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Factors That Influence Phagocytosis In Vitro

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I. FACTORS THAT INFLUENCE PHAGOCYTOSIS IN VITRO

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These studies were carried out 1946-1951 at the University of Rochester, Louisiana State University, and the University of Minnesota under contract with the office of Naval Research, Department of the Navy (NR-130-762).

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The ingestion of microorganisms and particulate matter by phagocytes, and the constant association of these cells with inflammation of all types suggest that further information concerning the activity of these cells will be helpful in understanding many disease processes. Many attempts have been made to devise methods for measuring the rate at which phagocytic cells are able to ingest particulate matter, and to determine the subsequent fate of the cells and of the phagocytized material.

In vivo techniques have yielded much valuable information concerning cellular immunity, but the constant changes in fluid and cells in the living body make evaluation of the activity of individual cells difficult.

Investigations of the functioning of phagocytic cells per se have turned to in vivo methods. The many different techniques devised attest to the inadequacies of each. Commonly, fluid containing phagocytes is mixed with a suspension of bacteria, and after agitation for various intervals of time, microscopic examination permits counting of the numbers of bacteria within phagocytic cells, and the relative number of cells with and without ingested bacteria. The phagocytic indices commonly employed represent quantitative measurements of the degree of activity of the phagocytes. These indices fall into three categories: (1) An index for the per cent phagocytosis to reflect the percentage of phagocytes which contain any bacteria; (2) The average number of bacteria per cell, considering only the cells with bacteria; and (3) the total number of bacteria phagocytized by 100 cells.

Published reports indicate that with phagocytes suspended in saline, no phagocytosis occurs; but, with the subsequent addition of plasma, phagocytic activity is observed.[1] In the presence of specific antibodies, an increased number of phagocytes are seen to contain bacteria, and increased numbers are usually found within single cells. Similarly altered phagocytic activity is reported to occur with variations in temperature, and in pH, and with the addition of a wide variety of chemical substances.[2] However, even under apparently standard conditions, wide variation in phagocytic activity is observed so that for other than gross changes, large groups of determinations are necessary for significant results, and often results are inconclusive or contradictory. Thus, it is apparent that more exact quantitative techniques are needed.

The present studies began as part of a series of investigations concerning the relationship of streptococcal infection to rheumatic fever. Phagocytosis was employed as a natural mechanism, whereby streptococcal constituents might be released to damage or to sensitize host tissues. This concept led to observations upon the role of phagocytosis in streptococcal disease and in other Gram positive bacterial infections. When standard techniques for normal specimens demonstrated disturbingly wide variations in the phagocytic indices, attention was turned toward the mechanisms concerned in the process of phagocytosis, and towards means for establishing accurate measurements.

Materials and Methods

Bacteria Three bacterial species were employed in these studies:

Diplococcus pneumoniae, Streptococcus pyogenes, (B hemolytic streptococcus) and Micrococcus pyogenes, var aureus. (Staphylococcus aureus)

Hemolytic streptococcus, strain CO, Group A, type 19 was used as either a glossy or matt variant.

The staphylococcus strain was isolated from a patient at the Strong Memorial Hospital, New York dead of a staphylococcal septicemia. This species was included because it had been reported that phagocytosis of staphylococcus was enhanced following exposure to x-ray[3,4], as well as because of the technical ease of its use.

Pneumococcus, type 27, was included, as a third Gram positive coccus, for comparative purposes, and because this pneumococcal type demonstrated non-specific capsular swelling in the presence of acute phase protein.[5]

All organisms were cultured in standard enriched types of laboratory broth. In all experiments except the first, where 18 hour cultures of the pneumococcus were employed, the bacteria were utilized as concentrated resuspended 4 hour old cultures. This was done to provide bacteria uniformly in the early logarithmic growth phase, and, by suspending in fresh broth, to remove any metabolic products of the bacteria which might be present in the culture fluid.

Bacterial counts on the suspensions employed were carried out in all experiments, by the serial-dilution-plating technique.

Bacterial counts, by the serial-dilution-plating technique, were carried out on all suspensions prior to use in phagocytosis.

Phagocytes. In all instances, phagocytes were made available as specimens of freshly drawn whole blood, kept fluid by the addition of heparin, 0.05 ml. per 5 ml. of blood, except where otherwise indicated. All specimens were routinely used within 30 minutes of the time of withdrawal. Rabbits and human subjects were employed. The effects upon phagocytosis resulting from the anti-coagulant are described in this paper. Total white cell and differential counts were carried out with all specimens, prior to use in phagocytosis.

Technique for Mixing Phagocytes and Bacteria

The technique of Fern[6] was employed because of its simplicity and ready adaptation to quantitative measurement. For phagocytic determinations, 0.5 ml. of blood, and 0.1 ml. of bacterial suspension were added to standard test tubes, which were placed on a circular platform inclined to a 45° angle and rotated at 9 revolutions per minute. The amounts of 0.5 ml. of blood, and 0.1 ml. of bacteria suspension were kept constant in all experiments, quantitative variations being achieved by altering the concentration of bacteria in the 0.1 ml. aliquot. The maintenance of constant temperature, throughout was assured by keeping the rotating machine in a walk-in incubator at 37°C. The phagocytic mixture was agitated for an interval of 30 minutes, except where otherwise specified.

Measurement of Results

Following the period of agitation of the blood-bacteria mixture, phagocytic indices were determined for all specimens and total white cell counts and bacterial counts where indicated.

The phagocytic indices were determined from smears stained with the Cross modification of the Pappenheim stain. Microscopically, the first 100 phagocytes (polymorphonuclear leucocytes and monocytes) encountered were recorded as containing, or not containing, bacteria with the specific number within single cells counted. From this information, three phagocytic indices were determined.

1. Per cent phagocytosis: the number of phagocytes, of the 100 counted, that had ingested bacteria.

2. Bacteria per cell, based only on phagocytes that contained bacteria: The average number of bacteria within cells which contain any bacteria.

3. Bacteria per 100 cells: the total number of bacteria taken up by 100 phagocytes, including all cells, with and without bacteria. This represents essentially a combination of the first and second indices.

For the smears employed for determination of phagocytic indices, the number of lymphocytes encountered while 100 phagocytes were tabulated provided a differential count of the leucocytes following phagocytosis. Total white cell counts, by standard laboratory methods, were carried out on the blood-bacteria mixture following phagocytosis in some instances.

Bacterial counts, by the serial-dilution-plating technique, were carried out upon the blood-bacteria mixture, and after phagocytosis had occurred, when indicated a bacterial count was made for the whole blood specimen. The plasma specimens, from the same blood sample, were treated exactly as the blood specimens to determine any effects upon the bacterial population which might result from factors in the non-cellular fraction of the blood.

Experimental

At the onset, a group of phagocytic determinations were carried out with normal rabbit blood and pneumococcus, type 27, to establish a technique for measurement of phagocytosis, and to provide, for comparison, a normal range of values. However, as an increasing number of determinations persistently showed indices for per cent phagocytosis ranging from 0 to 80%, it became apparent that an impracticably large group of determinations would be necessary in any experiment for statistically significant results. The observations from this pilot study led to the experiments which follow.

Experiment 1. 18 normal rabbits provided 140 blood samples over a period of 120 days. Indices were in most instances determined for from 2 to 6 blood samples simultaneously with aliquots from the same bacterial suspension. Coagulation of blood was prevented by the addition of heparin. Pneumococcus, type 27, was utilized as a concentrated, resuspended 18 hour culture. Total white cell and differential counts, and viable bacterial counts were carried out on all specimens prior to the mixture of blood and bacteria. Following the 30 minute phagocytic interval, smears were prepared from each specimen for the determination of the three phagocytic indices and for a differential leucocyte count. A summary of results is presented in Table I.

Table I demonstrates that the phagocytic indices for per cent phagocytosis ranged from 0 to 80%. The bacteria per cell indices paralleled the figures for per cent phagocytosis in all instances. Of the 18 rabbits tested, 2 persistently showed phagocytic indices which were much lower than those achieved by other rabbits tested simultaneously with aliquots from the same bacterial suspensions.

These two animals differed from the others in no detectable way. When several animals were tested together on separate days, parallel variations in the phagocytic indices were noted, with all high on some days, low on others. For this experimental group, no correlation could be demonstrated between the phagocytic indices and the numbers of bacteria added.

The results of this experiment indicated that additional factors in the technique required evaluation and control for achievement of more accurate and reproducable results.

The second experiment represents another pilot group of phagocytic determinations, but includes certain modifications which were suggested by the results of the first group. Again the purpose was to establish an accurate technique for the measurement of phagocytosis and to determine a normal range of values for comparative studies. The replacement of rabbit blood by human blood resulted in the advantages of more constant total leucocyte counts, a higher and more constant percentage of polymorphonuclear leucocytes, and greater certainty in morphologic identification of the leucocytes. Two other organisms, in addition to the pneumococcus, were

tested, i.e., hemolytic streptococcus and staphylococcus. The latter was employed in two dilutions, one ten times the concentration of the other. The bacteria were utilized as concentrated washed suspensions of young bacteria of uniform age.

Experiment 2. Eight healthy young men over a period of 37 days provided 36 blood specimens, each tested with three or four bacterial suspensions for a total of 183 phagocytic determinations. Heparin was used to prevent coagulation of the blood. Bacterial suspensions of pneumococcus, streptococcus, and staphylococcus were prepared following transfer in series as 8, 8, 8, and 4 hour cultures. The latter culture was centrifuged and resuspended in fresh broth to provide organisms uniformly in the early logarithmic growth phase. Blood cell and bacterial counts were made prior to mixture for phagocytosis. Following the 30 minute interval, smears were prepared for the determination of phagocytic indices and leucocyte differentials.

Table I indicates the distribution of the indices for per cent phagocytosis for each of the four bacterial suspensions employed. A wide distribution between 0 and 100% is again apparent, with all three organisms. However, it is readily noted that where the lesser concentrations of the staphylococcus were employed, indices are low, and where the more concentrated suspensions were used, relatively high indices are obtained. Similarly, for all three organisms, a relationship could be demonstrated between the magnitude of the phagocytic index and the numbers of bacteria added. With all three organisms, an irregular decrease in the percentage of polymorphonuclear cells and monocytes in the cell differential was observed following phagocytosis.

These results again demonstrate a wide range in the phagocytic indices, as found in the first experiment. However, the variations in phagocytic indices, in relation to the numbers of bacteria added, suggested the importance of strict quantitative control in measurement of phagocytosis in vitro. Accordingly, Experiments 3 and 4 were designed to directly measure this factor.

Quantitative Measurement of Phagocytosis

The third experiment was designed to measure phagocytic activity in the presence of different numbers of bacteria. Ten phagocytic tubes were simultaneously prepared, containing the same specimens of blood and total volumes, but different concentrations of bacteria. In addition to phagocytic indices, the determination of differential leucocyte counts and bacterial counts permitted measurement of the changes in the white cells and bacterial population following phagocytosis. The staphylococcus was selected of the three species under study as the most readily adaptable to quantitative procedures.

Experiment 3. Four healthy young men provided 12 separate blood specimens over a period of 49 days. Coagulation for each specimen was prevented by the addition of heparin. The staphylococcus, for each group, was prepared as a washed suspension of 4-hour-old organisms. From the original concentrated bacterial suspension, a series of 1:1 dilutions was carried out, resulting in a row of 10 tubes, each containing successively one half the concentration of the previous tube. The concentrations ranged from hundreds of millions to tens of thousands. From each tube, 0.1 ml. of suspension was added to 0.5 ml. of blood in a standard phagocytic tube, and a second 0.1 ml. aliquot to a tube containing 0.3 ml. of plasma. Bacterial counts, and total white cell and differential counts were carried out prior to the phagocytic interval on the blood and bacterial specimens. Following the 30 minute period of agitation, smears were prepared from each blood-bacteria specimen for the determination of phagocytic indices and differential counts, and serial dilution plating bacterial counts were established for each blood-bacteria admixture and for each plasma-bacteria specimen. A group of individuals worked simultaneously, so that all measurements could be made at the time intervals indicated. The results for the 12 group determinations are pre-

sented in Table III.

It can be seen that the findings in Table III are tabulated according to the numbers of bacteria which were added, from 100,000 to 50,000,000. The indices for per cent phagocytosis are distributed from a mean of 1.6 found when 100,000 bacteria were added to 89.6 when 50 million were added, with gradated values intervening. For each bacterial concentration, the indices achieved with the 12 blood specimens are remarkably constant. When fewer than 800,000 bacteria were added, all values for per cent phagocytosis are below 20. When more than 13 million were added, all values are above 60%.

The indices for the average number of bacteria per cell, considering only those cells containing bacteria, range from 1.8 when 100,000 bacteria were added to 15.8 when 50 million were added. In relation to the percent phagocytosis indices, the average numbers of bacteria per cell remain below 6 until the range where over 50% of the phagocytes contained bacteria. Above this, there is a rapid increase which apparently was limited by the disruption of phagocytes that had occurred when the greater numbers of bacteria had been added.

The total number of bacteria phagocytized by 100 phagocytes, including the leucocytes with and without bacteria, is demonstrated to be directly proportional to the numbers of bacteria added. For example, when 100,000 bacteria were added, the total number phagocytized by 100 cells was 5; with 200,000, 10; with 400,000, 20, and so on. This relationship was particularly noteworthy in the ranges where large numbers of bacteria were added, and the disruption of cells was excessive.

The differential white cell counts showed a decrease in the percentage of polymorphonuclear leucocytes and monocytes in the specimens to which large numbers of bacteria were added and that decrease was most marked with the greatest numbers.

In some instances, it was not possible to find an adequate number of phagocytic cells on a smear to permit determination of indices. It is of interest that the lymphocytes appeared to be unchanged in appearance and number on the smears.

The results of viable bacterial counts upon the plasma control specimens indicated that no change had occurred in the bacterial populations which had been mixed by agitation with the non-cellular portion of the blood. For all phagocytic tubes, with all concentrations of bacteria added, it was noted that the remaining bacterial count was approximately 20% of the number that had been added.

Table IV presents the calculations that effect the changes that occurred in the phagocytes and bacteria. For each original bacterial suspension, prior to its addition to a blood sample, quantitative counts determined by the viable serial dilution plating method were checked in a number of instances by the microscopic Petroff-Hausser chamber counting method. In all instances, it was noted that values for the microscopic count were approximately twice the values achieved by the viable technique. These findings were in accord with the observation that staphylococci occur commonly in pairs. Thus, for the present calculations, the figures for numbers of bacteria added, obtained by the serial dilution plating techniques, were multiplied by 2. From the figures for the numbers of bacteria added, and for the numbers found present after the phagocytic interval, the decrement of bacteria resulting from phagocytosis can be calculated. With the figures for the calculated numbers of phagocytic cells present, and for the average number of bacteria per phagocyte (based on all phagocytes, with and without bacteria), the total number of bacteria present within phagocytic cells at the end of the phagocytic interval can be calculated. When little disruption of phagocytes had occurred, the relationship between the figures for bacterial decrement and for the total number of bacteria within phagocytes is remarkably close. In the specimens where greater numbers of bacteria were added, and disruption of cells

was marked, the bacteria within phagocytes represents only a small part of the total decrement. It is apparent, from these figures, that disruption of phagocytic cells was associated with a destruction of large numbers of bacteria.

Experiment 4 repeats the determinations of Experiment 3, with certain modifications, utilizing blood specimens from a single individual over a period of 13 months. In the preparation of the bacterial suspensions, the use of 1:4 instead of 1:1 serial dilutions provided a wider range of bacterial concentrations with a smaller number of specimens. Total white cell counts were carried out for all blood bacterial mixtures following the phagocytic interval. Plasma controls for the bacterial counts were not included.

Experiment 4. A single healthy male adult provided 10 blood specimens at irregular intervals over a 13 month period. Heparin was used to prevent coagulation of blood and the same strain of staphylococcus was employed as the test organism. Resuspended bacterial concentrates, containing 4-hour-old organisms, were prepared as previously described. A 1:4 dilution in series were made for a total of six tubes. From each dilution 0.1 ml. was added to a 0.5 ml. aliquot of whole blood, following which the entire group was agitated simultaneously for 30 minutes. Smears were prepared for the determination of phagocytic indices and leucocyte differentials. Total white cell counts were carried out for each of the phagocytic tubes. Total bacterial counts were performed on only half of the tubes following phagocytosis, and no plasma controls were included. The results are presented in Table V.

It may be seen from Table V,a) that the findings closely corroborate the conclusions of Experiment 3, that relate phagocytic indices and bacterial changes; b) that total white cell and differential counts substantiate the conclusion that little change occurs in the numbers of lymphocytes present, and c) that the decrease in total white count, when large numbers of bacteria were added, reflected a disruption of phagocytic cells.

Timing of Phagocytosis

In the previous experiments, the measurements were routinely carried out after the phagocytes and bacteria had been allowed to interact for a 30 minute interval. Experiments were planned to determine phagocytic activity at other time intervals to assess for control purposes the importance of this factor by accurate *in vitro* measurements, and, if possible, to establish additional information concerning the kinetics of the phagocytic process and the fate of the cells and bacteria. Pilot experiments comparing phagocytosis after 30 minutes and 3 hours revealed inconclusive results. The following experiment was designed to measure phagocytosis after intervals ranging from 3 minutes to 24 hours. Three group experiments were carried out, differing only in the time intervals selected, to provide a wide range of values. In each group, a graduated series of staphylococcal concentrations were employed so that any variations in phagocytic activity, in relationship to the numbers of bacteria made available, as described in Experiment 3 and Experiment 4, might be established at different time intervals. The following data demonstrate the remarkable speed of phagocytosis, the continuation of phagocytic activity over several hours, or as long as intact phagocytes remain, and for all specimens an early decrease in the viable bacterial counts, followed by a rapid proliferation of organisms.

Experiment 5. A single adult male provided 3 blood specimens over a period of 2 months, each utilized for a group experiment. The groups were similar except for the time intervals employed. The group to be described measured phagocytosis at 5 minutes, 30 minutes, and 1, 2, 3, 4, and 10 hours. In the other groups, measurements were carried out at 3, 5, 15, and 45 minutes, and 3 hours, and at 5, 15, and 45 minutes, and 24 hours. Six 1:4 serial dilutions were carried out from an initial concentrated suspension of washed stapnylococci. From each concentration, a 0.1 ml. aliquot was added to each of 7 tubes con-

taining 0.5 ml. of heparinized blood. The phagocytic tubes were placed simultaneously upon the agitator. After the indicated intervals, one tube containing each of the bacterial concentrations was removed, and smears, white cell counts and bacterial counts were carried out simultaneously by a group of workers. Phagocytic smears were prepared, and total white cell counts were carried out for all specimens. Bacterial counts were done for only three of the specimens in each group because of the limitations of time.

The results of this experiment are presented in Table VI. Table VI-A indicates the total number of bacteria ingested by 100 phagocytes, with different numbers of bacteria added and at the various time intervals. Where 300 million bacteria were added, over 1,000 bacteria were taken up by 100 phagocytes. At this point, 85% of the phagocytes contained bacteria. A rapid destruction of cells occurred within 30 to 60 minutes, and the phagocytes that remained continued to ingest bacteria. Associated with these changes, the number of bacteria present showed a rapid decrease up to one hour following which there was a rapid proliferation of organisms. When fewer bacteria were added, the changes were progressively slower, to result for the lower bacterial concentrations, in phagocytic indices that increased up to 3 or 4 hours, and the persistance of intact phagocytic cells for 10 hours. For control purposes, tubes that contained blood but no bacteria, which were included to assess any alterations in the cells from mechanical agitation, revealed little change in the numbers of intact phagocytic cells at 10 hours, but nearly all were disrupted at 24 hours.

The results of Experiment 5 make it apparent that in such phagocytic studies, the numbers of bacteria added, and the time interval allowed for agitation of phagocytic cells and bacteria must be exactly known. For example, where large numbers of bacteria were added phagocytosis was so rapid that slight differences in the time interval allowed for phagocytosis might have been responsible for the great variation in phagocytic indices. Results determined at intervals of 1 hour are complicated by the proliferation of bacteria and the disruption of phagocytic cells. In view of the variations of phagocytosis observed with different bacterial species, any conclusions from the findings presented herein must be limited to the organism involved until observations are made with other bacteria.

Effect of Anticoagulants

In the use of whole blood as the source of leucocytes for measurement of phagocytosis, coagulation of the blood must be prevented. Sodium citrate, as employed by Glenn[3,4] in his studies on the effect of Roentgen irradiation upon phagocytosis, was utilized in a small group of the pilot experiments of the present series. When wide variations in results were observed it seemed possible that the effects might be influenced markedly by the anticoagulant. Accordingly, three methods commonly employed for the prevention of coagulation were investigated for their effect upon phagocytic activity of white cells. The information was sought in an attempt to establish more accurate methods for the measurement of phagocytosis and to study factors involved in the process of phagocytosis. The three experiments that follow compare phagocytic activity of blood specimens which were prevented from coagulating: a) by the addition of heparin; b) by the removal of calcium ions by the addition of sodium citrate; and c) by defibrination. In each of these methods, coagulation is prevented by a different mechanism. Phagocytosis of three bacterial species is considered.

Experiment 6. Eight rabbits, over a period of 38 days, provided samples of blood for 15 group determinations. Each blood sample, immediately on being withdrawn, was distributed among 3 flasks containing: a) 0.05 ml. of a 1:1000 solution of heparin per 5 ml. of blood; b) 15 mgm. of sodium citrate, as a fine powder, per 5 ml. of blood; and c) 15-20 sterile 5 mm. glass beads, with shaking for 10 minutes, for defibrination. Suspensions of the three bacterial species, i.e., staphylococcus, streptococcus, and pneumococcus,

prepared as previously described, were employed in single concentrations. Each of the three portions of blood was tested with each of the bacterial suspensions for a total of 9 phagocytic tubes, tested simultaneously. 0.5 ml. of blood and 0.1 ml. of bacterial suspension were employed routinely. Prior to phagocytosis, total white cell and differential counts were done for each of the blood specimens, and serial dilution plating counts for each of the bacterial suspensions. The blood-bacterial mixtures were agitated for 30 minutes, following which smears were prepared for determination of phagocytic indices and differential leucocyte counts. Table VII demonstrates the results of the total white cell and differential counts for the original blood specimens, prior to phagocytosis.

Table VII shows that the results for the heparinized and citrated specimens were approximately the same, and that the variations fell within the range of technical error. However, for all defibrinated specimens, a decrease in the total white cell count and the numbers of phagocytes present was observed, but the numbers of lymphocytes remained relatively constant. Moreover, the decrease in numbers of phagocytic cells varied from 20 to 80 per cent, as compared to the heparinized and citrated specimens. These experiments make it clear that citrate exerted an irregularly depressant effect upon phagocytosis, and that defibrination resulted in a selective decrease in the numbers of phagocytic cells.

Table VIII presents for all 15 group determinations the indices for per cent phagocytosis for the three types of blood and the three bacterial species. It is seen for all three bacterial species, that the phagocytic indices for heparin and defibrination were approximately equal, but the indices achieved with the citrated blood specimens were, with a single exception, well below the figures for the other blood types. The depression in phatocytic index when the pneumococcus was employed, was most pronounced, and for the streptococcus, the values more nearly approached the findings for the other two anticoagulants. The reason for this difference was not apparent. Moreover, it may be seen for the heparinized and defibrinated blood specimens that the irregular depression of the percentage of phagocytic cells in the differential counts following phagocytosis was most marked for defibrinated specimens. Finally, for citrated blood, little change had occurred from the figures that were established prior to phagocytosis. In these results, no constant relationship of phagocytic indices to the numbers of bacteria was made apparent.

The irregular depression of numbers of phagocytes in blood specimens which had been prepared by defibrination made this method for prevention of coagulation unsatisfactory for use in phagocytic studies, and its use was discontinued. With the observation of the depressant effect of citration upon the phagocytic activity of rabbit blood cells, the following experiment was similarly designed to compare for human blood phagocytosis with heparinized and citrated human blood. Similar depression of phagocytic activity by citration was found.

Experiment 7. Twenty blood specimens from four human subjects were tested over a period of 17 days. Each blood specimen, upon its withdrawal was divided among flasks that contained citrate and heparin in the same concentrations as in the previous experiment, and each blood specimen was tested for phagocytic activity, by utilizing each of the three bacterial species as employed previously. Total white cell and differential counts were carried out on each of the blood specimens, and serial dilution plating counts on each of the bacterial specimens prior to phagocytosis. Following the 30 minute interval of agitation, smears were prepared for phagocytic indices and differential leucocyte counts.

Table IX presents the indices for per cent phagocytosis, in relation to the numbers of bacteria added. The heparinized specimens, for all three bacterial species, yielded phagocytic indices that were higher, particularly

where greater numbers of bacteria were added. The citrated specimens, containing staphylococcus or pneumococcus, revealed little phagocytosis regardless of the numbers of bacteria added, and for the streptococcus, definite but irregular depression of phagocytic activity had occurred. These findings made it evident that citrate exerted, for human blood, depressant effect upon phagotycosis, which again was most marked for the pneumococcus and more irregular for the streptococcus.

With the demonstration that accurate quantitative measurements were made possible by the use of serial concentrations of bacteria for phagocytic measurements an experiment was designed to re-evaluate the depressant effect of the citrate by this method. In a series of six tests, 1:4 serial dilutions of staphylococci were prepared as in previous experiments, and a 0.1 ml. aliquot from each concentration was added to each 0.5 ml. blood specimen containing a) 3 mg. of citrate per ml. of blood (the concentration previously employed); b) 10 mg. of citrate per ml. of blood; and c) various other concentrations. In contrast to the results of the two previous experiments, practically no depression was noted with the specimens containing 3 mg. of citrate per ml., slight depression in the specimens containing 10 mg. per ml. and irregular results for the others containing up to 100 mgm. per ml. of blood.

Experiment 8. Six blood specimens were provided by a single individual, one of the subjects of the previous group. Each specimen was divided into several parts, each prevented from clotting by: a) heparin, as a control; b) citrate in a quantity of 3 mg. per ml.; c) citrate 10 mg. per ml.; d) various other higher concentrations of citrate. For each group test, a series of 6 bacterial concentrations were prepared with a dilution factor of 1:4, and an 0.1 ml. aliquot added from each concentration to each 0.5 ml. portion from each of the blood preparations. Total white cell and differential counts were carried out for each blood specimen, and serial dilution plating counts for the bacterial suspensions, prior to the initiation of phagocytosis. Following the 30 minute interval, smears were prepared for phagocytic indices and differential leucocyte counts.

Little depression of phagocytosis by the citrated blood was observed in relation to the heparinized control, or to the results achieved with heparin in previous experiments. For the specimens containing 3 mg. of citrate per ml. a minimal detectable difference was found from the heparin levels. The specimens containing 10 mg. per ml. showed slight uniform suppression, but not nearly to the degree observed in the previous experiments. The more concentrated specimens yielded very irregular results.

The apparent contradiction between the results of Experiment 8, as compared to those of Experiment 6 and Experiment 7, cannot be adequately explained by any of the experimental data. However, the sodium citrate had been obtained from a different supply house in a different city than that employed in Experiment 6 and Experiment 7. When Experiment 8 was carried out, none of the previous citrate specimens were available for re-testing. Thus, no explanation is offered for these unusual results, other than the need to consider the possibility that some factor other than the citrate *per se* was responsible for the pronounced depressive effect.

Phagocytosis with Bacteria of Different Ages

Bacteria, in their growth cycle, demonstrate variations in morphology and physiology. Since bacteria of different ages had been employed in the phagocytic studies which were carried out in other laboratories, and in view of the erratic results of Experiment 1 in which 18-hour cultures were employed, an experiment was carried out to learn whether alterations in phagocytic activity would result from the use of bacteria of different ages.

Experiment 9. A single blood specimen from a human subject was employed in heparainzed form. Beginning 36 hours prior

to the time of the experiment, culture tubes were inoculated at measured intervals with loopfuls of staphylococci in the logarithmic growth phase to make available, at a given time, five bacterial cultures containing organisms grown for 36, 18, 10, 4, and 2 hours. Each culture was centrifuged and resuspended to remove any metabolic bacterial products in the culture fluid. Serial 1:4 dilutions of the initial bacterial concentrate were prepared for a total of 5 concentrations for each of the 5 bacterial ages. From each of the 25 bacterial suspensions thus produced, 0.1 ml. was added to 0.5 ml. of blood. Prior to phagocytosis, the most concentrated suspension for each bacterial age was subjected to a viable serial dilution plating count, and a microscopic chamber count, since it was anticipated that in the older ages of bacteria, a percentage of the organisms would be non-viable. However, for all specimens, as previously, the microscopic counts were found to be approximately 2 times as large as those for the viable technique.

This experiment revealed no significant difference in the measurements of phagocytosis for the 5 ages indicated, or for any of the concentrations.

DISCUSSION

The studies described consider some of the factors that operate in the measurement in vitro of phagocytosis. As demonstrated in the first two experiments, where phagocytic indices from 0 to 100 were found under what have commonly been accepted as standard normal conditions, indices for phagocytosis were too variable for any accuracy in measurement. By the control of several additional factors, it was possible to reproduce accurately measurements for phagocytic activity, in terms either of phagocytic indices or of the alterations that were observed in the phagocytic cells and the bacteria.

The great importance of rigid quantitation of the bacteria and cells is demonstrated. Experiment 3 and Experiment 4 demonstrated that phagocytic activity becomes a direct function of the numbers of bacteria added when the number of cells for a fixed volume is held constant. With control of this factor, blood specimens from different individuals and from the same individual on widely separate occasions show consistent values for phagocytic activity. Moreover, it was demonstrated under the same circumstances that the number of bacteria taken up by 100 cells is directly proportional to the number of bacteria added. For example, it was found that the addition of twice the number of bacteria resulted in the phagocytosis of twice the number of bacteria. This relationship held until the phagocytic cells began to show marked disruption.

The three bacterial indices showed roughly parallel variations. When an increase resulted in the figures for per cent phagocytosis, the numbers for bacteria per cell indices increased as well. However, in the quantitative studies, it was demonstrated that as greater numbers of bacteria were added, the percentage of cells that contained bacteria increased more rapidly than the average number of bacteria taken up by each of the cells. However, subsequently, as the percentage of cells that contained bacteria are greater than 50, the average number of bacteria per cell increased progressively and more rapidly. Disruption of phagocytic cells was observed when cells containing 15 or more bacteria made their appearance. The quantitative studies make it apparent, at least for the circumstances described, that the presence of large numbers of bacteria in a cell precedes the disruption of the cell and that both bacteria and cell are destroyed. It appears that all circulating polymorphonuclear leucocytes and monocytes are actively phagocytic.

Under the experimental circumstances described, bacteria were unaffected by incubation in free plasma in contrast to whole blood in which active phagocytosis resulted uniformly in depression of bacterial numbers after the half hour interval. The degree of decrease in bacterial numbers was found to vary with the time interval, the lowest point being at about one hour. In no instance was there complete destruction of bacteria, even when very few were added. The rapid increase

in numbers of bacteria appears to be prevented by the presence of a sufficient number of phagocytic cells, since rapid growth of bacterial numbers was observed as the number of cells decreased. The experiments that were concerned with the timing of phagocytosis again gave evidence that phagocytosis is a function of frequency of contact between cells. For example, when large numbers of bacteria were added, phagocytosis and an associated disruption of cells occurred rapidly. When smaller numbers of bacteria were added, phagocytosis occurred more slowly but continued over a longer period of time. However, even with proliferation of bacteria, the degree of phagocytosis does not reach that observed where greater numbers of bacteria are added. The rapidity of phagocytosis is rather remarkable. Thus, when 400 million bacteria were added, the percentage of cells within 3 minutes that contained bacteria approached 100 and as many as 2,000 bacteria were found to have been ingested by 100 phagocytic cells. The variations that were observed in the phagocytic measurements carried out at different time intervals make essential careful and rigid control of this factor. It is apparent that any interpretation of a phagocytic index must be considered in terms of numbers of bacteria added and the time interval allowed for phagocytosis.

The anticoagulant employed for whole blood was found to exert an influence upon phagocytosis. Defibrination resulted in a loss of from 20 to 80 per cent of phagocytic cells but the cells that remained were capable of normal phagocytic activity. Two different samples of sodium citrate were employed to make up suspensions for anticoagulant purposes. It was found that one sample made up in optimal concentration exerted a profound and the other sample a slight suppressant effect upon phagocytic activity. While the reason for this difference was not learned, caution is indicated in the use of citrate as an anticoagulant in phagocytic studies.

In a single study, the age of the staphylococcus employed, from 36 to 2 hours was without effect upon measurements of phagocytosis. Since washed organisms were used, the effect of any non-viable bacterial products in the culture broth is not considered.

It is of interest that the techniques for studying phagocytosis when applied to blood from rabbits and humans that had been exposed to measured doses of x-ray failed to show alterations in phagocytic activity. Moreover, repetition of the technique employed by Glenn[3,4] did not result in confirmation of his finding of a significant increase in phagocytic indices following roentgen irradiation. Since he employed bacterial suspensions in unknown concentrations, it appears that his results may be explained by the fortuitous use on successive days following exposure of specimens in different concentrations.

Summary

1. The employment of standard techniques for the measurement of phagocytosis in vitro resulted in an unsatisfactorily wide range of "normal" values.
2. The importance of rigid quantitation of the factors that operate in phagocytic measurements is demonstrated and discussed. Phagocytic indices, and the fate of the phagocytic cells and bacteria are considered.
3. The rate at which phagocytosis occurs is shown to vary with concentration of bacteria present, being amazingly rapid when large numbers of bacteria are added.
4. The effects exerted by the anticoagulant are considered.
5. In a single experiment, the phagocytic activity of cells was not measurably influenced by the age of the bacteria that underwent phagocytosis.

References

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6. Fenn, W.O.: The Phagocytosis of Solid Particles.: J.Gen.Phys.5:311:1922-23.

TABLE I

Distribution of Phagocytic Indices (Per Cent Phagocytosis) for 140 Determinations with blood from 18 normal rabbits and Pneumococcus type 16.

Indices for Per Cent Phagocytosis	Number of Determinations	Per Cent of Total Number of Determinations
91 - 100	-	-
81 - 90	-	-
71 - 80	1	0.7
61 - 70	6	4.1
51 - 60	6	4.1
41 - 50	11	8.9
31 - 40	19	13.6
21 - 30	29	20.3
11 - 20	29	20.3
0 - 10	39	28.0

TABLE II

Distribution of Phagocytic Indices (Per Cent Phagocytosis) for 124 Determinations Carried Out with 36 Blood Specimens from 8 Human Subjects, utilizing 4 Bacterial Suspensions.

Indices for Per Cent Phagocytosis	Number of Determinations			
	Concentrated Staphylococcus	Dilute Staphylococcus	Streptococcus	Pneumococcus
91 -100	1	-	21	7
81 - 90	6	-	7	10
71 - 80	8	-	10	4
61 - 70	9	-	5	5
51 - 60	10	-	2	6
41 - 50	8	2	2	6
31 - 40	9	5	3	4
21 - 30	3	3	1	5
11 - 20	-	11	-	3
0 - 10	-	9	-	1

TABLE III

Data for Measurements of Phagocytosis utilizing Graded Numbers of Bacteria (Staphylococcus) with 13 Blood Specimens from 4 Normal Human Subjects

Numbers of Bacteria Added (Viable Count)	Per Cent Phagocytosis		Bacteria Per Cell (Cells with Bacteria)		Bacteria Per 100 Phagocytes		Per Cent of Phagocytes in Cell Differential After Phagocytosis		Viable Bacteria in Phagocytic Tubes (x1000)		Viable Bacteria in Plasma Controls (x1000)	
	Mean	Range*	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
100,000	1.6	0.0- 3.0	1.8	0.5- 3.0	5.3	1.2- 10.0	76.2	73-83	17.6	4- 27	114	52- 170
200,000	2.6	1.0- 4.5	3.0	2.0- 3.5	8.7	3.0- 15.0	77.9	70-88	29.6	7- 47	172	125- 250
400,000	6.4	3.5-12.0	3.2	2.5- 4.5	20.1	8.0- 36.0	75.9	66-82	97.0	26- 160	386	130- 650
800,000	12.8	8.0-20.0	3.5	3.0- 4.0	44.3	20.0- 75.0	73.4	62-86	240.0	90- 420	991	330- 2500
1,600,000	23.2	14.0-33.0	4.0	3.0- 5.0	93.2	40.0- 170.0	72.8	58-85	414.0	190- 700	1703	260- 6300
3,200,000	39.3	25.0-59.0	4.3	3.0- 6.0	189.2	105.0- 280.0	71.4	61-85	643.0	320- 1200	2200	1750- 5400
6,400,000	58.8	42.0-78.0	6.2	5.0- 8.0	375.8	260.0- 540.0	65.4	50-85	1380.0	600- 3000	2910	1110- 7500
12,800,000	69.9	60.0-81.0	8.1	6.0-10.5	608.0	400.0-1000.0	52.1	24-73	3600.0	1250- 7000	8200	1250-32000
25,600,000	84.4	75.0-93.0	11.6	7.5-15.5	992.0	650.0-1400.0	43.4	25-60	4010.0	1300- 9200	7550	1300-25000
51,200,000	89.6	81.0-99.0	15.8	13.5-17.5	1450.0	1200.0-1700.0	39.2	26-51	6940.0	1000-22000	14500	1400-50000

*Range indicates minimal and maximal figures in each group.

TABLE IV

Data for Measurements of Phagocytosis utilizing Graded Numbers of Bacteria (Staphylococci) with 10 Blood Specimens from a Single Normal Man over a Period of 18 Months

Numbers of Bacteria Added (Viable Count)	Per Cent Phagocytosis		Bacteria per Cell (Cells with Bacteria		Bacteria per 100 Phagocytes		Total Number of Phagocytes per cmm. after Phagocytosis		Viable Bacteria Phagocytic Tubes (x1000)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
100,000	1.7	1.7- 1.7	2.1	0.8- 4.3	4.9	2.5- 7.0	5500	4500-6500	31.3	17- 54
200,000	3.9	2.0- 5.0	3.3	1.5- 5.5	11.4	5.0- 23.0	5200	4500-6300	51.5	24- 100
400,000	6.5	4.0-10.0	3.9	1.8- 7.0	23.0	9.5- 44.0	5200	4600-5800	158.8	40- 280
800,000	12.2	8.0-16.0	4.0	2.0- 6.0	43.8	19.0- 66.0	5150	4600-6100	310.0	60- 520
1,600,000	21.8	15.0-30.0	4.2	2.0- 5.5	83.2	32.0- 120.0	4700	4300-5400	569.0	100- 1,000
3,200,000	35.0	25.0-41.0	4.8	2.5- 6.0	154.0	75.0- 210.0	4100	3400-4600	1110.0	150- 2,500
6,400,000	54.2	42.0-66.0	6.2	4.3- 8.5	308.0	190.0- 420.0	3100	2600-3500	1850.0	280- 4,800
12,800,000	71.8	57.0-81.0	8.5	7.0-12.5	571.0	420.0- 800.0	2300	1900-3000	2650.0	380- 6,500
25,600,000	86.1	75.0-97.0	11.7	6.8-16.5	1011.0	680.0-1600.0	1800	1500-2400	2900.0	400- 5,500
51,200,000	94.0	89.0-98.0	16.3	5.0-25.0	1557.0	1110.0-2000.0	1400	800-1900	4000.0	320-14,000
102,000,000	96.0	94.0-99.0	25.6	18.0-36.5	2457.0	1800.0-3600.0	1350	800-1800	5800.0	1000-20,000
204,000,000	96.8	94.0-99.0	34.1	27.5-42.5	3075.0	2200.0-4000.0	975	900-1100	5100.0	1400-13,000

TABLE V

Calculations for numbers of bacteria phagocytized and destroyed by measured numbers of phagocytic cells, and changes in cells and bacteria remaining.

	Bacteria added - viable count x2	Bacteria present after phagocytosis viable count x2	Bacterial decrement	Total number of phagocytes after phagocytosis	Bacteria ingested per cell based on all cells	Total bacteria within phagocytes	Bacterial decrement minus bacteria within phagocytes
1	200,000	35,000	165,000	2,850,000	.053	151,000	14,000
2	400,000	60,000	340,000	2,850,000	.098	280,000	63,000
3	800,000	195,000	605,000	2,850,000	.208	590,000	15,000
4	1,600,000	480,000	1,120,000	2,750,000	.443	1,210,000	- 90,000
5	3,200,000	830,000	2,370,000	2,730,000	.932	2,550,000	- 180,000
6	6,400,000	1,290,000	5,110,000	2,680,000	1.89	5,060,000	50,000
7	12,800,000	2,740,000	10,060,000	2,450,000	3.76	9,910,000	150,000
8	25,600,000	7,190,000	18,410,000	2,150,000	6.08	13,125,000	5,285,000
9	51,200,000	8,020,000	43,180,000	1,540,000	9.92	15,270,000	27,910,000
10	102,400,000	13,880,000	88,520,000	1,140,000	14.5	16,530,000	72,000,000

TABLE VI - A

Phagocytic Indices (Bacteria Phagocytized by 100 Cells) after Different Time Intervals with a Single Specimen of Human Blood and Graded Numbers of Bacteria

Numbers of Bacteria Added	Phagocytic Time Intervals						
	5 Min.	30 Min.	1 Hour	2 Hrs.	3 Hrs.	4 Hrs.	10 Hrs.
100,000	0	6	9	19	66	93	46*
500,000	1	36	29	128	102	157	108*
2,500,000	4	174	251	445	287	195	242*
12,500,000	52	527	550	498	665	500*	**
62,500,000	197	1667	928	1030	1483*	836*	**
312,000,000	1036	2461	3620	3425	4030*	4360*	**

* Inadequate number of phagocytic cells

** No phagocytic cells found

TABLE VI - B

Percentages of Phagocytic Cells in Differential Leucocyte Counts Following Phagocytosis for Different Time Intervals with a Single Specimen of Human Blood and Graded Numbers of Bacteria

Numbers of Bacteria Added	Phagocytic Time Intervals						
	5 Min.	30 Min.	1 Hour	2 Hrs.	3 Hrs.	4 Hrs.	10 Hrs.
100,000	80	79	77	77	68	70	72
500,000	80	78	74	71	74	74	77
2,500,000	77	75	75	68	42	34	52
12,500,000	79	68	42	42	31	3	5
62,500,000	79	33	30	30	5	9	3
312,000,000	65	33	20	20	8	4	0
No Bacteria	78	74	66	69	61	71	68

TABLE VI - C

Viable Bacterial Counts Following Phagocytosis for Different Time Intervals with a Single Specimen of Human Blood and Graded Numbers of Bacteria

Numbers of Bacteria Added	Phagocytic Time Intervals						
	5 Min.	30 Min.	1 Hour	2 Hrs.	3 Hrs.	4 Hrs.	10 Hrs.
100000	-	-	-	-	-	-	-
500000	360000	127000	75000	150000	135000	190000	210000
2500000	-	-	-	-	-	-	-
12500000	5200000	920000	70000	100000	1400000	15000000	60000000
62500000	-	-	-	-	-	-	-
312000000	240000000	1700000	400000	84000000	110000000	200000000	320000000

TABLE VII

Values for Total Phagocyte and Lymphocyte Counts for Heparinized, Citrated, and Defibrinated Portions of 15 Rabbit Blood Specimens

Specimen Number	HEPARIN		CITRATE		DEFIBRINATION	
	Phagocytic Cells per cmm.	Lymphocytes per cmm.	Phagocytic Cells per cmm.	Lymphocytes per cmm.	Phagocytic Cells per cmm.	Lymphocytes per cmm.
1	2400	2000	2000	2200	900	2200
2	6000	6000	7600	4400	1300	4200
3	5200	8000	7300	5400	3600	7600
4	4700	1800	3000	2000	1300	2200
5	2700	3300	3500	3000	700	2800
6	5200	3600	3800	4200	900	2600
7	4200	4200	2500	4000	1000	3000
8	6300	3600	4900	4400	1600	3400
9	6400	7600	6000	8000	2000	6000
10	3800	2600	2800	3200	1000	3800
11	6600	5400	6000	5000	1250	4600
12	3300	4200	3500	3300	900	2900
13	5300	3700	7800	2200	2700	3500
14	3400	2900	3700	3100	1400	2600
15	4200	6000	3800	3200	800	5200

TABLE VIII

Phagocytic Indices (Per Cent Phagocytosis) for 15 Rabbit Blood Specimens with 3 Types of Anti-coagulant and 3 Species of Bacteria

Specimen Number	STAPHYLOCOCCUS			STREPTOCOCCUS			PNEUMOCOCCUS		
	Hep.	Cit.	Def.	Hep.	Cit.	Def.	Hep.	Cit.	Def.
1	60	5	56	34	14	53	-	-	-
2	48	26	44	44	5	28	20	0	9
3	59	6	56	48	4	16	4	1	8
4	54	6	30	28	20	24	29	10	14
5	59	14	44	44	24	24	12	0	23
6	53	8	36	40	9	23	29	2	16
7	40	10	56	23	16	50	63	1	36
8	44	2	52	80	5	72	51	1	40
9	53	12	50	64	12	61	16	4	25
10	20	3	84	52	46	52	43	3	40
11	43	12	75	36	50	-	54	1	48
12	62	0	80	52	1	16	20	0	22
13	58	3	80	38	1	28	9	2	12
14	35	1	54	65	16	67	36	10	30
15	59	2	55	68	3	77	36	1	28

TABLE IX

Phagocytic Indices (Per Cent Phagocytosis) for 20 Blood Specimens from 4 Human Subjects with 2 Types of Anti-coagulant and 3 Bacterial Species - Considered in Relation to Numbers of Bacteria Added in:

STAPHYLOCOCCUS

	Numbers of Bacteria Added ($\times 10^6$)																			
	2.5		3.0		3.5		5.0		6.0		10		11		17		20		40	
Heparin	32	41	42	33	52	40	27	55	43	38	39	37	36	42	38	25	58	42	88	84
Citrate	5	6	7	11	7	4	2	3	10	4	3	4	0	2	8	1	13	11	48	27

STREPTOCOCCUS

	Numbers of Bacteria Added ($\times 10^6$)																			
	0.05		0.1		0.5		1.0		1.2		1.3		2.0		2.0		2.5		3.0	
Heparin	21	36	34	56	40	35	67	75	70	69	62	77	78	79	72	80	82	79	78	92
Citrate	2	10	16	14	3	4	4	8	12	4	7	3	9	4	5	0	8	8	10	10

PNEUMOCOCCUS

	Numbers of Bacteria Added ($\times 10^6$)																			
	0.25		0.25		0.4		0.5		1.2		4.0		7.0		10.0		12		35	
Heparin	7	12	26	16	38	40	21	18	46	43	64	61	24	50	50	61	81	67	89	84
Citrate	4	0	3	0	0	0	1	2	0	0	1	0	9	3	3	2	1	2	0	1

II. MEDICAL SCHOOL NEWS

Coming Events

Feb. 19	Minnesota Pathological Society Meeting; "Physiological and Therapeutic Significance of Levo-Arterenol -- the Principle Hormone of the Adrenal Medulla," Dr. M. L. Tainter, Director, Sterling-Winthrop Research Institute, Rensselaer, New York; Owre Amphitheater; 8:00 p.m.
Feb. 25-27	Continuation Course in Clinical Dietetics for Clinical Dietitians
Feb. 26	Special Lecture: "The Epidemiology and Control of Pertussis," Robert Cruickshank; Professor of Bacteriology, the Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London; Owre Amphitheater; 4:00 p.m.
Feb. 28 - Mar. 1	Continuation Course in Dermatology for General Physicians
March 3-5	Continuation Course in Radiology for General Physicians

* * *

Continuation Courses

Clinical Dietetics will be the subject of a continuation course to be given by the University of Minnesota February 25 to 27, 1952, at the Center for Continuation Study. As its name implies, the course is intended for dietitians who are actively engaged in the practice of clinical dietetics. The visiting faculty members for the course will be Dr. James M. Hundley, Consultant, Nutrition, Health, and Special Weapons Defense Division, Federal Civil Defense Administration, Washington, D.C.; and Dr. E. Neige Todhunter, Head, Department of Food and Nutrition, University of Alabama School of Home Economics, University, Alabama. The remainder of the faculty will include full-time and clinical members of the staff of the University of Minnesota.

The University of Minnesota will present a continuation course in Dermatology February 28 to March 1, 1952, at the Center for Continuation Study. This course is intended primarily for physicians who are in general practice. Emphasis will be placed on the diagnosis and specific treatment of common dermatological disorders. We are fortunate in having Dr. Allen C. Curtis, Professor and Chairman, Department of Dermatology and Syphilology, University of Michigan Medical School, Ann Arbor; and Dr. A. Rostenberg, Jr., Professor, Department of Dermatology, and Associate Professor, Allergy Unit, University of Illinois Medical School, as visiting faculty members for this course. The other faculty members will include clinical members of the staff of the University of Minnesota Medical School and the Mayo Foundation.

Faculty News

Dr. Francis Gilman Blake, 64, Sterling Professor of Medicine and former Dean of Yale University School of Medicine, Physician-in-Chief of the Grace-New Haven Hospital, and Civilian Director of Army Medical Research, died in Walter Reed Hospital on Friday, February 1. Dr. Blake was Assistant Professor of Medicine at the University of Minnesota in 1917-19.

Dr. W. Lane Williams, Associate Professor of Anatomy, attended the meetings of the American Federation for Clinical Research and the Southern Society for Clinical Research in Atlanta on January 18-19, and presented a paper (with Dr. F. W. Hoffbauer and R. B. Aronsohn) on "Histochemical Studies of Human Liver Biopsies."

III. UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
WEEKLY CALENDAR OF EVENTS

Physicians Welcome

February 18 - 23, 1952

Monday, February 18

Medical School and University Hospitals

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; W-612, U. H.

10:00 - 12:00 Neurology Rounds; A. B. Baker and Staff; Station 50, U. H.

11:30 - Tumor Conference; Doctors Kremen, Moore, and Stenstrom, Todd Amphitheater, U. H.

12:15 - 1:20 Obstetrics and Gynecology Journal Club; Staff Dining Room, U. H.

12:30 - Physiology Seminar; Metabolism of Isotopic Lactate by the Blood Perfused Heart; H. Meade Cavert; 214 Millard Hall.

1:30 - 2:30 Pediatric-Neurological Rounds; R. Jensen, A. B. Baker and Staff; U. H.

4:00 - Pediatric Seminar; Carbohydrate Metabolism in Scurvy; Charles Stewart; Sixth Floor West, U. H.

4:30 - 5:30 Dermatological Seminar; M-346, U. H.

4:30 - Public Health Seminar; 15 Owre Hall.

5:00 - 6:00 Urology-Roentgenology Conference; C. D. Creevy, O. J. Baggenstoss, and Staff; Eustis Amphitheater.

Minneapolis General Hospital

7:30 - Fracture Grand Rounds; Dr. Zierold; Sta. A.

10:30 - 12:00 Tuberculosis and Contagion Rounds; Thomas Lowry; Station M.

11:00 - Pediatric Rounds; Franklin H. Top; 7th Floor.

12:30 - Surgery Grand Rounds; Dr. Zierold; Sta. A.

1:00 - X-ray Conference; Classroom, 4th Floor.

1:30 - Pediatric Rounds; Robert Ulstrom; 4th Floor.

Veterans Administration Hospital

9:00 - G. I. Rounds; R. V. Ebert, J. A. Wilson, Norman Shrifter; Bldg. I.

11:30 - X-ray Conference; Conference Room; Bldg. I.

2:00 - Psychosomatic Rounds; Bldg. 5.

3:30 - Psychosomatic Rounds; C. K. Aldrich; Bldg. I.

Tuesday, February 19

Medical School and University Hospitals

8:30 - Conference on Diet Endocrines and Cancer; M. B. Visscher; 116 Millard Hall.

9:00 - 9:50 Roentgenology-Pediatric Conference; L. G. Rigler, I. McQuarrie and Staff; Eustis Amphitheater, U. H.

9:00 - 12:00 Cardiovascular Rounds; Station 30, U. H.

12:00 - 1:30 Selected Topics, Permeability and Metabolism; Nathan Lifson; 129 Millard Hall.

12:30 - 1:20 Pathology Conference; Autopsies; J. R. Dawson and Staff; 102 I. A.

3:15 - 4:20 Gynecology Chart Conference; J. L. McKelvey and Staff; Station 54, U.H.

4:00 - 5:00 Pediatric Rounds on Wards; I. McQuarrie and Staff; U. H.

4:30 - 5:30 Clinical-Medical-Pathological Conference; Todd Amphitheater, U. H.

5:00 - 6:00 X-ray Conference; Presentation of Cases by Veterans Hospital Staff; Drs. Fink, O'Loughlin, et al.; Eustis Amphitheater, U. H.

* 8:00 p.m. Minnesota Pathological Society Meeting; The Physiological and Therapeutic Significance of Levo-Arterenol -- the Principle Hormone of the Adrenal Medulla; M. L. Tainter, Director, Sterling-Winthrop Research Institute; Owre Amphitheater.

Ancker Hospital

8:00 - 9:00 Fracture Conference; Auditorium.

1:00 - 2:30 X-ray Surgery Conference; Auditorium.

Minneapolis General Hospital

8:00 - Pediatric Rounds; Spencer F. Brown; 5th Floor.

10:30 - 12:00 Medicine Rounds; Thomas Lowry and Staff; Station F.

11:00 - Pediatric Rounds; Erling S. Platou; 7th Floor.

Veterans Administration Hospital

7:30 - Anesthesiology Conference; Conference Room, Bldg. I.

8:30 - Infectious Disease Rounds; Dr. Hall.

8:45 - Surgery Journal Club; Conference Room, Bldg. I.

9:00 - Liver Rounds; Drs. Nesbitt and MacDonald.

9:30 - Surgery-Pathology Conference; Conference Room, Bldg. I.

10:30 - Surgery Tumor Conference; Conference Room, Bldg. I.

1:00 - Surgery Chest Conference; T. Kinsella and Wm. Tucker; Conference Room, Bldg. I.

2:00 - 2:50 Dermatology and Syphilology Conference; H. E. Michelson and Staff Bldg. III.

3:30 - 4:20 Autopsy Conference; E.T. Bell and Donald Gleason; Conference Room, Bldg.I.

Wednesday, February 20

Medical School and University Hospitals

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-109, U. H.

8:00 - 9:00 Roentgenology-Surgical-Pathological Conference; Allen Judd and L. G. Rigler; Todd Amphitheater, U. H.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; Surgery Case; O. H. Wangensteen, C. J. Watson and Staff; Todd Amphitheater, U. H.

12:30 - 1:20 Radio-Isotope Seminar; 12 Owre Hall.

1:30 - Conference on Circulatory and Renal Systems Problems; M. B. Visscher; 116 Millard Hall.

5:00 - 5:50 Urology-Pathological Conference; C. D. Creevy and Staff; Eustis Amphitheater, U. H.

5:00 - 6:00 Vascular Conference; Todd Amphitheater, U. H.

5:00 - 7:00 Dermatology Clinical Seminar; Dining Room, U. H.

7:00 - 8:00 Dermatology Journal Club; Dining Room, U. H.

8:00 - 10:00 Dermatological-Pathology Conference; Review of Histopathology Section; R. Goltz; Todd Amphitheater, U. H.

Ancker Hospital

8:30 - 9:30 Clinico-Pathological Conference; Auditorium.

3:30 - 4:30 Journal Club; Surgery Office.

Minneapolis General Hospital

8:00 - Pediatric Allergy Rounds; Lloyd Nelson; 4th Floor.

10:30 - 12:00 Medicine Rounds; Thomas Lowry and Staff; Station D.

11:00 - Pediatric Rounds; Franklin H. Top; 7th Floor.

12:30 - Pediatric Staff Meeting; Septicemia in the Newborn; Richard T. Smith; 4th Floor Annex.

12:30 - EKG Conference; Boyd Thomes and Staff; 302 Harrington Hall.

1:30 - Pediatric Rounds; E. J. Huenekens and Robert Ulstrom; 4th Floor.

12:00 - Surgery-Physiology Conference; Dr. Zierold, E. B. Brown; Classroom.

Veterans Administration Hospital

8:30 - 10:00 Orthopedic X-ray Conference; Conference Room, Bldg. I.

8:30 - 12:00 Neurology Rehabilitation and Case Conference; A. B. Baker.

2:00 - 4:00 Infectious Disease Rounds; Main Conference Room, Bldg. I.

4:00 - 5:00 Infectious Disease Conference; W. Spink; Conference Room, Bldg. I.

7:00 p.m. Lectures in Basic Science of Orthopedics; Conference Room, Bldg. I.

Thursday, February 21

Medical School and University Hospitals

8:00 - 9:00 Vascular Rounds; Davitt Felder and Staff Members from the Departments of Medicine, Surgery, Physical Medicine, and Dermatology; Heart Hospital Amphitheater.

Thursday, February 21 (Cont.)

Medical School and University Hospitals (Cont.)

9:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Todd Amphitheater, U. H.

12:30 - Physiological Chemistry Seminar; Ion Binding Properties of Cartilage; K. R. Woods; 214 Millard Hall.

1:30 - 4:00 Cardiology X-ray Conference; Heart Hospital Theatre.

3:30 - Medicine-Pediatric Infectious Disease Conference; Heart Hospital Auditorium.

4:00 - 5:00 Physiology-Surgery Conference; Todd Amphitheater, U. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

5:00 - 6:00 X-ray Seminar; Angiography in Sweden; Robert Bronson; Eustis Amphitheater, U. H.

7:30 - 9:30 Pediatric Cardiology Conference and Journal Club; Review of Current Literature 1st hour and Review of Patients 2nd Hour; 206 Temporary West Hospital.

Minneapolis General Hospital

8:00 - Pediatric Rounds; Spencer F. Brown; 5th Floor.

8:30 - Neurology Rounds; William Heilig; 4th Floor.

11:00 - Pediatric Rounds; Erling S. Platou; 7th Floor.

1:00 - Fracture-X-ray Conference; Dr. Zierold; Classroom.

Veterans Administration Hospital

8:00 - Surgery Ward Rounds; Lyle Hay and Staff; Ward 11.

9:15 - Surgery Grand Rounds; Conference Room, Bldg. I.

11:00 - Surgery Roentgen Conference; Conference Room, Bldg. I.

Friday, February 22 (HOLIDAY)

Saturday, February 23

Medical School and University Hospitals

7:45 - 8:50 Orthopedic X-ray Conference; W. H. Cole and Staff; M-109, U. H.

9:00 - 10:30 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amphitheater.

9:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; Heart Hospital Amphitheater

10:00 - 11:30 Surgery Conference; Todd Amphitheater, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff Station 44, U. H.

Minneapolis General Hospital

8:00 - Pediatric Rounds; George Lund; 5th Floor.

11:00 - 12:00 Medical - X-ray Conference; O. Lipschultz, Thomas Lowry, and Staff; Main Classroom.

11:00 - Pediatric Clinic; C. D. May and Floyd Denny; Classroom, 4th Floor.

Veterans Administration Hospital

8:00 - Proctology Rounds; W. C. Bernstein and Staff; Bldg. III.

8:30 - Hematology Rounds; P. Hagen and E. F. Englund.

*Indicates special meeting. All other meetings occur regularly each week at the same time on the same day. Meeting place may vary from week to week for some conferences.