# THE PREPARATION OF SERUMS AND ANTITOXINS.*

BY CHARLES M. TWINING, BERKELEY, CAL.

The paper presents the subject of serum manufacture in considerable detail, including selection of the animal, difficulties encountered in manufacturing, preservation of the serums, etc.

The subject of serum and antitoxin manufacture has been presented many times before, yet I believe that perhaps it has sometimes been treated in a too general or too technical way, which may have made the subject one of little interest to you.

It is my purpose in this paper to take up the very practical side of the subject, to show the many difficulties which may lie in the steps that at first glance may seem very simple, and perhaps show clearly that the man who prepares the serums has his troubles, as has the man who sells them.

Taking diphtheria antitoxin as an example of the best known and most important product, we are all familiar with the fact that at the start it is necessary to prepare a toxin of the diphtheria bacillus for the treatment of the horses which are to produce the antitoxin, and in the production of this toxin lies some of the most particular work the serum maker has to do.

Very often the bacilli of diphtheria taken from the throat of very malignant cases of diphtheria in the human, and which must produce large quantities of toxin in the infected person, do not produce appreciable quantities of toxin when grown on artificial culture media. This fact has led to the adoption, by practically all producers of diphtheria antitoxin, of a particular culture of the organism isolated at the Laboratory of the New York Board of Health several years ago and known as the "Park Williams Bacillus," or Diphtheria Bacillus No. 8. This organism, when grown under proper conditions, usually produces a toxin of very high potency, yet sometimes without apparent cause the toxicity may drop to a very low point.

Ordinarily the bacillus is grown upon a culture medium made from veal and containing a relatively high peptone content, as the toxin production seems to be dependent to a large extent upon the quality and quantity of the peptone present. After the organism has grown for a period of time upon a medium which seems to be best suited for the production of toxin, it may without apparent reason suddenly refuse to produce a toxin of high potency, and in spite of every effort remain at a low level for some time, only to revert again to its original power. There is, of course, some reason for this change, but just what causes may be at work is something very difficult or impossible to determine.

When we remember that it is quite necessary to use a toxin of high potency to obtain an antitoxin of high unitage it becomes evident that this particular point in the process of manufacture is a very important one.

A toxin which will kill a 250-gramme guinea-pig within four days in a dose of $\frac{1}{300}$ to $\frac{1}{500}$ of a cubic centimetre is a very satisfactory one, and sufficient quantities of such a toxin may be easily injected under the skin of the horse to produce the maximum amount of antitoxin in the blood without injecting an excessive quantity of fluid.

The length of time of growth of the bacillus in the incubator is one important

* Presented to the Scientific Section, A. Ph. A., San Francisco meeting.

point to be determined, since if it is grown for too short a time its full toxicity is not obtained, while if grown too long the toxin formed at first is gradually destroyed and the whole becomes less toxic.

Ordinarily the bacillus is grown for six or seven days at a temperature of 37° C. to 38° C., and in the dark, since light has a serious effect upon toxin, both in its forming and afterward.

The culture medium should be in flasks having a large surface in proportion to quantity, as this organism is grown principally on the surface, and the greater the growth the greater the toxicity within certain limits.

After growth has proceeded for the proper length of time the flasks are removed from the incubator and from 0.2 percent to 0.4 percent of carbolic acid or trikresol is added to kill the organisms and preserve the finished toxin. This toxin is then filtered through a germ-proof filter, usually the Berkefeld filter, to remove the dead bacilli, since all we desire is the poison it has produced.

The toxin is stored in full amber bottles in the dark, but at best the toxicity drops quickly at first, and for best results fresh toxin should be used for the horse inoculations.

The selection of the horse is another problem about which every serum maker has his own ideas, but, as a rule, young horses four to eight years old are the most suitable and will take more toxin in a given time than older animals. However, there are exceptions to this rule, as shown by the fact that one horse over fourteen years old yielded a serum testing 1800 units per cubic centimetre, and in doing so received subcutaneous inoculations in a period of six weeks of over one million minimum lethal doses for the guinea-pig; that is, the aggregate of the toxin this horse received in six weeks would have killed more than a million guinea-pigs, each weighing 250 grammes.

As a rule, mares are preferred for serum work, since they are more easily cared for and the swelling produced by the inoculation of the toxin is not so apt to produce complications.

The methods used in immunizing the horse with toxin vary. Sometimes the toxin is weakened by heat or chemicals before injecting the first dose or first few doses. Again, the start is made with extremely small doses, as for instance $\frac{1}{100}$ of a cubic centimetre of the toxin, which represents perhaps three to five minimum lethal doses for the guinea-pig. Sometimes the horse is given an inoculation of diphtheria antitoxin just before or at the time of giving the first injection of toxin, in which case the initial dose is larger, and may be as high as 1000 minimum lethal dose.

These inoculations are usually made under the skin of the side rather than in the neck, since the swellings are probably not so painful when occurring on the side.

The apparatus used in injecting varies with the different workers, but is usually a graduated jar fitted with siphon and tube leading to the hypodermic needle; the application of air-pressure by a pump or bulb to the interior of the tube forces the toxin out, and the quantity may be read as the injection progresses.

The quantity of toxin which may be administered to a horse varies greatly with different animals, but if we accept as a standard a 300 M. L. D. per Cc. toxin, the largest quantity given at any time does not usually exceed 500 Cc. Starting with 2 or 3 Cc. of such a toxin, it may require from three to six months to bring the horse up to his greatest tolerance, and a horse usually produces a commercially valuable antitoxin within six months if at all. It is, of course, impossible to predetermine the value of a horse as an antitoxin producer; varying results are

constantly obtained, as may be shown by the fact that of a series of five horses treated in exactly the same way the antitoxin produced in a given time may run all the way from a point so low as to be of no practical value to one that tests 1000 to 1500 units per cubic centimetre, which is considered very satisfactory. It is this fact which makes the cost of production of the antitoxins very erratic and which may for one period of time show a handsome profit and for another period a loss, yet which in the end, with careful work, averages fairly close to an estimate which can be made by an experienced worker.

After the immunization has progressed for a period of two or three months, depending upon the amount of toxin given, the horse is bled from the jugular vein to determine the progress made.

The bleeding is usually made with a cannula having a diameter of 10 to 12 mm., often having a side-arm for greater asepsis. The setting of the trocar and cannula into the vein is easily done by one experienced in the work, and the animal usually makes little objection to the procedure. The amount of blood drawn from a horse in regular work varies with the weight and condition of the animal, ranging from four to eight litres, or even more in the case of heavy horses in good condition. Bleedings are usually made every thirty days, though longer or shorter intervals may be used, depending upon the condition of the animal.

The blood is drawn into sterile glass tubes having a diameter of 50 mm. and a length of 500 mm., in which the blood clots and forces out the straw-colored serum, which is the part of value containing the antitoxin. It is probable that the clot contains some antitoxin, but it cannot be readily removed, so is discarded.

After drawing, the tubes are placed in cold storage to allow the clot to form, which requires from 24 to 48 hours, differing more or less in different horses and at different periods. Sometimes the clot forms solidly and but little serum separates. Again, the separation of serum may be very great. We will recognize that commercially this is a point of very great importance, since upon the quantity of serum obtained, as well as the quality, depends the value of the animal. Upon rare occasions the amount of serum obtained is so small as to condemn the animal as a producer of serum. In case of poor separation of serum various methods to overcome the trouble have been used.

Feeding the horse various substances which may cause a freer clotting and consequent greater squeezing out of serum has been tried with more or less success. It has also been noted that in many instances lack of green feed has resulted in diminished separation of serum, which may be overcome by supplying such feed.

Another method is the placing of sterile weights upon the clot to force it down and by mechanical pressure squeeze out the serum. Such a method obviously has the disadvantage of causing greater risk of contamination.

After the serum separates it is drawn from the tubes, by the use of suction, to amber stock bottles and preserved with either chloroform or trikresol. While serum without preservative will precipitate more or less, it is this preservative which is largely responsible for the sediment or precipitate often found in commercial serums. Trikresol is more efficient as a preservative, but produces more precipitate.

It is possible, of course, to prepare a serum without preservation, but, since this entails considerably more work and has, in a practical way, little advantage, the preservative is almost universally used.

The final step in the preparation of the serum is to filter through a germ-proof filter, though this is sometimes omitted, since it is possible, even probable, that at times the filter may remove some of the active principles present. This can be

measured accurately in the case of diphtheria antitoxin, but not so with many other serums, so it may be advantageous to omit this step in some cases, especially since careful drawing and handling of the serum will almost invariably avoid contamination.

Before taking up the practical side of the purity and potency testing of diphtheria antitoxin I would call your attention to the fact that most of the diphtheria antitoxin marketed in the United States is of the purified or concentrated type, which differs materially from the natural serum type formerly supplied. In the preparation of the concentrated type of diphtheria antitoxin the blood from the immunized animal, instead of being drawn in tubes and allowed to coagulate, is drawn into bottles containing a solution of sodium citrate, which prevents clotting but allows the corpuscles to settle, leaving the plasma containing the antitoxin as a supernatant fluid, which may be decanted when the sedimentation is complete.

By this method a greater yield per horse is obtained, this increase amounting sometimes to as high as 40 percent. It should be noted, however, that such a fluid is not suited for direct inoculation into the patient, as is the case with the serum, but it must be processed before use.

Starting with the plasma, there are several methods of concentration now in use; however, the general principle underlying each is the precipitation of the antitoxin-bearing globulins in the plasma by the use of ammonium sulphate or heat and ammonium sulphate, the elimination of as great a quantity of inert solids as possible, and the resolution of the antitoxin in the smallest quantity of water possible. A small quantity of salt is added to the finished product to make it isotonic.

The concentrated form has much to recommend it, both from the standpoint of the manufacturer and the patient, the most important of which is that a much greater number of units may be administered in a given volume in the case of the concentrated than in the old type, and the rashes do not occur quite so frequently following the use of this form.

From the standpoint of the serum maker the concentrated form is preferable, since antitoxins of such low potency as not to be of value in the serum state may be concentrated to a point where they are quite acceptable.

Formerly 1000 units per cubic centimetre were considered a high potency antitoxin, while since the advent of the concentrated form unitage as high as 4800 units per cubic centimetre have been obtained; by this I mean actual material ready for use, not experimental material, which, though it may be of greater potency, may also not be in a form for practical use.

One of the factors which must be carefully considered is the solid content of the finished product, which should not exceed 20 percent. This, in fact, is one of the factors which determines the limits of potency to which a concentrated antitoxin may be raised, since the greater the quantity of antitoxin-bearing solids in the fluid the greater the specific gravity and the slower the absorption by the patient.

The tests for purity put upon diphtheria antitoxin, as well as upon other sera, are exhaustive and are quite severe.

The first test is usually made by taking 5 Cc. of material from each lot before adding any preservative, which is injected into a young guinea-pig, the animal being watched carefully for ten days.

When ready to filter the antitoxin, each filter is tested for efficiency before being used. The Berkefeld filter is used, which, as you may know, is a cylinder of diatomaceous earth having pores so small that ordinary bacteria cannot pass through, though fluids of relatively low specific gravity may do so. These cylin-

ders, properly prepared, are sterilized in steam under pressure, and when cool are set up in the usual way and a culture of some harmless organism, such as the *Bacillus prodigiosus,* is placed in position and filtration started. A small amount of the filtrate is collected and planted on sterile culture media to determine whether or not any of the organisms got through. If so, the filter is discarded. If not, it is again sterilized in steam and used for the filtration of the antitoxin.

After the filtration of the antitoxin is completed several "plants" from the finished material are made on sterile culture media to determine its freedom from living bacteria. These tests remain in the incubator for seven days, which will determine without question the purity or contamination of the material.

Again, before the antitoxin is filled a small amount is removed from the stock bottle and tested for purity on appropriate media, both aërobically and anaërobically.

After filling the syringes or bottles another cultural test is put upon two or more finished packages from each lot.

The tests for potency require a great deal of careful work, so I shall not attempt to give you a very detailed description of all the steps, but only the most important ones.

The first step requires the very accurate standardization of the toxin in the determination of what is termed the L + dose, which is the quantity of toxin necessary to kill a 250-gramme guinea-pig when mixed with one "unit" of antitoxin. Broadly speaking, a unit of antitoxin will neutralize 100 M. L. D. of toxin, so the L + dose is broadly 101 M. L. D. (I say "broadly" because these statements are not strictly correct, several factors affecting the statement.) To determine the strength of the antitoxin to be tested, a series of guinea-pigs weighing 250 grammes are selected, appropriately marked to distinguish them, and each pig receives one L + dose, together with a quantity of serum previously determined and mixed with it. For instance, if a guinea-pig received one L + dose of toxin and 1 Cc. of antitoxin, which pig lived for just 96 hours, we would say that the serum tested one unit per cubic centimetre; however, if the same results were accomplished with $\frac{1}{100}$ of a cubic centimetre, it must necessarily be 100 times as strong, or 100 units per cubic centimetre; or, if $\frac{1}{500}$ of a cubic centimetre only was required, then it must contain 500 units per cubic centimetre.

Many things may affect the accuracy of the test unless great care is used, as may be shown by the fact that guinea-pigs from one source may be more resistant than from another source, and thereby discordant results may be obtained unless the source of supply be carefully watched. This has led many careful workers to establish breeding-pens of their own, with a careful watch kept to preserve the breeds selected.

The United States Government supplies a standard antitoxin, against which each serum maker supplying serum in the United States must standardize his toxin, which in turn is used to determine the unitage of the antitoxin produced.

Since each horse reacts differently to the treatment given him and produces more or less antitoxin, depending upon this treatment, the testing of the antitoxin may sometimes consume considerable time and a large number of animals.

The final potency tests should be put upon the antitoxin after filtration, since some of the antitoxin may be removed by the filter. This is especially so in the case of the concentrated type, since the specific gravity of this form is greater than the serum form, and it often contains a greater amount of precipitate, which may carry some of the antitoxin and reduce the potency by being held back in the filter.

When all the tests are completed the material may be filled into appropriate

containers, which in the United States is almost invariably a form of syringe which can be easily prepared for use. The American physician demands convenience, and the manufacturers have been quick to supply this demand. These syringes must, of course, be quite sterile before filling the antitoxin into them, and the process of filling is one where great care must be observed.

Various forms of apparatus have been devised for the aseptic filling of antitoxin, especially since the concentrated form has come into such general use, as it may at times be rather heavy in consistency and harder to fill than the serum.

While gravity may sometimes be effective in running the material from the stock bottle to the syringe through a siphon and tube, usually arrangement is made to apply pressure. A form of apparatus is also used which automatically measures the amount as it goes to the syringe.

Since antitoxin decreases in potency with age, the amount put into a package must be in excess of its labelled unitage in direct proportion to the length of time set for its market life. For instance, let us say we are to put up a 1000-unit package of antitoxin and we want to put a " return date " upon this package one year hence. Knowing that antitoxin may decrease in unitage from 10 percent to 25 percent per year, we must put into the package an excess of 25 percent to be assured that it will test up to its labelled unitage at the end of that year.

Both diphtheria and tetanus antitoxin may be evaporated in a vacuum and dispensed in a dry form, which retains its potency for many years. However, the time and labor of getting the dry powder into solution make this form of value only in remote places where the serum is used but rarely and the keeping quality of the powder is the greatest consideration.

The method of making tetanus antitoxin does not materially differ from that of making diphtheria antitoxin, both of them being true antitoxins. The making of tetanus toxin is perhaps easier than that of diphtheria, though the method is quite different, in that, while the diphtheria bacillus requires a liberal supply of oxygen for its best toxin production, the tetanus bacillus must be grown in the absence of oxygen, since it is what we term an " anaërobe " and grows only when oxygen is excluded. This explains why deep, dirty wounds like nail punctures most often produce tetanus, since the germ is placed deeply enough to exclude the air, where it can grow and produce toxin in the body.

The strength of tetanus toxin is very much greater than that of diphtheria, as shown by the fact that of a good toxin a dose of $\frac{1}{10000}$ to $\frac{1}{20000}$ of a cubic centimetre is fatal to the guinea-pig. The toxicity may run much higher than this, though for regular work this strength answers nicely.

The testing of tetanus antitoxin differs from that of diphtheria antitoxin in that the guinea-pigs used have a standard weight of 350 grammes instead of 250 grammes, and the unit is the quantity which will protect the animal against approximately 1000 minimum fatal doses of the toxin, so it is comparatively ten times as strong as the unit of diphtheria antitoxin.

This will explain in some measure the reason why the apparent strength of tetanus antitoxin is not so great as the antitoxin of diphtheria, since a 5000-unit package of the former usually requires a larger quantity of fluid. However, 5000 units of tetanus antitoxin really corresponds to 50,000 units of diphtheria antitoxin, so its strength is comparatively much greater.

The United States Government supplies standard tetanus toxin to be used in testing the antitoxin, and all such antitoxin is so tested.

The concentration of tetanus antitoxin is carried out in much the same way as described in the making of diphtheria antitoxin, and, since it is used in large

quantities in the treatment of tetanus, a high concentration is much to be desired.

Tetanus antitoxin is sometimes supplied in a dry form to be dusted upon the surface of wounds suspected of being infected with the tetanus bacillus.

I have here described the two antitoxic serums which are of the greatest importance of any of the serums. These are the only true antitoxins of any importance.

When a patient is infected with the diphtheria bacillus the organism usually localizes in the throat and, by producing quantities of toxin, poisons the patient more or less rapidly, but the germ usually remains only at this point, and the poison, is what causes in most cases the symptoms of the disease and death where death occurs. Also, in tetanus, the germ usually localizes at a point where a wound has been inflicted, and from that point it elaborates toxin which poisons the nervous system.

Now note that it is only these two diseases that act in this way, and, since they kill by their toxin, then the proper treatment is by the neutralization of the toxin, this being done by the antitoxin. However, the bacteria causing other diseases do not form toxin under artificial conditions, nor is it known that they produce a definite toxin in the body, so we must proceed with the making of a serum by using the living, virulent bacteria themselves in the treatment of the horses used to produce serum.

Such serums are not antitoxins, but are known as antibacterial serums, and as an example, perhaps, antistreptococcic serum is the best known. The process of making this serum differs in detail only with different serum makers, but the first step is the procuring of streptococci from human patients affected with the several diseases due to this organism. This may be erysipelas, septicæmia, various suppurative conditions, etc., and the greater the number of different cases from which these are gotten the greater the range of efficiency of the serum in treatment, presumably.

Usually the first few inoculations of the horse are of the killed bacteria, followed by increasing amounts of the live, virulent streptococci.

Sometimes the serum maker places his dependence upon one strain of the organism; that is, the streptococcus from one case of one disease, which has been passed through guinea-pigs or rabbits many times until it is very virulent. Whether serum so made is as efficient as one made by using many strains not so virulent but from a wide range of diseases due to the streptococcus is a disputed question, but the greater part of the serum made in America is of the many-strain type, which is therefore termed a polyvalent serum.

Quite naturally the effects upon the horses are greater in using the living bacteria than from the use of toxin, since the effect of the toxin is self-limited, because it cannot increase in quantity or virulence, and can be counteracted by antitoxin if the reaction is too severe, while with the live organisms, which can increase in number and virulence, great care must be used, as they may cause death, as would any natural disease due to the same cause, therefore it is necessary to build up the horses' immunity slowly and carefully, all of which requires much time; so the production of any antibacterial serum of proper potency requires more time than the production of either tetanus or diphtheria antitoxin.

The testing of the efficiency of antibacterial sera cannot be done with the accuracy that obtains in the testing of the antitoxins, since it is not known upon just what principles their curative action depends.

The tests put upon antibacterial sera may be a determination of the opsonin content, which is the quantity of a substance that prepares the bacteria causing a disease so they may be easily destroyed by the phagocytes or white blood-corpuscles.

The agglutination test is used to a considerable extent, this test showing the power of the antibacterial serum to agglutinate or clump the disease-producing bacteria.

Another test of some value is the determination of the quantity of bacteriolysin, or substance which acts by dissolving the pathogenic bacteria.

Tests upon small animals have been used to a considerable extent by first ascertaining the minimum amount of the organism, against which the serum is protective, which is fatal to a mouse or guinea-pig. Then a series of these animals are given this minimum fatal dose and varying quantities of the serum under test. The serum should protect in a quantity arbitrarily set as a standard. However, it is extremely difficult to determine the minimum fatal dose of a living organism because of the great difference in susceptibility and resistance among animals of the same lot, even when carefully selected. As a matter of fact, some tests of this kind would tend to show that even a very well made serum had no protective power, which may indeed be the case in so far as the mouse or guinea-pig is concerned, yet this cannot be considered a measure of its effectiveness for the human.

I cite antistreptococcic serum as an example because it is the best known and is a good type of antibacterial serum. Others, like antipneumococcic, antimeningococcic, anti-anthrax, anti-dysenteric, and antigonococcic serum, are used to some extent, and are made in much the same way. Antimeningococcic serum has given some very nice results during the last few years. The making of antimeningococcic serum is attended with probably more danger to the horse than the others, since to produce a serum of high potency it is necessary to force the treatment of the horse to the limit of tolerance. In the making of antimeningococcic serum, in addition to dead and living meningococci, we use autolysates of the organism, which is the germ grown in appropriate culture media and allowed to remain there until dissolved by the action of its own poisonous products.

The preservation of antimeningococcic serum presents a problem which is not present in most other serum work, in that this serum is administered almost entirely by inoculation into the spinal canal; therefore the kind and amount of preservative are of great importance.

Quantities of trikresol which are quite harmless when injected subcutaneously or intravenously are, when injected intraspinally, more or less dangerous. Therefore it is necessary to use a smaller quantity of preservative in this serum than in others, usually 0.1 to 0.2 percent trikresol or 0.1 percent chloroform.

When chloroform is used it may be volatilized by the application of mild heat to the serum before using. This, however, entails considerable work and is seldom done.

Antivenomous serum is another type of serum which has been used in this country to some extent, especially in the treatment of rattlesnake bite. This serum is prepared by treating rabbits, goats, or horses with gradually-increasing doses of venom from poisonous snakes, particularly the cobra and rattlesnake. The effect of venom when injected into the animal is immediate, and great care must be used in graduating the doses. Heat is sometimes used to attenuate the venom before injecting it. It will be noted that such a serum is not an antitoxin of the type of diphtheria or tetanus antitoxin, nor is it an antibacterial serum in any sense, but, as its name implies, a serum opposed to venom.

The venom from the cobra causes a dissolving of the blood-corpuscles of the patient bitten; also, it affects the nervous system considerably, while the effect of rattlesnake bite is shown principally upon the endothelium of the blood-vessels, which accounts for the severe hemorrhages usually seen in such cases. Therefore it would appear that the antitoxin for one type of venom would not be effective

in the treatment of bite carrying the other type. It should therefore be borne in mind that the most effective serum for each type of bite is the one made by the use of that particular venom. Serum may be made by using varied venoms in the treatment of the serum-producing animal, making a polyvalent antivenomous serum.

Another type of serum is that known as antipollen serum or pollen antitoxin (hay-fever serum).

The pollen of certain plants is the cause of certain very distressing symptoms in some persons, and in an endeavor to obtain an anti-substance a serum has been produced by the treatment of horses with this pollen, properly prepared. The pollen is mixed with sodium chloride solution containing a preservative, and this mixture is precipitated with alcohol and the precipitate is dissolved in salt solution.

The results from the use of this serum have been, on the whole, fairly good in properly-selected cases.

It should be noted that such a serum is applied locally by dropping into the eye or spraying into the nostrils or throat instead of by subcutaneous, intravenous, or intraspinal inoculation, used in administering other serums.

Normal serum, as its name implies, has no specific qualities and is simply the serum obtained from the normal blood of the human or from various animals, as the rabbit, sheep, or horse.

The serum of this type in greatest use is from the horse, and is prepared as are the other serums, except that the horse receives no specific treatment. The serum is useful in supplying those substances which facilitate clotting of the blood, and in the treatment of some types of hemorrhages in the human it is of great value.

Efforts are sometimes made to increase the quantities of these clot-forming substances in the blood of the horse by special feeding, which is probably of little avail; also by frequent bleedings we may obtain a stimulation of the production of such substances, and, therefore, perhaps, a horse which has been bled many times may produce a better normal serum than one bled but seldom.

The fact that a few of the serums have been quite effective has led to the preparation of many that have been of little or no value. These are of so little importance that they are not here mentioned. Among these, however, are a few of some interest because they are of a still different type from those before described.

One of the most absurd is an anti-alcoholic serum for the treatment of persons who imbibe too freely. Such a serum has been advocated, made by treating horses with large quantities of alcohol by the mouth, presuming that after a period of such treatment the blood of the horse will contain antibodies effective in treating alcoholics.

One is constrained to remark that if this theory were sound some very effective serum might be obtained by bleeding certain human beings without any further treatment.

No doubt many obstacles now standing in the way of the production of effective sera will be removed by the constant and conscientious efforts of the many who are now engaged in this work, and it is not too much to predict that some day we will have serum of one type or another that will be of sufficient specificity for each of the diseases due to bacteria to reduce the mortality in such diseases, as diphtheria antitoxin has in the last twenty years reduced the diphtheria mortality.

That the methods now used in the production of antibacterial sera must be changed or greatly augmented there is but little doubt. Along what lines such changes may be made must be left to one whose power of visualization is greater than mine.