I.

THE CURATIVE INFLUENCE OF EXTRACTS OF LEUCOCYTES UPON INFECTIONS IN ANIMALS.*

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1. THEORIES AND FACTS OF INFECTION AND IMMUNITY.

Before passing to the experimental part of our work, it may be well to consider certain matters of fact and theory relating to the subject of immunity. It would be a task, however, entirely apart from the requirements of this paper, to give even a fragmentary review of the literature of immunity and to attempt to sift the observations, experiments, data, and conclusions which have been educed in the study of the processes of protection against invading microörganisms, and of the establishment of cure and of post-infection immunity.

To those familiar with these questions, it may be taken for granted, certain fundamental conceptions and facts stand prominently forth—That, on the one hand, the theory of phagocytosis has been strengthened by the test of time, and the recent work on opsonins has but added a stimulus to further observations and experiments explanatory of the stimulation and mechanism of this well-known phenomenon. On the other hand, that the immense amount of work in elucidation of the part played by the fluids of the body has furnished an invaluable set of data in explanation of the method of production and action of antitoxins and the so-called immune bodies of the serum and plasma.

In spite, however, of the mass of data thus collected in the study of the processes involved in meeting infection and the establishment of cure and immunity, actual advances in serumtherapy and biological methods of treatment during this period have not kept pace with theory; and the successful production of antisera for diphtheria and tetanus and a few other diseases in which strong diffusible toxins play the major part stands out in bold relief against the practically

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universal failure when the same methods are attempted in the production of sera for the treatment of other germ diseases, notably those whose symptoms are not so definitely referable to the action of soluble toxins.

It is to these diseases, in which the bacterial poisons are supposed to be principally endotoxins, that our attention is chiefly directed in this paper.

Attempts to produce curative sera by the repeated inoculation of animals of various species with the inciting micro-organisms of these endotoxin diseases, as well as the injection of various strains of a given microörganism into the same animal to produce the so-called polyvalent sera, have thus far failed to lead to satisfying results. No more have the extracts of these microörganisms, obtained by pressure, freezing, and breaking up, or by osmosis or autolysis, when used for immunization instead of the intact organism, given results which have so far led to the practical use of such sera in the treatment of disease in man.

These sera have not apparently been antitoxic in the usually accepted sense and depend, so far as theory goes, for any curative value they may be supposed to possess upon their germicidal and bacteriolytic power and possibly, in the light of the opsonin theory, upon the opsonins they may carry and thus stimulate phagocytosis. That these sera are capable of protecting an animal from a many times lethal dose of an infecting organism, when mixed with it in surprisingly minute quantities is, of course, known to all, but that any consistent curative effects, other than merely local, have been definitely established as due to such germicidal action, after an infection has once been established, may justly be questioned. Something must, therefore, be lacking in these sera, either a toxin neutralizing body, or, as has been suggested, a complementary body necessary for the activation of the bactericidal or bacteriolytic immune bodies. In experiments in vitro, complements may, as is well known, be furnished by fresh normal sera of various kinds, but in the body of infected animals and man must be furnished, if at all, by the plasma or cells.

Even if experiments had demonstrated conclusively that bodies existing in drawn sera were present in the same condition and amounts in the blood plasma — a condition which we have no right definitely to postulate — we have reason to believe that the complement, at least so far as it is free in the plasma, is not present in sufficient amount to render efficient the intermediate body; and, so far as our knowledge of complement in sera is concerned, it is not, as is the immune body, increased during disease or in response to inoculations. Certain incomplete immune bodies, therefore, even if they are produced in excess and exist free in the plasma, are, in the absence of activating bodies, powerless against the invading or infecting organism. We have no convincing evidence, then, that the complementing body or cytase is normally at liberty in the plasma, and likewise we have no evidence that the opsonins are free in the circulating blood. Metchnikoff, in fact, claims that the cytases — micro and macro — are not normally free in the plasma, but are contained in the leucocytes and only given up by them under stress of death or injury, such as may occur from unusual osmotic conditions or in the formation of serum, and that, if the immune sera are directly destructive of organisms in the body they are so only by virtue of their combination with the products of injured or destroyed leucocytes.

However this may actually be, test and experiment have shown that animals and man suffering from a true infection running to a fatal termination may and often do furnish sera capable of strong bactericidal and bacteriolytic action (when combined with normal sera containing complement) if the disease has run a sufficiently long course, and yet, in spite of this fact, they succumb.

In the light of these and other facts which might be cited it has long seemed to me that one must give pause in attempts to produce beneficial effects by injecting still further amounts of bacteriolytic or similar bodies, and must seek further for an explanation of the exact methods and processes of the cure effected in those animals and man who do

survive an infection. Failure to solve these problems on lines hitherto followed should not discourage us, however, while we know that the mechanism of the animal body suffices to protect the animal even against enormous doses of injected organisms, without serious histological changes or marked systemic symptoms, if these organisms be given at proper intervals and in gradually increasing amounts. The conclusion that this power must reside in increased digestive and neutralizing or poison-destroying powers of the animal organisms cannot well be avoided, and these functions of the animal mechanism will probably be found to reside in some group of cells which are not only able to take up and digest the introduced or infecting microörganisms but are also able to neutralize the poisonous products resulting from the metabolism or destruction of the microörganisms and to thus protect the more sensitive and specialized cells from the action of such poisons.

Bearing directly on this point the brilliant researches of Metchnikoff have focussed attention upon phagocytosis and the actions of the various cells taking part in the scavenging and removal of foreign bodies and organisms from the infected animal and man. These researches have followed faithfully the steps involved in infection and the gradual return of the body to the normal state; in other words, have disclosed the story of infection and cure as seen under the microscope and revealed by staining reactions, especially as these processes unfold themselves in localized or local infections such as those going on in the peritoneal or pleural cavities or under the skin. Conclusions of the utmost importance have been drawn from these phenomena as to the active participation and functions of the white cells in protection of the animal or human body against infection.

In addition to these controlled animal experiments we have knowledge of cellular activities in man gained from morphological investigation of inflammatory exudates and of infected tissues and organs, and also a knowledge, in many diseases, of the changes and fluctuations in the white cell content of

the blood as influenced by the stage and character of the disease, all of which factors point a guiding finger to the forces acting for the protection and relief of the infected animal or man.

It is our belief that if one studies intelligently in each disease the character of the exudates and cellular changes, no matter where found, and in the different stages of the disease, that these will give strong clues to the major cellular forces acting in defence of the invaded organism, and may thus lead to a logical course of biological treatment. In determining this course, however, one must recognize that mere morphological studies are simply indicative, and that imagination and interpretation of function, backed by experiment, must really supply the key to the invisible physiological forces at play, and give us access to those storehouses of energy and supply whereby the ailing and losing animal organism may be artificially reinforced.

Possibly the animal body ideally protected in the time of bacterial invasion is one in which some set of cells — phagocytes — are immediately ready and able to take up the bacterial invaders and destroy them, and within their own bodies to neutralize any poisons secreted by such invaders or arising from their destruction by digestion, and this without serious harm to the ingesting cells, or — failing this full immunity from serious harm — that these ingesting cells should in their turn be taken up and with their noxious contents be digested by other scavenging cells, with a minimum liberation of the substances which could injure the body cells dedicated to specialized functions. And this is apparently what does occur in the case of the diseases which we are considering, for in experimental peritoneal infections not severe enough to cause death, the bacteria are sooner or later ingested, usually by polynuclear leucocytes, and these when injured by their bacterial contents are, in their turn, ingested by the larger mononuclear cells, which thus aid in the process of digestion and absorption or fixing of the harmful agent.

OBSERVATIONS AND THEORETICAL CONSIDERATIONS LEADING DIRECTLY TO THE EXPERIMENTAL WORK UPON LEUCOCYTE EXTRACTS AND INFECTIONS.

An apparently valid observation made in my laboratory* during the early part of 1907, that there was a difference between the phagocytic power of corpuscles from certain persons suffering from infection and the phagocytic power of corpuscles from normal man, led me to conclude that it was not a matter of indifference, as assumed by A. E. Wright, what might be the source of corpuscles used in opsonic tests, and from this I formulated a set of experiments which I thought might demonstrate that corpuscles of an infected animal, especially one suffering from systemic infection following intravenous inoculations, passed through a gradual change independent of the so-called opsonic content of its serum — a change, first of depression in phagocytic function as the height of the infection was reached, and then, of gradual exaltation of functions as the animal regained normal and passed into the immune state.

Unfortunately, although such experiments were actually undertaken by Dr. North and myself, they were interrupted.

In thinking over this work I came to the conclusion that in many diseases we are probably dealing with an immunity a large part of whose mechanism is individually cellular, not only in the sense of phagocytosis and digestion, but in the neutralization of poisons given rise to by the disintegration of the bacteria — a mechanism in which the protecting cells *must* intervene and, unaided by bodies in the plasma, neutralize within themselves the poisonous products of the invading microörganisms.

It was this thought that gave rise to the further idea of aiding the leucocytes by furnishing them as directly as possible with the weapons which were being taken away from them in their fight with invading microörganisms, and to thus protect them from destruction and give them an opportunity to recuperate and carry on successfully their struggle against the invading germs. These weapons, whatever might

* Dr. Charles E. North — personal communication.

be their nature, I assumed might possibly be furnished by an extract of the active substances of the leucocytes themselves — substances not ordinarily given up to the plasma or serum — and I also assumed that extracts would be more efficacious than living leucocytes themselves, introduced into the infected animal, since being diffusible they would probably be distributed impartially to all parts of the body by the circulatory mechanism and, as quickly as absorption would permit, relieve the fatigued leucocytes and protect, by any toxin-neutralizing or other power they might possess, the cells of highly specialized functions.

This idea of immunity differs from one that simply assumes the cells as the source of all immune bodies — which logically seems to be the case — in that it takes into consideration the presence and production in the leucocytes of agents, which are not normally given up to the plasma, but which are constantly able to reproduce themselves and carry on the functions of digestion or neutralization simply for the benefit and protection of the individual cell, while not being secreted or excreted by the cells for the more general benefit of the cell community at large.

Thus we have a differentiation of immune agents into those which by virtue of their liberation and overproduction by the cells, such as the antitoxins, amboceptors, and agglutinins, etc., are free in the plasma and thus, when active, are immediately available for the protection of all the body cells; and into those agents by which certain cells primarily nourish and protect themselves, and are only of benefit to the cell community at large by virtue of the direct intervention of these cells between the invading germs and their products and the highly specialized cells requiring protection.

It seems, then, that when these sources of protection are overtaxed or fail to act efficiently on account of some inherent weakness or untoward circumstance of location, that the most reasonable course is, if possible, to support the chief army of attack as indicated by a study of the exudates and pathological changes in the disease in man and animals, and to endeavor to supply those products which are

most heavily taxed in the fight, in other words, to introduce into the infected animal or man the *substances* composing the chief cells or all the cells of an exudate in the most available and diffusible form, and as little changed by manipulation as possible. Such substances, if they become free from the cells by extraction, might serve to neutralize poisons in the blood, might alone or in combination with bodies already present in the blood act deleteriously on the bacteria, and thus protect and augment the activities of the flagging leucocytes by supplying them with their own weapons in the fight against the invading organisms. And further, the extracts of such exudates from previously immunized animals might even better serve this purpose, since their cells probably have in their own fight against the same organisms gained increased powers, as is evidenced by the ability of such immunized animals to safely dispose of immense numbers of organisms without serious harm or loss of weight. Also, as a further adjuvant, immune sera might be found serviceable in some cases, especially early in the disease and when non-immune cells are being used, although it is our belief that sufficient immune bodies (bactericidal or bacteriolytic) are often present, and in sufficient amount even early in the disease, if the animal economy has not been entirely overwhelmed by an enormous primary dose of the infecting organisms or their poisons.

The leucocytes seem to lend themselves more than any other body cells to these more generalized functions, and no further exposition of the reason for selecting them for such experimentation is necessary.

The complete scheme of experimentation mapped out was as follows:

To determine,

(*a.*) The effect of extracts of leucocytes of normal animals on infections.

(*b.*) The effect of extracts of leucocytes of immune animals on infections.

(*c.*) The effect of immune serum alone, and combined with (*a*) and (*b*), on infections. And

(*d.*) The effect of extracts of the blood forming organs — bone-marrow, spleen, and lymph nodes — and of mononuclear leucocytes of normal and immune animals, alone or combined with immune serum.

Extracts of spleen and lymph nodes, and of mononuclear leucocytes were included not only because they might be found to have a toxin neutralizing effect, but because Metchnikoff has stated that mononuclear leucocytes are more active in phagocyting certain organisms than the polynuclears, such for instance as the bacilli of tuberculosis and certain organisms giving rise to chronic infections.

It is obvious that such a scheme of experimentation is a broad one, and that some of its phases have been attacked from various sides by other writers, notably Petterson, to whose excellent papers the reader is referred.

Petterson, however, has been chiefly interested, apparently, in elucidating the direct destructive action of certain leucocyte extracts upon bacteria, and in bringing out differences between the serum alexines (complement) and the bactericidal and bacteriolytic bodies of the leucocytes, in other words, with the visible mechanism of immunity as shown in vitro and in the peritoneal cavity. Our work, on the other hand, has had as its immediate object the practical determination of the *curative* effects of such extracts, the best method of extracting, etc., and the most available animals to use for material not only as to supply and ease of handling but as to the character of the extract obtained. In this connection it was apparent, from the first, that not all species may serve this purpose, since distinct differences have been shown to obtain not only in bactericidal power of serum from different species of animals, but in their leucocytes, and it was only fair to suppose that the functions of leucocytes from different species, such for instance as the rabbit and dog, might differ,

so that conclusions drawn from experiment with one might not be applicable to the other.

And again it might well be that experiments successful in certain animal species might not succeed in others, for instance, rabbit extracts might protect rabbits, but not other animals, since the reactions given might not be simple toxin neutralizing ones, but might require certain definite complementing actions to take place before the desired result could be obtained, and any of these might only obtain in certain species combinations.

That these and other points require investigation before the full value of the work thus outlined may justly be determined even from its more empirical side, is evident to the writer, and the different phases of investigation are being taken up as rapidly as may be, and will be treated of in separate papers. In the present paper, however, only certain fundamental experiments will be brought out that they may serve as a demonstration of certain facts and as a basis for our further work.

EXPERIMENTAL WORK.

The investigations set forth in the present paper were undertaken to determine the influence, and especially the curative influence, if any, of extracts of leucocytes upon infections.

Practical work. — Animals used: The animals used for obtaining the leucocytes and for most of the experiments were rabbits, although dog leucocytes have also been experimented with, and guinea-pigs have served in some instances as test animals.

Preparation of extracts. — The leucocytes have been obtained for the most part by double pleural inoculations with aleuronat, and the amount obtained after twenty-four hours from rabbits has usually been from thirty to sixty cubic

centimeters of turbid, often blood-stained fluid. This has been quickly centrifugalized and the serum decanted. The cells then at times washed in normal NaCl solution, or directly subjected to the extracting fluid, which is added in amounts about equal to the fluid poured off.

It is evident that the extracts must vary in strength, as there is no means by which they can be exactly standardized, principally on account of the red blood cells which are often present in the exudates.

Although extractions were first attempted by rapid freezings and thawings in .85 per cent NaCl solution, this was abandoned, and in the work detailed in this paper the extracts were obtained by thoroughly emulsifying the cells in distilled water, and allowing them to stand for a few hours at 37.5° C., and then at ice-box temperature until used. In most instances not only the clear supernatant fluid has been injected into animals, but also the cell residue which may easily be emulsified by shaking. We have done this so that the animal would have the benefit of all the cell products, since our methods of extraction are as yet too crude for us to feel certain that all active substances are freed from the cell by them.

It is a fact worthy of note that rabbit leucocytes and those of the dog act differently in the presence of distilled water. Rabbit leucocytes are not markedly disrupted, morphologically, by their new environment of distilled water, and no matter how small or large the quantity of water added, there is no tendency to a gelatinization. Dog corpuscles, on the other hand, seem to go into solution in distilled water, for after emulsifying in small amounts of water and then adding up to ten to fifteen cubic centimeters to one cubic centimeter volume of cells and placing at 37.5° C., a gelatinous clot-like mass forms, which soon, however, seems to dissolve and leave only a comparatively slight residue. Rabbit cells do not show this phenomenon unless placed in alkali or in strong NaCl solutions, and then do not tend to go again into solution when water is added. This solution of dog polymorphonuclear leucocytes is extremely interesting, and

renders the preparation of extracts from dog exudates one of great ease. It however seems to indicate a physiological as well as physical difference from rabbit leucocytes. This question will be discussed later.

2. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON STAPHYLOCOCCUS INFECTIONS IN RABBITS.

Our first experiments were made with Staphylococcus pyogenes aureus. The culture was a very virulent one for rabbits, and the doses of leucocyte extract were small. Brief protocols are given.

Experiment I. — March 13, 1907. At noon two rabbits, weighing respectively one thousand two hundred and one thousand four hundred grams, were inoculated subcutaneously in the abdomen with one-half of a twenty-four-hour agar culture of staphylococcus Pr. II. The one-thousand two-hundred-gram animal died within eighteen hours. The one weighing one thousand four hundred grams still survived and was given small doses — 1.5 cubic centimeters of NaCl leucocyte extract obtained by freezing and thawing — at 3 P.M. and at 4 P.M. of March 14. The animal's temperature was falling at the time of injections and no reaction was noted. Animal probably moribund at time. Died within thirty-six hours.

The experiment was without value except in establishing the virulence of our culture, since treatment was commenced very late, and after the death of the control.

Experiment II. — March 15, 1907. 11.30 A.M. Two rabbits, a gray weighing one thousand five hundred grams, and a maltese weighing one thousand five hundred and fifty-five grams, received subcutaneously in the ear one-fifth of a twenty-four-hour culture of staphylococcus Pr. II.

The gray, one-thousand-five-hundred-gram rabbit, was left without treatment. Its temperature reached 105.9° F. by 6 P.M. On March 16 at 8 A.M. its temperature was 102° F. There was no local lesion of note and the animal died at 1 P.M.

The maltese rabbit reached a temperature of 105.4° F. at 4.20 P.M., when it was given two cubic centimeters of an aqueous extract of rabbit corpuscles intraperitoneally. At 5 P.M. temperature was at 105° F. On March 16 at 8 A.M. its temperature was 105°, and at 12 M. 104° F. There was extensive local edema; animal otherwise in good condition. At 12.30 P.M. two cubic centimeters more extract were given. Temperature 1.30 P.M. 103.2°, then a gradual rise to 103.8° F. at 5 P.M. March

17, animal in good condition, eating. March 18, good general condition. Local infection a distinct abscess with discharging pus. Temperature normal. Animal practically normal on fifth day in temperature, appearance, and weight.

This experiment seemed to indicate that possibly the extract had some influence in localizing the infection and thus saving the animal.

Experiment III. — March 18, 1907. In this experiment three rabbits were inoculated subcutaneously in the ear with one-fifth of a twenty-four-hour agar culture of staphylococcus Pr. II. None of the animals died. The control weighing two thousand four hundred grams lost most weight, being one hundred and thirty grams lighter at the end of eight days, while the treated animals, weighing two thousand two hundred and eighteen grams and two thousand one hundred and forty-four grams, respectively, were normal in weight at the end of eight days. The treated animals received only small doses of extract, the first doses being given five hours after infecting — two cubic centimeters subcutaneously in one case and one cubic centimeter intraperitoneally in the other, — and at the end of twenty-four hours the same doses were repeated. Little or no effect was noted on the temperature, and there was little difference in the local lesions, the conservation of the weight of the treated animals being the only noteworthy feature of the experiment.

Experiment IV. — March 22, 1907. 10.15 A.M. Two rabbits, one thousand nine hundred and forty grams and one thousand nine hundred and thirty grams, were each given subcutaneously in the left ear one small twenty-four-hour agar culture of staphylococcus Pr. II.

The one-thousand-nine-hundred-and-thirty-gram rabbit received treatment — two cubic centimeters aqueous extract after five hours, and one cubic centimeter after seven hours — intraperitoneally. A drop of about one degree was noted after each injection at the end of one hour.

On March 23, as the animals did not seem particularly sick, another staphylococcus inoculation of one-half of a twenty-four-hour agar culture was given to each, in the right ear. Two hours afterward the rabbit undergoing treatment received two cubic centimeters of aqueous extract intraperitoneally — no drop in temperature was noted. On March 25 both rabbits were given an intravenous dose of a one-fifth agar culture of staphylococcus. The control died at 6 P.M. of March 28, six days after the first inoculation and three days after the intravenous inoculation. The treated animal received small daily intraperitoneal and subcutaneous injections of extract, which temporarily lowered the temperature, but from the twenty-eighth of March to the first of April ran a continuously high temperature, 104° to 105° F., which then dropped subnormal and

afterwards assumed a swinging character, the animal gradually emaciating. Chloroformed on April 5, having survived the control by eight days.

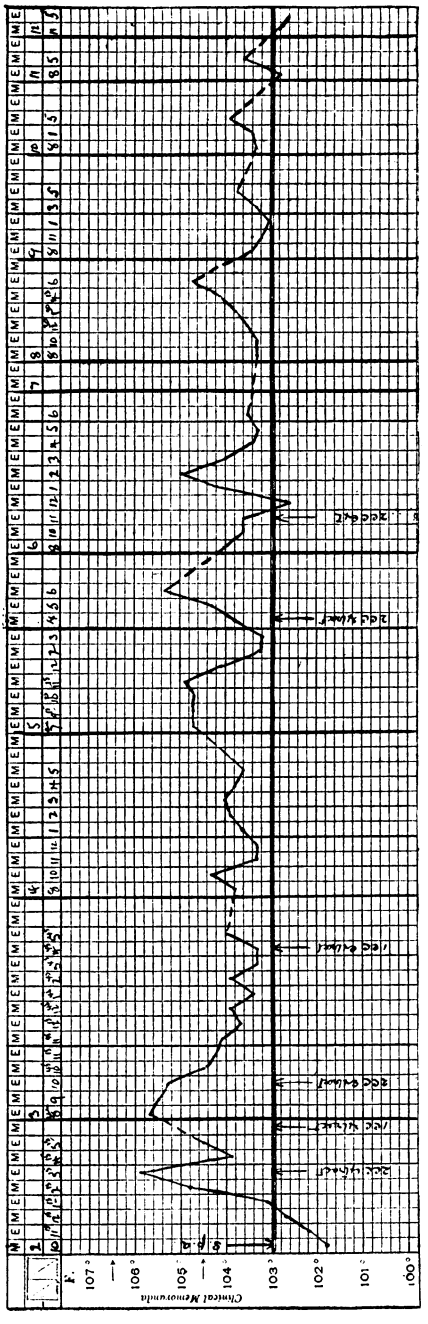
At autopsy the control animal showed on gross examination no visible abscesses in the voluntary muscles. There were abscesses in the kidneys and in the liver, and in the pericardium purulent fluid, and sanguineous fluid in the pleural cavities.

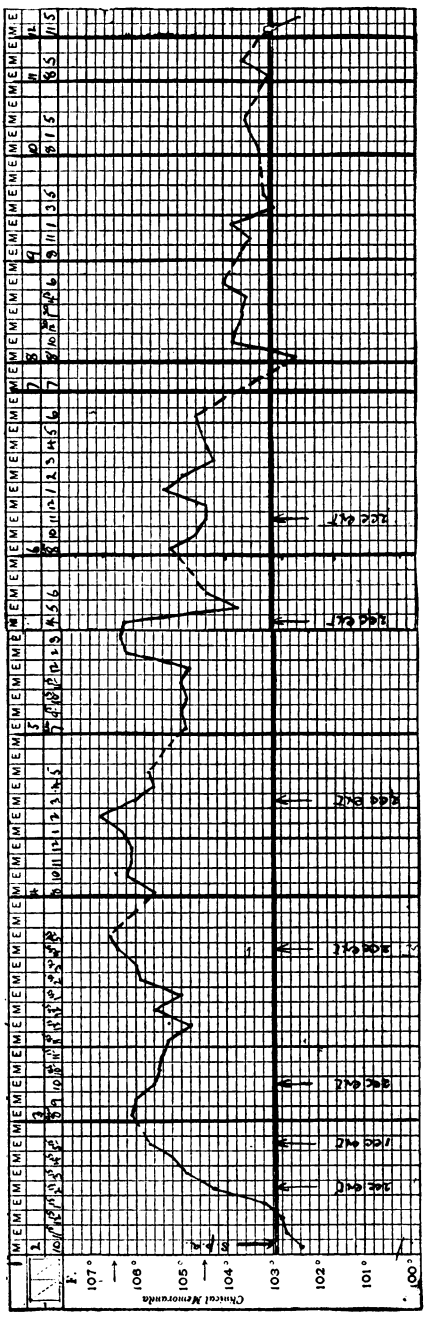
The treated animal had abscesses in the ventricles of the heart, small amount of pericardial fluid, no fluid in pleural cavities; liver, no gross lesions; kidneys, masses of abscesses. Large abscesses in voluntary muscles. Peritoneum normal. Pus in joints of front paws.

Here again the treatment with leucocyte extract apparently had a marked effect on the course of the infection, although given in small amounts and not earlier than five hours, even after the intravenous injection of the staphylococci. The disease was apparently changed from a rather acute septicemia to a fairly chronic pyemia, as evidenced by the anatomical picture at autopsy.

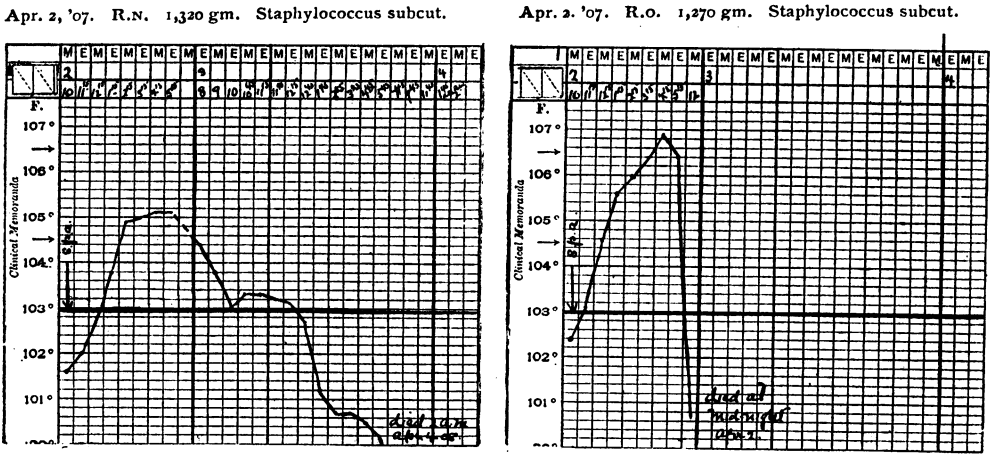
Experiment V. — April 2, 1907. As the culture used in the foregoing experiments showed signs of attenuation, the culture employed in this experiment was one whose virulence had presumably been increased by passage through a rabbit.

Four rabbits were given subcutaneously in the ear one-fifth of a twenty-four-hour agar culture Pr. II. R. I. at 10 A.M. The controls weighed one thousand three hundred and twenty grams and one thousand two hundred and seventy grams. The smallest control died in fourteen hours, and the largest in forty hours. The treated animals weighed one thousand one hundred and sixty grams and one thousand one hundred and five grams, and both survived. The smallest received intraperitoneally two cubic centimeters of extract after four hours, the largest after five hours. The largest animal showed a remission of temperature of two degrees within an hour after being treated, and then the temperature slowly rose again; the smaller animal did not show a remission, but its temperature was not so high as in the animal showing the remission. After seven hours each rabbit again received extract, and then daily for three or four hours. Neither animal lost much weight, the smallest one losing the most, about seventy-five grams. At the expiration of about eight to ten days even their temperatures, always apt to be above normal when animals are frequently handled, had reached normal. (See appended charts.)





Staphylococcus. Exp. V. Temperature charts of treated rabbits. Animals survived.



Staphylococcus. Exp. V. Temperature charts of control, untreated rabbits.

The results of this experiment are self-evident. The test, as indicated by the rapid death of the controls, was a severe one. The doses of leucocyte extract, although small, were given intraperitoneally and were probably quickly absorbed, as indicated by the change in temperature in one of the animals. They were probably efficacious in preventing a rapid systemic infection, although some systemic invasion probably took place.

Experiment VI. — April 4, 1907. 5 P.M. Four rabbits received one-fifth agar culture of staphylococcus Pr. II. R. I. subcutaneously in the ear. The controls weighed one thousand seven hundred and forty grams and one thousand five hundred and seven grams. The one-thousand-seven-hundred-and-forty-gram control died in eighteen hours. There had been some hemorrhage at the point of inoculation and it was thought that some of the injection had gone intravenously. This may account for the death of this animal while the lighter one did not show severe infection and was in apparently good condition on the fifth day following.

The treated animals did not receive any protection until the lapse of sixteen hours (April 5), and then only two cubic centimeters of extract intraperitoneally. These animals weighed one thousand four hundred and seventy grams and one thousand four hundred and fifteen grams. The heaviest showed a drop in temperature of three degrees (105° to 102°), while the lighter one's temperature fell only about one degree. The temperature rose slowly again and after five hours the protective dose

was repeated, the temperature again falling about one degree. On the following day the treatment was repeated, the temperature in one case dropping to below normal, but rising again within three hours. As the remaining control animal did not die, and was running nearly a normal temperature on the fifth day, a fresh infection was attempted, one-fourth of a twenty-four-hour agar culture being given each animal *intravenously* at 10 A.M. By 3 P.M. the temperatures of all the animals were above 106° F. The control and the one-thousand-four-hundred-and-seventy-gram-treated animal died during the night, in spite of the fact that the latter animal received two cubic centimeters of aqueous extract intraperitoneally five hours after the staphylococcus inoculation, and dropped two and one-half degrees in temperature within three hours.

The other treated animal, one thousand four hundred and fifteen grams, was treated with small doses intraperitoneally and survived until the twenty-fifth of April, when it was chloroformed, having lost six hundred grams in weight and being evidently in a lethal condition. This animal survived the control fifteen days.

At autopsy the control animal showed a few small abscesses in the voluntary muscles and some large ones in the kidney. In the ventricles of the heart were numerous pin-head abscesses. The treated animal, which died at the same time as the control, instead of showing evidences of prolonged infection, as did the control, which was evidently systemically infected from its first dose, gave a picture of acute septicemia. There were no macroscopic lesions in the heart, kidneys, or liver, or in the voluntary muscles. Organisms were cultivated from the blood.

In the case of the animal which survived fifteen days and received continuous treatment, the picture was one of great emaciation. The heart, lungs, and liver were free from abscesses. No fluid in the body cavities. Spleen seemed normal. In the left kidney was a fibrous patch possibly from an abscess. The right kidney was swollen and contained many abscesses. There was an abscess posterior to the kidney apparently becoming encapsulated. Organisms were recovered from the kidney pus, but not from the heart's blood.

It is worthy of note that this animal, following the intravenous inoculation, had shown signs of involvement of the joint of one of its front paws. This disappeared, however, during treatment.

This experiment seemed very instructive to us and almost to warrant the conclusion that the animal was saved from a rapid septicemia and generalized pyemia. The lesion was practically confined to the kidney, the organ most susceptible to staphylococcus infection, and the animal probably succumbed to disturbed metabolism and elimination, and, possibly, to chronic poisoning due to the localized infection.

Experiment VII. — May 1, 1907. This experiment does not properly belong in this series, but is given, not only because the animals really serve as further controls for the immediately preceding experiments, but as indicating possibly a slighter efficiency of dog corpuscle extract as compared with that of rabbits, at least when used in the treatment of rabbits. The infecting dose was, however, very severe and the experiment was not controlled with rabbit leucocyte extract so that little weight is to be attached to the outcome of the experiment.

Three animals, weighing one thousand six hundred and fifty grams, one thousand four hundred and ninety grams, and one thousand three hundred and eighty grams, were given one-fifth of an agar culture of staphylococcus Pr. II. R. I. (same doses as in immediately preceding experiment) intravenously at 10 A.M. At 11 A.M. the one-thousand-three-hundred-and-eighty-gram rabbit was given two cubic centimeters of dog leucocyte extract subcutaneously, which was followed by a four-tenths drop in temperature, and at 3 P.M. (five hours) two cubic centimeters more, followed by a six-tenths drop. Temperature, however, steadily climbed thereafter, and was over 106° F. at 6 P.M. The one-thousand-four-hundred-and-ninety-gram animal was treated with two cubic centimeters at 3 P.M. (five hours). Its temperature had not risen over one-half degree, but showed a remission of five-tenths and then began to rise steadily to 104.6° F. at 6 P.M. At 6 P.M. the control's temperature, after an initial fall at two o'clock, had risen abruptly to 105.8° F.

All of the animals were found dead in the morning (about eighteen hours).

No conclusions can legitimately be drawn directly from this experiment but, compared with the preceding, one might suppose dog leucocyte extract less efficient than that of rabbits.

No further staphylococcus experiments were undertaken at this time. Further experiments with this special infection are reserved for a later paper, in which the effect of treatment with immune leucocytes and immune serum will also be considered. Here we are chiefly interested in the effect of leucocyte extract (from normal animals) on various infections.

If we analyze our whole series it immediately becomes apparent that animals receiving subcutaneous injections of rapidly fatal doses of Staphylococcus pyogenes aureus can generally be saved by treatment with the extract of normal leucocytes of rabbits even in small doses, especially when

these are given intraperitoneally. Thus, in Experiments II., V., and VI., we find four control animals out of five dying in twenty-six, fourteen, forty, and eighteen hours, while the five treated animals survived, although never receiving treatment before the lapse of four hours, and in Experiment VI. not before sixteen hours. In Experiment VI. one control, however, also lived. The animals, however, in VI. were observed only five days before being given an intravenous injection of staphylococcus.

When intravenous injections were practiced the results were different, but treated animals usually survived the controls many days, and presented modified histological pictures.

Thus, in Experiment IV. we find our control dying in three days, while the treated animal survived eleven days.

In Experiment VI. the control died in eighteen hours, as did also one of the treated animals, while the other lived twenty days and showed an extremely favorable histological picture.

Such encouraging results from a novel method of treatment naturally led to tests on animals suffering from other infections.

3. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON TYPHOID INFECTIONS IN RABBITS.

Typhoid infections, if indeed we may really call them such, in rabbits are essentially different from infections caused by such organisms as staphylococci, streptococci, and pneumococci. The animals seem rather to suffer an acute intoxication, from which they either die within a very limited time and organisms may then be recovered from them, or they recover completely, or go into a state of cachexia, with gradually increasing emaciation, followed by death, but without organisms in the blood or organs. Death apparently is caused by cellular changes and disturbed metabolism induced by the primary violent poisoning, which is due, probably,

to the rather abrupt dissolution of the injected organisms by bacteriolysis and a liberation of their body poisons.

In man a hypoleucocytosis is characteristic of the typical fever, and changes indicating an activity of mononuclear cells are to be observed upon histological examination of involved tissues. The typhoid bacillus, however, in the semi-immune or immune (*i.e.*, patient following typical typhoid fever) is, nevertheless, associated with local purulent (polynuclear) inflammations and abscesses, which must, it would seem, be interpreted as endeavors to rid the system of these lingering invaders.

Such considerations would naturally lead one to hesitate in prophesying as to the effects of polynuclear leucocyte extracts upon the course of the disease, and it is fully recognized by the writer that experiments with mononuclear leucocytes or with spleen or lymphoid tissue extracts, either alone or combined in treatment with immune serum, might well have a more decided influence than polymorphonuclear leucocyte extracts alone.

Experiment I. — April 8, 1907. Three rabbits, weighing one thousand one hundred and twenty-five, one thousand one hundred and eight, one thousand one hundred and four grams respectively, received at 10.20 A.M. one-third of a twenty-four-hour agar culture of typhoid "70" intravenously. The one-thousand-one-hundred-and-four-gram rabbit received two cubic centimeters of aqueous extract of leucocytes at 11 A.M. and at 5 P.M. intraperitoneally; and the one-thousand-one-hundred-and-eight-gram animaltwo cubic centimeters intrap eritoneally at 3 P.M. Little or no drop in temperature followed these injections. The control animal, one thousand one hundred and twenty-five grams, showed, however, a temperature of 106.5° F. by six o'clock, while the temperatures of the other animals were only 104.8° and 104.3° F.

April 9. No treatment given. The control's temperature fell fairly steadily throughout the day from 105.8° at 8 A.M. to 104.6° at 6 P.M. The one-thousand-one-hundred-and-eight-gram rabbit (treated five hours) was at 105.7° at 8 A.M., 106.3° at 1 P.M., and 105.7 at 6 P.M.

The one-thousand-one-hundred-and-four-gram animal (treatment after one hour) was at 105.5° at 8 A.M., 105.1° at 1 P.M., and 104.4 at 6 P.M.

April 10. At 8 A.M. the control was at 104° F., the one-thousand-one hundred-and-eight-gram at 104.7° F., and the one-thousand-one-hundred-and-four-gram at 103.3, which was practically normal.

At 10.20 A.M. the animals all received an intravenous injection, one-third of an agar culture of typhoid "70."

The control's temperature reached 106° F. at 1 P.M., then fell gradually to 105° at 3 P.M., and was 105.5 at 6 P.M. From this time on its temperature ranged continuously between 104° and 106° F. until April 15 when it fell to 103.4° at 6 P.M.

The one-thousand-one-hundred-and-eight-gram rabbit's temperature reached only 105° by 3 P.M., when it received two cubic centimeters of cell extract intraperitoneally, temperature remitting about a half degree, and returning to 105° F. by six o'clock. From this time on the temperature ranged between 104.4° and 106.2° at 4 P.M. on the twelfth of April, when two cubic centimeters more of the extract was given intraperitoneally, the temperature dropping a degree in one hour and remaining between 104° and 105° until the fifteenth, when it dropped below 104° F.

The temperature of the one-thousand-one-hundred-and-four-gram rabbit rose abruptly from 103.3° F. to 105.1° within one hour, when two cubic centimeters of extract were given intraperitoneally. An hour later the temperature fell to 103°, over two degrees, and then rose gradually to 105.7° F. at 4 P.M., steadily falling from this time on and reaching 102.8 (normal) the following morning at 11 A.M. The range was then principally between 103° and 104, touching 103° on the fifteenth, no treatment being given.

On April 16 the dose of typhoid bacilli given intravenously was repeated, it being our hope in this way to simulate the typical infection of man by giving such apparently sub-lethal doses at comparatively frequent intervals.

Up to this time a careful record of the weights of the animals showed the following:

Control.	Treated within 5 Hours.	Treated within 1 Hour.
April 8. 1,125 grams.	1,108 grams.	1,104 grams.
" 11. 1,035 "	1,114 "	1,135 "
" 12. 1,030 "	1,160 "	1,200 "
" 13. 1,010 "	1,125 "	1,190 "
" 15. 1,085 "	1,148 "	1,225 "

Here it is seen that both of the treated animals had actually gained weight, the one receiving the treatment within one hour after infection having gained one hundred and twenty grams and the other one forty grams, while the control, although apparently picking up by the fifteenth, had lost fifty grams.

After the injection of the typhoid bacilli the control's temperature went up to 106° F., then gradually down to 103° F. on the nineteenth. The

one-thousand-one-hundred-and-eight-gram animal's temperature reached 106.5° F. by 3.30 P.M., at which time three cubic centimeters of cell extract were given. At 4.30 P.M. the temperature was 104.1°, and it ranged between this and 103.8° until the nineteenth.

The temperature of the one-thousand-one-hundred-and-four-gram animal (always treated within one hour) shot abruptly to 106.4° from 103.2° within one hour, when three cubic centimeters of extract were given and the temperature fell to 104.2° by one hour, and then gradually climbed to 107° F. by 4.30 P.M., but returned to 103.2° (normal) the next morning, and then ranged mostly below 104° F. until April 19, that is, remained practically flat.

April 19. Again the animals were given a dose of typhoid bacilli, this time one-third of an agar culture of a different strain, "Mallon." Following this inoculation the temperature of the control fluctuated and was even below 103° F. two hours after inoculation. From this time on the temperature rose gradually, reaching 106° F. on the twenty-first, then ranged between 104° and 105° F. till the twenty-fourth, when it commenced to fall, at times ranging higher, the animal dying on April 27, nineteen days after the first inoculation. Autopsy showed extreme emaciation, kidneys large and anemic, spleen of normal size. Clear fluid in peritoneal cavity. No organisms on cultivation.

The one-thousand-one-hundred-and-eight-gram rabbit showed a rise after the injection of two degrees within three hours, and after four and one-half hours received one and one-half cubic centimeters of extract intraperitoneally with little influence on the temperature. The following morning the temperature was as low as 102.8° and from then on ranged practically between 103° and 104°, the animal remaining in good condition, weighing one thousand one hundred and forty grams on May 1 when records were discontinued.

The one-thousand-one-hundred-and-four-gram rabbit's temperature rose from 103.2° at 12 M., the time of typhoid injection, to 107.5° at 2 P.M., when the animal received two cubic centimeters of extract intraperitoneally. At 6 P.M. the temperature was 106.4° F., but the following A.M. touched 103° (normal), and ranged between 103° and 104° until May 1 when records were discontinued.

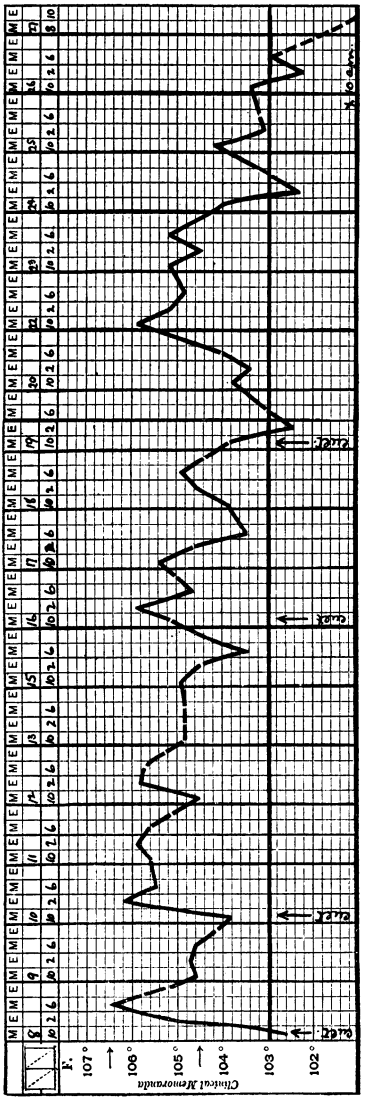
The weight records from the fifteenth on were as follows:

Control (1,125 Grams).	Treated within 5 Hours (1,108 Grams).	Treated within 1 Hour (1,104 Grams).
April 15. 1,085 grams.	1,148 grams.	1,225 grams.
(Injection.)	(Injection.)	(Injection.)
" 17. 1,020 grams.	1,130 grams.	1,185 grams.
" 18. 1,000 "	1,188 "	1,250 "
" 19. 968 "	1,160 "	1,228 "
(Injection.)	(Injection.)	(Injection.)
" 22. 935 grams.	1,100 grams.	1,135 grams.
" 24. 900 "	1,100 "	1,170 "
" 26. 865 "	1,085 "	1,210 "
" 29. Dead.	1,070 "	1,265 "
May 1. —	1,140 "	1,305 "

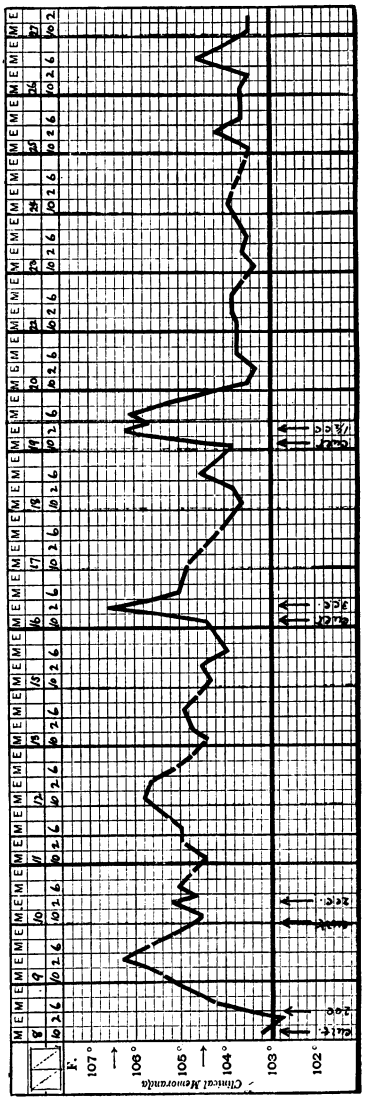
The animals, as is usual after toxic doses of typhoid bacilli, showed signs of poisoning, remaining quiet and refusing all food for some hours. The animals receiving protection with leucocyte extract, shortly after this treatment, always seemed much worse off than the control, and to the inexperienced would appear the most likely to die. This might possibly be due to a more rapid liberation of toxic substances by enhanced bacteriolytic processes, either brought about by a fuller complementing of immune bodies by the extract or to special digestive bodies of the leucocytic extract. That the poisoning in reality was fundamentally less severe than in the more normal appearing control is, however, evidenced by the rapid return of the treated animals to normal condition and weight, the weights following a perfectly logical order — untreated animal, animal treated late, animal treated early.

Another point of interest is the effect of the infecting doses on the temperatures of the animals. Leaving out of account the immediate effect of leucocytic extract on the temperature it is to be noted that following the later injections of typhoid bacilli the temperature of the untreated

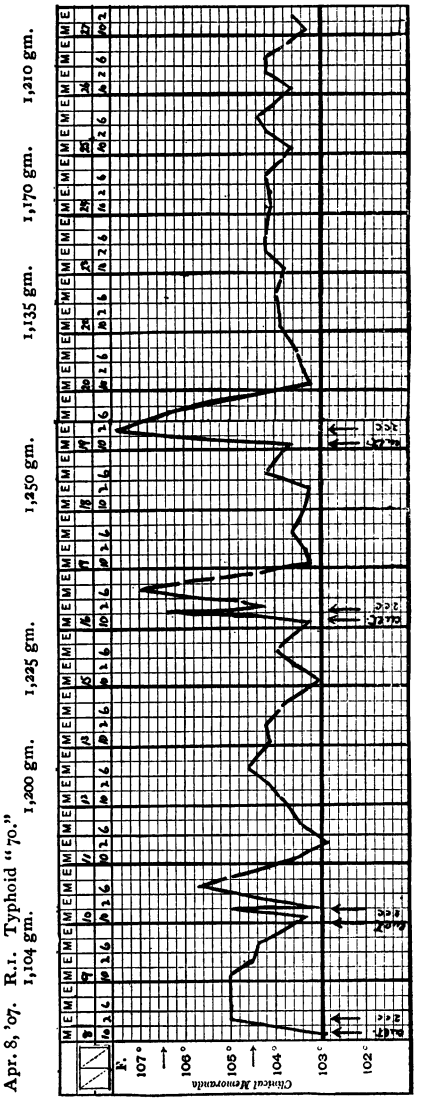
animal went up gradually and remained high, while the temperatures of the animal always receiving early treatment, at the later injections of bacilli, shot up abruptly and then returned rapidly to practically normal. There is a grading of these curves as there is of the weights — untreated animal, animal treated late, animal treated early. (See temperature charts.)



Typhoid. Exp. I. Temperature chart of untreated rabbit. Death. (103° C. is a fair normal temperature.)



Typhoid. Exp. I. Temperature chart of rabbit treated late. Animal lived.



Typhoid. Exp. I. Temperature chart of rabbit treated early. Animal lived.

No organisms were alive in the control, so that the animal died from poisoning and interference probably with metabolism and excretion due to cellular changes.

Experiment II. — April 17, 1907. This experiment will not be given in detail, as it largely repeats the foregoing one. The infecting dose was double the previous dose, and was repeated on the second day. The organism was culture "70" and the dose two-thirds of a twenty-four-hour agar culture. One animal was treated at the end of one hour, and one at the end of five hours. The dose of organisms was so large that its primary effect (after a very transient rise) was an abrupt lowering of the temperature followed by a rise shown at its height towards evening and the next morning.

The effect of the extract in the early treated animal was not a further lowering of the temperature, but an arrest and more abrupt rise than in the control and in the animal treated after five hours. Furthermore, both the control and the later treated animal had bad diarrhea within two hours, but the animal treated in one hour did not have diarrhea. The weights are again of interest and confirm our observations in Experiment I.

Control.	Treated late.	Treated early.
April 17. 1,030 grams.	1,020 grams.	980 grams.
" (Inoculation.)	(Inoculation.)	(Inoculation.)
" 18. 935 grams.	923 grams.	903 grams.
" (Inoculation.)	(Inoculation.)	(Inoculation.)
" 19. 907 grams.	880 grams.	922 grams.
" 22. 910 "	915 "	960 "
" 24. 890 "	900 "	940 "
" 26. 930 "	930 "	985 "
" 30. 880 "	945 "	1,015 "
May 1. 920 "	990 "	1,055 "
" 4. 840 "	965 "	1,010 "
" 7. 892 "	950 "	1,055 "

Here, slight fluctuations are noted, due no doubt to animals having been fed, at times, before noting temperature but not at others. The rule holds good, nevertheless — the animal receiving early treatment only lost weight transiently, rapidly regaining it, while the control lost weight more permanently, and the animal treated later held an intermediate position.

Another interesting observation was made in this experiment. The control and the animal receiving late treatment showed, on the day following the second injection of typhoid bacilli, a marked cyanosis of the ears and multiple petechial hemorrhages in the ears, suggesting the rose spots of typhoid fever. The animal treated early did not show these. The same appearances have been noted in animals receiving intravenous doses of meningococci, but not in connection with any other organism. This is a rather striking fact as both of these diseases in man are characterized by the appearance of such spots.

Experiment III. — May 5, 1908. As both of the earlier experiments (1907) had been performed with the same culture of B. typhosus, the present experiment was undertaken to see if the same result could be obtained when an organism of a different strain was used.

11 A.M. Three rabbits, weighing one thousand eighty, one thousand forty-four, and one thousand thirty grams respectively, were given an intravenous dose of one-third of a twenty-four-hour agar culture of typhoid " 12."

Two of the animals — one thousand eighty grams and one thousand thirty grams — showed an almost immediate drop in temperature of nearly three degrees. The one-thousand-forty-four-gram rabbit showed a slow but steady rise to 105° F., and was therefore presumably the most resistant animal and was kept as control. The one-thousand-thirty-gram rabbit received five cubic centimeters of extract at the end of one hour and the one-thousand-eighty-gram the same dose at the end of five hours. The control progressively lost weight and died on the nineteenth (*i.e.*, after fourteen

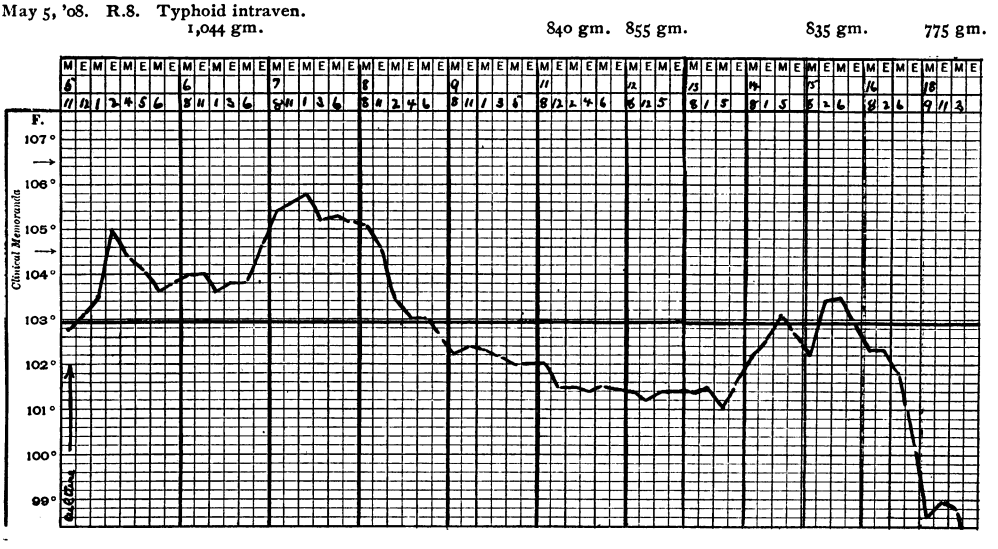
days), weighing only seven hundred and seventy-five grams, in spite of the fact that it rallied and ate voraciously even up to the eighteenth of May.

At autopsy, bloody fluid was found in the peritoneum containing pus cells and bacilli. There was an abscess around the left kidney. Cultures from the heart's blood were negative. Organisms, which proved to be typhoid bacilli, were recovered from the peritoneal fluid and the kidney abscess.

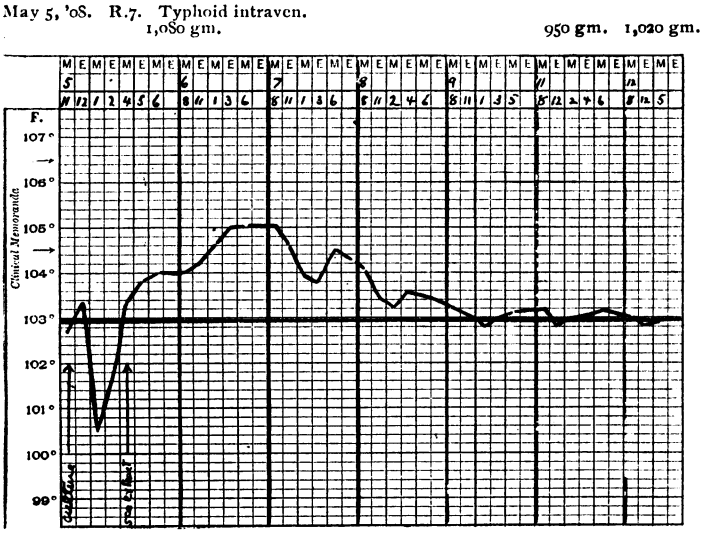
The treated animals lost weight transiently, the later treated one regaining weight more slowly than the one treated early. At the time of the death of the control the one-thousand-thirty-gram rabbit weighed one thousand one hundred grams, and the one-thousand-eighty-gram rabbit weighed one thousand ninety-three grams.

Two of the animals had hemorrhagic spots in the ears.

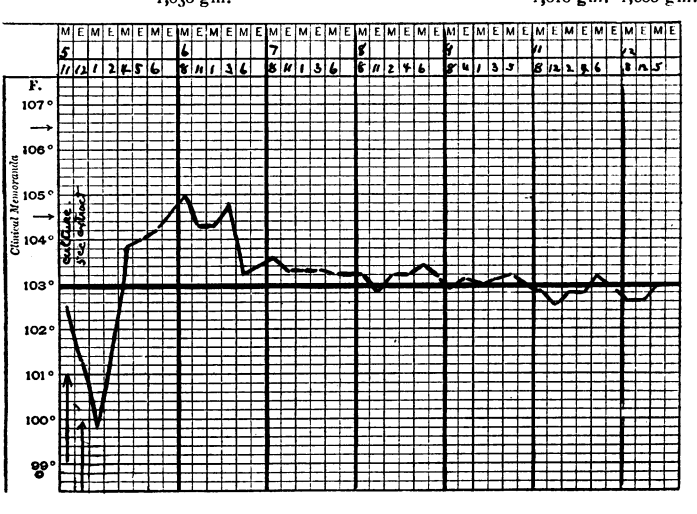
The charts in this experiment are very interesting and show distinctly the influence of the extract upon the course of the disease.



Typhoid. Exp. III. Temperature chart of untreated rabbit. Animal died in fourteen days.



Typhoid. Exp. III. Temperature chart of rabbit treated late. Animal lived.



Typhoid. Exp. III. Temperature chart of rabbit treated early. Note rapid return of temperature to normal. Animal lived.

These three experiments with leucocyte extract on typhoid infections in rabbits are sufficient for illustration, and the conclusion from them seems unavoidable, that leucocyte extracts have a markedly beneficial modifying action on the course of typhoid infections or poisonings in rabbits.

The same holds true of infections in guinea-pigs treated with rabbit leucocyte extracts, but apart from noting the fact here that subcutaneous injections, used curatively, are active in guinea-pigs, it does not seem of sufficient import to detail such experiments at this time.

Whether this action is to be attributed to products from polymorphonuclear or mononuclear cells can of course not be definitely stated, since all the exudates of course contain a certain percentage of mononuclear cells. It is not unlikely, however, the polymorphonuclear play an important part.

4. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON PNEUMOCOCCUS INFECTIONS IN RABBITS.

My first experiments with the pneumococcus were begun in April of 1907. Although these earliest experiments indicated that a modifying influence on pneumococcic infections, especially on temperature, was exerted by leucocyte extracts, they were unsatisfactory and were for the time abandoned.

These unsatisfactory results were due to the chance use of an extremely virulent organism and to the fact that small doses only of extract were employed at that time. Experiments with pneumococci were not resumed until early in 1908. In the meantime, leucocyte extracts had, however, been employed by us in pneumococcic infection in man with such encouraging results that the experiments about to be described were undertaken with much interest.

Experiment I. — Feb. 17, 1908. 11.15 A.M. Six rabbits, weighing one thousand four hundred and thirty, one thousand four hundred and twenty-five, one thousand four hundred, one thousand four hundred, one thousand three hundred and fifty-five, and one thousand three hundred and

twenty-five grams respectively, were given intravenously one cubic centimeter each of a twenty-four-hour ascitic broth culture of pneumococci "Ac."

The heaviest rabbits, one thousand four hundred and thirty grams and one thousand four hundred and twenty-five grams, were kept as controls. The two one-thousand-four-hundred-gram animals had been given, thirty minutes before receiving the pneumococcus, five cubic centimeters each, subcutaneously, of leucocyte extract. (This is the only instance in all our series of experiments that a prophylactic dose was given.) Notwithstanding this prophylactic treatment the temperatures of these animals, as well as that of all the others, rose consistently and steadily towards 105° F.

After the lapse of five hours the two remaining rabbits, one thousand three hundred and fifty-five grams and one thousand three hundred and twenty-five grams, were given subcutaneously five cubic centimeters of leucocyte extract (4 P.M.). At the expiration of two hours the one-thousand-three-hundred-and-fifty-five-gram animal showed a fall of temperature from 105.3° F. to 104° F., thus indicating a fairly rapid absorption of active substances. The one-thousand-three-hundred-and-twenty-five-gram rabbit's temperature did not fall.

No other treatment was given on this day.

February 18. The controls were still alive. The one-thousand-four-hundred-and-thirty-gram control's temperature reached 106° F. at 3 P.M., but fell to 105° by six o'clock and the animal was very sick. The temperature of the other control fell from 104.8° in the A.M. to 103° at 6 P.M., animal evidently dying.

All the treated animals seemed in fair condition with temperatures, however, ranging above 105° F. as a rule. All of them received treatments with the exception of one of the one-thousand-four-hundred-gram animals. These details are indicated on the charts.

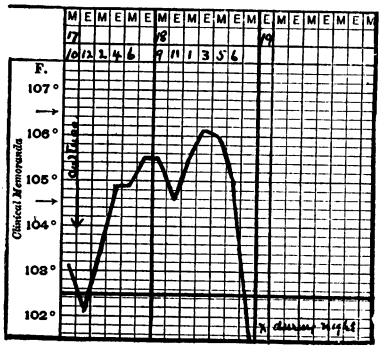
February 19. Both controls were found dead early in the morning (within thirty-six hours).

All the treated animals in fair condition, but temperatures high. No treatment given.

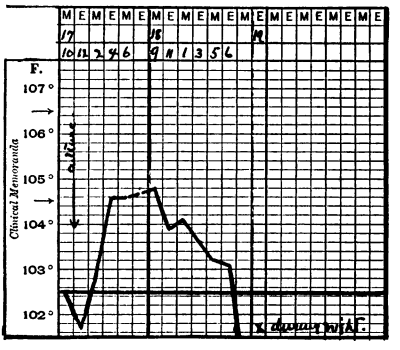
February 20 and 21. All the treated animals, with the exception of one of the one-thousand-four-hundred-gram animals, were doing very well, and in two the temperature fell to 104° during the twentieth, but ascended again on the twenty-first. The one-thousand-four-hundred-gram animal referred to as not doing well had from the first done badly, and had received two treatments on the eighteenth. On the twentieth its appearance was so bad that ten cubic centimeters of extract were given. The temperature dropped in an hour one degree and a half. In spite of this treatment it died during the night of the twenty-first (one hundred and eight hours). At autopsy there were evident signs of a double pleurisy, pericarditis, and peritonitis, but no pneumonia.

February 22. The temperatures of all the animals came down practically to normal, and continued from this time on practically flat. Animals in good condition, and eating well, from twenty-second on.

Feb. 17, '08. R.1. 1,430 gm. Pneumococcus.

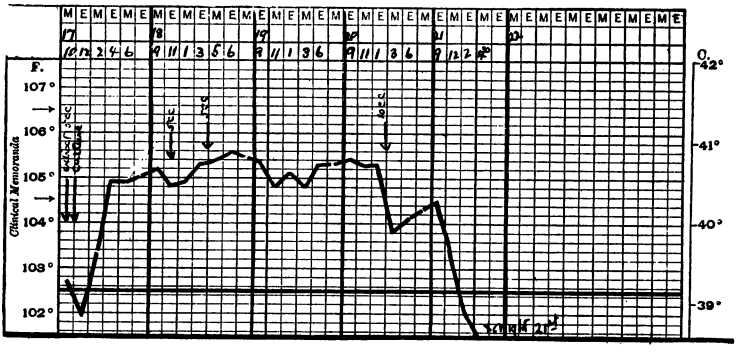


Feb. 17, '08. R.2. 1,425 gm. Pneumococcus.

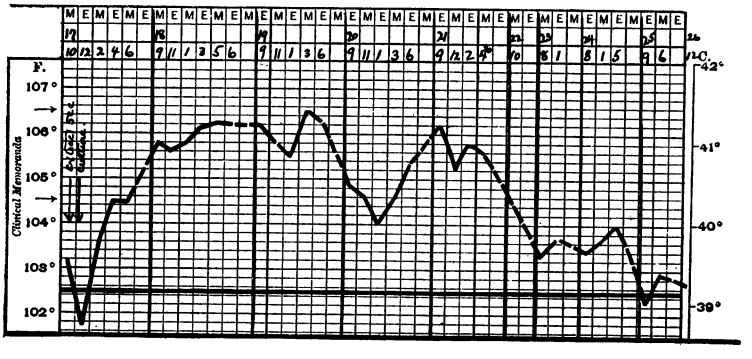


Pneumococcus. Exp. I. Temperature charts of untreated animals. Organisms given intravenously.

Feb. 17, '08. R.3. 1,400 gm. Pneumococcus.

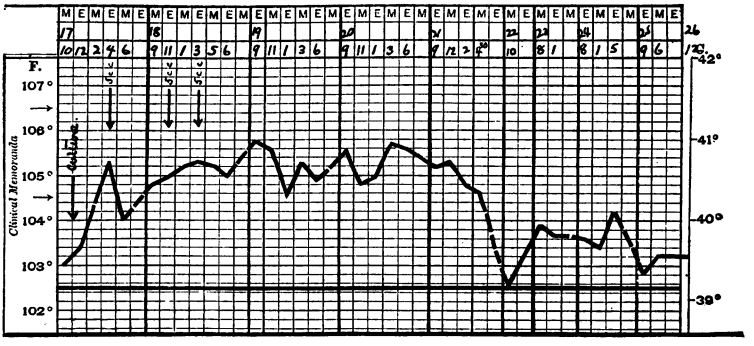


Feb. 17, '08. R.4. 1,400 gm. Pneumococcus.

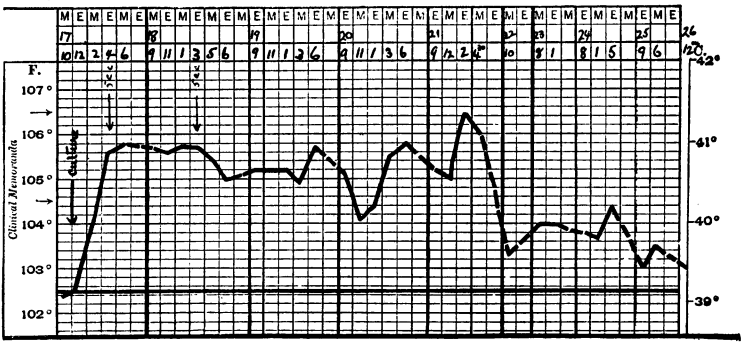


Pneumococcus. Exp. I. Temperature charts of rabbits receiving a prophylactic injection of extract.

Feb. 17, '08. R.5. 1,355 gm. Pneumococcus.



Feb. 17, '08. R.6. 1,325 gm. Pneumococcus.



Pneumococcus. Exp. I. Temperature charts of rabbits treated after five hours.

This experiment seemed to indicate a marked influence of leucocyte extract on pneumococcus septicemia in rabbits. The organism used was undoubtedly a virulent one, as indicated by the controls, both of which died in thirty-six hours. Seventy-five per cent of the treated rabbits recovered — two of them not having been given extract until the lapse of nearly five hours, while the other surviving one was only given a single prophylactic dose one-half hour before being infected, and had no further treatment. The one treated animal which died nevertheless survived the controls by over seventy hours (three days).

Experiment II. — Feb. 24, 1908. 11 A.M. Six rabbits, weighing one thousand two hundred and ten, one thousand one hundred and

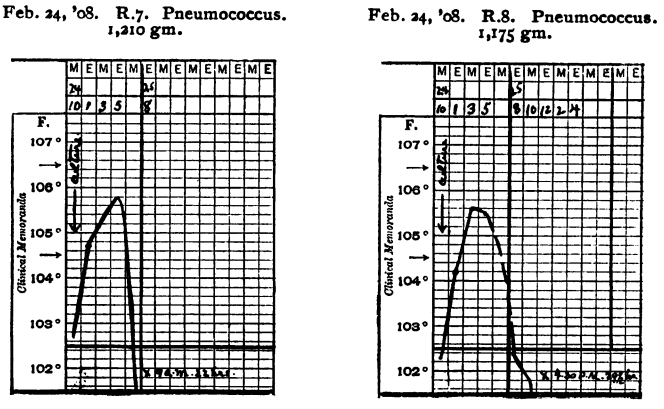
seventy-five, one thousand one hundred and sixty, one thousand seventy, one thousand sixty, and one thousand fifty grams respectively, were each given intravenously two cubic centimeters of a twenty-four-hour ascitic broth culture of pneumococcus "Ac." It is to be noted in this experiment that all the rabbits were smaller than in Experiment I. and that the dose was doubled. Two animals were treated at the end of four hours and two after six hours.

The controls died, one (one thousand two hundred and ten grams) in twenty-two hours, the other (one thousand one hundred and seventy-five grams) in twenty-nine and one-half hours.

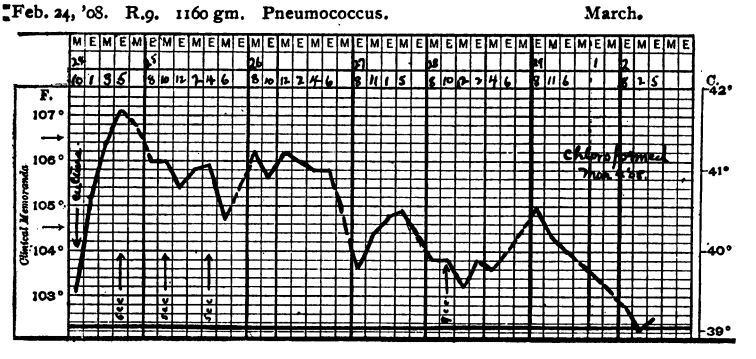
One of the animals (one thousand fifty grams), treated in four hours, died in twenty-two hours, the other (one thousand seventy grams) at the end of seventy-one hours.

Of the two animals treated after six hours one survived, being normal on the seventh day, but the other one died (chloroformed) on the ninth day. The death of this animal was not directly due to pneumococcus infection, for the rabbit had contracted a common laboratory ailment which is often fatal, and known to us locally as "wet-mouth." This supposition was confirmed at autopsy, for there were no gross lesions of lungs, pleura, pericardium or peritoneum, and no fluid in the body cavities and no organisms by stain or by cultivation.

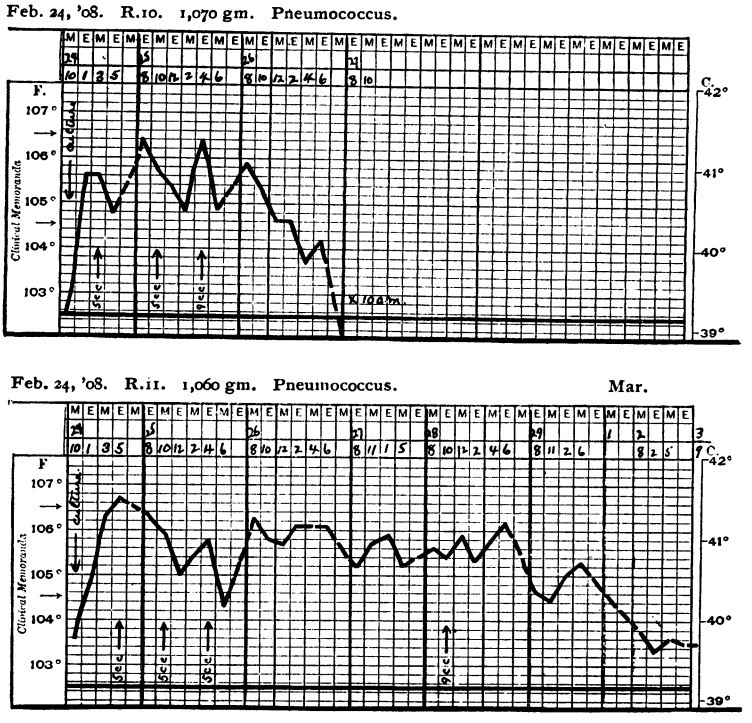
The test undertaken in this experiment was an extremely severe one, and again shows the influence of the extract. Both controls died, one in twenty-two hours, one in twenty-nine and one-half hours. Of the four treated animals, two died notwithstanding that they were treated — one died in twenty-two hours, the other one, however, only after three days — while the other two survived the infection, one recovering completely, the other one dying in nine days, but probably from an intercurrent disease, and with sterile blood and organs. It is also to be noted that all protective injections were made subcutaneously and, as indicated on the charts, markedly influenced the temperature in most instances. The doses of extract were usually five cubic centimeters. (See charts.)



Pneumococcus. Exp. II. Temperature charts of the untreated rabbits.



Pneumococcus. Exp. II. Temperature chart of treated animal.

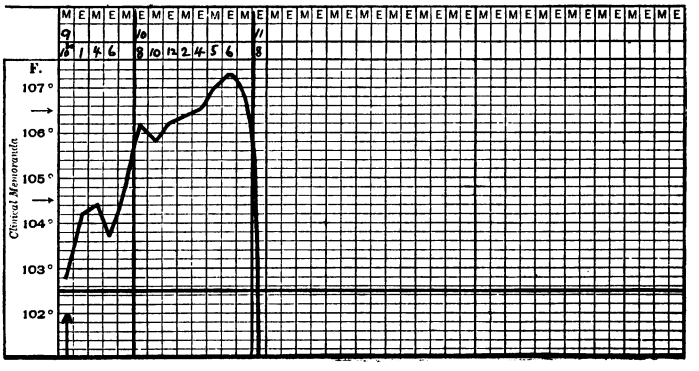


Pneumococcus. Exp. II. Temperature charts of the treated rabbits.

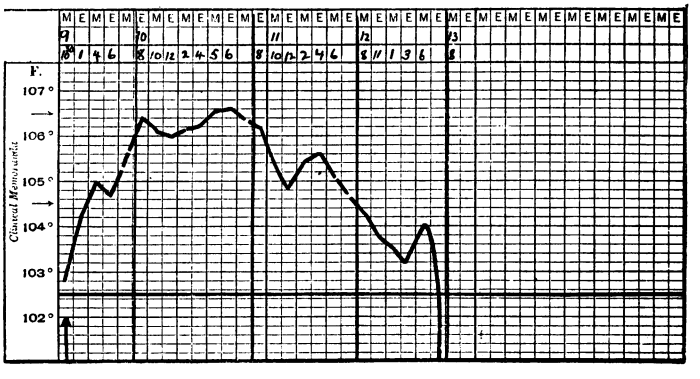
Experiment III. — March 9, 1908. 11 A.M. Six rabbits, weighing one thousand four hundred and forty, one thousand three hundred, one thousand two hundred and ninety, one thousand two hundred and seventy, one thousand two hundred and forty, and one thousand fifty grams respectively, were each given intravenously one cubic centimeter of a twenty-four-hour ascitic broth culture of pneumococcus "Ac." In this experiment we returned to the one cubic centimeter dose of culture and used animals slightly heavier than in Experiment II. All treatment was subcutaneously given, and no animal was treated before twenty-four hours after infection.

The controls, one thousand four hundred and forty grams and one thousand three hundred grams, died in forty-five and ninety-six hours respectively. All of the treated animals survived, only two of them, however, received more than one dose of five cubic centimeters of leucocyte extract. The doses and times of giving them are noted on the charts, where the effects of the extract on the temperature are also apparent. The temperatures of all were practically normal on the fifth to sixth day.

Mar. 9, '08. R.13. 1,440 gm. Pneumococcus.

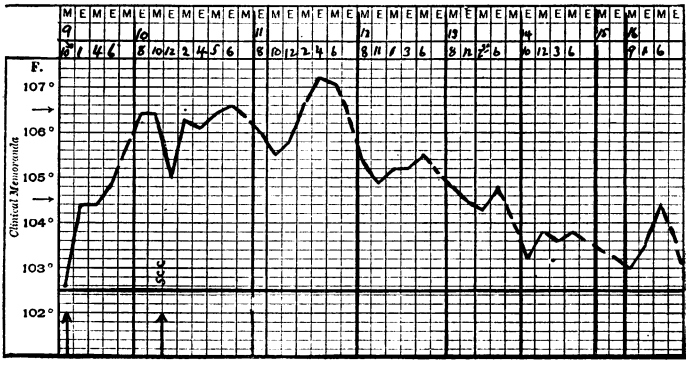


Mar. 9, '08. R.14. 1,300 gm. Pneumococcus.

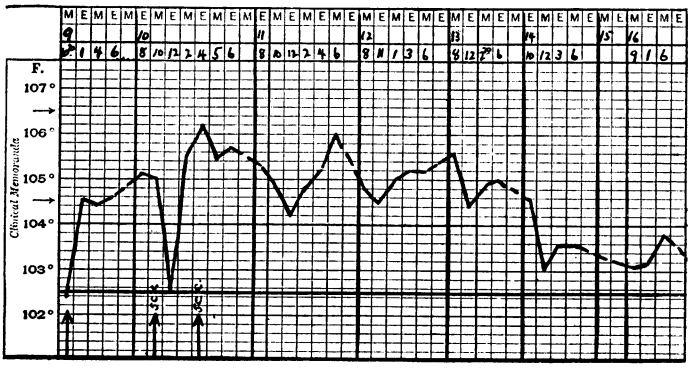


Pneumococcus. Exp. III. Temperature charts of the untreated rabbits.

Mar. 9, '08. R.15. 1,290 gm. Pneumococcus.

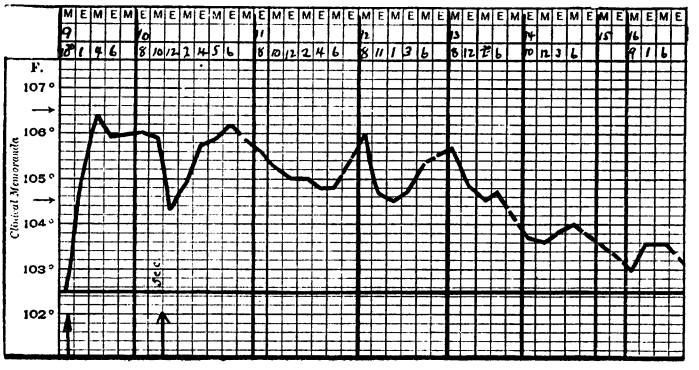


Mar. 9, '08. R.16. 1,270 gm. Pneumococcus.

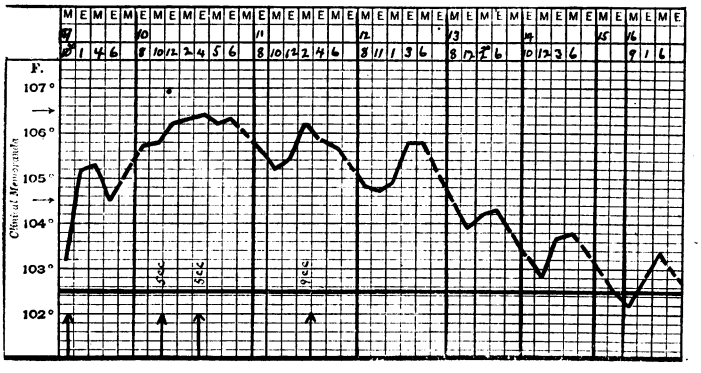


Pneumococcus. Exp. III. Temperature charts of rabbits treated twenty-four hours after intravenous inoculation.

Mar. 9, '08. R.17. 1,240 gm. Pneumococcus.



Mar. 9, '08. R.18. 1,050 gm. Pneumococcus.



Pneumococcus. Exp. III. Temperature charts of rabbits treated twenty-four hours after intravenous inoculation.

The effect of the extract is so self-evident in the experiment that no analytical remarks are necessary. Attention is especially, however, directed to the fact that the animals were saved from an infection fatal to one of the controls in forty-five hours, treatment not having been commenced until half of this time — twenty-four hours — had elapsed.

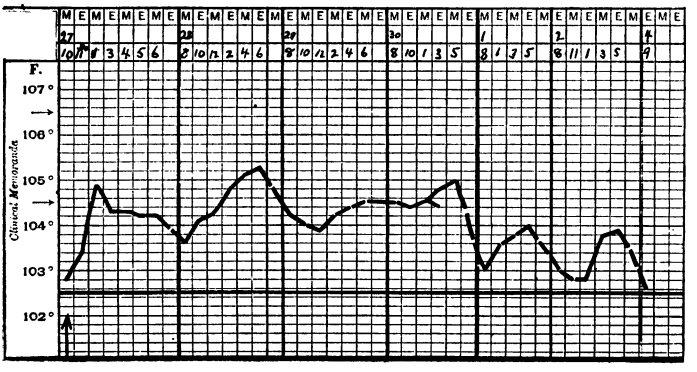
In order to demonstrate that such happenings were not peculiar to the given strain of organisms used, the following experiment is here given :

Experiment IV. — April 27, 1908. 10.30 A.M. Six rabbits, weighing one thousand three hundred and fifty, one thousand three hundred and fifty, one thousand three hundred and twenty-five, one thousand three hundred, one thousand two hundred and fifty, and one thousand two hundred and thirty grams respectively, were each given intravenously one cubic centimeter of a twenty-four-hour serum broth culture of pneumococcus "P." This organism had recently been isolated from a fatal case of pneumococcus meningitis. The organism heretofore used was from a pneumonic lung at autopsy.

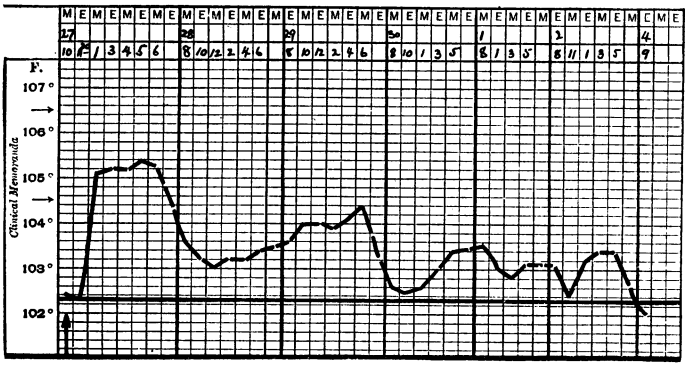
None of the animals died of the infection with this new organism, but the charts are interesting and illustrate beautifully the effect of leucocyte extract on the course of this more benign infection. It is worthy of note that the extract here used was over one month old (having been kept in the ice-chest) and was free from the usual red tinge, the exudate having been free from red cells.

In the charts one may observe the abrupt fall of the temperatures, veritable crises in some cases, following the absorption of the extract, which in each instance was given subcutaneously.

Apr. 27, '08. R.56. 1,350 gm. Pneumococcus "P." May.

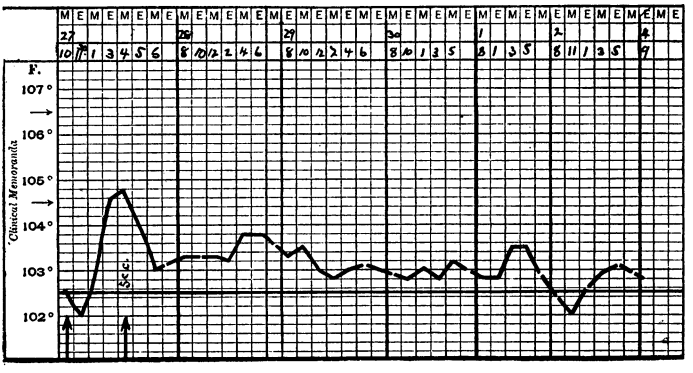


Apr. 27, '08. R.57. 1,350 gm. Pneumococcus "P." May.

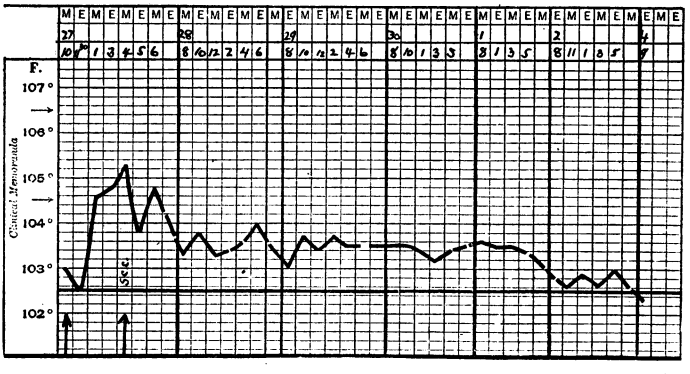


Pneumococcus. Exp. IV. Temperature charts of untreated rabbits surviving a mild infection.

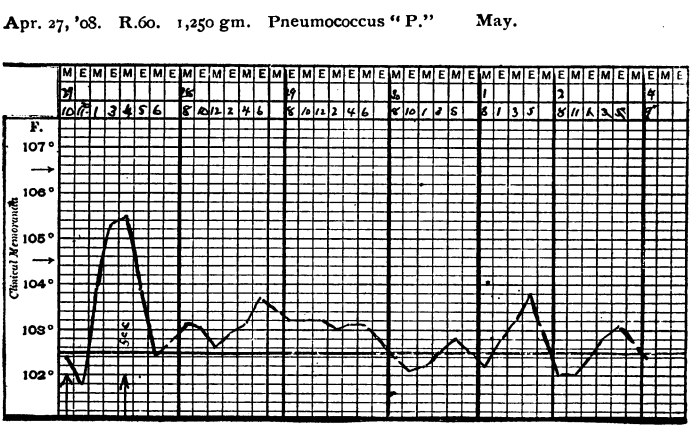
Apr. 27, '08. R.58. 1,325 gm. Pneumococcus "P." May.

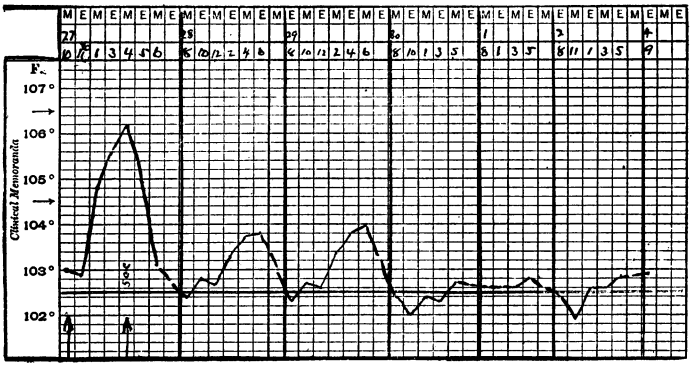


Apr. 27, '08. R.59. 1,300 gm. Pneumococcus "P." May.



Pneumococcus. Exp. IV. Temperature charts of rabbits treated after six hours. Type of infection mild.





Pneumococcus. Exp. IV. Temperature charts of rabbits treated after six hours. Type of infection mild.

It may not be altogether illogical to suppose that there may be times during an infection when leucocytes, having emigrated from their normal environment and being under adverse or unusual conditions, such as probably exist in the exudate in the lung during pneumonia, may be disintegrated in numbers and by a rapid neutralization of poisons in their immediate vicinity or by the reabsorption of their products into the general circulation bring about the abrupt

terminations of infections known as crises; or at other times by their cyclical destruction give rise to the heretofore inexplicable swinging temperatures so common in certain septic conditions.

It seems unnecessary to further illustrate this phase of the subject although data are at hand.

The following experiments are given as illustrative of certain other points which seem to us to demand some mention in the present paper.

Experiment V. — March 26, 1908. 11.15 A.M. Seven rabbits, weighing one thousand three hundred and ninety, one thousand three hundred and sixty-five, one thousand three hundred and fifty, one thousand two hundred and ninety-five, one thousand two hundred and ninety-five, one thousand two hundred and seventy, and one thousand one hundred and sixty grams respectively, were each given intravenously one cubic centimeter of a twenty-four-hour serum broth culture of pneumococcus "Ac."

No rabbit was given treatment of any kind until 10.30 o'clock the following morning, after practically twenty-four hours. The following table shows the treatment given each animal and the result:

Date.	Animal.	Weight.	Culture Inoculated.	Hour of Treatment.		Result.	Treated with.
				24-hr.	48-hr.		
1908.							
March 26.	R. 19	1,390	Pn. "Ac" 1 cc. intravenously.	.....	.....	36 hrs.	
"	R. 20	1,365	Pn. "Ac" 1 cc. intravenously.	.....	.....	36 "	
"	R. 21	1,350	Pn. "Ac" 1 cc. intravenously.	5 cc.	.....	36 "	Received H_2O suspension of cell residue after two previous H_2O extractions. Made to volume just before inoculation.
"	R. 22	1,295	Pn. "Ac" 1 cc. intravenously.	5 "	5 cc.	84 "	Received clear supernatant fluid composing second H_2O extraction.
"	R. 23	1,295	Pn. "Ac" 1 cc. intravenously.	5 "	5 "	132 "	Received clear supernatant fluid composing first H_2O extraction.
"	R. 24	1,270	Pn. "Ac" 1 cc. intravenously.	5 "	5 "	Survived.	Received regular aqueous extract containing emulsified cells. Made from same extracts as preparations above.
"	R. 25	1,160	Pn. "Ac" 1 cc. intravenously.	5 "	.....	36 hrs.	Received same as R. 24.

The experiment was planned to determine if the cell residue played much part in saving the animals and whether our aqueous extracts were saturated. The cells were, therefore, subjected to two extractions with distilled water, and then rapidly emulsified in a third equal volume of distilled water and given to the animal in volumes equal to the injections of the other extracts.

From the table it is seen that the controls died in thirty-six hours, as did also the animal receiving the suspended cell detritus, this animal having shown no temperature depression worthy of note following the injection. The animal receiving the second aqueous extraction lived for eighty-four hours, and the one given the first aqueous extraction survived for one hundred and thirty-two hours. The one-thousand-two-hundred-and-seventy-gram animal which received the regular aqueous extract plus the cell

detritus (as usually given) survived, while the small one-thousand-one-hundred-and-sixty-gram (two hundred and thirty grams lighter than the control animal) died in thirty-six hours. The death of this animal does not entirely invalidate the experiment, for the culture was very active and the animal much under weight. Leaving this animal out of consideration, the grading of the effect of the different materials used for treatment is interesting. No apparent effect with the doubly washed cell detritus, a survival of eighty-four hours of the animal receiving the second aqueous extract, and of one hundred and thirty-two hours of the one receiving the first extract, while the animal receiving the regular mixture of cell detritus and extract lived. The temperature charts show extremely little difference between the "regular" extract and the clear supernatant fluid, and probably the determining amount is represented by the slight protective power shown by the second aqueous extract.

The conclusion is probably warranted that most of the protective and curative bodies are free in the aqueous fluid, the rapidity with which the substances are absorbed, as indicated by their influence on temperature, also supports this view.

Other experiments on the effect of extraction by various methods will be given in a separate paper.

Experiment VI. — March 31, 1908. In this experiment the effects of immune serum and extracts from the leucocytes of immune animals as compared with the extract from leucocytes of normal animals was undertaken. The plan and details of the experiment are shown in the table:

Date.	Animal.	Weight.	Culture.	Hour of Treatment.		Result.	Remarks.
				24-hr.	48-hr.		
1908.							
March 31.	R. 26	1,420	Pn. "Ac" 1 cc.	.....	.....	52 hrs.	Control.
"	R. 27	1,380	" " "	.....	.....	24 "	"
"	R. 28	1,360	" " "	2½ cc.	.....	36 "	Serum from immune Pn. 4.
"	R. 29	1,360	" " "	5 "	.....	36 "	Serum from immune Pn. 4.
"	R. 30	1,340	" " "	2½ "	.....	36 "	Serum from immune Pn. 6.
"	R. 31	1,340	" " "	5 "	5 cc.	60 "	Serum from immune Pn. 6.
"	R. 32	1,300	" " "	1 "	.....	36 "	Extract from immune Pn. 4.
"	R. 33	1,280	" " "	2½ "	.....	46 "	Extract from immune Pn. 4.
"	R. 34	1,275	" " "	1 "	5 cc.	Survived.	Extract from immune Pn. 6.
"	R. 35	1,275	" " "	2½ "	.....	36 hrs.	Extract from immune Pn. 6.
"	R. 36	1,225	" " "	2½ "	5 cc.	Survived.	Extract normal 24-III.
"	R. 37	1,205	" " "	5 "	.....	36 hrs.	" " "

The immune leucocytes were recovered from two rabbits immunized against pneumococci and the immune serum was from these same animals, Pn. 4 and Pn. 6, which were bled to death at the time of taking the leucocytes from the pleural cavities.

Record of immunization of Rabbit **Pn. 4**:

Feb. 4, 1908. One live agar culture of pneumococcus "R" subcutaneously.

" 20, " Five cubic centimeters of twenty-four-hour serum broth culture subcutaneously.

March 5, " Five cubic centimeters of twenty-four-hour serum broth culture subcutaneously.

" 18, " Aleuronat given.

" 19, " Bled to death and exudate obtained.

Record of immunization of Rabbit Pn. 6:

Feb. 8, 1908. Five cubic centimeters of a serum broth culture of pneumococcus "Ac" heated to 60° C. subcutaneously.

Feb.	19, 1908.	Five cubic centimeters of a serum broth culture of "Ac" heated to 60° C. intravenously.
March	6, "	Same dose repeated.
"	16, "	Aleuronat given.
"	17, "	Bled to death and exudate taken.

The animals for the test received intravenous inoculations of one cubic centimeter of a twenty-four-hour serum broth culture of pneumococcus "Ac." The serum tests were favored by being made on the heaviest animals. The immune leucocyte extracts were given to the medium weight and the regular extract to the lightest animals. Treatment at end of twenty-four hours. The controls died in fifty-two and twenty-four hours.

The animals receiving 2.5 cubic centimeters of immune sera, Pn. 4 and Pn. 6, died in thirty-six hours.

Of those receiving five cubic centimeters of the sera the one receiving Pn. 4 died in thirty-six hours, and the one receiving Pn. 6 died in sixty hours, having also been given five cubic centimeters more serum at the end of forty-eight hours.

Of those receiving the extract from immune animals, Pn. 4, the one treated with one cubic centimeter died in thirty-six hours, the one with 2.5 cubic centimeters died in forty-six hours.

The animal receiving one cubic centimeter of Pn. 6 immune extract and then five cubic centimeters more at the end of forty-eight hours survived.

The other animal treated with 2.5 cubic centimeters of Pn. 6 extract died in thirty-six hours, so that it was probably due much to the native resistance of the animal getting the one cubic centimeter that it lived to receive five cubic centimeters more protection at forty-eight hours and then survived.

In the case of the animals receiving normal extract the lightest one, receiving five cubic centimeters at twenty-four hours, died in thirty-six hours. The other one, given 2.5 cubic centimeters at twenty-four hours, lived to receive five cubic centimeters more at forty-eight hours and survived.

The only point cleared up by this experiment is the inefficiency of moderate (really large compared with the extract) doses of immune serum in combating pneumococcus infection as compared with leucocyte extracts.

Little is to be learned from the comparison of the "immune" leucocyte extracts and the normal extracts, especially when it is to be remembered that it is impossible to represent strength by exact quantities. In the case of "immune" extract Pn. 4, the corpuscles may not have been really in an immune state at the stage when the exudate was taken, and, at any rate, the primary doses were small, and the animals did not survive for the forty-eight-hour treatment. In the case of the Pn. 6 extract the result was about the same as that shown by the use of normal extract — fifty per cent of recoveries — *i.e.*, the animals surviving to receive treatment in forty-eight hours.

This and other experiments with leucocytes from immunized animals have made it clear to the writer that it is a matter that will require extensive experimentation to determine the time most suitable for withdrawing leucocytes from an animal after immunizing doses of organisms or bacterial extracts. It is possible that the augmentation of beneficial substances, if they do occur at all in leucocytes, is of a very transient nature.

Experiment VII. — May 19, 1908. This experiment was undertaken with two objects in view —

(*a.*) To determine whether living leucocytes taken perfectly fresh from one animal (simply being centrifugalized and emulsified in normal salt solution) and introduced subcutaneously or intraperitoneally into an infected animal, had any influence on the course of the systemic infection, and

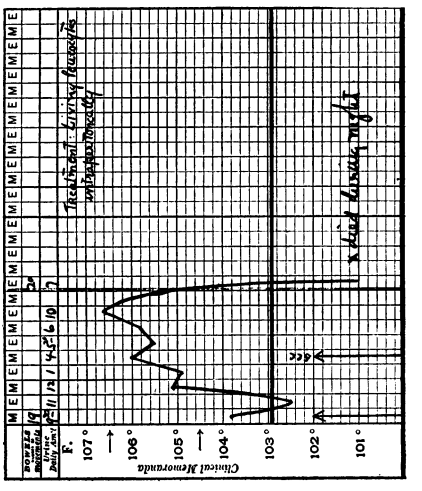
(*b.*) What effect heating at 60° C. for one hour had on the curative influences of leucocyte extract.

This experiment is also of interest as a different culture of pneumococcus from those employed in the former experiments was used. The animals received, intravenously, one

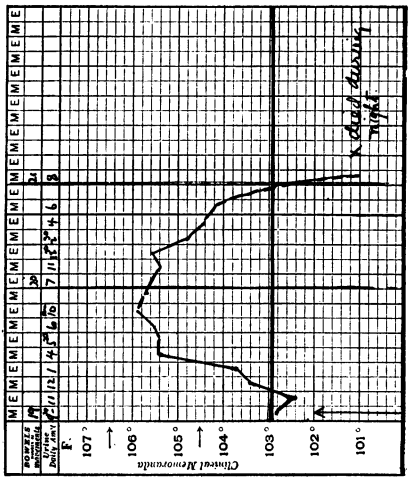
cubic centimeter of a twenty-four-hour broth culture of pneumococcus "$L_2 R_1$." Thus:

	Treatment.	Result.
R. 62. 1,360 grams.	.....	Died in 36 hours.
R. 63. 1,345 "	.....	" " 36 "
R. 64. 1,310 "	5 cc. cell emulsion subcutaneously.	" " 20 "
R. 65. 1,300 "	5 cc. cell emulsion intraperitoneally.	" " 20 "
R. 68. 1,260 "	5 cc. normal extract heated to 60°–63° C. subcutaneously.	" " 30 "
R. 69. 1,195 "	5 cc. normal extract subcutaneously.	" " 28 "
R. 70. 1,170 "	5 cc. normal extract heated to 60°–63° C. intraperitoneally.	" " 50 "
R. 73. 1,130 "	5 cc. normal extract intraperitoneally.	Survived.

The infection was evidently a severe one. Only one animal of the series survived, the one receiving normal leucocyte extract intraperitoneally. In this animal the drop in temperature following injection of extract was remarkable — four degrees in less than one hour (from 104.7° to 100.4° F.). In the animal receiving heated extract intraperitoneally there was also a drop of two degrees, showing that the heating had not entirely destroyed its activity (see charts), although it was undoubtedly weakened. The same extract was, of course, used for the heated and unheated test.

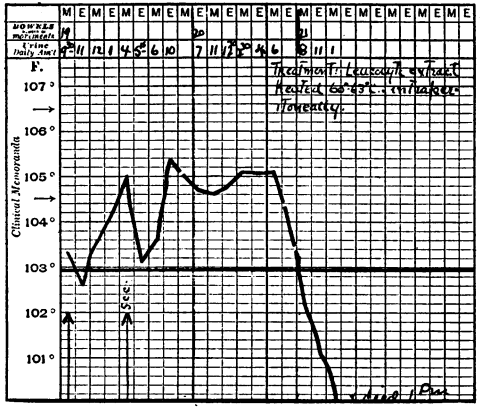


Pneumococcus. Exp. VII. Temperature chart of rabbit receiving living leucocytes intraperitoneally.



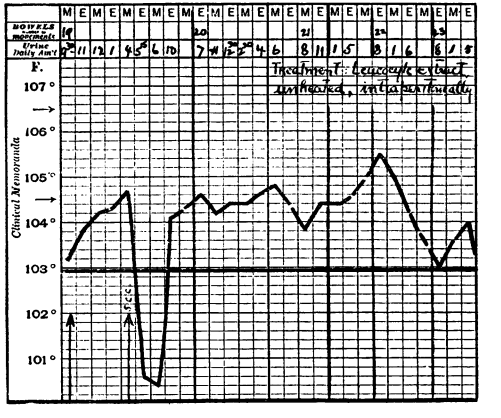
Pneumococcus. Exp. VII. Temperature chart of untreated control rabbit. Inoculation intravenously.

May 19, '08. R.70. 1,170 gm. Pneumococcus "L2R1."



Pneumococcus. Exp. VII. Temperature chart of rabbit receiving treatment, intraperitoneally, with leucocyte extract heated to 60°–63° C.

May 19, '08. R.71. 1,130 gm. Pneumococcus "L2R1."



Pneumococcus. Exp. VII. Temperature chart of rabbit receiving treatment, intraperitoneally, with regular unheated leucocyte extract. Animal survived.

The living leucocytes emulsified in normal salt had absolutely no effect, as administered, on the temperature or course of the disease — the animals dying even before the controls (see chart) — and this tends to support our supposition that even if they were active in absorbing toxins and giving up necessary substances to the plasma their action is too slow to be of avail unless they were introduced intravenously — a procedure certainly not to be thought of in the treatment of man — or were used in local infections such as occur in the pleural or peritoneal cavities or, possibly, in the subdural spaces.

Experiment VIII. — April 8, 1908. Up to the time of this experiment no definite determination of the degree of virulence of the culture "Ac" used in most of the tests had been made. No control had, however, survived a dose of one cubic centimeter of a twenty-four-hour serum broth culture given intravenously.

As is so well known, differences in weight of animals have so much influence on the outcome and course of infection that the exact virulence is hard to determine, and, further, in all experiments a certain percentage of deaths are undoubtedly due to preëxisting but not obvious conditions of the animals and should justly be allowed for in all series of tests. In other words, if all animals were in equally good and normal condition a much higher percentage of recoveries could certainly be counted on in the use of any beneficial therapeutic measure.

This present test was, therefore, made to get some idea of the approximate virulence of the organism used for most of our infections. The animals used were a little above the usual weight and the dose given intravenously was graded from .5 cubic centimeter to .01 cubic centimeter of a twenty-four-hour serum broth culture of "Ac." Thus:

Weight.	Dose.	Result.
1,525 grams.	1/2 cc.	Died in 7 days.
1,520 "	1/4 "	" " 7 "
1,480 "	1/10 "	" " 8 "
1,450 "	1/20 "	" " 4½ "
1,300 "	1/40 "	Survived, normal on 8th day.
1,300 "	1/100 "	" " " 5th "

It was probable that our animals in previous tests had been saved from at least twenty times the fatal dose, and possibly more, as the culture had been cultivated solely on artificial media during the full time of experimentation and may have been more virulent at the beginning.

EXPERIMENTS ON PNEUMOCOCCIC INFECTIONS WITH EXTRACTS FROM LEUCOCYTES OF THE NORMAL DOG.

Only one experiment on this phase of our work will be given here.

On April 13, 1908, six rabbits, weighing one thousand six hundred and twenty, one thousand six hundred and ten, one thousand five hundred and sixty, one thousand five hundred and fifty, one thousand five hundred and fifty, one thousand five hundred and thirty grams respectively, were given 1.5 cubic centimeters of a twenty-four-hour serum broth culture of pneumococcus "Ac" intravenously. The two heaviest animals were, as usual, held as controls. The other animals were treated subcutaneously with a freshly prepared and strong aqueous extract of leucocytes from a healthy, normal dog. Two animals, one thousand five hundred and fifty grams and one thousand five hundred and thirty grams, were given five cubic centimeters after five hours, and a slight fall in temperature was noted in each instance. The remaining one-thousand-five-hundred-and-fifty-gram animal and the one-thousand-five-hundred-and-sixty-gram animal received

five cubic centimeters of extract after twenty-four hours with practically no effect on temperature. No further treatment was given.

Both of the control animals survived, possibly on account of their greater weight — only fifty grams, however, in one instance, and the heaviest control being only ninety grams heavier than the lightest treated — while all but one of the treated animals died. Of the two animals treated after five hours, one died in ninety-eight hours, the lighter one in forty-eight hours. Of the animals treated in twenty-four hours, the heaviest one died in thirty-six hours, the other one survived, one might almost say, in spite of the treatment. It is, in fact, difficult to judge of the value or none-value of dog leucocyte extracts from the experiment. It seems, however, that they had no favorable effect on the infection in rabbits as the control animals (only very little heavier), although distinctly sick, survived the dose with apparent ease.

On the other hand, from the experiment one might even be justified in concluding that their effect was harmful.

From the writer's experience in this and other attempts to determine the value of dog leucocytes in treating various infections in rabbits he feels justified in assuming that they are certainly by no means as efficient as those of rabbits. No experiments, however, have been tried on dogs with their own leucocyte extracts, and up to the present the writer has not felt justified in attempting their use in man.

SUMMARY OF RESULTS OF PNEUMOCOCCUS INFECTION EXPERIMENTS.

If, in the series of experiments on pneumococcus infections detailed in the foregoing pages, we consider the animals treated with the extract of leucocytes of normal rabbits, we find that in such animals an infection, surely fatal in untreated rabbits, becomes significantly modified in such treated animals even if this treatment be delayed many hours. Thus, out of eight control animals used in four experiments in which the infecting dose was the same, all died, averaging only forty-five hours of life after being infected. Of the animals treated

— some as late as twenty-four hours after infection — nine out of twelve survived the infection; three died with an average life of sixty hours after infection, two of them not having received treatment until the expiration of twenty-four hours.

When the infecting dose was double the one just mentioned, the controls (2) averaged only twenty-five hours, while one animal out of four of the treated survived, but the three dying averaged one hundred and one hours of life after being infected. These are not selected examples, but are records of events as they developed in our regular research tests, and have been fully confirmed by experiments undertaken in elucidation of other points, and are unmistakably indicative of the powerful beneficial action of such extracts on pneumococcus septicemia in rabbits.*

On the other hand, living leucocytes, introduced subcutaneously, or even peritoneally, have little or no effect on systemic infections.

5. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON STREPTOCOCCUS INFECTIONS IN RABBITS.

Many strains of streptococci, no matter what their source, whether from slight or severe infections in man, are primarily not very virulent for rabbits, even when administered in large amounts. This is one of the distinguishing marks of the streptococci as a class as compared with pneumococci, which organisms, as a rule, no matter what their source, whether recovered while leading a presumably harmless parasitic life in the mouth of man, or from a patient suffering from a severe pneumonia or fatal septicemia, are quite regularly fatal to rabbits when administered in fairly small amounts.

When streptococci are primarily virulent for rabbits or become so after repeated passages through these animals,

* The results of the treatment of pneumonia in man by such extracts are given in another article in this number of the Journal.

the character of the resulting infection is, however, seldom to be distinguished from that given rise to by pneumococci, and animal experiments are considered of minor import in distinguishing one of these organisms from the other.

The writer has maintained, since his first studies on the physiology of pneumococci and streptococci, that there were certain distinct differences in the fermentative abilities of these two organisms, which were sufficient for purposes of identification, *i.e.*, that pneumococci fermented inulin while streptococci did not. The fact that pneumococci may at times have not enough kinetic fermentative energy to permit of immediate identification by this method has not seemed worthy of discussion, for examples of suppression of function are too numerous for the ordinarily well-informed biologist to consider such a phenomenon greatly worthy of note. On the other hand, the actual assumption of fundamentally new functions, however, is so rare that the report of such occurrences, not based on the most careful studies or investigation, may well be accepted with extreme caution, especially when they contradict a long series of previous observations. The statement, therefore, that certain strains of Streptococcus pyogenes may ferment inulin is so contradictory of the writer's personal observation through years that he again asserts his belief that conclusions to this effect are more than likely based on insufficient data, and that the organisms thus described are much more likely aberrant types of the inulin fermenting species than members of the non-inulin fermenting species that have assumed this character. The question is here referred to on account of the absolute necessity, which became apparent while carrying on the following experiments, of knowing the species to which an organism belongs before attempting to draw conclusions from animal experiments which may be carried on with it, and because the experiments here detailed indicate certain marked differences between pneumococci and, at least, certain streptococci, which may aid us in solving the question of identity.

The experiments on the influence of leucocyte extracts on streptococcus infections have, therefore, a double interest for us, on the one hand the fundamental one, whether or not they change favorably the course of the disease, and on the other hand whether the result is exactly comparable to that found to obtain in pneumococcus infections.

The culture used in the experiments was isolated from the throat of a scarlet fever patient. It was at first not virulent for rabbits; four cubic centimeters of a twenty-four-hour serum broth culture gave rise to a high temperature, but was not fatal to an eight-hundred-and-eighty-gram rabbit. This rabbit having apparently recovered and its temperature being normal, on the third day, an intraperitoneal dose of five cubic centimeters was given and the animal chloroformed after twenty-four hours. Four and one-half cubic centimeters of a serum broth culture of the organism received from the peritoneum of this animal were given intravenously to a seven-hundred-gram rabbit, which died from it in eighteen hours with organisms in its blood.

Two cubic centimeters of a culture from the heart's blood of this animal were given to a third, which died in twenty hours, with organisms in the blood. The organism from this animal was then cultivated on serum broth and used for the tests. Inulin fermentation tests and stains for capsule gave negative results, and all characters confirmed our opinion that the organism was a true streptococcus.

Experiment I. — March 23, 1908. At 10.30 A.M. six rabbits, weighing one thousand one hundred, one thousand seventy-five, one thousand ten, nine hundred and ninety, nine hundred and sixty, and nine hundred and thirty grams, were given intravenously one cubic centimeter of a twenty-four-hour serum broth culture of the streptococcus.

The temperature of all rose steadily and consistently to 106° F. and tended to remain high unless interfered with by the injection of leucocyte extract, the maximum depression occurring after each injection of the extract at the end of about two hours. Two of the animals, the nine hundred and ninety grams and the nine hundred and thirty grams, received treatment after five hours. The temperature of the nine hundred and ninety grams did not fall within two hours (6 P.M.), but was at 104.2° the following morning. The temperature of the nine hundred and

thirty grams fell a degree and a half by 6 P.M. (two hours), but was at 105.8° F. the following morning.

The two remaining rabbits, one thousand ten grams and nine hundred and sixty grams, were given five cubic centimeters of extract subcutaneously at the end of twenty-four hours. The temperature of the one-thousand-ten-gram animal fell within two hours from 106.2° to 103°, but rose again to 106.6° by 4 P.M. The temperature of the nine-hundred-and-sixty-gram animal fell from 104.8° to 102.8° within two hours, and then rose steadily to 106°. The subsequent course of the disease and its treatment may best be followed on the charts. Attention is directed to the marked influence of the extract on the temperature.

The controls died, one in seventy hours (one thousand one hundred grams) and one in thirty-six hours (one thousand seventy-five grams). There were no marked lesions, but the spleen of the one dying in seventy hours was enlarged. Organisms were present in the blood of each and the picture was one of septicemia.

Of the animals treated in five hours, but with no subsequent treatment, one survived (nine hundred and thirty grams), and one (nine hundred and ninety grams) died in thirteen and one-half days.

During the course of the disease both of these animals developed involvement of the joints, especially the joints of the front paws; in the case of the animal which recovered, distinct abscesses formed at the joints of the front paws and at one joint of a hind leg.

At autopsy of the animal dying in thirteen and one-half days there was great emaciation but no gross internal lesions; organisms, however, were recovered from the heart's blood. There was still involvement of the joints at death.

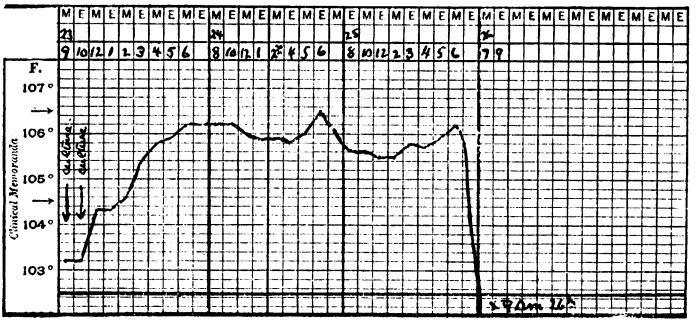
Both of the animals treated after twenty-four hours died, one (one thousand ten grams) in one hundred and eight hours, and one in five and one-half days. The latter animal was autopsied. There was bloody fluid in the peritoneum, the lungs were congested, and there was some fluid in the pleural cavities. The spleen was very large. Organisms were recovered from the blood.

The results are as follows:

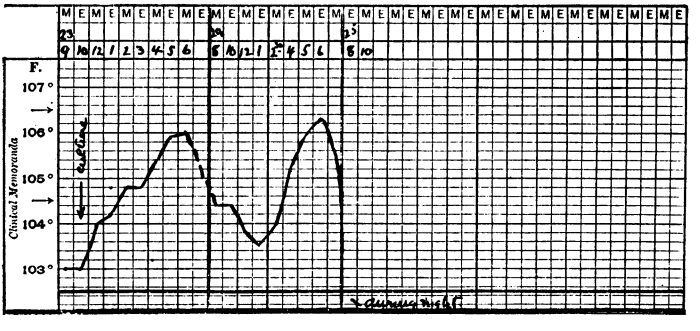
R. 1.	1,100 grams.	Died in 70 hours.	Controls.
R. 2.	1,075 "	" " 36 "	
R. 3.	1,010 "	" " 108 "	Treated, 24 hours.
R. 5.	960 "	" " 5½ days.	
R. 4.	990 "	" " 13½ "	Treated, 5 "
R. 6.	930 "	Survived.	

Such a result leaves little doubt of the beneficial action of the leucocyte extract on the streptococcus infection in rabbits, and this is seen not only in the prolongation of the life of the treated animals but also in the marked effect upon the temperature. (See charts.)

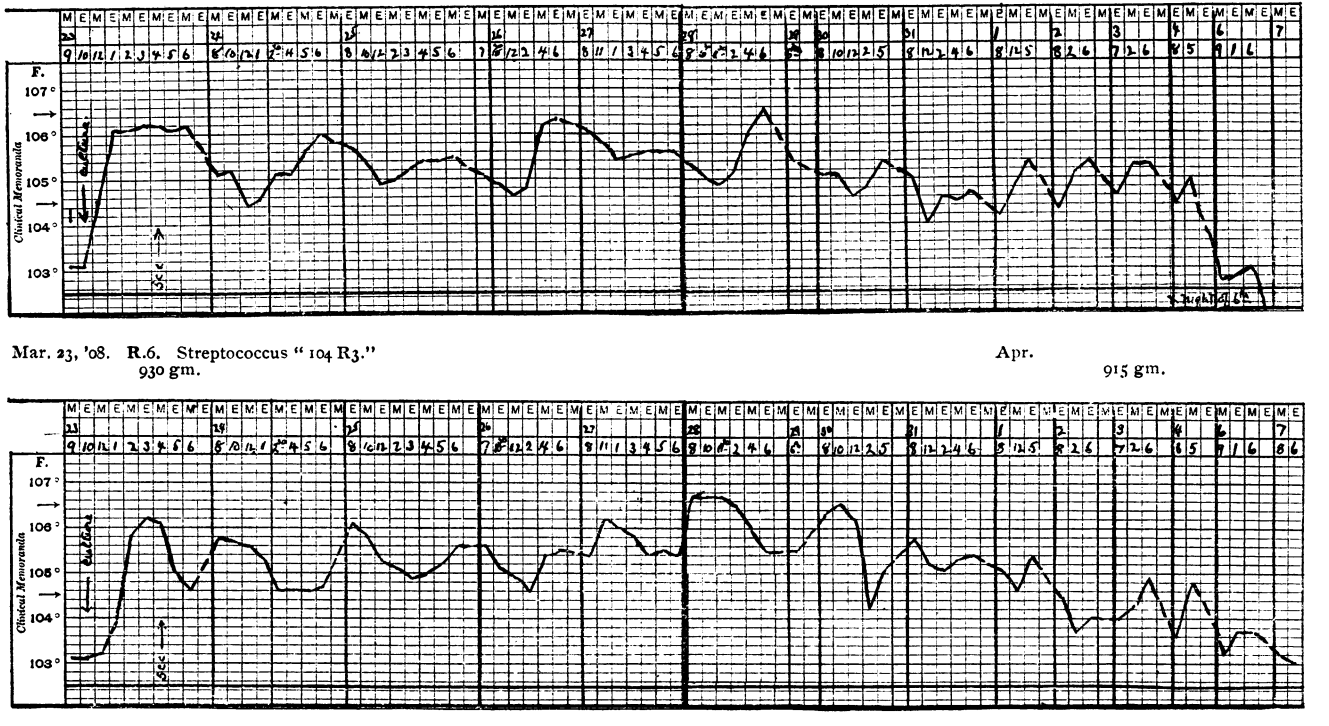
Mar. 23, '0S. R.1, 1,100 gm. Streptococcus "104 R3."



Mar. 23, '0S. R.2. 1,075 gm. Streptococcus "104 R3."

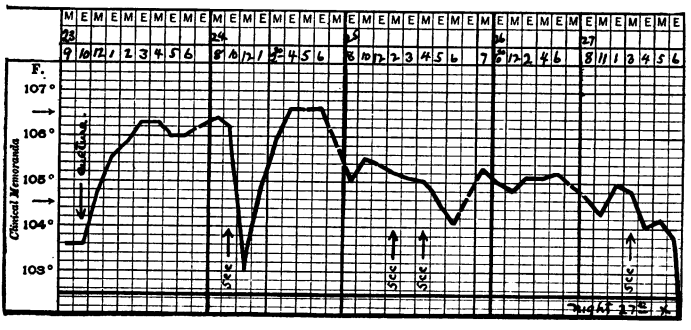


Streptococcus. Exp. I. Temperature charts of untreated rabbits, receiving inoculation intravenously.

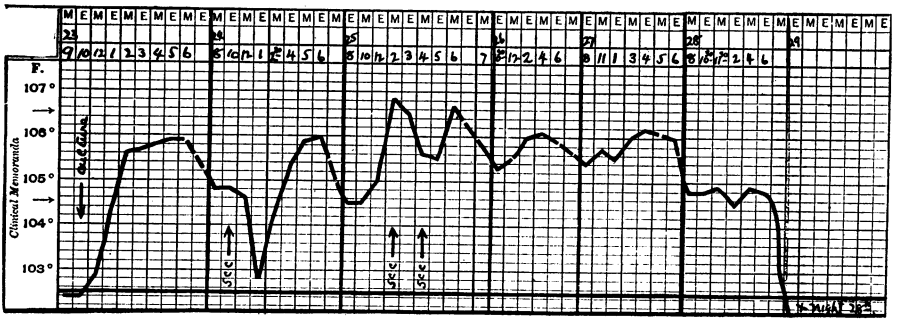


Streptococcus. Exp. I. Temperature charts of rabbits treated five hours after inoculation. R.6 survived, R.4 died.

Mar. 23, '08. R.3. 1,010 gm. Streptococcus "104 R3."



Mar. 23, '08. R.5. 960 gm. Streptococcus "104 R3."



Streptococcus. Exp. I. Temperature charts of rabbits treated twenty-four hours after inoculation. Note marked drop in temperature. Both animals eventually died.

Experiment II. — April 6, 1908. 11 A.M. Six fair-sized rabbits were given intravenously 1.5 cubic centimeters of a twenty-four-hour serum broth culture of the same streptococcus used in Experiment I. The results may best be seen by reference to the accompanying table:

R. 7. 1,455 grams.	Died in 30 hours.	Controls.
R. 8. 1,455 "	" " 45 "	
R. 9. 1,435 "	" " 9 1/4 days.	Treated in 6 hours; 5 cc. subcutaneously.
R. 10. 1,435 "	" " 60 hours.	
R. 11. 1,435 "	" " 7 days.	Treated in 6 hours; 10 cc. subcutaneously.
R. 12. 1,435 "	" " 36 hours.	

The results of this experiment confirm those of Experiment I. The dose of infecting organisms was evidently very severe, as was evidenced by a moderate primary rise in temperature followed by a distinct fall, in all cases, to normal or below. This was followed by rallying and rise of temperature to 105° or 106° F. The animals receiving treatment showed another fall in temperature by the end of two hours after injection of the leucocyte extract. The fact that the animals receiving ten cubic centimeters did not recover, or even outlive those receiving only five, may indicate that the primary poisoning was too severe in its effects to admit of recovery, or that in spite of the greater amount of extract that the animals received they lacked a sufficient amount of some protective element not furnished in the extract — suffered, for instance, from a paucity of or interrupted production of some interbody (amboceptor) necessary for the inhibition or destruction of the invading organisms. Further experiments may show that the deficient body can be furnished by immune serum.

In both of the animals surviving until the third day joint involvement manifested itself.

The regularity with which these articular or peri-articular involvements occurred with this special organism indicates that a difference may exist in the character of the infections

caused by streptococci and those caused by pneumococci. In our many observations on pneumococcus infections, both in treated and untreated animals, I have never observed this tendency to local manifestation. If further observations on other carefully identified strains of Streptococcus pyogenes show this to be a constant occurrence, it must certainly indicate a fundamental physiological difference and may aid us in definitely establishing the identity of certain of the organisms which have been reported as varying in cultural and fermentative characters from their type, and assuming characters typical of pneumococci.

Comparing these streptococcus experiments with those made with staphylococci we find that the streptococci although localizing at certain points — not unlikely points susceptible to injury from damp cages and constantly maintained cramped positions of sick animals — do not tend, so far as our experiments go, to give rise to the multiple abscesses which constitute the lesions of pyemia, so frequent a result of staphylococcus infection.

6. INFLUENCE OF EXTRACTS OF LEUCOCYTES FROM NORMAL RABBITS ON MENINGOCOCCUS INFECTIONS IN RABBITS.

The favorable impression, which the results of the writer's experiments on the effect of leucocyte extracts on staphylococcus infections and on the course of typhoid infections in animals had made upon him, led him as early as April, 1907, to attempt the treatment of another disease — acute cerebrospinal meningitis — and this time in man, on what was largely theoretical grounds.

A boy of fifteen had been admitted to Roosevelt Hospital suffering from cerebro-spinal meningitis due to the meningococcus. On April twenty-third all hope of his recovery had been given up by the attending physician and house staff, and the writer requested permission to give him an injection. Only small primary subcutaneous doses were given, as this

was our first attempt to administer leucocyte extract in man. It was found that no bad effects followed its administration and that the extract was very rapidly absorbed. The history of this case is given in a following clinical paper, but our observations were so encouraging and the result of this treatment, on the profound intoxication from which the boy was suffering, was apparently so marked that we were encouraged, not only to treat other cases of the infection, but to thoroughly investigate the influence of extract on the course of this infection in animals.

After several tentative experiments which convinced us that leucocyte extracts influenced meningococcus infections, or rather poisoning, in animals, more thorough experiments were begun in the summer of 1907.

Rabbits suffer a marked intoxication, if not, indeed, in some instances, a true infection, when given sufficient quantities of almost any race of meningococcus intravenously. During the epidemic of cerebro-spinal meningitis occurring in New York several years ago the writer proved this to his satisfaction many times, so that for the purpose of the following experiments he had no hesitation in selecting rabbits as the test animals. The only care necessary was to have the organisms in proper condition to be administered intravenously. Agar cultures were used and, after being as thoroughly emulsified in salt solution as possible, they were always filtered through thin layers of absorbent cotton to remove the larger masses. Any chance of the organism masses serving as emboli was thus largely eliminated. The dose necessary for satisfactory results was often as large as four or five agar cultures.

Experiment I. — Aug. 9, 1907. At 11.15 A.M. three rabbits, weighing seven hundred and seventy-five, seven hundred and thirty, and seven hundred and twenty-five grams respectively, were given a full agar culture of meningococcus "James" intravenously. The temperature of the control, seven hundred and seventy-five grams, rose abruptly to 107.4° F. by two o'clock, and the animal died during the night.

The seven-hundred-and-thirty-gram rabbit was given two cubic centimeters of extract subcutaneously at 12.15 o'clock, *i.e.*, one hour after infection. At 4 P.M. its temperature reached its maximum — 105° — and

then fell to 104.5 F. by 5 P.M. The following morning the animal was in good condition and eating a little; temperature practically normal — 103° F. This animal survived.

The seven-hundred-and-twenty-five-gram rabbit was given two cubic centimeters of extract at 2.15 P.M., *i.e.*, after three hours. Its temperature reached its maximum at 3 P.M. — 106°, and fell to 104° by five o'clock, another two cubic centimeters of extract having been administered at 4.15 P.M. The temperature remained around 105° F. on the following day, and in spite of subsequent injections the animal died at the end of four days. The notes on this experiment indicate an intercurrent infection of "snuffles;" meningococci were, however, recovered from the heart's blood and probably should be looked upon as the cause of death. The undoubted presence of meningococci in the blood of the animal after four days is very unusual and indicates that possibly all the effects noted after such inoculation are not always to be referred to intoxication from endo-toxins alone.

We have shown to us in this experiment, as in the case of staphylococcus, typhoid, and pneumococcus infections, apparently at least, an influence on the course of the infection or poisoning by the leucocyte extract.

Experiment II. — Oct. 25, 1907. In this experiment four rabbits were each given intravenously four full agar cultures of meningococcus "Margie." The two treated rabbits received a dose of five cubic centimeters of extract subcutaneously about five minutes before being given the culture intravenously. This was not really a prophylactic experiment as little absorption could take place in so short a time.

The animals weighed one thousand four hundred and thirty-five, one thousand four hundred and twenty, one thousand three hundred and fifty-five, and one thousand three hundred and five grams and were inoculated at ten o'clock. The one-thousand-four-hundred-and-thirty-five-gram animal — one of the controls — had bad diarrhea by eleven o'clock, its temperature fell from the beginning, the animal dying with a temperature of 94° at 2 P.M., *i.e.*, in four hours. The other one-thousand-four-hundred-and-twenty-gram control had bad diarrhea within an hour and a falling temperature which reached 99.6° F. by 11.30 A.M., and then began to ascend. The following morning its temperature was 106° F., but on the next day the temperature fell rapidly and the animal died with a low temperature at the end of fifty-three hours.

Neither of the treated animals showed a fall of over one and a half degrees, and the temperature then ascended slowly to about 105° F. by evening. The animals did not have diarrhea. Both of these animals survived, although they were evidently poisoned by their infection and took several days to reach a normal condition. By November first they were

normal in every way. On the twenty-eighth, it is to be noted, they were each given five cubic centimeters of extract subcutaneously, although it was not apparent that this was necessary to save their lives.

This experiment shows, even better than Experiment I., the beneficial effect of treatment with leucocyte extract.

Experiment III. — Oct. 31, 1907. 12.30 P.M. Three animals, weighing one thousand three hundred and eighty, one thousand three hundred and seventy, and one thousand two hundred and fifty grams, were each given intravenously four full agar cultures of meningococcus "Margie." The control weighed one thousand three hundred and eighty grams. Its temperature rose steadily to 106° F. by 6 o'clock, and during the next day ranged higher, 107.2° at mid-day. The animal died during the second night — thirty-six hours.

The one-thousand-three-hundred-and-seventy-gram animal showed a drop of about one degree after infection and had diarrhea; as its temperature rose again during the afternoon to 106° it was decided to defer treatment until the day following. The animal's temperature was still high the next morning, and at 10 A.M. five cubic centimeters of extract were given, the temperature remitting about one-half degree and then ascending a little again. The animal was distinctly sick and somnolent. The day following (November 2) its temperature had fallen to 103.7° and from then on to normal, the animal recovering its spirits and appetite and remaining well.

The one-thousand-two-hundred-and-fifty-gram rabbit showed, after infection, an immediate rise of one degree in temperature. From then on the temperature fell so that, at the expiration of four hours, five cubic centimeters of extract were given subcutaneously. The animal was very ill; the temperature after the extract injection still fell, and on the following morning was at 99° F. at 8 o'clock, the animal being very ill and having had diarrhea during the night. Extract was given at nine o'clock and the temperature rose steadily to 104° F., where it remained during the day. The animal was much improved on November second, and from then on its temperature was practically normal and the animal remained well.

This experiment is a further confirmation of the impression gained from the preceding experiments that leucocyte extracts have a distinctly beneficial effect on meningococcus infections; the treatment of one animal having even been deferred to twenty-four hours in an infection proving fatal in thirty-six hours in a heavier control. The fact that the other animal with a continuously falling temperature, which is

usually indicative of profound poisoning and impending death, lived proves conclusively to the writer the marked contribution of the leucocyte extract to the result. The experiments were, in fact, so conclusive and so confirmative of results obtained in man that a simple repetition of them was considered unnecessary.

Other experiments were, however, undertaken with other objects in view, which further confirm the results already obtained.

The two following experiments may be given together, as the object of them was practically the same:

COMPARISON OF CURATIVE EFFECT OF IMMUNE SERUM, IMMUNE LEUCOCYTE EXTRACT AND NORMAL LEUCOCYTE EXTRACT ON MENINGOCOCCUS INFECTION IN RABBITS.

Experiments IV. and V. — November 14 and 20, 1907. Nineteen rabbits were used in these two experiments. Five of them served as controls and the infecting dose was the same in both experiments — three and one-half full agar cultures of meningococcus "Margie" given intravenously.

In Experiment IV. the treatment was commenced in five hours, in Experiment V. in twenty-one hours. The plan of Experiment V. required the use of so many animals that, two of the heaviest animals having died before the lapse of twenty-one hours, this was considered more than sufficient indication of the markedly lethal character of the dose, and the remainder were given treatment (Experiment of Nov. 20, 1907).

In the other experiment (IV., November 14) three animals served as controls, a light one dying in five hours (*i.e.*, before it was planned to commence treatment), one in twenty-one hours, and a large animal, nearly four hundred grams heavier than the next in weight of the series, died in thirteen days, so that there is little probability that any of the other animals would have survived had they not been treated.

The scheme and results of these two experiments may be easily seen in the appended table:

	Hour of Treatment.				Result.	Remarks.
	5-hr.	21-hr.	24-hr.	72-hr.		
Controls:						
Nov. 14. R. 11, 1,640.	.....	.....	.....	.....	13 days.	100% deaths.
" " R. 12, 1,275.	.....	.....	.....	.....	21 hours.	
" " R. 18, 1,085.	.....	.....	.....	.....	5 "	
" 20. R. 21, 1,375.	.....	.....	.....	.....	18 "	
" " R. 23, 1,285.	.....	.....	.....	.....	6 "	
Animals treated with normal extract:						
Nov. 14. R. 14, 1,240.	2½ cc.	.....	2½ cc.	.....	Survived.	50% recoveries.
" " R. 16, 1,170.	5 cc.	.....	.....	.....	18 hours.	
" 20. R. 24, 1,260.	.....	5 cc.	.....	.....	Survived.	
" " R. 27, 1,187.	.....	5 cc.	.....	.....	8½ days.	
Animals treated with immune extract:						
Nov. 14. R. 20, 1,040.	5 cc.	.....	.....	.....	Survived.	66⅔% recoveries.
" 20. R. 25, 1,255.	.....	5 cc.	.....	.....	Survived.	
" " R. 28, 1,165.	.....	5 cc.	.. ..	5 cc.	9½ days.	
Animals treated with immune serum:						
Nov. 14. R. 13, 1,255.	2½ cc.	.....	2½ cc.	.....	70 hours.	20% recoveries.
" " R. 15, 1,220.	5 cc.	.....	.....	.....	Survived.	
" 20. R. 22, 1,320.	.....	5 cc.	.....	.....	36 hours.	
" " R. 26, 1,190.	.....	5 cc.	.....	.....	70 hours.	
" " R. 29, 1,032.	.....	5 cc.	....	5 cc.	8½ days.	
Animals treated with mmune serum and extract:						
Nov. 14. R. 17, 1,110.	2½ cc. extract. 2½ cc. serum.			.....	18 hours.	Immune serum and normal extract.
" " R. 19, 1,045.	2½ cc. extract. 2½ cc. serum.			.....	84 hours.	Immune serum and immune extract.

The rabbit furnishing the immune leucocyte extract was the same from which the immune serum was obtained —

Record of immunization of rabbit "9"

Sept. 25, 1907.	1 agar culture meningococcus "Margie" intravenously.	
" 30, "	1 " " " " "	
Oct. 5, "	2 " " " " "	
" 11, "	3 " " " " "	
" 21, "	5 " " " " "	
Nov. 6, "	Aleuronat given.	
" 7, "	Bled to death and exudate taken,	

so that the result of this comparison is very interesting. The test was an unusually severe one in both experiments. No control, with the exception of the very heavy one of November fourteenth, which should not be taken into account, lived over twenty-one hours. In the animals receiving normal leucocyte extract we have fifty per cent of recoveries in each experiment; and one out of the two animals which died lived for nearly eight and one-half days. In the case of the animals receiving immune leucocyte extract we have the only animal so treated after five hours living, and fifty per cent of those treated after twenty-one hours, thus giving sixty-six and one-third per cent recoveries, while the only animal of this series which died survived nine and one-half days.

Of the animals receiving immune serum fifty per cent of those treated in five hours died, while one hundred per cent of those treated after twenty-one hours died, although one survived eight and one-half days after having received further treatment at the end of seventy-two hours. The percentage of recoveries was only twenty per cent. The amounts of serum used must certainly be considered large and the animal furnishing it was fairly highly immunized.

The most interesting point in the experiment is the apparent, if not real, superiority of immune leucocyte extract over normal. This of course may possibly be simply a matter of concentration, for as before remarked there is no way at present of standardizing these extracts, but results obtained by use of the immune extracts in man make us feel that in the case of meningococcus immune extracts, at least, this difference is a real one.

No conclusion can be drawn from the experiments in which

immune serum and extracts were given to the same animal. The administration of the serum and extract was simultaneous, but into different places in the animals, so as to avoid any possible diversion of complement, the serum undoubtedly being absorbed more slowly than the extract.

Not enough of these tests were made to determine whether the administration of immune serum with the extract was in any way beneficial, but that it was not markedly so is evident from the comparatively early death of the two animals —the amounts given, however, were small and the animals very ill.

Some of the animals in the experiments showed hemorrhagic spots in the ears similar to those noted in rabbits suffering from typhoid infections.

SUMMARY OF EXPERIMENTS WITH MENINGOCOCCUS INFECTIONS.

If we analyze briefly the results of our experiments on rabbits infected with meningococcus we find the following:

In every experiment the controls died.

The total number of control animals used in the experiments, in which the treated animals received normal leucocyte or immune leucocyte extract, was nine. In one of the experiments one of the animals was greatly over weight and should not have been used. The animal died, however, in thirteen days. Leaving this animal out of account, we have eight controls averaging one thousand two hundred and fifty-four grams, with an average life of twenty hours after infection.

Of the treated animals there were thirteen. Nine of these recovered and four died, over seventy per cent of recoveries.

The average weight of the nine animals which recovered was one thousand two hundred grams, and of the four which died one thousand sixty-two grams, with an average life after infection of 5.7 days.

The majority of the animals did not receive treatment until the expiration of five hours after inoculation, and a number

of them not until twenty-one to twenty-four hours, some of the controls having at times died before these animals were treated with leucocyte extract. Severer tests could hardly be devised, and when results of such tests are compared with those obtained with the use of serum, they point strongly to the value of leucocyte extracts in the treatment of this infection.

GENERAL SUMMARY AND DISCUSSION.

In the earlier sections of this paper certain facts and hypotheses of immunity were discussed, and reasons were advanced in support of the idea that leucocytes play a dominant part in the protection of the animal economy; a part, which in many infections, especially those in which poisoning is supposed to depend upon endotoxins, necessitates the direct intervention of the leucocytes themselves between the invading microörganisms and their poisons and the more highly specialized cells of the animal.

It was assumed that there were two classes of bodies active in protection and immunity, one class, such as the antitoxins, the bacteriolysins (amboceptor), agglutinins, etc., which are readily overproduced and given off or secreted by the cells producing them, and are thus present free in the blood stream and equally available for the protection of all cells of the body; and another class of bodies serving directly for the protection of the individual cells possessing them, and only indirectly, through the intervention of these cells, for the protection of the other cells of the animal economy, but not as a customary thing given off into the blood stream during the life of the cells possessing them.

From this assumption it was argued that these substances — digestive, poison-neutralizing and complementary — might possibly be liberated from leucocytes by methods of extraction, and introduced in this free condition into infected animals or man, and that they might thus act not only in protecting the flagging leucocytes, and permitting them to recuperate, but also as a shield for the more specialized cells.

This hypothesis was put to test in experiments with the inciting organisms of some of our most prevalent infections, and the results have apparently justified our expectations.

Animals suffering from severe septicemias and poisonings, following intravenous injections of such organisms as staphylococci, streptococci, pneumococci, typhoid bacilli, and meningococci,* have shown the beneficial effect of treatments with extracts of leucocytes, and have, in many instances, survived infections fatal to the control animals in thirty-six hours, even when treatment has been delayed as late as twenty-four hours.

The action of the extracted substances is evidenced in many instances by a marked fall in temperature, and by a conservation or rapid return to normal of the animal's weight.

Animals receiving treatment with the extracts often appear, however, for a time, much sicker than the controls. This is especially true of typhoid and meningococcus infected rabbits and, to a certain extent, even of pneumococcus infected animals.

What the exact action of the extracted substance is, is, of course, at present largely a matter of conjecture. The fact that treated animals in some instances appear more intoxicated than the untreated may indicate an enhanced bacteriolytic action and liberation of endotoxins, thus suggesting the presence in the extracts of complementing bodies, or of digestive bodies peculiar to the leucocytes. These bodies may, of course, be present and play an active part, but the strongest impression given to one carefully following the experiments and noting the immediate effect on temperature and the conservation and quick return to normal weight of the treated animals is that the principal substance at work is one active in neutralizing poisons, and thus able to relieve the animal economy and give the phagocyting cells an

* The report of our investigations on tuberculosis and dysentery and cholera infections in animals will soon be ready for publication. It may be said here, however, that infections due to the various strains of the dysentery bacilli and to the Spirillum of Asiatic cholera are markedly influenced by leucocyte extracts.

opportunity to carry on their work of ingesting the micro-organisms and thus permanently rendering them harmless.

Freshly obtained living leucocytes, when introduced into an infected animal, even intraperitoneally, are practically without effect on systemic infections. The lives of the animals are not lengthened and these intact leucocytes seem to have no influence on the temperatures.

Intravenous injections of living leucocytes have not been tried, since the results of such a procedure are of purely academic interest, being entirely outside the realm of possibility in the treatment of human infections. The use of living leucocytes in the treatment of local infections, such as those of the pleura or peritoneum, or even subdural infections, is of course possible, but of limited application, both theoretically and practically, and their beneficial action would probably be due to the simple regeneration of the phagocyte army, or to extracts which, unsuspectedly, accompanied the supposedly intact leucocytes introduced.

CONCLUSIONS.

Extracts of leucocytes from normal rabbits have a distinct modifying and curative action when given subcutaneously or intraperitoneally to rabbits, even on systemic infections which are rapidly fatal in untreated rabbits.

Rabbit leucocyte extracts are also active in guinea-pigs, saving them from fatal infections.

Extracts of leucocytes from immunized animals seem, in some instances at least, to possess even greater curative powers than extracts from leucocytes of normal animals.

The action of the leucocyte extract may be due to the enhancement of the bacteriolytic action of the animal's plasma by the introduction of complement or to the action of digestive substances usually not liberated from the leucocytes; but is most likely chiefly due to poison-neutralizing or destroying bodies, which act on the endotoxins, *i.e.*, endo-antitoxins or antiendotoxins, and thus relieve the leucocytes of the animal from fatal poisoning and protect the higher cells of the animal so that their functions are not deranged.

If these protective bodies are, as we suppose, poison-neutralizing in their action, it does not seem improper to refer to them as endoantitoxins, since they are apparently antibodies, which are not normally given up to the plasma by the cells possessing them, and in this peculiarity correspond to the bodies against which, it seems, they are chiefly directed, and which being fixed constituents of the bacterial cell are, therefore, referred to as endotoxins. It would seem, then, more logical to refer to these nondiffusible bodies of the animal cell as endoantitoxins than as antiendotoxins.

It does not seem unlikely, then, that extracts of leucocytes (polymorphonuclear and mononuclear), and possibly of the blood-forming organs, furnish us with means of combating infections incited by those microörganisms generally looked upon as giving rise to endotoxin poisonings, and which have steadily refused to yield to the action of immune sera alone.

[It is not only a pleasure to the writer, but a duty, to mention the invaluable services of his constant and faithful assistant in this work, James May, through whose careful observation of the animals and skill in the preparation of the extracts this series of experiments was largely made possible.]