The Coexistence of the Genes for Hemoglobin E and α Thalassemia in Thais, with Resultant Suppression of Hemoglobin E Synthesis

SOODSARKORN TUCHINDA,[1] DONALD L. RUCKNAGEL,[2] VIRGINIA MINNICH,[3] URAPOL BOONYAPRAKOB,[1] KAMPANAD BALANKURA,[1] AND VINAI SUVATEE[1]

[1]*Department of Pediatrics, Faculty of Medical Sciences, Siriraj Hospital, Bangkok, Thailand.*
[2]*Department of Human Genetics, University of Michigan Medical School, Ann Arbor.*
[3]*Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri.*

OF THE LARGE NUMBER OF INHERITED ABNORMALITIES of the major component of human hemoglobin (Hb A) characterized to date, most have been shown to differ from normal hemoglobin by a single amino acid substitution in either the α or β polypeptide chains of which Hb A (commonly represented as $\alpha^{A}{}_{2}\beta^{A}{}_{2}$) is composed. Exceptions to this phenomenon are hemoglobins H and Bart's, both usually found in large amounts in association with thalassemia and now known to be tetramers of β or γ chains, respectively (Jones *et al.*, 1959; Hunt and Lehmann, 1959), the γ chain being the unique subunit of fetal hemoglobin (commonly represented as $\alpha^{A}{}_{2}\gamma^{F}{}_{2}$).

From the outset the mode of inheritance of Hb H has been obscure. In the first family study reported, one of the parents of children with Hb H had unequivocal morphologic findings of thalassemia; the other parent was believed to be normal, and neither parent's blood contained Hb H. These observations led to the initial hypothesis that the gene for Hb H, presumably carried by the normal parent, is expressed only on a background of thalassemia (Motulsky, 1956). Parent to offspring transmission of Hb H has subsequently been demonstrated but only in a minority of reported families, further complicating the genetic interpretation. The presence of Hb Bart's concomitantly with Hb H and with the hematologic abnormalities of thalassemia (Ramot *et al.*, 1959; Huehns *et al.*, 1960), has supported the concept that these homogeneous polymers are produced as a consequence of a deficiency of α polypeptide chain synthesis relative to that of β and γ chains (Jones *et al.*, 1959). The variety of thalassemia accompanied by the presence of Hb H or Hb Bart's has been referred to as α thalassemia (Ingram and Stretton, 1959). Henceforth in this report, the term Hb H disease will be used for the specific syndrome of hypochromic microcytic anemia, inclusion bodies, and Hb H with or without Hb Bart's.

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Elevated concentrations of a minor hemoglobin component, Hb A_2, which is represented as $\alpha^A{}_2\delta^A{}_{2_2}$, have been demonstrated in the erythrocytes of most but not all cases of so-called "classical" thalassemia minor (Kunkel and Wallenius, 1955; Gerald and Diamond, 1958). Variation in the severity of the anemia and in the hemoglobin electrophoretic pattern when thalassemia exists in combination with an abnormal hemoglobin has led to the recognition of thalassemia as a generic term for a heterogeneous group of inherited microcytic hypochromic anemias without hemoglobin electrophoretic abnormalities per se (Itano, Bergren, and Sturgeon, 1956; Zuelzer, Neel, and Robinson, 1956). Studies of the offspring of individuals with combinations of β chain structural mutants and thalassemia genes indicate that thalassemia characterized by high concentrations of Hb A_2 is due to a gene allelic with or closely linked to the β chain structural locus. The electrophoretic pattern of such heterozygotes reveals "interaction" or suppression of Hb A synthesis to the point of its complete absence in the erythrocytes of some sickle cell–thalassemics. By contrast, in three families in which the sickle cell or Hb C–thalassemia of one parent was manifested by synthesis of less than 50% of the abnormal hemoglobin, the segregation data suggest that such thalassemia is not allelic with the Hb_β structural locus (summarized in Rucknagel and Neel, 1961).

Thailand is a particularly favorable country in which to study the interrelationships of the aforementioned entities because of the high frequency of α thalassemia, "classical" or β thalassemia, and Hb E, a structural mutant of the Hb_β locus (Minnich *et al.*, 1954; Minnich *et al.*, 1956; Na-Nakorn, Minnich, and Chernoff, 1956). Here one would anticipate finding all possible combinations of these entities in reasonably high frequency. This report concerns hematologic and biochemical studies of five Thai families ascertained through children having severe anemia and hepatosplenomegaly and possessing Hb Bart's and approximately 15% Hb E. Other siblings possessed Hb H with or without Hb Bart's. In these families, one parent exhibited at most only minimal evidence of thalassemia and normal or low Hb A_2 levels. In the three families in which measurements were possible, the blood of the other parent contained only 21 to 25% Hb E, at the lower end of the range of values observed for Hb E in heterozygotes. The interpretation of these data is that the parent without Hb E is heterozygous for the α thalassemia gene and the other parent heterozygous for both $Hb_\beta{}^E$ and α thalassemia genes, the latter resulting in suppression of Hb E synthesis. The children with only 15% Hb E are considered to have two α thalassemia genes as well as one for Hb E. The parents of these sibships are compared with parents of children having classical thalassemia–Hb E. Additional hematologic data on parents of children with Hb H disease are presented for comparison. It is concluded that thalassemia manifested by H and Bart's hemoglobins (Hb H disease) is a consequence of two probably allelic and nearly recessive genes (t_1,t_2), although the possibility of homozygosity for a single autosomal recessive gene (t_1t_1) with variable expression cannot be excluded. Segregation in one of the families presented indicates that the α thalassemia locus is not closely linked

to the Hb_β structural locus. A preliminary report of these data has been presented previously (Tuchinda *et al.*, 1962).

MATERIALS AND METHODS

Routine hematologic techniques were employed for the red and white blood cell counts, hemoglobin, and packed cell volume. Reticulocytes and hemoglobin H inclusion bodies were enumerated in vital brilliant cresyl blue wet preparations (Minnich *et al.*, 1954). Target cells were estimated by counting the number in 1000 red blood cells in peripheral blood films using Wright's stain.

Hemolyzates were prepared and the hemoglobin electrophoretic pattern was determined first by paper electrophoresis (Smith and Conley, 1953) in Thailand. Fresh sterile blood samples, collected in ACD solution, were sent to St. Louis by air. The time between collection and arrival varied between four and seven days. Hemolyzates were prepared immediately upon arrival in St. Louis. Fetal hemoglobin was determined by the method of Singer, Chernoff, and Singer (1951). Either carbonmonoxy- or cyanmethemoglobin was used for electrophoresis.

Hemoglobin fractions were quantified and isolated by starch block electrophoresis (Kunkel *et al.*, 1957) and were concentrated after elution from starch either by ultrafiltration (Sober *et al.*, 1956) or by employing Carbowax 4000 (Union Carbide). Electrophoresis followed by concentrating was repeated until a single band was observed on starch gel electrophoresis (Smithies, 1959). Isolated hemoglobin fractions were used for fingerprinting (Ingram, 1958; Katz, Dreyer, and Anfinsen, 1959; Chernoff and Liu, 1961) and for hybridization studies (Singer and Itano, 1959; Gammack *et al.*, 1961). Normal Hb A_1 and A_2 were prepared in the same manner, except that the latter was finally concentrated and purified by carboxymethylcellulose column chromatography (Huisman, Martis, and Dozy, 1958). Bart's hemoglobin was separated from Hb A_3 by electrophoresis on starch block at pH 7.0 or by amberlite IRC-50 resin column chromatography (Allen, Schroeder, and Balog, 1958).

Clinical and laboratory data were collected on 22 individuals in five families in which at least one child had, in addition to Hb A, a low concentration of Hb E and Hb Bart's.

Hematologic values and hemoglobin electrophoretic patterns were obtained on three other groups for comparison with the major family studies: (1) both parents of three patients with Hb H disease; (2) both parents of four propositi with thalassemia-Hb E disease manifested by high concentration of Hbs E and F (Chernoff *et al.*, 1956 and a Hb E trait subject studied in St. Louis; and (3) one family in which the father had Hb H disease and the mother presumably homozygous Hb E disease.

FAMILY STUDIES

Families with Children Having Hemoglobins E and Bart's

The following five families are presented, each because at least one child had anemia, hepatosplenomegaly, and an abnormal hemoglobin electrophor-

TABLE 1. BLOOD FINDINGS OF PROPOSITI AND THEIR FAMILIES

Family Ref.	Member	Age years	Hb g/100 ml	RBC $\times 10^6$ /mm³	Packed cell Vol. %	MCV μ^3	MCHC %	Reticulocytes %	Alkali-resistant Hb %	Hemoglobin components				Erythrocyte Morphology				Target cells %
										A_2 %	E %	H %	Bart's %	aniso	poik	hypo	micro	
1.1	Father		13.6	4.90	40	82	34	0.6	1.6	1.8	0	0	0	+	+	++	0	.5
			14.4†	5.00	42	84	34	1.2										
1.2	Mother		11.7	3.82	33	87	35	1.2	1.6	—	25.0	0	0	+	+	0	0	0
1.3	Son	5	6.8	3.02	23	77	29	2.4	3.4	—	13.1	0	7.7	++	++	+++	0	23
1.4	Daughter	3	7.0	3.37	27	80	26	3.2	12.8	—	11.6	0	17.0	+++	++	+++	0	35
1.5	Son	2	10.1	5.10	35	69	29	0.3	2.0	2.7	0	0	0	+	+	+	+	1
1.6	Son	8/12	10.9	4.80	30	63	36	0.6	2.7	2.2	0	0	0	++	++	+++	++	2
2.1	Father	52	13.0	5.19	42	80	31	0.1	1.2	1.8	0	0	0	+	+	++	+	6
2.2	Mother	45	12.3	5.86	39	67	32	2.0	0.9	—	21.3	0	0	+	0	++	+	60
2.3	Daughter	21	13.9	4.60	40	88	35	1.3	0.7	1.4	0	0	0	—	—	—	—	—
2.4	Son	19	10.4	4.90	40	82	26	3.2	1.7	1.3	0	15.0	*	—	—	—	—	—
2.5	Son	17	13.6	4.60	41	89	33	1.6	1.5	2.1	0	0	0	—	—	—	—	—
2.6	Son	14	12.0	5.01	40	80	30	2.4	1.1	—	28.2	0	0	++	+	++	+	10
2.7	Daughter	12	8.9	3.89	31	80	29	6.0	4.0	—	13.1	0	8.0	++	+	++	++	56
2.8	Daughter	8	7.9	2.89	31.5	108	25	8.0	8.0	2.6	0	13.8	*	++	++	+++	**	15
3.1	Father		13.0	5.50	46	84	28	0.2	1.0	—	22.2	0	0	0	0	++	0	0
3.2	Mother		11.9	4.26	36.5	86	33	2.6	1.0	3.0	0	0	0	++	+	++	0	1
			12.4‡	4.05	35.5	88	35											
3.3	Daughter	7	8.6	3.20	31	96	28	3.8	7.4	—	15.3	0	10.7	+++	+	+++	**	33
4.1	Mother		9.9	4.36	32	73	31	1.2	1.5	2.2	0	0	0	+	+	++	0	+
			9.8‖	4.50	34	75	29	1.8										
4.2	Son	12	7.3	4.23	34	80	22	4.8	10.0	—	15.9	0	7.9	+++	++	+++	++	30
5.1	Father		14.1	6.73	47	70	30	0.8	—	Hb A (paper)		—	—	—	—	—	—	—
5.2	Mother		13.0	4.51	38	84	34	0.8	—	Hb AE (paper)		—	—	+	+	0	0	0
5.3	Son	8	6.2	3.60	28	78	22	10.4	8.8	—	15.5	0	14.0	+++	++	+++	0	22

†Counts obtained following 30 days of oral iron therapy.
‡Counts obtained following 38 days of oral iron therapy.
‖Counts obtained following 17 days of oral iron therapy.
*Small amount of Hb Bart's present which could not be accurately quantified.
**Macrocytosis.
0 Not present.
— Not tested.

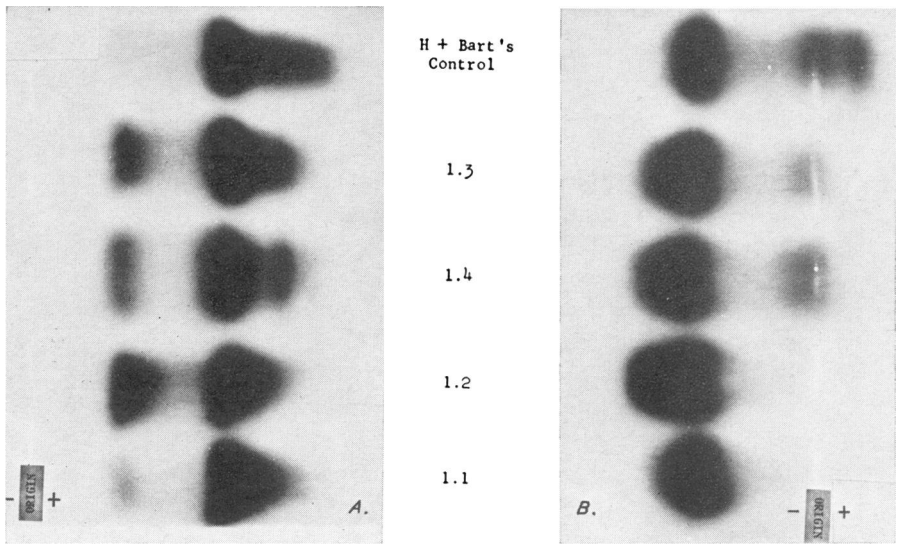

FIG. 1. Starch block electrophoresis of hemolyzates of members of Family 1, Table 1. The sample designated H + Bart's is from an unrelated individual. A. Electrophoresis performed in barbital buffer, 0.05 ionic strength, pH 8.6. B. Electrophoresis at pH 7.0 in 0.05 M phosphate buffer.

etic pattern revealing, in addition to Hb A, less than 20% Hb E and 7 to 17% Hb Bart's. Laboratory data are included in Table 1 and representative starch block electrophoretic patterns in Figs. 1A, B.

Family 1

The father (Ref. 1.1), pure Thai, was healthy. His hemoglobin concentration was 13.6 g/100 ml; red blood cell count 4,900,000/mm^3; packed cell volume 40%; MCV 82 μ^3 and MCHC 34%; and reticulocytes 0.6%. The stained red blood cells were slightly hypochromic with slight anisopoikilocytosis and 0.5% target cells. His hemoglobin pattern was normal with 1.8% Hb A_2 (Fig. 1). The alkali-resistant hemoglobin was 1.6%.

The mother (Ref. 1.2), ¼ Chinese and ¾ Thai, was also healthy. Her significant laboratory data included hemoglobin concentration of 11.7 g/100 ml and normal corpuscular constants; only slight anisopoikilocytosis and basophilic stippling were observed on her stained blood film. On starch block electrophoresis, Hb E accounted for 25% and Hb A 75% of the total hemoglobin (Fig. 1). The alkali resistant hemoglobin was 1.6%.

The propositus (Ref. 1.3), a boy five years of age, was admitted to the pediatric service at Siriraj Hospital, February 1959, because of fever of four days duration and symptoms of anemia. Pertinent history included frequent attacks of upper respiratory infection, mild jaundice, and progressive weakness. Physical findings were edema of both feet, hyperemic enlarged tonsils, liver enlargement to two fingerbreadths below the right costal margin, and splenomegaly to three fingerbreadths below the left costal margin. The significant laboratory findings were: hemoglobin concentration 6.8 g/100 ml; RBC

3,020,000/mm^3; packed cell volume 23%; reticulocytes 2.4%; direct bilirubin 0.4 and indirect 1.0 mg/100 ml; 7% BSP retention at 45 minutes; serum globulin 1.5 and albumin 3.2 g/100 ml. The stained blood film showed marked anisopoikilocytosis, extreme hypochromia, basophilic stippling, polychromasia, four nucleated red cells/100 WBC, and 23% target cells. In addition to Hb A, 13.1% of Hb E and 7.7% of Hb Bart's were present on starch block electrophoresis (Fig. 1). Alkali-resistant hemoglobin was 3.4%.

The second child (Ref. 1.4), a girl three years of age, was followed as an outpatient. Physical examination revealed a liver palpable three fingerbreadths and spleen four fingerbreadths below costal margins. Her hematologic values were similar to those obtained on her older brother (Table 1). Her blood film was also similar and contained 35% target cells. Her hemolyzate, in addition to Hb A, had 11.6% Hb E and 17.0% Hb Bart's (Fig. 1). Hb F determined by alkali denaturation was 12.8%.

The third child (Ref. 1.5), a boy two years of age, was seen in the Out-Patient Department because of frequent upper respiratory infections, bronchitis, and diarrhea. His spleen and liver were both palpable one fingerbreadth below their respective costal margins. His hemoglobin concentration was 10.1 g/100 ml; RBC 5,100,000/mm^3; packed cell volume 35%; MCV 69 μ^3 and MCHC 29%. His standard blood film revealed slight anisopoikilocytosis, hypochromia, microcytosis, basophilic stippling, and approximately 1.0% target cells. His electrophoretic pattern was normal, with a Hb A_2 value of 2.7%. Hemoglobin resistant to alkali denaturation was 2.0%.

The fourth child (Ref. 1.6), a boy eight months of age, born in June 1960, had findings very similar to those found in the third child (Table 1).

Comment. The father was apparently both clinically and hematologically normal, except for mild anisopoikilocytosis. The pertinent finding in the mother was a Hb E value of only 25%, which we believe is at the lower extreme for values in Hb E trait. The two oldest children were unlike either parent in that they had severe anemia, hepatosplenomegaly, and marked erythrocytic abnormalities similar to those seen in Cooley's anemia. They had even lower concentrations of Hb E than their mother and in addition Hb Bart's. These findings suggest that the father is heterozygous for a nearly recessive α thalassemia gene and that the mother is heterozygous for α thalassemia and for Hb_β^E. Thus the first two children most likely possess two α thalassemia genes and one Hb E gene. The two younger children with hepatosplenomegaly and normal electrophoretic patterns could be α thalassemia heterozygotes receiving the abnormal gene from either parent. However, nutritional factors have not been excluded. The interpreted pedigree of this family is diagrammatically presented in Fig. 2A.

Family 2

The father (Ref. 2.1), 52 years of age and pure Thai, was clinically normal. His hemoglobin concentration was 13.0 g/100 ml; RBC 5,190,000/mm^3; packed cell volume 42%; MCV 80 μ^3; MCHC 31%; and reticulocytes 0.1%. The red blood cells on fixed films showed slight anisopoikilocytosis, hypochromia,

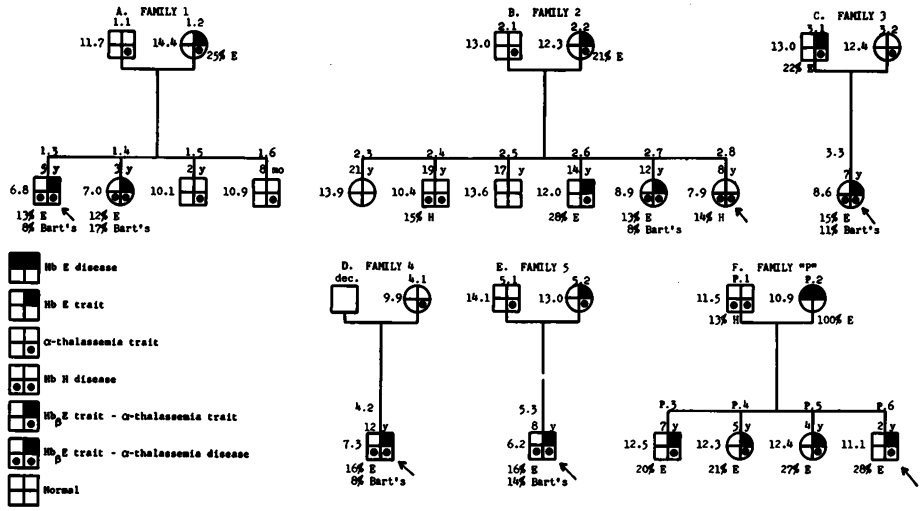

FIG. 2. Genetic interpretation of the families presented in Table 1 and the "P" family of Table 4. Figures above the individuals refer to ages and reference numbers, those to the left to their total hemoglobin concentrations (g/100 ml).

and 6% target cells (Fig. 3A). The Hb A_2 content was 1.8%, and the alkali-resistant hemoglobin 1.2%.

The mother (Ref. 2.2), 45 years of age and pure Thai, was healthy. Her hemoglobin concentration was 12.3 g/100 ml; RBC 5,860,000/mm^3; packed cell volume 39%; MCV 67 μ^3; MCHC 32%; and reticulocytes 2.0%. Her blood film showed hypochromic microcytic red blood cells and 60% target cells (Fig. 3B). The hemoglobin components obtained on electrophoresis were Hb E, 21.3%, and A, 78.7%. Alkali-resistant hemoglobin was 0.9%.

There were six children. The proposita (Ref. 2.8), a girl eight years of age, was seen in the Out-Patient Department for symptoms of anemia. Physical examination revealed hepatosplenomegaly. Her hemoglobin concentration was 7.9 g/100 ml; RBC 2,890,000/mm^3; packed cell volume 31.5%; MCV 108 μ^3; MCHC 25%; and reticulocytes 8.0%. A slide of peripheral blood (Fig. 3D) showed moderate anisopoikilocytosis, macrocytosis, hypochromia, and 15% target cells. On vital staining with brilliant cresyl blue, 95% of the erythrocytes contained inclusion bodies. Hemoglobin fractions obtained on starch block electrophoresis were Hb A_2, 2.6%; Hb H, 13.8%; and Hb A. Hb Bart's was observed on the hemoglobin electrophoretic patterns but could not be measured accurately. The alkali-resistant fraction was 8.0%.

A brother (Ref. 2.4), 19 years of age, had a similar blood picture, although his anemia was not as severe (10.4 g Hb/100 ml). His red blood cells also showed inclusion bodies following incubation with brilliant cresyl blue; 15% of the total hemoglobin was Hb H, 1.7% Hb A_2, and the remainder Hb A. The hemoglobin resistant to alkali denaturation was 1.7%.

A sister (Ref. 2.7), 12 years of age, also had hepatosplenomegaly. Her hemoglobin concentration was 8.9 g/100 ml; RBC 3,890,000/mm^3; packed cell

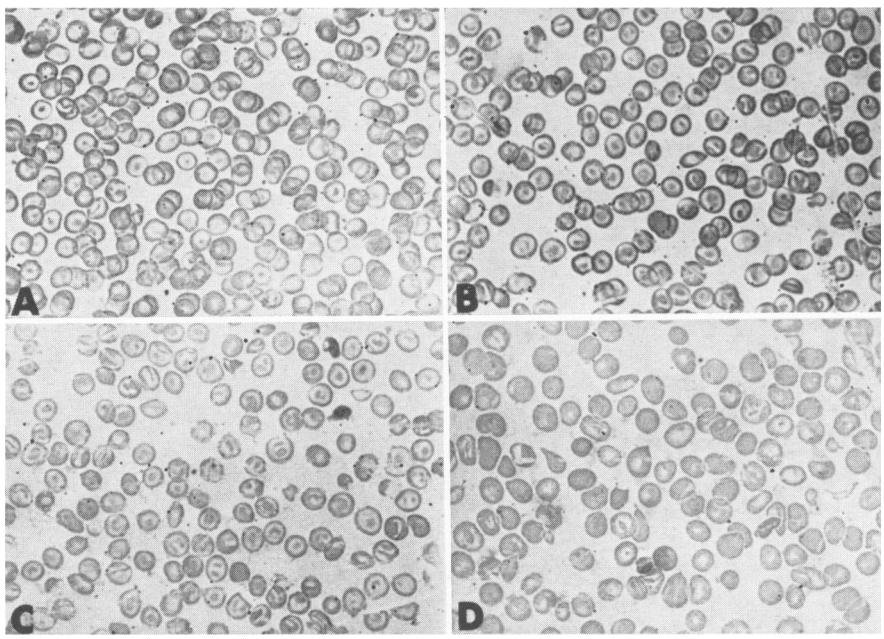

FIG. 3. Peripheral blood films of individuals of Family 2, Table 1. A, father; B, mother; C, Ref. 2.7, having Hb E and Hb Bart's; D, Ref. 2.8, having Hb H disease.

volume 31%; MCV 80 μ^3; MCHC 29%; and reticulocytes 6.0%. Moderate anisocytosis, hypochromia, 56% target cells, basophilic stippling, polychromasia, and nucleated red cells were seen on a stained slide of peripheral blood (Fig. 3C). No erythrocytic inclusions were seen in wet preparations following incubation with brilliant cresyl blue. Her hemoglobin electrophoretic pattern was remarkable for 13.1% Hb E and 8.0% Hb Bart's; the remainder was Hb A. The alkali-resistant hemoglobin was 4.0%.

A brother (Ref. 2.6), 14 years of age and clinically healthy, had a hemoglobin concentration of 12.0 g/100 ml; RBC 5,090,000/mm^3; packed cell volume 47%; MCV 80 μ^3; MCHC 30%; and reticulocytes 2.4%. On fixed films his red blood cells appeared hypochromic and microcytic, and 10% were target cells. On starch block electrophoresis 28.2% of his hemoglobin was Hb E, the remainder Hb A. The amount resistant to alkali denaturation was 1.1%.

Two members of the family, a girl 21 (Ref. 2.3) and a boy (Ref. 2.5) 17 years of age, had hematologic values within normal limits; the hemoglobin concentrations were 13.9 and 13.6 g/100 ml respectively. The hemoglobin electrophoretic patterns were also normal. The former had 1.4% Hb A_2 and the latter 2.1% Hb A_2.

Comment. The phenotypes found in this remarkable family are: Two children have Hbs A and H; one has Hbs E, A, and Bart's; one has Hbs E and A; and two have only Hb A. The father is apparently normal except for the presence of hypochromia and target cells observed on fixed preparations. The mother's blood has 21.3% Hb E, well below that expected in Hb E trait, numerous target cells, microcytosis, and hypochromia. These findings suggest

that each of the three severely anemic children have received modifier or α thalassemia genes from both parents, and one has received Hb_β^E in addition. Moreover, the Hb E segregates independently from the thalassemia gene. The one boy with reticulocytosis and only 28% Hb E appears, like his mother, to be heterozygous for both Hb_β^E and for α thalassemia genes. Two children are apparently normal, although the Hb A_2 value of one (Ref. 2.3) may be indicative of α thalassemia trait. The proposed interpretation is presented diagrammatically in the pedigree (Fig. 2B).

Family 3

The father (Ref. 3.1), of pure Thai descent, was clinically normal. His hemoglobin concentration was 13.0 g/100 ml; RBC 5,500,000/mm^3; packed cell volume 46%; MCV 84 μ^3; MCHC 28%; and reticulocytes 0.2%. On starch block electrophoresis, Hb E accounted for 22.2% of the total hemoglobin and Hb A 77.8%. Alkali-resistant hemoglobin was 1.0%. Blood films were normal except for slight hypochromia (Fig. 4A).

The mother (Ref. 3.2) was also of pure Thai ancestry. Her hemoglobin concentration was 11.9 g/100 ml; RBC 4,260,000/mm^3; packed cell volume 36.5%; MCV 86 μ^3; MCHC 33%; and reticulocytes 2.6%. Stained preparations of her blood showed anisopoikilocytosis, hypochromia, basophilic stippling, and 1% target cells (Fig. 4B). The electrophoretic pattern was normal, and Hb A_2 accounted for 3.0% of the total hemoglobin. Alkali-resistant hemoglobin was within normal limits. Following oral iron therapy for 38 days, her hemoglobin rose to 12.4 g/100 ml, while her red blood count remained essentially the same.

The proposita (Ref. 3.3) was a seven year old girl who developed frequent attacks of fever and marked jaundice at age four and became progressively weaker. At the time of her first admission on February 8, 1961, her temperature was 39.5° C. Physical examination revealed pharyngitis, generalized edema, marked pallor, slight jaundice, and hemic murmur. Her liver was palpable one fingerbreadth and her spleen five fingerbreadths below the costal margins. Her hemoglobin concentration was 8.6 g/100 ml; red blood cell count 3,200,000/mm^3; white blood cell count 6,700/mm^3, and reticulocyte count 3.8%. Wright's stained preparations of her peripheral blood revealed 39 nucleated red cells/100 WBC, marked anisopoikilocytosis, hypochromia, 33% target cells, Howell-Jolly bodies, and polychromasia (Fig. 4C). The direct bilirubin was 0.65 mg/100 ml and the indirect 3.1 mg/100 ml. The serum globulin was 2.1 and albumin 3.9 g/100 ml. She was treated with seven blood transfusions of 200 ml each and penicillin. One month later her hemoglobin was 8.6 g/100 ml. She was discharged and followed in the Out-Patient Department. In November 1961 (without intervening transfusions), Hb E accounted for 15.3 and Hb Bart's 10.7% of the total hemoglobin. The fetal hemoglobin determined by alkali denaturation was 7.4%.

There were two other children in the family, a boy ten and a girl eight years of age, who were said to be healthy but who were not examined.

Comment. The hypochromia and low Hb E concentration in the father

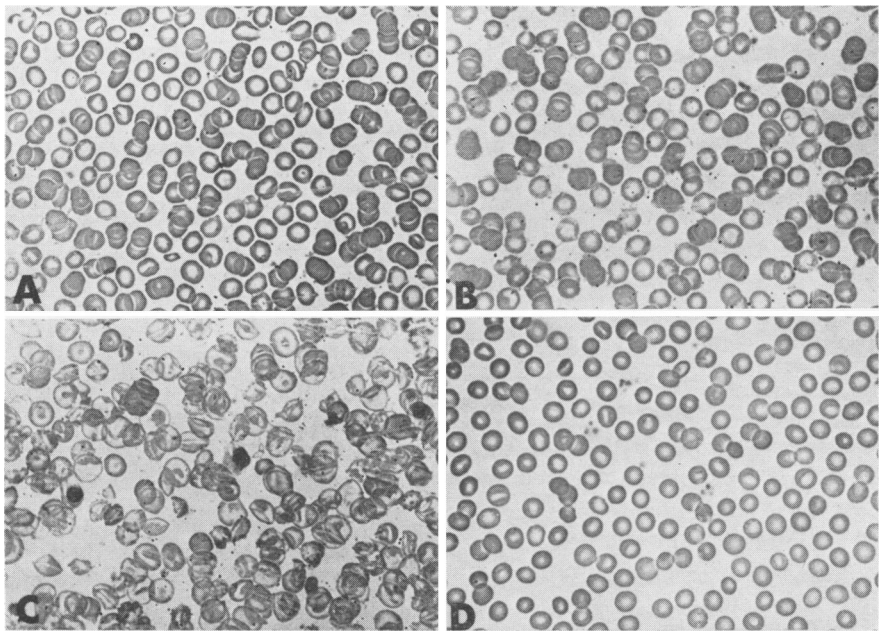

FIG. 4. Peripheral blood film of members of Family 3, Table 1. A, father; B, mother; C, Ref. 3.3, possessing Hb E + Bart's; D, normal blood for comparison.

despite 13 g Hb/100 ml is more likely due to double heterozygosity for α thalassemia and Hb_{β}^{E} genes than to nutritional factors. In the mother reticulocytosis and morphologic abnormalities without evidence of iron deficiency also point toward thalassemia trait. The profound anemia in the child plus the very unusual electrophoretic findings are likewise consistent with the presence of two thalassemia genes as well as the one for Hb E (Fig. 2C).

Family 4

The father, who was of pure Thai descent, had died of fever. The mother (Ref. 4.1), also pure Thai, was healthy. Her hemoglobin initially was 9.9 g/100 ml, and after 17 days of oral iron therapy it was 9.8 g/100 ml. Before treatment the MCV was 73 μ^3 and the MCHC 31%. Following therapy the MCV was 75 μ^3 and the MCHC 29%. Stained preparations of her blood revealed slight anisopoikilocytosis, hypochromia, basophilic stippling, and a few target cells. Her electrophoretic pattern was normal; Hb A_2 accounted for 2.2% of the total hemoglobin. Alkali-resistant hemoglobin was 1.5%.

The propositus (Ref. 4.2), a boy 12 years of age, was admitted to the hospital in September 1957 because of frequent attacks of fever and progressive weakness. Physical examination revealed a child of normal size for his age, a liver extending three cm and spleen six cm below the costal margins. Hemoglobin concentration was 6.4 g/100 ml, red blood cells 4,500,000/mm^3, and reticulocytes 18.0%. Wright's stained films of peripheral blood showed 30 nucleated red cells/100 WBC, marked anisopoikilocytosis, hypochromia, polychromasia, and 30% target cells. Paper electrophoresis of hemoglobin revealed

Hb E, Hb A, and a fast component. Alkali-resistant hemoglobin was 14.7%. No inclusions were seen in the erythrocytes following incubation with brilliant cresyl blue. The patient was given seven blood transfusions of 200 ml each, and one month later his spleen was removed. Following splenectomy his hemoglobin stayed between 8 and 9 g/100 ml without transfusions. In November 1959, the hemoglobin concentration was 7.3 g/100 ml. Hb E accounted for 15.9 and Bart's 7.9% of the total hemoglobin. Alkali-resistant hemoglobin was 10%.

The patient had an older brother who was said to be healthy but who was not examined.

Comment. It is difficult to arrive at any definite conclusions from this family because the father is dead (Fig. 2D). However, the mother had more marked hematologic abnormalities suggesting thalassemia than did any of the non-E parents in the first three families. Her red blood cells were both hypochromic and microcytic. The propositus's clinical and hematologic findings were like those found in children in the other three families with low concentration of Hb E and Hb Bart's.

Family 5

The family lived in Ubol in the northeast section of Thailand. The father and mother were seen only one time when they brought their child to Bangkok because the mother had noticed an abdominal mass three months previously.

The father (Ref. 5.1), half Thai and half Burmese, was healthy. His hemoglobin concentration was 14.1 g/100 ml; RBC 6,730,000/mm^3; packed cell volume 47%; MCV 70 μ^3 and MCHC 30%. The reticulocyte count was 0.8%. Only Hb A was seen on paper electrophoresis of his hemoglobin. The mother (Ref. 5.2) was of pure Thai descent. Her hemoglobin concentration was 13.0 g/100 ml and her other hematologic values were within normal limits. Hbs E and A were seen on paper electrophoresis, the latter in excess.

The propositus (Ref. 5.3), a boy eight years of age, had a history of weakness, susceptibility to fatigue, and slight jaundice of four to five years duration. The physical examination revealed an alert, intelligent child, small for his age. Slight jaundice and pallor were evident. His liver was palpated one fingerbreadth and spleen four fingerbreadths below the costal margins. An X ray of the skull and long bones showed evidence of hemolytic anemia. His hemoglobin concentration was 6.2 g/100 ml; red blood cell count 3,600,000/mm^3; packed cell volume 28%; MCV 78 μ^3; MCHC 22%; WBC 11,700/mm^3; reticulocytes 10.4%; and fecal urobilinogen 308 mg/day. Peripheral blood film showed marked anisopoikilocytosis, hypochromia, rare nucleated red cells, and 22% target cells. In addition to Hb A, there was 15.5% Hb E and 14.0% Hb Bart's. Alkali-resistant hemoglobin was 8.8%. The patient had received no transfusions nor was hospital care necessary. He had three sisters who were said to be healthy but who were not studied.

Comment. The father showed stigmata of thalassemia trait—erythrocytosis, microcytosis, and hypochromia—despite having 14 g Hb/100 ml. His hemoglobin pattern was normal on paper electrophoresis, but the Hb A_2 was not

TABLE 2. HEMATOLOGIC DATA OBTAINED ON PARENTS OF PATIENTS WITH HEMOGLOBIN H DISEASE

Relationship		Hb g/100 ml	RBC $\times 10^6$ /mm³	Packed cell vol. %	MCV μ^3	MCHC %	Alkali-resistant Hb %	Hb A_2 %	Osmotic fragility
Tha.	Father*	13.6	4.74	42	88	32	0.1	2.5	.42–.26
	Mother	11.4	4.69	35.5	76	32	0.5	2.1	.48–.14
Som.	Father	12.1	4.50	43	96	28	1.2	1.8	—
	Mother	11.2	4.60	40.5	88	28	0.6	2.0	—
Sir.	Father	12.7	4.30	45	104	28	0.7	2.1	—
	Mother	11.9	4.09	38	94	31	1.6	2.6	—
		11.4	4.56	39.5	87	29†			

*Trace of Hb H seen on starch block electrophoresis at pH 7.0.
†Counts obtained after oral iron treatment for 30 days.
—Not tested.

determined. The mother had normal hematologic values, but Hb E was found on paper electrophoresis. The propositus is like the other patients with a low concentration of Hb E and Bart's, and we assume that he is homozygous for α thalassemia and heterozygous for Hb_β^E.

Parents of Children with Hb H Disease

To contribute further to understanding the mode of inheritance of Hb H, the parents of three families in which at least one child had Hb H disease are presented for comparison (Table 2). In the first family (Tha.), ascertained because Bart's hemoglobin was found in the cord blood of one child (Tuchinda *et al.*, 1959), the mother had microcytic erythrocytes both on film and by erythrocytic indices. Her red blood cells and those of her husband (normal morphologically) had decreased osmotic fragility. In two families (Som. and Sir.), both parents had hypochromia. All had normal Hb A_2 values ranging from 1.8 to 2.6% of the total hemoglobin. Alkali-resistant hemoglobin was within normal limits. The lowest hemoglobin concentration was 11.2 g/100 ml. Although both parents in each family had some slight evidence for thalassemia, no single abnormal laboratory test was common to all; therefore, we believe that the α thalassemia gene may be nearly recessive.

Parents of Children with β Thalassemia–Hb E Disease

In contrast to the low Hb A_2 values seen in the parents of patients with Hb H disease are the high Hb A_2 values seen in parents of patients with classical thalassemia–Hb E disease. Four non-Hb E parents were tested; the Hb A_2 values ranged from 4.6 to 5.9% (Table 3). Alkali-resistant hemoglobin ranged from normal to 3.1%. Their mates with Hb E had from 30 to 33.1% of the abnormal hemoglobin. Nutritional factors cannot be ruled out as cause for hypochromia in two of these, nor in any of the entire group, as serum iron values were not available. We were able to study another case of Hb E trait in a doctor presently working in St. Louis. Hematologic values were all within normal limits, with 13.6 g Hb/100 ml. Her hemolyzate contained 30.8% of Hb E on starch block electrophoresis.

TABLE 3. HEMATOLOGIC DATA OBTAINED ON PARENTS OF PATIENTS WITH THALASSEMIA–HEMOGLOBIN E DISEASE*

Relationship	Hb g/100 ml	RBC $\times 10^6$ /mm³	Packed cell vol. %	MCV μ^3	MCHC %	Alkali-resistant Hb %	Hb A_2 %	Hb E %
Nit. Father	9.8	4.20	36	86	27	1.1	—	32.9
Mother	12.5	4.90	42	86	30	1.0	5.2	0
Sud. Father	13.6	6.40	44	69	31	1.5	—	33.1
Mother	9.7	5.20	32	62	30	1.7	4.7	0
Pra. Father	9.4	4.90	42	86	22	0.7	—	30.0
Mother	12.0	5.20	37.5	72	32	3.1	4.6	0
Jid. Father	13.8	4.90	43.5	89	32	1.0	—	30.0
Mother	12.1	4.20	36	86	34	1.6	5.4	0

*Patients have high concentrations of hemoglobins E and F; Hb A not seen on paper electrophoresis. —Not tested.

Mating of Hemoglobin H Disease and Homozygous Hb_β^E Subjects

Supporting the hypothesis that Hb H disease is the result of two nearly recessive allelic thalassemia genes and that heterozygosity for such α thalassemia and Hb E genes lowers the concentration of Hb E in the blood is one additional family ("P", Table 4 and Fig. 2F). This family was ascertained in the study of the prevalence of Hb Bart's in cord blood in Thai babies by Tuchinda *et al.* (1959), inasmuch as the cord blood of the fourth child of this mating contained Hb Bart's which subsequently disappeared. The father's hemolyzate had Hb H and the mother's only Hb E; she was presumably homozygous for the gene, since her anemia was not severe enough to suspect Hb E–thalassemia. Two of the children had 20.3 and 20.8% Hb E, which is definitely lower than that seen in Hb E trait subjects, and two had approximately 27% Hb E. All four children had microcytosis without anemia.

If Hb H disease is a result of the homozygous state for a single gene, t_1t_1, or of heterozygosity for two allelic thalassemia genes, t_1t_2, all of the children should have at least one of these genes, which appears to be the case. Offspring with 21 and 27% Hb E suggest that the parent with Hb H has two different thalassemia genes which suppress Hb E to varying degrees in the offspring.

Hemoglobin Identification

The Hb E component in the proposita of Family 3 (Table 1, Ref. 3.3), which also contained Hb A_2 as a contaminant, was isolated by starch block electrophoresis and fingerprinted. By two techniques the peptide corresponding to peptide βTpIV was missing and replaced by two new peptides (E_1 and E_2 of Fig. 5) created by substitution of lysine for a glutamic acid residue, forming an additional cleavage point (Hunt and Ingram, 1960; Chernoff and Liu, 1961). Thus the slow electrophoretic component is predominantly Hb E.

In an attempt to detect the presence of the β^{E}_4 polymer, which might be expected to migrate electrophoretically with Hb A, this component from the same child's blood was hybridized with Hb $D_{\alpha\ \text{St. Louis}}$ (Minnich *et al.*,

TABLE 4. HEMATOLOGIC FINDINGS IN THE "P" FAMILY

Family ref.	Member	Age years	Hb g/100 ml	RBC $\times 10^6$ /mm³	Packed cell vol. %	MCV μ^3	MCHC %	Reticulocytes %	Alkali-resistant Hb %	Hemoglobin components			Erythrocyte Morphology				Target cells
										A_2 %	E %	H %	aniso	poik	hypoch	micro	
P. 1	Father		11.5	6.00	39.5	66	29	—	2.6	1.5	0	13.4	++	++	++	+	9
P. 2	Mother		10.9	5.18	30.5	59	36	1.4	1.8	—	100.0	0	++	+	0	+	20
P. 3	Son	7	12.5	5.57	36.5	66	34	0.7	1.7	—	20.3	0	++	+	++	0	13
P. 4	Daughter	5	12.3	5.66	33.5	60	37	1.0	1.4	—	20.8	0	+	+	++	0	7
P. 5	Daughter	4	12.4	6.04	38	63	33	1.5	1.4	—	27.2	0	+	+	0	+	2
P. 6	Son	2	11.1	4.28	30	70	37	0.4	2.2	—	27.6	0	+	+	++	—	6

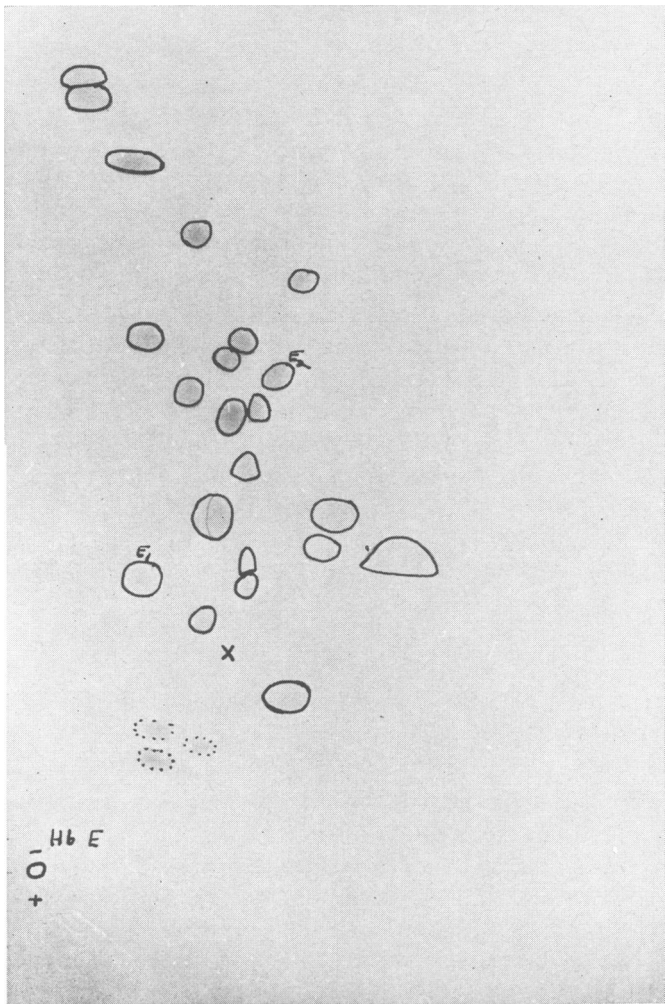

FIG. 5. Fingerprint of the Hb E component from a child also having Hb Bart's (Ref. 3.3, Table 1). Chromatography was performed in the horizontal dimension, electrophoresis at pH 3.5 in the vertical. The peptides designated E_1 and E_2 are identical to those obtained by Chernoff and Liu (1961) from Hb E. X marks the location of peptide βTpIV of Hb A from which these were derived.

1962). If the polymer were present, one would hope to see a hybrid component ($\alpha^{D}{}_2\beta^{E}{}_2$) with a +6 net charge. None was detected on agar gel and starch gel electrophoresis.

DISCUSSION

The chemical basis of thalassemia remains an enigma despite the rapidity with which the primary structure of the hemoglobin molecule and the role of the gene in directing protein synthesis have been elucidated. Three phenomena implicate thalassemia with the hemoglobinopathies. One of the

earliest observations was the alteration of the amount of abnormal hemoglobin in the blood of individuals heterozygous for an abnormal hemoglobin and a thalassemia gene compared with heterozygotes for the hemoglobin mutant only (Itano, Bergren, and Sturgeon, 1956; Zuelzer, Neel, and Robinson, 1956). In most of the latter, the abnormal hemoglobin constitutes approximately 40% of the total hemoglobin. In the majority of cases of sickle cell–thalassemia reported, the amount of Hb S has ranged from 75 to nearly 100%. Matings of such "interacting" sickle cell–thalassemia persons with normal individuals produce only two types of offspring—sickle cell trait and thalassemia minor—suggesting that the two genes are allelic or closely linked (Neel, 1958; Rucknagel and Neel, 1961). Secondly, the erythrocytes of persons with thalassemia trait (or minor) contain approximately 5% of the minor component, Hb A_2, compared with only ca. 2.5% in normal blood. Finally, fetal hemoglobin is commonly elevated in persons homozygous and heterozygous for the thalassemia genes. There is as yet no good explanation for the increases in Hb A_2 and F in thalassemia or for the fact that some subjects with "interacting" sickle cell–thalassemia have 20% Hb A and others none. By analogy with the modulating mechanisms controlling protein synthesis in microörganisms, it has been postulated that the thalassemia genes represent mutations at "controller" or regulator loci (Neel, 1961; Motulsky, 1962).

Individuals possessing Hb H have also been considered to have thalassemia, inasmuch as they manifest numerous stigmata of thalassemia (Rigas, Koler, and Osgood, 1956; Minnich *et al.*, 1956). Splenomegaly, hypochromic and microcytic anemia, leptocytosis, erythroblastosis, and unique intraerythrocytic inclusions are constant features (Minnich *et al.*, 1954). The detection with sufficiently sensitive methods of Hb Bart's (Ramot *et al.*, 1959; Huehns *et al.*, 1960) in a number of individuals possessing Hb H disease, as the above syndrome is called, has led to the current notion that both hemoglobins are formed in response to suppression of synthesis of α chains by thalassemia genes (Jones *et al.*, 1959). Thus, the designation α thalassemia (Ingram and Stretton, 1959).

In earlier studies of families of patients with Hb H, one parent showed stigmata of thalassemia minor, the other was normal, but neither possessed Hb H. This led to speculation that thalassemia was necessary for the Hb H gene to be expressed (Rigas, Koler, and Osgood, 1956; Motulsky, 1956). To date, of the 14 reported families having Hb H offspring in which both parents have been studied and neither parent has Hb H, both parents have had some evidence of thalassemia in seven families (Fessas and Papaspyrou, 1957; Lie-Injo *et al.*, 1957; Ager and Lehmann, 1958; Hedenberg *et al.*, 1958; and Quattrin *et al.*, 1961); in six families only one parent had evidence of thalassemia, the other being apparently normal (Gouttas *et al.*, 1955; Minnich *et al.*, 1956; Wolff, Michaels, and Von Hoffe, 1958; Koler and Rigas, 1961; Lie-Injo, Tjoa, and Kho, 1961; and Sturgeon *et al.*, 1962); and in one family both parents were normal (Bingle, Huehns, and Prankerd, 1958). The amounts of Hb A_2 in the blood of these "thalassemia"

parents are normal (Hedenberg *et al.*, 1958; Dittman *et al.*, 1960; Quattrin *et al.*, 1961), with one exception (Choremis *et al.*, 1959), demonstrating that the thalassemia gene associated with Hb H is different from the so-called classical thalassemia in which the Hb A_2 content is elevated. Several modes of inheritance for Hb H have been considered. Numerous examples of parent to offspring transmission suggest "dominant" inheritance (Gouttas *et al.*, 1955; Minnich *et al.*, 1956; Bingle, Huehns, and Prankerd, 1958; de Traverse, Le Xuan, and Coquelet, 1960; Dittman *et al.*, 1960). The possibility that Hb H individuals represent the homozygous state for a recessive gene or contain at least two thalassemia genes has also been postulated (Huehns *et al.*, 1960; Lie-Injo, Tjoa, and Kho, 1961; Koler and Rigas, 1961; Sturgeon *et al.*, 1962).

Considerably less information is available regarding the interaction of α thalassemia and structural mutants involving the α polypeptide chains. The only Hb_α^I–thalassemia case reported has a normal amount of Hb A_2 and approximately 70% Hb I (Atwater *et al.*, 1960). Since the thalassemia involved was not associated with an elevation of the Hb A_2 fraction, and thus on the basis of current thinking is α thalassemia, this suggests that the so-called α thalassemia enhances the expression and Hb_α mutants in a manner analogous to that of "classical" thalassemia and Hb_β mutants. A similar interaction occurs with Hb_α^Q and α thalassemia (Vella *et al.*, 1958; Dormandy, Lock, and Lehmann, 1961). The concept of α thalassemia rests upon chemical evidence; there is no *genetic* evidence, i.e., offspring of matings of double heterozygotes for α thalassemia and Hb_α structural mutants with normal spouses, to allow complete analogy of α thalassemia to β or "classical" thalassemia.

In the families described in Table 1, children in the same family have Hb H disease without Hb E, whereas others have Hb Bart's and severe anemia along with Hb E. Because of the severity of their disease, the children with Hb H disease have presumably received two thalassemia genes, one from each parent. The children with Hb E and Hb Bart's are similar clinically and hematologically to those with Hb H disease, suggesting that they too have received two α thalassemia genes as well as the gene for Hb E. The two thalassemia genes have therefore suppressed the synthesis of Hb E considerably. In the discussion to follow, variation in the relative amounts of abnormal hemoglobin components is equated with variation in the rate of synthesis, with the realization that other mechanisms might also explain the observations, i.e., unequal distribution of Hb E among erythrocytes with shortening of the erythrocyte survival time proportional to the amount of Hb E within a cell might be manifested as a decrease in Hb E in whole blood, despite over-all net increase in Hb E synthesis.

Some evidence exists for thalassemia in the non-Hb E parents of families 2, 3, and 4 (Table 1), but the hematologic findings are not as striking as those which ordinarily are associated with thalassemia. In the four parents shown to have Hb E (Table 1), the hematological indices were not sufficiently different from those of apparently uncomplicated Hb E trait individuals

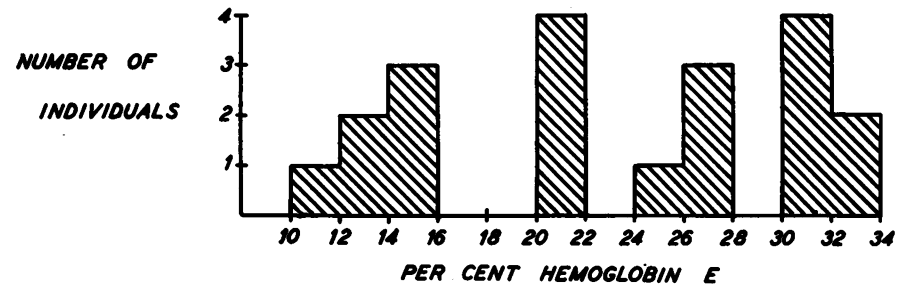

FIG. 6. Frequency distribution of amount of Hb E in the blood of individuals of Tables 1, 3, and 4, and one additional case of Hb E trait, showing the apparent presence of three or four modes. This correlates with the genetic evidence suggesting that Hb_{β}^{E} may exist in combination with two α thalassemia genes (left), one each of two different α thalassemia genes (center) or as a simple heterozygote (extreme right).

(Table 3) to suggest the presence of a thalassemia gene. The distribution of the amount of Hb E in the members of the various families described is depicted in Fig. 6. The variation in the amount of Hb E is not due to technique inasmuch as the mean difference between duplicate starch block analyses on 16 blood samples was only 2%. The four parents with simple Hb E trait (Table 3) possessed more than 30% Hb E. The parents with Hb E of Table 1 possessed less than 30% Hb E (mean 22.8%), at the lower end of the range for heterozygotes, suggesting that they carry a thalassemia gene which suppresses the synthesis of Hb E but to a lesser extent than in their anemic children.

The interpretation of the "P" family (Table 4, Fig. 4F) is also consistent with that given the five families presented in Table 1. In this family one parent has Hb H disease and the other is apparently homozygous for Hb_{β}^{E}. All four of the children had less than 30% Hb E, suggesting that they received a thalassemia gene from the father which suppressed the expression of the Hb_{β}^{E} gene from the mother. The apparent existence of modal values of Hb E at about 21 and 27% (Table 4 and Fig. 6) in this family and in the other families of Table 1 suggests that Hb H disease might be the result of two different α thalassemia genes, each of which, when combined in the heterozygous state with a Hb E gene, suppresses Hb E formation to varying degrees. This admittedly may be overinterpreting the apparent trimodality of the distribution of Hb E among nonanemic heterozygotes (Fig. 6). To establish this conclusively, much more family data will be required.

According to current concepts, the so-called α and β thalassemia genes act to suppress rather specifically the polypeptide chain synthesis governed by the structural loci to which they are presumably linked. That this is an oversimplification of the problem is amply demonstrated by the suppression of β^{E} polypeptide chains in the children with Hb E and Bart's and the enhancement of γ chain synthesis by the α thalassemia genes. The differential effect of the modifier, or α thalassemia, genes segregating in these families is shown in Table 5 in which the absolute amount of hemoglobin as α^{A}, β^{A}, β^{E}, and γ^{F}

TABLE 5. ABSOLUTE CONCENTRATION OF HEMOGLOBIN POLYPEPTIDE CHAINS IN HB E–α THALASSEMIA SYNDROMES

	Total Hb conc. g/100 ml	E %	H %	Bart's %	Polypeptide chains g/100 ml*				
					α_2	β_2^A	β_2^E	γ_2^F	Total non-α chains
Normal	15					7.5	7.5		7.5
Hb E trait	13.6	35			6.8	4.5	2.3		6.8
Hb H disease	10.4		15		4.4	6.0			6.0
Hb E–Bart's	6.8	13.1		7.7	3.1	2.6	0.4	0.6	3.6
Hb E trait–α thalassemia trait	11.7	25			5.9	4.4	1.5		5.9
α thalassemia trait	13.0		—		6.5	6.5			6.5

*e.g.: Absolute concentration of β^A chains = total Hb conc. $\left(\frac{\%\text{Hb A}}{2} + \%\text{ Hb H}\right)$.

chains is calculated for each of the phenotypes considered. The small amount of γ chains normally present as fetal hemoglobin was neglected for simplification. Thus, 100 ml of blood from normal individuals with 15.0 g hemoglobin contains 7.5 g each of α and β polypeptide chains. A Hb E trait person has 4.5 g β^A and 2.3 g β^E chains combined with 6.8 g α^A chains. In the children with Hb E and Hb Bart's, 3.6 g of non-α chains are present compared with only 3.1 g of α chains. But β^E synthesis is only 1/5 that of β^A and 0.6 g γ chains are produced as Hb Bart's. This suppression of β^E chain synthesis indicates that the effect of the α thalassemia gene is not quite as specific as envisioned in the currently held model but governs the activity of the other structural loci as well.

A possible precedent for suppression of the Hb_β locus is provided by three previously reported families in which individuals heterozygous for an abnormal hemoglobin and thalassemia have less than 50% of Hb S or C (Zuelzer and Kaplan, 1954; Aksoy and Lehman, 1957; Cohen *et al.*, 1959). The amount of the abnormal hemoglobin varied between 22 and 29% in those bloods in which it was measured, and in the last family cited by Cohen *et al.*, the Hb A_2 fraction was found not to be elevated. Moreover, the thalassemia genes segregated independently of the Hb_β structural genes, indicative of non-β thalassemia (Rucknagel and Neel, 1961). The absence of Hb H in members of these families makes identity of the thalassemia with that found in Thailand uncertain. If the relationships between α and β thalassemia and the hemoglobin structural loci are analogous, one would also predict that the coexistence of β thalassemia and a Hb_α structural mutant would result in depression of the amount of abnormal hemoglobin present.

Lie-Injo (1962) has attributed the large amounts of Hb Bart's and smaller amounts of Hb H found in the blood of some erythroblastotic stillborn fetuses in Malaya to homozygosity for α thalassemia. In comparing these findings with ours the following considerations can be applied. First, the difference between the stillborn fetuses and the Hb H–thalassemia observed may simply reflect the range of expression of homozygosity for the α thalassemia genes. Alternatively, it is possible that the erythroblastotic fetuses are homozygous for the same α thalassemia gene, whereas the Hb H disease observed in

children and adults is due to heterozygosity for two different mutant genes, as is suggested by the distribution of Hb E above. In any event, we do not consider these data to conflict seriously with those of Lie-Injo. The hematologic evidence for thalassemia among the parents of the erythroblastotic fetuses is no more certain in her families than in the cases presented here. Clearly much more genetic data and information are needed regarding environmental variables (Pearson and McFarland, 1962) in formation of Hb H and Bart's.

If this interpretation is correct, these segregation data also demonstrate that the α thalassemia and Hb_β structural loci are not linked, since the Hb E parent of Family 2 would have transmitted both the Hb_β^E and α thalassemia genes to offspring 2.7 (Fig. 2) but only the thalassemia gene to the children with Hb H disease (2.4 and 2.8). This does not constitute proof of linkage of the thalassemia gene to the Hb_α structural locus, however. Thus the α thalassemia gene might more certainly be considered as non-β thalassemia. In the absence of an interaction effect upon the electrophoretic pattern of a coexisting hemoglobinopathy, this type of thalassemia gene is nearly recessive if not completely so. Evidence of nonlinkage with the Hb_β locus is also incompatible with the recent hypothesis of Nance (1963), which postulates that the α thalassemia gene is a fusion product due to unequal crossing over between the Hb_β and Hb_δ loci. Although the genetic evidence does not allow differentiation between close linkage and allelism of the Hb_β structural locus and classical or β thalassemia, the current evidence points to thalassemia as a result of mutation at a regulatory locus closely linked to the hemoglobin structural loci (Neel, 1961; Motulsky, 1962), two of which, Hb_β and Hb_δ, are already known to be within 10 map units of each other (Boyer *et al.*, 1963). Our findings suggest that thalassemia is analogous to the regulator loci of microörganisms whose effect is mediated through cytoplasmic repressor substances capable of regulating synthesis at more than one structural locus.

The coexistence of Hb_β mutants and α thalassemia genes necessitates extension of the hemoglobin terminology to encompass this combination. The following, based upon the already existing nomenclature, allows definition of both the type of thalassemia and the hemoglobin mutant (Table 6). Thus the parents described herein are designated Hb_β^E trait–α thalassemia trait and α thalassemia trait, respectively. The designation of the six possible genotypes from such matings are presented in Table 5, along with the expected ratio and the observed numbers of each among the 13 children examined. As could be expected, because of the method of ascertainment, six children had Hb_β^E trait–α thalassemia disease. Two children had Hb H disease or α thalassemia disease. Only one child had 25% Hb E or Hb E trait–α thalassemia trait. Sickle cell–thalassemia might be designated sickle cell (or Hb S) trait–β thalassemia trait. If Hb H disease proves to result from double heterozygosity for two different α thalassemia genes, it might be designated $\alpha_{1,2}$ thalassemia disease. Likewise, heterozygosity for two β thalassemia genes might be designated $\beta_{1,2}$ thalassemia disease.

TABLE 6. DISTRIBUTION OF OFFSPRING FROM POOLED α THALASSEMIA TRAIT × HEMOGLOBIN E–α THALASSEMIA TRAIT MATINGS

Genotype of offspring	Designation	Number observed	Proportion expected
Hb_β^A/Hb_β^E, Th^T/Th^T	Hemoglobin E trait–α thalassemia disease	6	1/8
Hb_β^A/Hb_β^E, Th^N/Th^T	Hemoglobin E trait–α thalassemia trait	1	2/8
Hb_β^A/Hb_β^E, Th^N/Th^N	Hemoglobin E trait	0	1/8
Hb_β^A/Hb_β^A, Th^T/Th^T	α thalassemia disease (Hb H disease)	2	1/8
Hb_β^A/Hb_β^A, Th^N/Th^T	α thalassemia trait	2	2/8
Hb_β^A/Hb_β^A, Th^N/Th^N	Normal	2	1/8
		13	

Two children had minor morphologic abnormalities suggestive of α thalassemia trait, but in the absence of serum iron determinations, it is difficult to exclude the possibility that they were normal. Only one child was apparently normal. Unfortunately, in this small series there were no children with amounts of Hb E large enough to be designated as Hb E trait.

It is to be emphasized that the preceding interpretation and discussion are predicated upon the assumptions that the variations in the amount of Hb E are not due to laboratory error, nutritional deficiency, or other environmental factors and are of genetic significance. Other interpretations are possible but less probable. Hb Bart's in the children with Hb E could be due to interaction of a single thalassemia gene with Hb_β^E. This would not explain the presence of Hb H in two children without Hb E. The possibility that the Hb E component of the propositi was in fact Hb A_2 and not Hb E has been excluded by demonstrating that the fingerprint of this component is identical to that found by Chernoff and Liu (1961) and did not contain peptides of Hb A_2. Thus the earlier statement of Aksoy, Lehmann, and Lie-Injo (1957) that Hb A_2 can be elevated to as much as 12% and that Hb E does not exist in less than 20% concentration in Hb E trait must be revised. In fact, the case to which they refer may be identical to the entity described here; hematologic or genetic details were not presented. The presence of a single gene facilitating polymerization of non-α chains is possible, but no β^E_4 polymer was detected in the component with the mobility of Hb A, and this would also leave unexplained the absence of Hb H and Hb Bart's in the parents. Others believe that the α and β thalassemia mutations result in a degenerate deoxyribonucleic acid triplet code word, impairing the rate at which the α or β polypeptide chains, respectively, are synthesized without altering the primary amino acid sequence per se (Itano, 1962; Ingram, 1963).

The above alterations are compatible with such a hypothesis, provided one also invokes secondary hypotheses to explain the depression of both α and β polypeptide chains and the increase in the amount of γ chains synthesized.

The ultimate proof of the genetic interpretation, which is based on a rather small body of information, will require considerably more family data. It should also be subject to test by the methods of population genetics.

SUMMARY

Five Thai families were ascertained through children with apparent thalassemia major. Their blood contained approximately 15% hemoglobin E and 10% Bart's hemoglobin in addition to hemoglobin A. The identity of Hb E was verified by fingerprinting. Other siblings had Hb H disease, thalassemia minor with normal amounts of Hb A_2, or were normal. One sibling's blood possessed only 28% Hb E. One parent's blood showed minimal evidence for thalassemia with normal amounts of Hb A_2; the other parent's blood contained only about 25% Hb E. The children with Hbs E and Bart's are interpreted as having two α thalassemia genes in addition to the Hb_β^E gene. The individuals with less than 30% Hb E are considered to have one Hb_β^E and one α thalassemia gene and those with Hb H disease to have two possibly different α thalassemia genes. Other family data, including the children of a woman with homozygous Hb E disease and a father with Hb H disease, are presented in support of this interpretation.

In the former families, the α thalassemia and Hb_β^E genes segregate independently. The α thalassemia genes decrease the amount of β^E polypeptide chains relative to β^A as well as the amount of α chains relative to the non-α chains. These observations are interpreted as evidence that thalassemia genes are analogous to the regulator genes of microbial genetic systems.

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