will show whether liquid nitrogen is not only more convenient but also more efficient than glycerol.

One red cell aldolase, one transaminase and two TPN dependent dehydrogenases have been measured in these blood samples before and after storage for various periods. The level of the aldolase, and to a lesser extent that of the transaminase, fell significantly during storage but the concentration of the dehydrogenases was maintained. These findings may have a bearing on the use for transfusion of blood stored by the present method.

The method reported is rather more expensive than the glycerol method but more convenient in use. In particular, the rate of loss of cells during storage is much reduced, and recovery of cells for use is much quicker. It is suggested that liquid nitrogen represents a suitable alternative to glycerol for the smaller laboratory, allowing them to have their own red cell panel readily available.

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SERUM IMMUNO-CONGLUTININ IN MULTIPLE SCLEROSIS, HASHIMOTO'S DISEASE, AND RHEUMATOID ARTHRITIS

BY

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The titre of immuno-conglutinin in the serum rises after antigenic stimulation. While its role in the mechanism of immunity is not as yet understood, it is believed that the immuno-conglutinin response is a non-specific indicator of antigenic stimulation. Since there is a common belief that multiple sclerosis may fall within the category of "autoimmune" disease (Miller and Schapira, 1959) it was thought worth while investigating the serum and spinal fluid level of immuno-conglutinin at various stages of the disease and comparing these with those to be found in an accepted "autoimmune" disease such as Hashimoto's thyroiditis. Since cases of rheumatic disease also afford evidence of an altered immunological state (as shown by a positive Rose–Waaler test) a series of such cases was also investigated.

Materials and Methods

Sera for testing, free from macroscopic haemolysis, were stored at −20° C. Complement was inactivated by heating to 56° C. for 30 minutes, and the sera were then absorbed with 50% volume of packed washed sheep red cells (0.25 ml. of packed cells to 0.5 ml. of serum) for 20 minutes at room temperature (20° C.).

Sheep red cells and bovine and horse serum were freshly obtained from a slaughterhouse. Early experimental results were found not to be reproducible, and it was only when fresh sheep erythrocytes (not more than one week old) and fresh bovine and horse sera were used that a standardized and reliable method was evolved.

Immuno-conglutinin was titrated by the method of Coombs, Coombs, and Ingram (1961). To 0.1 ml. of doubling dilutions of serum in saline was added 0.1 ml. of alexinated cells and 0.1 ml. of saline. The tubes were then incubated at 37° C. for 30 minutes, spun lightly, and read macroscopically in the manner suggested by Coombs *et al.* after resuspending in the supernatant fluid. The end-point was taken as the last tube showing discrete visible agglutination. Negative controls using alexinated cells, prepared with inactivated horse serum only, were put up with each serum to eliminate error due to non-specific clumping.

Alexinated cells were prepared as follows. Sheep red cells were first sensitized with bovine antibody by incubating equal volumes of a 5% suspension and a 1:2 dilution of heat-inactivated bovine serum (30 minutes at 56° C.) at 37° C. for 15 minutes. This suspension was centrifuged, washed once in saline, and suspended in saline to give a 5% suspension. Red-cell concentrations were prepared by accurate dilution from a stock suspension of known packed cell volume.

Alexination was then carried out as follows: 2 ml. of the above suspension sensitized cells, 2 ml. of horse serum complement, 2 ml. of heat-inactivated horse serum, and 14 ml. of saline were mixed and incubated at 37° C. for 15 minutes. This suspension of cells was used without washing, and in our hands proved stable and not liable to non-specific agglutination.

Results

Sera from 43 normal blood donors, collected over the same period as the pathological sera, were used as controls. The distribution of immuno-conglutinin titre is shown in Fig. A.; the statistical significance of results is set out in the Table.

Sera from 27 cases of multiple sclerosis which fall into two groups—chronic cases seen at follow-up examinations; and new cases presenting in an acute episode and where a confident clinical diagnosis could be made—were distributed very similarly to the normal (Figs. B

and C). Statistical analysis showed no difference between normal controls and multiple sclerotic patients or between "chronic" and "acute" cases.

Statistical Significance of Results

Sera Compared			Statistical Significance of Difference
Acute M.S.	against	Normal	N.S. P>0·7
Chronic M.S.	,,	,,	,, P>0·9
Acute M.S.	,,	Chronic M.S.	,, P>0·7
Hashimoto's disease	,,	Normal	P<0·001
Rheumatoid arthritis	,,	,,	P<0·001*
"Non-rheumatoid arthritis	,,	,,	P<0·001
Rheumatoid arthritis	,,	Non-rheumatoid arthritis	P<0·01*

* Cochran's modified "t" test for unequal variance was applied.

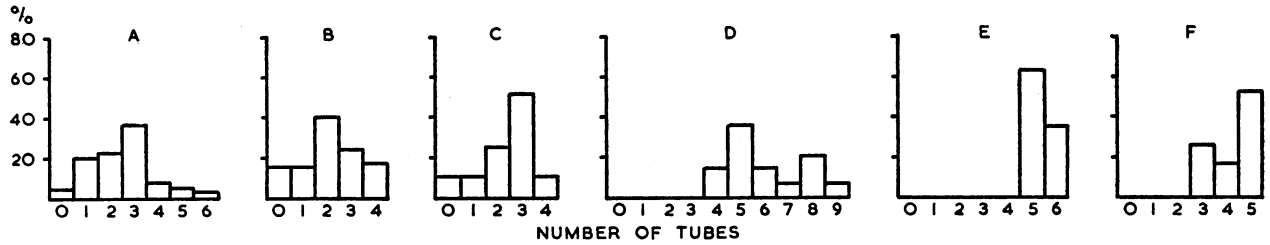
All cases of clinical rheumatic disease had a raised immuno-conglutinin level independent of the result of the Rose–Waaler test, and this may indicate some stimulus not detected by the common serological tests applied in rheumatoid disease.

Summary

Serum immuno-conglutinin levels have been studied to assess the presence or absence of antigenic stimulus.

The serum from patients with multiple sclerosis showed normal immuno-conglutinin levels and distribution.

Immuno-conglutinin was markedly raised in Hashimoto's disease and in rheumatoid arthritis.



Distribution of immuno-conglutinin titres. FIG. A, Normal. FIG. B, Acute multiple sclerosis. FIG. C, Chronic multiple sclerosis. FIG. D, Hashimoto's disease. FIG. E, Classical rheumatoid arthritis. FIG. F, Other "rheumatic" disease.

Sera from 14 patients with Hashimoto's disease serologically and/or pathologically confirmed showed markedly raised immuno-conglutinin titre, distributed as in Fig. D. These were mostly cases with very high antibody titres (tanned red-cell test), and in view of the small size of this group no attempt was made to correlate the immuno-conglutinin and antithyroglobulin titres.

The 22 patients with rheumatic disease (Figs. E and F) were divisible into those with true rheumatoid arthritis (11) and those with conditions such as osteogenic arthropathy, ankylosing spondylitis, etc. (11). The former of these two subgroups had a significantly higher conglutinin titre than the latter ($P<0.01$), but both differ significantly from normal ($P<0.001$).

Tests on cerebrospinal fluids gave uniformly negative results.

Discussion

Our normal distribution of immuno-conglutinin differs from that given by Marks and Coombs (1957) but corresponds more closely to some of their analyses for individual months. However, as both the normal controls' and the patients' sera were obtained at the same time it is perhaps justifiable to assume that the incidence of minor infection influencing the immuno-conglutinin titre is the same in the two groups.

To the primary question posed—namely, whether cases of multiple sclerosis show an elevated immuno-conglutinin titre in the blood—we are able to elicit a definitely negative answer in both acute and chronic cases. This contrasted sharply with our findings in Hashimoto's disease, and the test thus gave similar results to the globulin-consumption test applied by Field and Ridley (1960) to these two diseases.

The results obtained with sera from cases of Hashimoto's disease gave the results one would expect from a confirmed auto-antibody disorder; high immuno-conglutinin levels were found in all cases we examined. It would be of interest to reinvestigate all these cases after varying periods of treatment to observe variations of immuno-conglutinin with depression of antibody titre and clinical improvement.

We are indebted to Dr. E. J. Field for suggesting the investigation and defining its scope and purpose; to Drs. Henry Miller, Malcolm Thompson, and G. S. Owen for sera; and to Mrs. D. Weightman for statistical analyses.

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Preliminary Communications

Treatment of Herpes Simplex with 5-Iodo-2'-deoxyuridine

The numerous and continuing studies on the prophylaxis and treatment of herpes simplex suggest that there is no satisfactory therapeutic regime for this recurrent virus infection. Immunological techniques, either by repeated autoinoculation of successive lesions as advocated by Hruszek (1933) or by multiple smallpox vaccinations as demonstrated by Schiff and Kern (1954) and earlier workers, are no longer regarded by most dermatologists as acceptable or effective forms of treatment.

Lazar (1956) showed that a new disease site could be initiated after the use of unmodified material containing the live virus, and Kern and Schiff (1959) have negatived their earlier work by a controlled trial where they managed to "cure" nearly as many patients with a heat-killed inactive vaccine as with the active vaccine. These workers suggest that any benefit obtained may have been due to inoculation of foreign protein or other substances within the vaccine or to "suggestion." Of more recent interest are the observations of Alexander (1962), who showed that temperature increases were