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PERSISTENCE OF HAEMOGLOBIN "BART'S" BEYOND INFANCY IN A CHILD WITH THALASSAEMIA

BY

C. CHOREMIS, M.D.

L. ZANNOS-MARIOLEA, M.D.

Paediatric Clinic, University of Athens

J. A. M. AGER, M.B., B.S.

Jenner Laboratory, St. Thomas's Hospital, London

AND

H. LEHMANN, M.D., Sc.D., M.R.C.P., F.R.I.C.

Department of Pathology, St. Bartholomew's Hospital, London

Haemoglobin "Bart's" (Ager and Lehmann, 1958) was found in a newborn infant of mixed ancestry (Spanish, West-Indian, Negro, Chinese). Its electrophoretic mobility at alkaline pH was slower than that of haemoglobin H and faster than that of haemoglobin J, resembling that of haemoglobin N. On chromatography at pH 6 it moved faster than any hitherto known variant. This pigment was adjudged to be an abnormal foetal haemoglobin because of its absence in both parents, because its resistance to alkali denaturation was intermediate between that of haemoglobin A and haemoglobin F, and because its ultra-violet spectrum was of the foetal type. Haemoglobin "Bart's" was seen to disappear as the child grew older, and at the age of 6 months it had disappeared completely.

The possibility was considered that a haemoglobin discovered in early infancy in a Greek child (Fessas and Papaspyrou, 1957) was identical with "Bart's." This pigment migrated more slowly than J on paper electrophoresis at alkaline pH, and was not alkali-resistant. These two findings might have been due to the fact that the haemoglobins of Fessas and Papaspyrou had been in about half the concentration of that of the "Bart's" component. However, dilution of a haemoglobin containing 24% "Bart's" and a mixture of foetal and adult haemoglobin, with an equal amount of haemoglobin A, did not alter the mobility of the fast component. Samples of infants' blood containing haemoglobin "Bart's" and sent to us by Dr. F. Vella from Singapore, Dr. S. Tuchinda from Thailand, and Dr. R. Schneider from Galveston, Texas, contained amounts of "Bart's" varying from < 10% to > 20%. The mobility on paper electrophoresis at alkaline pH was in all cases greater than that of J, and the isolated fraction was denatured more slowly than haemoglobin A and faster than haemoglobin F.

By the courtesy of Dr. F. Vella we have recently examined a haemoglobin from an infant suffering from anaemia. There was 15% of a "fast-moving" haemoglobin whose ultra-violet spectrum was of the foetal type. This haemoglobin moved on paper and starch electrophoresis at alkaline pH similar to J and more slowly than "Bart's." It may well be possible that we were here dealing with an abnormally large proportion of the pigment described by Fessas and Papaspyrou (1957). The haemoglobin sample was in the carboxy form and could therefore not be tested for resistance to alkali denaturation. Some of the fast-moving fraction was eluted from filter paper after electrophoresis, oxygenated for two hours, treated with sodium metabisulphite, and again briefly reoxygenated. This sample showed then an increased resistance to alkali denaturation, which had not been noted in their foetal variant by Fessas and Papaspyrou. However, we do not think that too much reliance can be placed on a result under these circumstances.

The present communication is intended to report yet another resemblance between haemoglobin "Bart's" and haemoglobin F—its persistence beyond the age of infancy in a child with thalassaemia. The patient, a boy aged 9 years, showed some degree of infantilism

FIG. 1.—Stained film of the patient's blood. Moderate hypochromia, aniso-poikilocytosis, some macrocytes, and a few target cells are present.

FIG. 2.—Hanging strip paper electrophoresis at pH 8.6, comparing the patient's haemoglobin with haemoglobin from the original infant with haemoglobin "Bart's," then aged 3 months, and showing 15% of the abnormal foetal pigment. The difference in the position between the two slow-moving fractions (A+F) is due to the higher haemoglobin F content in the baby's blood.

(height 118 cm., instead of 130 cm.; weight 20 kg. instead of 28–30 kg.). The facies was of the type seen in Cooley's anaemia and in microdrepanocytic disease. Haemoglobin concentration was 6.7 g./100 ml.; red cells 2,500,000/c.mm.; packed cell volume 22%; indirect serum bilirubin 0.75 mg./100 ml.; reticulocytes 2%. No inclusion bodies were formed on incubation of the red cells with cresyl violet. The red cells showed aniso-poikilocytosis and hypochromia and macrocytes (Fig. 1). The spleen was 3 cm. palpable; the liver was just palpable. X-ray examination of the skull showed widening of the diploic space, absence of the outer plate, and diffuse osteoporosis without, however, "brush appearance." The long bones showed no abnormality.

When the haemoglobin was examined a fast-moving fraction was seen on paper electrophoresis at pH 8.6 which moved more slowly than H and faster than J (Fig. 2). On elution from paper it was found to have an ultra-violet spectrum of the foetal haemoglobin type (Fig. 3). The same fraction separated from A and F

FIG. 3.—Ultra-violet spectrum. ●—●: Pure haemoglobin F eluted from amberlite chromatography column at pH 6. ●---●: "Fast-moving" component of patient's haemoglobin eluted from starch after electrophoresis at pH 8.6.

on paper electrophoresis at pH 6.5, on starch electrophoresis at pH 6, and on "amberlite" chromatography at pH 6 (Fig. 4). In all these procedures a direct comparison with the original "Bart's" haemoglobin showed them to behave identically. Of the total haemoglobin, 10% was found to be alkali-resistant in the denaturation test of Singer, Chernoff, and Singer (1951). The total amount of foetal haemoglobins was, however, higher, and, combining the observations made on chromatography and electrophoresis, it could be calculated to be 13%, of which 7% was haemoglobin F and 6% haemoglobin "Bart's."

Both parents showed evidence of thalassaemia in their blood. The father's blood could be examined fully. From the mother only one specimen of citrated blood could be obtained, but a satisfactory dry film could not be prepared. The father's film showed a mild hypochromia and numerous target cells (Fig. 5). No alkali-resistant haemoglobin was found in either parent and no haemoglobin "Bart's" was discovered on electrophoresis. However, the haemoglobin A₂ fraction was >3% in both (3.8 and 4.2% respectively). A failure of foetal haemoglobin to persist into adult life is not unusual in thalassaemia heterozygotes.

It seems, therefore, that the patient is suffering from thalassaemia major, and that in his case both haemoglobin F and "Bart's" have persisted beyond the age of infancy.

A haemoglobinopathy with which this condition might be confused is haemoglobin H disease. The blood picture is similar; and as in haemoglobin H disease, so here, a "fast-moving" haemoglobin is found in the patient but not in either of the parents, and there is evidence that the patient could have inherited thalassaemia.

The failure of the red cells to form inclusion bodies in cresyl violet solution in the cold; the resistance of the haemoglobin to cold denaturation; and, of course, the comparison of the fast fraction with haemoglobin H, will serve to distinguish the two conditions.

FIG. 4.—Amberlite resin chromatography at pH 6, comparing the patient's haemoglobin with one consisting of haemoglobins A and H. Haemoglobin "Bart's" is seen to move faster than haemoglobin H in this separation.

FIG. 5.—Stained film of blood of patient's father. Numerous target cells can be seen.

Summary

In a 9-year-old child with thalassaemia haemoglobins A and F and also haemoglobin "Bart's" were found. Hitherto "Bart's" has been demonstrated only in early infancy, and its persistence into a later age in conjunction with thalassaemia is further evidence in favour of its being a variant of foetal haemoglobin.

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