HAEMOLYTIC ANAEMIA

BY

MERVYN GATMAN* AND LEONARD HAMILTON

From the Department of Medicine, University of Cambridge

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The application of the technique described by Coombs and others (1945) has made it possible to demonstrate serological differences between cases of congenital and acquired haemolytic anaemia. This test determines the red cell agglutinating effect of serum from rabbits immunized against human serum globulins. Agglutination of the sensitized cells with a rabbit anti-human-globulin serum is taken to mean that the red cells have adsorbed an immune antibody globulin from the plasma; this adsorbed globulin reacts with the antiglobulin rabbit antibody causing agglutination. Boorman and others (1946) demonstrated that washed red cells from patients with acquired haemolytic anaemia were agglutinated with rabbit anti-human-globulin serum, whereas similarly treated cells in seventeen cases of congenital haemolytic anaemia were not. Evans and others (1947) found that the red cells of two patients with acquired haemolytic anaemia were readily agglutinated by an immune rabbit serum containing an anti-human-globulin antibody. Moreover, the antibody adsorbed on the sensitized red cells could be eluted and reabsorbed on to normal group O cells.

The antibody has been shown to be lipoidophilic, but has never been identified as due to any specific antigen. Under the title of "symptomatic haemolytic anaemia" many cases in the older literature are attributed to such causes as syphilis, tuberculosis, streptococcal septicaemia, paratyphoid fever, and cirrhosis of the liver; and more recently haemolytic anaemias have been described in association with Hodgkin's disease, sarcoma of the spleen, reticulo-endotheliosis, leukaemia, sarcoid, carcinoma of the pancreas, and Salmonella infection.

This case of haemolytic anaemia is reported because of the observation that in a patient with a history strongly suggestive of syphilis the eluted antibody gave a positive Kahn reaction even

though the serum Kahn and Wassermann were both negative. The subsequent improvement with antisyphilitic treatment affords further support to the specific aetiology of the haemolytic process.

Case Report

The patient was a male aged 69 years, and married. He was admitted to Addenbrooke's Hospital, Cambridge, on May 21, 1946, complaining of shortness of breath and general weakness since May, 1944. There was no relevant family history.

In 1922, in Marseilles, he had contracted a venereal infection which had resulted in a swelling on his penis. This was treated by a French doctor with local application of lotion, and the swelling disappeared. He was not given any injections, and there is no record of a Wassermann reaction having been done.

In 1936 he had a swelling of the right side of his scrotum, which was accompanied by a slight pain in the right testicle. He was treated with lotion, and the swelling disappeared.

In 1938 a friend noticed that he was jaundiced. He felt well, and he had neither nausea nor loss of appetite, but he thought he ought to remain in bed until the yellow colour had disappeared, which it did in three weeks. He had no recollection of the colour of his stools or urine at this time.

In 1939 he had a vesicular eruption on the right side of the face which lasted six weeks and left a residual scarring.

The present illness began in February, 1944, when for a brief period he felt tired and breathless. He saw his doctor, but the condition improved rapidly and he returned to work. By May, 1944, he found he was again getting short of breath when walking and he felt tired, especially after going upstairs, but these symptoms did not progress. He had had no tingling in hands or feet, and he had been free from cough, palpitations, and pain in the chest. He was given daily liver injections by his doctor. After these he noticed that his urine was red and soupy with sediment. This continued for the next nine to twelve months, but his urine had been orange-yellow during the year before admission. His stools for the same period had been yellow ochre in colour, bulkier than formerly, and offensive. He had not noticed any greasiness in them.

* Elmore Research Student

TABLE I
BLOOD EXAMINATION: MAY 21, 1946

Red cells	1.68 millions/c.mm.
Haemoglobin	6.15 g./100 ml.
M.C. Hb	36.6 $\gamma\gamma$
M.C.V.	116.1 μ
M.C. Hb conc.	31.5%
Reticulocytes	58%
Leucocytes	11.9 thousands/c.mm.
<i>Differential W.B.C.</i>	
Neutrophils:	
Myelocytes	None
Juvenile cells	12/c.mm.
Stab cells	477 "
Segmented cells	8,805 "
Eosinophils	595 "
Basophils	298 "
Lymphocytes (large)	665 "
Lymphocytes (small)	1,012 "
Monocytes	59 "
Platelets	487,000 c.mm.
<i>Film:</i>	
Stippled cells	Moderate
Anisocytosis	Moderate
Poikilocytosis	Slight
Macrocytosis	Moderate
Abnormal cells	
Normoblasts	119/c.mm.
Arneth 1	5%
" 2	11%
" 3	29%
" 4	35%
" 5	20%

His doctor had noticed yellowness in his eyes for two years, but he himself had never noticed a similar colour in his skin during this period. He had had no bleeding from the gums or nose, and no bruising or spots, nor had he noticed any increased tendency to bleed. Since December, 1945, he had had a gnawing pain in the upper abdomen which came on two or three hours after meals. The pain began in the upper left quadrant and radiated over to the left side or occasionally over the middle upper abdomen. The pain was relieved by taking further food and by alkalis. Despite this new complaint he had felt that during the past five months his breathlessness and tiredness had been better.

He had increased frequency of micturition at night and a marked decrease in his exercise tolerance.

When examined, his conjunctivae were yellow and he had a mild icteric tinge to his skin. His mucous membranes were pale, but he had no angular stomatitis and his tongue was natural. His pupils were normal and reacted to light and accommodation, and no abnormality in the central nervous system was detected. His respiratory and cardiovascular systems were normal.

There was some distension of the abdomen without any tenderness, and the liver was enlarged from one to

two fingers' breadth below the costal margin and was not tender to deep palpation. His spleen, palpable below the costal margin, was firm but not tender. No other abnormal masses were felt, and no definite scarring could be detected on the penis. There were no significant lymph nodes palpable. He had a staphylococcal urinary infection which responded to treatment with sulphathiazole.

Special Investigations.—Electrocardiograms and radiographs of chest, abdomen, and bones were normal.

Urine.—Reaction, acid; specific gravity, 1019; sugar, nil; albumin, a trace; urobilin, strong positive. Deposit showed 50 red blood cells and 10 leucocytes per field, no casts or crystals, but profuse staphylococci.

Faeces.—Yellow ochre in colour; urobilin, 1.940 mg. per 100 g.

Blood.—N.P.N., 51 mg. per 100 ml.; total proteins, 6.4 mg.; albumin, 4.3 mg.; globulin, 2.1 mg.; fibrinogen, 0.46 g.; cholesterol, 140 mg.

Laboratory investigations showed that the van den Bergh test was a direct negative, but the quantitative test gave a reading of 4.0 mg. azobilirubin per 100 ml. of serum.

The results of a full blood examination are set out in Table I. The red cell distribution curve is shown

FIG. 1.—Red cell distribution curve. Arithmetic mean = 7.23μ ; standard deviation $\sigma = 0.965 \mu$; coefficient of variation 13.3 per cent.

FIG. 2.—Quantitative fragility test.

FIG. 3.—Sedimentation rate.

in Fig. 1, the results of the fragility test in Fig. 2, and the sedimentation rate in Fig. 3.

The Wassermann and Kahn reactions were negative, as also were the Donath-Landsteiner reactions. The blood was group B and Rh-negative. There

was slight autohaemolysis when the patient's cells were in contact with his fresh serum at room temperature.

The patient's cells were washed three times in saline and added to rabbit antiglobulin serum; this gave a strong agglutination; that is, the antibody, γ globulin, was absorbed on the patient's cells.

The patient's serum (freshly centrifuged immediately after withdrawal) was absorbed by cells of the same group, which were then washed three times in saline. When these cells were added to rabbit antiglobulin serum there was slight agglutination only (that is, there was a trace of antibody in the serum).

The Kahn reaction, on the eluate obtained after preliminary heating of the patient's cells in contact with saline at 56° C. for 30 minutes, was positive ++. The Wassermann reaction remained negative. Controls with normal cells under similar conditions were negative.

Saline eluate obtained from the patient's cells was added to washed group O normal cells. These group O cells agglutinated with anti-globulin serum at 37° C. at room temperature, and at 4° C.

Progress.—The staphylococcal urinary infection improved with sulphathiazole. The patient was given a course of iodides and bismuth as it was considered that arsenic or penicillin might provoke a serious Herxheimer reaction and it was recommended that he be given a series of transfusions. For this purpose he went to Southmead Hospital in July, 1946. At this time his haemoglobin was 5.6 g. A transfusion of 1 pint of whole blood and 2 pints of packed cells raised his haemoglobin to 10.9 g., the transfusion being without reaction or abnormal incident. The transfused cells did not survive long in his circulation, and seven days later the haemoglobin level had again dropped to 6.7 g.

Twelve days after the first transfusion he was given a further transfusion of 3 pints of packed cells, again without reaction, and his haemoglobin level rose to 9.5 g., but a fortnight later was back at 7.4 g.

He had no further transfusions until September, 1946, when he had two, each of 1 pint of whole blood. The first pint raised his haemoglobin level from 6.0 to 7.0 g., and a week after the second pint his haemoglobin was 7.7 g. From that time onwards his anaemia improved and his haemoglobin level showed a steady rise up to 10.5 g. on Nov. 1, 1946 (Fig. 4). Every time blood was taken it was tested with rabbit anti-human-globulin serum, and it was always strongly agglutinated. He was seen at intervals subsequently, the last time being on May 26, when his haemoglobin was 12.7 g. and the red cell count 3.64 million per c.mm. of blood. On May 31 an estimation of the sternal bone marrow was made, and the results are summarized in Table II. Apart from some complaints of epigastric discomfort he was free from unpleasant symptoms and there had been no relapse, although the haemolytic process was still to some extent active.

FIG. 4.—Haemoglobin levels: data supplied by Tovey and Hackett.

Discussion

This case had been previously diagnosed and treated for several years as refractory pernicious anaemia, though when the patient was admitted to Addenbrooke's Hospital the appearances were those of severe haemolytic anaemia with a macrocytic tendency, the bone marrow being of the macronormoblastic type. The red-cell distribution curve showed double peaks, the spherocytic cells being microcytic. The plasma, urine, and

faeces were those of haemolytic anaemia. This clearly illustrates that the haemolytic syndrome may be a cause of macrocytic anaemia.

It is interesting to note that there was a staphylococcal urinary infection. This has been known to produce a haemolytic anaemia, but in this case the treatment of the urinary infection left the haemolytic process unaffected and it appears probable, therefore, that this infection was not the aetiological factor.

The history of venereal disease, and the fact that a lipoidophilic antibody is present in syphilis, suggests as a possible explanation of the haemolytic process that the invasion of the erythropoietic tissue by the *Treponema pallidum* resulted in the production of an antibody sufficiently specific to be selectively adsorbed on to the red cells and yet capable of producing a positive Kahn reaction. The fact that the antibodies were almost completely adsorbed on to the red cells may explain why the Wassermann and Kahn reactions were

the red cells by means of saline at 56° C. The antibody could then be reabsorbed on to heterologous red cells. This eluted antibody caused marked flocculation of the Kahn antigen as performed in the standard Kahn reaction. Controls using normal red cells under the same conditions produced negative results. Moreover, the washed red cells of the patient were strongly agglutinated with rabbit anti-human-globulin serum. Coombs (1947) examined the red cells from ten consecutive patients diagnosed as syphilitics with positive Wassermann reactions and no haemolytic anaemia. He found no evidence of red cell sensitization and he observed similar negative findings in three cases of pernicious anaemia and in one case of macrocytic anaemia associated with chronic nephritis.

It is unusual for an acquired haemolytic anaemia of such long standing and in a patient of this age to have responded to a few transfusions. Following the initial rapid haemolysis of the transfused cells there has been a continuous and steady improvement in the patient's condition. It is reasonable to assume that the antisyphilitic treatment played a significant role in this recovery.

Summary

A case of acquired haemolytic anaemia in an old subject with a probable syphilitic aetiology was found to respond to simple antisyphilitic treatment and a few transfusions. The syphilitic reaction was demonstrated by eluting the antibody.

We are grateful to Sir Lionel Whitby for allowing us to publish a report of this case, which was under his care, and to Dr. Tovey and Dr. Hackett, South-West Regional Transfusion Centre, for the follow-up data.

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TABLE II

STERNAL BONE MARROW: MAY 31, 1946

Total nucleated cell count	187,000/c.mm.
LEUCOCYTE SERIES:	%
Myeloblast	0.5
Promyelocyte	1.0
Neutrophil:	
Myelocyte	5.75
Juvenile	5.5
Mature	16.25
Eosinophil:	
Myelocyte	1.5
Mature	1.5
Basophil:	
Myelocyte	—
Mature	0.25
Lymphocyte	3.0
Monocyte	—
Plasma cells	1.75
NUCLEATED RED CELLS:	
Haemocytoblast	1.75
Pro-erythroblast	3.5
Early normoblast	30.0
Intermed. normoblast }	
Late normoblast	
Intermed. megaloblast }	21.25
Late megaloblast	6.5
Myeloid: erythroid ratio ..	1:2

both negative. Very little antibody was found in the serum, but it could be eluted readily from