A POSITIVE COOMBS REACTION IN PERNICIOUS ANAEMIA

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Following the description by Dacie (1950) of cold "incomplete" auto-antibodies which exist in the sera of many apparently normal people, it may be of interest to record a case of anaemia in which such an antibody caused a temporary haematological puzzle.

As reported by Dacie, these antibodies can be demonstrated if a suspension of red blood cells in their own serum is chilled in a refrigerator (2–5° C.) for about one hour, and the red cells are washed three times in saline at 37° C. to remove the serum and to elute any cold auto-agglutinins and then mixed with anti-human-globulin rabbit serum for a Coombs test (Coombs *et al.*, 1945a, 1945b). If present, the incomplete auto-antibodies are apparently not eluted at 37° C., and their adsorption on the red cells is shown by a positive result. Sometimes a positive Coombs reaction can be obtained if a weak cell suspension in serum is allowed to stand at room temperature. Heating a serum at 56° C. for 5–30 minutes prevents the action of the antibodies on subsequent chilling.

Case Report

A 38-year-old farm labourer, with a three-months history of increasing weakness and breathlessness, was sent to hospital for a blood examination. Two years previously, at the Dorset County Hospital, Dorchester, he was found to have a macrocytic anaemia with poikilocytosis and anisocytosis, histamine-fast achlorhydria, a megaloblastic marrow, normal red-cell osmotic fragility, slightly raised serum bilirubin, and absence of reticulocytes. Pernicious anaemia was diagnosed, and the blood showed a satisfactory return to normal after injections of liver. Later he was given oral proteolysed liver for maintenance, but stopped this of his own accord early in 1949.

When first seen in January, 1950, he showed marked pallor with no other abnormal physical signs, and his blood picture was: haemoglobin, 31% (4.6 g./100 ml.); red cells, 1,310,000 per c.mm.; colour index, 1.2; leucocytes, 5,100 per c.mm. (normal differential); reticulocytes, < 1%; nucleated red cells, 8 per 100 leucocytes; P.C.V., 16%; M.C.V., 122 μ^3; M.C.H.C., 28%; serum bilirubin, 1 mg. per 100 ml. The red cells showed marked anisocytosis and poikilocytosis, and also marked auto-agglutination at room temperature.

In view of the previous findings the diagnosis of pernicious anaemia in relapse was accepted, and liver injections were started immediately. The finding of auto-agglutination caused us to do further tests, the results of which are shown in Table I. On January 26 the red-cell osmotic fragility (Dacie and Vaughan, 1938) was found to be normal, haemolysis beginning in 0.42% NaCl and being complete in 0.28%.

The patient was seen at the Westminster Memorial Hospital, Shaftesbury, so that, although the cell counts were done the same day, the clotted blood samples for the cold auto-agglutination and Coombs tests, on reaching Salisbury in the evening, were placed overnight in a refrigerator. The samples were allowed to return to room temperature before testing. The cold auto-agglutinin in the serum was titrated against the patient's cells in tubes with saline dilutions of the serum for two hours at 4° C. and the titre determined microscopically. Red cells removed from the clot were washed three times with saline at 37° C., and the direct Coombs test was performed on a tile at room temperature. The Coombs serum, prepared at this laboratory, had a titre of 1,000 against "Rh-coated" cells, contained no detectable red-cell agglutinins, and was used in a 1/30 saline dilution. The agglutination obtained by gentle rocking on a tile for five minutes was classified thus:

+ + + : Agglutination rapidly appearing and proceeding to complete agglutination.

+ + : Agglutination appearing at about 1 minute and becoming marked.

+ : Agglutination appearing at about 2 minutes.

Weak + : Agglutination appearing at about 3 minutes.

TABLE I

Date	Hb % (100% = 14·8 g./100 ml.)	Red Cells (mil./c.mm.)	P.C.V.%	Reticulocytes %	Wassermann	Kahn	Titre of Cold Auto-agglutinin	Direct Coombs Test*
Jan. 20	31	1·31†	16	< 1			32	+ +
,, 24	40	1·60†	20·5	11·5				
,, 26	50	2·27‡	27	26·2	+		32	+ + (90% positive)
Feb. 3	58	2·67‡			+ +	+	32	
,, 17	70	4·14		< 1	± ±	+	8–16	+ (52% positive)
March 3	70	3·76		< 1	+		2–4	+ (58% positive)
,, 17	84	4·42	42		± ±	±	2	Weak +
,, 30	84	4·71		< 1	± ±	−		Weak + (33% positive)
April 6	90	4·92			−	−	2	Very weak +
May 4	90	5·04						
June 1	94	4·72						
,, 29	100	5·08						—
July 28	90	4·98						—
Sept. 18	104	5·06					2–4	—

* Cells from clotted samples after overnight refrigeration (see text).
† Strong auto-agglutination at room temperature.
‡ Weak auto-agglutination at room temperature.
The percentages in parentheses are the proportion of cells determined to be "Coombs positive" by a differential agglutination technique (Selwyn and Hackett, 1949).

In view of the positive results obtained in the Wassermann, Kahn, Coombs, and cold auto-agglutination tests, we wondered if an acquired haemolytic anaemia was present, secondary to a syphilitic infection, and whether the absence of reticulocytes was due to depression of bone-marrow activity. (A positive direct Coombs reaction has now been described in many cases of acquired haemolytic anaemia—Boorman *et al.*, 1946; Loutit and Mollison, 1946; Sturgeon, 1947; Wagley *et al.*, 1948; Evans and Duane, 1949.) But with liver injections as the only treatment a reticulocytosis appeared and disappeared again, whilst the red cells increased in number—a rise of 1,360,000 per c.mm. in the first two weeks, which conforms to an "average response" (Della Vida, 1942) of pernicious anaemia to liver therapy. As the red-cell count increased, the serological findings became weaker, though they had yet to be explained, as the diagnosis of pernicious anaemia still seemed to be correct.

On March 30 further tests were done (as suggested by Dr. P. L. Mollison): blood was taken into a warmed syringe and a few drops were immediately mixed with saline at 37° C. to form a 2% cell suspension. These cells, after three washes in saline at 37° C., gave a negative direct Coombs reaction. The remainder of the blood, after clotting and standing at 4° C. for one hour, yielded cells giving a weak positive Coombs reaction.

On September 18, when the red-cell picture had returned to normal, cells and serum were removed from a clotted blood sample at room temperature without previous refrigeration and were tested as shown in Table II. It was found that a "coating" antibody active at 4° C. was still present, but a higher serum–cell proportion than that in the whole blood was needed for its demonstration. Also, heating the serum at 56° C. prevented the action of this antibody.

TABLE II

	Test Mixtures Incubated at 4° C. for 1 Hour, the Red Cells then Washed Three Times at 37° C.	Coombs Test on Tile
1	5 drops 5% cell suspension in saline	−
2	5 drops 5% cell suspension in saline + 5 drops fresh serum	+
3	5 drops 5% cell suspension in saline + 5 drops serum after incubation at 56° C. for 5 minutes	−
4	5 drops 5% cell suspension in saline made from clotted sample after 1 hour at 4° C. ..	−

The results of these further tests led us to believe that the patient's serum contained a cold incomplete auto-antibody, which caused the positive Coombs reactions on the patient's cells through the clotted blood samples' being chilled in a refrigerator. Moreover, it seems that the potency of both the cold auto-agglutinin and the incomplete auto-antibody was increased during the period of anaemia, when the erythropoietic system was deficient in the liver factor. This deficiency was also associated with positive Wassermann and Kahn reactions by the serum. The patient's history contains no suggestion of a syphilitic infection, and we can offer no explanation of these findings except to postulate that, as the liver-factor deficiency in some way caused an increased production of a non-specific haem-agglutinin and a presumably non-specific incomplete red-cell antibody, it also caused the production of a non-specific antibody capable of giving positive Wassermann and Kahn reactions. As these latter reactions became negative with no treatment except liver injections, we feel they should be regarded as false. We are aware of a comparable case (Rubinstein, 1948)—one of acute haemolytic anaemia with transient positive Wassermann reactions during a haemolytic crisis. In cases of primary atypical or virus pneumonia the serum often contains strong cold auto-agglutinins and occasionally gives transient positive syphilitic serological reactions (Florman and Weiss, 1945). It seems reasonable to expect that factors causing a rise in titre of cold auto-agglutinins would cause a parallel increase in the cold incomplete auto-antibodies, and so one might find such a combination of serological reactions in a case of virus pneumonia as we found in our case. But the clinical picture of our case at no time suggested a virus pneumonia, and the diagnosis remains as pernicious anaemia in relapse. The patient has now felt perfectly fit and the blood has remained normal for the past six months with maintenance liver injections.

Finally, we wish to draw attention to the added complication of a direct Coombs reaction on the red cells of a patient with suspected acquired haemolytic anaemia, in that one must now ensure that a positive result is not due to cold incomplete auto-antibodies in the patient's serum.

Summary

Details are given of a case of pernicious anaemia in relapse, in which the serum gave false positive Wassermann and Kahn reactions and contained a cold incomplete auto-antibody causing the red cells to give a positive Coombs reaction.

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A CASE OF PAINFUL SWELLING OF THE HAND

BY

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There is no intention to suggest, even by implication, that the surgical procedures described below constitute a cure for more than a very occasional case of poly-arthritis or of neuritis of the upper limb; but so dramatic a release from pain and swelling and so rapid a return of function are worthy of record.

Case History

The patient, a woman aged 56, had been vigorous and healthy before 1941, living in the cold dry air of the north-east of England. In 1941, in an air raid, she received a severe emotional shock, and from this time her general health began to deteriorate. In 1943 she felt tingling sensations in the fingers of both hands. The pain was intermittent and worse at night. A few months after its onset the nocturnal pain became so severe that she was unable to sleep. Her hands were always red and, in her own words, began to look old. She felt as if two heavy weights were tied to her wrists. Because of the swelling of her hands she was advised to become a blood donor, and during the next few years she gave six blood donations. Repeated venesection did not give relief. Very soon the inability to get a good night's rest sapped her general health, and as a result she became emotional and psychoneurosis was suspected.

Within a few months of the onset of the illness the pain had travelled to the palms of the hands, up the arms to the shoulder-joints, and across the front of the chest and back. By 1946 she found it impossible to lie on either side while in bed, the slightest pressure against her shoulders being intolerable. No special position gave relief. From 1946 to 1948 the condition slowly worsened, and by 1948 both her hands were swollen and painful. As the swelling and the pain in the right hand increased, the condition in the left hand improved, and was resolved by June, 1949. Full investigations had been carried out in another hospital, and the blood sedimentation rate was found to be normal on two occasions.

When I saw her in October, 1949, the whole of her right arm was swollen, the shape of the hand being as shown in Fig. 1. Movement at any joint from the shoulder downwards

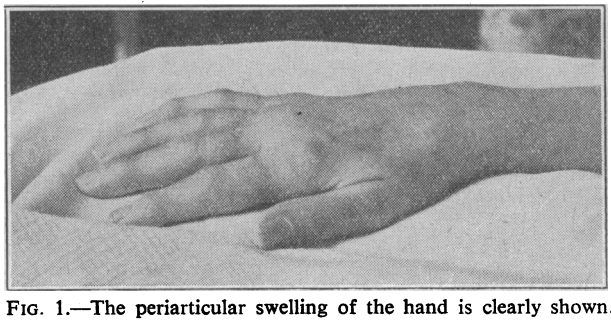

FIG. 1.—The periarticular swelling of the hand is clearly shown.