SYPHILITIC PAROXYSMAL COLD HAEMOGLOBINURIA

CASE REPORT AND STUDY OF THE COOMBS TEST

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A case of congenital syphilis which presented with paroxysmal cold haemoglobinuria is described.

The patient had aortic incompetence, and the significance of this finding is discussed.

The Coombs test was studied, and positive results appear to be due to interaction between antiglobulin serum and complement adsorbed on to the test cells, this adsorption being promoted by the cold antibodies.

Syphilitic paroxysmal cold haemoglobinuria is an uncommon condition, and, unlike many of the other haemolytic diseases, the serological mechanisms involved are by no means clear. Thus, according to Darmady and Davenport (1954), the Coombs test is negative, although Jordan, Pillemer, and Dingle (1951) had stated that in the presence of an excess of complement the indirect Coombs test is positive. Dacie (1954) thought that in this condition the optimum concentration of antiglobulin serum was considerably greater than that needed for routine blood grouping, and some batches would therefore give false negative results.

Dacie, Crookston, and Christenson (1957) studied a group of patients with cold incomplete antibodies, and considered that a positive Coombs test was due to interaction between the antiglobulin serum and the cold antibody. They studied one patient with cold haemoglobinuria and Donath-Landsteiner antibodies, and found that he behaved similarly.

We report here a patient with congenital syphilis, who presented with paroxysmal cold haemoglobinuria and in whom detailed studies of the Coombs test were made.

Case Report

Private T.C., aged 19 years, was admitted to Catterick Military Hospital in April, 1957, because he had been passing red urine.

He had been perfectly well until the age of 14 when, after playing in the snow for two or three hours, he felt generally unwell, hot and shivery, and when he

passed urine he saw that it was dark red. There was no frequency or dysuria, no loin pain, and he did not think that he was jaundiced. Over the next 36 hours the urine gradually cleared, but he was admitted to the local hospital for investigation. Urine examination showed no abnormality, and, although an intravenous pyelogram had failed to outline the right upper calyces, retrograde pyelography showed no abnormality. Cystoscopy was normal.

Several weeks later, again following exposure to cold, when he had been shovelling snow, he passed red urine for about 12 hours. When aged 15 he left school and started work in a railway workshop. While so employed he suffered three further attacks in which he passed red urine, each one following exposure to cold. The patient had been so sure of this relationship that he had changed his job for one in the warmer surroundings of a printing works.

In March, 1957, he was called up for National Service and passed the routine medical examinations satisfactorily. One week later, however, following a three-hour spell of fatigues during which time he was peeling potatoes, and his hands were immersed in cold water, he again felt unwell, shivered, and passed red urine. He was admitted to a surgical ward, but no abnormality was found on clinical examination; as the urine did not contain red cells no further investigations were done and he was returned to his unit. Two days before his readmission he had again been working in the open air on a cold day and had thereafter passed dark urine.

There was no other relevant history and, in particular, there was no illness resembling acute rheumatism. His parents, aged 42, were both well, as were his 16-year-old brother and 14-year-old sister. His mother had had no miscarriages or stillbirths.

Examination showed a fit-looking boy, of below-average intelligence and of average physique. The

upper incisor teeth were peg-shaped, but the molars were normal. Other relevant findings were as follows:

Cardiovascular System.—Blood pressure was 145/55 mm. Hg. No cardiac enlargement was found, but a loud diminuendo diastolic murmur was heard down the left sternal border. A chest film and screening were normal, as was an electrocardiogram.

Central Nervous System.—The pupils were equal and regular, reacting well on convergence but very sluggishly to light. Ophthalmoscopic examination showed signs of old choroido-retinitis. Reflexes were normal.

Laboratory Investigations.—In order to carry out any test involving serum, it was necessary to allow separation to take place at 37° C. to prevent haemolysis.

Haematological Investigation.—Haemoglobin was 94% (13.9 g./100 ml.), white cells 7,600/c.mm. (differential count normal), red cells normochromic and normocytic, reticulocytes 0.6%.

Urine.—On admission the urine was dark brown and free from red cells, but spectroscopy showed strong absorption bands of oxyhaemoglobin. Twenty-four hours after admission the urine was normal.

Serum bilirubin was less than 0.5 mg./100 ml.

Schumm's test for methaemalbumin was positive.

Ham's test was negative.

Cold agglutinin titre was 1 in 8.

Serum protein electrophoresis was normal.

The Wassermann reaction was positive 1 in 320, and Kahn and Price precipitation reactions were positive 320 u.

A *Treponema pallidum* immobilization test was positive. The cerebrospinal fluid contained 4 lymphocytes/c.mm., protein 110 mg./100 ml. The Lange curve was 1555210000. The Wassermann reaction was positive 1 in 80.

Cooling the limbs by immersion in ice-cold water for 20 minutes was followed by the appearance, 30 minutes later, of haemoglobin in the urine. This had disappeared by three hours. The serum bilirubin level rose to 5.3 mg./100 ml. two hours after cooling, but had returned to normal levels within 24 hours. The patient was symptom-free throughout, and there was no change in the peripheral blood count.

The results of studies on the Donath-Landsteiner and Coombs tests, which were positive throughout, are described below.

Investigation of Patient's Family.—Blood taken from the patient's mother gave a positive Wassermann reaction, but that from the two younger children was negative. The father refused to have the test performed.

Treatment.—The patient was given bismuth, 0.2 g. intramuscularly, twice weekly, and a mixture of potassium iodide, gr. 10, and liquor hydrarg. perchlor. B.P., 1 drachm three times daily, for two weeks.

Subsequently procaine penicillin, 1 mega unit, was given intramuscularly daily for three weeks. The patient remained perfectly well throughout the course of treatment, and indeed throughout his entire stay in hospital.

After treatment he was boarded out of the Army and returned home. During the following year he remained symptom-free, with no recurrence of the haemoglobinuria.

Special Tests

Donath-Landsteiner Reaction.—Results obtained with serum from this case confirmed the results of other workers. The methods used were those described by Darmady and Davenport (1954).

(1) The maximum titre observed at any time was 1 in 8 serum dilution. (2) Negative results were consistently observed unless complement was present in the cold phase as well as in the warm phase. (3) Although positive results were obtained when sensitization was carried out at 4° C., no haemolysis was observed if sensitization was carried out at 22° C. and 37° C.

The Coombs Test.—Patient's serum was used fresh after separation at 37° C. or subsequently heated for half an hour at 56° C.

Normal fresh human serum was used as complement absorbed three times with fresh Group O cells at 4° C. for one hour.

Guinea-pig complement was pooled, normal, fresh serum absorbed three times with fresh Group O cells at 4° C.

Three batches of rabbit antihuman globulin serum from different sources were employed. These had all proved equally satisfactory in the identification of rhesus antibodies.

Anti-guinea-pig globulin serum was inactivated at 56° C. for half an hour, diluted, and absorbed three times with Group O cells at 4° C.

For test cells, fresh Group O cells, washed and made up to a 2% suspension, were used.

Human γ globulin was the preparation supplied for prophylactic immunization by the Lister Institute.

The methods described by Dacie (1954) and Dacie, Crookston, and Christenson (1957) were used.

Results

Effect of Different Batches of Antiglobulin Serum.—Three different batches of antiglobulin serum, A, B, and C, were used. The sera were first used at a dilution which was optimal for Rhesus testing; with antiglobulin serum A the direct Coombs test on the patient's cells was negative, with serum B it was doubtful, and with serum C unequivocally positive. The antiglobulin sera were then used at a higher concentration (a dilution which was just sufficient to ensure that unsensitized cells were not agglutin-

ated). Sera A and B now gave an unequivocally positive direct Coombs test, and the positive test previously obtained with serum C became stronger (judged by a more rapid agglutination and the appearance of larger cell aggregates). Similar results were obtained in the indirect Coombs test. With test cells sensitized in the cold by undiluted patient's serum, a positive Coombs test was obtained with all three antiglobulin sera, provided that they were used in the more concentrated form, the strongest result being obtained with serum C. When the test cells were sensitized with the patient's serum diluted 1/16, only antiglobulin serum C gave a positive result.

Effect of Variation in Temperature During Sensitization.—Using fresh normal patient's serum, without added complement, it was shown that a positive indirect Coombs test could best be demonstrated if the sensitization was performed at 0° C., was just present at 12° C., but was negative if sensitized at 20° C. and 27° C.

Need for Complement.—When heated patient's serum was used alone in an attempt to sensitize the cells, the Coombs test was negative. However, if doubling dilutions of the heated patient's serum were carried out in fresh human serum, which provided complement, the Coombs test was positive to a titre of 1/16. Adequate amounts of complement were present in the fresh unheated patient's serum because this same titre was obtained when test cells were sensitized with dilutions of unheated serum in saline, and it was not enhanced by the addition of normal serum as a further source of complement.

Effect of γ Globulin Added to Antiglobulin Serum.—The effect of adding increasing dilutions of human γ globulin to the antiglobulin serum used in the direct Coombs test on the patient's cells was studied and compared with the effect on the indirect Coombs test carried out on a Rhesus-sensitized system. The direct Coombs test was inhibited on the patient's cells only when a large amount of γ globulin was added, whereas with Rhesus-sensitized cells the Coombs test was inhibited by very small amounts of γ globulin. In the case of the direct Coombs test on the patient's cells, no inhibition occurred until the final dilution of the 10% γ globulin, added to the antiglobulin serum, was less than 1/32, whereas with Rhesus-sensitized cells inhibition was complete with dilution up to 1/2,000.

Indirect Coombs Test Sensitizing with Alternative Sources of Complement.—Test cells were sensitized by doubling dilutions of the patient's

heated serum carried out first in fresh human serum and then in fresh guinea-pig serum as alternative sources of complement. The Coombs test was carried out in the normal way, using antihuman globulin serum, and also by substituting for the antihuman serum a rabbit serum prepared against guinea-pig serum (anti-guinea-pig globulin serum). The results are set out in the Table. When human complement was used

TABLE
AGGLUTINATION BY ANTIGLOBULIN SERA OF RED CELLS SENSITIZED BY DILUTIONS OF PATIENT'S HEATED SERUM CARRIED OUT WITH HUMAN OR GUINEA-PIG SERUM AS COMPLEMENT

Antiglobulin Serum	Serum Added to Antiglobulin Serum	Serum Used as Source of Complement	Dilution of Patient's Heated Serum Used for Sensitization				
			1/2	1/4	1/8	1/16	1/32
Antihuman	None	Human	++	++	++	+	—
Anti-guinea-pig			++	++	+	—	—
Antihuman	Guinea-pig	"	++	++	++	+	—
Anti-guinea-pig			++	++	+	—	—
Antihuman	Human	Guinea-pig	++	+	—	—	—
Anti-guinea-pig	None		++	++	++	+	—
Antihuman	"	"	++	++	++	+	—
Anti-guinea-pig			++	++	+	—	—
Antihuman	Guinea-pig	"	—	+	—	—	—
Anti-guinea-pig	Human	"	++	++	+	—	—
			+	+			

during sensitization, there was strong agglutination with antihuman serum and a weaker reaction with anti-guinea-pig serum. When guinea-pig complement was used, the strength of the reaction was reversed, the strong reaction occurring with the anti-guinea-pig serum.

The tests were then repeated using antihuman globulin serum to which guinea-pig serum had been added, and anti-guinea-pig globulin serum to which human serum had been added. The effect was to abolish the weaker reactions altogether, while leaving the strong reaction unchanged. It was concluded that the weak reactions were presumably due to cross-reactions between the antiglobulin serum and heterologous serum components.

Discussion

From the clinical viewpoint, this case presents two interesting features. The first of these relates to the patient's previous history, for it will be recalled that he was investigated on two occasions for "haematuria." This is a common story in such patients, and serves to emphasize the importance of examining the urine for pigments as well as red cells before discounting the patient's story. The second, and more important, feature was the presence of a diminuendo diastolic bruit

and wide pulse pressure. It seems possible that this patient had syphilitic aortic incompetence, as there was no evidence of previous acute rheumatism, yet there was clear evidence that he had congenital syphilis, and that his teeth, central nervous system, retinae, and antigen-antibody mechanisms were affected. There is no conclusive recorded evidence that such patients develop aortitis, but Bowie and Simpson (1953) described a 38-year-old male congenital syphilitic with aortic incompetence. Although their patient had had rheumatic fever at the age of 19, an aortic aneurysm was present, and the evidence was strongly in favour of a diagnosis of syphilitic aortitis.

The serological studies showed that our patient's Donath-Landsteiner antibody behaved in the Coombs test in a similar manner to the cold antibodies occurring in other conditions (Dacie *et al.*, 1954). Thus the positive Coombs test was due to the interaction of the antiglobulin serum with complement adsorbed on to the test cells. The Donath-Landsteiner antibody did not appear to interact with the antiglobulin serum but exerted its effect by causing complement to be adsorbed on to the cells.

This concept is consistent with some of the other findings: the importance of using anti-globulin serum at a higher concentration than the usual optimum for Rhesus testing; the variability of the results of the Coombs test with different batches of serum, and the persistence of positive results after the addition of γ globulin to the antiglobulin serum. These apparently anomalous results accord well with the idea that it is some additional antibody, other than the specific anti-globulin antibody, that is responsible for the positive results observed.

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