STUDIES ON THE BACTERICIDAL PROPERTIES OF THE SYNOVIAL FLUID

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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^-6) 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 10^6 and 10^7 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 Eberthella 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 x 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 X 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 fol-
lowing 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 re-
peatedly.

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 spec-
cific 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 streptoc-
 cocci 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 rheuma-
toid 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. 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 strepto-
cocci or E. coli, respectively. In one instance, the
blood plasma, but not the synovial fluid, was
bactericidal. There was no difference in the bac-

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

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**TABLE I**

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

<table>
<thead>
<tr>
<th>Lesion of joint</th>
<th>Synovial fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number examined</td>
</tr>
<tr>
<td>(A) Infectious and rheumatoid arthritis</td>
<td>15</td>
</tr>
<tr>
<td>(a) Non-specific infectious</td>
<td>2</td>
</tr>
<tr>
<td>(b) Gonorrheal</td>
<td>1</td>
</tr>
<tr>
<td>(c) Syphilitic</td>
<td>2</td>
</tr>
<tr>
<td>(d) Rheumatoid*</td>
<td>10</td>
</tr>
<tr>
<td>Total (A)</td>
<td>15</td>
</tr>
<tr>
<td>(B) Non-infectious arthritis</td>
<td>20</td>
</tr>
<tr>
<td>(e) Intermittent hydroarthrosis</td>
<td>2</td>
</tr>
<tr>
<td>(f) Traumatic</td>
<td>6</td>
</tr>
<tr>
<td>(g) Hypertrophic</td>
<td>12</td>
</tr>
<tr>
<td>Total (B)</td>
<td>20</td>
</tr>
<tr>
<td>(C) Of unknown origin†</td>
<td>5</td>
</tr>
<tr>
<td>Total (A + B + C)</td>
<td>40</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Lesion of joint</th>
<th>Total number of cases examined</th>
<th>Fluid and plasma bactericidal for streptococcus</th>
<th>Fluid only bactericidal for streptococcus</th>
<th>Fluid and plasma non-bactericidal for <em>E. coli</em></th>
<th>Fluid only bactericidal for <em>E. coli</em></th>
<th>Plasma only bactericidal for <em>E. coli</em></th>
<th>Fluid and plasma non-bactericidal for <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non specific infectious</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Syphilitic</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rheumatoid*</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Intermittent</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hydroarthrosis</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Traumatic</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hypertrophic</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

* One specimen was not tested for streptococci.

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

<table>
<thead>
<tr>
<th>Medium</th>
<th>Number of colonies after rotation at 37.5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovial fluid, unheated</td>
<td>0</td>
</tr>
<tr>
<td>Synovial fluid, 56°C; 30 minutes</td>
<td>0</td>
</tr>
<tr>
<td>Synovial fluid, 56°C; 60 minutes</td>
<td>—</td>
</tr>
<tr>
<td>Broth</td>
<td>∞</td>
</tr>
</tbody>
</table>

∞ = 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 streptococcidal 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 streptococcidial. 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 conceivable 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-gonorrhreal patients may show the same variations in bacterial killing power to various gonococcal strains as do patients with acute or chronic gonorrhrea." 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 streptococcidial 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 streptococcidial 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 streptococcidial activity. However, there can be no question that streptococcidial activity cannot be as easily and regularly demonstrated as some previous reports indicated (11, 12). Even fluids with high leukocyte counts were devoid of streptococcidial 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 streptococcidial 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 streptococcical. Thus it can be stated that most of the synovial fluids were practically free of streptococcical 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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**BIBLIOGRAPHY**


23. de Gara, P. F., Unpublished observations.