Alpha-chain Thalassemia and Hydrops Fetalis in Malaya: Report of Five Cases

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A FAST-MOVING hemoglobin component, "Bart's" hemoglobin—probably identical with Fessas and Papaspyrou hemoglobin—has been found by the author to occur in 3.3 per cent Chinese newborn infants in Indonesia. The incidence is much lower in Indonesian newborns, about 0.4 per cent. This hemoglobin disappears during the first months of life, and is therefore not found in adults. "Bart's" hemoglobin has only rarely been found in disease states, and the author has found it in two cases of chronic hemolytic anemia (to be published). Because this hemoglobin is not rare in healthy newborns, it may be questioned why it is not found more often in disease conditions. Assuming that the patients die too early to be detected, we have therefore screened diseased and stillborn babies in an attempt to correlate this hemoglobin with disease and fetal death. A correlation was indeed found between the presence of a large amount of this hemoglobin and a well defined type of clinical picture. Four cases of severe hydrops and erythroblastosis fetalis have been reported in association with "Bart's" hemoglobin from Indonesia.

Studying the incidence of this fast moving hemoglobin in newborns in Malaya, the author, in collaboration with Dr. Ti, found an even higher incidence, 5.1 per cent, of this component in Chinese newborns in Kuala Lumpur. Examining stillborn and diseased fetuses in this area, she again found five cases of severe hydrops and erythroblastosis in association with this hemoglobin.

It has been postulated, that these cases of hydrops fetalis might be instances of homozygous a-chain thalassemia, based on the theory of Ingram and Stretton that in thalassemia, either the a-chain or the b-chain production of hemoglobin might be impaired.

This paper is a clinical and hematologic report on the Malayan cases.

METHOD AND MATERIALS

Routine hematologic examinations were carried out according to standard methods. Paper electrophoretic analysis of hemoglobins was carried out by the method of Smith and Conley, using veronal buffer at pH 8.6, ionic strength 0.06, and phosphate buffer, pH 6.5.

Chromatographic study was done by the method described by Huisman and Jonxis, using ion-exchange resin IRC-50.

Alkali-resistant hemoglobin was examined by the method of Singer, Chernoff and Singer.

Stillborn and diseased fetuses were obtained directly from the labor room of the Obstetric Department of the General Hospital, Kuala Lumpur, or indirectly from the same source by way of the mortuary of the hospital. This survey was carried out from January to July 1961, and then again during the month of October of the same year.

Blood was obtained from the umbilical cord or by heart puncture.
I51, showing severe erythroblastosis, many thin cells and target cells (1100 X).

The hemoglobin of 54 diseased and stillborn babies of different races, most of them Chinese, was studied by the paper electrophoretic method.

Only five cases of hydrops fetalis were found and all were associated with a large amount of "Bart's" hemoglobin. This hemoglobin was not found in other abnormal conditions, nor in stillborn babies of normal appearance. This number does not reflect the true incidence of hydrops among stillborn babies, since most of the babies stillborn from known causes were not examined and the author was usually only called for abnormal looking stillborn fetuses.

Case Reports

Case 1. Fetus Chan was born prematurely on January 2, 1961 at the age of about 32 weeks. It was a male fetus with severe generalized hydrops, enlarged liver and spleen, and ascites. It looked pale and died immediately after birth. The placenta was large and friable. Hematologic study of blood from the umbilical cord gave the following data: Hb 6.4 Gm. per cent, RBC 2.53 million/cu. mm., PCV 40.5 per cent MCV 160 cu. µ, MCH 25.3 µg., MCHC 15.8 per cent, nucleated cells 121,400 cu. mm., reticulocytes 16.4 per cent. There was severe erythroblastosis of the blood (fig. 1). The cells were very thin, and there were numerous target cells. When a drop of blood was left on a slide under a coverslip, the red blood cells showed sickling after a few hours at room temperature (fig. 2). In brilliant cresyl blue, several red blood cells showed intracellular crystals. Hemoglobin analysis showed the presence of a large amount of Hb "Bart's" (fig. 3). A small amount of what seemed to be Hb A on electrophoresis at pH 8.6, and a trace of Hb H were also present. Alkali-resistant hemoglobin was 70.2 per cent; serum bilirubin direct 0.4 mg. per cent, indirect 1.8 mg. per cent. The Kahn reaction was negative. Blood group was O, Rh+.
Fig. 2.—Wet unstained preparation of the blood of Case 1, showing sickling of the red blood cells. No Hb S was detectable in the hemolysate (1100 X).

Direct Coombs’ test was negative. Postmortem examination showed numerous foci of extramedullary erythropoiesis in liver, spleen and placenta.

The mother, a pure Chinese woman of 39 years, had hypertension when she came in, with possible toxemia of pregnancy. There was no splenic or liver enlargement. Hematologic study showed: Hb 11.5 Gm. per cent, RBC 5.49 million cu. mm., PCV 38.0 per cent, MCV 69.2 cu. μ, MCH 20.9 μg., MCHC 30.2 per cent. WBC and reticulocytes were not increased. Kahn reaction was negative. Blood group O, Rh+ . Coombs’ test direct negative, indirect not done.

The father, a pure Chinese, was healthy and physically normal. He had no spleen or liver enlargement. Hematologic study showed: Hb 12.4 Gm. per cent, RBC 5.15 million cu. mm., PCV 40.5 per cent, MCV 78.6 cu. μ, MCH 24.1 μg., MCHC 30.6 per cent, WBC 9400 cu. mm., reticulocytes 0.6 per cent.

Fragility of erythrocytes to hypotonic saline solutions slightly decreased, initial at 0.48 per cent, complete at 0.24 per cent (control at 0.44 per cent and 0.28 per cent, respectively).

The first child in the family, a girl of 8 years, was healthy and physically normal. Hb was 12.8 Gm. per cent, RBC 5.40 million cu. mm., PCV 37.0 per cent, MCV 68.5 cu. μ, MCH 23.7 μg., MCHC 34.6 per cent. Fragility test: initial at 0.48 per cent, complete at 0.28 per cent.

Hemoglobin analysis of the blood of the mother, father and sister revealed only Hb A (fig. 3); there was no abnormal hemoglobin. The alkali-resistant hemoglobin was within normal limits, and the amount of Hb A2, judged from paper electrophoretic studies was not increased. The second child in the family, a boy of about 5 years, was living and healthy, but could not be examined.

Case 2. Fetus L. H. was stillborn on February 29, 1961 at the age of about 32 weeks. It was a male fetus with slight generalized hydrops, ascites and hepatosplenomegaly—the liver was especially enlarged (fig. 4). The placenta was large, edematous, pale and friable.
Blood was obtained by heart puncture, but since it was obtained after death, the hematologic indices are probably unreliable. Hematologic study showed: Hb 9.4 Gm. per cent, RBC 4.42 million/cu. mm., PCV 68.0 per cent, MCV 153.8 cu. µ, MCH 21.3 µg., MCHC 13.8 per cent, nucleated cells 123,400/cu. mm., reticulocytes 10.4 per cent. Many red blood cells showed intracellular crystals in brilliant cresyl blue. There was enormous erythroblastosis of the blood. Many thin cells and target cells were seen. A wet blood preparation showed sickling of the erythrocytes when left at room temperature for several hours. Analysis showed the hemoglobin to consist almost entirely of Hb "Bart's." Hb H was thought to be present on examination by paper electrophoresis, but could not be demonstrated by the chromatographic method—probably the amount of Hb H was too small. Alkali resistant hemoglobin was 57.2 per cent. Serum bilirubin direct negative, indirect 2.8 mg. per cent. Kahn reaction was negative. Blood group was A, Rh+. Direct Coombs' test was negative.

Histologic study of the liver, spleen and placenta showed numerous foci of erythropoiesis. Hemosiderin deposits were seen in the spleen and liver.

The mother was pure Chinese woman, 39 years old. She was slightly anemic when admitted, but otherwise normal, with neither spleen nor liver enlarged. After delivery she was given 2 pints of blood because of her anemic appearance and the following day her blood data were: Hb 8.5 Gm. per cent, RBC 3.73 million/cu. mm., PCV 28.0 per cent, MCV 75.1 cu. µ, MCH 22.8 µg., MCHC 30.4 per cent, reticulocytes 2.0 per cent, WBC 14,700 per cu. mm. Hemoglobin analysis showed only normal adult Hb A. There was no increase of alkali-resistant hemoglobin. There was no increase of Hb A₂ on paper electrophoresis. Blood group was A, Rh+, direct and indirect Coombs' tests were negative.

The father and seven other children in the family could not be examined, but were said to be healthy.
Case 3. Fetus A. L. was stillborn by breech delivery on March 22, 1961 at the age of about 30 weeks. It was a female fetus with edema in the face and legs, while the abdomen was protuberant. The liver was much enlarged, the spleen only slightly so. There was some icteric fluid in the abdominal cavity. The placenta was not available for examination.

Blood taken from the heart gave the following findings: Hb 11.1 Gm. per cent, RBC 4.88 million/cu. mm., PCV 84.0 per cent, MCV 172.1 cu. μ, MCH 17.2 μg., MCHC 13.2 per cent, nucleated blood cells 195,000/cu. mm., reticulocytes 17.8 per cent. There was poikilo- and anisocytosis of the red blood cells. Many thin and target cells were seen. Erythroblasts were numerous. Wet blood preparations showed sickling of the erythrocytes when left at room temperature for several hours.

Hemoglobin analysis showed a large amount of Hb "Bart's," and probably a small amount of Hb H and Hb A. Dr. T. H. J. Huisman, who examined the fast-moving component by CMC-chromatography, alkali denaturation, spectral analysis, starch gel electrophoresis and hybridization experiments, found it indeed to be Hb "Bart's." Alkali resistant hemoglobin was 60.4 per cent. Serum bilirubin was not done because of slight hemolysis.

Blood group was O, Rh+, and direct Coombs' test negative. Extramedullary erythropoiesis was found in the liver and spleen, with hemosiderin deposits chiefly in the liver. The mother, a pure Chinese of 19 years, was anemic, with edema of the legs. There was no fever. Her spleen and liver were not enlarged. Hematologic study showed: Hb 8.5 Gm. per cent, RBC 4.34 million/cu. mm., PCV 30.5 per cent, MCV 70.3 cu. μ, MCH 19.6 μg., MCHC 27.9 per cent. Reticulocytes 1.3 per cent, WBC normal. Hemoglobin analysis: only Hb A; alkali-resistant hemoglobin 0.4 per cent. Hb A₂ was not increased on paper electrophoresis. Serum bilirubin was not increased. Kahn reaction was negative. Blood group was O, Rh+. Coombs' tests were direct and indirect negative. Two weeks later the mother was examined again; Hb 9.5 Gm. per cent, RBC 4.36 million/cu. mm., PCV 30.5 per cent. There was increased resistance of the red blood cells to hypotonic saline solutions.

The father was a pure Chinese, 25 years old, who looked healthy and was physically normal. Hematologic study showed: Hb 14.1 Gm. per cent, RBC 7.06 million/cu. mm., PCV 48.0 per cent, MCV 68.0 cu. μ, MCH 20.0 μg., MCHC 29.4 per cent. There was increased resistance of red blood cells to hypotonic saline solutions. Hemoglobin analysis: only Hb A; alkali resistant hemoglobin 0.7 per cent. Hb A₂ component was not increased. Blood group was B, Rh+. There were no other children in the family.

Case 4. Fetus Fong was stillborn on May 29, 1961 at the age of about 28 weeks with gen-
eralized hydrops and an enormous ascites—the abdomen was like a balloon (fig. 5). The weight of the female fetus, including ascites fluid, was 1,545 Gm., while the yellow icteric ascites fluid weighed 750 Gm. The placenta was relatively large and friable, weighing 762 Gm. The liver was much enlarged, weighing 111 Gm., while the spleen was only 3.6 Gm. Hematologic examination could not be carried out properly, because the blood obtained by heart puncture showed small clots and slight hemolysis. A stained blood smear, however, showed numerous nucleated red blood cells, poikilo- and anisocytosis, polychromasia, and many thin and target cells. A drop of blood left on a slide under a coverslip showed sickling of the erythrocytes after several hours. The serum was very yellow, with a reddish tint because of slight hemolysis. Hemoglobin analysis showed a large amount of Hb "Bart’s," with a trace of a hemoglobin pigment having the mobility of adult Hb A on paper electrophoresis at pH 8.6. Blood group: O, Rh+. Direct Coombs’ test was negative.

Postmortem examination showed many cells of the liver, kidney and spleen to be autolyzed, but the presence of extensive extramedullary erythropoiesis could still be observed.

The mother was a pure Chinese woman from Canton, 23 years old. She was anemic, and thought to have toxemia on admission. Her spleen and liver were not enlarged. Hemoglobin was 7.4 Gm. per cent, RBC 3.72 million cu. mm., PCV 27.0 per cent, MCV 72.5 cu. μ, MCH 19.9 μg., MCHC 27.4 per cent, WBC 23,400 cu. mm., reticulocytes 2.0 per cent. There was poikilo- and anisocytosis of the peripheral blood. Resistance of the red blood cells to hypotonic saline solutions slightly increased. Hemoglobin analysis: Hb A; alkali-resistant hemoglobin 0.4 per cent. There was no increase of Hb A₂. Bone marrow was normoblastic. Blood group was O, Rh+. Direct and indirect Coombs’ tests were negative.

The father was a pure Chinese from Canton, 26 years old. He was healthy and physically normal. Hematologic study showed: Hb 13.2 Gm. per cent, RBC 6.70 million cu. mm., PCV 45.0 per cent, MCV 67.1 cu. μ, MCH 19.7 μg., MCHC 29.3 per cent, reticulocytes 1 per cent, WBC 7800/cu. mm. There was slight aniso- and poikilocytosis. Erythrocyte resistance to hypotonic saline solutions was slightly increased. Hemoglobin analysis: Hb A; alkali resistant Hb 0.5 per cent. Hb A₂ was not increased. Blood group was A, Rh+. The present baby was her fifth. Her second child was prematurely stillborn and the other three living children could not be examined.
Case 5. Fetus Lam was prematurely stillborn in October 1961 at the age of about 34 weeks. The female fetus showed generalized hydrops and ascites. The liver was much enlarged, the spleen scarcely so.

Blood taken from the heart showed some hemolysis. Hematologic study showed: Hb 10.0 Gm. per cent, RBC 3.30 million/cu. mm., nucleated cells 192,000/cu. mm., consisting mainly of nucleated red blood cells. There was enormous erythroblastosis, many of the erythrocytes being very young. There was poikilo- and anisocytosis, with many thin cells and target cells. A drop of blood left on a slide under a coverslip showed sickling of many red blood cells after several hours. Hemoglobin analysis: the hemoglobin consisted almost entirely of Hb "Bart's." A trace of Hb A was also present. No Hb H could be detected. Alkali-resistant hemoglobin: 57.0 per cent. Blood group was AB, Rh+. Direct Coombs' test was negative.

The mother, a pure Chinese woman 23 years old, was anemic but otherwise physically normal. Her spleen and liver were not enlarged. Hematologic study showed: Hb 7.6 Gm. per cent, RBC 3.45 million/cu. mm., PCV 23.0 per cent. MCV 66.7 μ, MCH 22.0 μg., MCHC 33.0 per cent, WBC 14,500/cu. mm., reticulocytes 1.5 per cent. There was some aniso- and poikilocytosis. Hemoglobin analysis showed only adult Hb A, and no increase of Hb A₂. Alkali resistant hemoglobin was 0.5 per cent. Kahn reaction was negative. Blood group was B, Rh+. Direct Coombs' test was negative, indirect not done. Other members of the family could not be examined.

**DISCUSSION**

The four cases of hydrops fetalis reported from Indonesia²⁴ and the five described in this paper were all of Chinese origin and showed essentially the same picture—generalized hydrops, ascites and hepatic enlargement. The spleen, however, was not always enlarged. In contrast to the finding in hydrops fetalis due to iso-immunization, the spleen was often relatively small or even not at all enlarged. There was severe erythroblastosis of the peripheral blood, with reticulocytosis, target cells and thin cells. In general the MCV in the fetuses was very high. This was probably due to the presence of an enormous number of young nucleated red cells and young reticulocytes. In three of the Indonesian cases and in all five cases described in this paper in which the phenomenon was studied, the red cells showed an interesting sickling phenomenon. As already mentioned,¹⁰ the mechanism of the sickling in these fetuses is different from that due to Hb S. Low oxygen tension seems not to be the precipitating factor in this phenomenon, while the addition of brilliant cresyl blue seems to inhibit sickling. What role it plays in vivo cannot be said yet. In each of the nine cases found to date, there was a large amount of hemoglobin “Bart’s” in the blood. In six, Hb H could also be detected. Postmortem examination showed extramedullary erythropoiesis in many organs. The symptoms found in the fetuses described in this paper indicate a severe hemolytic condition. Although iso-immunization of the mother cannot be entirely ruled out, it is very improbable. All mothers were Rh positive as were the fetuses; as regards the ABO blood groups, in four of the cases (Cases 1, 2, 3 and 4), the fetus was of the same blood group as the mother. In all fetuses the direct Coombs' test was negative, while abnormal hemoglobin production was demonstrated in all five cases. Besides, the appearance of the red blood cells was entirely different from that usually seen in isoimmunization.

As mentioned above, “Bart's” hemoglobin has also been found in low concentration in a proportion of healthy newborn infants without clinical symp-
toms. It has been postulated,\textsuperscript{3} that the difference between the low concentration of this hemoglobin in the healthy newborn and the high concentration in the diseased fetuses, and the absence or presence of clinical symptoms, is based upon the inheritance of one or two abnormal genes. We have also postulated\textsuperscript{4,6} that these cases of hydrops fetalis might be instances of homozygous $\alpha$-chain thalassemia based on the theory of Ingram and Stretton\textsuperscript{11} that in thalassemia either the $\alpha$-chain or $\beta$-chain production of the hemoglobin might be impaired. In homozygous $\alpha$-chain thalassemia, severe impairment of $\alpha$-chain production in the formation of fetal hemoglobin ($Hb \ \alpha_2 \ \gamma_2$), would lead to surplus $\gamma$-chains which would form "Bart's" hemoglobin ($Hb \ \gamma_4$), and impairment of $\alpha$-chain production in the formation of $Hb \ \alpha$ ($Hb \ \alpha_2 \ \beta_2$), would lead to surplus $\beta$-chains which would form $HbH$ ($Hb \ \beta_4$). Since in the fetal and neonatal period, $Hb \ \mathrm{F}$ is usually the predominant type of hemoglobin produced, severe impairment of $\alpha$-chain production in this period would lead to a large surplus of $\gamma$-chains and therefore to a large amount of $Hb$ "Bart's." Only a small amount of $Hb \ \mathrm{H}$ would be expected from the impairment of $\alpha$-chain production in the formation of $Hb \ \alpha$. The presence of $Hb \ \mathrm{H}$ could not be detected in all of our cases. Probably the amount of $Hb \ \mathrm{H}$ is sometimes so low that it was not detected by the conventional methods used. Besides, $Hb \ \mathrm{H}$ is known to be unstable. Since there is ample evidence that there is severe depression of $\alpha$-chain production and, in addition, the hematologic symptoms in the fetuses resembled thalassemia, these cases should be considered $\alpha$-chain thalassemia. Judging from the extreme depression of the $\alpha$-chain production and the severity of the clinical and hematologic symptoms in the fetuses, one would suspect that they represented the homozygous condition for this abnormal gene. However, the homozygosity should be proved by finding the trait condition in both parents. Unfortunately, the criteria for $\alpha$-chain thalassemia trait cannot be sharply defined yet, since only a few families with this condition have been described. It is clear, however, that it should fit the theory of $\alpha$-chain depression and should, therefore, not show an increase of $Hb \ \mathrm{F}$ or $Hb \ \alpha_2$ contrary to the finding in $\beta$-chain thalassemia.

In the parents examined, no abnormal hemoglobin could be demonstrated. It is possible that in the trait condition the impairment of the $\alpha$-chain production is so slight that the surplus of $\beta$-chains leading to the formation of $Hb \ \mathrm{H}$ ($Hb \ \beta_4$) was not detected with the methods used. However, the microcytosis often seen in the parents, although not always obvious, might be the expression of the presence of a single gene. However, while we found clear microcytosis in all the parents of our cases from Indonesia, this microcytosis was not always clear-cut in all parents of the cases described here. For instance, the MCV of 78.6 cu. $\mu$ in the father of Case 1 is at the lower limit of normal. The MCV of 75.1 cu. $\mu$ in the mother of Case 2 was obtained after blood transfusion, and therefore cannot be evaluated. It is a pity that serum iron estimations could not be carried out to eliminate the possibility of iron deficiency. Although iron deficiency cannot be eliminated as a cause of this microcytosis in the parents, iron deficiency was certainly not the cause of the disease in the fetuses.

From the fact that all cases of hydrops encountered in this survey were as-
associated with abnormal hemoglobin production, and that in none was there evidence of isoimmunization as a cause of the disease, it seems that, in this area, abnormal hemoglobin metabolism is a more important factor in the causation of hydrops and erythroblastosis than Rh or ABO isoimmunization, at least in the Chinese. This has also been shown for Indonesia. It would not be surprising to find the same situation in China and other Southeast Asian countries.

Summary

Five cases of severe hydrops and erythroblastosis fetalis in association with a large amount of Hb “Bart’s,” all of Chinese origin, are described. The following characteristic clinical and hematologic symptoms were found. There were generalized hydrops, ascites and gross enlargement of the liver. The spleen, however, was not always enlarged. The placenta was large and friable. Severe erythroblastosis of the blood was always found, with reticulocytosis, many target cells and thin cells. The MCV of the red cells was very high. The cells showed an interesting sickling phenomenon. No evidence of isoimmunization was found. In eight parents examined, no abnormal hemoglobin was detected, and alkali-resistant hemoglobin and hemoglobin A2 were not found to be increased. Their blood showed microcytosis of the red cells except in one father and one mother. In this mother, however, the blood was examined after a blood transfusion. It is thought probable that these were cases of homozygous α-chain thalassemia.

Summary in Interlingua

Es describite cinque casos de sever hydropisis e erythroblastosis fetalis in association con grande quantitates de hemoglobina Bart, omnes in subjectos de origine chinese. Esseva trovate le sequente characteristic symptomas clinic e hematologic: Esseva presente generalisate hydropisis, ascites, e grossier alargamento hepatic. Tamen, le splen non esseva allargate uniformemente. Le placenta esseva grande e friabile. Sever erythroblastosis del sanguine esseva etiam trovate, insimul con reticulocytosis, numeroso cellulas a oculo de ave, e tenue cellulas. Le volumine corpuscular medie del erythrocytos esseva altissime. Le cellulas exhibiva un interessante phenomeno de falciformation. Esseva trovate nulle evidentia de iso-immunisation. Octo parentes esseva examinate, e nulle de istes habeva un hemoglobina anormal, e nulle augmento del hemoglobina alcali-resistente e de hemoglobina A2 esseva constatate. Le sanguine de iste subjectos monstrava microcytosis del erythrocytos, con le exceptione de un patre e de un matre. Tamen, il debe esser notate que in iste matre le sanguine esseva examinate post un transfusion. Es opinate que iste casos esseva possibilemente casos homozygotic de thalassemia a catena α.

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REFERENCES

6. —: Significance of the “Bart’s” or Fessas & Papaspyrou haemoglobin. Tenth Pacific Science Congress, Honolulu, August 1961.

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