ATYPICAL SPHEROCYTOSIS IN AN AFRICAN GIRL

PHILIP LANZKOWSKY,* M.D. (CAPE TOWN), M.R.C.P. (EDIN.), D.C.H. (ENG.), *Paediatric Haematology Unit, Department of Child Health, University of Cape Town and Red Cross War Memorial Children's Hospital, Rondebosch*

Hereditary spherocytosis is best known as a disorder affecting people of European origin, although it is by no means confined to this group. The disease has been reported in Egyptians by Salah,[1] in Filipinos by Stransky and Dauis-Lawas,[2] and Kline and Holman[3] in a comprehensive search of the literature reported that 42 *bona fide* cases had been described in Negroes. In the African this condition is considered to be a distinctly rare entity. Gelfand[4] has not seen it in an African in the Rhodesias, and Foy and Kondi[5] recorded, without mentioning any details, one typical case of hereditary spherocytosis in Kenya. In the South African Bantu, Merskey and Baskind[6] and Gon[7] each reported a case of chronic haemolytic anaemia resembling acholuric jaundice. In neither case was a family study carried out and in the former, comprehensive techniques for the exclusion of antibodies had not been evolved at the time of recording. Metz[8] was the first to report a Bantu family where the diagnosis of hereditary spherocytosis could be established and he mentioned a further case in a Bantu male. Recently, Spector and Metz[9] recorded another Bantu family with hereditary spherocytosis. The true incidence of this disease in races other than European is not known. Whether the paucity of reports in Africans indicates that the disorder is rare in this race, or results from failure in diagnosis, or in reporting of known cases, is not possible to assess. In view of the apparent rarity of this disorder in Africans, this paper presents a case in a Bantu girl which, for reasons to be mentioned later in the report, is considered to be a case of atypical spherocytosis or 'type-B' of Young, Izzo, Altman and Swisher.[10]

*Present address: Dept. of Pediatric Hematology, Cornell University Medical Centre, 525 East 68th Street, New York, NY 10021, USA.

METHODS

Routine haematological studies were performed by standard methods.[11] Autohaemolysis studies were done by the method described by Cartwright;[12] glucose utilization studies by the

method described by Tanaka, Valentine and Miwa;[13] and the blood glucose was determined by the Somogyi method. The serum iron,[14] iron-binding capacity,[15] urinary urobilinogen,[16] faecal urobilinogen,[17] serum haptoglobins,[18] haemoglobin electrophoresis,[19] foetal haemoglobin determination,[20] and the glucose-6-phosphate dehydrogenase[21] estimations were all performed by established methods.

CASE REPORT

R.M., a 10-year-old Bantu girl, was admitted to the Queenstown Hospital in February 1963 on account of gross anaemia and splenomegaly. The haemoglobin was found to be 2·6 G/100 ml. and she was transfused with 2 pints of blood. During the interval between this admission and her referral to the Red Cross War Memorial Children's Hospital, Cape Town, she was given 1,400 mg. of iron-dextran complex (Imferon) intramuscularly. No malarial parasites were detected on the smear. The initial diagnosis was iron-deficiency anaemia and she was given a further 600 mg. of intramuscular iron-dextran complex.

Family history. Her parents and 4 siblings had no history of anaemia or jaundice and when examined by Dr. G. J. Lötter, District Surgeon of Queenstown, they did not have anaemia or splenomegaly. The father's blood was available for study and was found to be completely normal, as was the osmotic fragility.

Physical examination showed a well-developed, well-nourished Bantu girl with slight pallor and scleral icterus. Her height was 4 ft. 8 in. and weight 61 lb. She had a splenomegaly of 6 cm. and the liver was palpable 1·5 cm. below the costal margin. Cardiomegaly was present and a pre-systolic sound, confirmed on phonocardiography, was audible. Radiological examination of the chest showed moderate cardiac enlargement.

The relevant *haematologic findings* are shown in Table I. Many of the red cell inclusions stained with prussian blue for iron. It was not possible to transfer these inclusions by incubating the patient's serum with normal compatible erythrocytes at 37°C for 24 or 48 hours. The bone marrow aspirate showed erythroid hyperplasia in keeping with haemolytic anaemia, and staining for iron showed increased iron content.

TABLE I. HAEMATOLOGIC FINDINGS

	Before splenectomy	*After splenectomy*
Haemoglobin G/100 ml.	9·2	13·7
Volume of packed erythrocytes %	26	38
Mean corpuscular haemoglobin concentration %	35	36
Reticulocytes %	17	1·2
Spherocytes	++++	++++
Polychromatophilia	++	–
Basophilic stippling	+++	++
Howell-Jolly bodies	+	++
Pappenheimer bodies	+++	+++
Serum iron μg/100 ml.	96·4	
Total iron-binding capacity μg/100 ml.	304·0	
Saturation %	32	
Serum bilirubin (mg./100 ml.): total	3·3	0·7
direct	0·4	0·2
Urinary urobilinogen mg./2 hours	10	Nil
Faecal urobilinogen mg.%	495	82
Plasma methaemoglobin	Absent	
Plasma methaemalbumin	Absent	
Plasma sulphaemoglobin	Absent	
Serum haptoglobin mg.%	24·0	—
Mechanical fragility %	3·7	4·2
T½ chromium 51-labelled erythrocyte survival time (autotransfusion).	12 days	30 days
Antibody tests:		
(*a*) Direct antiglobulin (Coombs test)	– Negative	
(*b*) Indirect Coombs test	– Negative	
(*c*) Ham's acid-serum test	– Negative	
(*d*) Auto-antibodies at 37°C	– Negative	
(*e*) Auto-antibodies at 22°C	– Negative	
(*f*) Auto-agglutinins at 4°C	– Negative	
(*g*) Cold agglutinin titre	– ¼	
Haemoglobin electrophoresis	AA	
Foetal haemoglobin %	0·6	
Haemoglobin A_2%	3·0	
Glucose-6-phosphate dehydrogenase	0·2 units	
Wassermann and Berger tests	Negative	
Urinary lead	Absent	

The red cell osmotic fragility and autohaemolysis estimations were markedly increased and the results are shown in Tables II and III. Unlike the typical cases of hereditary spherocytosis,

TABLE II. OSMOTIC FRAGILITY STUDIES BEFORE AND AFTER SPLENECTOMY

	Percent haemolysis			
	Pre-incubation		*After 24 hours' incubation at 37°C*	
%*NaCl*	*Before splenectomy*	*After splenectomy*	*Before splenectomy*	*After splenectomy*
0·10	100	100	100	100
0·20	98	100	98	100
0·30	97	100	98	98
0·35	96	100	98	96
0·40	94	95	97	96
0·45	93	95	97	95
0·50	85	92	97	95
0·55	58	67	95	95
0·60	45	52	90	93
0·65	38	20	85	86
0·75	13	2	74	78
0·85	1	0	65	50

TABLE III. AUTOHAEMOLYSIS STUDIES BEFORE AND AFTER SPLENECTOMY

	Percent haemolysis			
Whole blood plus 0·1 ml.	*After 24 hours' incubation at 37°C*		*After 48 hours' incubation at 37°C*	
	Before splenectomy	*After splenectomy*	*Before splenectomy*	*After splenectomy*
0·9% NaCl	6·9	2·6	46·9	22·5
10% Glucose	9·4	1·2	31·4	5·6
0·2 M Adenosine	10·8	0·9	23·2	1·9
0·2 M ATP	1·5	0·6	11·1	2·5

the addition of glucose or adenosine to the incubating blood in the autohaemolysis studies did not markedly retard haemolysis and, indeed, appeared to aggravate it after 24 hours' incubation. Slight retardation of haemolysis occurred with those additives after 48 hours' incubation. The presence of adenosine triphosphate (ATP) in a concentration of 0·1 M in the incubating blood appeared to retard haemolysis significantly, both after 24 and 48 hours' incubation.

Glucose utilization tests performed on two separate occasions showed a mean glucose utilization by the erythrocytes of 16·6 and 16·2 mg./100 ml./hour respectively.

Red cell survival, following an autotransfusion of radioactive chromium-labelled erythrocytes, gave a $T\frac{1}{2}$ of 12 days (normal 28 ± 2 days)—Fig. 1. The organ-scanning data (Fig. 2) indicated that the excessive destruction of erythrocytes was taking place in the spleen. When compatible donor's labelled erythrocytes were injected into the patient the $T\frac{1}{2}$ was 24 days (Fig. 1) and surface counting of the radioactivity over the

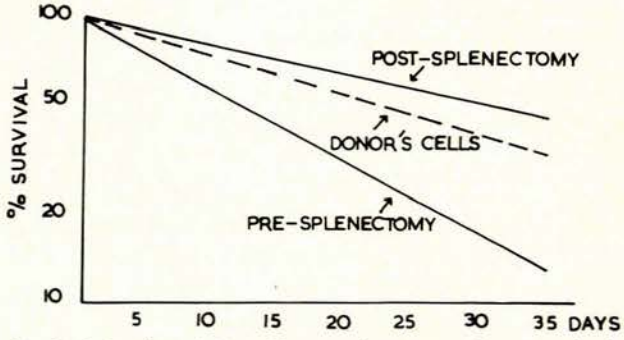

Fig. 1. Red cell survival studies, pre-splenectomy, of autotransfused patient's and of transfused normal donor's erythrocytes, and, post-splenectomy, of autotransfused patient's erythrocytes.

spleen showed that no excessive destruction of compatible donor's erythrocytes was taking place there (Fig. 2).

Treatment

Splenectomy was performed by Prof. J. H. Louw on 20 April 1964. The spleen was enlarged, weighing 360 G. Histological examination showed tremendous engorgement of the red pulp, mainly affecting the interstitial pulp. Venous sinuses were

also dilated and engorged. Haemosiderin-laden macrophages were abundant.

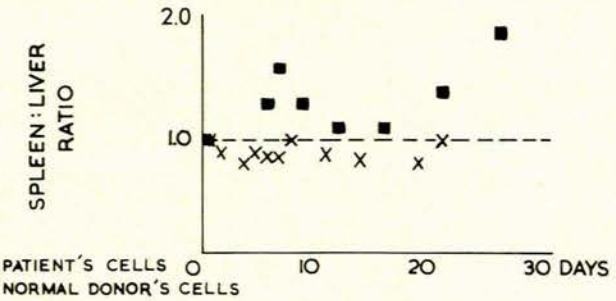

Fig. 2. Organ-scanning data following injection of radioactive labelled patient's erythrocytes and normal compatible donor's erythrocytes.

The post-splenectomy course was uneventful. The haematological status improved and the data are shown in Table I. The osmotic fragility was unchanged (Table II). There was considerable improvement in the autohaemolysis studies (Table III). The latter in the post-splenectomy period behaved in a manner similar to typical hereditary spherocytosis in so far as the presence of glucose and adenosine in the incubating blood markedly retarded haemolysis. The red cell survival was now within normal limits, the $T\frac{1}{2}$ being 30 days (Fig. 1). Within one month postoperatively, her cardiac size returned to normal and no abnormal heart sounds or murmurs were audible.

DISCUSSION

In typical cases of hereditary spherocytosis the family history is positive and autohaemolysis proceeds at an abnormally fast rate. Dacie[22] showed that in 21 patients with hereditary spherocytosis the range of haemolysis at 48 hours, without additional glucose, was 7·5 - 47·5% with a mean of 28·5%. Dacie[22] and other workers[10] showed that haemolysis was markedly slowed in the presence of glucose and that adenosine was also effective in retarding haemolysis.

In atypical spherocytosis (group B) described by Young and his co-workers[10] in 1956, the disorder differed in that the rate of autohaemolysis of the patient's erythrocytes was not reduced by the addition of glucose. They described 2 patients under this title. After splenectomy, from which the patients derived striking clinical benefit, glucose had as marked an effect in reducing autohaemolysis as in typical cases of hereditary spherocytosis. The reason for these discrepancies is unknown. Dacie[22] has made similar observations in 2 children of different families whose parents were apparently normal, as well as in an adult with a positive family history who had the nephrotic syndrome and amyloid disease in addition to hereditary spherocytosis. The patient described in this paper appears to be a further case of atypical spherocytosis behaving in the same way on incubation with glucose before and after splenectomy, as did the cases previously recorded.[10,22] These observations suggest that the biochemical abnormality in hereditary spherocytosis may not be uniform, and support the concept that more than one type of the disorder exists. Previous authors have not, however, recorded the effect of the addition of adenosine or ATP to the blood in the autohaemolysis studies in their cases.

The exact abnormalities in hereditary spherocytosis have not yet been defined. Since ATP considerably reduced autohaemolysis in this case, it suggests that these cells lack sufficient ATP as an energy source and when an exogenous supply of this energy is provided, the abnormal cells are better equipped to withstand autohaemolysis. Since ATP does not easily penetrate the erythrocyte membrane[23] the added ATP probably undergoes hydrolysis to ADP by the action of the phosphatases of the plasma.[24] The ADP thus formed may then be converted in the erythrocyte membrane to ATP and AMP by adenylate kinase.[25] Some of the ATP enters the cell and reconstitutes, at least in part, the ATP supply of the cell and thus may reduce autohaemolysis. Failure to produce adequate amounts of ATP appears to be the essential lesion in this case. Adequate regeneration of the ATP is necessary to make available energy-rich phosphate bonds for the maintenance of the biconcave shape of the erythrocyte. The precise lesion in the erythrocyte responsible for inadequate ATP regeneration has not been determined in this case and, indeed, may differ from case to case in clinical hereditary spherocytosis.

The inclusions observed in the red cells of the patient described in this paper, before splenectomy, are considered to be as a result of 2 G of intramuscular iron-dextran complex injected into a person who was not iron-deficient but in fact had a disease which caused iron overload. Many of these inclusions stained positively with prussian blue, indicating their iron content.

SUMMARY

Attention is drawn to the rarity of hereditary spherocytosis in the African. A case of atypical spherocytosis is described in a Bantu girl.

Thanks are due to Dr. M. C. Botha and his staff, for some of the studies included in this paper; Dr. D. McKenzie, for providing the facilities of his pathology laboratory; and Miss R. Schein, for her technical assistance. I would like to acknowledge the assistance of Dr. M. I. Papilsky, of Queenstown, for providing the early clinical and haematologic data on this patient.

This work was supported by the CSIR Clinical Nutrition Research Unit, the University of Cape Town and the US Public Health Service Research Grant AM—03995NTN.

REFERENCES

1. Salah, M. (1936): J. Egypt. Med. Soc., **19**, 205.
2. Stransky, E. and Dauis-Lawas, D. F. (1952): J. Philipp. Med. Assoc., **28**, 647.
3. Kline, A. H. and Holman, G. H. (1957): Amer. J. Dis. Child., **94**, 609.
4. Gelfand, M. (1957): *The Sick African*, 3rd ed. Cape Town: Juta.
5. Foy, H. and Kondi, A. (1956): Cent. Afr. J. Med., **2**, 254.
6. Merskey, C. and Baskind, E. (1946): S. Afr. Med. J., **20**, 230.
7. Gon, F. (1959): *Ibid.*, **33**, 87.
8. Metz, J. (1959): *Ibid.*, **33**, 284.
9. Spector, I. and Metz, J. (1963): *Ibid.*, **37**, 211.
10. Young, L. E., Izzo, M. J., Altman, K. I. and Swisher, S. N. (1956): Blood, **11**, 977.
11. Dacie, J. V. and Lewis, S. M. (1963): *Practical Haematology*, 3rd ed. London: Churchill.
12. Cartwright, G. E. (1963): *Diagnostic Laboratory Hematology*, 3rd ed., p. 214. New York: Grune & Stratton.
13. Tanaka, K. R., Valentine, W. N. and Miwa, S. (1962): Blood, **19**, 267.
14. Trinder, P. (1956): J. Clin. Path., **9**, 170.
15. Henry, R. J., Sobel, C. and Chiamori, N. (1958): Clin. chim. Acta, **3**, 532.
16. Watson, C. J. (1936): Amer. J. Clin. Path., **6**, 458.
17. MacLaglan, N. F. (1946): Brit. J. Exp. Path., **27**, 190.
18. Owen, J. A., Better, F. C. and Hoban, J. (1960): J. Clin. Path., **13**, 163.
19. Lehmann, H. (1960): The Association of Clinical Pathologists, Broadsheet No. 27.
20. Singer, K., Chernoff, A. I. and Singer, L. (1951): Blood, **6**, 413.
21. Desforges, J. F., Kalaw, E. and Gilchrist, P. (1960): J. Lab. Clin. Med., **55**, 757.
22. Dacie, J. F. (1960): *The Haemolytic Anaemias: Part I—The Congenital Anaemias*, 2nd ed., p. 102. London: Churchill.
23. Gabrio, B. W., Finch, C. A. and Huennekens, F. M. (1956): Blood, **2**, 103.
24. Surgenor, D. M., Hunter, M. J. and Brown, R. K. (1953): In *Blood Cells and Plasma Proteins*, p. 315. New York: Academic Press Inc.
25. Kashket, S. and Denstedt, O. F. (1958): Canad. J. Biochem., **36**, 1057.