring (14) and its close relatives (15, 16), the primary structure of individual globin chains in the thalassemia syndromes is normal (1–3). Although imbalanced globin chain synthesis has occasionally been associated with unstable hemoglobin variants, such

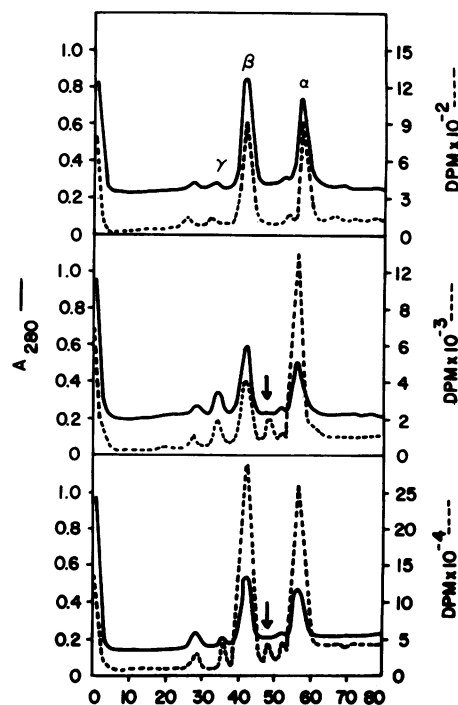


FIGURE 1 Chain separation by CM-cellulose column chromatography of hemolysates of peripheral blood and bone marrow samples incubated at 37°C in the presence of [³H]-leucine for 1 h. The upper panel represents the peripheral blood of a normal control. The middle panel represents the peripheral blood of the proband. The lower panel represents the bone marrow of the proband. β^{Ind} is indicated by the arrow, A₂₈₀ by the solid lines, and disintegrations per minute (DPM) by the dashed lines. The positions of the γ-, β^A-, and α-chains of hemoglobin are indicated in the upper panel. These columns were run from the same gradient and monitored simultaneously in a multichannel recording spectrophotometer. The bone marrow sample was stored at -80°C and run 3 mo later. It differed from experiments on fresh samples (e.g., Table III) in that the β^{Ind}:β^A ratio was 0.05 instead of 0.15.

TABLE IV
Kinetic Biosynthesis Studies of Reticulocytes from Patients with Hb Indianapolis

Individual	Incubation time <i>min</i>	Stroma-free hemolysate				Stroma
		$\beta^A:\alpha$	$\beta^{Ind}:\alpha$	$\beta^{Ind}:\beta^A$	$\beta^A + \beta^{Ind}:\alpha$	$\beta^{Ind}:\alpha$
Proband	1.25	0.35	0.28	0.80	0.63	2.1
	2.5	0.36	0.24	0.67	0.60	1.6
	5.0	0.36	0.22	0.61	0.58	0.81
	10	0.37	0.18	0.49	0.54	0.72
	20	0.35	0.16	0.46	0.51	0.74
Father	1.25	0.42	0.23	0.55	0.65	2.8
	2.5	0.42	0.21	0.50	0.63	1.9
	5.0	0.40	0.17	0.43	0.57	—
	10	0.41	0.16	0.39	0.57	—
	20	0.40	0.13	0.32	0.53	0.68

cases do not appear to have the clinical and hematologic manifestations of thalassemia (13). The patients with Hb Indianapolis, therefore, appear to be unique; they have the phenotype of homozygous or possibly severe heterozygous β -thalassemia which is the result of the production of a β -chain variant of extreme instability. Although synthesized at what appears to be a near normal rate for heterozygous β -chain variants (32), the β^{Ind} chain is rapidly degraded to the point where it is undetectable in erythrocyte hemolysates by hemoglobin electrophoresis, isoelectric focusing, or by its absorbance at 280 nm on CM-cellulose column chromatography.

Clinically and hematologically, both patients resemble individuals with homozygous β -thalassemia, rather than those with typical heterozygous β -thalassemia (1, 2) or the inclusion body variety of heterozygous

TABLE V
Pulse-Chase Studies of Reticulocytes from Patients with Hb Indianapolis

Individual	Chase period <i>min</i>	Stroma-free hemolysate				Stroma
		$\beta^A:\alpha$	$\beta^{Ind}:\alpha$	$\beta^{Ind}:\beta^A$	$\beta^A + \beta^{Ind}:\alpha$	$\beta^{Ind}:\alpha$
Proband	0.0*	0.34	0.21	0.62	0.54	1.9
	2.5	0.35	0.17	0.48	0.52	0.91
	5.0	0.37	0.14	0.38	0.51	—
	10	0.39	0.10	0.26	0.49	0.32
	20	0.39	0.06	0.15	0.45	0.25
Father	0.0*	0.37	0.17	0.46	0.54	3.2
	2.5	0.36	0.13	0.36	0.49	—
	5.0	0.38	0.09	0.24	0.47	0.61
	10	0.36	0.07	0.19	0.43	0.23
	20	0.37	0.03	0.08	0.40	0.12

* 5.0-min pulse period.

TABLE VI
HLA Typing of Patients with Hb Indianapolis and Family Members

	HLA
Proband	A9, B12, CW4/A28, B12
Father	A9, B12, CW4/AW26, BW38
Mother	A2, TT/A28, B12
Brother	A2, TT/AW26, BW38
Sister	A28, B12/AW26, BW38
Grandfather (paternal)	A9, B12, CW4/A2, B12
Grandmother (paternal)	A1, unknown/AW26, BW38

β -thalassemia (33, 34). The ferrokinetic studies are typical of individuals with the ineffective erythropoiesis most characteristic of homozygous β -thalassemia (18, 27). Yet, the family evaluation, biosynthetic studies, and hemoglobin studies are all indicative of heterozygosity for the abnormal β -chain allele with direct transmission of the disorder from the father to daughter. Family studies also indicated that the gene for Hb Indianapolis arose from a spontaneous mutation, because paternity could not be ruled out in the paternal grandfather. Unstable hemoglobins arising from spontaneous mutations are not uncommon, whereas thalassemias arising from this mechanism are extremely rare (2, 35, 36).

It is also difficult to explain why heterozygosity for Hb Indianapolis results in a severe hemolytic anemia, whereas heterozygous β^0 -thalassemia, which like heterozygous β^{Ind} has only half the usual number of β -chains available, is most often a disorder with minimal clinical and hematologic abnormalities. The family studies make it very unlikely that the so-called silent carrier type of β -thalassemia (37) is present in this kindred, accounting for the clinical findings in both patients with Hb Indianapolis. The pathophysiology of thalassemia derives not so much from the effects of diminished synthesis of a globin chain, as from the damage to developing erythroblasts by the precipitates of the chain left in excess (1-3). The efficiency of cellular mechanisms for the removal of these redundant chains may play some role in the determination of the pathologic consequences of a thalassemic gene. Globin synthesis and family studies indicate that both patients have about half the usual amount of β -chain. Similar genetic and synthesis studies in β^0 -thalassemia are most often not associated with the clinical and hematologic findings noted in our patients. It may be that the unstable β^{Ind} chain, during the course of its intracellular destruction, causes cellular damage in excess of that found by the relatively mild excess of α -chain noted in heterozygous β^0 -thalassemia. This is suggested by the finding that the Heinz bodies isolated from cells of these patients consisted of both β^{Ind} and α -chains. Heinz bodies, of any

composition, are usually not found in the cells of patients with heterozygous β^0 -thalassemia.

The catabolism of Hb Indianapolis may be inferred from the kinetic and pulse-chase studies. The kinetic studies indicated that there is either rapid loss of β^{ind} or a rapid decline in its synthesis relative to the other chains. The pulse-chase studies clearly showed the former mechanism to be operative. The half-life of the β^{ind} chain in the stroma-free hemolysate appeared to be between 5 and 10 min. By 20 min of chase, the radioactivity associated with this chain was essentially absent. Because the $\beta^{\text{A}}:\alpha$ ratio remained nearly constant in the pulse-chase studies, and no β^{A} was found in the stroma, it may be concluded that very few β^{A} or α -chains become associated with the membrane during the chase. If this is the case, then the abnormal chain is also lost very rapidly from the ghosts. It is impossible in the present study to determine the rate of its loss, because it can only be related to the α -chain, whose rate of loss is unknown.

The kinetic and pulse-chase studies suggest that β^{ind} is rapidly precipitated from the soluble cell phase to the erythrocyte ghosts where it is rapidly catabolized. It is tempting to speculate that this extremely rapid precipitation and catabolism are responsible for the clinical findings in this hemoglobinopathy.

There have been numerous descriptions of unstable β -chain variants associated with hemolytic anemia (38). In most, there are only mild-to-moderate degrees of anemia with the proportion of 25–30% unstable hemoglobin detectable in hemolysates. The $\alpha:\beta$ biosynthetic ratios are nearly balanced, the variant β -chain is synthesized at a rate equal to that of the β^{A} chain, and the erythrocytes are normocytic and have a normal hemoglobin content (2). Hb Bushwick ($\beta^{\text{T}}\text{gly} \rightarrow \text{val}$) appears to be one of the most unstable β -chain variants described to date (39). It was present at a level of 1–2% of the total hemoglobin. Synthesis studies demonstrated its rapid post-translational destruction, but, in common with other patients having unstable β -variants, the affected individuals, despite evidence for hemolytic disease, were not anemic and had normal erythrocyte indices, and the synthesis of α - and β -chains in reticulocytes was equal (39). These findings led to the postulate that the β -allele in *trans* to the β -Bushwick gene was able to compensate by increasing its output. Such "compensation" is not found in reticulocytes of β -thalassemia nor does it appear to be present in reticulocytes of our patients with Hb Indianapolis.

As mentioned, biosynthetic studies of most unstable variants have revealed equal or nearly equal production of α - and non- α -chains (2, 31). There is a slight excess synthesis of α -chains in Hbs Koln (40, 41), Hammersmith (42), Bristol (43), Riverdale-Bronx (44, 45), and Abraham Lincoln (46). The $\beta:\alpha$ ratios in these hemoglobins range from 0.8 to 0.9. For two of these hemoglobins (Hbs

Koln and Abraham Lincoln), experimental evidence suggested that this slight imbalance in globin chain synthesis is the result of early degradation of the unstable β -chain (41, 46).

An exception to the rule of balanced globin synthesis in unstable hemoglobin disorders is the case of Hb Leiden (31). In this β -chain variant, in which the glutamic acid residue at position six or seven is deleted, an excess of α -chains relative to β -chains was found with $\beta:\alpha$ ratios between 0.47 and 0.63 in reticulocytes and 0.82 in marrow. Hb Leiden represents about 24% of the total hemoglobin and is associated with very mild hemolysis, and normal erythrocyte indices. It was not clear why Hb Leiden differed from other unstable hemoglobins in regard to globin chain synthesis, although it was postulated that excessive numbers of α -chains might be made (31).

Hb Indianapolis is similar to Hb Leiden with regard to globin synthesis. In Hb Indianapolis, the $\beta^{\text{A}}:\alpha$ total radioactivity ratios were consistently around 0.4; these ratios were about 0.3 in Hb Leiden. If the β^{A} and α -chains were synthesized at normal rates, the $\beta^{\text{A}}:\alpha$ ratio would be expected to be 0.5. The low ratio in Hb Indianapolis patients is all the more puzzling because of the binding of α but not β^{A} to the stroma. As in the case of Hb Leiden, we have no experimental evidence to support a mechanism for this imbalance.

Structural abnormality of the affected globin chain was an early hypothesis concerning the molecular pathology of the thalassemia syndromes. The structural abnormality in Hb Indianapolis certainly produces the phenotype of severe β -thalassemia. As there were no standard laboratory techniques that suggested the presence of an abnormality in the structure of the β -globin chain, it is likely that similar disturbances in the primary sequence of globin chains may be responsible for some cases previously thought to be one of the heterogeneous varieties of β -thalassemia.

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