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# STUDIES ON THE BACTERICIDAL PROPERTIES OF THE SYNOVIAL FLUID<sup>1</sup>

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Studies on normal synovial fluid and on joint effusions under pathological conditions have been reported (1, 2). In 1919, Labor and von Balogh (3) detected antibodies in the joint cavity during inflammations of the joints. Their results have been confirmed (4 to 6). Positive gonorrheal complement fixations and positive Wassermann reactions in joint fluids have also been reported (1).

The bactericidal activity of various body fluids has been repeatedly investigated (5, 7 to 9), but the only communication of similar studies on synovial fluid was made by Spink and Keefer (10). These authors studied the killing power for gonococci of 44 synovial fluids from 18 patients with gonorrheal and from 13 patients with non-gonorrheal arthritis. Fluids which contained living gonococci and those from patients with non-gonorrheal arthritis were not bactericidal for gonococci. Sterile synovial fluids from patients with gonorrheal arthritis killed the homologous strain of gonococcus. Their bactericidal power was the same or slightly weaker than that of the blood.

According to Collins (11), "the joint fluid in rheumatoid arthritis, on account of its content of polymorphs, is highly bactericidal." Sweetapple (12) pointed out that "synovial fluid is strongly antibacterial while fresh, but excellent pabulum for bacteria when stale." Neither author offered experimental proof for these statements. In rabbits with experimental streptococcal arthritis, synovial fluid obtained more than 3 to 6 weeks after the infection is usually sterile (13).

The object in undertaking the present investigation was to study the bactericidal activity for gram-positive cocci and gram-negative bacteria of the synovial fluid of patients with rheumatoid arthritis and other joint diseases.

It was thought that the detection of a "specific" bactericidal activity for hemolytic streptococci in the synovial effusions from patients with rheumatoid arthritis would support the theory of the streptococcus etiology of this disease. Attempts to isolate a specific microorganism from the synovial fluid in rheumatoid arthritis have been unsuccessful in a high percentage (14), but there is inferential evidence that hemolytic streptococci may be of etiological significance in this disease.

Whenever possible, oxalated blood plasma was tested for its bactericidal activity simultaneously with the joint fluid of the same patient.

In addition, cultures were made and the complement content, as well as the agglutinin titre for streptococci and for *E. coli*, determined. Certain physicochemical and cytological properties of the synovial fluid were also studied.

## MATERIALS AND METHODS

Synovial fluid was obtained by aseptic puncture of the affected joint, usually the knee, of 40 patients with rheumatoid arthritis or other joint diseases.

*Cultures for the bactericidal test.* Ten-fold dilutions (up to 10<sup>6</sup>) of 18-hour broth cultures of a beta hemolytic streptococcus (Strain AB 13) and an *Escherichia coli* were made in broth. The number of organisms in the last dilutions was determined by making pour plates with 0.1 cc. portions of those dilutions. The number of colonies grown in the 10<sup>6</sup> and 10<sup>5</sup> dilutions usually ranged between 20 and 200. These dilutions were used in the experiments. Similar dilutions of overnight broth cultures of 2 other strains of beta hemolytic streptococci, a hemolytic staphylococcus aureus, a Type I pneumococcus, another strain of *E. coli*, and an *Eberithella typhi* (H and O strains) were used in some of the experiments.

*Bactericidal test.* One-half cc. portions of the materials to be tested were mixed in sterile 10 × 100 mm. pyrex glass tubes with 0.1 cc. of suitable, freshly diluted broth cultures of the microorganisms. The tubes were sealed and fastened to a square box on a rotating machine of the type described by Todd (15). After rotation for 24 hours, at 37.5° C., at 8 to 10 r.p.m., 0.3 cc. of the contents of the tubes was plated out with agar, and 0.1 cc. was transferred into tubes of broth. Colony counts were made after 24 and 48 hours. If the rotation time was prolonged to 48 hours, the results did not

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change, but if it was reduced to only 6 hours, frequently the microorganisms were not killed.

In about one-half of the experiments, the mixtures were rotated in sterile 20 × 70 mm. vials, closed with a sterile rubber cap through which a sterile injection needle with a sterile cotton plug had been inserted. Sterile tuberculin syringes were used to fill the vials and to withdraw the contents after rotation. The "open" vials offer the following advantages: (1) The mixtures are tested under aerobic conditions, (2) the process of sealing of the tubes is eliminated, and (3) the vials can be used repeatedly.

The results with sealed tubes and open vials were equivalent in all the experiments with *E. coli*. In some experiments with streptococci in broth, however, fewer colonies developed on agar from the "open" vials than from sealed tubes.

*Other examinations of the fluids.* Cultures were made on liquid and solid media. Complement was titrated and agglutinin titres for streptococci and for *E. coli* were determined by the usual methods.

The mucin content was examined by the qualitative "sac and tube" test (1). The relative viscosity was estimated with Ostwald's viscosimeter (16). The specific gravity was determined by the drop method (17). The hydrogen ion concentration at the beginning of the experiment was determined by the bicolor method (18). For cytological studies, total and differential cell counts, and Giemsa and Wright stains, were made.

#### RESULTS

The bactericidal properties of synovial effusions for hemolytic streptococci and *E. coli*, in relation

to the type of joint disease, are shown in Table I. Thirty-nine fluids were obtained from the knee joint and one from an elbow joint.

Bactericidal properties for hemolytic streptococci were detected in 4 of 37 fluids (10.8 per cent), for *E. coli*, in 34 of 39 fluids (87.1 per cent). Bactericidal properties for hemolytic staphylococci and for Type I pneumococci were absent in 6 fluids from patients with infectious and rheumatoid arthritis, and in 4 fluids from those with non-infectious arthritis. These fluids were not streptococcal.

A strong bactericidal power for *E. typhi* was detected in 10 fluids which also killed *E. coli*.

Simultaneous studies of the bactericidal activity of the synovial fluid and oxalated blood plasma were made with materials from 15 patients.<sup>2</sup> The results are given in Table II.

Three synovial fluids killed streptococci and 6 other effusions were bactericidal for *E. coli*, but the blood of the same patients did not kill streptococci or *E. coli*, respectively. In one instance, the blood plasma, but not the synovial fluid, was bactericidal. There was no difference in the bac-

<sup>2</sup> It should be noted that the bactericidal activities of oxalated blood plasma were compared with those of native synovial fluid.

TABLE I  
*Bactericidal properties for hemolytic streptococci and Escherichia coli of human synovial fluid obtained from patients with various joint diseases*

Lesion of joint	Synovial fluids				
	Number examined	Number which were bactericidal for streptococci	Number which were non-bactericidal for streptococci	Number which were bactericidal for <i>E. coli</i>	Number which were non-bactericidal for <i>E. coli</i>
(A) Infectious and rheumatoid arthritis					
(a) Non-specific infectious.....	2	1	1	2	
(b) Gonorrheal.....	1		1	1	
(c) Syphilitic.....	2		2	2	
(d) Rheumatoid*.....	10	2	7	7	3
Total (A).....	15	3	11	12	3
(B) Non-infectious arthritis					
(e) Intermittent hydroarthrosis.....	2	1	1	2	
(f) Traumatic.....	6		6	5	1
(g) Hypertrophic.....	12		12	11	1
Total (B).....	20	1	19	18	2
(C) Of unknown origin†.....	5		3	4	
Total (A + B + C).....	40	4 (10.8 per cent)	33	34 (87.1 per cent)	5

\* One specimen was not tested for streptococci.

† Two specimens were not tested for streptococci, a third was not tested for *E. coli*.

TABLE II

Comparison of bactericidal properties for hemolytic streptococci and *Escherichia coli* in synovial fluid and blood plasma of 15 patients with various joint diseases

Lesion of joint	Total number of cases examined	Number of cases with						
		Fluid and plasma bactericidal for streptococcus	Fluid only bactericidal for streptococcus	Fluid and plasma non-bactericidal for streptococcus	Fluid and plasma bactericidal for <i>E. coli</i>	Fluid only bactericidal for <i>E. coli</i>	Plasma only bactericidal for <i>E. coli</i>	Fluid and plasma non-bactericidal for <i>E. coli</i>
Non specific infectious. . .	1		1		1			
Syphilitic. . . . .	2			2		2		
Rheumatoid*. . . . .	6	1	1	3	3	1	1	1
Intermittent hydroarthrosis. . . . .	2		1	1	2			
Traumatic. . . . .	1			1		1		
Hypertrophic. . . . .	3			3	1	2		
Total. . . . .	15	1	3	10	7	6	1	1

\* One specimen was not tested for streptococci.

tericidal properties of blood plasma and synovial fluid of 11 patients when tested with streptococci, and of 8, when tested with *E. coli*.

The effect of undiluted and of diluted synovial fluid on increasing concentrations of *E. coli* is presented in Table III.

TABLE III

Effect of dilution of synovial fluid on bactericidal properties for various concentrations of *Escherichia coli*

Dilution of culture	Number of colonies			
	Synovial fluid undiluted	Synovial fluid 1 : 5	Synovial fluid 1 : 10	Broth
10 <sup>2</sup>	∞	—	—	∞
10 <sup>4</sup>	1000	∞	—	∞
10 <sup>5</sup>	9	∞	∞	∞
10 <sup>6</sup>	0	0	∞	∞
10 <sup>6*</sup>	90	90	90	90

\* Number of organisms inoculated into 10<sup>6</sup> dilution.  
∞ = innumerable colonies.  
— = no test made.

Five-fold dilutions in broth of synovial fluid did not reduce the killing power for small amounts of *E. coli*. No bactericidal activity was present in ten-fold diluted synovial fluid. With increasing concentrations of *E. coli*, the bactericidal properties of the synovial fluid diminished almost quantitatively. Similar observations were made on the bactericidal activity for streptococci of the few fluids which could be tested.

The influence of inactivation by heat on the killing power of synovial fluid for *E. coli* is given in Table IV.

TABLE IV

Effect of heating of synovial fluid on bactericidal properties for *Escherichia coli*

Medium	Number of colonies after rotation at 37.5° C.		
	Fluid 1	Fluid 2	Fluid 3
Synovial fluid, unheated. . . . .	0	0	0
Synovial fluid, 56° C.; 30 minutes. . . . .	0	25	0 (48 hours)
Synovial fluid, 56° C.; 60 minutes. . . . .	—	550	8 (96 hours)
Broth. . . . .	∞	∞	∞

∞ = innumerable colonies.  
— = no test made.

Inactivation in a water bath at 56° C. for 30 minutes did not alter the bactericidal activity for *E. coli* of one fluid, but diminished that of 2 other fluids. Heating at 56° C. for 60 minutes caused a further reduction, but not a complete destruction, of the bactericidal properties of fluid 2. Retardation of bacterial growth by inactivated synovial fluid was observed in specimen 3. Inactivation at 56° C. for 30 minutes completely destroyed the bactericidal activity for streptococci of the few fluids which could be tested.

Eighteen synovial fluids were frozen and kept for 6 months in dry ice at approximately — 50° C. The bactericidal activity for *E. coli* was retested frequently. It was found almost unaltered after 180 days of storage. The bactericidal properties of 2 fluids for streptococci were completely lost after storage in dry ice for 3 and 12 days, respectively.

Bacteriological cultures of all fluids used in this study were sterile.

Complement titrations were made on 20 specimens of fresh synovial fluid and of effusions stored in dry ice for periods up to 75 days. The titre of 14 fluids varied between 0.025 and 0.1 cc. (average 0.05 cc.), while that of 6 fluids was above 0.1 cc. The complement titre of stored fluids gradually decreased within 2 months; the bactericidal activity for *E. coli*, however, remained practically unaltered for at least 6 months. The complement titre was very low in 3 of the 5 fluids not bactericidal for *E. coli*. The titre of the other 2 fluids was not tested. The streptococcal fluids had a normal complement titre.

Agglutinins for streptococci were found in 2 synovial fluids in final dilutions of 1:40; these fluids were not streptococcal. No agglutinins for streptococci were detected in the other fluids. The agglutination reactions with *E. coli* were always negative.

The mucin reaction was always positive. The relative viscosity of the fluids varied between 2.9 and 39.1, the specific gravity, between 1.018 and 1.026, and the hydrogen ion concentration at the beginning of the experiment, between pH 7.4 and pH 8.2.

Cytological examinations revealed wide variations in total and differential cell count as reported by previous authors (1). The lowest total cell count was 595 per c.mm., the highest 51,300 per c.mm.

There was no relation between viscosity, specific gravity, hydrogen ion concentration, or cytology of the synovial fluids and their bactericidal properties for hemolytic streptococci or *E. coli*.

#### COMMENT

Most of the synovial fluids from various joint diseases were strongly bactericidal for *E. coli* and *E. typhi*. This activity was closely related to the complement content of the synovial fluid, but heating at 56° C. for 30 to 60 minutes did not completely destroy the bactericidal properties. It has been shown (19) that the third and fourth components of complement require a temperature of 62° to 65° C. for inactivation. No experiments with inactivation at this higher temperature were done in this study. It seems con-

ceivable that the heat-stable components of the complement may be responsible for the bactericidal activity of "inactivated" synovial fluid.

The bactericidal activity for gonococci of gonorrheal synovial fluid was thought to be due to specific antibodies (10). According to Bauer (20), however, "blood from non-gonorrheal patients may show the same variations in bacterial killing power to various gonococcal strains as do patients with acute or chronic gonorrhea." In this study, no agglutinins for *E. coli* or *E. typhi* were found in the fluids which killed these organisms. Bactericidal activity for these microorganisms was detected in the synovial fluid from patients with a variety of joint diseases. It is unlikely that specific antibodies were responsible for this activity.

There is evidence (21) that strains of streptococci vary in susceptibility to bactericidal action of serum and that there is not a good correlation between *in vivo* evidence of streptococcal power of patients' blood and *in vitro* activity of sera. Variations in hydrogen ion concentration and in oxidation-reduction potential play important parts in inhibiting the streptococcal activity of serum (22). No significant variation in susceptibility was noted in the 3 strains used in our experiments, but this does not mean that other strains of greater susceptibility would not have been encountered if a greater number of strains had been tested with synovial fluid, or that wider ranges of hydrogen ion concentration and oxidation-reduction potential might not have brought to light more fluids with streptococcal activity. However, there can be no question that streptococcal activity cannot be as easily and regularly demonstrated as some previous reports indicated (11, 12). Even fluids with high leukocyte counts were devoid of streptococcal activity.

Bactericidal properties for hemolytic streptococci were detected in 2 of 9 synovial fluids from patients with rheumatoid arthritis, and in 2 of 28 fluids from other joint diseases. These results do not justify the conclusion that streptococcal antibodies are usually present in synovial effusions of patients with rheumatoid arthritis, or that their presence is specific for this disease.

From the studies of synovial effusions, it would appear that the bactericidal activities for gram-positive cocci are distinctly different in nature

from those for gram-negative bacteria. The bactericidal activity for gram-positive cocci may be related to the combined action of many phagocytic cells and antibodies. In our studies, evidence for this hypothesis was obtained from a few experiments on the synovial fluid of animals which had received intravenous or intra-articular injections of a sterile irritant or of living or killed hemolytic streptococci (23). All the human synovial fluids examined contained phagocytic cells but only a few effusions were streptococidal. Thus it can be stated that most of the synovial fluids were practically free of streptococidal antibodies. The bactericidal activity for gram-negative bacteria is closely related to the complement content of the synovial fluid and may, therefore, be expected to be present in effusions containing sufficient amounts of complement.

## SUMMARY

The bactericidal properties of 40 sterile synovial effusions from patients with rheumatoid arthritis or other joint diseases were studied. Four of 37 synovial fluids (10.8 per cent) killed hemolytic streptococci, and 34 of 39 synovial fluids (87.1 per cent) were bactericidal for *E. coli*. The killing power of the synovial fluid usually was stronger than that of the oxalated blood plasma. Bactericidal properties for hemolytic streptococci were detected in only 2 of 9 synovial fluids in rheumatoid arthritis (22.2 per cent), and 2 of 28 synovial fluids in other joint diseases (7.1 per cent).

The bactericidal activity for *E. coli*, but not for hemolytic streptococci, was related to the complement content of the synovial fluid; it was not completely destroyed by heating at 56° C. for 30 to 60 minutes, and remained almost unaltered for 6 months in frozen synovial fluid.

There was no relation between viscosity, specific gravity, hydrogen ion concentration at the beginning of the experiment, or cytology of human synovial effusions and their bactericidal activities for hemolytic streptococci or *Escherichia coli*.

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