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Failure of Streptococcal Antibodies to Influence Chemotaxis of Leukocytes.*

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Is chemotaxis of leukocytes a specific immunological reaction; is it, like phagocytosis and agglutination, enhanced by the presence of bacteriotropins, or, on the contrary, does chemotaxis occur just as well in the absence of specific antibodies, in the normal as in the immunized animal? It is well known that polymorphonuclear leukocytes display positive chemotaxis to most or all kinds of bacteria, and advance toward them by amœboid movement. However, it is not known whether chemotaxis is aided by the presence of specific antibodies.

To obtain information on this question, the chemotactic response of rabbits' polymorphonuclear leukocytes to streptococci was observed *in vitro,* and measurements were made to find whether the reaction is increased by the addition of streptococcal antibodies.

The method used in these experiments has been described in detail elsewhere.[1] Essentially it consists in observing with the microscope the direction of locomotion of polymorphonuclear leukocytes in proximity to a clump of bacteria. The bacteria, grown in liquid medium, are washed twice in distilled water, and a small loopful of the concentrated bacterial suspension is placed on a glass slide. After drying, the bacteria form a flat, round or oval mass about 1 mm in diameter. Leukocytes are obtained from the peritoneal cavity of the rabbit by injecting 150 cc of physiological saline and removing it 4 hours later; it then contains great numbers of polymorphonuclears. These are concentrated by gentle centrifugation and are then mixed with plasma obtained from the heart blood of the same animal. No anticoagulant is used. The suspension is made in such a way that each high-power field of the microscope contains from 10 to 25 leukocytes. A drop of the plasma-leukocyte suspension, while still liquid, is placed on a coverslip and superimposed on the glass slide, with the bacterial clump in the center of the preparation. This is sealed to prevent evaporation and is observed with the microscope at

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1 McCutcheon, M., Coman, D. R., and Dixon, H. M., *Arch. Path.,* 1939, **27**, 61.

37.5°C. The image of a microscopical field containing a portion of the bacterial clump and a number of leukocytes is projected on paper by means of a drawing ocular, and the position of each leukocyte is recorded at intervals of a minute for 10 minutes. The distance of each leukocyte from the bacterial clump at the first observation is measured, and these distances are added. Similarly the sum of the distances at the last observation is found. The difference between these sums is divided by the total minutes of observation for all the leukocytes, thus giving the value of chemotropism in microns per minute for each microscopical field. This value has a positive sign if the cells taken collectively move toward the bacteria, a negative sign, if away. In strong positive chemotropism, a value of the order of +10 microns per minute is expected, while in strong negative chemotropism an equally high negative value is obtained.

Since the object of these experiments was to find out whether acquired antibodies increase the attraction of leukocytes to bacteria, a strain of organisms was selected which did not attract leukocytes strongly in the absence of such antibodies; in this way any increase in attraction due to antibodies would be more easily detectable. A suitable organism was obtained from the Department of Bacteriology, *Streptococcus hemolyticus* (strain 1048). It had already been found[1] that this organism attracts leukocytes for only a few minutes under the present conditions. We now planned experiments to show whether attraction would be increased in intensity or prolonged in time by the presence of agglutinating and phagocytosis-promoting substances.

In the first series of experiments, bacteria were sensitized with immune rabbit serum; leukocytes and plasma were obtained from normal animals. Sensitization of streptococci of this strain greatly increases phagocytosis.[2] Our experiments gave an opportunity to observe whether conditions that increase phagocytosis also increase chemotaxis.

Lyophilized serum was prepared by Dr. David Lackman of the Department of Bacteriology, and was used in the dilution of 1:256, which was the limiting dilution of maximal agglutination. Streptococci, usually from 18-hour cultures in neopeptone broth, were sensitized from 30 to 90 minutes at 37°C. Then they were washed with distilled water, and a loopful placed on a glass slide, as already described. As control, a loopful of bacteria treated with normal rabbit serum was placed on the same slide about one cm from the

[2] Mudd, S., Czarnetzky, E. J., Lackman, D., and Pettit, H., *J. Immunol.*, 1938, **34**, 117.

sensitized organisms. Thus the chemotactic effects of sensitized and unsensitized streptococci could be compared in the same preparation.

Ten-minute records were then made of the movements of leukocytes near the sensitized and unsensitized bacteria in turn. These observations were repeated after half an hour. During the first period of observation, chemotaxis was moderately strong toward both sensitized and unsensitized bacteria. In 14 preparations the mean value of chemotaxis was, with sensitized streptococci, +7.9 microns per minute; with unsensitized bacteria, +7.4 microns per minute. The difference is obviously not significant.

During the second period of observation, in 10 preparations, chemotaxis brought about by sensitized bacteria fell to +3.9 microns per minute; with bacteria treated with normal serum a value was obtained of +2.1 microns per minute. The difference with its standard error is 1.8 ± 1.11 and is not statistically significant. It is concluded that under these conditions, sensitization of bacteria by specific antibodies did not increase the intensity of chemotaxis nor prolong the duration of the reaction.

The second series of experiments was designed to reproduce more closely conditions in actual infections, that is, the antibodies were present in the cell-plasma suspension surrounding the bacteria. Unsensitized bacteria were used, and cells and plasma were obtained from rabbits immunized by repeated inoculation with living hemolytic streptococci (strain 1048). The pooled serum of the 6 rabbits used was found by Dr. Lackman to have an agglutinating titer of 1:4096. In control experiments, cells and plasma were obtained from normal rabbits. The sera of none of the normal rabbits, tested separately, had agglutinating power. The bacteria in these experiments were from relatively old cultures (several days), since it appeared that bacteria from an old culture exerted less attraction than younger organisms, and therefore gave better opportunity to detect any increase in chemotaxis brought about by antibodies.

Nineteen pairs of preparations were observed. During the first period of observation, with cells and plasma of immunized animals the mean value of chemotaxis was +3.7 microns per minute; in preparations made with cells and plasma of normal animals, +4.5 microns per minute. The difference with its standard error is 0.8 ± 0.83, and is not significant. During the second period of observation, chemotaxis ceased in both types of preparation, the leukocytes appearing to move at random. In 15 preparations with cells and plasma of immunized animals, the mean value of chemotaxis was +0.8 microns per minute, in preparations made from normal animals, —0.9 microns per minute. The difference is not sig-

nificant. It is concluded that under the conditions of these experiments the presence of agglutinating and phagocytosis-promoting antibodies in the plasma fails to increase or prolong chemotaxis.

Thus no evidence has been obtained from these experiments favoring the view that chemotaxis is dependent upon or is increased by the presence of agglutinating and phagocytosis-promoting antibodies. It seems rather that chemotaxis is brought about primarily by substances produced by the organisms,[3] and that such substances are given off by all kinds of bacteria.[4] If this is true, chemotaxis is not a specific immune reaction as are, in part, phagocytosis and agglutination, but a non-specific response. It appears that antibodies play their part only after the cell has been attracted to the bacteria; then, by facilitating the spread of cell on particle,[5] antibodies aid in bringing about phagocytosis.

Summary. Sensitization of a strain of hemolytic streptococcus with rabbit antiserum did not increase the chemotactic attraction of these bacteria for rabbit polymorphonuclear leukocytes *in vitro.* Also the chemotactic response to hemolytic streptococci was no greater when leukocytes and plasma were obtained from immunized rabbits than when they were obtained from normal animals. Under these conditions, chemotaxis, unlike phagocytosis and agglutination, is not increased by specific antibodies, but appears to be rather a non-specific response of leukocytes toward microörganisms.

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Studies on *H. pertussis.** I. Liberation by Sonic Vibration of a Soluble Component That Absorbs Phase I Agglutinins.

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(Introduced by D. W. Bronk.)

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The antibacterial action of protective antisera has been shown to involve combination with antigens present on the bacterial surface.

[3] Dixon, H. M., and McCutcheon, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 378.

[4] McCutcheon, M., and Dixon, H. M., *Arch. Path.*, 1936, **21**, 749.

[5] Mudd, S., McCutcheon, M., and Lucke, B., *Physiol. Rev.*, 1934, **14**, 210.

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