While we have used this method of sterilization only in tissue fibrinogen it offers very interesting possibilities in the ever-growing field of protein preparations for injection because we are able by it to obtain a sterile product without permanently affecting its nature.

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BIOCHEMICAL LABORATORIES
OF THE WM. S. MERRELL CO.,
CINCINNATI, OHIO.

ABSTRACT OF DISCUSSION.

The author was asked if the mercuric bichloride left in the solution would be sufficient to sterilize the product. The author replied that this was impossible for the concentration of mercuric chloride in the finished product was only 1 in 25,000 while 1 to 800 is a minimum quantity to affect the sterilization of anthrax bacillus.

The author was further asked whether the method had been applied to vaccines. In answer thereto he said that it offered a very interesting possibility for the sterilization of vaccines without heat, but he had not completed experimentation and, therefore, was not in position to give a definite answer.

PNEUMOCOCCUS ANTIBODY SOLUTION.*

With Special Reference to Its Nature, Preparation, Administration and Effect.

BY PAUL S. PITTENGER.

Antibody solutions are a new classification of biologic products just beginning to come into use.

Their importance cannot be overestimated as clinical data (1, 2, 3) already indicate that Pneumococcus Antibody Solution will no doubt prove to be the greatest discovery in the biologic field since the discovery of Diphtheria Antitoxin.

Immediately following the discovery of Diphtheria Antitoxin and publication of clinical data showing the remarkable reduction in mortality as a result of its use, pharmacists were called upon by physician and layman alike for all kinds of information in reference to its nature, preparation, administration and effect.

Clinical data have already been published by competent clinicians (1) showing that the lobar pneumonia mortality has been reduced to one-half the usual rate in cases receiving early treatment with Pneumococcus Antibody Solution.

It is to be expected, therefore, that we as pharmacists will within the near future be called upon to supply this new product and give information in reference to it.

In view of the fact that few physicians or pharmacists are as yet familiar with this latest classification of biologic products, I thought that a paper on *Antibody Solutions* would be acceptable at this time.

Pneumococcus Antibody Solution is the first of these solutions to be successfully produced on a manufacturing scale. I will therefore confine my remarks to a description of this product.

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* Scientific Section, A. Ph. A., Asheville meeting, 1923.

NATURE OF PNEUMOCOCCUS ANTIBODY SOLUTIONS.

The nature of Pneumococcus Antibody Solution can be best described by showing how it differs from Antipneumococcic Serum.

Antipneumococcic Serum is prepared by injecting horses intravenously with emulsions of killed Pneumococci, Types I, II, III, in gradually increasing doses until the horse establishes a very high degree of immunity, and it can be demonstrated by laboratory tests that there are considerable quantities of antibodies existing in the horse's blood.

It is then possible to start injecting the horse with infinitesimally small doses of living organisms of the same kind and types. These quantities can be gradually increased as the immunity is increased. Injections are continued during a period of several months after which the horse is said to be hyper-immune and is ready to be bled.

Approximately 50% of the horse's blood is serum which contains antibodies. This serum is tested for potency both by laboratory and animal tests.

When the serum is of such potency that it will protect a white mouse against one billion fatal doses of pneumococcus, it is generally held the serum has a titer sufficiently high to be of therapeutic value.

A small quantity of preservative is added and the serum sterilized by passing through Berkfeldt filter.

Although Antipneumococcic Serum can be made of such strength that it protects against one billion fatal doses of the pneumococcus, and is therefore a valuable agent in the treatment of pneumonia, it has certain disadvantages. The principal objection to the serum is the fact that it contains large quantities of horse protein and may therefore produce serum sickness or sensitize the patient so that subsequent serum injections are liable to occasionally produce anaphylaxis.

In Antipneumococcic Serum the serum itself is of a colloidal nature, and the protein is of little or no therapeutic value.

The serum merely acts as a menstruum for carrying the specific antibodies.

Pneumococcus Antibody Solution.—In an endeavor to get rid of the objectionable serum, biologic chemists have for years tried to separate the antibodies from the serum.

This separation was first accomplished on a successful manufacturing scale four years ago by Dr. F. M. Huntoon of the Mulford Laboratories. Huntoon's proctocols were published in the *Jour. of Immunology*, 6, 117, March 1921.

This separation was accomplished by a very ingenious procedure based upon the fact that if killed pneumococci are added to Antipneumococcic Serum, the antibodies in the serum will become attached to, or absorbed by the killed bacteria.

The bacteria with the contained antibodies can then be removed and washed free from serum, after which the antibodies themselves can be redissolved in water and separated from the dead bacteria, thus leaving a water solution of the antibodies containing only about 0.035 mg. of nitrogen per cc.

Pneumococcus Antibody Solution is therefore an aqueous solution of pneumococcus antibodies having the same protective value as the serum, but is practically free from all serum proteins and solids.

It is practically permanent in appearance and is of the color and density of distilled water.

The nature of this solution was described in a recent paper by Drs. Cecil and Larsen (2) as follows:

"The advantages of this preparation (Pneumococcus Antibody Solution) are:

"1. It is polyvalent; that is, it is aimed against the three fixed types of pneumococcus; and according to our figures, it has also shown itself to be of distinct value in the Type IV pneumonia. By reason of its polyvalent character, typing of the sputum is no longer absolutely necessary, and treatment of the patient can be instituted as soon as the diagnosis of Lobar Pneumonia has been made. This of course is of great advantage over serum."

"2. Pneumococcus Antibody Solution is practically free from horse protein, hence there is no danger of anaphylaxis or serum sickness. This removes a great source of worry for the physician and discomfort to the patient and there is therefore no reasonable limit to the amount of antibody solution that can be administered."

PREPARATION OF PNEUMOCOCCUS ANTIBODY SOLUTION.

The principle involved in the method of manufacturing this solution follows:

1. The antibodies are in suspension in the Antipneumococcic Serum.
2. Killed pneumococci are added to the serum and the antibodies are absorbed by the killed bacteria. In other words, we have a combination of antibodies with antigen (killed bacteria).
3. The antibody-antigen combination is then broken up by suspending the combination in salt solution, from which the killed bacteria are removed, allowing the antibodies to remain in suspension.
4. The antibody solutions are then sterilized by filtration and supplied without preservative, after it has been shown that the antibody solutions are equal in potency to the serum.

It will be noted, therefore, that if a serum is prepared by immunizing a horse with emulsions of Types I, II, III Pneumococci, an antibody solution can be prepared from this serum which will be practically serum-free and have the same protective value as the original serum from which it is prepared.

ADMINISTRATION OF PNEUMOCOCCUS ANTIBODY SOLUTION.

In the first clinical tests the solution was administered *intravenously*.

The results obtained in over four hundred cases treated by intravenous injection are reported in a paper by Drs. Cecil and Larsen (1) of one hundred and three cases in a paper by Dr. Connor (3).

It was found, however, that the intravenous injection was usually followed by rather severe general reactions.

Later investigations by Dr. Cecil have shown that equally good results can be obtained by *subcutaneous injection* without the severe reaction.

The subcutaneous method of administration is, therefore, to be preferred because this method has produced no untoward effects other than a mild local reaction.

The solution is promptly absorbed and can be administered to infants as well as aged patients.

Dosage.—The initial dose in mild cases is 50 cc. to 100 cc. subcutaneously; in severe cases, 100 cc. to 300 cc. Two doses are usually given the first day, from eight to twelve hours apart. This is usually followed by one or two doses on subsequent days according to the severity of symptoms.

As the solution is practically serum-free there is no danger of serum sickness

or anaphylaxis and as much as 1000 cc. have been administered to one patient within 24 hours.

There are apparently no contraindications as very sick patients have been treated with over 1000 cc. within 24 hours.

EFFECTS OF PNEUMOCOCCUS ANTIBODY SOLUTION.

The therapeutic efficacy of Pneumococcus Antibody Solution has been tested clinically in the wards of the Bellevue Hospital, New York, and the New York Hospital. These tests were carried out in comparison with the older methods of treating pneumonia as controls. In Bellevue Hospital the work was conducted by Dr. Russell L. Cecil and Dr. Nils P. Larsen. They were under the supervision of coöperative arrangement between Bellevue Hospital, the Influenza Commission appointed by the Metropolitan Life Insurance Co. at the suggestion of its vice-president, Dr. Leo. Frankel, Cornell University Medical College working in co-operation with the Hospital and the Mulford Co.

The preliminary results of the clinical study made by Drs. Cecil and Larsen have been published in the *Jour. A. M. A.* (1) and in a paper read before the Philadelphia College of Physicians and published in the *Weekly Roster and Medical Digest* (2).

A few abstracts from the latter paper by Dr. Cecil follow:

TABLE I.

"Comparison of Death Rate in Treated and Control Series.

	Cases treated with antibody.			Control cases.		
Type.	Cases.	Deaths.	Rate %.	Cases.	Deaths.	Rate %.
Pn 1.....	157	21	13.4	175	41	23.4
Pn 2.....	78	22	28.2	76	31	40.7
Pn 3.....	57	20	35	60	24	40
Pn 4.....	109	17	15.6	137	31	22.8
Total.....	401	80	19.9	448	127	28.3
Strep. Hemo.....	21	10	47.6	21	9	42.8
Strep. Vir.....	7	1	14.2	10	1	10

"The results of treatment with antibody were much more striking in cases that were treated early in the disease. For example, in Table II it will be noticed that the death rate for Type I, pneumonias treated during the first 48 hours of the

TABLE II.

"Death Rates for Pneumococcus Pneumonias Receiving Antibody on or before Third Day of Disease. Control Shows Death Rate for Pneumococcus Pneumonias Admitted to Control Wards on or before Third Day of Disease.

	Cases treated with antibody.			Control cases.		
Type.	Cases.	Deaths.	Rate %.	Cases.	Deaths.	Rate %.
Pn 1.....	56	5	8.9	68	16	23.5
Pn 2.....	24	5	20.8	25	8	32
Pn 3.....	10	1	10	19	7	36.8
Total.....	90	11	Ave. 12.2	112	31	Ave. 27.6
Pn IV.....	24	4	16.6	45	11	24.4
Total.....	114	15	Ave. 13.1	157	42	Ave. 26.7

disease, was only 8.9 per cent., while controls admitted to the hospital during the first 48 hours of the disease showed a death rate of 23 per cent. Similar reductions are noticed in the other types. Altogether 114 pneumococcus pneumonias treated with antibody during the first 48 hours of the disease showed a death rate

of only 13.1 per cent., whereas 157 pneumococcus pneumonias in the control series ran a death rate of 26.7 per cent., practically twice as high."

The published clinical data show that Pneumococcus Antibody Solutions are specific in the treatment of all types of pneumococcus pneumonia, but without effect in streptococcus or B. Friedlander pneumonias.

Dr. Cecil states that "Upon the evidence which has been presented we must conclude that Pneumococcus Antibody Solution is a therapeutic agent of considerable power. The effect is most striking when the antibody is administered early in the disease, but it may also be of value in the later stages by neutralizing the circulating toxins."

Antibody solutions have for the past two years been supplied to the leading pneumonia specialists throughout the United States for clinical study. The satisfactory results obtained by these specialists as shown by the above abstracts indicate that antibody solutions mark an important step in advance in the specific treatment of lobar pneumonia.

It is quite possible that other antibody solutions will be developed in the near future and it is to be expected therefore that within the near future we as pharmacists will be called upon to supply antibody solutions.

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RESEARCH LABORATORY,
H. K. MULFORD COMPANY,
PHILADELPHIA, PA.

ABSTRACT OF DISCUSSION.

A MEMBER: I would like to ask whether it would not be possible to find some absorbent for the antibodies, other than bacteria.

MR. PITTENGER: No other means have been found.

MR. SNYDER: There has been an unlimited amount of work done in this direction but I believe that this is the only workable method that has been discovered.

MR. KEBLER: Some very interesting things have been brought out in this paper which may eventually prove to be of great value to the medical profession, but I wish to caution the members against becoming over-enthusiastic. The matter of antibodies is not as yet thoroughly understood. I would therefore like to call attention to the unfortunate conclusions that are frequently made by men who are in this line of work.

I do not think that these men would deliberately make misstatements in regard to a discovery, but the trouble lies in the fact that they become absolutely enthusiastic over their subject and over-estimate the possibilities of what they have discovered. I do not want to criticize Dr. Pittenger, but it is the idea that I wish to emphasize.

A few years ago there was a great deal of work done and talk on the treatment of pneumonia with anti-pneumococcic serum. Dr. McCoy has told me that although they have gone into the subject very thoroughly and used the serum intravenously and intramuscularly, on a large number of cases they found the treatment to have very little merit or advantage over other methods.

We have all seen cases, conspicuous indeed, many of them, where men have gone astray, not wittingly but unwittingly, and I just want to add a word of caution about making assertions that later may be shown to be absolutely inaccurate.

MR. PITTENGER: In answer to Dr. Kebler, I would like to say that just for the particular

reason which he has so fully brought out, the investigation of the clinical value of Pneumococcus Antibody Solution was turned over to an absolutely disinterested commission.

This work was carried out in the wards of the Bellevue Hospital, New York, and the New York Hospital. The tests were under the supervision of the Influenza Commission consisting of Drs. Roseneau, Parke, McCoy, Frost, Jordan, Cecil, Frankel and Knight, with coöperative arrangement with Cornell Medical College, Metropolitan Life Insurance Co. and the Mulford Co.

In Bellevue Hospital the work was conducted by Dr. Cecil and laboratory attachés of U. S. Hygienic Laboratory.

I think this is probably the first instance in which a scientific discovery was turned over to such a commission with Government coöperation and investigation as to its clinical value.

We realize the fact that by selecting the cases, unusually favorable results may be obtained. In this investigation, however, the physicians in the hospital did not select their patients.

Arrangements were made for proper controls, that is to say, every other patient regardless of condition would be treated with Pneumococcus Antibody Solution, while alternate patients would receive the best known treatment without antibodies. As a result of this the data include patients received in the hospital in all stages of the disease.

In addition to the above hospitals, the serum has been furnished to pneumonia specialists in many of the large cities, and to hospitals throughout the United States. The results of these clinical tests are to be published.

Although we have had hundreds of requests for Antibody Solution it has not as yet been placed on the market for sale.

I can appreciate Dr. Kebler's point in view of the remarks of one of the Cleveland specialists who described a treatment for pneumonia which had given him wonderful results one season and was practically of no value the following season.

This investigation is, therefore, in the hands of our most eminent specialists and all data have been subject to control cases.

No statements will be made for this product that do not come from these specialists regarding its clinical value.

AN ADULTERANT OF QUEEN-OF-THE-MEADOW.*

BY OLIVER ATKINS FARWELL.

This drug is the herb, or, to speak more accurately, the leaves and flowering tops of *Eupatorium maculatum* Linn. In pharmaceutical literature it passes under the nomenclatorial name of *Eupatorium purpureum* Linn probably because when this name was adopted, *E. maculatum* had been reduced by botanists in general to a synonym of *E. purpureum* The leaves of *E. purpureum* are thin and even, not rugose, the stems are glabrous or whitish puberulent at the summit and the flowers are whitish or pale pink. In *E. maculatum* the leaves are thick and prominently rugose, the stems at the summit are densely glandular pubescent and the flowers are rose-purple. It is probable that both species enter into the make-up of the drug as found upon the market, but it has been my experience to find that it is mostly *E. maculata.* In general, this drug is very free from adulteration. Some lots of recent collection have been found to contain as much as 20% of Ironweed, *Vernonia Noveboracensis* Willd. It would be a difficult matter to say if it were an intentional adulteration. But the two plants are so unlike in the living state that it needs must stretch one's credulity to the limit to believe that the adulteration was accidental. If well spread out and mixed up with the genuine drug, the Ironweed might go undetected except through the closest scrutiny.

DEPARTMENT OF BOTANY,
PARKE, DAVIS & COMPANY, DETROIT, MICH.

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* Scientific Section, A. Ph. A., Asheville meeting, 1923.