cholesterol was followed by the same method as in the first group of animals. The results are summarized in Table II.

TABLE II
Blood Cholesterol in Sensitized Animals

Observed	Female 3400 gm.	Female 3150 gm.	Male 3250 gm.	Female 3550 gm.
5-15	174	273	170	207
5-16	155	269	197	173
5-17	149	275	181	—
5-17	0.1 cc. 1/10 O.T. Intraven.	0.5 cc. 1/10 O.T. Subcut.	1.0 cc. 1/10 O.T. Intraven.	1.0 cc. 1/10 O.T. Subcut.
5-18	255	330	375	156
5-19	325	415	675	415
5-21	373	547	266	357
5-22	200	—	145	110
5-23	231	347	162	150
5-24	195	168	110	100
5-25	148	165	107	83
5-29	215	225	150	165
6-1	250	235	153	230
6-3	275	287	265	150

The results indicate that these animals respond to the single injections of tuberculin with prompt though transient hypercholesterolemia. This is followed within a week by a return to normal value, which in some instances is first preceded by a fall below the normal level.

7538 C

Bactericidal Power of Blood in Chronic Arthritis.

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There is considerable evidence that chronic rheumatic joint disease, as well as acute rheumatic arthritis, is neither strictly a metabolic disturbance nor purely allergic in character but the result of hematogenous streptococcic infection of the joints. This evidence is of 4 types: the demonstration of streptococci in involved joints with typical structural alteration in direct relation to the actual distribution of the bacteria;[1, 2, 3] the streptococcemia which occurs at

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[2] Cecil, R. L., Nicholls, E. E., and Stainsby, W. S., *Arch. Int. Med.*, 1929, **43**, 571.

[3] Wetherby, M., and Clawson, B. J., *Am. J. Path.*, 1932, **8**, 283.

intervals during the active phases of the disease;[2, 4, 5] the presence of streptococcic antibodies in arthritic patients in concentrations greater than those usually found in normal individuals;[5, 6, 7, 8] and the production experimentally of lesions in the joints of animals by the use of intravenous injections of streptococcus cultures.[2, 10] On the other hand, other investigators have failed to corroborate some of these observations.[11–13]

This positive evidence has led to the wide use of agglutination and sedimentation tests as diagnostic procedures in chronic arthritis and to the use of vaccines for treatment of the disease. Our own experience and that of others[7] with the use of agglutination tests and determinations of sedimentation rates have led us to believe that these have little practical value in diagnosis or as indices of the results of treatment. In searching for a more reliable index of immunity to streptococcic infection, we have tested the whole or defibrinated blood of 30 patients and the serum of 38 patients from the Evanston Hospital Arthritis Clinic for bactericidal power. As controls, 31 tests on the defibrinated blood and 22 on the serum of normal individuals were done. Bactericidal tests were performed also on the blood and serum of 10 patients suffering from non-streptococcic infections and in patients with acute streptococcic sepsis. In most instances the sedimentation rate and the agglutinating titer were determined on samples of blood drawn at the same time for comparison with the results obtained in the bactericidal tests.

Preliminary experiments showed that there was little difference between the bactericidal power of heparinized blood and that of defibrinated blood if the defibrination was done carefully and with constant technique. The latter method was finally adopted for routine tests because the procedure was simple and inexpensive.

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[7] Dawson, M. H., Olmstead, Miriam, and Boots, R. H., *J. Immunol.*, 1932, **23**, 187.

[8] Clawson, B. J., Wetherby, M., Hilbert, E. H., and Hilleboe, H. E., *Am. J. Med. Sc.*, 1932, **184**, 758.

[9] Keefer, C. S., Meyers, W. K., and Oppel, T. W., *J. Clin. Invest.*, 1933, **12**, 267.

[10] Burbank, R., *Bull. N. Y. Acad. Med.*, 1929, **5**, 176.

[11] Nye, R. N., and Waxelbaum, E. A., *J. Exp. Med.*, 1930, **52**, 885.

[12] Dawson, M. H., Olmstead, Miriam, and Boots, R. H., *Arch. Int. Med.*, 1932, **119**, 173.

[13] Bernhardt, H., and Hench, P. S., *J. Infect. Dis.*, 1931, **49**, 489.

An eleven-year-old strain of *Streptococcus viridans* obtained from Dr. B. J. Clawson was used as the test organism chiefly because it had been used previously in our routine agglutination tests. Several of the strains that we have isolated from the blood of arthritic patients have been similar culturally and serologically to this one. Tests with recently isolated strains of streptococcus gave unreliable results by our method.

For the test 1 cc. of a suspension containing about 10,000 bacteria per cubic centimeter was used. This was added to 3 cc. of a 50% dilution of serum, or to 3 cc. of defibrinated blood, in a 7 cc. Wassermann tube. The tube was closed with a paraffined rubber stopper and placed in the rack of a mixing machine in a 37° incubator. Constant agitation was found to be necessary for these tests and a rotating machine was devised for this purpose.*

The degree of reduction of living organisms was determined by the plating method. For this a 0.5 cc. sample of the mixture was removed, plated with 10 cc. of dextrose agar immediately after the mixture was made and again after 4 hours and 8 hours of incubation in the agitating machine. The colony counts were made after 18 to 24 hours of incubation and recorded numerically when possible, or estimated roughly as 2 plus (4000±), 3 plus (6000±) and 4 plus (8000±) when too numerous for actual counting.

The results of the initial tests in arthritic and non-arthritic patients are summarized in Table I. For the sake of simplicity we have merely indicated as having bactericidal power those cases which showed inhibition after 4 hours of rotatory agitation in the incubator. The results varied from complete inhibition to slight (10%) reduction in the number of colonies.

In 38 arthritic patients the serum alone of only 2 cases (5%) exhibited bactericidal power, while in 3 cases (9%) among the controls definite bactericidal action was observed.

It is interesting that in the group of cases of active arthritis, all of which were of a severe type, only one blood failed to show definite inhibition of the strain used. The agglutination titer in these cases was well within normal limits and the sedimentation rates showed about the same average values and varied within narrower limits than in the normal cases.

In the group of 12 cases of arthritis that showed unequivocal clinical evidence of improvement, only 5 gave positive bactericidal tests. The sedimentation rates varied within the same limits as in the normals. Agglutination titers were not done at this time.

* Details of the technique and a description of the agitating machine are included in an accompanying paper.

TABLE I.
Bactericidal Power, Agglutination Titer and Sedimentation Rate in Arthritis and Non-arthritic Conditions.

				Agglutination Tier				Sedimentation Rate in mm. (60 min.)			
		Total cases	Cases with pos. Bc tests	Av. in pos. cases	Extremes in pos. cases	Av. in neg. cases	Extremes in neg. cases	Av. in pos. cases	Extremes in pos. cases	Av. in neg. cases	Extremes in neg. cases
Normal individuals		31	21 (68%)	96	20-320	44	20-180	11	2-27	15	5-24
Arthritis (untreated)											
Atrophic and mixed	Recovering	12	5 (41%)					17	9-26	14	6-24
	Active	12	11 (91%)	50	20-80			11	2-23		20
Hypertrophic		5	3 (60%)					17	14-19	11	5-21
Arthritis (treated-vaccine)											
Atrophic and mixed	Recovering	12	7 (58%)	1730	320-5120	1664	160-5120	18	4-29	12	6-19
	Active	2	2 (100%)	1600	640-2560			4	3-5		
Hypertrophic		6	3 (50%)	320	160-640	3630	640-5120	16	14-19	12	5-21
Streptococcic sepsis		2	2 (100%)	160	0-320						
Non-strep. infections											
	Acute	6	5 (83%)	104	0-320		80	14	4-28		26
	Chronic	4	3 (75%)	50	20-80			10	3-18		6

Of the 5 cases diagnosed as hypertrophic arthritis 3 gave positive bactericidal tests and the sedimentation rates were not significant.

The acute and chronic non-streptococcic infections were local lesions, some with suppuration but none showing systemic effects. In 3 of the chronic cases the infection was localized. In these cases agglutination tests yielded normal values and the sedimentation rate, while slightly greater in the acute infections, varied within narrow limits. The bactericidal effect of the defibrinated blood in the more acute cases is comparable with that in the active cases of infectious arthritis. This evidence supports the idea that the bactericidal property of whole blood or defibrinated blood as applied to gram positive cocci is not specific.

Both of the cases of acute streptococcic sepsis exhibited marked bactericidal power. In both instances *Streptococcus viridans* was recovered repeatedly in blood cultures. It is interesting that in the patients with acute streptococcic or non-streptococcic infections and in the cases of active arthritis, the bactericidal power of the blood was approximately the same. The agglutination titers in these 2 patients with streptococcus sepsis varied within normal limits, emphasizing again the lack of correlation between bactericidal power and the agglutination titer.

In the third division of the table the data accumulated from studies of 20 arthritic cases receiving intravenous vaccine therapy at the time of the initial bactericidal tests, are summarized. As in past experience we find generally increased agglutination titers without correlation between titer values and clinical improvement. The 2 most active cases showed the lowest sedimentation rates. Vaccine therapy apparently had no effect upon either bactericidal power or sedimentation rate.

Conclusions from such small groups of cases as presented in the table must necessarily be guarded. However, we consider that the following generalizations are justified in the light of previous findings of other workers and supplementary unpublished data from our own experience.

1. Tests of bactericidal power of blood by the method used are of little or no value in the diagnosis of chronic arthritis.

2. In general, the defibrinated blood from active cases of atrophic arthritis and acute streptococcic and non-streptococcic infections more often exhibits inhibitory properties against *Streptococcus viridans* than the blood from normal individuals, from patients with

chronic non-streptococcic infections, from quiescent or recovering cases of atrophic arthritis, or from hypertrophic arthritis.

3. Intravenous streptococcic vaccine therapy does not influence the bactericidal property of blood while the agglutinating titer may be greatly increased.

4. There is no correlation between streptococcic bactericidal property, agglutinating titer and sedimentation rate in the cases so far examined.

5. Serum alone usually shows little or no bactericidal power against *Streptococcus viridans,* irrespective of its agglutinating titer. In exceptional cases there may be definite inhibitory action.

7539 C

Importance of Continuous Agitation in Bactericidal Tests with Streptococci.

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Although the advantages of continuous agitation in phagocytosis experiments had been noted by earlier investigators,[1, 2, 3] Robertson and Sia[4] were apparently the first to use the rotatory-oscillation method extensively. All have observed that constant mixing during incubation promotes bactericidal action and produces more constant results than stationary incubation or intermittent agitation. Most of the previous work has been done with cultures of pneumococci and we were faced with the necessity of checking these factors with the streptococcus with which we were working.

An agitating machine utilizing the same principle as that in the machine used by Fenn[2] and later in the improved machine of Robertson and Sia[4] was designed and built for us by Mr. Wm. H. Hamilton, E. E. It consists of 3 brass flanges mounted rigidly on a motor driven shaft. Two of the flanges act as tube holders, having 5/8 in. perforations bored at uniform intervals near the periphery, while the third flange acts as a guard. The flanges will hold 18 tubes at

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