Papers Presented to Local Branches

THE PRODUCTION AND CARE OF ANTITOXINS AND VACCINES.*

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I have been asked by the President of your Association to read a paper this evening on the production of antitoxins and vaccines, and I have tried to arrange my remarks in order to have them of interest to the pharmacist.

The enormous progress that has been made in the past twenty years, and indeed I might say in the past five years, has brought so many new angles in the treatment of disease, that it well behooves the pharmacist of today to keep in touch with the production of biologicals. For I believe—and perhaps my belief will be shared by many of the gentlemen present—that the coming way to cure a disease will be to prevent it.

I refer, of course, by such a paradoxical remark, to the question of immunity —both the "natural" and "acquired." By "natural" we refer to the power of our bodies to resist infection. This power has probably been handed down by our forefathers to a certain point, and strengthened to a great extent by our exposure to attenuated forms of many prevalent pathogenic organisms. In a broad sense, "acquired" immunity, here, may be taken to mean both the condition of those who have passed through a mild or severe form of some disease and have produced their own protection against future invasion of that particular organism, and that of those in whom immunity has been produced, at least passively, by the injection of anti-bodies. These anti-bodies are usually contained in what we commonly know as antitoxins.

Of all antitoxins, the best known, and most widely used, is the diphtheria antitoxin. As the procedure in all is practically the same, it will be sufficient for me to explain the producer's side of this one only.

In 1883, the diphtheria bacillus was first described by Klebs, and in 1884, was cultivated on artificial media by Loeffler. Hence it is commonly known among bacteriologists, as the Klebs-Loeffler bacillus. In 1888, Roux and Yersin discovered the presence of toxin in broth cultures. This bacillus, and its power to produce toxin, is the foundation, the all-necessary starting point, in the production of antitoxin for diphtheria. We keep this bacillus constantly growing in the laboratory-incubators.

The culture now used by, practically, all the laboratories in the work, was obtained from a case of diphtheria, by Dr. Parks, of the New York City Board of Health, and has proved the strongest toxin-producer so far procured, although it has been grown for many years on artificial media.

After we have the bacillus, the next step is to get the maximum toxin-produc-

*Read before the City of Washington Branch, April, 1914.

tion. This is done by growing it on specially prepared peptonized beef-broth. This broth must be made from fresh beef, freed from fat. To the extracted juice is added, 1½ percent peptone and ½ percent NaCl. It is then titrated, using phenolphthalein as an indicator. The acidity is reduced to 0.8. It is then flasked and sterilized in an autoclave at a temperature of 116° to 120° C., or about ten pounds steam pressure. This exposure to heat renders the beef broth sterile. It is then ready for inoculation.

This is done by transferring a small portion of membranous growth from our stock culture. The development is very rapid. In fact, the whole surface of the medium will be covered by a heavy membrane after twelve to eighteen hours' growth at 37° C. The growth is allowed to continue for from eight to ten days. The flasks are then taken from the incubator and examined microscopically. If pure, they are then titrated and should be found alkaline to phenolphtalein.

Filtering this culture through a germ proof filter, is the next step. After filtering, the toxin is then tested on guinea pigs to determine its strength.

This is done by injecting varying amounts. For instance, three pigs are selected, each weighing from 250 to 300 grams. They are injected subcutaneously, No. 1, 2, and 3, with 0.005 cc., 0.008 cc., and 0.01 cc. of toxin, respectively. The pig that survives is counted out of the test; the one that dies on the fourth or fifth day is taken to indicate the M. L. D. or minimum lethal dose of this particular toxin.

It is now ready for injection into the horse. I will say here that great care is exercised in selecting the animals for an antitoxin stable. They are kept in a quarantine stable until they have satisfactorily passed the test for glanders and tuberculosis. Temperatures are taken night and morning of all horses. When a horse is finally pronounced ready, the initial dose of toxin is given. This dose is usually about 0.1 of a cubic centimeter, and sometimes gives a very decided reaction. I have known on several occasions where a horse weighing from 1000 to 1200 pounds would be killed in from twenty-four to thirty-six hours following an injection of only 0.2 cc. of diphtheria toxin. The doses are gradually increased, as the immunity is established, until the horse will stand 1500 or even 2000 cc. of strong toxin. This amount would be sufficient to kill 3000 or more non-immunes. In many cases the horse will stand from 500 to 700 cc. of toxin without decided reaction. The blood is drawn from the jugular vein. From this point we will discuss antitoxin.

The blood is drawn into sterile jars containing sodium citrate solution, in sufficient quantity to bring the content to 2 percent. This prevents the coagulation, and allows the corpuscles to settle to the bottom of the jar. The jars of blood are kept in the ice box until the serum or plasma is ready to be decanted. The decanting is done by vacuum. The plasma is drawn into sterile containers. It is now ready for precipitation.

The method of precipitation has recently been decidedly improved upon by Dr. Benzhaf, of the New York Department of Health, and his method is now in use in our laboratory. I will here set forth the routine:

We first measure our plasma. To the total quantity is added 50 percent of a 4 percent NaCl solution. To the total quantity resulting from this mixture, is

added approximately 32 percent of a *saturated solution* of ammonium sulphate. This mixture is allowed to stand—usually over night. It is then heated in a water bath built especially for the purpose. The temperature of the mixture is brought to 60° C. and held there for ten minutes. It is then removed from the bath and filtered while hot through filter paper. This precipitation, called "first fraction," has thrown down the euglobulin, which is collected on the filter paper, and, later, after being pressed to dryness, is discarded.

The filtrate is measured, and to the resulting volume is added, approximately, 38 percent ammonium sulphate in saturated solution. This we call our "second fraction" and results in the precipitation of the pseudo globulins, (and is high in antitoxin value). This precipitate is collected on the filter papers and is placed between absorbent or blotting paper and subjected to a great pressure in a press arranged for the purpose. By this means all moisture is extracted and the precipitate is scraped from the papers in the form of a dry, powder-like substance, which is immediately put into parchment bags and dialyzed in running water from six to ten days, during which time it is redissolved and practically freed from the salts which have been used in its precipitation.

The resulting antitoxin is now of a decided green color, and is ready for the preliminary filtration. This is accomplished by drawing it through a pulp paper by means of a vacuum pump. After this filtration, 0.8 percent sodium chloride is added and the antitoxin is ready to test.

The testing is an all-important feature and must be accurately and scientifically performed. The method of testing is as follows:

First. We must determine the L+ dose of our test toxin. The L+ dose is the quantity of poison, not only sufficient to neutralize one antitoxin unit, but it must contain excess sufficient to kill a 250 gram guinea pig on the fourth or fifth day. The exact dose now having been determined, this amount is mixed with varying dilutions of the antitoxin to be tested. For instance, a serum which is supposed to contain 300 to 400 units to the cc. is diluted as follows: 1:200, 1:250, 1:300, etc. One cc. of each of these dilutions is mixed with the L+ dose of the toxin in the injection syringes and allowed to stand at room-temperature for one hour, then injected subcutaneously into selected guinea pigs. If the animal receiving L+ plus the 1:250 dilution, lives, and the one receiving L+ plus the 1:300 dilution, dies, we know that the unit strength lies between these values and further tests will establish it almost accurately.

The strength determined, the antitoxin is filtered through germ-proof filters and one-tenth of one percent chloroform is added and thoroughly mixed.

This is now stock and is stored in the ice box at a temperature of 40 degrees where it is kept until filled into syringes and distributed to the pharmacist upon order.

I have here samples of antitoxin showing the various stages of its preparation, which I have tried, at least, to describe to you.

There are several other antitoxins, such as tetanus, antimeningococcus, streptococcus, and recently one has been developed for pneumonia at the Rockefeller Institute. A bulletin on this subject will soon be published.

In serums we have also the normal horse serum, which has been used extensively in the control of haemophilia with splendid results.

Vaccine now comes up for consideration. Until recently when one spoke of vaccine it meant only one thing to us all—smallpox vaccine. Since 1796 vaccination against smallpox has been practiced. This work was first done by Jenner, and his observations were published as far back as 1798. In spite of this lapse of time, the etiological factor which causes the disease is still unknown. We do know, however, that with successful vaccination and quarantine, the dreaded scourge has been robbed of many victims.

Vaccination today, is quite a different problem from the procedure of the past, and is gradually wearing down the prejudice held by so many, who were sufferers, either directly or indirectly, as a result of the unscientific method of charging our vaccine points directly from the lesions on the calf, or using the scab from the arm of one person to vaccinate another. In this way it was possible to carry infections and have very sore arms, or even much more serious consequences. Such danger at the present time is practically *nil.* For we now use great care in the selection of our seed lymph, and by so doing have now almost a pure vaccine. This is put on calves selected by and under daily inspection of a veterinarian. These animals are housed in a modern building, put up for this purpose alone, and kept in the best state of cleanliness. The calf, after vaccination, is kept under observation practically day and night, until the "take" is complete and the vaccine is ready to be removed and prepared for commercial use.

The "pulp," as we term the ripened vaccine as it comes from the calf, is brought to the laboratory and goes immediately into glycerin. The effect of the glycerin is to kill off the bacteria which have gotten into the vaccine during the period of incubation on the calf, leaving the true vaccine virus unharmed and still highly potent.

After weeks in glycerin the "pulp" is finely ground and fresh glycerin is added. It is then tested both bacteriologically and physiologically. The lymph having proved pure and potent is then charged on ivory points, or filled into capillary tubes, ready for use. The lymph will remain potent for a long time if kept under proper conditions. By "proper conditions," I mean that these points and tubes should be kept in a cool, dark place. These instructions, which are printed on every package, do not seem to be taken seriously by some pharmacists, and, consequently, the lymph dries and loses its potency much sooner than it should, and thence results the "failures" which not infrequently occur with all vaccines, a matter which is of no small moment to the physician, and of these cases, I assure you the producer is kept *well* and sometimes *harshly informed.* Within the past few days, a physician told me that he had to go to several places before he found one where vaccine was kept in an ice box. He had experienced trouble with vaccine kept on the shelf at room-temperature, and did not wish to have it repeated. I want to lay stress upon the fact that biological products should be kept in a cool and fairly constant temperature. If this rule is followed the results will be better for us all.

As an illustration of this, I will say that between February 5 and 19 of this year we had the opportunity to have our vaccine tested on about one hundred children in an institution of this city. The work was done by the physician of

the institution, and he reported 100 percent of "takes" in primary cases, with no commonly called "bad arms."

The recent development of bacterial vaccines has brought a new field, both to the laboratory and the pharmacist, and their use is rapidly becoming a routine with many physicians in treating furunculosis, otitis media, pyorrhea, gonorrhea—in fact, wherever pus production is present, and in septic conditions generally.

This treatment consists of making the vaccine from the bacteria or combination of organisms causing the pathological condition. This is done by growing the bacteria on suitable media, that is selecting the medium on which the best and most rapid growth takes place. After satisfactory development, the bacteria are washed off in sterile normal salt solution, and thoroughly shaken in a bottle containing beads. This is done to separate or thoroughly break up colonies or clumps of bacteria. This accomplished, they are exposed to heat sufficient to kill all the germs present. 0.1 percent to 0.2 percent trikresol is then added and the suspension, or emulsion as it is sometimes called, is allowed to stand over night.

The next day cultures are made on various media, both ærobic and anærobic, using from 1 cc. to 2 cc. for each tube. These are incubated for from two to four days, and, if they remain sterile, the vaccine is then ready to standardize, that is to count the bacteria in each cc. This procedure is the same as that used in determining the number of corpuscles in the blood.

After standardizing, any dilution desired lower than the original count can be made.

Not only does the injection of this vaccine control the infection, but produces an immunity that is more or less lasting against future recurrence.

This method of treatment is constantly being tried along new lines, and splendid results are being reported almost daily.

The theory is, that by the injection of the killed bodies of the bacteria, the opsonic index is raised and the body is able to successfully resist the invading bacteria. Raising the opsonic index means stimulating the action of the polynuclear leucocytes and preparing them to envelope the bacteria and look after their destruction. It might be said that you prepare your army to fight the foe.

This has been proved most conclusively in the use of typhoid vaccine in the Army and Navy, and also by the many injections made recently by the general practitioner. You are all, of course, more or less familiar with this work. To those of us who are following closely the study, it looks as if the practice would become even more general than the vaccination against smallpox.

In conclusion, I want to say, that the modern and very material advance in medical science, is being built on a more firm foundation, and the component parts of this foundation are, Anatomy, Physiology, Bacteriology, Pathology and Pharmacology. Let us each do what we can for their advancement and the benefit of mankind.