

EWING (J.)

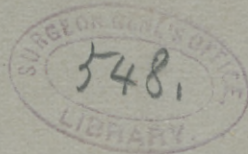
THE LEUCOCYTOSIS OF DIPHTHERIA
UNDER THE INFLUENCE OF
SERUM THERAPY

BY

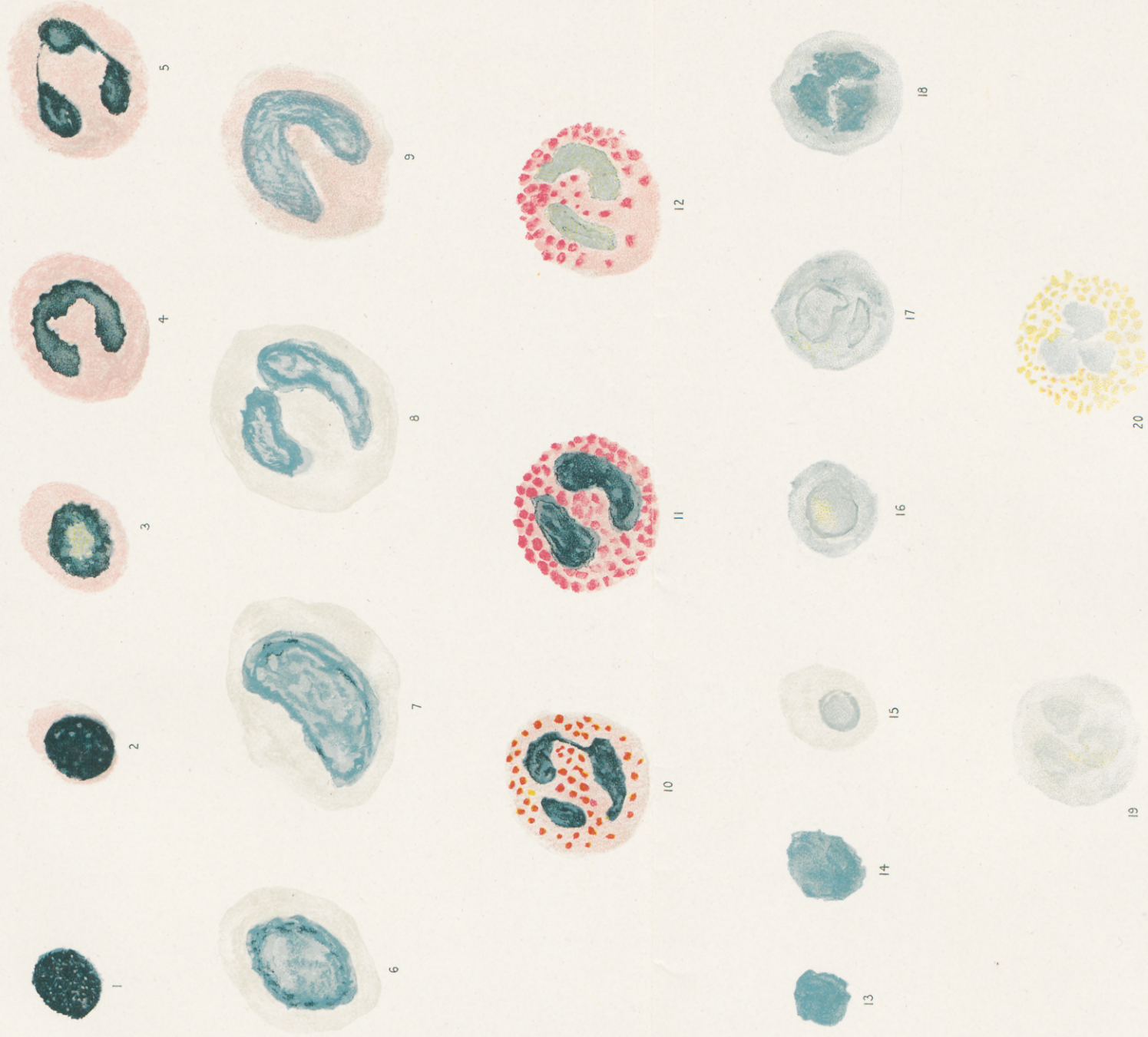
JAMES EWING, M. D.,

TUTOR IN HISTOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS
(SCHOOL OF MEDICINE OF COLUMBIA COLLEGE);
ASSISTANT PATHOLOGIST TO ROOSEVELT HOSPITAL, NEW YORK.

REPRINTED FROM
THE NEW YORK MEDICAL JOURNAL
AUGUST 10 AND 17, 1895



NEW YORK
D. APPLETON AND COMPANY
1895



DR. EWING'S ARTICLE ON LEUCOCYTES OF DIPHTHERIA.

1-12, Dry preparations. Eosin and hæmatoxylon. $\frac{1}{2}$ immersion lens.

13-20, Leucocytes as seen in salt solution tinged with gentian-violet.

1-5, Mononuclear and polynuclear leucocytes, with compact, deeply staining nuclei.

6-9, Stages of large mononuclear leucocytes.

10, Pseudo-eosinophile granules.

11, 12, Eosinophile cell.

18, Polynuclear, well-stained nuclei.

19, Polynuclear, poorly staining nuclei.

20, Eosinophile cell.

THE LEUCOCYTOSIS OF DIPHTHERIA
UNDER THE INFLUENCE OF
SERUM THERAPY

BY

JAMES EWING, M. D.,

TUTOR IN HISTOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS
(SCHOOL OF MEDICINE OF COLUMBIA COLLEGE);
ASSISTANT PATHOLOGIST TO ROOSEVELT HOSPITAL, NEW YORK.

REPRINTED FROM
THE NEW YORK MEDICAL JOURNAL
AUGUST 10 AND 17, 1895



NEW YORK
D. APPLETON AND COMPANY
1895

THE FUNDAMENTALS OF DENTISTRY
UNDER THE AUSPICES OF
SPECIAL THERAPY

COPYRIGHT, 1895,
BY D. APPLETON AND COMPANY

NEW YORK
D. APPLETON AND COMPANY
1895

THE LEUCOCYTOSIS OF DIPHTHERIA UNDER THE INFLUENCE OF SERUM THERAPY.

THE universal interest now centring upon the serum therapy of diphtheria has served to emphasize the present lack of knowledge of the leucocytosis of this disease. While probably not to be regarded as the ultimate agent in the production of immunity, the leucocyte has been certainly demonstrated as the instrument through which the immunizing principle secures a limitation of infectious processes. It might be anticipated, therefore, that in this malignant infection a careful study of the leucocyte, the organism's chief means of defense, would prove to be of some theoretical interest, or perhaps give valuable aid in following the clinical course of the disease. Especially under the influence of a new therapeutic agent claimed to limit the local process and possibly destroy the circulating poison, a more complete knowledge of the leucocytosis of diphtheria is demanded, in the hope of partially explaining the mode of action of the so-called "antitoxine" and the production of immunity in general.

The literature concerned with the leucocytosis of diphtheria is limited. In 1868, before the application of our present methods to the enumeration of blood-cells, Bouchut observed an "acute leucæmic condition" of the blood in diphtheria, by examining specimens under a cover glass.

In 1877, Bouchut and Dubrisay published ninety-three analyses of the blood of children ill with diphtheria. They noted a progressive febrile anæmia and, with few excep-

tions, a considerable leucocytosis. Their conclusions as to the presence and significance of this phenomenon in diphtheria not being supported by fourteen observations of Cuffer's, a more extensive study was undertaken, and in 1879 Bouchut reported a larger series of cases, in which the changes in the blood had been followed from day to day. As a result of this work, he concluded that when diphtheria remains a local process, it always terminates favorably and is unattended by leucocytosis; but when the local process is complicated by septicæmia, the disease always terminates fatally and a leucocytosis occurs which is the exact measure of the toxæmia. He apparently ignored, in these conclusions, evidence appearing in his own studies, that in some cases there is a moderate and decreasing leucocytosis accompanying marked and increasing septicæmia and a malignant course.

Pee, as cited by Rieder, found the leucocytosis of diphtheria less pronounced than that of ordinary follicular amygdalitis.

Rieder adds five analyses of his own, in three cases attended with moderate leucocytosis. He regards the examination of the blood as of no value in the diagnosis between diphtheria and benign pharyngitis. Further observation is necessary, he thinks, to support the observation of Bouchut that the leucocytosis of diphtheria increases with the severity of the disease, and that the examination of the blood may therefore be of value in prognosis.

Very recently, Morse published thirty analyses of the blood in as many cases of diphtheria occurring in children and adults at the Boston City Hospital. He found, as did Bouchut, that the leucocytosis of diphtheria increases with the degree of septic intoxication, and concluded that the examination of the blood is of no value in prognosis. In his cases, the leucocytosis corresponded usually, but not always, with the extent of the membrane, and subsided with or soon after its disappearance. The leucocytosis was not affected by glandular involvement, nor by pulmonary or renal complications. From a morphological study of the leucocytes, he found the increase to be due, in the majority of cases, to an excess of multinuclear neutrophile cells; but in several instances all varieties were present in

normal proportions, and in some convalescent cases a lymphocytosis was observed without glandular enlargement.

The serum therapy of diphtheria having been most extensively followed in Paris by Roux, observations on the influence of antitoxine upon the leucocytosis of diphtheria were naturally expected from that centre. Accordingly, Gabritschewsky recently reported a study of the leucocytosis of fourteen cases of diphtheria treated by antitoxine. His clinical observations led him to believe that "the leucocytosis of diphtheria follows a peculiar course which distinguishes it from the leucocytosis of many other infectious diseases," such as pneumonia. "Progressive leucocytosis in diphtheria is a bad prognostic sign, and the analysis of the blood may give useful indications concerning the value of treatment." In eleven successful cases and in one fatal case there was a progressive diminution of leucocytes following treatment by antitoxine. In two fatal cases the leucocytosis increased after the use of antitoxine. In a series of experiments conducted upon rabbits, he found the intraocular injection of the diphtheria bacillus to be followed, in the susceptible animal, by absence of local inflammatory reaction, by generalization of the poison, and death; but in the vaccinated animal there was an intense inflammatory reaction, which prevented the entrance of bacteria into the blood, and, through phagocytosis, soon effected a cure.

In sections through the tissues at the site of inoculation, the necrotic action of the bacillus was much more extensive, and phagocytosis less pronounced, in the susceptible than in the vaccinated animal. He believes that the necrotic action of the diphtheritic toxine affects all the cells of the organism and inhibits phagocytic activity, while therapeutic serum renders the cells less sensitive to the action of the toxine.

The literature thus reviewed seems to offer insufficient evidence from which to establish the relation of the leucocytosis to the clinical features of diphtheria. Bouchut alone was able to follow his cases with this end in view for a continued period. Further, the virulent nature of the diphtheritic poison makes this disease a specially favorable field for a morphological study of the leucocyte as affected

by a bacterial toxine. Finally, the influence of the anti-toxine upon leucocytosis and the morphology of the leucocyte has already been mentioned as deserving much more careful attention than the subject has yet received.

With these ends in view, the writer followed the changes in the blood of fifty-three cases of diphtheria received at the Willard Parker Hospital during the months of February and March, 1895. Access to these cases was secured through the kindness of Professor T. Mitchell Prudden, which the writer here takes pleasure in acknowledging.

The constant courtesy of Dr. Somerset removed much of the difficulty of obtaining specimens of blood from the patients, all of whom were under his immediate charge. The serum used in the hospital was prepared under the direction of Dr. Park and Dr. Fitzpatrick, and that employed in the experiments was kindly furnished by Dr. Ira T. Van Gieson.

Technique and Morphology of Leucocytes.—The specimens of blood were drawn in the usual way from the dried finger tip through a fine needle puncture.

The Thoma Zeiss erythrocytometer was employed in the examinations, the blood being diluted a hundred times in six-per-cent. salt solution tinged with gentian violet. A very weak solution of this dye is recommended to demonstrate some of the reactions of the leucocytes hereafter described.

One drop of saturated alcoholic solution of gentian violet added to fifty cubic centimetres of salt solution gave the most satisfactory staining fluid for this purpose. Not being able to secure the counting chamber designed by Zaffert, in which is outlined nine times the area of Thoma's instrument, a mechanical stage was used in order to count the number of leucocytes necessary to guarantee accurate results.

By this method forms were enumerated which may be classified and described as follows :

1. Small mononuclear cells, of which the nucleus is usually compact and deeply staining, and the protoplasm limited to a thin, faintly visible ring. By the examination of dry specimens stained with hæmatoxylin and eosin, this

class was found to include lymphocytes without visible protoplasm, ordinary uninuclear leucocytes with compact nucleus, transitional forms of uninuclear leucocytes in which the nucleus shows a tendency to become incurved or vesicular, and, finally, a few uninuclear leucocytes in process of direct nuclear division.

2. Large uninuclear leucocytes with vesicular, usually faintly staining, nucleus, and a considerable mass of clear protoplasm having no affinity for gentian violet. Dry preparations showed this class to consist of uninuclear leucocytes with neutrophile protoplasm, and a large round or slightly incurved nucleus, which is not distinctly vesicular, but which presents throughout a thin stratum of chromatin much condensed at its circumference. In the tables, the large and small mononuclear cells, as determined in salt solution, are combined for the sake of brevity.

3. Multinuclear leucocytes of which the nucleus stains distinctly with the above weak solution of gentian violet. Occasionally, in the protoplasm of these cells, a few greenish (eosinophile) granules were observed. In dry preparations the nuclei of these cells were always found compact and deeply stained with hæmatoxylin. The isolated eosinophile granules appeared deep red as usual, and some of these cells contained the pseudo-eosinophile granules mentioned below.

4. The enumeration of this class of cells was based solely upon a much-diminished or entire lack of affinity shown by the nuclei of many multinuclear leucocytes for gentian violet in the above-mentioned diluted solution. In dry preparations the nuclei of these cells appear less compact and stain rather less deeply with hæmatoxylin than the nuclei of multinuclear leucocytes found in normal human blood. Their protoplasm frequently contains granules of varying size which show an increased affinity for eosin, not sufficient to place them among eosinophile cells, but too evident to allow their classification among multinuclear neutrophile cells. Many of the forms enumerated by the writer in this fourth class are doubtless identical with the so-called pseudo-eosinophile cells, or with the amphophile cells of Ehrlich; for, notwithstanding the statement of Cantacuzene (*loc. cit.*, page 46) that pseudo-eosinophile

cells do not occur in human blood, treatment by eosin and methyl blue develops the violet color of such granules in every one of the pale leucocytes to be found in severe cases of diphtheria. It was not, however, the presence of these granules, which are invisible in salt solution, but the failure of staining quality in the nucleus, which determined this special classification. In fact, after treatment by eosin and methyl blue, nearly all the multinuclear leucocytes of the severe cases of diphtheria showed an abundance of pseudo-eosinophile granules. This observation accords with those of Cantacuzene, and of Everard, De-moor, and Massart, who found that in guinea-pigs and rabbits nearly all multinuclear leucocytes contain such granules within twenty-four hours after intraperitoneal injection of the cholera vibrio.

The formation of these granules had been shown by Metchnikoff to result from the process of intracellular digestion which occurs during phagocytosis. Examining the peritoneal exudate from vaccinated guinea-pigs after injection of the cholera vibrio, he found that the vibrios englobed by the leucocytes at first assumed a blue color with methyl blue, but soon began to show an affinity for acid dyes. Treated successively by eosin and methyl blue, many of the leucocytes were found to contain, side by side, a vibrio colored blue and another colored red. At a later stage, the bacteria were reduced to granulations so arranged as to reproduce the original form of the vibrio, and colored blue or violet or red, according to the stage of digestion. Finally, as the intracellular digestion was completed, the leucocytes of the peritoneal exudate were gorged with pseudo-eosinophile granules, resulting from the destruction of microbes. While this process in diphtheria may very well result in the formation of pseudo-eosinophile granules in the protoplasm of leucocytes, it is hardly a sufficient explanation of the occurrence of these granules in ninety per cent. of the intravesicular multinuclear leucocytes, nor does it explain the bleaching of the nuclei. To the necrotic action of the circulating toxins must be attributed a considerable influence in the production of these changes in the leucocytes of diphtheria.

5. The true eosinophile cells contain large greenish re-

fractive granules, which can be quite as positively identified in salt solution as by staining processes. The nuclei of these cells usually stain faintly with gentian violet, but occasionally they assume a color as dark as that of ordinary multinuclear leucocytes. Following the principle previously stated, the eosinophile cells with well-stained nuclei were added to the third class, and the same cells with poorly staining nuclei to the fourth.

6. Finally, the proportion of amœboid figures was found to greatly increase with the severity of the disease, and a separate enumeration was made of cells in this condition.

Dry preparations were submitted for three to five minutes to the action of a saturated alcoholic solution of eosin. Gage's hæmatoxylon was used as a nuclear stain, and the specimens were treated with this fluid for five minutes. For the demonstration of pseudo-eosinophile granules, the preparations were stained for five to ten minutes in a strong watery solution of methyl blue, after the usual treatment by eosin.

In the examination of stained preparations the following forms were enumerated:

1. Small lymphocytes with compact, deeply staining nucleus, and with or without a very thin ring of protoplasm.

2. Uninuclear leucocytes of which the nucleus is always deeply stained, in form either compact and spherical, or slightly incurved, or partially vesicular, and of which the protoplasm is abundant and neutrophile.

3. Large uninuclear leucocytes with a large circular or slightly irregular nucleus, staining well with hæmatoxylon at the periphery, less deeply at the centre, and with abundant neutrophile protoplasm.

4. Multinuclear leucocytes of which the nuclei are compact and deeply staining and the protoplasm more or less occupied by pseudo-eosinophile granules.

5. Multinuclear leucocytes of which the nuclei are larger, appearing vesicular in salt solution, staining deeply with hæmatoxylon at the periphery, but faintly at the centre. A sufficient reason for the separate enumeration of these cells was found in the fact that their variations cor-

responded to changes in the severity of the disease, and that injections of antitoxine were found to affect principally these and the large mononuclear forms.

6. Eosinophile cells, which, on account of their infrequent occurrence and lack of significance in acute infectious diseases, here deserve little attention.

The Variations of Leucocytosis and the Clinical Features of Diphtheria.—From the study of the present cases, it appears that the leucocytosis of diphtheria is considerably affected by many of the clinical features of the disease, but bears no invariable relation to any of them.

In a large majority but not in all of the severe cases a pronounced increase of leucocytes was found at the time of admission to the hospital. In its further course the leucocytosis was most affected probably by the local inflammatory reaction, as judged by the extent of the membrane, the hyperæmia, and the involvement of the cervical lymph nodes. In three cases in which the inflammatory reaction was slight and the membrane very limited in extent, the leucocytosis was very moderate in spite of a high febrile disturbance, and in five others in which there was extensive formation of membrane the leucocytosis was pronounced, but the rise of temperature moderate.

The variations in leucocytosis often corresponded with the course of the fever. This relation, however, held only during the first days of the disease, for after the climax of the systemic reaction there were many variations between the grade of leucocytosis and the height of the fever, some cases recovering with falling temperature but high leucocytosis, others dying with a rising temperature but steady decrease in the numbers of leucocytes.

In eleven cases in which the streptococcus as well as the Klebs Loeffler bacillus was identified morphologically in the cultures, there was no definite influence referable to the mixed infection. Many of these cases were very severe or fatal.

In ten fatal cases a complicating pneumonia was the cause of death. When the pulmonary lesion was rapidly fatal, this complication caused a rapid increase of leucocytosis, but when the termination was postponed for three or four days, a steady diminution of leucocytes was observed.

The influence of renal complications was not recognized either in the height or in the course of the leucocytosis. A pronounced lymphocytosis was a special feature in two severe cases, unaccompanied by any hyperplasia of the lymph nodes. There was here no evidence that the excessive increase of uninuclear leucocytes had any influence on the numbers of multinuclear cells, which throughout the disease appeared in the usual proportions.

A considerable hyperleucocytosis persisted in two cases long after the complete subsidence of fever and the disappearance of bacilli and local inflammation. This observation may be related to that of Chatenay, who found that the leucocytosis of animals undergoing successful vaccination was very slow in subsiding.

From the dry preparations it was noted that the numbers of uninuclear leucocytes were subject to periodical variations without apparent relation to clinical features of the disease. The proportions of multinuclear leucocytes usually increased with the severity of the symptoms and diminished during convalescence. In many of the fatal cases the percentage of multinuclear cells progressively declined or had never reached a high figure.

The low proportion of multinuclear cells often observed at an early period may be regarded as a relic of the toxic hypoleucocytosis produced at the onset of the disease. This feature was specially prominent in very severe cases.

In order to correctly interpret the variations of leucocytosis in diphtheria, it is necessary to consider the relations of the local inflammatory process, the degree of septic absorption, and the activity of the blood-producing function. It is necessary also to distinguish between local and intravascular leucocytosis. The latter does not represent the real war of the organism against infection, which can only be seen in sections through the tissues at the point of inoculation and by estimation of the local afflux of phagocytes. Intravascular leucocytosis represents only the activity of the blood-producing function in the attempt to remove from the system those bacterial products which have passed the barrier of leucocytes at the site of infection. It may also indicate to a less degree the number of

leucocytes developed in different organs and on their way to the local inflammatory focus.

From the experimental evidence accumulated in this and other local infectious diseases, it is known that the quantity of bacteria and products that reach the circulation at the beginning of an infectious process varies with the amount and virulence of the infection and the susceptibility of the animal. In susceptible animals a localized injection of pathogenic bacteria is followed by a considerable period of negative chemotaxis, during which there is a failure of phagocytosis and the injected material is free to enter the circulation. In refractory and inoculated animals this period of negative chemotaxis lasts a very few minutes or is practically absent, and the injected material is immediately attacked by phagocytes, probably influenced also by the bactericidal action of blood serum, and at once shut off from the circulation. In two animals of equal susceptibility the period of negative chemotaxis is longer in that one which receives the larger and more virulent injection. In very susceptible animals the period of negative chemotaxis may continue until the death of the animal. The inoculation of guinea-pigs with diphtheria is followed by such a course of intoxication. By further application of the same principles it is possible that, even after the initial period of negative chemotaxis has passed and leucocytosis and phagocytosis are well established, the injections may be so varied and increased that negative chemotaxis may be re-established, the blood-producing function exhausted, and the intravascular and local leucocytosis succeeded by progressive failure of both phenomena.

The death of the animal may, of course, occur at any stage of the process, either before intravascular leucocytosis has occurred, or at its height, or during its progressive failure (Metchnikoff, Pfeiffer, Wasserman, Isaëff, Sanarelli, Cantacuzene, etc.).

Many of the clinical variations of leucocytosis to be observed in diphtheria may be explained according to these experimental data.

A moderate local infection in the insusceptible adult may be unattended by general leucocytosis, as was several times observed in the present series of cases. Children

being more susceptible than adults, in them nearly all diphtheritic infections may be expected to produce an appreciable period of negative chemotaxis, allowing considerable absorption of toxins, with a corresponding leucocytosis. Accordingly, only one case of well-marked severity occurring in a child was observed to run its course without distinct leucocytosis.

With an intense local infection it might be anticipated that even in insusceptible adults a moderate quantity of bacterial products would reach the circulation and there excite leucocytosis. In the cases observed in adults, when the local infection was virulent enough to cause the formation of much membrane, there was also pronounced intravascular leucocytosis, corresponding in general to the severity of the local reaction.

With one possible exception, no case was found in which the period of negative chemotaxis continued until a fatal issue; but it seems probable that a more extended observation of malignant epidemics would produce an example of malignant diphtheria unattended by leucocytosis. The human subject being comparatively insusceptible to diphtheria, the period of positive chemotaxis, with high intravascular leucocytosis and intense local reaction, may be expected early in the disease. This is the usual clinical form of the affection in children, and most of the fatalities occur at a period marked by high leucocytosis and active inflammation. In the present series eleven of the fatal cases died during this stage.

It is more difficult to explain the course of the blood changes observed in two children who, after the leucocytosis had reached a considerable height, continued to do badly, one dying from sepsis, the other from pneumonia, while the numbers of intravascular leucocytes steadily decreased. So far as the writer can learn, leucocytoses of such a character have not been previously observed in diphtheria, nor have they been successfully reproduced experimentally. Both of these cases being prolonged examples of the disease and attended with extreme septic absorption, it is possible that the excessive quantity of toxin thrown into the circulation produced a condition of progressive toxic hypoleucocytosis, exhausted the blood-producing

function, and even destroyed many leucocytes already present in the blood. Evidences of leucocytolysis were not wanting in the blood drawn before death. In salt solution very few of the leucocytes could be stained, their outlines were very irregular, and amœboid figures were abundant, while in dry preparations there was an unusual number of broken nuclei without adherent protoplasm.

For the continued period of hypoleucocytosis observed in one case, in which the cervical lymph nodes were enormously swollen, and which was fatal on the nineteenth day from pneumonia, no plausible explanation offers itself.

The Analysis of the Blood in the Prognosis of Diphtheria.—In none of the cases was it found possible to attach any prognostic value to a simple enumeration of the leucocytes at any stage of the disease.

Previous writers have agreed that the examination of the blood can give little aid in the prognosis of diphtheria. Since the intravascular leucocytosis measures in general the degree of septic absorption, it may be inferred from a high leucocytosis in diphtheria, as in pneumonia, that the infection is severe, but not that the course of the disease will prove unfavorable. The highest leucocytoses of the present series were noted in two severe cases, both of which ultimately recovered, and, on the other hand, in two fatal cases there was no leucocytosis during the first few days of the disease. When, however, the blood changes are followed from day to day, it will be found that progressive increase of leucocytes is usually followed by death. Yet, contrary to the conclusion of Gabritschewsky, in some very septic fatal cases there was a steady decrease of leucocytosis up to the time of death. Some little assistance may be obtained from a study of dry preparations, from which it appears that in the favorable cases the percentage of multinuclear leucocytes is usually high and increasing, while in the fatal cases their proportion is lower and diminishing.

It must be concluded, therefore, that no valuable prognostic signs can be obtained from observations of the leucocytoses of diphtheria thus far considered. When, however, the blood is drawn in salt solution, tinged with gentian violet, and the staining qualities of the leucocytes are con-

sidered, a greater value can be at once placed upon the examination. From an analysis of the appended tables it will readily be seen that the numbers and percentages of the poorly staining leucocytes, and usually of amœboid figures, invariably increased with the unfavorable course of the disease, and without relation to the total numbers of leucocytes to be found in the blood. This failure of color reaction can be only imperfectly indicated by numerical estimates of stained and unstained leucocytes, for in severe cases all the multinuclear leucocytes were somewhat affected. Whatever its nature may be, evidence of the prognostic importance of this nuclear change was found in every severe case in which it was considered. In all the grave conditions the proportion of poorly stained leucocytes was large; if the patient improved, the leucocytes recovered their staining capacities; or, if the general condition grew worse, the staining reaction became weaker. Any considerable increase of poorly stained leucocytes, especially if accompanied by a decrease of well-stained cells, invariably heralded a grave or fatal turn in the disease. This evidence of the blood sometimes preceded any noticeable change in the patient, as judged by the usual clinical signs; more often the failing condition was evident at the time of the examination; occasionally the patient appeared to fail or improve a few hours before any change was observed in the leucocytes. In a few rapidly fatal cases in which mechanical stenosis or pneumonia was the immediate cause of death, this change in the leucocytes was less pronounced, but when septic absorption had continued for several days a high proportion of stainless leucocytes was always noted. While subject, then, to the limitations of most isolated clinical symptoms throughout these observations, the staining capacity of the leucocytes took its place among the features of the disease, and proved to give the most valuable prognostic sign obtainable from the examination of the blood.

The Influence of Antitoxine upon the Leucocytosis of Diphtheria.—The injection of antitoxine in nearly every instance produced marked and immediate effects upon the leucocytes. Of eighteen observations made just before and after the injections, in fifteen there was a considerable de-

crease in the numbers of leucocytes, most marked within twenty-five to forty minutes after the injection. Of the exceptions, two cases were very shortly fatal, and one was an adult, receiving only seven cubic centimetres of the serum. The duration of this period of hypoleucocytosis could not well be determined in patients profoundly ill, but in normal rabbits receiving an equal quantity of serum according to their weight, the hypoleucocytosis was found to persist for twenty-four or even forty-eight hours. In rabbits suffering from injections of virulent cultures of diphtheria it was observed to continue for three to five hours. The more powerful serum produced the greater decrease of leucocytes.

From the examination of dry preparations made before and after injections it became evident that the decrease of leucocytes was principally in the uninuclear and poorly staining multinuclear forms, and not in the ordinary polynuclear cells, which are most affected in toxic hypoleucocytosis. The multinuclear cells, however, were here found distinctly increased in proportion, though diminished in numbers. According to Lowit, in the hypoleucocytosis produced by cooling and exhaustion the uninuclear leucocytes are diminished, while the multinuclear cells are increased in proportion. Shock, on the contrary, reduces principally the multinuclear forms. It does not seem probable that the decrease of leucocytes observed after serum injections can be entirely attributed to any of these factors, for there is certainly as much shock as reduction of temperature or exhaustion attending the injection of serum, but probably not sufficient of any of these influences to noticeably affect the blood. In the writer's experiments two cubic centimetres of normal sheep's serum were injected subcutaneously in rabbits without noticeably affecting the blood. The injections were repeated with the same serum treated with solid camphor, the preservative agent used in the antitoxine, and still no considerable effect was observed in the blood. Under exactly the same conditions the injection of the same quantity of antitoxine markedly reduced the leucocytes in normal rabbit's blood, a reduction which became more pronounced with stronger serum. Unless the technique of these experiments was faulty, it may safely be

concluded that the hypoleucocytosis occurring after injections of antitoxine is due principally to the active immunizing agent contained in the serum, and not to shock, or cooling, or exhaustion, or the camphor dissolved in the serum, or the common constituents of all normal animal blood serum.

What, then, is the significance of this reduction of leucocytes following the injection of antitoxine? It has been noted by Isaëff, Cantacuzene, and others that the local afflux of leucocytes after intraperitoneal injection of the cholera germ is much greater in animals treated by preventive serum than in animals not receiving this treatment. Possibly this fact may offer a partial explanation of the hypoleucocytosis observed after injection of diphtheria antitoxine. If the serum does hasten the separation of membrane and reduce septic absorption, as seems probable from the weight of clinical testimony, it must do so largely by an increase of leucocytosis and inflammation at the site of infection. Whether an afflux of leucocytes to the site of the lesion occurs within half an hour after the injection of serum can only be proved by an enumeration of leucocytes in the exudate, or, better, in the inflamed tissues, before and after the injections.

A final possible factor in the blood changes referable to the antitoxine remains for consideration. Most histologists favor the belief that all leucocytes are developmental forms of a single series. As the antitoxine increases the proportion of multinuclear forms while diminishing that of the uninuclear elements, it is possible that some of the uninuclear cells are rapidly transformed into multinuclear leucocytes, thus increasing the phagocytic power of the blood. The length of time required is no objection to this hypothesis, for the production of multinuclear leucocytes is exceedingly rapid in experimental leucocytosis. But, in spite of the evident convenience of such an explanation, there seems to be no sufficient basis on which it can rest. Most of the recent evidence has told against the theory that all or any two forms of leucocytes belong to one series. Zenoni's work may be mentioned as one of the most convincing studies of this character, in which he found no connection whatever between the various forms of leu-

cocytes during the excessive variations in their numbers which follow repeated large hæmorrhages. Not wishing to enter further into this discussion, it may be said of the dry preparations studied in the present cases that no transitional forms were seen between the large uninuclear cells and the multinuclear cells with compact nucleus. Further, because of differences in the size, form, and staining quality of the nucleus, and in the quantity and staining reaction of the protoplasm, it is believed that no such transitional forms existed in these preparations. Between the small uninuclear cells with compact nucleus and the multinuclear leucocytes transitional forms were abundant, but these cells were much less affected by the injections than the large uninuclear elements were. While, therefore, it is possible that the increase in the proportion of multinuclear leucocytes after injections of antitoxine partly results from rapid development of these cells from uninuclear elements, the writer can offer no evidence that such a change actually occurs.

Consequently, the evidence at hand favors the belief that the hypoleucocytosis produced by the antitoxine is due to a negative chemotactic action residing in the immunizing principle contained in the serum. In the hypoleucocytosis produced experimentally by injection of chemical agents the leucocytes have been found to lodge in the visceral capillaries, frequently causing thrombi in the organs. Very large injections, however, are necessary to produce such lesions, and the small doses, comparable to an injection of antitoxine, cause such slight changes in the number of leucocytes in the capillaries that an increase can be determined only by careful enumerations (Goldschneider and Jacob, Werigo, etc.). Nevertheless, this negative chemotactic action of the antitoxine must be regarded with suspicion, until it is shown by experiment that the injection does not cause a lodgment of leucocytes in the visceral capillaries, and does not remove from the circulation a certain number of available phagocytes. When ten thousand to fifteen thousand leucocytes per cubic millimetre are suddenly removed from the circulation, and the patient dies within a short period, or in a few days, as occurred in at least three of the present cases, one must hesitate before

recommending the use of antitoxine after a very grave condition has been established in diphtheria.

In small and repeated doses the necessary quantity of serum could probably be administered without producing such a rapid and marked diminution of leucocytes.

Besides the reduction in the total number of leucocytes and increase in the proportion of multinuclear forms, the antitoxine strikingly influenced the staining capacity of the leucocytes, as seen in salt solution and less plainly in dry preparations. In several instances an improvement in the staining capacity of the leucocytes was apparently to be observed within thirty minutes after the injection; but the indications of these cases are to be distrusted, because, in the most evident example, a successful intubation was combined with the injection of antitoxine. In rabbits, after partially destroying the staining capacity of leucocytes by repeated injections of diphtheria cultures, the antitoxine seemed to produce an immediate improvement in the leucocytes but once in four experiments. Yet it is an interesting fact that five children in whom the numbers of stainless leucocytes did not diminish immediately after the injection all died. When, however, a period of twelve hours is allowed to intervene after the use of the serum, the examination of the blood in both clinical and experimental diphtheria left no doubt that in favorable cases, after the injection of antitoxine, the staining capacity of the leucocytes is greatly improved. This period may very well allow other influences than the antitoxine to affect the leucocytes, but the fact of the sequence remains unaltered. In the fatal cases the change in the leucocytes was entirely absent or less evident, and soon succeeded by increasing failure of staining reaction.

Significance of the Staining Reaction of Leucocytes.— Having followed the leucocytes and their affinity for gentian violet through many of the phases of diphtheria, and having noted the close relation of this property to the symptoms and prognosis of the disease, some questions arise concerning the significance of the staining capacity of the leucocytes and its possible importance in the process of immunization.

Does the affinity of the nuclei of leucocytes for certain

dyes depend upon the presence in the nuclei of some chemical principle essential to phagocytosis, and which is altered or destroyed by bacterial poisons? Does the condition of the nucleus bear any relation to the phenomena of chemotaxis? With all the evidence now accumulated in favor of the phagocytic theory, we do not yet know the ultimate reason why the leucocytes absorb and digest bacteria under some conditions and fail entirely to do so in others. A short review of the present theories of immunity will serve to place the subject in its proper bearing.

In 1891 Klemperer protected animals against fatal doses of the vibrio of Metchnikoff and of the pneumococcus of Fraenkel by injections of serum from patients cured of cholera and pneumonia. Behring and Kitasato secured the same result in tetanus and diphtheria. On this and other such evidence rests the antitoxic theory of immunity—that the serum of patients cured of a disease contains a substance which neutralizes the poison produced by that germ. The mode of destruction of the germ itself was not investigated in these experiments.

The bactericidal theory presents much stronger claims for consideration. In 1890 Behring and Nissen found that the vibrio of Metchnikoff grew luxuriantly in normal pig's serum, but soon perished in large numbers in the serum of immune animals. They maintained that the serum of inoculated animals contained unknown principles capable of killing the vibrio within or without the organism, and that this principle was the cause of immunity. Zäselein, in 1890, proved that the injected vibrio found its way into the blood, where, in the inoculated animal, it soon perished, but in the susceptible animal increased enormously. Studying the accompanying leucocytosis and observing no increase of leucocytes in the inoculated animal and extreme leucocytosis in the other, he concluded that phagocytosis was little concerned in the process of cure and that the blood serum alone was the bactericidal agent. Pfeiffer, in 1892, finding that after large intraperitoneal injections the peritoneal exudate contained very few vibrios at the time of death, inferred that, while the animal died of intoxication, the germs had not withstood the bactericidal action of the blood serum.

Pfeiffer and Wasserman, in 1893, corroborated the previous observation of Gamaleia, that fresh and inoculated animals are equally susceptible to the toxins produced by the vibrio of Metchnikoff. They believe that in the inoculated animal certain phenomena occur at the point of inoculation which prevent the formation of toxins. Neither is immunity due, in their opinion, to the bactericidal action of blood serum, which they found merely to retard the growth of bacteria, which later multiply abundantly. Phagocytosis they regard as a secondary process, for when the peritoneal cavity is infected with cultures sterilized by drying, and containing neither living germs nor toxins, phagocytosis still occurs. Further, in inoculated animals under the influence of opium, choleraic infection of the intestine is readily fatal, although phagocytosis is abundant and many pus cells are found in the intestine containing partially digested vibrios. The process of immunity, they conclude, is therefore neither bactericidal nor antitoxic nor phagocytic, but dependent upon very complex and still unknown factors.

Pfeiffer and Isaëff, in 1894, infected the peritoneal cavity in guinea-pigs with a cholera vibrio twenty-four hours after treatment with cholera serum. Examining subsequently the peritoneal exudate, they found that in all cases in which the serum acted as a specific, the germs perished within two or three hours, and they convinced themselves that the great majority of the germs were destroyed without phagocytosis.

Buchner presents evidence to show that the bactericidal action of the blood depends upon an unstable albuminoid compound, "alexine," contained principally in the leucocytes. By injecting a vegetable irritant, "*Aleuronmehl*," into the pleural cavity of the rabbit, he obtained a purulent exudate free from bacterial products, which was more actively bactericidal than normal blood. That the bactericidal action of this exudate is independent of phagocytosis he concludes from the fact that after freezing—treatment which must destroy all phagocytic power—the bactericidal action of the exudate is not at all impaired. Phagocytosis he therefore regards as a secondary process in the destruction of bacteria within the organism.

The phagocytic theory has been largely in the hands of the French school of scientists. In 1891 Metchnikoff, continuing the observations of many previous studies, corroborated the results of Behring and Nissen as to the bactericidal power of vaccinal serum, but maintained that the survival of bacteria within the organism was much greater than in culture media prepared from the serum of vaccinated animals. After intraocular injections, he found marked local phagocytosis in the refractory animal, but absence of this phenomenon in susceptible animals. In the inoculated subject the phagocytes had englobed the vibrios, and, by the examination of hanging drops, he proved that these absorbed bacteria were still active and capable of rapid growth in the supposed bactericidal serum. He proved also that the germs, when introduced into a refractory organism, underwent a distinct increase of virulence.

In 1893 he noted that the therapeutic power of cholera serum bore no relation to the favorable or unfavorable termination of the disease, the patient recovering at times when the blood was entirely lacking in therapeutic virtue.

In 1894 Isaëff found that the bactericidal power of the blood might not be established for three weeks after recovery from cholera, and was then little greater than that of normal serum. He secured varying grades of immunity in guinea-pigs from injections of salt, normal serum, nuclein, and tuberculin. Studying phagocytic reaction, he produced marked local afflux of leucocytes by intraperitoneal injection of each of these agents. After fatal bacterial injections the afflux of leucocytes failed to occur, and the period of negative chemotaxis was continued till the death of the animal. In guinea-pigs previously treated with immunizing serum, injections of bacteria were followed in ten minutes by local leucocytosis. In susceptible animals there was slight local leucocytosis, with no absorption of bacteria; in animals treated with normal serum the local reaction was marked, and many bacteria were taken up by the leucocytes, while in strongly immunized animals the inflammatory reaction was very pronounced and the phagocytosis rapid and complete.

Sanarelli, in a series of experiments conducted during

the past year, found that the vibrio of Metchnikoff perished in large numbers in vaccinal serum, but that many survived, and when washed in salt solution and freed from serum the remaining bacteria showed increased virulence.

Very recently, Cantacuzene has published the results of an extended study, and shown the necessity of an afflux of leucocytes to the intestinal tract in the cure of experimental cholera. He has proved that opium narcosis, which suppresses immunity in refractory animals, does so because it first suspends the activity of leucocytes.

Sufficient evidence has thus been reviewed to establish the essential character of phagocytosis and the probable bactericidal action of blood serum in the production of immunity.

The importance of the nucleus in the cellular reaction of the organism against infection has been indicated by Klemperer. After the observation of Kossel, that nucleinic acid destroys the cholera germ very rapidly, Klemperer demonstrated the presence of this acid in the nuclei of the intestinal epithelial cells by the green coloration produced with Ehrlich's triacid mixture. He regards the acid existing in the nuclei of epithelial cells as the true agent in immunity against cholera.

Lilienfeld and Posner have also demonstrated the presence of an acid in the nuclei of epithelial cells by the green color produced with methyl green. Though failing to see how an acid contained in the nuclei of epithelial cells can affect bacteria free in the intestinal lumen, they find that injections of nucleinic acid not only destroy the cholera germ, but enfeeble the action of the choleraic toxine.

Buchner maintains that the cellular principle concerned in immunity is not nucleinic acid, the bactericidal action of which is destroyed by cold but is unaffected by moderate elevations of temperature. The bactericidal power of the purulent exudate previously described is, however, not at all affected by freezing, is destroyed by a moderate degree of heat, and must therefore depend upon the presence of some principle other than nucleinic acid.

These observations, then, point to the possible importance of the nucleus as the special agent in the phagocytic

cellular reaction and defense of the organism against bacterial invasion. The variation of the staining qualities of leucocytes to be found in diphtheria is believed to offer further evidence that some principle exists in the nuclei of these cells which is very susceptible to the action of bacterial toxins, the normal presence of which is necessary to positive chemotaxis and phagocytosis, and which is conserved or restored by immunizing serum.

SUMMARY.

Diphtheria is usually attended by pronounced leucocytosis. The increase of leucocytes begins a few hours after the infection, probably appearing earlier in refractory individuals, and often being long delayed in susceptible cases with severe infection. In favorable cases the leucocytosis is greatest at the climax of the disease, and steadily declines during convalescence. There may, however, be prolonged hyperleucocytosis after other local and constitutional symptoms have subsided.

In unfavorable cases, the leucocytosis continues until death; but in somewhat prolonged cases, with much septic absorption, there may be an uninterrupted decrease of leucocytes continuing up to the fatal termination.

A complicating pneumonia usually causes a considerable increase in leucocytosis.

The degree of leucocytosis in diphtheria often varies with the fever, but much more frequently corresponds to the extent of the local lesion.

The intravascular leucocytosis of diphtheria measures exactly the systemic reaction against the toxic products circulating in the blood and absorbed from the site of infection.

High leucocytosis in diphtheria indicates a pronounced reaction against a severe infection, but is not necessarily an unfavorable prognostic sign.

Steadily decreasing leucocytosis usually, but not always, accompanies a favorable course in the disease.

Slight leucocytosis usually indicates a mild infection, but fatal cases may for several days show no increase, or even a decrease, of leucocytes.

The staining reaction of the leucocytes is an accurate

measure of the severity of a diphtheritic infection, and variations in this reaction often precede changes in other symptoms.

Antitoxine, within thirty minutes after its injection, causes a hypoleucocytosis, the reduction affecting specially the uninuclear leucocytes, while the proportion of well-stained multinuclear cells is increased. This action is due largely to the immunizing principle contained in the serum.

In favorable cases, after the injection of antitoxine, the leucocytosis never again reaches its original height. In severe and less favorable cases, the injection is followed in a few hours by hyperleucocytosis and fever, exceeding those symptoms as found in the original condition. In unfavorable cases, an injection of antitoxine may be followed immediately by rapid hyperleucocytosis or extreme hypoleucocytosis and death.

The reduction of leucocytes immediately succeeding the injection of antitoxine, especially in severe cases of diphtheria, is an undesirable feature of the action of this agent, and as far as possible should be avoided.

The multinuclear leucocytes found in the blood of favorable cases after treatment by antitoxine show increased affinity for gentian violet. This change may be observed within twelve hours after the injection, and the failure of its occurrence is a very unfavorable prognostic sign.

The variations in the staining reaction of leucocytes in diphtheria indicate that the nuclei of these cells contain a principle essential to phagocytosis and immunity in this disease.

No.	Age.	Day of disease.	Highest temperature.	Highest pulse.	LEUCOCYTES PER CUBIC MILLIMETRE.										Remarks.				
					Total.	Per cent. in salt solution.					Per cent. in dry preparations.								
						Mononuclear.	Well-stained polynuclear.	Poorty stained polynuclear.	Ameboid figures.	Small lymphocytes.	Mononuclear leucocytes.	Large mononuclear.	Vesicular nucleol.	Poly-nuclear.		Compact nucleol.	Eosinophile.		
1	3 yrs.	6th	103.2°	140	Condition on admission, grave; tonsils large; many small patches of membrane; much hyperemia; Klebs-Loeffler cocci. Injected, sixth day, 700 im. u.; seventh day, 600 im. u., ninth day, 600 im. u.; tenth day, 300 im. u. Died—sepsis.		
		7th	103.6	26,000	14	60	30	14	14	2	14	8	11	66	66	0			
		8th	104.4	45,500	24	44	130	44	14	6	8	5	4	77	77	0			
		9th	103.4	36,000	22	38	84	15	2	16	3	6	9	70	70	0			
		10th	102.6	28,500	28	58	16	1	16	6	12	6	12	65	65	0			
		11th	105.6	20,000	24	16	40	20	0	13	8	9	9	70	70	0			
		2	3 yrs.	4th	104.0	140	72,500	192	80	18	6	10	50	0	9	31		0	Condition grave; small amount of membrane on tonsils; laryngeal stenosis; intubation; Klebs-Loeffler, twenty-fourth day. Injected, third day, 500 im. u.; fourth day, 500 im. u.; thirteenth day, condition precarious. Albumin, five per cent. Recovery (?).
				5th	104.4	160	72,000	196	74	14	4	12	44	3	5	44		2	
				7th	102.0	160	41,500	84	62	12	8	12	40	3	2	38		2	
				9th	101.0	138	30,000	40	56	20	4	10	30	3	8	46		3	
				12th	104.5	156	24,000	34	54	8	2	8	34	3	2	52		3	
13th	104.6			172	17,750	13	36	20	13			
14th	105.0			170	18,500	10	56	8	2			
3	7 mos.	16th	105.4	160	20,000	8	60	10	4	Condition fair; no visible membrane; croupy cough; Klebs-Loeffler; cachexia; prolapsus recti. Injected, 17th			
		17th	104.0	140	15,000	6	48	6	2	4	18	8	3	64	3				
		6th	100.0	180	16,000	30	34	8	0	32	31	0	0	37	0				
		7th	22,500	46	16	10	2	35	27	5	0	33	0				
		8th	99.0	180	25,000	58	32	10	0	12	50	6	2	30	0				

4	16 mos.	10th 11th 12th 15th	99-4 99-4 101-4	190 ... 190 146	28,000 31,000 24,500 15,000	56 52 38 26	36 48 42 6	20 24 16 28	0 2 3 4	.. 5 .. 10 .. 33 .. 10	.. 34 .. 33 .. 5 .. 6	.. 9 .. 2 .. 53 .. 0	.. 31 .. 53 .. 0 .. 0	.. 1 .. 0 .. 1 .. 0	.. 1 .. 0 .. 1 .. 0	Death (ca- chexia), twentieth day.
		4th 5th 6th 40 min. after injection.	99-4 102-6 101-4	174 186 164 ...	12,500 35,000 29,000 14,000	8 18 24 12	24 108 84 36	14 14 8 8	4 4 6 2	.. 0 .. 10 .. 19 .. 21	.. 10 .. 6 .. 3 .. 2	.. 4 .. 4 .. 3 .. 2	.. 80 .. 80 .. 72 .. 72	.. 0 .. 0 .. 1 .. 0	.. 0 .. 0 .. 1 .. 0	Condition grave; no visible membrane, stenosis; intubation; Klebs-Loeffler cocci. Injected, fourth day, 500 im. u.; sixth day, 500 im. u.; ninth day, scarlatinoid rash. Died.
5	11 mos.	10th 11th 40 min. after injection. 12th	103-6 107-4 108-6	164 210 22,000 19,500 16 .. 16 .. 8 .. 8	.. 20 .. 84 .. 28 .. 28	.. 52 .. 42 .. 42 .. 28	.. 8 .. 8 .. 8 .. 8	.. 0 .. 6 .. 18 .. 18	.. 6 .. 6 .. 6 .. 6	.. 9 .. 9 .. 12 .. 12	.. 60 .. 59 .. 67 .. 71	.. 0 .. 0 .. 0 .. 0	.. 0 .. 0 .. 0 .. 0	Condition very grave; no visible membrane; tonsils large; profuse nasal discharge; probable pneumonia. Injected, tenth day, 500 im. u.; eleventh day, 500 im. u. Died.
6	11 mos.	4th 5th 1 hr. after anti- toxine.	102-4 105-0 108-6	180 200 41,500 26,500 16 .. 16 .. 6 .. 6	.. 84 .. 48 .. 48 .. 48	.. 64 .. 52 .. 52 .. 48	.. 6 .. 14 .. 14 .. 14	.. 6 .. 11 .. 11 .. 11	.. 8 .. 7 .. 7 .. 7	.. 12 .. 4 .. 0 .. 0	.. 70 .. 81 .. 70 .. 81	.. 0 .. 0 .. 0 .. 0	.. 0 .. 0 .. 0 .. 0	Condition very grave; small patches on tonsils; stenosis; intubation. Injected, fourth day, 500 im. u.; fifth day, im. u. Died.
7	4 yrs.	8th 9th 11th 12th	102-6 103-8 104-6 103-4	124 140 186 196 17,500 21,500 32,000	.. 12 .. 14 .. 14 .. 13	.. 10 .. 44 .. 44 .. 69	.. 5 .. 28 .. 46 .. 46	.. 12 .. 8 .. 12 .. 12	.. 10 .. 13 .. 6 .. 6	.. 5 .. 4 .. 10 .. 10	.. 12 .. 4 .. 10 .. 10	.. 60 .. 61 .. 70 .. 70	.. 1 .. 1 .. 1 .. 1	.. 1 .. 1 .. 1 .. 1	Condition very grave; thin membrane on tonsils; no hyperemia. Injected, eighth day, 600 im. u. Died, twelfth day—pneumonia.
8	16 mos.	4th 5th 6th	99-6 101-0 103-8	152 160 158 21,000 18,000	.. 13 .. 13 .. 12	.. 61 .. 44 .. 44	.. 8 .. 16 .. 16	.. 5 .. 8 .. 8	.. 2 .. 22 .. 22	.. 22 .. 12 .. 12	.. 5 .. 5 .. 5	.. 59 .. 59 .. 59	.. 0 .. 0 .. 0	.. 0 .. 0 .. 0	Condition grave; thin membrane on tonsils; extreme stenosis; intubation. Injected, fourth day, 400 im. u.;

12	8 mos.	5th 6th 7th	101.0 100.0 99.6	130 130 142	35,500 27,500 21,000	22 110 70 68	10 24 10 16	4 4 3	0 16 ..	14 13 ..	5 2 ..	3 6 ..	78 64 ..	0 0 ..	Condition poor; pharynx, tonsils, bleeding, raw; small dots of membrane. Injected, fifth day, 1,000 im. u. Passed from observation. Cachexia; suppuration. Died, forty-first day.
13	1½ yr.	5th to 25th 26th 27th	100.6 102.0 102.0 104.6	124 140 120 160 17,500 30,500 2 0 10 8 13 17 4 8 70 66 1 1	Condition fair; thin membrane on swollen tonsils. Injected, fifth day, 500 im. u.; seventh day, 500 im. u. Cachexia; cough; paralysis, palate. Died, twenty-eighth day.
14	5 yrs.	4th 5th 30 min. after anti- toxine. 6th	101.8 102.0 102.4	120 136 ... 140 27,500 13,000 48,500 2 3 2	.. 3 10 14	.. 16 10 5	.. 20 9 5	.. 59 68 72	0 0 0 0	Condition grave; thick membrane on tonsils, uvula; nose obstructed; diffuse swelling of neck. Injected, fourth day, 500 im. u.; fifth day, 400 im. u. Died, sixth day, suddenly.
15	1½ yr.	3d 4th 1 hr. after anti- toxine.	102.0 102.2	186 190 33,000 47,000 5 8	.. 12 8	.. 7 8	.. 7 6	.. 69 69	0 0 0	Condition very grave; thin membrane, left tonsil; stenosis; intubation. Injected, third day, 250 im. u.; fourth day, 400 im. u., followed by antimorsem (?) leucocytosis. Died, fourth day.
16	1½ yr.	9th 10th 11th 10 min. after anti- toxine. 12th 14th 16th 18th 21st	102.0 102.6 103.0 101.0 102.6 103.2 103.8 104.0	160 176 144 ... 132 152 154 170 190 24,000 20,000 18,500 13,500 18,500 12,500 11,000 16 3 30 43 6 11 4 2 44 40 0 1	Condition grave; thin membrane on tonsils; stenosis; intubation. Injected, ninth day, 500 im. u.; tenth day, 500 im. u. Pneumonia. Died, twenty-second day.

20	4½ yrs.	8th 9th	105·2 106·4	156 ...	24,000	10 ..	64 ..	22 ..	6 ..	0 ..	15 ..	5 ..	5 ..	75 ..	0 ..	inubation; Klebs-Loeffler, staphylococci, and streptococci. Injected, sixth day, 1,000 im. u. Died—pneumonia.	
21	1½ yr.	11th	99·8	124	33,000	Condition serious; small patch on left tonsil; stenosis; intubation. Injected, eleventh day, 1,000 im. u. Recovery.	
		12th	102·0	128	33,000	20	104	8	4	8	6	7	7	72	0		
		13th	100·4	124	28,000	20	86	6	2	3	15	10	8	64	0		
		14th	99·0	120	15,000	6	46	6	2		
		3d 10 min. after anti- toxine.	102·2	156 ...	18,000 27,000	.. 10	64 34	.. 5	2 0	30 21	3 2	1 7	64 70	0 0		Condition fair; no visible membrane; large ulcer of cheek. Injected, third day, 1,000 im. u. Died, thirty-third day, pneumonia.
22	2 yrs.	4th	102·6	150	15,000	24	24	14	3	14	24	2	2	66	0		
		6th	100·0	136	18,000	26	42	24	23	29	3	5	38	2			
		9th	99·6	140	11,500	16	20	10	2		
		11th	13,000	16	30	6	0	4	5	9	2	2	72	2	
		4th 5th 40 min. after anti- toxine.	100·4 104·6	132 160 ...	12,500 30,000 23,500 10 54 30 6	3 4 1	21 5 11	10 7 9	3 7 4	63 70 76	0 2 0		Condition serious; small patch on each tonsil; croupy cough. Injected, fourth day, 700 im. u.; fifth day, 500 im. u. Injection abscess. Eruptions; anaemia. Recovery.
23	4½ yrs.	7th	101·0	160	26,500	12	82	10	8	0	14	9	3	72	3		
		9th	100·2	140	12,500	6	30	18	2	10	14	8	8	60	0		
		11th	101·6	140	26,000	20	64	16	6		
		13th	103·4	146	25,000	16	74	10	2	6	10	2	1	80	1		
		16th	101·0	130	12,750	18	54	24	6	7	24	4	3	59	3		
23	4½ yrs.	5th	100·4	148	30,500	16	54	22	10	Condition grave; no visible membrane; tonsils large; stenosis; intubation. Injected, fifth day, 1,000 im. u. Recovery.	
		6th	100·4	160	24,000	6	68	22	8	4	17	7	1	70	1		
		7th	100·6	146	27,000	30	73	20	5	6	12	4	6	72	0		
		8th	99·2	114	17,500	9	36	29	8	0	6	12	7	74	0		
		10th 12th	98·0 98·4	112 100	18,000 22,000	8 10	54 78	10 4	2 0	.. 0	.. 6	.. 10	.. 5	.. 83	.. 2		
13th	21,500	6	68	8	2	0	8	11	2	79	0				

No.	Age.	Day of disease.	Highest temperature.	Highest pulse.	LEUCOCYTES PER CUBIC MILLIMETRE.										Remarks.	
					Total.	Per cent. in salt solution.			Per cent. in dry preparations.							
						Mononuclear.	Well-stained polynuclear.	Poorly stained polynuclear.	Ameboid figures.	Small lymphocytes.	Mononuclear leucocytes.	Large mononuclear.	Vesicular nuclei.	Polynuclear.		Compact nuclei.
24	3 yrs.	15th	99·6°	120	2	14	19	2	63	0	Condition fair; large patch on each tonsil, absent by thirteenth day; stenosis; intubation; Klebs-Loeffler bacillus absent after fourteenth day. Injected, eighth day, 700 im. u.; tenth day, 500 im. u. Recovery.	
		16th	98·8	120	0	8	11	2	78	1		
		18th	98·6	120	10	4	8	2	76	0		
		20th	99·6	130	11	6	4	6	73	0		
		22d	99·6	120	8	4	5
		26th	12	8	4
25	5 yrs.	29th	98·8	120	10	74	14	4	1	25	11	4	55	4	Condition fair; small patch on membrane of each tonsil; moderate stenosis. Injected, fifth day, 500 im. u. Recovery.	
		4th	101·0	130		
		5th	100·4	120	4	14	6		
		30 min. after anti-toxine.	4	11	3	69	0		
		70 min. after anti-toxine.		
		6th	99·8	110		
		8th	99·2	108	5	23	7	1	64		0
		10th	98·0	96	7	14	2	3	72		2
		12th	99·0	99	19	3	4	2	68		4
		19th	99·6	100	15	9

26	7 yrs.	3d 30 min. after anti- toxine.	99.8	128	10,500 7,000 7,000	0 8 ..	12 5 ..	20 9 ..	6 4 ..	62 76 ..	0 0 ..	Condition good; no visible membrane; Klebs-Loeffler cocci. Injected, third day, 500 im. u. Recovery.
27	2 yrs.	4th 6th 3d 4th 5th 6th 10th 12th	99.6 98.6 101.8 102.8 100.2 100.2 100.0 100.4	100 100 122 156 140 136 104 128	19,800 9,800 7,750 9,800 13,000 11,500 16,500 12,000 18 2 20 14 20 4 7 16 20 4 3 5 4 0 0 4 4 1 0	8 6 6 5 2 ..	11 22 29 36 26 ..	11 6 11 7 6 ..	1 2 2 4 4 ..	69 62 60 52 49 62 ..	0 0 0 2 2 ..	Condition grave; thin streaks on tonsils; fourth day, condition precarious; sixth day, condition good. Injected, third day, 500 im. u. Recovery.
28	2½ yrs.	5th 6th 9th 10th 11th 13th 20th	101.2 104.2 102.6 101.0 100.4 103.6 101.0	130 170 130 108 124 120 120	25,000 23,500 14,000 23,500 17,500 21,500 6 2 8 34 22 10 2 2 1	.. 17 4 23 17 1 1	.. 11 20 8 4 6 10 20	.. 7 8 7 4 10 10 10	.. 6 6 5 1 2 4 ..	59 58 51 72 79 64 0 4 4 2 2 1 ..	Condition grave; nose occluded; membrane at anterior nares; gangrenous odor; membrane on tonsils and pharynx; tenth day, scarlatinoid rash; fourteenth day, general desquamation. Injected, fifth day, 700 and 500 im. u.; sixth day, 700 im. u. Recovery.
29	7 yrs.	5th 6th 7th 8th 9th 11th 13th 15th 22d	101.0 100.0 100.4 99.8 99.0 99.0 99.0 99.0 98.0	122 130 144 144 108 100 98	26,000 35,500 21,000 16,500 18,000 24,500 25,000 10,000 8 5 2 18 .. 8 6 2	.. 12 13 11 8 .. 20 7 10	.. 2 8 9 6 .. 6 16 7	.. 4 7 1 8 .. 9 6 6 4	.. 72 66 76 60 .. 57 66 72	.. 2 1 1 0 .. 0 0 3	Condition grave; thick membrane on uvula, tonsils, and pharynx, appearing at anterior nares; absent on fifteenth day; Klebs-Loeffler streptococci. Injected, fifth day, 700 im. u.; sixth day, 700 im. u. Albumin, five to ten per cent. Recovery.

33	8 yrs.	5th 7th 10th 12th 15th 20th	102.6 99.6 99.0 100.4 100.2 99.0	122 120 112 100.4 100 120	31,500 7,000 12,000 15,000 17,500 9,800 14 24 0	1 1 4 .. 8 5	8 15 4 .. 10 12	17 14 7 .. 8 3	3 0 3 .. 2 1	71 70 82 .. 72 78	0 0 0 .. 0 0	Condition serious; thick patch on one tonsil till thirteenth day; neck swollen; albumin, fifteen to twenty per cent. Injected, fourth day, 400 im. u.; fifth day, 400 im. u. Recovery.
34	2½ yrs.	6th 8th 10th 12th 16th	100.0 100.0 102.0 100.2 100.2	140 130 120 120 120	22,500 15,000 17,000 22,000 12,500	9 0 10 12 4	11 4 27 22 16	13 13 6 6 7	13 11 6 6 7	6 11 4 2 5	59 71 56 58 67	2 1 2 2 1	Condition fair; distinct patch on each tonsil until ninth day; Klebs-Loeffler until twenty-eighth day. Injected, sixth day, 500 im. u. Recovery.
35	4 yrs.	8th 10th 12th 18th	99.6 99.0 99.0 98.6	116 100 98 96	16,000 12,500 11,000 10,500 36	5 8 8 8	9 6 16 11	10 7 8 3	10 7 8 4 5	7 1 4 5	68 78 64 73	0 0 0 0	Condition good; thin patches on tonsils till seventeenth day; Klebs-Loeffler till seventeenth day; albumin, five to twenty-five per cent. Injected, eighth day, 100 im. u. Recovery.
36	1½ yr.	7th 8th 10 min. after anti- toxine. 9th	100.8 101.4	124 170 33,500 26,500 9 20	.. 11 12	.. 14 1	.. 8 7	.. 8 7	.. 57 60	.. 1 1	Condition serious; no visible membrane; severe stenosis; intubation; scarlatina rash. Recovery.
37	2½ yrs.	11th 12th	99.2° 99.0	120 116	14,250 10,000	14 8	32 26	3 6	3 ..	16 ..	9 ..	7 ..	62 ..	3 ..	Condition good; thin secondary membrane on uvula and tonsils till fifteenth day; albumin, ten per cent. Injected, tenth day, 300 im. u. Recovery.
38	12 yrs.	9th 11th	99.6 98.6	98 72	10,000 16,000	8 16	24 38	8 8	.. 12	.. 20	.. 7	.. 3	.. 58	.. 0	Condition good; no visible membrane; Klebs-Loeffler cocci. Injection omitted. Recovery.

No.	Age.	Day of disease.	Highest temperature.	Highest pulse.	LEUCOCYTES PER CUBIC MILLIMETRE.											Remarks.		
					Per cent. in salt solution.						Per cent. in dry preparations.							
					Total.	Mononuclear.	Well-stained polynuclear.	Poorly stained polynuclear.	Ameboid figures.	Small lymphocytes.	Mononuclear leucocytes.	Large mononuclear.	Vesicular nucleus.	Polynuclear.	Compact nucleus.		Eosinophile.	
39	6 yrs.	4th 40 min. after anti- toxine. 5th	101·2°	126 ...	41,500 25,000	18 3	62 70	82 28	30 12	1 1	5 5	6 2	4 3	4 3	84 89	0 0	0 0	Condition grave; membrane on tonsils and uvula; severe stenosis; intubation; Klebs-Loeffler cocci. Injected, fourth day, 1,200 im. u. Recovery.
40	15 yrs.	2d 30 min. after anti- toxine.	101·2	96 ...	23,500 20,500	10 4	70 72	14 7	0 0	1 2	10 10	5 4	3 3	3 3	81 81	0 0	0 0	Condition good; much thick membrane over pharynx; Klebs-Loeffler cocci. Injected, 1,200 im. u. Recovery.
41	28 yrs.	2d 30 min. after anti- toxine. 3d	102·5	120 ...	17,500 10,000	2 6	52 28	12 6	2 0	0 0	4 5	12 7	6 3	6 3	78 85	0 0	0 0	Condition good; many small patches on tonsils. Injected, second day, 1,200 im. u. Recovery.
42	50 yrs.	3d 4th 6th 8th	102·6 101·2 99·6 100·8	96 96 100 90	25,000 14,500 14,500 11,000	22 16 24 16	151 24 24 28	32 18 10 0	6 4 2 0	2 4 ..	7 16 ..	8 8 ..	3 2 ..	3 2 ..	80 70 ..	0 ..	0 ..	Condition fair; large patch on tonsils and uvula until sixth day; Klebs-Loeffler till thirteenth day. Injected, second day, 600 im. u.; third day, 600 im. u. Recovery.

43	19 yrs.	3d 5th 7th 10th	102-2 101-0 100-4 99-4	100 102 88 84	46,000 12,000 14,000 10,500	10 3 7 3	9 7 8 0	4 8 0 0	9 7 0 0	64 75 88 ..	4 0 2 ..	Condition good; large patch on right tonsil; tonsils much swollen. Injected, third day, 600 im. u. Recovery.
44	22 yrs.	5th 6th 8th 10th 12th	100-0 99-8 98-0 98-0 98-0	114 100 104 90 80 22,000 16,500 16,000 13,000	4 6 8 8 8	6 8 11 26 7	8 8 11 7 0	2 2 2 2 2	.. 80 64 .. 57	0 2 2 .. 2	Condition good; small patches on tonsils; moderate general hyperaemia. Injected, sixth day, 600 im. u. Recovery.
45	19 yrs.	4th 30 min. after anti- toxine.	101-0	110 ..	14,000 11,500	6 6	10 5	17 14	6 3	61 72	0 0	Condition good; thin membrane on left tonsil; much general hyperaemia; Klebs-Loeffler cocci till eleventh day. Injected, 200 im. u. Recovery.
46	19 yrs.	5th 45 min. after anti- toxine.	100-8 100-8 99-4	108 96 100 ..	10,000 11,000 5,500 4,000	4 6 2 4	30 36 16 10	6 2 4 2	6 2 1 1	2 2 1 1	2 2 0 0	.. 72 50 68	0 2 0 2	Condition good; no visible membrane. Injected, fifth day, 200 im. u. Recovery.
47	17 yrs.	5th 6th 8th	100-0 99-0 98-0	110 96 80	13,500 20,000 18,000	1 8 5	2 4 11	21 20 16	0 2 0	76 66 67	0 0 0	Condition good; irregular streaks on tonsils. Injected, third day, 400 im. u. Recovery.
48	23 yrs.	6th 9th	101-8 100-2	100 110	8,250 8,500	6 6	21 28	6 0	2 0	6 10	15 5	53 71	0 1	Condition good; large patch on each tonsil till ninth day; much hyperaemia. Injected, sixth day, 200 im. u. Recovery.

No.	Age.	Day of disease.	Highest temperature.	Highest pulse.	LEUCOCYTES PER CUBIC MILLIMETRE.										Remarks.				
					Per cent. in salt solution.		Per cent. in dry preparations.												
					Total.	Mononuclear.	Well-stained polymuclear.	Poorly stained polymuclear.	Ameboid figures.	Small lymphocytes.	Mononuclear leucocytes.	Large mononuclear.	Vesicular nucleus.	Polymuclear.		Vesicular nucleus.	Polymuclear.	Compact nucleus.	Eosinophile.
49	22 yrs.	4th 30 min. after anti- toxine. 5th	104·0°	124 ...	10,500 11,000	8 6	32 41	2 0	2 0	2 0	2 4	6 4	3 4	5 5	5 84	5 87	0 0	0 0	Prostration moderate; no visible membrane on tonsils and palate; very hyperæmic, swollen. Injected, fourth day, 125 im. u. Recovery.
50	24 yrs.	6th 9th	100·0	92	9,000	2	30	4	4	4	3	9	10	4	73	1	1	Prostration slight; large patch on each tonsil. Injected, fifth day, 400 im. u. Recovery.	
51	24 yrs.	7th 8th	103·6° 103·2	110 100	8,000 8,500	4 4	26 28	2 2	4 2	4 2	6 ..	6 ..	11 ..	1 ..	75 ..	0 ..	0 ..	Prostration moderate; membrane exfoliated. Injection omitted. Recovery.	
52	28 yrs.	6th	101·0	100	14,000	14	42	0	2	2	7	12	7	1	72	1	1	Prostration slight; small patch of membrane on tonsils. Injection omitted. Recovery.	
53	24 yrs.	4th	99·0	80	5,000	8	12	0	0	0	Prostration slight; small patches on both tonsils. Injected, fourth day, 125 im. u. Recovery.	

Experiments with Injections of Diphtheria Cultures.—In four rabbits an attempt was made to produce the change in the staining qualities of the leucocytes such as was observed in clinical diphtheria.

For this purpose the animals received a subcutaneous injection of broth cultures of diphtheria, two or four days old, and one fortieth of a cubic centimetre of which killed a two-hundred-and-seventy-gramme guinea-pig in forty-eight hours. The attempt was only partially successful. While an increase in the numbers of stainless leucocytes

was noted continuously up to the death of the animals, the appearance of these cells did not perfectly resemble that of the stainless leucocytes found in the human subject. Such a result is, however, to be expected in view of the great difference in the conditions produced by such injections from those found in severe cases of pharyngeal diphtheria.

In one rabbit which survived, the staining reaction of the leucocytes was well restored after powerful doses of antitoxine.

Effect on Rabbits of Subcutaneous Injections of Virulent Cultures of Diphtheria.

No.	Date.	LEUCOCYTES EXAMINED IN SALT SOLUTION.						Treatment.
		Total.	Small uninuclear.	Large uninuclear.	Well-stained multinuclear.	Poorly stained multinuclear.	Anceboid figures.	
1	March 14th, before injection.	13,000	23	5	23	1	3	1 c. c. culture, two days old. 2·5 culture, four days old. 2·5 culture, two days old. 50 im. u. (Behring) antitoxine. Multinuclears all paler. Ante mortem.
	March 15th.....	9,000	10	2	23	1	4	
	March 16th.....	13,500	5	8	36	5	4	
	March 17th.....	13,750	8	4	38	5	5	
	4 hours after injection.....	11,750	7	1	24	15	5	
	35 minutes after antitoxine.....	6,250	4	1	8	12	3	
	March 18th.....	53,250	6	0	132	22	12	
	3 hours later.....	35,750	3	0	68	36	10	

2	March 14th, before injection.	12,000	28	3	21	1	2	1.5 c. c. culture, two days old.
	5 minutes later	8,750	17	7	8	3	0	
	5 hours later	7,000	4	2	24	4	2	
	March 15th.	8,250	11	2	15	5	2	2.5 c. c. culture, four days old.
	March 16th.	12,000	16	3	21	8	3	2 c. c. culture, four days old.
	March 17th.	20,000	15	3	50	12	7	
	March 18th.	22,750	18	3	55	15	6	2 c. c. two days old.
	March 19th.	55,250	28	0	112	81	17	Died, March 20th.
3	March 18th, before injection.	13,000	20	7	24	1	1	2 c. c. two days old.
	March 19th.	11,500	5	7	23	11	0	
	March 20th.	9,000	13	6	17	6	2	2 c. c., four days old.
	4 hours later	14,500	16	0	26	16	4	2 c. c. 40 im. u. antitoxine.
	30 minutes after antitoxine..	10,750	12	1	22	8	4	
	March 21st.	27,500	42	2	47	19	6	
	P. M.	43,250	47	1	109	16	6	2 c. c. 300 im. u. antitoxine.
	30 minutes after antitoxine..	33,500	19	7	107	11	5	
	March 22d.	40,000	4	2	104	52	15	2 c. c. 300 im. u. antitoxine.
	35 minutes after antitoxine..	24,500	10	4	40	44	3	
	4 hours after	19,250	7	1	52	17	2	2 c. c., two days old.
	March 23d.	36,000	6	3	101	34	7	Leucocytes well stained.
								Recovery.
4	March 18th, before injection.	9,250	13	5	19	0	0	2.5 c. c. culture, two days old.
	March 19th.	8,250	4	6	20	3	0	
	March 20th.	11,500	3	2	40	1	3	2 c. c., four days old.
	March 21st.	11,000	10	0	24	10	4	
	March 22d.	21,500	6	4	36	40	23	2 c. c. 300 im. u. antitoxine.
	30 minutes after antitoxine..	10,000	5	1	16	18	5	2 c. c. culture, four days old.
	March 23d.	25,000	2	4	42	52	20	Died, March 26th.

Bibliography.

- Bouchut. *Gaz. des hôpitaux*, November 20, 1879.
 Bouchut et Dubroisay. *Compt. rend.*, lxxxv, No. 3, 1877.
 Cuffer. *Revue mens.*, 1878, p. 519.
 Pee. *Untersuchung. ü. Leukocytose*, Berlin, 1890.
 Rieder. *Beiträge z. Kenntniss d. Leukocytose*, Leipsic, 1892.
 Limbeck. *Grundriss ein. klin. Path. d. Blutes, etc.*, Jena, 1892.
 Morse. *Boston Med. and Surg. Jour.*, March 7, 1895.
 Gabritschewsky. *Ann. de l'Institut Pasteur*, 1894.
 Zappert. *Zeitschr. f. klin. Med.*, 1893.
 Ehrlich. *Farb-Untersuchung, z. Histol. u. Klin. d. Blutes*, 1891.
 Cantacuzene. *Recherch. sur la destruction de vibr. chol.*, Paris, 1894.
 Everard, Demoor, Massart. *Ann. de l'Institut Pasteur*, 1893.
 Metchnikoff. *Ann. de l'Institut Pasteur*, 1887.
 Lowit. *Studien ü. Phys. u. Path. d. Blut. u. Lymph.*, Jena, 1892.
 Isaëff. *Untersuch. ü. künstliche Immunität gegen Chol., Zeit. f. Hyg.*, 1893.
 Zenoni. *Ziegler's Beiträge z. path. Anat., etc.*, p. 537, 1894.
 Kitasato. *Zeit. f. Hyg.*, 1891.
 Klemperer. *Berl. klin. Woch.*, 1892.
 Lazarus. *Berl. klin. Woch.*, 1892.
 Behring u. Nissen. *Zeit. f. Hyg.*, 1890.
 Zasslein. *Rivista Clinica. Archiv. ital. d. clin. med.*, 1890.
 Pfeiffer u. Wasserman. *Zeit. f. Hyg.*, 1893.
 Pfeiffer u. Isaëff. *Zeit. f. Hyg.*, 1894.
 Gamaleia. *Ann. de l'Institut Pasteur*, 1889.
 Metchnikoff. *Ann. de l'Institut Pasteur*, 1891-'94.
 Isaëff. *Zeit. f. Hyg.*, 1894.
 Sanarelli. *Ann. de l'Institut Pasteur*, 1893.
 Klemperer. *Semaine médicale*, 1894 (Soc. de méd., Berlin), p. 211.
 Kossel. *Arch. f. Anat. u. Phys. (phys. Abtheil)*, 1893, p. 157.
 Lilienfeld. *Arch. f. Anat. u. Phys. (phys. Abtheil)*, 1893, p. 391.
 Chatenay. *Les réactions leucocytaires v. toxines, etc.*, Paris, 1894.
 Goldscheider u. Jacob. *Zeitschr. f. klin. Med.*, Bd. xxv, No. 5, 1894.
 Werigo. *Ann. de l'Institut Pasteur*, 1892.
 Buchner. *München. med. Woch.*, 1894.

THE New York Medical Journal.

A WEEKLY REVIEW OF MEDICINE.

EDITED BY

FRANK P. FOSTER, M.D.

THE PHYSICIAN who would keep abreast with the advances in medical science must read a *live* weekly medical journal, in which scientific facts are presented in a clear manner; one for which the articles are written by men of learning, and by those who are good and accurate observers; a journal that is stripped of every feature irrelevant to medical science, and gives evidence of being carefully and conscientiously edited; one that bears upon every page the stamp of desire to elevate the standard of the profession of medicine. Such a journal fulfills its mission—that of educator—to the highest degree, for not only does it inform its readers of all that is new in theory and practice, but, by means of its correct editing, instructs them in the very important yet much-neglected art of expressing their thoughts and ideas in a clear and correct manner. Too much stress can not be laid upon this feature, so utterly ignored by the “average” medical periodical.

Without making invidious comparisons, it can be truthfully stated that no medical journal in this country occupies the place, in these particulars, that is held by THE NEW YORK MEDICAL JOURNAL. No other journal is edited with the care that is bestowed on this; none contains articles of such high scientific value, coming as they do from the pens of the brightest and most learned medical men of America. A glance at the list of contributors to any volume, or an examination of any issue of the JOURNAL, will attest the truth of these statements. It is a journal for the masses of the profession, for the country as well as for the city practitioner; it covers the entire range of medicine and surgery. A very important feature of the JOURNAL is the number and character of its illustrations, which are unequalled by those of any other journal in the world. They appear in frequent issues, whenever called for by the article which they accompany, and no expense is spared to make them of superior excellence.

Subscription price, \$5.00 per annum. Volumes begin in January and July.

PUBLISHED BY

D. APPLETON & CO., 72 Fifth Avenue, New York.

