**Serum-therapy in the light of the most recent investigations / by Frederick W. Stetson.**

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## Light of the Most Recent Investigations.

FREDERICK W. STEVENSON, M. D.

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FREDERICK W. STETSON, M. D.

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THE Trustees of the Fiske Fund, at the annual meeting of the Rhode Island Medical Society, held at Providence, June 5, 1902, announced that they had awarded a premium of two hundred dollars to an essay on "Serum-Therapy in the Light of the Most Recent Investigations," bearing the motto :

"Winslow."

The author was found to be FREDERICK W. STETSON, M. D., of Dorchester, Mass.

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# SERUM-THERAPY IN THE LIGHT OF THE MOST RECENT INVESTIGATIONS.

By serum-therapy is meant the treatment of disease by the administration to the patient of blood-serum, prepared or selected in a particular manner, with the object of producing a specific effect on that disease. Such administration is customarily carried out in the form of injections, either subcutaneously, into the lymph-spaces, whence the serum may find its way into the blood, or intravenously, directly into the blood itself. The class of diseases treated by this method is that dependent on the presence of a deleterious organic substance in the blood of the patient; and the value of the serum as a therapeutic agent is derived from the presence in it of certain elements which cause, directly or indirectly, a destruction or neutralization of these deleterious substances. Inasmuch as the diseases of this class are due to the presence of deleterious substances in the blood, which act, in causing the disease, by destroying the tissues of the organism, or by interfering with their function, the destruction or neutralization of these substances serves to allow the tissues to regain their normal activity, and thus the disease is cured.

The serum may be administered either before or after the harmful substances have entered the organism and produced the symptoms of the disease; its action is the same in both instances. When administered before, it serves to prevent their introduction into

the body, thus warding off a possible advent of the disease, or, in other words, rendering the organism immune to that disease. When administered afterwards, it serves to check their further increase within the body, thus annulling the severity of the disease, or bringing it to a termination.

The nature of the harmful substance, against which the therapeutic serum is designed to act, varies according to the nature of the disease dealt with. It may be the dead or living bodies of bacteria. It may be their products known as toxines. It may be of other vegetable origin; as in the case of abrin, derived from *abrus precatorius*, and ricin, from the castor bean, vegetable proteids, of which use has been made in causing poisoning experimentally in animals, and in successfully treating the disease thus induced by the use of their specific sera. Or it may be of animal origin, notably the snake poisons, which are known to be proteids, and the poisons normally existing in certain sera, as in that of the eel.

In what chemical group the toxines should be placed has been a matter of much discussion. There has been a tendency to regard all toxines as similar chemical substances. But it is probable that they are of varied composition, not only as shown by apparently successful isolation and analysis of some of them, but also because of their varied manner of production. Take, for instance, the conditions under which the toxines of diphtheria and of tetanus are produced, the former best aided by an abundance of oxygen, the latter dependent upon an absence of oxygen, and we see that the two may be substances wholly different from each other. Probably many of these toxines are alkaloids,

or, as they are often termed when produced in processes of decomposition outside of the system, ptomaines.

It is well recognized that bacteria are the agencies concerned in the production of many, if not all, ptomaines; and many of these ptomaines have been carefully studied and found to possess toxic properties in varying degrees. For illustration, we have tyrotoxicon, a poison obtained from cheese by Vaughan, cadaverine and putrescine, obtained from cultures of the cholera bacillus, typhotoxine, from the typhoid bacillus, tetanine, obtained from the bacillus of tetanus by Brieger, and an alkaloid from a culture of anthrax by Martin. These are poisonous alkaloids of known chemical composition produced by bacteria; but whether they are the specific toxines causing the diseases with which the bacteria are associated has not been proved. Still, it is fairly probable that these or similar alkaloids are the toxines concerned in certain diseases.

In the same way, other toxines may be ferments, and others of proteid nature, or toxalbumins; since we know that in other instances bacteria produce both of these classes of substances, Brieger and Fränkel have isolated a toxine from culture of the Klebs-Löffler bacillus of diphtheria, which they regarded at one time as a toxalbumin, but which Brieger regards now as non-proteid.

However, other properties of the toxine of diphtheria are definitely known. It is more or less unstable; heated in solution for several hours at 60° C. it loses almost wholly its toxicity. Exposure to air and light also weakens it, but drying renders it more stable. It may be precipitated from a filtered culture

fluid, in combination with the peptones and albumoses, by the addition of ammonium sulphate, and the mixture may then be dried in vacuo. It may also be precipitated by the addition of various other neutral salts, alcohol, zinc chloride, or by the formation of an insoluble phosphate in the fluid containing it (1). At present our knowledge of the nature of toxines is meagre, and a full understanding of the subject can only come after each has been studied separately.

The pathological changes which these harmful substances produce in the organism are as varied as the diseases themselves. While many different tissues or organs may be affected by one of these substances, either through impairment of function or through direct destruction; often particular tissues suffer the greatest injury from the substance. Thus, the effect of tetanus toxine is shown strikingly on nerve-cells, of the toxine of yellow-fever, on the cells of the liver; of plague, on the lymphoid tissues. And we know that different toxines may produce a different effect on the same tissues. Thus ricin causes an agglutination of the red corpuscles, while the toxine of eel serum causes their dissolution. The former is one of a numerous class called haemagglutinins; the latter belongs, also, to a numerous class occurring especially in normal blood-sera and called haemolysins. Indeed, this specific destructiveness of toxines has been carried still further experimentally, and sera have been produced which destroy definite cells, such as the ciliated epithelium of the trachea (15), the white corpuscles (16) or the spermatazoa (17).

Having taken a brief glance at the nature and variety of the substances against the action of which

in the organism serum-therapy is directed, let us turn to a consideration of the serum itself. Serum is that portion of the blood left after the removal of the corpuscles and the fibrin. As far as chemical analyses have been able to show, we know that it is composed of serum-globulin, serum-albumins, certain extractives and inorganic salts. That any one of these is the effective agent in treatment with serum is obviously not the case, for, as we shall see later, a particular kind of serum is required for the treatment of a given disease. There must exist, then, other elements in some sera, at least, which have to do with serum therapy. These may come under the classes enumerated above, *i. e.*, may be varieties of globulin, albumin, etc., or may be of other nature and have escaped chemical detection. However, that there are such elements, varying in different sera, we know from their physiological action.

According to their action, they have received various names. One class causes a dissolution of bacteria, and these are called bactericides. Another class, causing a clumping together or agglutination of bacteria, without necessarily their destruction, is called agglutinins. Still another class serves to prevent the blood corpuscles from being agglutinated by the haemaglutinins, and another class serves to prevent their dissolution by the haemolysins, receiving the name of antihaemolysins. Finally, there is that class of antitoxines proper which serves to counteract the effect of the toxines, those substances that act by injuring the various tissues of the organism. Inasmuch as the toxines have such a variety of selective actions, displaying, as I have previously shown, a tendency for

the destruction of nerve, liver, lymph-cells or other tissues, it is possible that the class of antitoxines embraces numerous classes of anti-elements, each having its specific protective action. Thus there may be included under the broad term antitoxines hitherto undiscovered antineurolysins, antihepatolysins, antilympholysins, etc., after the analogy of the already recognized antihaemolysins.

Now it has recently been shown by various investigations that *normal* serum contains many such protective elements in small amounts; that the sera of different species differ from each other in the varieties of elements they contain; and that among individuals of the same species there is a quantitative variation in these elements. In the case of the bactericides, it was shown in 1888 by Nutall that rabbits' blood was bactericidal for anthrax, the bacillus subtilis, and the bacterium megatherium (2). Nissen has demonstrated the same effect of rabbitt's blood upon cholera, typhoid, coccus aquatilis (3). Buchner found the serum of rabbits bactericidal for anthrax, hog cholera, typhoid, cholera, etc. (4), and at the same time that this effect was not an inherent quality of serum itself, for he found that ox and horse sera were quite ineffective toward the typhoid bacillus. Furthermore, that this bactericidal action toward different bacteria was not due to a single substance in the serum was demonstrated by an experiment of Nissens. In this he injected intravenously into a rabbit a great amount of the coccus aquatilis, and immediately drew off the blood from the vein. This blood had lost its bactericidal strength for this organism, while it remained unchanged for cholera and typhoid (3).

That the bactericidal effect of serum toward different varieties of bacteria is due to the presence of different substances in the serum was further verified by Bail. (5) He found on the not excessive addition of dead staphylococci to rabbits' serum, that, after the bacteria, and presumably attached to them their specific bactericides, had been centrifuged out, the serum was still bactericidal for typhoid bacilli, not, however, for staphylococci. The same experiment succeeded also when reversed, and with staphylococci and cholera, as well as typhoid and cholera. Neisser was able to show by this same absorption method that the bactericidal substances of rabbit serum are independent of the haemolytic substances. For by the addition of dead anthrax bacilli to rabbit serum and centrifuging, this lost its bactericidal power toward the bacillus without losing its haemolytic power toward goats' and sheep's blood corpuscles (6).

Recent experience has likewise shown that the agglutinins may exist preformed in normal serum. And the same sort of experiments as were carried on with the bactericides is adduced to prove that the agglutinins also are distinct elements, each specific for one variety of bacteria. Bordet, making use of the absorption method, showed that horse serum, which agglutinates cholera and typhoid, after being centrifuged with agglutinated bacteria of one species, loses its agglutinin for this species, but not for the others (7). Malkoff did the same later with red blood corpuscles (8). Normal goat serum agglutinates blood corpuscles of the rabbit, of the pigeon and of man without destroying them; other kinds of blood are not at all or only partly agglutinated. But among

individual goats there may be a great variation in the behavior of their sera. In Malkoff's experiments, when pigeon blood was added to goat serum, the agglutinins for both of the other kinds of blood were present in the centrifuged serum, while the agglutinin for pigeons' blood had disappeared. And these experiments succeeded with the three sorts of blood in all combinations, even when, by the addition of two kinds of blood, the two corresponding agglutinins were removed. While I previously classed these agglutinins of blood corpuscles with the toxines, among the substances harmful to the invaded organism, it is readily seen that they are analogous to the protective elements, and in this connection may be spoken of with them; inasmuch as they, like the agglutinins of bacteria, are substances existing in normal serum, and having a specific action on bodies foreign to that serum.

Similar experiments have been conducted with antihaemolysins. It is known that staphylococcus aureus and the bacillus of tetanus produce substances which dissolve the corpuscles of rabbits' blood. It is also known that horse serum exerts an anti-haemolytic action on both of these haemolysins (13), (14). Neisser has shown by an ingenious experiment that this action of the serum is due to two separate antihaemolysins, each having a specific effect on its corresponding haemolysin (6). In his experiment, he first established the complete destructive dose of these two haemolysins for one drop of rabbits' blood. Then, using the sera of four different horses, he found the amount required to neutralize each of these complete destructive doses. He found that serum No. 1

contained in the same quantity 10 times as much antihaemolytic power against staphylolysin as against tetanolysin, serum No. 2, .67 times, serum No. 3, more than 40 times, and serum No. 4, an equal amount. Inasmuch as the antihaemolytic power against both haemolysins was not proportionate in every serum, it must have depended upon the presence of two separate elements in varying proportions.

So, too, antiferments have been found in normal serum. Landsteiner, who also cites older literature, found antitryptic substances in normal rabbit, guinea pig and ox serum (9). Morgenroth found, in normal goat and horse serum, substances antagonistic to rennet and cynarase (10).

Likewise, normally existing anti-toxines are known. For Meade Bolton and later Cobbett found in a great percentage of normal horse sera diphtheria antitoxin in very variable quantities (11). Wasserman found in many normal human sera not inconsiderable amounts of diphtheria antitoxin (12). Neisser found in normal human sera a constant antagonistic substance for staphylotoxine (6).

Taking into account these recent investigations of normal serum, we are compelled to accept the view that serum normally contains a vast complexity of independent, though probably allied elements in variable amounts, usually minute. Some of these elements are protective, serving by their action on invading foreign substances to overcome these and prevent them from injuring the organism. An organism which is so protected by its normal serum is said to be naturally immune to the harmful substance. This natural immunity is thus seen to be an important safeguard of

nature, preventing the organism from succumbing to every toxine or variety of bacteria which might gain a foothold within it.

How great a part the protective action of the blood serum plays in conferring natural immunity must be an open question; though, from what we have just seen, it would seem to have the chief rôle in most cases. Until recently, this *humoral* theory, first promulgated by Buchner, was given little acceptance; it was supposed that the stable existence in the blood of such a complexity of substances, specific for each disease, would be an impossibility. But in the light of the above recorded observations, we see that the blood can and does contain many distinct elements, each with a separate function, not only protective against toxine or bacterium, but also affecting in various ways, — dissolving, or agglutinating, or preventing this result, as the case may be, — many different sorts of blood corpuscles or cells. And it is safe to assume, considering the possible combinations of cells and sera that may be experimented with, that only a beginning has been made in the discovery of such elements. We need not suppose, however, that the teleological significance of these elements in the serum is for the purpose of acting upon foreign substances which may be introduced into it. For what would be the object of the haemolysin in the serum of the eel, for instance? A more probable explanation of the presence of these elements is that they serve some nutritive function, or are the result of the normal metabolism of the organism; the fact that they are also protective or agglutinative or haemolytic probably merely being accidental.

But there is another form of immunity besides nat-

ural immunity, so familiar that it need only be mentioned, viz., acquired immunity. This form is brought about by a previously susceptible organism suffering from a given disease and thereby losing its susceptibility for that disease. Such a result is a usual occurrence in the history of most of the infectious diseases. Now this acquired immunity may be brought about artificially in several ways. The organism may be directly inoculated with the disease, as was at one time the custom in the process of immunizing against smallpox, or with a modified form of the disease, as in our present vaccinations against smallpox, or the organism may be accustomed to a toleration of large doses of the disease-producing substance by being subjected to a succession of gradually increasing doses, beginning with a comparatively harmless dose. Finally, an individual may be made immune, or the severity of the disease, when present, may be lessened, by the administration to it of an injection of the serum from another individual made immune by the preceding process, that of successively stronger injections of the virus. This last method is called *passive* immunizing, in distinction from the preceding or *active* immunizing.

These processes of passive and subsequent active immunizing form the basis of serum-therapy. In its practice an animal is rendered actively immune to a given disease, and injections of the serum of this animal are employed in rendering human beings passively immune to the same disease. The method as carried out in the case of the most successful employment of serum-therapy, viz., in the treatment of diphtheria, is substantially the following: (18) Cultures of diphtheria bacilli in alkaline bouillon are allowed to

grow for three or four weeks at a temperature of 37°C. After the given time has passed, the acidity primarily produced by the bacillus gives place to a much more intense alkilinity than originally existed, and with this alkilinity the ratio of toxine production corresponds. When "ripe" 0.4 per cent. of trikresol is added to the cultures, which are then filtered through porcelain. The filtering serves to prevent too much local irritation. If the bacillus employed is virulent, and the conditions of culture are favorable, the filtered culture should be so toxic that 0.1 cc. would be fatal to a 500 gram guinea-pig in twenty-four hours. Even under the most favorable circumstances it is difficult to obtain a toxine which will kill in less than thirty hours.

The animal now used to furnish the serum is the horse, which has the advantages of being easily immunized and of being large enough to furnish a considerable quantity of serum. In the beginning, a small dose of the toxine, about 1 cc. is given hypodermically to detect individual susceptibility. Horses vary much in this particular, light-colored horses being distinctly more susceptible than dark-colored ones. If well borne, the preliminary injection is followed in about eight days by a larger dose, in eight days more by a still larger one, and the increase is continued every eight days or so, according to the condition of the animal, until enormous quantities, 300 cc. are introduced at a time.

The toxine causes some local reaction, at first a distinct inflammation, later a painful œdema and a febrile reaction. This, together with the appetite and general condition, the temperature-curve, and the stability of the body-weight must all be taken into consideration,

so that the administration shall not be too rapid, and the animal be thrown into a condition of cachexia, with toxic instead of antitoxic blood. One of the principal things to be avoided is haste. Too frequent or too large dosage is almost certain to kill the animal.

When, because of the tolerance to large quantities of toxine, the horse seems to possess antitoxic blood, a "twitch" is applied to the upper lip, the eyes are blindfolded, a small incision is made through the skin, a trocar thrust into the jugular vein, and the blood allowed to flow into sterile bottles. It is then allowed to coagulate, and remains upon ice for two days or so, that the clear serum may be pipetted off. This serum is the antitoxic serum. It does not always materialize according to the desires of the experimenter, sometimes proving unexpectedly strong in a short time, sometimes unexpectedly weak after months of patient preparation.

The strength of the serum is expressed in what are known as immunizing units. This denomination originated with Behring, whose original or *normal* serum was of such strength that 0.1 cc. of it would protect against the ten-times fatal dose of toxine when simultaneously injected into guinea-pigs. Each cubic centimeter of this normal serum he called an *immunizing* unit. Later it was shown that the strength of the serum could easily be increased tenfold, so that 0.01 cc. of the serum would protect the guinea-pig against the ten-times fatal dose. Each cubic centimeter of this stronger serum was described as an *antitoxic* unit, and of course contained ten *immunizing* units. Still later it was shown that the limits were by no means reached, and he succeeded in making sera of as much as 300

times the normal strength, each cubic centimeter of which contained 300 immunizing or 30 antitoxic units.

This serum, then, when injected under the skin of a patient suffering from diphtheria, in varying amounts, according to the severity of the case, serves to abort the disease. Its action depends upon the presence in it of diphtheria antitoxine, which was produced in increased amounts in the body of the horse by the action of the injected toxine, and which in turn serves to counteract the diphtheria toxine circulating in the body of the patient.

It may be of interest to consider briefly the steps which led up to this valuable discovery of the production of passive immunity. Although protective inoculations against smallpox had been practiced since ancient times, and vaccination, introduced by Jenner in 1768, had been generally adopted, the true significance of these practices remained unknown, owing to our ignorance of the bacterial cause of disease, and the application to other diseases of the principles thus hinted at could not be attempted. The first discoveries along this line were made by Pasteur. In his studies of the bacillus of chicken cholera in 1880, he found that when cultures were allowed to remain undisturbed for several months, their virulence was greatly lessened, and new cultures planted from these were also attenuated. By inoculating chickens with these attenuated cultures, using successfully cultures of increasing virulence, he succeeded in rendering them immune to attacks of the disease. Later various other methods of attenuating bacteria were discovered, and this principle of protective inoculations was applied to other diseases. It was found that heat, light, and various

chemicals were capable of producing attenuation in cultures, and that the injection of attenuated cultures would afford protection against the disease represented by that culture.

Another important advance was the discovery of Salmon and Smith, in 1886, that sterilized cultures of hog cholera bacilli, injected into pigeons, would render them immune to this disease in the same manner that attenuated cultures of the bacilli themselves would; thereby showing that immunity depended upon the action of the soluble products of the bacteria, and not upon that of the germs themselves. In the study of these soluble products thus aroused, Roux and Yersin in 1888 discovered the toxine of diphtheria, showing that filtered fluid cultures were as pathogenic as the bacteria themselves.

Although several years previously Farran had used the blood serum from individuals who had recovered from cholera in treating patients with the same disease, the logical step in the advancement of our knowledge of immunity was taken by Foa and Bonome in 1887. These experimenters found that immunity to injections of proteus vulgaris could be conferred upon rabbits through intravenous injections of the blood of other rabbits which had recently succumbed to this organism. The report of their experiment furnishes the first recorded instance of passive immunity.

Behring, in the following year, contributed to our knowledge of the subject, by showing that, in some cases at least, immunity was due to the direct destructive action of the serum of the immune animal on the bacteria themselves. He found that the serum of rats, which are naturally immune to anthrax, caused

the degeneration of anthrax bacilli which were exposed to it, while at the same time these would grow luxuriantly in the blood and serum of other animals not immune to the disease, such as the mouse, guinea-pig and ox (19). Further researches along this line by Behring and Nissen showed that this law of the destructiveness or non-destructiveness toward given bacteria of the sera of animals immune or susceptible to them hold good for many, though not for all, species (20.)

That immunity was not necessarily dependent upon the presence of toxines in the organism, such as might have been the case in the preceding instances of immunization, was shown by Ogato and Jasuhara in 1890, who used the blood of a naturally immune animal in conferring immunity upon a susceptible animal. They were able to protect mice from anthrax by means of injections of the blood of the dog, which is naturally immune to anthrax. As in this case no previous injections of toxines had been made, the inference must be drawn that dog's blood contains some substance hostile to anthrax, in other words, an antibody or anti-toxine or bactericide.

Similar evidence, confirmatory of the existence of antitoxines, was afforded in the same year by the experiments of Behring and Kitasato. In these it was demonstrated that the blood of rabbits immunized to tetanus or diphtheria by the injection of filtered cultures of those bacteria, when added to a culture of one or the other of those germs, so neutralized the culture that it failed to kill animals into which it was injected (22).

Finally, in 1891, Tizzoni and Cantanni succeeded in fully establishing the process of immunization by put-

ting to practical test the elaborated anti-toxine in conferring immunity upon other animals. They first rendered dogs immune to large doses of tetanus toxine by giving them successively increasing doses, at first in small amounts. Then with injections of small amounts of serum from these immunized dogs, they were able to render white mice immune to otherwise fatal doses of the toxine. These experiments led Behring to apply the same principles to the treatment of diphtheria, and upon him falls the honor of inaugurating serum-therapy in its application to human beings. Having tested the effectiveness of his antitoxic serum upon animals, he successfully used it in the treatment of persons suffering from diphtheria, and published the first reports of cases so treated in 1893. Later the so-called Behring's Law, according to which the blood serum of animals which have been immunized to a particular infectious disease is capable of conferring the same immunity upon other individuals, became recognized as operable experimentally for many of the pathogenic organisms. Through Ehrlich's experiments with the poisonous vegetable albumins abrin and ricin, it was shown that this law availed not only for the toxines produced by micro-organisms but also for those produced by plants (21). Calmette, finally extended the application of the law to animal poisons in his experiments with snake venom (51).

Having seen that when an organism is exposed to the action of various noxious substances, there may be produced in its serum either an increase of already existing antagonistic substances, or a development of such substances, previously lacking in that serum, let us examine into the theories that have been deduced to account for

the origin of these protective bodies. One theory seeks to explain their origin by regarding them as excreted products of the bacteria themselves. This view was advanced by Buchner, (23), and by Behring and Knorr, who adduced as evidence the fact that bouillon cultures of tetanus, after having been rendered inocuous by heating to 65°, are, under certain circumstances, directly antitoxic toward tetanus toxine, like the antitoxine itself (24). Smirnow, indeed, by the aid of electricity, has produced anti-toxine directly from toxine (25). Emmerich and Löw hold that these products are enzymes, which in the body unite with albumin to form an immunizing proteid which retains their bactericidal property. In favor of this view are the facts of the gradual cessation of growth of fluid cultures of bacteria, despite the abundance of nutritive medium, and the healing by means of bacterial products or unfiltered cultures. Certain of these enzymes have also antitoxic as well as bactericidal properties, certain have only the latter, some not only toward the same species of bacteria but also toward others. In this connection is especially to be mentioned the enzyme of bacillus pyocyaneus, which is said to be destructive for anthrax, diphtheria, plague and other infectious germs (26).

While this theory may explain the origin of antitoxines in some instances, it will not hold for many cases, where the bacteria have no entrance into the organism in which the antitoxine is produced, as in the case of the production of diphtheria antitoxine by injections of the toxines of diphtheria alone. Here the antitoxine must in some way be produced from the body tissues, and its production is most plausibly ex-

plained by the "side chain theory" of Ehrlich. According to this theory the protoplasmic cells produce a substance, "side chains," which physiologically may act as a special form of nutriment to the cell. When produced in excess, it becomes detached from the cell and assimilated by the blood. When toxines are introduced into the system, they act by fastening upon the side-chains of that sort of cells for which they have a special affinity, and subvert their normal function. If this involves many or vital elements, sickness or death ensue. In other cases, the affected side chains are cast off and eliminated, and then replaced by a new formation. By such repeated destruction and regeneration of side chains, an overcompensatory tendency to reproduction is ultimately brought about. The blood then becomes stocked with these superfluous side chains; so that upon a fresh advent of toxine, either in the same body or after the blood has been introduced into another body, this unites so readily with the free side chains, that those of the living protoplasm are not attacked, and the disease is prevented.

Corroborative of this theory are experiments of Wasserman's. He assumed that, inasmuch as the toxine of tetanus exerted its effect on the brain and spinal cord, the side chains which compose its antitoxine originated in the cells of those structures. If this were the case, injections of the normal side chains, *i. e.*, of brain and cord tissue, ought to answer the purpose of injections of the artificially produced side chains, *i. e.*, of antitoxine. Such in his experiments proved to be the case. Tissues of the cord and brain of man, the guinea-pig, rabbit, pigeon and horse all served as a protection against the toxine when given

within twenty-four hours before injection, or several hours after (27). Further experiments along this line would seem to show that these side chains are not always cast off into the general circulation, but remain in contact with the cells, or at least within the organs where they originated. For Wasserman found that although the organs of healthy animals did not protect against typhoid injection in animals, injections of cord lymph-nodes, spleen and thymus of animals which had some days previously received injections of dead or living typhoid bacilli protected against typhoid infection when made in other animals; although blood, brain, muscle, liver and kidneys, afforded no such protection (28).

This side chain theory of Ehrlich, while affording a clever speculation as to the origin of toxines and allied substances, was unable to furnish an explanation of all the phenomena connected with the behavior of these bodies. Accordingly, in the case of the bactericides, and with equal propriety this might be applied to the case of the other antagonistic substances, a further hypothesis, the double-element theory of Ehrlich and Morgenroth was adduced (29). This theory assumes that, for the effectiveness of bactericidal sera, two separate substances are required, one the so-called immune or connecting bodies (Twischenkörper), the other the so-called complement or end bodies (Endkörper). The end body is a sort of destructive ferment, which dissolves the bacteria, and is contained in the blood serum of every normal organism. Buchner has given it the name of alexine. The connecting body, on the other hand, serves only to fasten the end body to the bacterial cell, and is not present in appreciable amount

in the normal organism. It exists in greater quantities only after artificial immunizing, or after spontaneous recovery from the specific infection; and is found in the immune-serum, which is composed of it almost exclusively, as can readily be shown by experiment. Hitherto attention was paid only to one of these two factors, the immune-serum or connecting body, and no thought was taken as to whether in a given case there was present a sufficient amount of the end body. Great pains were taken to supply the organism with sufficient connecting bodies for the given injection, whereas the much needed ferment or end bodies may have been exhausted.

In order more fully to understand the application of this theory, we may compare the connecting body to a double cog-wheel with two sets of gearing, one set of which fits similar cogs on the bacterium, the other, corresponding cogs on the end body. It may then be readily understood how the connecting bodies may differ among themselves, having two opportunities for variation, according to their valence toward the varieties of end bodies, or toward the varieties of bacteria. For instance, in any given connecting body molecule, one end fits only its specific bacterium, *e. g.*, a typhoid bacillus, whence the specificness of the serum which contains that form of molecule. In like manner, the other end of the connecting body is so fashioned that only certain end bodies can be attached to it; so that not every complement *i. e.*, the normal serum of not every species of animal is adapted to any immune-serum. For example, if we have obtained typhoid immune-serum by immunizing a horse, we cannot say that the serum of any normal animal, *e. g.*,

that of a goat, will increase the effect of this upon a typhoid infection; for we do not know *a priori* whether the end bodies of the goat serum will fit the connecting bodies produced in the horse serum. This task of finding the proper complements for specific immune-sera is one of the important ones for future investigation.

From the foregoing it would seem most reasonable to employ as complement the serum of the same species as the infected animal. For, inasmuch as the specific immune-serum alone protects the animal against small doses of the infection, it would seem to follow that the end bodies of that animal were best adapted to the connecting bodies of that particular serum. However, this does not always hold true. Very often the newly introduced end bodies of the same species, *e. g.*, fresh, normal guinea-pig serum in an infected guinea pig, lose very easily their effectiveness. Possibly under these circumstances, substances exist which either directly destroy or link themselves to the end bodies.

Wasserman illustrates the double element theory, showing the effectiveness of combined immune-serum and complement, in a series of experiments with guinea-pigs subjected to injections of typhoid cultures. Where either serum alone is injected into the guinea-pig, the animal dies and its body contains living typhoid bacilli. Where both are injected, the animal recovers (29). He holds that along these lines we may derive practical benefit from the bactericidal sera applied to human serum-therapy, particularly in typhoid, cholera and plague.

Two phenomena have been cited as showing the

fallacy of the side chain theory (30). One is the method in which diphtheria antitoxine may be produced. When a horse has been actively immunized until his serum contains a large number of antitoxine units, a very small dose of toxine will suffice to increase greatly the number of antitoxine units. It is argued that, according to the side chain theory, the toxine of this last dose should be appropriated by a portion of the great number of side chains that are free in the serum of the animal, instead of attacking the body cells and stimulating them to the production of more antitoxine. The other phenomenon is what is known as the *paradox reaction*. If toxine be given to a recently actively immunized animal at too short intervals or in too rapidly progressive amounts, the animal will succumb to the poison, in spite of the fact that the blood of its body contains a great amount of the antitoxine, an amount, a small part of which suffices to protect a fresh animal, through passive immunity, against any injurious effect of even greater poisonous doses. Behring has formulated the paradox reaction in speaking of tetanus immunizing. "Actively immunized horses with $2\frac{1}{2}$ antitoxine units in 1 cc. of blood are less immune than those passively immunized with $\frac{1}{500}$ antitoxine units per cc." The opponents of the side chain theory maintain that if it were true, the free side chains of the actively immunized animal ought to unite with the toxine, and protect the tissues of the animal against its action. Instead, the animal succumbs to the toxine as though no side chains had been formed. If side chains are effective at other times, why are they not at this time, when, according to the theory, the blood of the animal is stocked with

an amount greatly in excess of that required to produce immunity?

But this phenomenon of the paradox reaction seems to afford good evidence that something is lacking in the actively immunized animal to render the side chains effective, while it is present in the passively immunized animal. Apply the double element theory to this case, and the side chain theory will remain perfectly tenable. We may suppose that, in the actively immunized animal, the complement existing normally in the blood has been exhausted by the rapid successive introductions of toxine, which, at the first, united with the complement at the same time that the toxine united with the connecting bodies or free side chains. Without the presence of the complement, the free side chains are then ineffective against the toxine, and the latter is free to attack the vital cells. In the passively immunized animal, on the contrary, the normal amount of complement is present, and serves to render the introduced connecting bodies or immune serum effective. In the same way, the double element theory serves to explain the first-mentioned phenomenon, where, although large amounts of side chains, according to the theory, are present, a small amount of toxine, instead of being rendered inert by them, is effective in stimulating the cells which produce the side chains to increased productiveness. Here, also, owing to repeated introductions of toxine, the complement has been exhausted, and the newly introduced toxine, therefore, is not attacked by the connecting bodies alone.

Having considered the nature of serum-therapy and the theories of its action, let us turn to the diseases in which it has been employed, and consider its present

and prospective value in each. Of the various antagonistic elements which may exist in the serum, viz., agglutinins, antihaemolysins, antitoxines, and bactericides, the last two are the only ones of practical therapeutic importance. The diseases in which the antitoxines have been used with considerable success are diphtheria, tetanus, and snake poisoning. The bactericides have thus far been less successful in the treatment of disease in man, though experimentally in animals they have been demonstrated to be of value, and give promise of being applied in human therapeutics. Among the sera containing bactericides are those of cholera, typhoid, pneumococcus, steptococcus, plague, and anthrax. Attempts have also been made to obtain antitoxic cholera and typhoid sera; and these have succeeded to a certain extent, although they are not more effective therapeutically than the bactericidal sera (29).

*Use in Diphtheria.* That serum-therapy has proved itself of great value in reducing the mortality of diphtheria, and diminishing the severity of the disease is now admitted by all intelligent observers. As proof of its value, it is but necessary to study the death rate from this disease in the cities and hospitals of the civilized world, and to note its reduction since the introduction of diphtheria antitoxine.

Before antitoxine was used the death rate of diphtheria varied from 30 to 50 per cent. According to Lenox Browne, in 11,598 cases treated in the Asylums' Board Hospitals, London, from 1888 to 1894, inclusive, the death rate was 30.3 per cent. From 1895 to 1898, in the same hospitals, 20,382 patients were treated with antitoxine, and the death rate fell to

18.4 per cent. In the Boston City Hospital, before the use of antitoxine the death rate was 46 per cent., after the use of antitoxine, 12.9 per cent (31). In Bayeux's work on diphtheria, the death rate is given as 55 per cent. before the advent of antitoxine, and 16 per cent. after its use. The rate of 16 per cent. is based upon an analysis of more than 200,000 cases. Siegert states that the mortality in the German and Austro-Hungarian Hospitals for five years previous to the use of antitoxine was 40 per cent., after its use, 16 per cent. (32). In 1,163 cases in Heubner's clinic in Berlin, the mortality fell under the use of antitoxine from 54.4 per cent. to 15 per cent. (33).

The benefit from the use of anti-toxine in operative cases, intubation and tracheotomy, is also pronounced. Before the use of antitoxine, the death rate in operative cases was very high, from 75 to 87 per cent. Since the advent of antitoxine, the mortality in these cases has been greatly reduced. In 313 cases of tracheotomy in the Asylums' Board Hospitals in London the death rate was 38 per cent. In the Belvidere Fever Hospital, Glasgow, the death rate in operative cases for the year ending May 31, 1899, was 41.9 per cent. In the Willard Parker Hospital, New York, there were 737 cases of intubation from 1895 to 1900, with a mortality of 63 per cent. In the last two years the rate was 52 per cent. In the Municipal Hospital in Philadelphia, the rate in 165 cases was 58.8 per cent. In the Boston City Hospital the rate for 1898 was 46 per cent., for 1899, 34 per cent. (31). In the German and Austro-Hungarian Hospitals, before the use of antitoxine, the mortality in operative cases was 60 per cent., after its use, 34 per cent. (32). In 370 intubations reported by O'Dwyer, from his personal

experience, the percentage of mortality was 72.4 before antitoxine was used: while in 59 intubation cases after it was used, the mortality was 23.7 per cent. (34).

The claim has been made by the opponents of antitoxine that, coincident with its introduction, the practice of establishing the diagnonsis of diphtheria by bacteriological examinations came into vogue, and that, in consequence, mild cases of diphtheria are now classified as such, while they were not before. They claim that this increase in the number of mild cases of diphtheria reported is the cause of the diminished death rate, and not the use of antitoxine. That this objection is invalid is shown in several ways. Most of the statistics given deal with hospital cases; and such cases are, as a rule, severe ones, and would have been recognized as diphtheria before the use of cultures for diagnosis. The statistics relating to laryngeal diphtheria can certainly not have been affected by the advent of laboratory diagnosis.

Then, again, if, instead of comparing the number of fatal cases with the number ill with diphtheria, we compare them with the total population, we see that there has been an actual decrease in the mortality from diphtheria. This could in no way be due to a former error in diagnosing the mild cases. The following table gives the ratio of mortality per 10,000 of the living for the city of Boston from the years 1888 to 1899:

| | | | |
|---|---|---|---|
| 1888 | 13.88 | 1894 | 18.03. |
| 1889 | 15.65 | 1895 | 11.73. |
| 1890 | 10.30 | 1896 | 9.80. |
| 1891 | 6.23 | 1897 | 7.7. |
| 1892 | 10.29 | 1898 | 3.2. |
| 1893 | 11.45 | 1899 | 4.8. |

It will be seen that since 1895, when antitoxine began to be used in Boston in the treatment of diphtheria, there has been a decided diminution in the actual death rate from the disease.

In Berlin for the four years from 1890 to 1893, the percentage of mortality from diphtheria was 35.34; that for the four years from 1894 to 1897 inclusive, when antitoxine was used, was 18.08. In an analysis of the ratio of mortality in 266 German cities, it was found that the ratio of mortality per 10,000 of the living for the years from 1886 to 1893, before antitoxine was used, varied from 13 to 8.4, while the ratio from 1894 to 1897 varied from 10.1 to 3.5. Its progressive diminution coincident with the increasing use of antitoxine is seen from the following figures: Ratio for 1894, 10.1; for 1895, 5.3; for 1896, 4.3; for 1897, 3.5. This diminution has been too marked and too gradual to be explained by any change in the type of the disease.

A similar reduction in the number of deaths has occurred in Paris. From 1890 to 1893, the deaths from diphtheria varied from 1,639 in 1890 to 1,262 in 1893. Following the advent of antitoxine, the number of deaths in 1894 fell to 993, in 1895, to 411; in 1896 it was 445, and in 1897, 274 (34).

In addition to the foregoing statistics, as evidence of the positive value of antitoxine in diphtheria, we may well heed the testimony of those who have had a large experience with the disease both before and after the introduction of this therapeutic measure. Such observers have generally been impressed with the favorable change in the course of the disease produced by the administration of antitoxine. Innumer-

able cases might be cited illustrative of this; but I can not do better than to quote the experience of McCullom, Resident Physician for the South Department of the Boston City Hospital: "No case of diphtheria in the acute stage should be considered hopeless. Antitoxine should be administered in each and every instance. It has been my experience during the past few years to see so many patients, apparently hopelessly ill, recover, that my convictions are firm on this subject. When one sees a patient with membrane covering the tonsils and uvula, profuse sanious discharge from the nose, spots of ecchymosis on the body and extremities, cold clammy hands and feet, a feeble pulse and the nauseous odor of diphtheria; and finds that after the administration of 10,000 units of antitoxine in two doses the condition of the patient improves slightly, that after 10,000 units more have been been given there is a marked abatement in the severity of the symptoms, that when an additional 10,000 units have been given the patient is apparently out of danger, and eventually recovers, one must believe in the curative power of antitoxine." (31).

Claims have been made that the use of antitoxine is dangerous; but clinical evidence does not warrant this statement. One alleged element of danger is an injurious effect on the heart; cases of sudden death have been said to be more common after its administration. A study of diphtheria before antitoxine was used shows that heart complications were exceedingly frequent, more so than at the present time. They were recognized long before antitoxine was used. Mollard and Regand proved by their experiments on dogs and rabbits injected with fatal doses of diphtheria

toxine, that the process in the heart is similar both in symptoms and in pathological appearances to that found in human diphtheria. Different observers as far back as 1878 found marked changes in the nervous structure of the heart and in the muscle itself, due to the absorption of the diphtheritic toxine. It is a significant fact that, according to McCullom, the majority of the diphtheria patients who died from heart complications at the Boston City Hospital, had been ill three or four days before antitoxine was administered; and in consequence the bacilli of diphtheria had had sufficient time to generate toxine enough to cause degeneration of the nervous structures of the heart. There was not the slightest evidence, either from the clinical study of the cases, or from the result of autopsies, that antitoxine had any injurious effect upon either the muscle of the heart or its nervous structures.

Albuminuria is another of the alleged dangers from the use of antitoxine. This, however, is also a symptom long recognized as occurring in diphtheria. An analysis of the urine in 173 cases treated at the Boston City Hospital was made before and after the administration of antitoxine. In these 173 cases it was found that in 99 instances albumin was absent both before and after this agent was used, without doubt due to the fact that the antitoxine was given before enough toxine had been generated to cause albuminuria. In 33 cases, the albumin was about the same after the use of antitoxine as before it; in 25, it was diminished; in 16, it was slightly increased, but not to a sufficient extent to cause any special anxiety. This would not justify the conclusion that the albuminuria was due to the antitoxine and not to the disease (34).

Paralysis, too, although occurring in the severe cases, was not so prominent as it would have been in an equal number of cases treated without antitoxine (31).

The only annoying sequelæ which can be attributed to antitoxine are urticaria and arthralgia, and these are always transitory, and never endanger the life of the patient. Bayeux, in his analysis of 200,000 cases, asserts that not a single death has been scientifically demonstrated to be due to the use of antitoxine.

From this review of facts showing the brilliant results from the use of the serum, we see that it is with perfect justice that diphtheria antitoxine has been hailed as one of the most valuable additions to medicine of the nineteenth century.

*Use in Tetanus.* In determining the value of the antitoxine treatment of tetanus, it is necessary to bear in mind that the disease manifests itself in two different forms, the acute or severe, and the subacute or mild form. Authorities generally agree that cases may be classed as acute where the incubation period is under ten days, or where the onset is rapid and the spasms severe; and as subacute where the incubation period is ten days or over, or where the onset is slow and the spasms are mild. It is recognized, however, that these conditions are merely relative, there being no marked differentiation between the two classes; and that cases with a long incubation period may suddenly develop severe symptoms and run an acute course, while cases which have an incubation period of a few days may develop slowly and deserve to be classed as subacute. Inasmuch as there is a great difference in the mortality of the two classes of cases,

it is desirable, in compiling statistics on the benefit of treatment, to consider the two classes of cases separately as well as in total, since in the latter case those of one class might preponderate and thus influence the result.

The death-rate in tetanus has been variously stated by different authors. In acute cases it has been given as from 78 to 96.6 per cent.; in sub-acute cases as from 17.8 to 87.5 per cent., the average for all cases varying from 21 to 87.5 per cent. (35). The largest number of cases collected is 1,222 war cases (36), with a mortality of 88.6 per cent., and 280 cases occurring in time of peace with 76 per cent. mortality (37), making 1,502 cases with a mortality of 87.7 per cent. Lambert (35), estimates the mortality for acute cases as 80 per cent., for sub-acute cases as 40 per cent., and for all cases as 60 per cent.

Lambert in 1897 collected a total of 114 cases treated with the antitoxine of tetanus with a mortality of 46, or 40.35 per cent. Loeper and Oppenheim (38) have collected 210 cases published since then, with a mortality of 97, and I have added 23 other cases selected at random with a mortality of 8. This forms a total of 347 cases, with a mortality of 151, or 43.5 per cent.

Of these later cases, however, 68 were treated by the method of intracerebral injections, a method which has come into vogue since the time of Lambert's paper. The principal upon which the treatment by intracerebral injections is based is that expounded by Wasserman — that the toxine of tetanus has a special affinity for the nerve cells, and produces its harmful results by uniting with them in preference to other

tissues of the body. Roux and Borrel, seizing upon this idea, were able to protect tetanized guinea-pigs by injections directly into their brains with doses of antitoxine insufficient to afford protection when injected into other parts of the body. This method of treating tetanus was then inaugurated in the treatment of cases in man, and met with favorable reports.

Loeper and Oppenheim (38), however, have collected the reported cases thus treated, and have found that out of 59 cases there were 43 deaths, a mortality of 73 per cent. Among the 29 cases with an incubation under 11 days, there were 24 deaths, a mortality of 82 per cent.; and in the 15 milder cases there were 9 deaths, a mortality of 60 per cent. Thus the mortality is seen to be greater than that in cases not treated by the use of antitoxine; viz.: 60 per cent. for all cases, 80 per cent. for acute cases, and 40 per cent. for subacute cases.

When the mortality in this method of treatment is compared with that where subcutaneous injections are used, the result is seen to be vastly inferior to that obtained from the latter method. In 144 cases treated by subcutaneous injections collected by these authors, there were 51 deaths, a mortality of only 35.4 per cent. Among the 80 acute cases, there were 35 deaths, a mortality of 43.8 per cent.; and among the cases less severe, amounting to 42, there were 7 deaths, a mortality of 16.7 per cent. Loeper and Oppenheim have thus shown that, while theoretically and experimentally the intracerebral method is the most rational one, clinically it has no value over treatment without antitoxine, and is vastly inferior to the use of antitoxine subcutaneously.

Their explanation of this fact is that the lesion produced by the injection in the brain, which by all other observers has been regarded as of trivial consequence, is of sufficient magnitude to aid in the unfavorable course of the disease; and they adduce as proof of this the autopsy records in many of their collected cases, and the records of mental and cerebral symptoms in those which recovered. Whatever the explanation, the high mortality of the intracerebral method is a sufficient cause for condemning it as a mode of administering antitoxine in tetanus, and for eliminating the cases thus treated from our statistics bearing upon the efficacy of the serum-treatment of this disease.

Subtracting, then, the 68 cases thus treated, with 47 deaths, from our total of 347 cases and 151 deaths, we have a total of 279 cases, and 104 deaths, or a mortality of 37.3 per cent. In dividing these cases into acute and subacute, I am obliged to follow the criteria adopted by Lambert, as his list of cases was not published, and the division of his cases must be accepted as made by him. He includes in the first class all cases with an incubation period of eight days or less, and with a rapid onset of the symptoms, or those with a longer period of incubation but with an intensely rapid onset. In the class of sub-acute cases, he includes those with an incubation period of nine days or more, or those with a shorter incubation where the onset was slow. Lambert also further excludes cases which have died with intercurrent diseases, cases which died twenty-four hours after treatment was begun, and all mild cases of recovery which did not receive treatment until the tenth to fifteenth days of

the disease; but as such cases are not excluded in the statistics of tetanus treated by former methods, which we must use for comparison, it seems unfair to exclude them from the present statistics. Therefore I shall not do so, but shall adopt those of Lambert's figures from which he has not excluded these cases. He has thus selected 47 acute cases with 35 deaths, a mortality of 74.5 per cent.; and 61 subacute cases with 10 deaths, a mortality of 16.4 per cent. From the later cases, excluding those treated by sub-dural injections, and those in which the incubation period was not known, I have been able to select 33 acute cases with 19 deaths, and 62 subacute cases with 9 deaths. This gives a total of 80 acute cases with 54 deaths, or a mortality of 67.5 per cent.; and 123 subacute cases with 19 deaths, or a mortality of 15.4 per cent.

It will thus be seen that in the light of a larger number of cases the mortality as determined by Lambert still holds true; and, furthermore, that this mortality, when compared with the mortality when antitoxine is not used, shows that the use of antitoxine is of distinct value, and a marked step in the advancement of the treatment of tetanus. The following table will give an opportunity for comparing these statistics:

| | Estimated mortality without antitoxine. | Mortality with antitoxine as determined by Lambert. | Present mortality with anti-toxine. |
|---|---|---|---|
| Total cases | 60 per cent | 40.35 per cent | 37.3 per cent. |
| Acute cases | 80 per cent | 74.5 per cent | 67.5 per cent. |
| Sub-Acute cases | 40 per cent | 16.4 per cent | 15.4 per cent. |

Additional testimony to the value of antitoxine in tetanus is received when we consider certain individual cases. While it apparently has no effect in many of the grave cases, occasionally one is encountered where

recovery under its use seems nothing short of miraculous. Such a one is that reported by Mixter (39), which was of extremely severe type, and in which enormous doses of antitoxine were used, 3,400 cc. in all, an average of about 285 cc. a day. The author states that all other cases at the Massachusetts General Hospital approaching that one in severity have died; and as treatment varied from that of the other cases only in the large doses of antitoxine given, it seemed probable that this was responsible for the recovery. Another striking instance was seen in the treatment of a subacute case by Abbe (40). After his case, which was of slow onset, had begun to assume a serious aspect, with stiffness extending to upper and lower extremities and two pronounced spasms with opisthotonos, 50 cc. of antitoxine were given by subcutaneous injections daily for a week. During this week there was gradual and continuous improvement. When the antitoxine was stopped, improvement ceased, but continued when the injections were resumed.

A further proof of the value of the antitoxine is seen in the results of preventive inoculations. Various observers have employed these in cases of injury where tetanus has been prevalent, and all agree as to the marked lessening of the number of tetanus cases developing after its use.

We may conclude, then, that serum-therapy as applied to tetanus is an established success; and, with the employment of earlier treatment, larger doses, sera of greater antitoxic strength and more general intravenous or sub-dural injections, all of which procedures are being advocated by the different authorities, we may hope for a still greater lessening of the death-rate in this disease.

*Use in Snake Poisoning.* The results with the antitoxine treatment of snake-bite have been most encouraging. While the subject may yet be regarded as being in the experimental stage, and not enough cases have been published from which to draw conclusions by means of statistics, there have still been a good number of instances where recovery has rapidly followed the use of the antitoxine in cases which seemed absolutely hopeless. Inasmuch as the venom of serpents acts with great rapidity in causing destructive lesions, the antitoxine, to be effective, must be injected very soon after the bite is received. Where this has been done, its use has been most satisfactory ; where this can not be done, if the bite is that of a deadly serpent and contains the usual amount of venom, the case is beyond human aid.

The antitoxine used is that devised by Calmette, produced in the serum of horses, by accustoming the animals to increasing doses of cobra and crotalus venom, which has been freed from one of its poisonous principles by heating. The toxine destroyed by heat is that which causes local destructive lesions ; that which remains and evolves the antitoxine is that which causes the nervous lesions, and to which death from snake-bite is due. This latter toxine is found in varying proportions in the venom of all serpents; hence the antivenene of Calmette is of use in all forms of snake-bite. Since, however, the venom of serpents like the cobra contains larger proportions of this toxine, while that of serpents like the rattlesnake, viper, and other American varieties contain only small proportions of it, antivenene is of greater value in treating bites of tropical serpents, and of less value in treating those of our own country.

*Use in Plague.* Two varieties of serum have been used in the treatment of plague; that of Lüstig and that of Yersin. Both of these owe their therapeutic properties to bactericides. Lüstig's serum is prepared by treating the horse with a nucleo-proteid obtained from dead plague microbes. In India, where the disease has been most virulent, the mortality of plague as determined from 5,952 cases was 79.4 per cent. In 475 cases occurring at the same time, the mortality under treatment with Lüstig's serum was reduced to 60.64 per cent.

(41.) Yersin's serum is prepared by gradually immunizing the horse to a very virulent culture of the germ. When Yersin introduced his serum in 1896, he treated 23 cases without a single death. In the epidemic at Oporto, this serum was used with a mortality of only 14 per cent., while in the cases treated without serum it was at least 70 per cent. In Alexandria, Yersin's serum treatment was tried in 11 cases with 7 recoveries (42). In Glasgow it was used in 9 cases, with recovery in 5 (43). Although this is too small a series of cases from which to draw conclusions, the observer was able to record that, where administered intravenously, in each instance there followed an improvement easily noticed by both physician and patient.

While the balance of opinion is in favor of the positive value of antiplague serum, I must note the experience to the contrary of one observer. Clemon (44) used Yersin's serum in 50 cases, and compared its effects with the result in 50 control cases. The mortality was the same in both series; in 6 cases the serum was injected almost directly after the appear-

ance of the disease, and all six died. On the whole, however, we must conclude that anti-plague serum is of distinct value; though it at present, like the antitoxines of tetanus and snake poison, has not become so nearly a specific as has that of diphtheria. Its need, too, has been greatly lessened by the success of Haffkine in producing from cultures of the plague bacillus a virus for prophylactic inoculations against plague.

*Use in Streptococcus Infections.* The value of serum-therapy in streptococcus infections remains extremely doubtful. This method of treatment has had, since its inception, many enthusiastic upholders; but it has also, after extended trials, met with considerable opposition; and at present its title to the attainment of definite results is generally regarded with *scepticism*. The sera employed are produced according to the method of Marmorek, introduced in 1895 (53). The essential principle of his method consists in the use of horse or human serum in the media on which the streptococci are grown, so that cultures do not deteriorate in virulence as they do on other media. In this manner, cultures which have attained a high degree of virulence by being successively passed through animals may be kept at that potency, and injected in successive doses in the animal to be immunized. The action of the resulting therapeutic serum is directed against the bacteria themselves, not against their toxines.

The results obtained with Marmorek's serum in experiments with animals have been tested by numerous observers. Many of them failed to find that it had any value in protecting the animals against strepto-

coccus infection. Still others obtained positive results from it in varying degrees. These discrepancies are explained by the work of Méry and Lorraine, Courment, Desse, Van de Velde (45), and others, who have shown that, while a serum produced from a certain streptococcus may be extremely efficacious against infections due to that particular organism, it may be absolutely inert against infections due to streptococci from other sources. Van de Velde even asserts that the serum obtained from an individual streptococcus is of little or no value in treating a given infection, as the chances are against the infection being of the same reactive nature as the organism from which the serum was made. He accordingly advocates the use of a "polyvalent" serum obtained by means of a number of streptococci from different sources, so that the chances will be greater of its being effective, no matter what sort of infection may be present. This variation in the reaction of different streptococci to a given serum but coincides with our present knowledge of the variation in other characteristics among streptococci. For while it is now accepted that the streptococci all belong to one group of organisms, it is known that they may be made to change their characteristics in many ways, as in their virulence, their behavior on culture media, their staining reactions, and their morphology (56).

These experiments are further borne out by clinical experience, possibly serving to explain why some cases do well after injections of the serum, while others are uninfluenced by it. Such a case is that of Bruce (46), where a patient with septic endocarditis received several injections of antistreptococcic serum without

alteration in his condition, while after an interval he was given injections of the serum from another source, and showed an immediate and striking improvement. Another cause of such varying results experimentally and clinically may be due to deterioration of the serum by keeping, which has been proven to take place (47).

The most striking laboratory demonstration of the inefficiency of Marmorek's serum is that given by Koch and Petruschky (48). These authors found that cultures of streptococci highly virulent for rabbits, among them that from which Marmorek's serum was made, could be injected in man with impunity, apparently proving that the streptococci of septicaemia in animals were not of the same variety as those of human septicaemia, and that in consequence the bactericides derived from these would probably not be of avail in combating human infection. Petruschky verified this inference by trying preventive injections of Marmorek's and other sera in the case of a patient with malignant disease, in whom he had succeeded in producing erysipelas at will by the use of cultures of his own. Injections twenty-four hours previous to the inoculation which produced the attack of erysipelas apparently had no effect on the course of the disease.

The diseases in which antistreptococcus serum has been employed to any extent are puerpal fever, general sepsis, erysipelas, and scarlet fever.

A committee appointed by the American Gynecological Society reported in 1899 a series of 352 cases of puerperal sepsis treated by the use of anti-streptococcus serum (49). In 101 of these cases the presence of the streptococcus was verified by bacteriological examination; and in this series there were 33 deaths, a

mortality of 32.7 per cent. In the remaining 251 cases, where no bacteriological examination had been made, 40 patients died, a mortality of 15.8 per cent. The total death-rate was 20.7 per cent. As statistics have shown that the percentage of cases of puerperal septicaemia due to the streptococcoccus is only about 20 per cent. (49), the series where no bacteriological examination had been made should be disregarded, leaving the mortality of the first series, 32.7 per cent., as the most exact estimate of the value of this treatment.

One of the members of the committee, Williams, reported 23 cases of streptococcus infection which he had seen in the preceding three years and which had not received treatment with antistreptococcus serum, with only 1 death, a mortality of 4.35 per cent. Kronig of Leipzig reported 56 cases similarly treated, with a mortality of only 4 per cent. Inasmuch as these cases cover a considerable period of time and are drawn from all varieties of surrounding conditions, being taken from both hospital and private practice, the mortality may be taken as a fair estimate of the death rate in streptococcus infection when treated by the method of these observers. This mortality is seen to be much lower than had been previously supposed; other estimates have placed it at 25 per cent. Comparing, then, the mortality of the cases treated by the use of the serum, 32.7 per cent., with 4 or 5 per cent., the mortality of cases not so treated, we are compelled to abandon any faith held in the curative powers of the serum.

But we can not attribute this comparatively high mortality entirely to the serum itself, since in very few of the cases were ill results observed to follow

immediately its use. It is more properly accounted for by the committee partly from the assumption that many of the cases may have been unusually severe ones, the milder being treated by ordinary means, but chiefly from the fact that most of these cases were reported by French clinicians, who do not hesitate to employ curettage freely. In many of their individual reports it was stated that curettage had been employed. As neither Williams nor Kronig had employed curettage in the treatment of their cases, this difference in the methods of treatment is, in the opinion of the committee, sufficient to account for the difference in mortality. That such is the case is substantiated by the experience of Savor, who treated 15 cases of streptococcus infection with the serum, but without curettage, and had only one death. This mortality is far below any other series of cases treated with antistreptococcus serum, and can only be explained from the fact that curettage was not used.

The statistics thus collected afford pretty conclusive evidence that antistreptococcus serum, as now prepared has little or no value in the treatment of puerperal streptococcus infection. That it has little prophylactic value is apparent from the experience of Wallich (55). He administered the serum to 383 women suspected of infection on entrance to the hospital, but nevertheless 58 of them developed well-marked symptoms of infection.

The number of reported cases of general sepsis treated by means of antistreptococcus serum is too small to allow of drawing any conclusions from the mortality statistics. For conclusions drawn in this manner, from a small number of cases, except in dis-

eases which follow a fairly constant course and which offer little chance of being influenced by other circumstances, are unreliable. In general sepsis, however, there is no typical form of the disease, the prognosis is always uncertain, and, in some apparently unfavorable cases, unlooked for and lasting improvement may unexpectedly set in. It is true that in certain cases improvement has followed the use of serum-therapy; whether brought about by it or not it is impossible to determine.

The largest series of erysipelas cases is that of Marmorek (54). Out of 413 cases treated with his serum, he had a mortality of 3.87 per cent. In a previous series in the same service treated without serum, the mortality was 5.12 per cent. Marmorek noted in these cases a definite improvement in general condition after five to twelve hours, often with a rapid fall of temperature and pulse, and an improvement in the local condition which was often rapid and permanent. It is well recognized, however, that such rapid improvement often occurs in the course of erysipelas; and the difference in mortality is not so great that it can not be regarded as accidental or due to other causes. According to Therese the ordinary mortality varies from 1 to 5 or 6 per cent.

In scarlet fever the results of serum-treatment have been unsatisfactory. No large series of cases has been reported, and the mortality has been high, as admitted by Marmorek himself.

There is a difference of opinion among observers as to the harmlessness of the serum. A few have attributed serious collapse or even the death of the patient to its use (57). Numerous authors have had experience

with abscesses forming at the seat of the injection, some claiming these to be due to streptococci which were found in the serum itself. Erythema and urticaria following its use are of comparatively common occurrence, as they are in the use of other sera. On the other hand, many observers have employed it in large numbers of cases without observing any serious effects from it. Wallich used it in 400 cases without getting ill effects from its use (55).

That abscesses and urticaria are directly attributable to the serum there can be no doubt. How harmful an influence the abscesses may have on the course of the disease is open to question. If the serum is beneficial, the abscesses would seem to be a lesser evil, to be tolerated when they occur. If due to streptococci in the serum, they could be avoided with the use of more carefully prepared or tested sera. Urticaria, of course, need not be reckoned with. That symptoms of collapse or death should be attributed to the serum are matters of personal opinion, and the fact that such symptoms are attributed only in the cases of certain observers, while in other large series of cases no such symptoms have occurred, would lead us to discredit the correctness of these deductions. As the matter stands, then, we are not justified in regarding the use of the serum as especially harmful.

From the foregoing results we may conclude that the value of antistreptococcus serum as an aid in the treatment of streptococcus infections has not been demonstrated. Laboratory experiments, too, would throw grave doubt upon the efficiency of Marmorek's serum. Although some individual cases have done well under its use, we should hesitate before drawing

conclusions as to the part played by the serum in their recovery, as reasoning from single cases is notoriously fallacious. Inasmuch as the serum is not harmful, its trial should still be continued, as the number of reported cases is still too small for pronouncing final judgment. As Cotton (58) points out, the dose given may in many cases have been too small. A dose of one-seventh the body-weight is required to protect against ten times the fatal dose of toxine in a rabbit, corresponding to 10 cc. in man, and we have no means of knowing how great an amount of toxine may be present in a given infection. Hence, it is better to give too large than too small doses; especially as there is such a variation in the strength of different makes of serum, and it is so liable to deteriorate by keeping. Furthermore, as pointed out by Bruce (46), where one brand of serum may prove ineffectual, another may be beneficial. In consideration, then, of the possible benefit to be derived from it, the serum should be given in every severe case of streptococcus infection, as one possible additional means of combating the disease.

*Use in Pneumonia.* Antipneumococcic serum has been employed to a slight extent in the treatment of pneumonia, and, while its use is still in the experimental stage, it gives promise of being of no small value. The serum is bactericidal as well as antitoxic, as is seen from the method of its preparation. The animal used in preparing it is the horse, and the process of immunizing the creature to sterile cultures of the bacterium is similar to that employed in the production of diphtheria antitoxine. However, after the horse has acquired a certain degree of immunity to the pneumotoxine, he is subjected to injections of

living pneumococci in gradually increasing doses. His serum is then able to cope with living pneumococci when present in rabbits or other animals.

While the number of cases of pneumonia treated with this serum is perhaps too small for the drawing of definite conclusions, the mortality of these cases is certainly promising in showing a possible benefit from such treatment. Wilson and Page (52) have collected all the reported cases thus treated up to July, 1891, amounting to 162. Of these, 27 died, a mortality of 16.6 per cent.

In consideration of the facts that these were not selected cases, that in some of them serum-treatment was begun late in the disease, and that the ordinary mortality in pneumonia varies from 20 to 40 per cent. I can not agree with the conclusions of these authors that, owing to the extreme variations in the course of the disease these statistics are wholly unreliable, and that there is no encouragement for continuing the use of serum-treatment. Without doubt we should reserve our final judgment until a much greater number of cases has been collected; but, at the same time, with this beginning the outlook seems encouraging, and warrants a further trial of antipneumococcus serum.

*Use in Anthrax.* The treatment of anthrax by the use of serum has met with considerable success in Italy. Sclavo has succeeded in obtaining from sheep, subjected to progressive inoculations of anthrax bacilli, a serum which is efficient against the disease when produced in rabbits or sheep. In a recent article on the subject (59), he refers to a score of cases in man treated with this serum, most of which recovered. He is hopeful that with the employment of intravenous

instead of subcutaneous injections of the serum the proportion of recoveries will be greater.

No definite achievements have yet been recorded with the use of serum-therapy in cholera and typhoid. Work on these diseases is still largely in the hands of laboratory investigators, notably Pfeiffer, who has produced a serum effective against cholera infections in animals, and Chantemesse, who has produced one effective against typhoid. The so-called serum, used with some success in preventive inoculations against typhoid, is not a serum, but a liquid culture of the typhoid bacillus, and would more properly be classed as a virus. The tuberculin, employed in the treatment of tuberculosis, is a similar product. Serum-therapy has also been attempted in the treatment of other diseases, as cancer, yellow fever, leprosy, syphilis, and rabies, but with results too vague to afford conclusions. We may conclude, then, that it has secured a recognized position in the treatment of diphtheria, tetanus, and snake-poisoning; and will probably soon attain this position in the treatment of pneumonia, plague, and anthrax; while, with a perfection of methods, its satisfactory employment in other diseases, notably streptococcus infection, typhoid, and cholera is among future possibilities.

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# APPENDIX.

Table of additional cases of tetanus treated by tetanus antitoxine.

| | Author. | Incubation. | Method of injection. | Result. |
|---|---|---|---|---|
| 1. | Abbe, Case I.... | 16 d. | Subcutaneous. | Death. |
| 2. | Abbe, Case II... | 6 d. | " | " |
| 3. | Abbe, Case III.. | 5 d. | " | " |
| 4. | Abbe, Case VI.. | 11 d. | " | Recovery. |
| 5. | Wise..... | 8 d. (grave). | " | " |
| 6. | Holsti..... | 2 or 3 wks. | " | " |
| 7. | Meyer..... | 7 d. | " | " |
| 8. | Leyden ..... | Not given (mild). | Subdural. | " |
| 9. | Von Leyden.... | 10 d. | " | " |
| 10. | Laplace..... | 10 d. | " | " |
| 11. | Collier..... | 9 d. (acute). | " | " |
| 12. | Jabonlay..... | 8 d. | " | Death. |
| 13. | Jacob..... | 6 d. | " | Recovery. |
| 14. | Schultze..... | 3 wks. | " | " |
| 15. | Abbe, Case IV.. | 7 d. | Intracerebral. | " |
| 16. | Abbe, Case V... | 8 d. | " | Death. |
| 17. | Abbe, Case VII. | 9 d. | " | Recovery. |
| 18. | Abbe, Case VIII. | 6 d. | " | Death. |
| 19. | Abbe, Case IX.. | 19 d. | " | Recovery. |
| 20. | Johnson..... | 6 d. | " | " |
| 21. | Gimlette..... | 6 d. | " | " |
| 22. | Rogers..... | 18 d. | " | Death. |
| 23. | Fowler..... | 3 d. | " | " |