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Spermagglutination by Bacteria.

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The agglutination of spermatozoa under the influence of different factors, such as acids, alkalis, salts of the heavy metals, CO_2, dyes, substances derived from ova, etc., was observed by various authors. By studying the action of bacteria on spermatozoa *in vitro* we discovered a new agglutinating factor in some strains of *B. coli.*

For our experiments we used: 1. A saline suspension of spermatozoa of guinea pigs, rats, and rabbits (epididymis specimens), and human (condom specimens). 2. A broth culture of *B. coli* or a saline emulsion of the bacterial growth from an agar slant. The test was performed on a glass slide by mixing a drop of the sperm-suspension with a drop of the culture. In a control drop of sperm without bacteria, the single spermatozoa were evenly distributed in the fluid. But after mixing with the bacteria, the spermatozoa were almost instantaneously agglutinated and formed flocculi and clumps which could easily be seen with the naked eye. Microscopic examination with low and high dry power showed that the spermatozoa were clumped together into big net-like formations. At the same time most of the spermatozoa were immobilized. A few of them, although included in the clumped net-work, were still slightly motile, but after a few minutes they lost all signs of motility. Spermatozoa mixed with *B. coli* culture in a tube formed clumps which in a short time settled down, leaving the supernatant fluid clear and transparent The reaction was irreversible. Addition of acids or alkalis, or shaking did not disintegrate the clumps. The agglutination by bacteria did not depend on the pH of the medium. The clumping under the influence of bacteria took place in solutions of various pH covering the range from 3 to 10. Dead spermatozoa (heated at 56° for 1 hour or killed by chloroform) were susceptible to clumping by bacteria to the same degree as living ones.

The active principle is contained within the bodies of the bacteria. A broth culture passed through a Berkefeld filter does not affect the spermatozoa. On the other hand, the sediment of a broth culture after repeated washing with saline, still preserved its agglutinating power. Boiling the culture for 1 minute at 100°C. destroys the active principle, but heating at 60°C. for a prolonged time (24 hours and more) does not affect it at all, although the bac-

teria themselves are killed at this temperature. Subjecting the liquid bacterial culture to a vacuum at room temperature for one hour to expell gases does not interfere with the agglutinating power of the bacteria. Thus, the possible influence of CO_2 as the agglutinating agent can be excluded.

The age of the bacterial culture is of no significance. Those several months old are as active as one 24 hours old.

Addition of dyes (*e. g.*, trypaflavin solution 1:1000) also agglutinates the spermatozoa. But while this agglutination by dyes does not take place in the presence of normal serum,[1] the agglutination by bacteria does not suffer by the addition of serum.

Among many strains of *B. coli*, isolated on various occasions from feces and urine of patients, only few have been found to be active. Various other bacteria (strains of streptococci, staphylococci, pneumococci, gonococci, *B. typhi, B. paratyphi, B. proteus, B. pyocyaneus, B. subtilis, B. tuberculosis*) tested against spermatozoa gave negative results.

The sperm agglutination by bacteria is in all aspects analogous to the phenomena described by Lillie[2] as "sperma mass coagulation" and by Loeb[3] as "real sperm agglutination" and considered by them as detrimental to the fertilizing power.

Therefore the presence of spermagglutinating strains of bacteria in the vagina and in the cervix of the uterus should be taken into consideration as a potential factor of sterility.

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[1] Rosenthal, L., and Hornick, O., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 516.

[2] Lillie, F. R., *Biol. Bull.*, 1915, **28**, 19.

[3] Loeb, L., *J. Exp. Zool.*, 1914, **17**, 126; *Pflüger's Arch.*, 1904, **104**, 335.