responses in subhuman primate fetus and neonate. Metabolism, *20:* 805 (1971).
12. Felig, P.: The glucose alanine cycle. Metabolism, *22:* 179 (1973).
13. Gresham, E. L., James, E. J., Raye, J. R., Battaglia, F. C., Makowski, E. L., and Meshia, G.: Production and excretion of urea by the fetal lamb. Pediatrics, *50:* 372 (1972).
14. Hobel, C. J., Emmanouilides, G. C., Townsend, D. E., and Yashiro, K.: Ligation of one umbilical artery in the fetal lamb. Obstet. Gynecol., *36:* 582, (1970).
15. Lambert, A. E., Junod, A., Stauffacher, W., Jeanrenaud, B., and Renold, A. D.: Organ culture of fetal rat pancreas. I. Insulin release induced by caffeine and by sugars and some derivatives. Biochim. Biophys. Acta, *184:* 529 (1969).
16. Marks, V.: An improved glucose oxidase method for determining blood, CSF, and urine glucose levels. Clin. Chim. Acta, *4:* 395, (1959).
17. Milner, R. D. G., and Hales, C. N.: Effect of intravenous glucose on concentration of insulin in maternal and umbilical cord plasma. Brit. Med. J., *1:* 284, (1965).
18. Mintz, D. H., Chez, R. A., and Horger, E. O.: Fetal insulin and growth hormone metabolism in the subhuman primate. J. Clin. Invest., *48:* 176 (1969).
19. Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: Two antibody system. Diabetes, *12:* 115 (1963).
20. Muller, W. A., Faloona, G. R., and Unger, R. H.: The effect of alanine on glucagon secretion. J. Clin. Invest. *50:* 2215 (1971).
21. Obenshain, S. S., Adam, P. A. J., King, K. C., Teramo, K., Rairio, K. O., Raiha, N., and Schwartz, R.: Human fetal insulin response to sustained maternal hyperglycemia. New Engl. J. Med., *283:* 566 (1970).
22. Oh, W., Omori, K., Emmanoulides, G. C., and Erenberg, A.: Somatic growth, umbilical blood flow and substrate transfer in fetal sheep (submitted for publication).
23. Sperling, M. A., Delamater, P. V., Phelps, D., Fisher, R. H., Oh, W., and Fisher, D. A.: Spontaneous and amino acid stimulated glucagon secretion in the immediate postnatal period: Relation to glucose and insulin. J. Clin. Invest., *53:* 1159 (1974).
24. Sperling, M. A., Erenberg, A., Fisher R. H., Jr., Oh, W., and Fisher, D. A.: Placental transfer of glucagon in sheep. Endocrinology, *93:* 1435 (1973).
25. Willes, R. F., Boda, J. M., and Manns, J. G.: Insulin secretion by the ovine fetus in utero. Endocrinology, *84:* 520 (1969).
26. Wise, J. K., Hendler, R., and Felig, P.: Evaluation of alpha-cell function by infusion of alanine in normal, diabetic and obese subjects. New Engl. J. Med., *288:* 487 (1973).
27. Wise, J. K., Santokh, S. L., Hendler, R., and Felig, P.: Evidence of stimulation of glucagon secretion by alanine in the human fetus at term. J. Clin. Endocrinol. Metab., *37:* 345 (1973).
28. FBA Pharmaceutical, New York; 0.1 ml = 1,000 kallikrein units.
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Hemoglobin Barts
neonate
α-thalassemia

α-Thalassemia in Negro Infants

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Extract

A total of 104 of 693 Negro infants (15.0%) had moderate or small amounts of hemoglobin Barts visible on starch gel electrophoresis. Moderate amounts were found in 21 infants (3.0%) and small amounts in 83 infants (12.0%). In 17 Negro infants judged to have moderate amounts of Hb Barts, the quantitation showed 2.0–9.3% with a mean of 5.4 ± 2.1 (1 SD). A significant decrease in mean cell volume and mean cell hemoglobin was found in the Negro neonate with more than 2% Hb Barts studied at 4 days of age. In 10 Negro infants with more than 2.0% Hb Barts studied at 4 days of age, the $\alpha/(\beta + \gamma)$ ratio was 0.97 ± 0.06 (1 SD) (range 0.88–1.06). In nine infants aged 5–24 months who had more than 2.0% Hb Barts in the newborn period, including six infants studied in the first group, the mean α/β ratio was 0.74 ± 0.06, (range 0.65–0.83). Each of the nine infants with more than 2.0% Hb Barts at birth had marked microcytosis and hypochromia at 5–24 months despite adequate iron therapy. Two newborn infants with moderate levels of Hb Barts at birth (8.2% and 6.8%) and balanced total globin synthesis had no free radioactive α chain by gel filtration studies. Our studies indicate clearly that the presence of more than 2% Hb Barts in the newborn period denotes the presence of α-thalassemia trait.

Speculation

In a group of Negro newborn infants, 3% had more than 2% Hb Barts. These infants had the genetic disorder, α-thalassemia trait. An additional 12% of the infants had elevated levels of Hb Barts between 1% and 2%. This group may also have an α-thalassemia disorder, as has been shown in other racial groups. The absence of hydrops fetalis due to α-thalassemia in Negro neonates suggests that the molecular defect of α-thalassemia detected in Negro neonates differs from that seen in Orientals in that it is not associated with a complete absence of α-chain synthesis.

α-Thalassemia is a common genetic disorder of hemoglobin synthesis which occurs in many ethnic groups, including Chinese (18), Thai (23, 34), Italians (18), and American Negroes (27). Extensive population studies in Thailand have shown a correlation between the α-thalassemia syndromes and the amount of Hb Barts present in the neonatal period (22, 23, 33, 34). Hb Barts is a tetramer of the γ-globin chain (γ_4), a polypeptide which is found in association with α chain in fetal hemoglobin (Hb F-$\alpha_2\gamma_2$).

The significance of Hb Barts in Negro neonates is controversial. Weatherall (35) has claimed that the presence of

5–10% Hb Barts in Negro neonates indicates inherited α-thalassemia, while Esan (6) has suggested that this finding is a nonthalassemic developmental abnormality. We have performed a prospective evaluation of a large group of Negro infants using both hematologic and globin synthesis studies to investigate the significance of Hb Barts in the newborn period (7). Our studies indicate clearly that the presence of more than 2% Hb Barts in the neonatal period denotes the presence of α-thalassemia trait. This conclusion is supported by erythrocyte indices at birth and later in childhood, by globin synthesis studies, and by measurements of free α chain pools in the neonatal period.

METHODS

DETECTION OF HB BARTS

Capillary blood was obtained by heel puncture from 693 Negro and 110 Caucasian full term newborn infants from November 1970 to February 1972. One heparinized microhematocrit tube was filled with blood, and the blood was transferred to a test tube, 10 X 75 mm. The erythrocytes were washed two times with 0.85% saline, hemolyzed with 2.0 ml distilled water, and centrifuged at 700 X *g* for 5 min. The supernatant hemolysate was standardized by appropriate dilution and analyzed by vertical descending starch gel electrophoresis at pH 8.6 (30), with 10–18 samples from neonates on each gel. Benzidine-stained gels were visually examined for Hb Barts, which was recorded as being present in moderate, small, or trace amounts, or as being absent. The gels were always examined by one of two observers within 15–30 min after staining, when the Hb A_2 band in the normal control was clearly visible. Moderate amounts of Hb Barts appeared to be equal or greater than the amount of Hb A_2 in the normal adult control. In most patients with moderate amounts and in many with lesser amounts of Hb Barts, the percentage of Hb Barts was determined by chromatography of hemolysate on carboxymethyl Sephadex at pH 6.6 (14). The rapidly eluting hemoglobin was Hb Barts. The identity of this fraction was confirmed in several patients with moderate or small amounts of Hb Barts by urea-gel electrophoresis (9) and by "fingerprinting" of a tryptic digest (2).

HEMATOLOGIC STUDIES

Hematologic studies were done by standard methods (4). Hb A_2 levels were determined by starch granule electrophoresis (10). Hb F levels were determined by alkali denaturation (29).

MEASUREMENTS OF GLOBIN CHAIN SYNTHESIS

Globin synthesis was studied by methods described previously (5, 26). Peripheral blood was incubated with (^{14}C)-leucine for 2 hr, the α, β, and γ globin chains were separated by chromatography on carboxymethyl cellulose at pH 6.5 in 8 M urea with a sodium phosphate gradient, and the radioactivity and optical density of each tube were determined. In the newborn infants, relative synthesis of globin chains was expressed as the $\alpha/(\beta + \gamma)$ ratio of radioactivities. In the older infants and adults, synthesis was expressed as the α/β ratio of specific activities. In this latter group, where small amounts of radioactivity are incorporated into globin, the use of ratios of specific activities determined at the heights of each peak yields accurate and reproducible results (17, 26).

STUDIES OF FREE α CHAIN POOL

Peripheral blood tagged with (^{14}C)leucine was also analyzed by gel filtration chromatography (11). Three newborn infants with no visible Hb Barts in the newborn period and two with moderate amounts of Hb Barts were studied. One-milliliter aliquots of peripheral blood hemolysate were applied immediately after incubation to Sephadex G-100 columns kept at 4°. Two-milliliter fractions were eluted with a phosphate buffer, 0.1 M, pH 7.0, at a rate of 3 ml/hr. Radioactivity of a 0.2-ml sample of each fraction was determined in a liquid scintillation counter. The largest peak of radioactivity coincided with the hemoglobin peak. In control infants a small peak of radioactivity eluted immediately after the hemoglobin peak. The tubes comprising the major hemoglobin peak and the smaller second peak were pooled separately. Globin was prepared from each peak and was separated by carboxymethyl cellulose chromatography as described above. The relative size of the free α chain pool was calculated from α chain radioactivities in the separated peaks (11).

RESULTS

DETECTION OF HB BARTS

A total of 104 of the 693 Negro infants (15.0%) had moderate or small amounts of Hb Barts visible on starch gel electrophoresis. Moderate amounts were found in 21 infants (3.0%) and small amounts in 83 infants (12.0%). In addition, 187 infants had trace amounts visible. A total of 5 of the 110 Caucasian infants (4.5%) had moderate or small amounts of Hb Barts on electrophoresis. Moderate amounts were found in 2 infants (1.8%) and small amounts in 3 infants (2.7%). In addition, trace amounts were seen in 19 infants. One of the Caucasian infants with moderate amounts of Hb Barts was of Italian extraction and the other was an Ashkenazic Jew. Newborn infants with elevated levels of Hb Barts have been reported previously from both of these ethnic groups (3, 12).

Visual estimation of Hb Barts on starch gel as well as quantitation of Hb Barts by chromatography was performed on hemolysates of venous blood drawn from 38 infants including all infants on whom globin synthesis studies were done. The results (Fig. 1) indicate that visual estimation of Hb Barts from a benzidine-stained starch gel can distinguish grossly between different levels of the hemoglobin. In 17 Negro infants and 1 Italian infant judged to have moderate amounts of Hb Barts, the quantitation showed 2.0–9.3%, with a mean of 5.4 ± 2.1 (1 SD). In 11 Negro infants and 1 Chinese with small amounts there was 1.1–1.7% Hb Barts with a mean of 1.4% ± 0.2 (1 SD). The quantitative amounts of Hb Barts did not differ between the group judged to have trace amounts (0.3–0.9%) and the group without visible Hb Barts (0.2–0.9%). The group with trace amounts was therefore considered to be part of the normal group. None of the infants

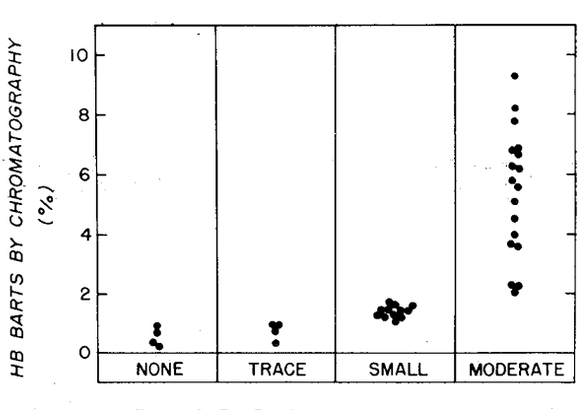

Fig. 1. Comparison of the visual estimation of Hb Barts by starch gel electrophoresis and quantitation by chromatography on carboxymethyl-50 Sephadex. Each point represents a different newborn infant.

Table 1. *Hematologic values in newborn period*

Hematologic value	Hb Barts >2.0%	Absent Hb Barts	*P*
Hemoglobin (g/100 ml)	16.3 ±1.4 (1 SD)	18.3 ±3.3	>0.05
Hematocrit (%)	51.9 ±4.2	58.1 ±10.3	>0.05
Erythrocytes (× 10^{-6}/mm^3)	5.2 ±0.4	5.1 ±0.9	>0.05
Reticulocytes (%)	9.2 ±2.2	9.7 ±3.4	>0.05
Mean cell volume (μm^3)	99.0 ±5.6	111.1 ±10.4	<0.01
Mean cell hemoglobin (pg)	31.0 ±2.3	35.7 ±3.2	<0.01
Mean cell hemoglobin concentration (%)	31.4 ±1.0	31.5 ±1.0	>0.05

with moderate amounts of Hb Barts (greater than 2.0%) had Hb S, Hb C, or another abnormal hemoglobin. The hematologic and globin synthesis studies described below were limited to infants with greater than 2% Hb Barts and to control subjects without visible Hb Barts.

HEMATOLOGIC STUDIES

Hamatologic values were determined on 10 infants with greater than 2.0% Hb Barts and on 10 normal Negro neonates without visible Hb Barts (Table 1). Each infant was studied at 2–4 days of age. Only mean cell volume (MCV) and mean cell hemoglobin (MCH) differed significantly between the two groups. In general, there was a greater degree of poikilocytosis and anisocytosis seen in the peripheral blood smears of the infants with moderate levels of Hb Barts.

GLOBIN SYNTHESIS STUDIES AND SUBSEQUENT HEMATOLOGIC STUDIES

The results of the studies of globin synthesis are shown in Table 2. In 10 Negro infants with more than 2.0% Hb Barts studied at 4 days of age, the $\alpha/(\beta+\gamma)$ ratio was 0.97 ± 0.06 (1 SD) (range 0.88–1.06). A representative chromatogram is shown in Figure 2. Four newborn infants without visible Hb Barts had $\alpha/(\beta+\gamma)$ ratios of 1.01, 1.02, 1.13, and 1.14. The last two infants had sickle cell trait and an abnormal γ chain, respectively. In nine infants aged 5–24 months who had more than 2.0% Hb Barts in the newborn period, including six infants studied in the first group, the mean α/β ratio was 0.74 ± 0.06 (range 0.65–0.83). Hb Barts and Hb H were not found at the time of these studies. Each of these infants had an adequate trial of oral therapy with ferrous sulfate before these studies. Three infants who did not have visible Hb Barts in the newborn period had α/β ratios of 0.85, 0.87, and 0.88 at 1–2 years of age. We have been able to find reported studies of globin synthesis on only two other normal infants less than 2 years old and past the newborn period. A 5-month old had an α/β ratio of approximately 0.98, and a 2-year old had a "normal" ratio (8, 15).

Each of the nine infants with more than 2.0% Hb Barts at birth had marked microcytosis and hypochromia at 5–24 months despite adequate iron therapy. The means of the hematologic values of these infants were hemoglobin concentration of 10.9 g/100 ml (9.6–12.5), MCV of 63.3 μm^3 (54.9–70.0), and MCH of 20.4 pg (16.3–22.5). None of the infants had a high Hb A_2 level.

One infant with 7.8% Hb Barts at birth was studied when she was 3 days, 3 weeks, 11 weeks, and 7 months of age, with $\alpha/(\beta+\gamma)$ or α/β ratios of 1.02, 9.52, 0.57, and 0.72, respectively. Another infant, a male, with 6.2% at birth was studied at 4 days, 6 months and 9 months of age with ratios of 0.89, 0.83, and 0.71, respectively. At 6 months of age, after adequate iron administration, this boy's hemoglobin was 9.9 g/100 ml, MCV 69.4 μm^3, and MCH 20.6 pg.

A nonthalassemic adult control group had a mean α/β ratio of 1.01 ± 0.05 (1 SD). A group of 14 adult Negro patients with heterozygous α-thalassemia previously studied in our laboratory (27) had a mean α/β ratio of 0.84 ± 0.07 (1 SD). Chinese and Italian patients with α-thalassemia trait had a mean α/β ratio of 0.77 ± 0.04 and the silent carriers had a mean ratio of 0.87 ± 0.06 (18).

Table 2. *Globin synthesis studies*

	Neonates		
Hb Barts at birth	$\alpha/(\beta+\gamma)$	α Chain pool, %	Older infants, α/β
>2%	0.97 ± 0.06 (1 SD)	Absent	0.74 ± 0.06
None	1.07 (1.01–1.14)	5.0 (3.7–6.3)	0.87 (0.83–0.88)

Globin synthesis studies were performed on five mothers of children with greater than 2.0% Hb Barts. The fathers were not readily available for these studies. The mean α/β ratio in this group was 0.77 (0.72–0.85). Four of the mothers had normal erythrocyte indices, while one mother had a MCV of 80.7 μm^3, MCH of 23.0 pg, and an α/β ratio of 0.80. Her serum iron and iron-binding capacity were normal. Each mother had normal percentages of Hb A_2 and Hb F.

STUDIES OF FREE α CHAIN POOL

Peripheral blood from three control newborn infants who had no visible Hb Barts in the newborn period were analyzed by gel filtration chromatography. A small radioactive peak eluted after the hemoglobin peak in each study. The hemoglobin peak in each study contained radioactivity associated with γ, β, and α chains, whereas the smaller peak contained almost entirely α chain radioactivity. The $\alpha/(\beta+\gamma)$ ratio of total radioactivity comprising the hemoglobin peak and the smaller subsequent peak was close to one in the three normal neonates. The newly synthesized free radioactive α-chains comprised 5.7, 6.3, and 3.7% of the total α chains in the three neonates without Hb Barts. These values are similar to those found in bone marrow of three adult control subjects whose mean was 6.2% (3.7–9.8%) (11). Two newborn infants with moderate levels of Hb Barts at birth (8.2% and 6.8%) and balanced total globin synthesis had no free radioactive α chain by gel filtration studies. Two adults with inherited Hb H disease (Negro, Chinese) and one with acquired Hb H disease also lacked any free radioactive α chain in similar studies of peripheral blood.

DISCUSSION

Four major α-thalassemia syndromes have been identified in oriental populations: hydrops fetalis due to homozygous α-thalassemia, Hb H disease, α-thalassemia trait, and the silent carrier. In this population group the percentage of Hb Barts in hemolysates from newborn infants indicates the type of α-thalassemia present. In the silent carrier there is approximately 1–2% Hb Barts, in α-thalassemia trait 5–6%, in Hb H disease 25%, and in hydrops fetalis more than 80% (23, 34). In the heterozygous conditions (α-thalassemia trait or the silent

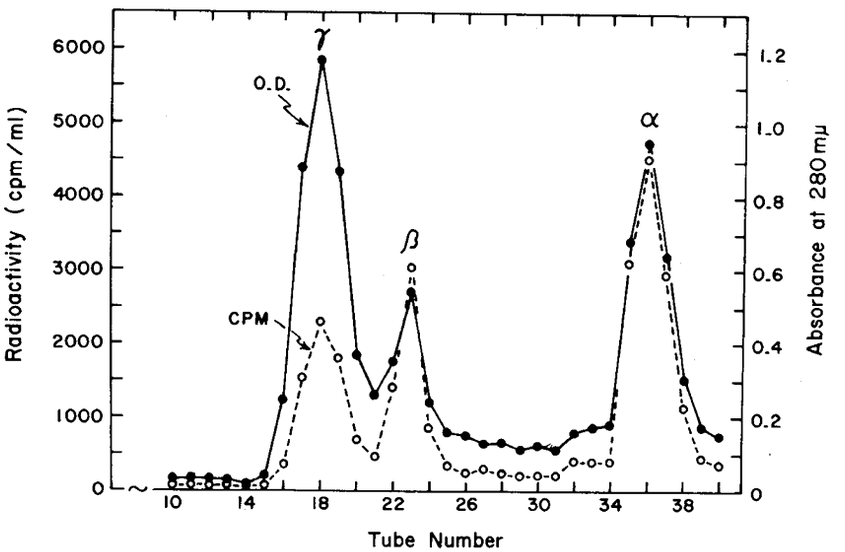

Fig. 2. Chromatogram of globin from a newborn Negro infant with 6.7% hemoglobin Barts. The $\alpha/(\beta+\gamma)$ ratio is 0.95.

carrier) the excess of γ chains disappears by 4–6 months of age and is not replaced by equivalent amounts of Hb H (β_4).

Several previous population studies have detected Hb Barts in Negro neonates. Studies in the United States in St. Louis (19), Atlanta (21), Baltimore (35), and Galveston (25) have found Hb Barts in 2.0–30.0% of Negro infants. Studies of newborn Africans in Nigeria (6) and the Congo (32) found 5.1% and 17.9% of infants with Hb Barts. The techniques used in these studies were of varying sensitivity, and precise quantitation of Hb Barts was only occasionally performed (35). The correlation of levels of Hb Barts in neonates with complete hematologic and globin synthesis studies has not been investigated previously.

The present study is limited to the group of infants with more than 2.0% Hb Barts, comparable with the Thai group with α-thalassemia trait. A significant decrease in MCV and MCH was found in the Negro neonates, while other hematologic variables were within normal limits. The mean hemoglobin level and hematocrit were also decreased in this group, but the difference was not statistically significant for the number of infants studied. A recent study has demonstrated similar hematologic abnormalities by showing that six of nine Negro neonates with low MCV and MCH values had Hb Barts levels above 2% in the newborn period (24).

Globin synthesis ratios were within normal limits in the newborn period in all the infants studied. Globin synthesis studies have not been reported for newborns of other racial groups with genetically proven α-thalassemia. The one Italian newborn with 3.7% Hb Barts in our study had an $\alpha/(\beta+\gamma)$ ratio of 0.89, and one Chinese infant, the son of a patient with Hb H disease, had 1.3% Hb Barts and a ratio of 1.05. The radioactivity ratios of the 2 Caucasian neonates with α-thalassemia trait are similar to the $\alpha/(\beta+\gamma)$ ratios determined in two groups of 12 and 3 nonthalassemic Caucasian neonates studied by other workers (8, 15). Balanced globin synthesis in the newborn period in heterozygous α-thalassemia may be a common finding, perhaps related to the major readjustments occurring with the switchover from fetal to adult hemoglobin synthesis. In β-thalassemia trait, where the globin synthesis defect is greater (28), the disorder may be detected in the neonate by analysis of specific activity ratios (16).

Restudy of the infants at 5–24 months showed that all had deficient α chain synthesis, in the range of α-thalassemia trait. There was no overlap in globin synthesis ratios between these infants and the control groups. Each infant had mild anemia, decreased MCV and MCH, and significant abnormalities of erythrocyte morphology which persisted despite iron administration. The two children with several repeated studies showed continued abnormalities of erythrocyte morphology and globin synthesis. The α/β ratio was decreased in the mothers who were studied, despite normal erythrocyte indices in four of the five. In an earlier study of α-thalassemia in the adult Negro in which heterozygotes were identified by genetic relations, several carriers had normal erythrocytes indices (27). One family which had been followed for many years (1, 27) had two children with high Hb Barts in the newborn period, but who had normal erythrocyte indices and synthesis ratios at 9 and 12 years of age. It will be of interest to see whether the children in the present study will develop more nearly normal values as they grow older.

Israeli workers have studied a large group of Yemenite and Iraqi Jewish children at 1–6 years of age who had 1–3% Hb Barts (12 children) or 5–6% Hb Barts at birth (9 children) and found that both groups had deficient α chain synthesis (36). The mean globin synthesis ratio of the group with 5–6% Hb Barts was 0.64 ± 0.05 and that of the group with lesser amounts Hb Barts was 0.76 ± 0.08, both values overlapping the range of values determined in the Negro children with more than 2% Hb Barts we have studied. A large group of Negro neonates (12.0%) had 1–2% Hb Barts, corresponding to the Thai "silent carrier" group. Further studies will be needed to determine whether this level of Hb Barts represents a form of α-thalassemia in the Negro.

Measurement of free radioactive α chains by gel filtration chromatography combined with globin chain separation is a sensitive method of detecting variations in the free α-chain pool in different thalassemia syndromes. A small pool of free α chains is present in human peripheral blood (13, 20, 31) and in bone marrows of adults with no known thalassemia defects (11). We found a similar small pool of free α chains in the peripheral blood of three newborn infants without detectable Hb Barts. In contrast, a large percentage of total α chain radioactivity is present in the free α-chain pools of all subjects with heterozygous and homozygous β-thalassemia, including sickle β-thalassemia, Hb Lepore-β-thalassemia, Hb Lepore trait, $\alpha\beta$-thalassemia, β-thalassemia trait, and Cooley's anemia (11). A patient with $\alpha\beta$-thalassemia had 14.1% of α chain radioactivity in the free α chain pool, a value intermediate between that of control subjects (6.2%) and patients with β-thalassemia trait (35.9%). This finding shows the modifying effect of α-thalassemia on the free α chain pool (11). In the present study, two newborn infants with moderate levels of Hb Barts lacked free α chains in their peripheral blood despite balanced globin synthesis. There were also no free α chains

detected in three adults with Hb H disease and decreased α/β ratios. The measurement of α chain pools in bone marrow cells in older children and adults may provide a sensitive means for the diagnosis of α-thalassemia. The lack of free α chains in the neonates confirms the hematologic and globin synthesis data presented here showing that Negro neonates with greater than 2% Hb Barts have α-thalassemia.

SUMMARY

Hemoglobin Barts in the neonate is associated in many ethnic groups with α-thalassemia. Its significance in Negro neonates has been controversial. In 693 Negro neonates we found 21 infants (3.0%) with Hb Barts greater than 2% of the total hemoglobin. These infants had decreased MCV and MCH levels in both the newborn period and at 5–24 months of age despite iron therapy.

Despite balanced globin synthesis in all neonates studied including those with elevated levels of Hb Barts, those with elevated levels of Hb Barts lacked a free radioactive α chain pool, while those without Hb Barts had normal α chain pools.

Nine neonates with more than 2% Hb Barts at birth who were studied at 5–24 months, and several of their mothers had decreased α/β synthesis ratios, which indicated the presence of a persistent defect in α chain synthesis. These hematologic data, globin synthesis ratios, and α chain pool studies indicate clearly that Negro infants with greater than 2% Hb Barts have α-thalassemia.

REFERENCES AND NOTES

1. Atwater, J., Schwartz, I. R., Erslev, A. J., Montgomery, T. L., and Tocantins, L. M.: Sickling of erythrocytes in a patient with thalassemia-hemoglobin-I disease. New Engl. J. Med., *263:* 1215 (1960).
2. Baglioni, C.: The fusion of two peptide chains in hemoglobin Lepore and its interpretation as a genetic deletion. Proc. Nat. Acad. Sci. U. S. A., *48:* 1880 (1962).
3. Bianco, I., Graziani, B., Salvini, P., Mastromonaco, I., and Silvestroni, E.: Frequence et caractéres de l'alpha–microcytémie dans les populations de la Sardaigne Septentrionale. Nouv. Rev. Franc. Hématol., *12:* 191 (1972).
4. Cartwright, G. E.: Diagnostic Laboratory Hematology (Grune & Stratton, Inc., New York, 1963).
5. Clegg, J. B., Naughton, M. A., and Weatherall, D. J.: An improved method for the characterization of human hemoglobin mutants: Identification of $\alpha_2 \beta_2{}^{95}$GLU, hemoglobin N (Baltimore). Nature, *207:* 945 (1965).
6. Esan, F. G. J.: Hemoglobin Barts in newborn Nigerians. Brit. J. Haematol., *22:* 73 (1972).
7. Friedman, S., Atwater, J., and Schwartz, E.: Hemoglobin Barts and alpha thalassemia in the Negro newborn. Pediat. Res., *6:* 366 (1972).
8. Gaburro, D., Volpato, S., and Vigi, V.: Diagnosis of beta-thalassemia in the newborn by means of hemoglobin synthesis. Acta Paediat. Scand., *59:* 523 (1970).
9. Garrick, M. G., Balzer, R. H., Jr., and Carlton, J. P.: An improved method for electrophoretic characterization of globin chains from hemolysates, purified hemoglobins and fractions selected from chromatographic separations of chains. Anal. Biochem., *34:* 312 (1970).
10. Gerald, P. S., and Diamond, L. K.: The diagnosis of thalassemia trait by starch block electrophoresis of the hemoglobin. Blood, *13:* 61 (1958).
11. Gill, F. M., and Schwartz, E.: Free α-globin pool in human bone marrow. J. Clin. Invest., *52:* 3057 (1973).
12. Goldschmidt, E., Cohen, T., Isacsohn, M., and Freier, S.: Incidence of hemoglobin Barts in a sample of newborn from Israel. Acta. Genet. Basel, *18:* 361 (1968).
13. Huehns, E. R., and Modell, C. B.: Hemoglobin synthesis in thalassemia. Trans. Roy. Soc. Trop. Med. Hyg., *61:* 157 (1967).
14. Huisman, T. H. J.: Human hemoglobins. In: J. J. Yunis: Biochemical methods in red cell genetics, pp. 455–471 (Academic Press, New York, 1969).
15. Kan, Y. W., Forget, B. G., and Nathan, D. G.: Gamma-beta thalassemia: A cause of hemolytic disease of the newborn. New Engl. J. Med., *286:* 129 (1972).
16. Kan, Y. W., and Nathan, D. G.: Beta thalassemia trait: Detection at birth. Science, *161:* 589 (1968).
17. Kan, Y. W., and Nathan, D. G.: Mild thalassemia: The result of interactions of alpha and beta thalassemia genes. J. Clin. Invest., *49:* 635 (1970).
18. Kan, Y. W., Schwartz, E., and Nathan, D. G.: Globin chain synthesis in the alpha thalassemia syndromes. J. Clin. Invest., *47:* 2515 (1969).
19. Minnich, V., Cordonnier, J. J., William, W. K., and Moore, C. V.: Alpha, beta, and gamma hemoglobin polypeptide chains during the neonatal period with a description of a fetal form of hemoglobin Dα St. Louis. Blood, *19:* 137 (1962).
20. Modell, C. B., Latter, A., Steadman, J. H., and Huehns, E. R.: Hemoglobin synthesis in beta thalassemia. Brit. J. Haematol., *17:* 485 (1969).
21. Morton, B. F., Thompson, R. D., Cozy, A. M., Nechtman, C. M., Nichols, E., and Huisman, T. H. J.: Inhomogeneity of hemoglobin. VI. The minor hemoglobin components of cord blood. Blood, *20:* 302 (1962).
22. Na-Nakorn, S., and Wasi, P.: Alpha thalassemia in northern Thailand. Amer. J. Hum. Genet., *22:* 645 (1970).
23. Na-Nakorn, S., Wasi, P., Pornpatkul, M., and Pootrakul, S.: Further evidence for a genetic basis of hemoglobin H disease from newborn offspring of patients. Nature, *223:* 59 (1969).
24. Schmaier, A. H., Maurer, H. M., Johnston, C. L., and Scott, R. B.: Alpha thalassemia in neonates by mean corpuscular volume and mean corpuscular hemoglobin determination. J. Pediat., *83:* 794 (1973).
25. Schneider, R. G., Haggard, M. F., and Gustavson, L. P.: Hemoglobin Barts in newborns with adult genotypes AA, AS and AC. Blood, *28:* 38 (1971).
26. Schwartz, E.: The silent carrier of beta thalassemia. New Engl. J. Med., *281:* 1327 (1972).
27. Schwartz, E., and Atwater, J.: Alpha thalassemia in the American Negro. J. Clin. Invest., *51:* 412 (1972).
28. Schwartz, E., Kan, Y. W., and Nathan, D. G.: Globin chain synthesis in the alpha-thalassemia heterozygotes. Ann. N.Y. Acad. Sci., *165:* 288 (1968).
29. Singer, K., Chernoff, A. I., and Singer, L.: Studies on abnormal hemoglobins. II. Their identification by means of the method of fractional denaturation. Blood, *6:* 429 (1951).
30. Smithies, O.: An improved procedure for starch gel electrophoresis: Further variations in the serum proteins of normal individuals. Biochem. J., *71:* 585 (1959).
31. Tavill, A. S., Grayzel, A. I., London, I. M., Williams, M. K., and Vanderhoff, G. A.: The role of heme in the synthesis and assembly of hemoglobin. J. Biol. Chem., *243:* 4987 (1968).
32. Van Baelen, H., Vandepitte, J., and Cornu, G.: Routine detection of sickle cell anemia and Hemoglobin Barts in Congolese neonates. Trop. Geograph. Med., *21:* 412 (1969).
33. Wasi, P., Na-Nakorn, S., Pootrakul, S., Sookanek, M., Disthansongchan, P., Pornpatkul, M., and Panich, V.: Alpha- and beta-thalassemia in Thailand. Ann. N.Y. Acad. Sci., *165:* 60 (1969).
34. Wasi, P., Na-Nakorn, S., and Suingdumrong, A.: Hemoglobin H in Thailand: A genetic study. Nature, *204:* 907 (1964).
35. Weatherall, D. J.: Abnormal hemoglobins in the neonatal period and their relationship to thalassemia. Brit. J. Haematol., *9:* 265 (1963).
36. Zaizov, R., Kirschmann, C., Matoth, Y., and Adam, A.: The genetics of α-thalassemia in Yemenite and Iraqi Jews. Israel J. Med. Sci., *9:* 1457 (1973).
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39. Informed consent was obtained from all adults and the mothers of all infants included in this study.
40. Requests for reprints should be addressed to: Shlomo Friedman, M.D., Children's Hospital of Philadelphia, 1 Children's Center, 34th and Civic Center Blvd., Philadelphia, Pa. 19104 (USA).
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