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Studies in Chemotropism. Source of Substances Attracting Polymorphonuclear Leukocytes to Bacteria.*

HAROLD M. DIXON AND MORTON MCCUTCHEON.

From the Department of Pathology, University of Pennsylvania Medical School.

One of the outstanding phenomena in bacterial infections is the migration of polymorphonuclear leukocytes into the infected area. This leukocytic reaction, known as chemotropism or chemotaxis, is presumably brought about by the release of some substance in the infected area; but what this substance is, whether it comes from bacteria or from injured cells of the host or from both, is uncertain.

It seemed probable from previous experiments[1] that the source of the attractive substance is, at least in part, the bacteria themselves. The present experiments were designed to test this hypothesis more rigorously.

In infections *in vivo,* products of cell- and tissue-injury cannot be excluded, but *in vitro,* experiments can be planned in such a way that the only possible source of attraction is the bacteria. If, under these conditions, leukocytes show positive chemotropism, we would conclude that bacteria do indeed give off attractive substances.

In the present experiments a clump of *Staphylococcus albus* was taken from an agar slant and placed on a glass slide, where the bacteria formed a small circle 0.1 to 0.2 mm. in diameter. The leukocytes were obtained by injecting 150 cc. of isotonic NaCl into the peritoneal cavity of the rabbit. The solution was withdrawn after 4 hours and leukocytes were concentrated by light centrifugation. The cells were then suspended in plasma of the same animal.

Since the purpose of these experiments was to find out whether leukocytes show chemotropism to bacteria in the absence of injured tissue-cells, it was essential to prevent any of the leukocytes from coming into contact with and phagocytizing the bacteria, because thereby the leukocytes might be injured and give off substances that would attract other leukocytes.[2] The problem, therefore, was to isolate the bacteria from the leukocytes. This was done by plac-

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1 McCutcheon, M., and Dixon, H. M., *Arch. Path.*, 1936, **21**, 749.

2 McCutcheon, M., Wartman, W. B., and Dixon, H. M., *Arch. Path.*, 1934, **17**, 607.

ing a minute drop of liquid plasma (without cells) on a coverglass and superimposing this drop on the bacteria, so that the plasma formed a disk with the clump of bacteria in the center. After this drop of plasma had coagulated, a suspension of leukocytes was allowed to run in between slide and coverslip, filling the space except that already occupied by the coagulated plasma. The preparation was sealed with petrolatum and observed with the microscope, at 37°C.

A representative experiment is illustrated in Fig. 1. By means of a drawing-ocular, a microscopic field was projected on a piece of paper and the outline of the bacterial clump was recorded. The broken line indicates the interface between the circle of plasma originally free from leukocytes, to the right, and the plasma containing leukocytes, to the left. The position of each leukocyte at the first observation, taken about 15 minutes after making the preparation, is indicated by an open circle. It is seen that 2 leukocytes had already moved into the inner ring of plasma. The path of each cell was now recorded for 15 minutes. During this time, all but one leukocyte moved closer to the bacteria.

The method used for evaluating chemotropism has been described in an earlier paper.[3] It consists in determining how nearly straight a cell moves toward a source of attraction. The cell designated "A" in Fig. 1 will be used as an illustration. At the first observation this cell was 123 microns from the edge of the bacterial clump, at the last observation, 78 microns. The difference, 45 microns, is divided by the length of the actual path which the leukocyte travelled in the same time. This was 75 microns. The quotient +0.6, is the value of chemotropism. If the cell had approached the bacteria in a straight line the value would have been +1, which is the maximal positive value of chemotropism; if it had moved directly away, the value would have been −1, *i. e.*, maximal negative chemotropism.

The average value for the 17 cells shown in Fig. 1 is +.65, a fairly high value. In 9 similar preparations, positive chemotropism was shown in each case, these observations being made before any leukocyte had reached the bacteria. Combining the results of all 10 experiments, the value of the mean and standard deviation for 184 leukocytes was $+0.53 \pm 0.33$. Nine control preparations, made in the same way except that bacteria were omitted, showed only slight positive chemotropism; for 162 cells the mean and standard deviations were $+0.15 \pm 0.42$.

From these values we conclude that leukocytes were attracted,

[3] Dixon, H. M., and McCutcheon, M., *Arch. Path.*, 1935, **19**, 679.

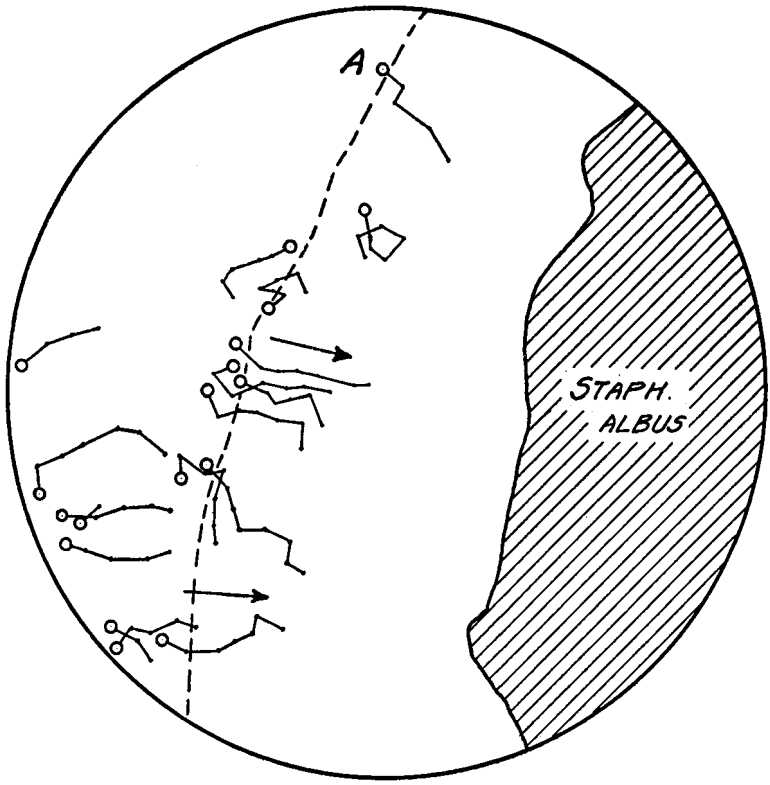

FIG. 1.

Camera lucida record of the paths of 17 leukocytes observed for 15 minutes, before any cells had reached the bacteria. The cells with one exception are moving toward the bacteria. The mean value of chemotropism is +0.65.

usually strongly, by *Staph. albus* even when no products of tissue-injury were present. This result is consistent with the hypothesis that the attractive substance is derived from the bacteria themselves.

It was now of interest to find out whether chemotropism would be increased after some of the leukocytes had reached the bacteria and had begun to phagocytize them. It seemed possible that phagocytes, gorged with bacteria, would suffer injury and give off chemotropic substances, which would increase the effectiveness of the bacterial products. Therefore the following additional observations were made on the same 10 preparations used in the experiments reported above. After phagocytosis had begun, the paths of other leukocytes were analyzed as they approached the bacteria. The mean and standard deviation for 260 cells was $+0.51 \pm 0.34$, a value

that is not significantly different from that obtained before any leukocytes reached the bacteria. Therefore phagocytizing leukocytes did not increase the chemotropic effect of the bacteria, presumably because the leukocytes were not injured.

We conclude that in these experiments the source of attraction was the bacteria themselves. The nature of the attracting substance and the possibility that attraction by bacteria may be due in part to substances adsorbed from the culture-medium are now being investigated.

Though in these experiments we obtained chemotropism in the absence of damaged tissue-cells, yet under other conditions, products of tissue-injury are important sources of chemotropism (the literature has been reviewed by Silverman[4]).

Summary. Experiments were designed to show whether bacteria chemotropically attract leukocytes directly, or only indirectly as the result of injuring cells and tissues and causing these to liberate chemotropic substances. Clumps of *Staph. albus* on a glass slide were separated from a suspension of rabbit's leukocytes by a zone of cell-free plasma. Leukocytes moved into the inner ring of plasma and made their way to the bacteria, showing strong positive chemotropism. Values of chemotropism obtained before any leukocytes reached the bacteria represent the reaction to bacteria alone, no tissue-cells being present in the clump of bacteria. After a number of leukocytes reached the bacteria and began to phagocytize them, the value of chemotropism was determined for other leukocytes *en route* to the bacteria. The two values of chemotropism were not significantly different. It is concluded that leukocytes may react chemotropically to substances given off directly by bacteria.

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Action of Pentamethylenetetrazol (Metrazol) on Splanchnic Circulation of the Dog.

J. ERNEST NADLER AND A. R. BERGER. (Introduced by A. C. DeGraff.)

From the Department of Therapeutics, New York University College of Medicine.

The analeptic drug pentamethylenetetrazol (Metrazol) used in the treatment of cardiovascular conditions has been reported[1] to

[4] Silverman, D., *Arch. Path.*, 1938, **25**, 40.

[1] Camp, W. J. R., *J. Pharm. and Exp. Therap.*, 1928, **33**, 81.