mented to a greater degree in the lower extremities than in the upper. The amplitude of pulsation showed less change than did the skin temperature or volume changes of the digits.

The greatest rise in temperature was seen in the toes. The temperature of the skin of the calf, subcutaneous tissues, and deep muscle of the calf showed a decreasing temperature effect in the order shown. The rectal temperature and temperature of the forehead showed the least change.

Benzyl-imidazoline was not effective in raising the skin temperature of the foot, forehead, or hand after indirect heating of one arm.

Benzyl-imidazoline shock was seen on 2 occasions.

The action of benzyl-imidazoline is complex, and the increased flow to the extremities may result from a combination of effects including peripheral vasodilatation, and, possibly, cardiac stimulation.

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Differential Sheep Cell Agglutination Test in Rheumatoid Arthritis.*

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One of the greatest obstacles to the objective study and evaluation of therapy in rheumatoid arthritis has been the lack of a simple and reliable laboratory test for the measurement of rheumatoid activity. Many tests have[1] been proposed in the past and several have proven useful adjuncts to diagnosis and evaluation of a patient but no one test alone is sufficiently significant to serve as sole guide for therapy. Recently Rose, Ragan *et al.*[2,3] have proposed a new simple test for activity in rheumatoid arthritis and have claimed for it a high degree of specificity and reliability. These authors discovered that the serum of patients with rheumatoid arthritis in the active stage contains a substance which agglutinates sheep erythrocytes sensitized with specific amboceptor to much higher titer than normal sheep cells. The test consists in a comparison of the titers to which normal and sensitized sheep cells are agglutinated by patients' serum. The results are expressed as "Differential titer" *i.e.* the algebraic difference between the agglutination titers observed with normal (NS) and sensitized sheep erythrocytes (SS). In patients with active rheumatoid arthritis this differential agglutination titer was found by Rose *et al.* to be "never lower than 16 and usually considerably higher." In other diseases examined (with exception of one case of ankylosing spondylitis) the differential titer was 16 or less.

In order to accumulate data on the validity and possible limitations of this laboratory test, sera from a number of patients were examined in this laboratory. The present paper reports the results from 160 patients living in Northern California, about half of whom had rheumatoid arthritis.

Materials and Methods. The technique described by Rose *et al.*[2] for the differential sheep cell agglutination test was followed in all details. Sera of patients were obtained from freshly drawn venous blood and were tested usually within 18 hours, always within 72 hours of collection. Red blood cells were obtained from a number of different sheep but all gave identical results when tested with the same sera. Special attention was paid to careful preparation of glassware to avoid any

* Thanks are due to many physicians who cooperated in this study by furnishing patients or sera and offering advice.

1 Collins, D. H., *Practitioner*, 1948, **161**, 180.

2 Rose, H. M., Ragan, C., Pearce, C., and Lipman, M. O., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 1.

3 Editorial, *J. Am. Med. Assn.*, 1948, **138**, 514.

TABLE I.
Differential Sheep Cell Agglutination Test Applied to Sera of 160 Patients.

Clinical diagnosis	No.	"Positive"* No.	"Positive"* %	Differential agglutination titer 0-2	4	8	16	32	64	128	256+
Rheumatoid arthritis											
Marked activity	20	13	65.0	6	1	0	3	6	3	1	0
Mild-moderate activity	37	5	13.5	23	4	6	2	3	0	0	0
No activity	21	0	—	19	2	0	0	0	0	0	0
Other arthritis											
Ankylosing spondylitis	10	1	10.0	9	0	0	0	1	0	0	0
Rheumatic fever	5	0	—	5	0	0	0	0	0	0	0
Miscellaneous	15	0	—	14	1	0	0	0	0	0	0
Other diseases											
Visceral angiitis	5	0	—	5	0	0	0	0	0	0	0
Infectious mononucleosis	7	0	—	7	0	0	0	0	0	0	0
Lymphoma (Hodgkin's, leukemia)	8	0	—	8	0	0	0	0	0	0	0
Hepatitis	24	1	4.1	21	1	1	1	0	0	0	0
Lues	8	0	—	8	0	0	0	0	0	0	0

* "Positive": A differential titer of 16 or over.

false results which might be attributable to remaining traces of soap or detergent.

For sera adsorbed with boiled guinea pig kidney and boiled beef erythrocytes the technique of Davidsohn[4] was followed, except that 1 part of serum was mixed with only 3 parts of 20% suspensions of adsorbent. Because of this, some adsorptions were incomplete.

The diagnosis of rheumatoid arthritis was established in patients on the basis of the opinions of several physicians based on typical clinical observations and laboratory findings. Patients were grouped, relative to the activity of their disease, as "marked," "moderate," and "none."

Results. Sera from 160 patients, 78 of whom carried the diagnosis of rheumatoid arthritis were examined. Table I shows their clinical diagnoses and the results of the differential sheep cell agglutination test carried out on their sera. Among the patients with rheumatoid arthritis, one-third of the group with marked activity had titers in the "normal" range, and two-thirds had differential titers of 16 or more. Almost half of the rheumatoid patients were classified as mildly or moderately active. All of them had pain, progressive limitation of motion, joint changes, increased perspiration, an elevated erythrocyte sedimentation rate and often anemia. Their differential agglutination titers extended over a wide range but only in a few patients were they 16 or higher. In the 21 patients with arrested, inactive rheumatoid arthritis the test was uniformly negative. Eighty-two patients comprised the control group as seen in Table I. Six of the 10 cases of ankylosing spondylitis were definitely active. The single case showing a high differential titer did not appear to be different from the others and had no peripheral joint involvement. No other group showed any tendency toward elevated differential titers except a few patients with hepatitis in whom abnormal globulins might be expected. Two patients with titers of 8 and 16 suffered from severe cirrhosis with reversal of the albumin-globulin ratio.

Thus differential agglutination titers of 16 or more were encountered mainly in patients with active rheumatoid arthritis, but only a small proportion of such patients had high titer sera. The impression was gained that high differential titers occurred predominantly in the most severely ill rheumatoid patients.

It was of interest to determine whether the differential sheep cell agglutination test varied with the treatment and clinical course of the disease. In Table II are shown the results obtained with sera of patients on whom tests were repeated over a period of 3-5 months. In

[4] Davidsohn, I., *J. Am. Med. Assn.*, 1937, **108**, 289.

TABLE II.
Differential Sheep Cell Agglutination Titer in Relation to Clinical Status of Patients Observed During a Period of Time.

Patient	Date	Clinical impression	Therapy	Differential aggl. titer
H	8/24/48	R.A.,* marked activity	Gold	128
	11/ 9	" " "	"	128
	12/21	" slight improvement	"	128
	2/ 1/49	" moderate activity	"	64
C	9/21/48	R.A., marked activity	"	16
	12/21	" " "	"	16
	1/25/49	" moderate activity	"	8
M	9/21/48	R.A., " "	"	16
	12/21	" " "	"	16
	1/25/49	" " "	"	16
L	8/10/48	R.A., " "	"	32
	12/21	" greatly improved	"	4
	1/25/49	" no activity	"	4
Ma	9/21/48	R.A., marked activity	Rest	32
	12/21	" minimal activity	"	4
B	9/21/48	R.A., marked activity	Gold	64
	12/21	" greatly improved	"	8
	1/25/49	" questionable activity continued improvement	"	2

* Rheumatoid arthritis.

these few patients tested and over a short period of time the differential titer did reflect the course of the disease (Table II).

Rose[2] demonstrated by electrophoretic analysis that the substance responsible for agglutination of sensitized sheep cells was a globulin in the beta-gamma fraction of serum. The heterophile antibody present in normal serum is adsorbed by guinea pig kidney. In serum from infectious mononucleosis the heterophile antibody can be removed by adsorption with boiled beef erythrocytes. Table III shows the agglutination of normal and sensitized sheep erythrocytes by adsorbed sera. The normal heterophile antibody of sera from active rheumatoid arthritis was adsorbed by guinea pig kidney. However, neither boiled beef erythrocytes nor guinea pig kidney removed the substance responsible for agglutination of sensitized sheep cells, thus differentiating it from the heterophile antibodies present in sera from normal individuals, from patients with infectious mononucleosis, or from persons who have been injected with horse serum. Thus the differential titer of sera from patients with active rheumatoid arthritis could frequently be increased by adsorbing with boiled guinea pig kidney, prior to the test.

The stability of the substance responsible for agglutination of sensitized sheep erythrocytes was investigated. With sterile sera kept at 4°C both absolute and differential agglutination titers remained essentially stable for 4-20 days. Minimal bacterial contamination quickly destroyed the activity. When stored at —70°C in the dry ice chest in sealed ampoules individual or pooled sera maintained their titer for at least 3 months.

Discussion. The results obtained confirm the claims of Rose *et al.*[2] that there is a substance in the serum of some patients with rheumatoid arthritis which agglutinates sensitized sheep erythrocytes. Adsorption of such sera with boiled guinea pig kidney or beef cells emphasizes the difference between this substance and other components of the serum which agglutinate sheep cells.

There are, however, important differences between the results reported by Rose *et al.* and those observed in the present series. In the New York series all patients with active

TABLE III.
Differential Sheep Cell Agglutination Test Applied to Sera Absorbed with Boiled Beef Erythrocytes and Boiled Guinea Pig Kidney.

Serum	Absorbed with	NS*	SS†	Differential aggl. titer
Normal human	Unabsorbed	8‡	16	2
	Beef erythrocytes	16	16	0
	Guinea pig kidney	0	0	0
Infectious mononucleosis	Unabsorbed	4096	4096	0
	Beef erythrocytes	64	128	2
	Guinea pig kidney	4096	4096	0
Rheumatoid arthritis Marked activity	Unabsorbed	32	2048	64
	Beef erythrocytes	16	2048	128
	Guinea pig kidney	4	2048	512

* Normal sheep erythrocytes.
† Sheep erythrocytes sensitied with specific amboceptor.
‡ Reciprocal of highest dilution giving 2+ agglutination of erythrocytes.

rheumatoid arthritis had differential titers of 16 or more, while in the group of cases in Northern California high differential titers were associated mainly with severe rheumatoid disease and did not occur in many others with obvious activity. Since Rose's method was adhered to in all details these differences in results might represent variations in patients rather than differences in technique. Perhaps the rheumatoid arthritis observed in Northern California is a somewhat milder disease than that on the East Coast. Some cases in the present series, included under the diagnosis of rheumatoid arthritis, despite a typical clinical picture, may actually have had some other disease entity. On the other hand it must be pointed out that on the whole the sensitivity of the test, as carried out in this laboratory, was somewhat less than that indicated in Rose's paper, with both absolute and differential titers at lower levels. It is not clear at this time what technical feature might be responsible for these reported differences.

Rose *et al.* reported that rheumatoid arthritis was the only one of the disorders, sometimes attributed to "hypersensitivity," which produced elevated differential agglutination titers while rheumatic fever and visceral angiitis failed to do so. Our results agree in this respect. One case of ankylosing spondylitis with a high differential titer had, in contrast to Rose's observation, no peripheral joint involvement.

In the group studied the differential sheep cell agglutination test was of value in following the course of patients who initially had high differential titers. Titers observed over a period of 3-5 months in most cases reflected the clinical status. In patients with unequivocal improvement, the differential titer fell long before any change in the erythrocyte sedimentation rate. The test might thus be helpful as an objective aid for the evaluation of the results of treatment.

Summary. Results obtained with the differential sheep cell agglutination test of Rose *et al.* in a series of 160 patients are reported. High differential titers were observed almost solely in sera of patients with severe rheumatoid arthritis. In many others, with milder yet active disease, no significant titers were obtained. Adsorption with boiled guinea pig kidney frequently increased the differential titer of sera from active cases of rheumatoid arthritis. In patients with initial differential titers of 16 or more, tested over 3-5 months, the titers paralleled the clinical course.