# THE CHEMOTACTIC EFFECT OF OSMOSIS UPON LEUCOCYTES[1].

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This paper presents the results of a preliminary study of conditions affecting the movement of leucocytes. It demonstrates experimental facts which confirm the assumptions that osmotic force present in aqueous solutions and the permeability of leucocytes for water are the factors responsible for the movement of these cells from place to place.

## I.

The action of soluble substances in solution upon the movements of unicellular plants and animals has been extensively investigated and a voluminous literature has accumulated upon the subject. Solutions of different chemical substances undoubtedly have a varying effect upon the movements of these cells. The exact nature of the chemical or physical action involved has not, however, been satisfactorily demonstrated.

Pfeffer, who was one of the earliest workers in this field, at first attributed the movements of certain one-celled plants to osmosis. He later, after Stahl (1884), concluded that the stimulus was chemical in origin and gave the phenomenon its present name of "chemotaxis." His conclusions were extensively corroborated by various workers, but the development of physical chemistry and its application to the study of biological phenomena have done much to diminish the significance of the experiments upon which these conclusions were based.

Jean Massart, in 1889, demonstrated that the movements of bacteria and other plant cells were affected by soluble substances in solution. He concluded that the effect of solutions of different substances varies with the molecular weight and chemical structure of the substance. He said, "The repulsion exerted by these solutions is inversely proportional to the molecular weight and directly proportional to the isotonic coefficient. The substances which form exceptions are those which easily penetrate the cells in question." The conclusions of this author apparently show a direct connection between the molecular force which causes diffusion and the movement of the cells

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he studied. The proportionality to the isotonic coefficient also shows this motion is modified by the permeability of the cell membrane.

Several physical hypotheses have been developed with regard to the activities of amoebae. Certain of these which appear to be most accepted at present, have for their basis physical phenomena observed in a study of artificial amoebae. These artificial amoebae are drops of liquid suspended in another liquid in which they are insoluble. By producing local variations in surface tension at the surface layer of such a droplet it may be made to move in a manner strikingly similar to movements observed with the living amoebae.

While the analogy between the phenomena thus produced is a close one, the conditions under which they are observed are in fact quite dissimilar. A drop of liquid suspended in another liquid in which it is insoluble, can hardly be likened to a cell which is made up mostly of water and suspended in the same fluid. Local variations in surface tension and consequent formation of pseudopodia are haphazard and due to slight variations in tension at points upon the surface layer of the droplets. Even though these variations in surface tension occurring within the surface layers of the liquid droplets do account for tactile movements in living cells, it is unlikely that these forces either have sufficient magnitude or proper application to account for such motion as is indicated by the migration of leucocytes toward an infected area. In order to produce translation for a considerable distance in one direction, a sustained force of considerable magnitude is necessary in order to overcome the inertia of the liquid droplet or cell and the resistance offered by the liquid in which it is suspended and the surfaces with which it may be in contact. A careful analysis of these surface tension hypotheses has been made by Jennings (1915) with regard to the activities of amoebae and by Wells (1918) with regard to the movement of leucocytes.

If the experimental facts, based upon actual work with leucocytes, alone are considered, precedence must be given to the hypothesis that chemical affinity is the source of the stimulus which either produces or induces movements of translation of leucocytes. Among the most important studies upon this phase of the subject were those of Buchner (1890), Massart and Bordet (1891) and Gabritschewsky (1890). These workers used glass capillaries which were filled with solutions of the substances to be tested. They were sealed at one end and placed under the skin or in the peritoneal cavity of experimental animals. After several hours the capillaries were removed, washed, and examined as to their content of leucocytes. The presence of leucocytes within the tubes was regarded as indicating a positive chemotaxis and their absence a neutral condition or repulsion. By the use of this technique a great variety of substances were tested, only a few of which showed a definite attraction of leucocytes. The best attraction was obtained with bacteria and their extracts when these were free from toxin. Solutions of peptone, urea, dextrin, glucose and a few others also attracted leucocytes, while alcohol, acids, alkalies and salts repelled them. Sicherer used a technique *in vitro* in which

the capillaries containing the test solutions were suspended, with their open end just under the surface of a suspension of leucocytes. His results were similar to those obtained by the workers mentioned above but they were criticized by Pfoehl (1898) who maintained that the chemical nature of the phenomenon was not proven, and on the contrary concluded that the apparent results obtained were due to reactions of a physical nature.

## II.

At the beginning of the present study the technique *in vitro* of Sicherer (1899) was adopted on account of its simplicity and the possibility it gives for studying the physical conditions present. The substances used as test solutions were bacterial toxins and antitoxins, normal animal sera, broth medium, potassium tartrate and sodium chloride representing neutral salts, citric acid, quinine hydrochloride, glycerine, alcohol and 0·85 per cent. sodium chloride, which latter was included as a control upon the solution in which the leucocytes were suspended. The purpose of the experiment was to determine the chemotactic properties of the different substances, first in the form of their most concentrated solutions and second of gradually decreasing concentrations of the same substances.

Leucocytes were obtained by intraperitoneal injection of aleuronat into guinea-pigs. The leucocytes were washed twice and suspended in physiological salt solution (0·85 per cent.). Capillary glass tubes were filled with the various increasing dilutions of the substances to be tested, sealed at one end, and placed open-end down into the saline suspension of leucocytes. The suspensions of leucocytes were contained in small agglutination tubes and were about 1 cm. deep. A separate tube was used for each capillary. A temperature of 37·5° C. was used throughout. After one hour the capillaries were examined with a microscope, the height to which the leucocytes had risen was measured and the tube broken and its contents stained with Wright's stain.

All of these substances except alcohol and physiological salt solution showed strong attraction for leucocytes as represented by an accumulation of leucocytes within the capillary tubes. This evidence of attraction disappeared gradually and with varying rapidity as the concentration of the test solutions decreased. An examination of the densities of those dilutions of the test solutions in which no leucocytes accumulated revealed the fact that these dilutions possessed either the same or a lesser density than was possessed by the suspension of leucocytes. On the other hand, in every case where an accumulation of leucocytes was found within the capillaries, the densities of the test solutions were greater than the density of the leucocyte suspension.

On account of the vertical position of the capillaries above the suspension of leucocytes, it was thought that the leucocytes in contact with the denser solution at the opening of the capillaries might possess an increased buoyancy and as a consequence rise into the capillaries under the influence of gravity. However, in no case did the leucocytes accumulate at the upper end of the capillaries as would be expected if this were the cause. Nevertheless some of the tests were repeated with the capillaries in a horizontal position.

## Table I.

Experiments *in vitro* demonstrating the accumulation of leucocytes in capillaries containing solutions at higher density than the solution in which they were suspended, and their absence from capillaries containing these same solutions at the same or a lower density than the leucocyte suspension.

| Test Solutions | Dilutions in Capillaries | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1–0 | 1–5 | 1–10 | 1–20 | 1–40 | 1–80 | 1–160 | 1–320 | 1–640 | 1–1280 | 1–2560 | 1–5120 |
| *Potassium tartrate ... | + + + | + + + | + + + | + + + | + | – | – | – | – | – | – | – |
| *Citric acid ... ... | + + + | + + + | + + + | + + | + | – | – | – | – | – | – | – |
| *Quinine hydrochloride ... | + + + | – | – | – | – | – | – | – | – | – | – | – |
| Normal rabbit serum ... | + + + | + + + | + + + | + + | + | – | – | – | – | – | – | – |
| Normal horse serum ... | + + + | + + + | + + + | + + + | + + | + | – | – | – | – | – | – |
| Diphtheria antitoxin ... | + + + | + + + | + + + | + + + | + + | + | – | – | – | – | – | – |
| Tetanus antitoxin ... | + + + | + + + | + + + | + + + | + + | + | – | – | – | – | – | – |
| Diphtheria toxin ... | + + + | + + | + | – | – | – | – | – | – | – | – | – |
| Diphtheria toxin heated | + + + | + + | + | – | – | – | – | – | – | – | – | – |
| Tetanus toxin ... ... | + + | + | – | – | – | – | – | – | – | – | – | – |
| Tetanus toxin heated ... | + + | + | – | – | – | – | – | – | – | – | – | – |
| Broth used for toxin prod. | + + + | + + | – | – | – | – | – | – | – | – | – | – |
| Physiological salt ... | – | – | – | – | – | – | – | – | – | – | – | – |
| *Sodium chloride ... | + + + | + + + | + + + | + + | – | – | – | – | – | – | – | – |
| Alcohol ... ... ... | – | – | – | – | – | – | – | – | – | – | – | – |
| Glycerine ... ... | + + + | + | – | – | – | – | – | – | – | – | – | – |

+ + + = More than 19 mm. rise. + + = More than 4 mm. rise. + = More than 1 mm. rise.

* Dilutions of potassium tartrate, citric acid, quinine hydrochloride and sodium chloride were made up from saturated solutions.

In thermostat for one hour at 37·5° C.

Leucocytes suspended in 0·85 per cent. NaCl.

A cell was constructed about 18 mm. square and 5 mm. deep. The bottom of the cell was a three inch glass slide. The top was an inch cover-slip and the sides were built up with paraffin. A single capillary containing the test solution was thrust through a hole in the paraffin wall and parallel to the glass slide which formed the base. This was sealed in with a hot needle and the leucocyte suspension filled into the cell. The cover-slip was now sealed in place and the whole observed under the microscope in a 37·5° C. thermostat.

These tests gave results identical with those observed in the preceding experiment. The leucocytes accumulated only in those tubes which contained solutions possessing a greater density than that of the leucocyte suspension.

In order to more fully establish the effect of difference of density upon the movement of leucocytes a greater variety of substances were tested especially including several which according to previous investigators possessed positively chemotactic properties. Bacterial suspensions and extracts, starch, aleuronat, dextrose, lactose and other substances were used. Solutions or suspensions were prepared and their density adjusted to the same density as that of the suspension of leucocytes. Two groups of tests were performed, one in which the density was that of 0·85 per cent. sodium chloride solution at 37·5° C. and the other in which the density was adjusted to that of guinea-pig serum at the same temperature.

Two floating equilibria were constructed. One was adjusted to equilibrium at a temperature of 37·5° C. in an 0·85 per cent. solution of sodium chloride. The other was adjusted

at the same temperature but in normal guinea-pig serum. These equilibria were small glass balloons weighted with mercury and adjusted by fusing glass to the tips until they would neither rise nor sink in the solutions at 37·5° C. Test solutions of the various substances were prepared and adjusted to the same density as the leucocyte suspensions by means of the equilibria. The test solutions were filled into glass capillaries and set up as in the first set of experiments. The tests were carried out in a 37·5° C. thermostat. The capillaries were examined from time to time during 22·5 hours to determine whether a rise of leucocytes into the capillary tubes occurred.

Nine substances, namely, echinacea, phytolacca, quinine hydrochloride, urea, citric acid, acetic acid, dextrose, lactose and sodium chloride, were prepared from saturated solutions of these substances, in concentrations varying between 1 in 50 to 1 in 1000. The density of each dilution was adjusted

## Table II.

Experiments *in vitro* demonstrating the fact that when solutions to be tested for chemotactic action, contained in capillary tubes, are adjusted to the same density as the solution in which the leucocytes are suspended, no leucocytes accumulate within the capillaries.

| Test Solutions | | Dilutions in Capillaries | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1–50 | 1–50 | 1–50 | 1–100 | 1–100 | 1–100 | 1–500 | 1–500 | 1–500 | 1–1000 | 1–1000 | 1–1000 |
| Echinacea | * | – | – | – | – | – | – | – | – | – | – | – | – |
| Phytolacca | * | – | – | – | – | – | – | – | – | – | – | – | – |
| Quinine hydrochloride | + | – | – | – | – | – | – | – | – | – | – | – | – |
| Urea | + | – | – | – | – | – | – | – | – | – | – | – | – |
| Citric acid | + | – | – | – | – | – | – | – | – | – | – | – | – |
| Acetic acid | + | – | – | – | – | – | – | – | – | – | – | – | – |
| Dextrose | + | – | – | – | – | – | – | – | – | – | – | – | – |
| Lactose | + | – | – | – | – | – | – | – | – | – | – | – | – |
| Sodium chloride | + | – | – | – | – | – | – | – | – | – | – | – | – |

| | Distilled water extract | | | Filtered suspension | | | Supernatant fluid | | | Diluted with $H_2O$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aleuronat | ... | ... | ... | – | – | – | ... | ... | ... | ... | ... | ... |
| Starch | ... | ... | ... | ... | ... | ... | – | – | – | ... | ... | ... |
| Diphtheria toxin | ... | ... | ... | ... | ... | ... | ... | ... | ... | – | – | – |
| *B. typhosus* | – | – | – | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| *Staph. albus* | – | – | – | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| *B. coli* | – | – | – | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| *Staph. aureus* | – | – | – | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| *B. pyocyaneus* | – | – | – | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Normal horse serum | ... | ... | ... | ... | ... | ... | ... | ... | ... | – | – | – |
| Normal rabbit serum | ... | ... | ... | ... | ... | ... | ... | ... | ... | – | – | – |
| Tetanus antitoxin | ... | ... | ... | ... | ... | ... | ... | ... | ... | – | – | – |
| Dysentery antitoxin | ... | ... | ... | ... | ... | ... | ... | ... | ... | – | – | – |
| Diphtheria antitoxin | ... | ... | ... | ... | ... | ... | ... | ... | ... | – | – | – |

* = made from approximate normal tinctures.

\+ = made from saturated solutions.

Leucocytes suspended in 0·85 per cent. NaCl.

Test solutions are adjusted to the same density as 0·85 per cent. NaCl by means of the floating equilibrium.

Test solutions made from saturated solutions adjusted by dilution with water or with saline solution.

Temperature of exp. 37·5° C. Time 3 to 22½ hours.

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to the desired density by the addition of water or salt solution. The bacterial emulsions and extracts and the normal sera, toxins and antitoxins were used in their concentrated form, the adjustment being made as before with water or saline.

None of the capillaries during any period of the experiment, which was continued for twenty-two and one-half hours, showed an accumulation of leucocytes. In these tests the chemical nature of the test substance certainly does not affect the movement of leucocytes. In Table II are recorded only the results obtained with densities adjusted to the density of 0·85 per cent. sodium chloride solution. The results obtained with the densities adjusted to the density of normal guinea-pig serum were absolutely identical, and for that reason have not been tabulated.

In view of the facts determined by use of the above technique, some tests were carried out with capillary tubes containing the test solutions inserted subcutaneously in guinea-pigs. Four test substances were chosen, three of which, according to Buchner and Gabritschewsky, were positively chemotactic, namely urea, peptone and *B. pyocyaneus* protein; the other, sodium chloride, was negatively chemotactic. Solutions of three different densities of each substance were tested. The densities chosen were normal guinea-pig serum density, and densities greater and less than this.

An aqueous extract of *B. pyocyaneus*, an aqueous solution of urea, one of peptone and one of common salt were prepared. Three dilutions of each were adjusted by means of the floating equilibrium; one to guinea-pig serum density, one of greater and one of lesser density. These dilutions were filled into capillary tubes 1·5 mm. in diameter and 12 mm. long and inserted under the skin of the abdomen of guinea-pigs. After eight hours they were removed and the contents examined for leucocytes.

It was impossible to obtain reliable results with this technique. The glass capillaries were often broken and in other cases fibrin-like plugs closed the openings so that the leucocytes could not penetrate even if an attraction was exerted by the test solution. The results obtained with solutions of sodium chloride and urea in guinea-pig no. 1 and the results with peptone in guinea-pig no. 3 show definitely the greater accumulation of leucocytes in those capillaries containing the more concentrated solutions of these substances.

## III.

The irregularities encountered by use of the capillary tube technique in animals led us to repeat the work of Buchner (1890) with bacterial protein, which has been generally accepted as demonstrating the chemical nature of the chemotaxis of leucocytes.

*B. pyocyaneus* was cultivated on potato slants at room temperature for 72 hours. On account of the scanty growth, the organisms were later cultivated on beef infusion agar at 37·5° C. for 24 hours, there being no apparent difference in the bacterial extract obtained. The bacterial mass was scraped from the media and rubbed up in about 50 times its volume of 0·5 per cent. caustic soda solution. This was left at 6° C. for 18 hours, a clear solution resulting. This solution was made faintly acid with N/10 hydrochloric acid, and the volu-

Table III.

Guinea-pig experiments *in vitro* demonstrating the effect of varying the density of the test solutions within capillary tubes inserted subcutaneously in guinea-pigs and removed after 8 hours.

| Content of capillary | Pig Number I | Pig Number II | Pig Number III | Pig Number IV |
|---|---|---|---|---|
| Salt solution | | | | |
| 1. Isotonic | + | Capillaries of salt solution lost and confused with urea. Two of the six were recovered but not identified | + | Two capillaries lost, third not identified |
| 2. Serum density | + + | | − air bubble at entrance | |
| 3. Saturated solution | + + + | | − " " | |
| Urea solution | | | | |
| 1. Isotonic | + | Ditto | − | + |
| 2. Serum density | + + | | + + | + |
| 3. Saturated solution | + + + | | + + | + + + |
| Peptone solution | | | | |
| 1. Isotonic | − | + | + | + |
| 2. Serum density | + dense fibrin mass | + | + + | + dense fibrin mass |
| 3. Saturated solution | + + capillary broken | + + capillary broken | + + + | + + + |
| *Pyocyaneus* extract | | | | |
| 1. Less than serum density | + | − | Two capillaries lost, third not identified | + |
| 2. Serum density* | + + | + | | + dense fibrin mass |
| 3. Greater than serum density* | Capillary lost | + | | + + + |

* = Density adjusted by addition of salt solution.
− = No leucocytes present.
+ = Few leucocytes in open end of capillary.
+ + = Leucocytes approximately 6 mm.
+ + + = Leucocytes entire length of capillary—12 mm.

minous precipitate formed was subsequently separated by centrifugalization. This precipitate was rubbed up in a little water and a small quantity of 0·5 per cent. caustic soda solution added, just sufficient to bring it into solution. This solution was dark brown and clear and gelatinized in the cold as described by Buchner.

The dissolved protein was sealed in freshly drawn out spindle shaped glass tubes 5 cm. long and about 6 mm. wide at the middle, and sterilized by boiling in a water bath for one hour.

A space on a rabbit's back about 4 inches square was shaved and a little slit made through the skin. The capillaries could be easily pushed through this slit and moved forward about 25 cm. from the point of insertion and the tips broken off. Control tubes were filled with isotonic salt solution. These capillaries were left in place for from four to 48 hours. They were then removed and examined macroscopically and with a hand-lens. The tubes were then broken into sections and the contents of the sections examined with a dark field and by staining.

Contrary to the results reported by Buchner no leucocytes accumulated in any of the capillaries containing either bacterial protein or isotonic salt solution. At first what appeared to be a cloud of leucocytes was observed in the tubes, but a closer examination showed this to be a precipitate which formed in the protein regardless of whether the tubes were placed in the animal or simply in the incubator for a similar period of time. The particles of this precipitate were exactly the size and had the general appearance of leucocytes, but were much more highly illuminated in dark field preparations,

and staining and microscopic examination showed them to be semi-crystalline in nature. Even when stained with Wright's stain they resembled leucocytes. At the openings, and extending a millimeter or two into the capillaries, plugs of fibrin-like clot containing a few leucocytes were invariably formed, both in the tubes containing bacterial protein and in those containing isotonic salt solution.

It was thought that positive results might be obtained if the capillaries containing the bacterial protein were placed in the peritoneal cavity. For this reason similar capillaries were filled with the protein and placed in the peritoneal cavities of rabbits and guinea-pigs. In some instances aleuronat or distilled water was injected into the cavity from four to five hours before the tubes containing the test solutions were inserted so as to have a considerable quantity of leucocytes present. In no case was an accumulation of leucocytes found within the capillaries other than the small plug at the opening and this was almost invariably present.

These same or similar plugs were observed by Buchner who seemed to regard them with the few leucocytes they contained as evidence of chemotaxis. He observed these plugs however only with positively chemotactic substances while our experiments showed them to be present regardless of the nature of the test solution.

## IV.

As a result of our work with a capillary tube technique in animal experiments it was concluded that such a procedure was unsuitable for a study of the effect of substances in solution upon the movement of leucocytes, and that results by its use should be regarded with considerable scepticism. The experiments, on the other hand, by the use of capillaries *in vitro* gave what seemed to be reliable results and definitely indicated that the movement of leucocytes was due to some physical property which depended upon a difference in density between two solutions in contact with each other.

The early observations of Massart (1889), previously mentioned, regarding the effect of dissolved substances upon the movement of certain bacteria and plant cells, indicated that diffusing substances had a repelling action upon these cells and that this repulsion was in some manner dependent upon the isotonic coefficient. In other words, diffusion and the permeability of the bacteria for the diffusing substance were responsible somehow for their repulsion from the test solutions.

Now diffusion obviously occurs in solutions of different density in contact with each other, and especially when the solvent is the same for each solution. This diffusion takes place in two directions, the dissolved substances diffusing in one direction and the solvent in the opposite direction.

Animal and vegetable cells are in all cases easily permeable to water, while their permeability for dissolved substances differs considerably although it is always less than their permeability for water.

With these facts in mind the following conception was formulated as defining the conditions to be observed in a study of the action of soluble substances in solution upon the movement of leucocytes.

(1) That there is no inherent force within the leucocyte which can cause it to move considerable distances in a short space of time.

(2) That the force which produces this motion resides in the solution and is physical and not chemical in nature.

(3) That in a solution in which the solute is not at concentration equilibrium with its solvent, there exists a force of diffusion both upon the molecules of the solute and of the solvent.

(4) That the movement of leucocytes is from a less dense to a denser solution or from one of lesser to one of greater concentration in an aqueous medium and that this motion is due to the force of diffusion of the water molecules moving in a direction opposite to the force upon the molecules of solute.

(5) That a leucocyte is a particle of semi-permeable material which has taken up the solvent water in an amount far in excess of its mass as represented by protein and, as a consequence, moves by reason of the force of diffusion upon the molecules of water and in a direction opposite to the direction of action of the diffusing molecules of solute.

With this conception of the problem in mind the four following experiments were carried out.

## TEST NO. 1.

The first test shows that an artificial leucocyte which consisted of a cell in the shape of a sphere made of collodion and filled with water moves in water toward a source of diffusing saccharose.

A copper sterilizing pan five inches wide and one and one-half inches deep and ten inches long was divided into three compartments by means of parchment partitions. One of the end compartments was filled with a concentrated solution of saccharose and the other two with distilled water. A spherical sack of collodion one inch in diameter was filled with water, the opening tied with a silk thread and placed in the middle compartment.

After about 30 minutes this cell had moved over against the partition through which the saccharose was diffusing, and this occurred repeatedly during the course of the experiment regardless of where the cell was placed within the middle compartment.

## TEST NO. 2.

This test demonstrates the movement of leucocytes over a considerable distance toward a source of diffusing sodium chloride.

Ordinary six by five-eighths inch test tubes were drawn out at one end to a diameter of about 6 mm. They were bent to an angle of about 120 degrees at the beginning of the constriction. The constricted end was cut off about four inches from the bend.

Five batches of 1/2 per cent. aqueous agar were prepared containing respectively no sodium chloride, 0·5, 1, 2, and 3 per cent. sodium chloride.

The agar was then filled into the bulbs of the test tubes described and allowed to gelatinize. Washed guinea-pig leucocytes were suspended in 0·5 per cent. sodium chloride solution contained in a small crystallizing dish. The drawn out ends of the tubes were now filled with 0·5 per cent. saline and the open ends immersed in the suspension of leucocytes. The experiment was carried out in a thermostat at 37·5° C.

After four or more hours the leucocytes were found to have moved into the tubes containing the 2 and 3 per cent. sodium chloride for from three to four inches, while in the other tubes no leucocytes had accumulated.

TEST No. 3.

The following test is an actual demonstration of the presence of osmotic force and shows the effect of the diffusion of sodium chloride upon the rate of settling of leucocytes from a suspension.

A suspension of guinea-pig leucocytes was made in 0·5 per cent. sodium chloride solution. This was filled into two small test tubes, about 2 c.c. of suspension in each. At the surface of the suspension in each tube there was placed by means of wire loops a small particle of agar (about 3 mm. cross section). This agar was plain aqueous 1 per cent. agar in one tube and in the other was 1 per cent. agar containing 3 per cent. sodium chloride. These tubes were placed in the thermostat at 37 5° C.

The suspension contained in the tube with the plain aqueous agar settled rapidly to the bottom while that contained in the other tube had not settled in five hours.

This experiment was repeated using pellets of agar in place of the particles described above. These pellets rested upon constrictions made in the test tubes. The results were similar.

TEST No. 4.

The last test shows that an insoluble substance like casein has no effect upon the rate of settling of leucocytes from a suspension, but that when a soluble substance is produced by tryptic digestion, the same effect is observed as with sodium chloride.

This experiment was set up in the same manner as test no. 3, using two test tubes containing the leucocyte suspension. The agar pellets, however, contained in the one case powdered casein and in the other a mixture of casein and trypsin.

The leucocytes in the tubes containing the casein agar pellet settled quite rapidly to the bottom, while in the tube containing the casein trypsin agar mixture, the leucocytes remained suspended for more than six hours.

Regardless of any theoretical considerations these four experiments together with capillary tube experiments *in vitro* demonstrate that with the substances used, and in an aqueous medium, leucocytes move in a direction opposite to that in which the dissolved substances are diffusing. This supports the hypothesis that the movement of leucocytes is due to the force of osmosis which exists in a solution not at concentration equilibrium, and that the application of that force depends upon the greater permeability of leucocytes for water than for the dissolved substances.

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