Miscellaneous Manuscripts

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Chemotropism of Leucocytes in Relation to Their Rate of Locomotion.*

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A method was described[1, 2] for measuring the chemotropic reaction of polymorphonuclear leucocytes *in vitro*. Leucocytes were observed with the microscope and their paths recorded as they approached bacteria or other sources of attraction. In measuring the reaction to these attracting bodies only the direction of the path was taken into account, not the rate of locomotion. It seemed possible, however, that leucocytes might respond to such attraction not only by moving toward its source but by traveling at increased speed. In this case it would be desirable to take account both of direction and of velocity of approach in measuring the chemotropic reaction.

The point at issue has been touched on by previous observers,[3, 4, 5] who, however, did not make actual measurement to compare the rate of locomotion of leucocytes approaching a source of attraction with that of leucocytes moving at random. We have made such a comparison by means of a simple method which has been described by one of us.[6]

A minute clump of bacteria or of other particles is placed on a glass slide, where, after drying, it appears as a flat oval or circular body from 50 to 150 microns in diameter. A drop of blood, obtained by puncturing the finger, is placed on a coverslip, lowered

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

2 Dixon, H. M., and McCutcheon, M., *Arch. Path.*, 1935, **19**, 679.

3 Schade, H., and Mayr, K., *Krankheitsforschung*, 1930, **8**, 354.

4 Schultz, E., *Z. exp. Med.*, 1932, **84**, 609.

5 Philipsborn, E. v., *Fol. haematologica*, 1930, **43**, 142.

6 McCutcheon, M., *Am. J. Physiol.*, 1923, **66**, 180.

on the bacterial mass and allowed to spread between slide and coverslip. The preparation is sealed with petrolatum and examined with the high power of the microscope in a warm box at 37°C.

After waiting half an hour for the cells to develop their normal rate of locomotion,[6] one edge of the bacterial clump is brought into view; the image of the microscopic field is projected on a piece of paper by means of a drawing ocular, and the position of each polymorphonuclear leucocyte is recorded at half-minute intervals for 5 minutes or until the cell moves out of the field. The total path traversed by each cell is measured in millimeters and converted into microns by multiplying by a factor that depends on the magnification. In this way the rate of locomotion is obtained in microns per minute.

Chemotropism is computed by measuring the net approach of cell to source of attraction; that is, the final distance of the cell from the bacteria is subtracted from the original distance; the quantity thus obtained is divided by the total path traversed in the same time. This measure of chemotropism is independent of the rate of locomotion. Values of chemotropism may range from +1.00, maximal positive, to —1.00, maximal negative chemotropism; most cells have intermediate values, 0.00 representing random movement. The calculation has been explained more fully elsewhere.[2] Leucocytes in the same microscopic field as the bacteria yield, with few exceptions, high positive values for chemotropism.

After recording the paths of cells close to the bacteria, a field several millimeters distant is observed in the same way, the rate of locomotion and value of chemotropism being determined for each cell. Since the duration of the experiment has been found to affect the rate of locomotion,[7] we have alternated observations between fields close to the bacteria and fields remote from them.

The following sources of attraction have been used: *Staph. albus*

TABLE I.
Rate of Locomotion and Chemotropism of Human Polymorphonuclear Leucocytes.

		Near Source of Attraction			Remote from Source of Attraction		
Source of Attraction	No. of Experiments	No. of Cells	Rate of Locomotion Microns/min.	Mean Value of Chemotropism	No. of Cells	Rate of Locomotion Microns/min.	Mean Value of Chemotropism
Staph. albus	4	27	28 ± 7.6	+.71	23	32 ± 7.4	—.06
Staph. aureus	5	63	32 ± 5.6	+.63	37	39 ± 5.3	+.06
Collodion	7	49	35 ± 8.2	+.55	44	35 ± 8.1	—.07

[7] McCutcheon, M., *Am. J. Physiol.*, 1924, **69**, 279.

(from agar slants); *Staph. aureus* (also grown on agar but washed 3 times in distilled water); collodion particles (these particles, made by the method of Loeb,[8] are of about the size of bacteria; they were washed 3 times in distilled water). The results are given in the accompanying table. On the left side of the table are given values of chemotropism and rates of locomotion (with standard deviation) computed from the paths of cells in the same microscopic field as the source of attraction, while the values on the right side were obtained from cells remote from the source of attraction. The mean values for chemotropism are seen to be strongly positive in fields containing the source of attraction but approximate zero in remote fields; that is, in remote fields, leucocytes were moving at random. Comparing the rates of locomotion it is seen that in no case is the average rate of cells reacting chemotropically greater than that of cells moving at random. In the case of collodion particles the 2 rates are the same, 35 microns per minute; with *Staph. albus* the difference in rates is not significant, while with *Staph. aureus,* leucocytes in distant fields appear to have moved slightly faster than those near the bacteria (the reason for this is not clear). Thus our experiments give no evidence that cells reacting chemotropically travel faster than those moving at random.†

In interpreting these results, 3 possible sources of error have been held in mind. First, when bacteria are taken directly from the nutrient medium without being washed, they might carry with them sufficient salt to make hypertonic that part of the preparation immediately surrounding the bacteria. Consequently the rate of locomotion of leucocytes close to the bacteria might be reduced, thus neutralizing any tendency of attracted cells to move with increased velocity. This criticism would apply to the experiments with *Staph. albus* but not to those with the other particles, which were thoroughly washed with distilled water.

Second, the clump of bacteria or collodion might prevent slide and coverslip from coming as close together in the field of chemotropic attraction as elsewhere in the preparation. Such difference in thickness of the preparation might affect the rate of locomotion of leucocytes. Fortunately the arrangement of the erythrocytes, whether they occur singly, in twos or threes, or in rouleaux, gives an indication of the thickness of the preparation in any field. The rate of locomotion in thick and in thin fields was determined several

8 Loeb, J., *J. Gen. Physiol.*, 1922, **5**, 109.

†Our conclusions do not necessarily conflict with those of Schulz,[4] who stated that increase in rate occurred only *just before* the leucocyte reached the bacteria.

times, and consistent differences were not found, except in fields so thick that they were obviously unsuitable for observation.

Third, bacteria or other attracting bodies might give off substances toxic to leucocytes, reducing their rate of locomotion. Since the highest concentration of such substances would be in the zone immediately about the bacteria, any increase in velocity due to chemotropism might be masked by the toxic effect.

It appears impossible to rule out this objection entirely, but we have tried to meet it by using 3 different attractive bodies of which *Staph. albus* and thoroughly washed collodion particles would be expected not to prove toxic.

Summary and Conclusion. Quantitative evidence is presented that human polymorphonuclear leucocytes, when reacting to attraction by bacteria or other bodies *in vitro,* travel at no greater rate than do these cells when moving at random. It is therefore concluded that chemotropic response is one of direction only, while the rate of motion depends on such factors as temperature,[9] osmotic pressure of the medium, and on the internal condition of the cell.[5]

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Progestin Content of Human Placenta.

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The amount of progestational hormone in the human placenta is of interest not only because of contradictory reports as to the quantity present but because of the significance of this organ along with the corpus luteum as a source of luteal hormone during pregnancy.

Ehrhardt and Weigel[1] using implantation methods reported failure to demonstrate corpus luteum hormone in the human placenta. However, Ehrhardt[2] a few months later, using at this time placental extracts from which estrogenic fractions were separated, found as much as 10 (Clauberg) rabbit units in a single term placenta. Negative or weakly positive results were obtained in every instance when unseparated extracts were injected.

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9 McCutcheon, M., *Am. J. Physiol.*, 1923, **66**, 185.

1 Ehrhardt, K., and Weigel, W., *Endocrinol.*, 1933, **18**, 225.

2 Ehrhardt, K., *Münch. Med. Woch.*, 1934, **81**, 869.