Alpha-Thalassemia in Northern Thailand

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Alpha-thalassemia is a common trait in Thailand as evident from the large number of patients with hemoglobin (Hb) H disease (Na-Nakorn et al. 1965; Wasi et al. 1969) and with hydrops fetalis caused by homozygous α-thalassemia (Pootrakul et al. 1967*a*). Recently Thumasathit et al. (1968) reported 27 such hydropic fetuses observed in a period of two years at Chiengmai, a northern province of Thailand. This is probably the highest incidence of this disease so far reported anywhere. It indicates that α-thalassemia is extremely prevalent in that area. Precise data on the incidence of thalassemia, particularly of the α variety, are rarely known because of the difficulties in the diagnosis of the heterozygous forms. Detailed hematologic studies (Pornpatkul et al. 1969) of 275 obligatory heterozygotes for α-thalassemia trait suggest that heterozygosity for α-thalassemia may be associated with a normal blood picture. It is thus unreliable to obtain frequencies of α-thalassemia from hematologic examination of the population. However, α-thalassemia expresses in the neonatal period by the presence of significant amounts of Hb Bart's (γ_4). This pigment disappears approximately three months after birth (Pootrakul et al. 1967*b*). Examination of newborns for this abnormal hemoglobin by sensitive electrophoretic methods is, at this time, the most accurate and practical method in the diagnosis of α-thalassemia. We wish to report data from such studies in 287 cord blood samples from northern Thailand.

MATERIALS AND METHODS

In 1966 an experienced nurse-technician was stationed in Chiengmai to collect cord blood samples in EDTA and to record relevant data. The blood specimens were sent in ice by train to Bangkok, where they were examined on the following day. Hemoglobin types were identified by starch-gel electrophoresis (Smithies 1959) in tris-borate-EDTA buffer, pH 8.6, stained with orthodianisidine. Hb Bart's was quantitated by cellulose acetate electrophoresis in tris-borate-EDTA buffer without staining as described previously (Wasi, Disthasongchan, and Na-Nakorn 1968).

RESULTS

Hemoglobin Bart's was detected in 88 out of 287 newborns, an incidence of 30.66%. In the orthodianisidine-stained starch gels, the amounts of Hb Bart's distinctly segregated into three groups (fig. 1). The careful quantitative determination of Hb Bart's by cellulose-acetate electrophoresis in the 88 newborns with this pigment re-

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vealed a clearly trimodal distribution as depicted in figure 2. Data related to these three groups are shown in table 1.

Newborns without Hb Bart's had a mean body birth weight of 2,969 gm (SD = 352) and a mean placental weight of 543 gm (SD = 104). Birth weight and placental weight averages in the newborns with Hb Bart's were 2,885 gm (SD = 400) and 525 gm (SD = 94), respectively.

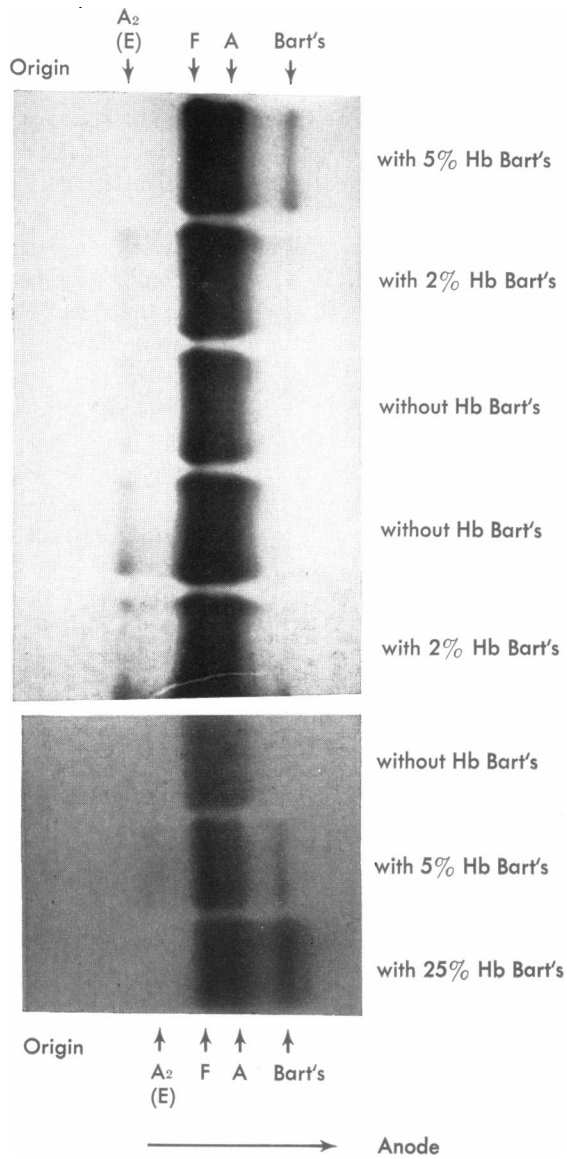

FIG. 1.—Starch-gel electrophoresis of the cord blood hemoglobin in tris-borate-EDTA buffer, *p*H 8.6, stained with orthodianisidine. The terms A_2, (E), F, and A refer to the relative mobility of Hb A_2, Hb E, Hb F, and Hb A.

DISCUSSION

Although it has been reported that minute amounts of Hb Bart's can be detected in fresh cord blood samples of normal newborn infants (Fessas and Mastrokalos 1959; Vella 1959; Dance and Huehns 1962; Weatherall 1963), this was not true in our starch-gel system (fig. 1) when used in several thousand cord blood samples. When Hb Bart's was detectable, it was practically always associated with α-thalassemia. The α-thalassemia gene suppresses the synthesis of the α-chain, thus leading to polymerization of the excessive γ-chains to a tetrameric form—Hb γ_4 or Hb Bart's.

Evidence indicates that there is more than one type of α-thalassemia. The study of α-thalassemia began with genetic observations on Hb H disease. Usually Hb H

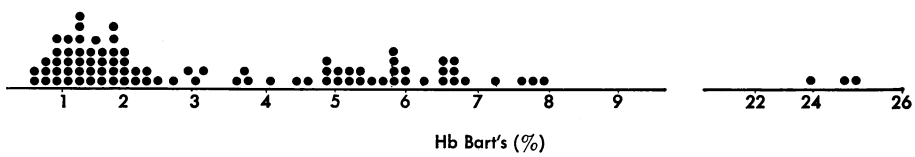

FIG. 2.—Distribution of the levels of Hb Bart's in 88 newborns

TABLE 1

NEWBORNS (88) WITH HB BART'S

AMOUNT OF HB BART'S	NO.	HB BART'S (%)		TRAIT	FREQUENCIES
		Mean	SD		
Trace.....	50	1.47	0.61	α-thalassemia$_2$	.1742
Small.....	35	5.80	1.1	α-thalassemia$_1$	.1219
Moderate.....	3	24.3	.....	α-thalassemia$_1$/ α-thalassemia$_2$	.0105
Total frequency of α-thalassemia.....	.....	.....	.....	.....	.3066

is not detectable in parents of patients with Hb H disease; yet, one of the parents has stigmata of thalassemia. Motulsky (1956) hypothesized that a recessive gene in addition to thalassemia was necessary for the occurrence of this disease. Huehns et al. (1960) suggested that two α-thalassemia genes were responsible for Hb H disease. Koler and Rigas (1961) examined several genetic mechanisms for this disease. Huehns (1962) also suggested that Hb H disease is caused by the interaction of a "severe" α-thalassemia gene with a "mild" α-thalassemia gene. Weatherall (1963) suggested that besides the α-thalassemia gene, another gene must be present for the development of Hb H disease. Wasi et al. (1964), from a large series of familial studies in Thailand, showed that Hb H disease occurs from the interaction of two α-thalassemia genes; one is the classical α-thalassemia (*α-thal*$_1$) and the other is a milder allele (*α-thal*$_2$). The latter is equivalent to the "recessive" gene conceived by Motulsky. The hypothesis that Hb H disease is the phenotype resulting from the genotype

α-*thal*$_1$/α-*thal*$_2$ is supported by additional evidence (Wasi, Na-Nakorn et al. 1968; Na-Nakorn et al. 1969; Wasi et al. 1969).

The level of Hb Bart's at birth is positively correlated with the severity of α-thalassemia (Pootrakul et al. 1967*b*). Hydropic fetuses homozygous for the severe thalassemia allele (α-*thal*$_1$) have practically 100% Hb Bart's because of complete inhibition of α-chain synthesis. Those who are born with 25% of Hb Bart's develop Hb H disease (α-*thal*$_1$/α-*thal*$_2$) or α-thal-Hb E disease ($Hb\beta^E/Hb\beta^A$, α-*thal*$_1$/α-*thal*$_2$) (Wasi, Sookanek, et al. 1967). Offspring of patients with Hb H disease inherit either the α-*thal*$_1$ or the α-*thal*$_2$ alleles; their levels of Hb Bart's at birth were found to segregate around the means of 5.7% and 1.53% (Na-Nakorn et al. 1969). It is believed that infants born with 5%–6% Hb Bart's have α-thalassemia$_1$ trait and those with 1%–2% of this hemoglobin have α-thalassemia$_2$ trait. In this series, the levels of Hb Bart's were trimodally distributed around the means of 1.47%, 5.80%, and 24.3%, corresponding to α-thalassemia$_2$, α-thalassemia$_1$, and α-thalassemia$_1$/α-thalassemia$_2$ trait, respectively. It should be pointed out that quantitation of small amounts of Hb Bart's may be difficult. We have been successful with the system described. It was important not to stain the cellulose acetate strips to prevent a large additional error from being introduced.

From the frequencies shown in table 1, the gene frequency for α-thalassemia$_1$ is $(35 + 3)/(287 \times 2)$, or .0662; the gene frequency for α-thalassemia$_2$ is $(50 + 3)/(287 \times 2)$, or .0923. Expectation for the frequency of α-thalassemia$_1$/α-thalassemia$_2$ according to the Hardy-Weinberg law, was $2\ pq = 2 \times .0662 \times .0923 = .0122$. Among 287 newborns, $.0122 \times 287$, or 3.5, were expected to have the α-thalassemia$_1$/α-thalassemia$_2$; three were found (those with 23%–25% Hb Bart's). Similarly, homozygosity for α-thalassemia$_1$ (hydrops with Hb Bart's) was expected to occur at a frequency of $(.0662)^2$, or .00438. Thumasathit et al. (1968) found that among 8,345 babies delivered at Chiengmai Hospital in two years, 33 were hydropic, and 27 out of 31 examined had homozygous α-thalassemia$_1$ disease. This would be equivalent to $27/31 \times 33$, or 28.7 Hb Bart's hydropic fetuses per 8,345 births. The expected number of fetuses with this disease from the predicted frequency was $.00438 \times 8345$, or 36.5. The observed and expected values were not significantly different ($P > .1$).

In this series of 287 newborns, homozygosity for α-thalassemia$_2$ was expected to be found in $287 \times (.0923)^2$, or 2.4 babies. So far, the phenotype for homozygous α-thalassemia$_2$ remains unidentified, and is believed to be similar to and included with α-thalassemia$_1$ heterozygosity.

It may be argued that the apparent trimodality of the levels of Hb Bart's shown in figure 2 could be interpreted differently. That is, the group with 0.5%–3% Hb Bart's may represent the tail of a normal distribution for Hb Bart's and not α-thalassemia$_2$ heterozygosity. Evidence that this group actually constitutes α-thalassemia$_2$ heterozygosity can be given. First, cord blood examination of offspring of patients with Hb H disease revealed two modes of Hb Bart's values segregating around 5.7% and 1.53%, respectively (Na-Nakorn et al. 1969). Those cases with a mean of 1.53% Hb Bart's most likely had α-thalassemia$_2$ trait and correspond to babies with putative α-thalassemia$_2$ heterozygosity with a mean of 1.47% in this study. Second, examinations of 1,408 newborns in Bangkok (Pootrakul et al. 1970)

revealed that the group with trace amounts of Hb Bart's had significantly lower mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values than newborns without detectable Hb Bart's, again suggesting that trace quantities of Hb Bart's indicate the presence of a thalassemia gene. Third, both in Bangkok and in the present series, the distributions fit the Hardy-Weinberg law if the data are interpreted as suggested.

The incidence of 30.66% for both types of α-thalassemia found in Chiengmai appears to be the highest so far reported. Similar studies on 1,408 cord blood samples in Bangkok revealed an incidence of 20.45% for all types of α-thalassemia (Wasi et al. 1969; Pootrakul et al. 1970).

The population of Thailand is quite heterogeneous ethnologically. A few thousand years ago the Thai lived in present southern China. They moved into northeastern India (Assam), Burma, Thailand, and Laos in several waves, but many remained scattered in southern China. Hb E is most frequent in northeastern Thailand (Flatz et al. 1965; Flatz 1967; Wasi, Na-Nakorn, and Suingdumrong 1967), reaching a heterozygote frequency of 50% in Surin province. This abnormal hemoglobin is very rare among the Chinese (Na-Nakorn 1959; McFadzean and Todd 1964). The Thai may have acquired Hb E after they moved into Southeast Asia. In Thailand, the frequencies of β-thalassemia appear to be reciprocally related to those of Hb E; its incidence is high in the north where that of Hb E is low. Besides the data for Bangkok and Chiengmai, the frequencies of α-thalassemia in other parts of Thailand remain unknown. The combination of Hb E with α-thalassemia will give rise to disease only when α-thalassemia is present in double dose (Tuchinda et al. 1964; Wasi, Sookanek, et al. 1967), unlike β-thalassemia where a single β-thalassemia gene will cause severe disease when present together with a single Hb E gene. Thus, it can be predicted that α-thalassemia genes should undergo milder selection than the β-thalassemia genes when combined with Hb E. If data on Thai populations living outside Thailand should one day become available, they will contribute appreciably toward a better understanding of the population dynamics of thalassemia.

SUMMARY

Starch-gel electrophoresis of 287 cord blood samples from Chiengmai, a northern province of Thailand, revealed hemoglobin (Hb) Bart's in 88 infants, an incidence of 30.66%. This approach gives the best estimate for the frequency of α-thalassemia in this population, representing the highest frequency of α-thalassemia ever reported.

Distribution of the levels of Hb Bart's in these 88 neonates, as determined by cellulose-acetate electrophoresis, was distinctly trimodal. The mean values for Hb Bart's in the three groups were 1.47% (SD = 0.61), 5.80% (SD = 1.1), and 24.3%. These groups are believed to represent α-thalassemia$_2$ trait, α-thalassemia$_1$ trait, and double heterozygosity for these two α-thalassemia alleles. The respective frequencies were .1742, .1219, and .0105. These values fit the expected frequencies for both Hb H disease (α-thalassemia$_1$/α-thalassemia$_2$) and for hydrops fetalis caused by homozygosity for α-thalassemia$_1$ in that area.

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AAAS Meeting—Human Genetics Symposium

The American Genetic Association is sponsoring a one-day symposium at the annual meeting of the American Association for the Advancement of Science, Section N—Medical Sciences, on "Advances in Human Genetics and Their Impact on Society" at 9 A.M. on December 28 at the Conrad Hilton Hotel, Parlor A, Chicago, Illinois. The speakers and discussants include Dr. Digamber S. Borgaonkar, Johns Hopkins Hospital; Dr. Kurt Hirschhorn, Mount Sinai School of Medicine; Dr. Neil Macintyre, Case Western Reserve University; Dr. Saleem Shah, National Institute of Mental Health; Professor Nathan Hershey, University of Pittsburgh; and Professor Elyce Zenoff Ferster, George Washington University.