A Modification of the Schleicher Technique for Detecting the "Erythrocyte Aggregation Factor" in Serum

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The "erythrocyte aggregation factor" in the plasma and serum of patients with acute lupus erythematosus demonstrated by Schleicher (*1*) could be detected by using a cold conglutination technique. In the sera of 17 out of 17 patients with the acute form of the disease this factor was present. Further, it could be shown that the EAF is present in clinical entities other than lupus erythematosus. It is of interest that strongly positive sera also gave positive liver function tests (Table 1).

The standard routine conglutination technique as used for blood crossmatching was employed for the demonstration of the erythrocyte aggregation factor. Commercial bovine albumin[2] was used in place of fresh egg white. The sodium chloride diluent was also omitted except in making the red blood cell suspension. The cold conglutination test is done as follows: In a Wassermann test tube are placed 3 drops of commercial albumin, 2 drops of the test serum, and 2 drops of a 10% washed red blood cell suspension in saline. The erythrocytes should be washed three times in saline before using them for the test. Group "O" Rh-positive red blood cells or the patient's cells can be used. The test is refrigerated for 2–24 hr. Immediately upon removal from the refrigerator, the test is centrifuged at 2,000 rpm for 1 min and read macroscopically as a cold agglutination test.

Controls should be run simultaneously, since clumping of erythrocytes may occur in negative sera. In a negative test, however, the cell clumps are easily broken up by gentle shaking. The clumping phenomenon in positive sera disappears at room temperature, but reappears when the test is placed in the refrigerator for 2–24 hr. Strongly positive sera caused clumping of the red blood cells in serum dilutions of 1:8 or 1:16. Sterile sera that gave a strong positive test gave a weak positive test when kept at room or refrigerator temperature for several days. Sterile negative serum incubated with *Staphylococcus aureus* or *Escherichia coli* cultures for 12 hr failed to produce a positive test, but in some instances a positive cephalin flocculation test resulted.

TABLE 1

RESULTS OF COLD CONGLUTINATION TEST IN ACUTE LUPUS ERYTHEMATOSUS AND OTHER CLINICAL CONDITIONS CORRELATED WITH LIVER FUNCTION TESTS

Patient	Diagnosis	Cold conglutination	Thymol turbidity	Cephalin flocculation, 48 hr
1	Lupus Erythematosus	Positive	Not done	Not done
2	" "	"	4	4+
3	" "	"	10	±
4	" "	"	10	4+
5	" "	"	6	Negative
6	Scleroderma?	"	10	4+
7	*E. coli* cystitis	"	10	4+
8	" "	"	10	4+
9	" "	"	9	3+
10*	" septicemia	"	Negative	Negative
11†	" "	"	Not done	Not done
12‡	Macrocytic hemolytic anemia	Weak positive	5	4+
13	Asthma (undetermined)	Positive	6	4+
14	Pneumonia (viral?)	"	7.5	Negative
15	Fracture of femur	"	5	3+
16	Rheumatic fever?	"	Negative	2+
17	Undiagnosed	"	7	3+
18	*Staphylococcus*, monilia infection	Negative	4	Negative
19	Infectious mononucleosis	"	Negative	±
20	Acute lymphatic leukemia	"	"	1+
21	Syphilis (Kline positive)	"	4	Negative
22	Streptococcal septicemia	"		
23§	*Staph. aureus* septicemia	"		
24§	" " "	"		
25	Hemolytic streptococcal septicemia	"		
26–200	Normal sera	"		

* 8% bromsulphalein retention.
† With methemoglobin.
‡ Methemoglobin.
§ Hemolytic, coagulase positive.

Since this report is of a preliminary nature, no correlation is attempted between the cold agglutination phenomenon and positive liver function tests. Studies are in progress to identify the factor causing the clumping of erythrocytes.

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[2] Armour Laboratories, Chicago, Ill.

Reference

1. SCHLEICHER, E. M. *Science*, **113**, 558 (1951).