AGGLUTINATION OF SENSITIZED SHEEP ERYTHROCYTES IN DISSEMINATED LUPUS ERYTHEMATOSUS

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(RECEIVED FOR PUBLICATION JANUARY 28, 1957)

In the course of our earlier investigations (Svartz, 1956a, b; Svartz and Schlossmann, 1953a, b, 1954, 1955, 1956) it was found that the cold precipitable protein fraction of serum from patients with active rheumatoid arthritis was able to agglutinate sheep erythrocytes sensitized with rabbit anti-sheep amboceptor. However, the haemagglutination titres of unfractionated sera, previously absorbed with normal sheep red cells—in the following pages denoted as whole serum—were sometimes found to be somewhat higher than those obtained with the cold precipitates, showing generally a single-tube difference in the end-point of agglutination. Comparative agglutination tests performed on cold precipitate from positive sera from patients with diseases other than rheumatoid arthritis showed haemagglutinating activity in the supernatant fluid but scarcely ever in the cold precipitable fraction. Thus, only a few cold precipitates other than those from rheumatoid arthritis showed haemagglutination, and then only those with a low titre. It should be added that in rheumatoid arthritis a varying amount of haemagglutinating factor could also be demonstrated in the supernatant fluid; the main point is that the rheumatoid factor (RF) can be precipitated in cold, whereas this happens only extremely rarely in the other collagen diseases.

On the basis of earlier observations, it seemed obvious that the haemagglutination test performed with whole serum should be considered to be a laboratory procedure of great value for making differential diagnoses between rheumatoid arthritis and other joint diseases and collagen diseases. The introduction of the haemagglutination test with cold precipitate seemed to allow of a much safer differential diagnosis between rheumatoid arthritis and other conditions. In other words it made the haemagglutination test more "specific" to rheumatoid arthritis.

Some recently published observations (Ziff, Brown, Lospalluto, Badin, and McEwen, 1956) suggest that patients with disseminated lupus

erythematosus quite frequently show clinical features of both disseminated lupus erythematosus and rheumatoid arthritis. Such patients showed positive L.E. cells as well as positive sheep red cell agglutination titres with both whole serum and cold precipitate. It should be kept in mind that the early stages of rheumatoid arthritis and atypical cases of disseminated lupus erythematosus not infrequently present diagnostic difficulties. Positive haemagglutination tests obtained with whole sera and re-dissolved cold precipitates from patients presumably suffering from disseminated lupus erythematosus with joint symptoms should be interpreted with caution. It is conceivable that such patients are suffering both from rheumatoid arthritis and lupus erythematosus, or from rheumatoid arthritis only, since L.E. cells can sometimes be demonstrated in true rheumatoid arthritis (Kievits, Goslings, and Schuit, 1956; Olhagen, 1957).

The purpose of the present paper is to report on the results of our haemagglutination tests hitherto obtained with both whole serum and cold precipitable fraction from patients in whom the diagnosis of disseminated lupus erythematosus had been ascertained through the follow-up.

Method

Cold precipitable substances may precipitate spontaneously in the refrigerated stocks, and small amounts of these substances may also precipitate during the process of clot formation. It is therefore necessary to keep the blood samples, drawn by vein puncture, at 37°C. during the period of clot formation. After this procedure the serum is separated from the clot at 20–22°C., and may be used for haemagglutination tests. The method of preparing cold precipitable fractions and testing them with sensitized sheep red cells is described elsewhere (Svartz and Schlossmann, 1954). Haemagglutination occurring in dilutions of 1:32 or more was considered to be positive in the series of whole serum, and 1:16 or more in the series performed with cold precipitate. In the present

study, a total of 64 patients with disseminated lupus erythematosus was tested.

Results

Table I shows the distribution of agglutination titres using whole serum from patients with disseminated lupus erythematosus.

When whole serum was employed for the haemagglutination reaction, 50 per cent. of the 64 patients with disseminated lupus erythematosus showed positive results. In 32 cases the serum was lacking in haemagglutinating activity or showed a haemagglutination titre below 1:32.

In Table II the haemagglutination titres obtained with the whole serum are compared with those obtained with the cold precipitable fraction. It appears that whole serum from thirty patients with disseminated lupus erythematosus showed haemagglutination titres from 1:16 to 1:1,024. Only one of the cold precipitable fractions showed a high haemagglutination titre, namely 1:256, while all other cold fractions were lacking in haemagglutinating activity or showed a titre below 1:16.

Discussion

The data in Table I demonstrate that whole serum from 32 out of 64 patients with the clinical diagnosis of disseminated lupus erythematosus showed haemagglutination titres of from 1:32 to 1:1,024. Our experience has proved that lupus erythematosus is the disease that, next to rheumatoid arthritis, shows the highest percentage of positive reactions (Svartz, 1956a, b).

Table II demonstrates the important fact that the cold precipitable fractions obtained from 29 out of thirty cases with lupus erythematosus disseminatus showed haemagglutination titres of only 1:8 or less, or failed to agglutinate sheep red cells. This pattern strongly supports the theory that a fundamental difference exists between the haemagglutinating activity of the cold precipitable fraction obtained from patients with rheumatoid arthritis and that from disseminated lupus erythematosus patients. As demonstrated by us, the haemagglutination of

TABLE II
AGGLUTINATION BY WHOLE SERUM AND COLD PRECIPITABLE FRACTIONS IN PATIENTS WITH DISSEMINATED LUPUS ERYTHEMATOSUS

Number of Controlled Samples	Agglutination Titre	
	Whole Serum	Cold Precipitable Fraction
5	1:16	0
7	1:32	0
4	1:64	0
2	1:64	1:4
2	1:128	0
2	1:128	1:4
2	1:256	0
1	1:256	1:4
1	1:256	1:8
1	1:256	1:256
1	1:512	1:4
1	1:512	1:8
1	1:1,024	0

whole sera and of cold precipitates from the same sera run parallel in rheumatoid arthritis.

It is demonstrated by our studies that, if the haemagglutination test is performed by means of cold precipitates from serum or joint exudate, the frequency of "false" positive reactions can be diminished. Thus, by means of the cold test, it seems possible to exclude from the group of rheumatoid arthritis conditions that are often otherwise clinically indistinguishable from that disease.

The frequency of haemagglutination in collagen diseases other than rheumatoid arthritis and lupus erythematosus seems to be much lower, but our material is still too small to permit the formation of a definite opinion. As far as may be judged at present, it seems probable that no collagen disease other than rheumatoid arthritis provokes haemagglutination with cold precipitate from sera. It would appear that the remarkably low haemagglutinating activity of cold precipitable fractions, as

TABLE I
AGGLUTINATION TITRES OF WHOLE SERUM IN DISSEMINATED LUPUS ERYTHEMATOSUS

Agglutination Titre	Negative Reaction			Positive Reaction						Total
	0	1:4 to 1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1,024	
Adults with Disseminated Lupus Erythematosus	6	15	11	11	8	5	5	2	1	64

compared with the results obtained with unfractionated sera in collagen diseases other than rheumatoid arthritis (Table II), provides a test which allows of a differentiation being made between these two types of conditions. A positive haemagglutination test with whole serum and a negative one with cold precipitate in a patient with joint symptoms argues in favour of the diagnosis of lupus erythematosus.

It should be noted that, in one of the patients presumably suffering from disseminated lupus erythematosus, a high haemagglutination titre of 1:256 was found with the cold precipitable fraction as well as with the whole serum. It is too early to judge, but it seems quite possible that this case may be considered to be one of disseminated lupus erythematosus with coexistent rheumatoid disease. Further evaluation of this question requires a more systematic study.

It is of interest that in one case of disseminated lupus erythematosus the whole serum showed a haemagglutination titre as high as 1:1,024, while the cold precipitable fraction entirely failed to agglutinate the sensitized sheep red cells (Table II). Nothing similar to this has ever been observed in any serum from patients with rheumatoid arthritis.

Summary

Experiments showed that 50 per cent. of whole sera from 64 patients with disseminated lupus erythematosus gave a positive agglutination reaction with sensitized sheep erythrocytes. When the sensitized sheep-cell test was performed with the cold precipitable fraction of serum instead of with whole serum the results were quite different. Thus, in those cases of lupus erythematosus in which a positive result was obtained with whole serum, the cold precipitate test was nearly always negative. Our studies provide evidence that the results of haemagglutination obtained with the cold precipitable fraction of sera from patients with disseminated lupus erythematosus differ remarkably from those obtained from patients with rheumatoid arthritis, in which the haemagglutination titres obtained with whole serum and those obtained with cold precipitate are closely parallel.

Stress is laid on the diagnostic value of the haemagglutination test by means of the cold precipitable fraction.

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Agglutination des érythrocytes sensibilisés de mouton dans le lupus érythémateux disséminé

RÉSUMÉ

Les expériences ci-dessus mentionnées ont montré que 50 % des sérums complets provenant de 64 malades atteints de lupus érythémateux disséminé donnait une réaction positive d'agglutination avec les érythrocytes sensibilisés de mouton. Quand la réaction des globules de mouton sensibilisés était faite avec la fraction précipitable froide du sérum plutôt qu'avec le sérum complet, les résultats étaient tout-à-fait différents. Ainsi, dans ces cas de lupus érythémateux dans lesquels un résultat positif était obtenu avec le sérum complet, la réaction avec le précipité froid était presque toujours négative. Nos études apportent des preuves que les résultats d'hémagglutination, obtenus avec la fraction précipitable froide de sérums provenant de cas de lupus érythémateux disséminé diffèrent remarquablement de ceux obtenus dans des cas d'arthrite rhumatoïdale, dans lesquels les titres d'hémagglutination avec le sérum complet et ceux avec le précipité froid sont étroitement parallèles.

On souligne la valeur diagnostique de la réaction d'hémagglutination à l'aide de la fraction précipitable froide.

Aglutinación de eritrocitos sensibilizados de oveja en el lupus eritematoso diseminado

SUMARIO

Las investigaciones sobredichas han revelado que el 50% de los sueros completos de 64 enfermos con lupus eritematoso diseminado dieron una reacción positiva de agglutinación con eritrocitos sensibilizados de oveja. Al proceder a esta reacción con la fracción precipitable fría del suero en lugar del suero completo, se obtuvo resultados muy diferentes. Así, en aquellos casos de lupus eritematoso en los cuales hubo un resultado positivo con el suero completo, el precipitado frío daba casi siempre una reacción negativa. Nuestras investigaciones muestran que los resultados de hemaglutinación con la fracción precipitable fría de los sueros de sujetos con lupus eritematoso diseminado difieren notablemente de los obtenidos en casos de artritis reumatoide, en los cuales los títulos de hemaglutinación con el suero completo y los con el precipitado frío revelan un paralelismo estrecho.

Se subraya el valor diagnóstico de la reacción de hemaglutinación con la ayuda de la fracción precipitable fría.