CONGENITAL HYPOPLASTIC ANAEMIA WITH AMINO-ACIDURIA IN A BANTU INFANT

J. G. A. DAVEL, M.R.C.P., F.R.C.S. (EDIN.), B.M., B.CH., M.A. (OXON.), M.Sc. (S.A.), D.C.H., *Department of Paediatrics, University of Pretoria;* and

R. E. CRONJE, M.B., B.CH., M.R.C.P. (EDIN.), *National Nutrition Research Institute, Pretoria*

In 1938 Diamond and Blackfan[1] reported a type of anaemia found in young infants and characterized by feeble erythropoiesis and normal leucocyte and platelet production. As early as 1936, however, Josephs[2] referred to a pure red-cell hypoplasia in 2 children. Since then several cases have been reported.[3-5] Treatment has consisted of repeated blood transfusions, but the disease has been considered to be incurable, although spontaneous remission has occurred. A good summary of the condition has been published by Smith.[13]

Since the first publications, 2 interesting developments have occurred. Firstly, an abnormal excretion of certain metabolites has been found in the urine of some of these patients, and the suggestion has been made that this may be due to a defect in the metabolism of tryptophan.[6,7] Secondly, it has been found that many of these cases can be favourably influenced by treatment with corticosteroids, especially if these are administered within 3 months of the onset of the disorder.[8,9] In the present paper the first case, in a South African Bantu infant, of congenital hypoplastic anaemia of the above type, with an associated amino-aciduria, is reported.

CASE REPORT

History

A 4-month-old male Bantu infant was admitted to the General Hospital, Pretoria, with dyspnoea and severe anaemia. The child was said to have been well until 3 days before admission, when dyspnoea developed. One day before admission the child began to cough and vomit. The mother had not noticed any blood in the stools, urine, vomitus or sputum. Although the mother had not had any complaints during pregnancy, she had received injections at an antenatal clinic owing to the finding of a positive Wassermann reaction. The birth of the child was normal. The baby progressed well after birth and was not jaundiced at any age. The mother and father were well, and 4 older siblings were healthy and had never been jaundiced or anaemic. The child was breast-fed and received a supplement of a weak solution of condensed milk. There had been no previous illnesses and no drug or herb had been given to the child at any time.

Physical Examination

The weight was 9 lb. The temperature was normal, the pulse rate 130 per minute, and the respirations 32 per minute. The facial appearance was not unusual in any way, and the state of nutrition was fair. The child was anaemic and obviously dyspnoeic. The liver was palpable 2 cm. below the costal margin in the mid-clavicular line, and the jugular vein filled to about 1 cm. above the manubrial notch in the sitting position. There was a short, soft, systolic murmur over the praecordium. No other abnormalities could be detected.

Laboratory Investigations

Urine. Microscopic, chemical and bacteriological examination of the urine showed no abnormality except a gross amino-aciduria. Chemical tests for urobilinogen and bilirubin were negative.

Faeces. There was no evidence of blood loss in the stools or of parasitic infestation.

Haematology. The blood haemoglobin concentration on admission was 1·15 G. per 100 ml., RBCs 540,000 per c.mm., WBCs 11,000 per c.mm. (polymorphs 57%, lymphocytes 37%, monocytes 3% and eosinophils 3%). The red cells showed anisocytosis and poikilocytosis, and were slightly hypochromic. There were 2 normoblasts per 100 nucleated cells in the peripheral blood, but no reticulocytes. Examination of the bone marrow on admission and on several occasions later showed virtually no erythroblastic activity. The megakaryocytes were increased in number, but other findings were essentially normal. Electrophoresis of the patient's haemoglobin revealed no abnormal components. The patient's blood group was A Rh+ and that of the mother O Rh+. With regard to red-cell fragility, haemolysis began at a concentration of 0·40% NaCl and was complete at a concentration of 0·24% NaCl. Coombs' test was negative.

Blood chemistry. The serum-protein concentration was normal (albumin 4·4 G. per 100 ml., globulin 2·6 G. per 100 ml.) and the electrophoretic pattern of the serum proteins showed no abnormality. The blood-urea concentration was 47 mg. per 100 ml. shortly after a blood transfusion, and 36 mg. per 100 ml. later. The serum-bilirubin level was 0·7 mg. per 100 ml. The serum-iron concentration was 160, 171 and 165 μg. per 100 ml. on 3 separate occasions. The total iron-binding capacity of the serum was 189 μg. per 100 ml. The Kolmer complement-fixation test and the Price precipitation test were negative.

Electrocardiographic examination. The pattern was one of tachycardia and left axis deviation.

X-ray examinations. X-ray examination of the chest showed generalized enlargement of the heart. The thymus was not enlarged. An intravenous pyelogram showed good dye excretion and no deformity of the pelvis or ureters.

Special tests of tryptophan metabolism. The serum α-amino-nitrogen concentration was 0·72 mg. per 100 ml. (normal values 2 - 4 mg. per 100 ml.). The urinary excretion of α-amino nitrogen was 160 mg. per 24 hours (26·6 mg. per kg. body weight per day). The 24-hour urinary excretion of xanthurenic acid was 0·5 mg., that of N-methyl-nicotinamide 2·12 mg., and that of 5-hydroxyindoleacetic acid 9·6 mg. Paper chromatography showed that the urine contained glycine, glutamine, alanine, histidine, tyrosine and threonine.

DL-tryptophan was then given to the child by mouth in a dose of 0·5 G. per kg. body weight. A 24-hour specimen of urine was again collected and analysed. The 24-hour excretion of α-amino-nitrogen had increased to 242·8 mg. (40·5 mg. per kg. body weight per day), that of xanthurenic acid to 2·3 mg., and that of N-methyl-nicotinamide to 6·36 mg. An attempt was made to detect various metabolic derivatives of tryptophan, such as anthranilic acid, without helpful results, because of technical difficulties.

Administration of 50 mg. of pyridoxine daily for 7 days produced no significant change in the amino-aciduria. After administration of riboflavin (100 mg. daily for 4 days), the urinary excretion of α-amino nitrogen was reduced to 101·6 mg. per day before loading, and 94·5 mg. per day after loading with tryptophan. However, the following amino acids were still found in the second specimen of urine: glycine, glutamine, alanine, serine, taurine, tyrosine, glutamic acid, and tryptophan.

Progress and Treatment

Within 3 days of admission, 300 ml. of whole blood were transfused, raising the haemoglobin level to 8·4 G. per 100 ml. Penicillin (500,000 units) was given intramuscularly 6-hourly for 5 days. Shortly after the blood transfusion the child's general condition improved and the dyspnoea and venous congestion disappeared. Iron was given by mouth until it was realized that the anaemia was due neither to blood loss nor to iron deficiency. Folic acid (15 mg. by mouth daily) plus

vitamin B_{12} (100 μg. intramuscularly per day) failed to influence the anaemia. The administration of riboflavin, pyridoxine and a multivitamin syrup was also ineffective. Testosterone propionate (25 mg. intramuscularly per day) with methyl-prednisolone (2 mg. *t.i.d.*) for a period of 6 weeks produced no change in the blood picture.

The haemoglobin was kept at reasonable levels by transfusions. Corticosteroids given alone also seemed to be without effect in small doses, but when, about 6 months after admission, the dosage was greatly increased to 5 mg. of prednisolone 4 times a day, the haemoglobin level rose spontaneously for the first time. Although the level subsequently fell, the decline was less steep than before. At this stage the platelet count in the peripheral blood rose remarkably and immature white cells appeared. The bone-marrow picture, however, showed no notable change. The steroid dosage was again increased to 30 mg. of prednisolone daily, and a definite and sustained increase in the haemoglobin concentration took place.

At this stage the child's general condition was good enough to warrant discharge from hospital, although a pronounced 'moon face' was present. Twenty-four days later the patient was seen at the outpatient department and the treatment was changed to dexamethasone, 1·6 mg. 4 times a day, on account of the 'moon face'. Two days later the child developed a diarrhoeal disorder and died a few hours after re-admission to hospital.

Postmortem Examination

At autopsy the lungs showed signs of early bronchopneumonia, and the colon showed signs of non-specific subacute colitis. Signs of mild transfusional siderosis were found in the spleen, and moderate siderosis in the liver. Examination of the adrenals showed cortical adenomatosis. The bone marrow was active and richly cellular, with both red and white cell elements present. There was no evidence of malignancy.

COMMENT

Hammond and Keighley[10] have enumerated the criteria for the diagnosis of congenital hypoplastic anaemia as: (1) onset of anaemia in early infancy, (2) specific erythroid hypoplasia of the bone marrow, (3) reticulocytopenia, and (4) absence of significant splenomegaly.

In our patient these criteria were fulfilled. There was no evidence of blood loss at any time. Transplacental blood loss could not account for the failure to respond to haematinics later or for the reticulocytopenia. Neonatal haemolysis caused by incompatibility between the mother's and child's erythrocytes has been described by Smith[11] in association with congenital hypoplastic anaemia. This cause could be ruled out in our patient. Evidence of haemolysis was not found. The bone-marrow examination did not suggest that the anaemia was due to a deficiency state and the condition failed to respond to the administration of a multivitamin syrup, folic acid, vitamin B_{12}, pyridoxine, riboflavin or iron. The serum-iron level was high and the serum iron-binding capacity low.

In the adult a condition of pure red-cell anaemia has been found in association with a tumour of the thymus,[12] and in children with renal lesions.[13,14] There was no radiological or postmortem evidence of pathology of the kidneys or the thymus. The blood-urea level was normal and, except for the amino-aciduria, the urine did not show any abnormality. Although the mother possibly had syphilis during her pregnancy, the child's serological tests for syphilis were negative, the bones were radiologically normal, and there was no response to a thorough course of penicillin.

The cardiac failure was certainly due to the gross anaemia, since the heart returned to normal as the haemoglobin concentration was raised, and at autopsy no cardiac lesion was found.

Several workers[6-8] have found evidence of excessive excretion of various metabolites of tryptophan in the urine in some of these cases. There is no agreement yet about the nature of the defect. Xanthurenic acid and other metabolites of tryptophan are excreted in excessive amounts in the urine of pyridoxine-deficient rats and humans.[14,15] Pyridoxine deficiency can also give rise to anaemia.[16] In our patient, however, there was no abnormal increase in xanthurenic-acid excretion after a loading dose of tryptophan. There did not seem to be an inability to convert tryptophan to N-methyl-nicotinamide, a reaction for which pyridoxine is essential.[17] In addition, the administration of pyridoxine had no effect on the peripheral blood picture.

There is a resemblance between the biochemical defect in these cases and that found in riboflavin-deficient animals. Altmann and Miller[6] reduced the excretion of anthranilic acid in one of their patients by administering riboflavin, without any improvement of the anaemia. Our patient also failed to improve after a short period of riboflavin therapy in which very large doses were employed.

Pearson and Cone[8] found the urinary excretion of 5-hydroxyindoleacetic acid to be normal. Here the excretion of this substance was also within normal limits.

An interesting feature was the excessive excretion of amino acids in the urine. According to different workers,[18-22] the normal variation in the α-amino-nitrogen concentration in the urine is between 1·86 and 6·5 mg. per kg. body weight per day in infants more than a few months old. Our patient excreted 26·6 mg. of α-amino nitrogen per kg. body weight per day, which increased to 40·5 mg. per kg. body weight per day after the tryptophan load. This increase did not seem to be due to overflow of the tryptophan as judged by the paper chromatogram.

While pyridoxine administration did not seem to affect this amino-aciduria, administration of riboflavin seemed to reduce the α-amino-nitrogen excretion, although the level was still above normal. The urinary excretion of free amino acids, according to the paper chromatograms obtained, also deviated from the normal pattern described by several workers.[23-26] This has not been reported by other writers. The plasma α-amino-nitrogen level was low, suggesting a renal tubular defect as the cause of the amino-aciduria.

Pearson and Cone[8] and Allen and Diamond[9] have reported the use of corticosteroids with good results. If the best results are to be achieved, treatment should be begun within 3 months of the onset of the disease. Large doses of corticosteroids may be necessary. No other treatment has been of avail apart from repeated blood transfusions. There does not seem to be a defect in the erythrocyte-stimulating factor in the serum of these patients.[10] In our patient, although treatment with corticosteroids was begun within 2 months of discovery, the dosages used were low and only when prednisolone was given in amounts of 20 - 30 mg. daily did the haemoglobin concentration rise. No other form of treatment had any effect.

The immediate cause of death was gastro-enteritis and bronchopneumonia. The child had received 1,140 ml. of blood during his life, and at autopsy there was some evi-

dence of transfusional siderosis of the liver and the spleen. The adenomatosis of the adrenal cortex was of a type not uncommonly found in children and was apparently not a cause of symptoms. Shortly before death the peripheral haemoglobin concentration had begun to rise and examination of the haematopoietic system at postmortem examination showed that both white and red cell elements were present, indicating the possible beneficial effect of steroid therapy in this patient.

SUMMARY

A case is presented of congenital hypoplastic anaemia associated with excessive amino-aciduria in a Bantu infant. Various biochemical investigations carried out are described and discussed. Considerable improvement in the condition was brought about by the administration of large doses of corticosteroids, but the patient succumbed to a fulminating attack of gastro-enteritis and bronchopneumonia. The postmortem findings are described.

We should like to thank Dr. J. J. Theron, of the National Nutrition Research Institute, for the studies of tryptophan metabolism and various other investigations, and Dr. L. de Villiers, of the Institute of Pathology, University of Pretoria, for his advice and help.

REFERENCES

1. Diamond, L. K. and Blackfan, K. D. (1938): Amer. J. Dis Child., **56,** 464.
2. Josephs, H. W. (1936): Medicine (Baltimore), **15,** 307.
3. Cathie, I. A. B. (1950): Arch. Dis. Childh., **25,** 313.
4. Smith, C. H. (1953): J. Pediat., **43,** 457.
5. Fisher, C. D. and Allen, F. M. B. (1953): Arch. Dis. Childh., **28,** 363.
6. Altmann, K. I. and Miller, G. (1953): Nature (Lond.), **172,** 868.
7. Smith, N. J., Price, J. M., Brown, R. R. and Moon, R. L. (1960): Amer. J. Dis. Child., **100,** 752.
8. Pearson, H. A. and Cone, T. E. (1957): Pediatrics, **19,** 192.
9. Allen, D. M. and Diamond, L. K. (1960): Amer. J. Dis. Child., **100,** 748.
10. Hammond, D. and Keighley, G. (1960): *Ibid.*, **100,** 466.
11. Smith, C. H. (1949): Blood, **4,** 697.
12. Clarckson, B. and Prockop, D. J. (1958): New. Engl. J. Med., **259,** 253.
13. Smith, C. H. (1959): J. Pediat., **54,** 609.
14. Dalgleish, C. E. (1952): Biochem. J., **52,** 3.
15. Greenberg, L. D., Bohr, D. F., McGrath, H. and Rinehart, J. F. (1949): Arch. Biochem., **21,** 237.
16. Snyderman, S. E., Carretero, R. and Holt, L. E. (1950): Fed. Proc., **9,** 371.
17. Snyderman, S. E., Holt, L. E., Carretero, R. and Jacobs, K. G. (1953): J. Clin. Nutr., **1,** 200.
18. Ghadimi, H. and Schwachman, H. (1960): Amer. J. Dis. Child., **99,** 457.
19. Huisman, T. H. J. (1954): Voeding, **15,** 527.
20. *Idem* (1957): Maandschr. Kindergeneesk., **25,** 247.
21. Fowler, D. I., Norton, P. M., Cheung, M. W. and Pratt, E. L. (1957): Arch. Biochem., **68,** 452.
22. Childs, B. (1952): Proc. Soc. Exp. Biol. (N.Y.), **81,** 225.
23. Berry, H. K. (1960): Pediatrics, **25,** 983.
24. Snyderman, S. E. (1958): *Ibid.*, **21,** 117.
25. Chisholm, J. J. and Harrison, H. E. (1960): Pediat. Clin. N. Amer., **7,** 333.
26. Woolf, L. I. and Norman, A. P. (1957): J. Pediat., **50,** 271.