

FURTHER EXPERIMENTS WITH THE INTRADERMAL PNEUMOCOCCUS INFECTION IN RABBITS.

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In a previous communication (1) we have described the characteristic symptom-complex brought about by infecting rabbits intradermally with Type I pneumococci. The following paper is a continuation of that report.

I.

Specific Serum Therapy.

Since we believe that the rabbit experiment as described in our first paper promises a method of determining the therapeutic potency of specific antipneumococcic serum more satisfactorily than the now prevalent mouse protection technic we have given considerable attention to the effects of serum administration on the course of the pneumococcus infection of rabbits, both in regard to the local and general phenomena.

The following points have already been established:

1. That animals recover promptly after the intravenous administration of large amounts of antipneumococcic serum.
2. That there are four important features of this induced recovery, as follow: (*a*) the immediate and permanent disappearance of organisms from the circulating blood; (*b*) a drop in temperature to normal levels within 24 hours; (*c*) the disappearance of organisms from the local lesion in the course of 4 to 20 hours; (*d*) loss of inflammatory color of the local lesion within 24 hours, usually accompanied by epidermal desquamation.
3. That for a given serum at a given stage of the disease there may be established a *minimal effective dosage* (M.E.D.) such that larger

amounts give no added therapeutic value and smaller amounts are insufficient to bring about recovery.

Minimal Effective Dosage at Various Stages of the Disease.—It is a common clinical observation that the amount of serum which might be used effectively on the 1st or 2nd day of lobar pneumonia is insufficient if given at a later time. Some clinicians have even stated that after the infection has progressed beyond a certain point no amount of serum will save.

The question of a quantitative element has been studied in the experimental "dermal pneumonia" of the rabbit by determining the minimal effective dose of a given serum for each of three stages of the disease. It is necessary to stress again the sharpness of the end-point in determinations of the effectiveness of therapeutic sera. The results of such an experiment are shown in Fig. 1.

This experiment shows that the amount of serum necessary for effective therapy increases very rapidly as the disease progresses and emphasizes the importance of early treatment. Even the time occupied in the ordinary typing process would greatly lessen or entirely eliminate the chances of successful therapy.

For this particular serum, if one assumed a definite relationship between therapeutic dose and body weight of the animal and transferred these proportions to man, the results would be as follow: In the standard rabbit (1500 gm.) we have found it necessary to use serum to the amount of 0.23 per cent of body weight at 24 hours, 0.47 per cent at 48 hours, and approximately 0.67 per cent at 72 hours. This would indicate that in a man of 70 kilos it would be necessary to use 150 cc. of this serum at 24 hours, 325 cc. at 48 hours, and approximately 475 cc. at 72 hours.

In the experimental rabbit condition it is not essential that the minimal effective level be exceeded at a single injection but if multiple injections are used these must not be too far apart and the total quantity must exceed the minimal effective amount for that period. Unless this effective level is exceeded at a given time absolutely no beneficial result has been obtained.

Bloomfield (2) advances the opinion that in lobar pneumonia the usefulness of serum depends upon the presence and seriousness of the bacteriemia. The rabbits which we have studied have shown some

variation in the severity of the bacteriemia at the time of treatment but the minimal effective dosage of the serum appears to be a fixed quantity for a given period and this does not vary with the severity of the bacteriemia. If such a correlation did exist it might be anticipated that ten times as much serum would be necessary at 48 hours

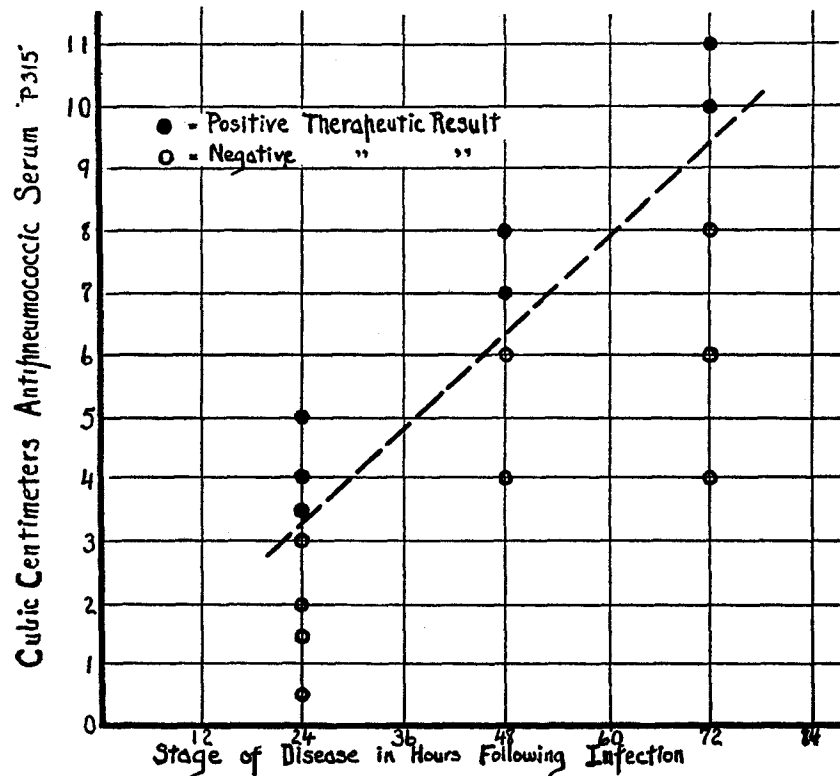


FIG. 1. Determinations of the minimal effective dosage of Antipneumococcic Serum P 315 at various stages of the disease. The diagonal line represents the suggested effective level of therapy.

as was necessary at 24 hours, for the number of bacteria per cc. of blood has often increased by a multiple of 10 or even of 100.

The contour of the suggested boundary curve, shown by the broken line in Fig. 1, suggests that there is a progressive rise in some element which unites quantitatively with the antiserum or quantitatively

antagonizes it. Proponents of the pneumonia toxin theory have advanced the idea that there may be a progressive accumulation of some toxic element in the tissues.

Decision as to the significance of the findings presented in Fig. 1 must await the solution of this question. Our results have shown, however, that there is an experimental basis for early and vigorous treatment of lobar pneumonia, and would seem to point to the immediate use of some sort of polyvalent serum, since the time required for typing greatly decreases chances for success with practicable amounts of serum.

The use of agglutinin titer or protective value of a specifically treated patient's serum in guiding further specific therapy has been suggested by Sutliff (3). The qualitative detection of agglutinins and protective substances in the treated rabbit indicates only that it has been treated; even if such tests are made quantitatively the information gives only the approximate dilution that the therapeutic serum has undergone with the rabbit's own serum. It is obvious that qualitative tests have little value since various antibody levels are necessary at various stages of the disease in order to bring about successful therapy.

The Comparison and Standardization of Antipneumococcic Sera on the Basis of Therapeutic Value.—The earlier workers used agglutinin titer as a basis for comparison of probable therapeutic efficacy of sera. This method has given way to a titration in which mouse protection is the indicator of protective value. The Hygienic Laboratory has established a certain standard and furnishes for comparative purposes a serum which fulfills this requirement. This standard is not based on any known quantitative therapeutic value for cases of lobar pneumonia.

Having found the minimal effective dosage at a given hour in a 1500 gm. rabbit to be a characteristic of the serum and of a constant value for that serum, experiments were planned by which this might be made the basis for the comparison of sera.

Seven sera were selected for comparison. The general plan of work consisted in determining for each of the sera the mouse-protective value, the agglutinin titer, and the therapeutic minimal effective dosage for rabbits at 24 hours.

In determining the mouse-protective value it was assumed that the so called virulence titer of a culture was in reality a measure of the number of minimal fatal doses of virulent pneumococci per cc. for mice. The culture used had a virulence such that 0.000,000,1 cc. would cause the death of a mouse within 4 days. With a definite dilution of this culture and varying quantities of the serum to be tested it was possible to arrive at a numerical value for the protective titer of each serum—this protective value being defined as the number of mouse-protective units per cc. of undiluted serum. The least amount of serum which will protect against a given dosage of pneumococci is determined. By dividing this amount by the number of M.L.D. of pneumococci used, a figure is obtained which represents a unit of serum. The number of such units per cc. of serum constitutes the value which we use for comparison. In every instance except one, 0.1 cc. of culture (100,000 M.L.D.) was employed against varying dilutions of serum. The mouse-protective value which represents the minimal Hygienic Laboratory standard is 5,000,000, according to this system of calculation, but it will be seen that the serum furnished by them for comparison shows a much higher figure.

The agglutinin titer was found by the ordinary technic, with heat-killed pneumococcus suspension as the agglutinin.

In determining the therapeutic value of each serum, rabbits were treated intravenously at 24 hours following infective intradermal inoculation. Careful studies such as detailed in the previous paper were made before and after treatment. In order to determine the approximate therapeutic zone a preliminary experiment was carried out with widely varying amounts of serum. Then a second experiment was conducted in which the amounts were less widely separated. The rabbit therapeutic value is expressed in minimal effective doses per cc. of serum.

The following sera were used for comparison.

1. Antipneumococcic serum (horse). Hygienic Laboratory Control P 7.
2. Antipneumococcic serum (horse). Massachusetts Antitoxin and Vaccine Laboratory, Lot P 315. This serum is of the type with which physicians are supplied.
3. Antipneumococcic serum (horse). Massachusetts Antitoxin and Vaccine Laboratory, Lot 400-31. An experimental serum prepared by the Zinsser-Grinnell blood clot method (4).
4. Antipneumococcic serum (horse). Lot 440-3-12. An experimental serum: a bleeding at 6 days following a single injection of killed pneumococci.
5. Concentrated pneumococcus antibodies (Felton). Type I, Lot 56. Supplied through the courtesy of Dr. L. Felton.
6. Serum from a rabbit which recovered without treatment. Bled 30 days after the infective inoculation (25 days after critical recovery). Lot 296.
7. Serum of a rabbit immunized by repeated injections of washed heat-killed pneumococci. Lot 301.

The results of the necessarily large number of experiments are condensed in Table I.

The great interest in this table lies in the fact that the mouse-protective doses listed in the column of mouse-protective values show the extraordinary potency of the serum in protecting a highly susceptible animal when serum and organisms are mixed before injection, an experimental condition which is comparable in absolutely no respect to the condition under which the serum is therapeutically used. In the second column under "Rabbit therapeutic value," also calculated for 1 cc., is shown the much diminished potency, though still considerable, when the serum is used in another highly susceptible animal under conditions which, we believe we are justified in assuming, simulate

TABLE I.
Comparison of Agglutinin Titer, Mouse-Protective Value, and Rabbit Therapeutic Value of Seven Sera.

Serum	Mouse-protective value (in terms of fatal doses protected against by 1 cc. of serum, if serum and organisms are mixed <i>in vitro</i> before injection)	Rabbit therapeutic value		Agglutinin titer
		Minimal effective dose of serum (amount necessary to bring about recovery if ad- ministered 24 hrs. after infection)	Minimal effective doses per cc. of serum	
1	500,000,000	3.0	0.33	80
2	50,000,000	3.5	0.29	40
3	50,000,000	3.5	0.29	40
4	500,000	>10.0	Less than 0.1	0
5	1,428,000,000	0.2	5.0	640
6	5,000,000	3.5	0.29	10
7	50,000,000	0.5	2.0	320

the condition in man, under which serum is therapeutically employed, as closely as this can be done in animal experiments.

It is not altogether surprising that there is not a complete parallelism between figures in the three columns but it is somewhat difficult to account for this in every case. The first point that may well be considered is the therapeutic value of homologous as against heterologous immune sera. Nos. 6 and 7 are from rabbits, and it is to be observed that these sera have a low mouse-protective value in comparison with their relatively high therapeutic value for rabbits. Thus, Serum 7 has only one-tenth the protective value of Serum 1, but its

therapeutic value is six times greater. Compared to Nos. 2 and 3, which have the same protective value, this serum has six or seven times the therapeutic value. The same comparisons hold good for Serum 6. It is obvious that an homologous immune serum, although low in protective value for an heterologous animal, does possess marked therapeutic value as contrasted to the immune serum of a third species. The cells of the treated animal are intimately concerned with any change in the status of the disease and the homologous serum probably owes its advantageous effect to the fact that such proteins are more readily absorbed by the cells.

A consideration of the therapeutic value of the four more potent antisera from horses (Nos. 1, 2, 3, and 5) shows that there is a more definite correlation with the agglutinin titer than with the mouse-protective value. There is a very striking dissimilarity in relative mouse-protective and rabbit therapeutic values.

It is suggested that a method of standardization of antipneumococcic serum, based on the rabbit therapeutic usage as outlined, might prove valuable as compared with the present system. Such a procedure would be no more difficult and only slightly more expensive than the mouse method and the results are quite as regular and definite. It would have the great advantage of measuring that property for which the serum is utilized. Our chief hesitancy in proposing such a method is that we do not know, nor is there any present method of determining, whether the therapeutic value for rabbits does represent a definite measure of therapeutic value in human lobar pneumonia.

II.

Non-Specific Therapy.

In many of these therapeutic experiments large amounts of foreign protein have been injected and it becomes important to separate the specific and non-specific effects. To this end the latter type of therapy has been studied in sixteen cases with normal horse and rabbit sera and typhoid vaccine as the non-specific agents. Various quantities were employed and the cases were usually treated at 24 hours following infection. Four of this series of sixteen survived but this survival rate does not sufficiently exceed that of untreated cases to be considered significant.

Following the intravenous injection, at 24 hours after infection, of 3 to 5 cc. of normal horse serum the temperature may be slightly elevated for a few hours. The number of white cells, already rather low, is not appreciably altered. The most striking result that has been

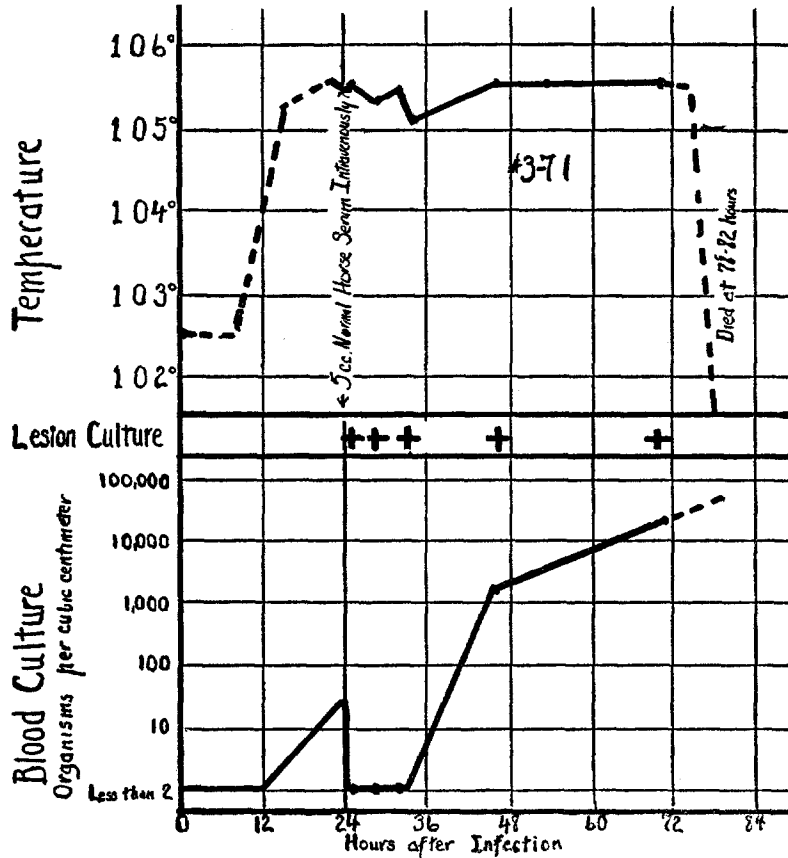


FIG. 2. Chart of findings on Rabbit 3-71 showing the effects of the injection of a non-specific agent.

observed is the immediate disappearance of pneumococci from the circulating blood. The blood may remain free of organisms for several hours but the bacteria then reappear in increasing numbers. A chart of such a case is shown in Fig. 2.

The normal horse serum used in these experiments had no protective action against pneumococci in mice and the result of the treatment of these rabbits, in so far as can be determined, must be due entirely to non-specific factors. This conclusion is supported by the finding that the same result is obtained if typhoid vaccine is administered intravenously.

It has been suggested that the immediate freeing of the blood stream of organisms in the specifically treated case may be due entirely to non-specific factors, but this is probably not the case. That the specific element alone is capable of bringing about this effect is shown in instances of successful intravenous therapy with the homologous (rabbit) immune serum as contrasted to entirely negative results with the homologous normal serum. The injection of normal rabbit serum appears to have no effect upon the number of circulating organisms.

These results show that the non-specific element involved in specific intravenous therapy with "raw" serum must be considered as a definite factor but negligible as compared to the specific element and probably having no bearing on the outcome of any case.

III.

Active Immunity.

In our previous communication (1) the relation of crisis to the development of active immunity in the experimental rabbit disease was discussed. In the following paragraphs we propose to present observations concerning active immunity in convalescent animals and in those previously vaccinated, and to discuss the problem of active immunization in pneumonia.

The Determination of Active Immunity.—Our experience leads us to believe that for the present the only reliable method of determining active immunity is by observing the response to reinfection. Preliminary experiments showed that convalescent rabbits, though immune, still develop a limited localized lesion at the site of intradermal inoculation of undiluted virulent pneumococcus broth culture, and for this reason, before an arbitrary reinfection dose could be selected, it was necessary to study the results obtained with varying amounts of culture.

The method of carrying out this experiment differed from that used in determining the susceptibility of the normal rabbit to varying intradermal dosage of pneumococci, for in the convalescent case the lesion obtained does not exhibit the tendency to spread that is seen in the normal animal. A number of tests may therefore be made on a single animal at the same time. The results of such an experiment are shown in Table II.

On the basis of this experiment it seemed that there would be no particular advantage attached to the use of any one dose for reinfection.

TABLE II.

Susceptibility of the "Immune" Rabbit to Varying Dosage of Pneumococci.

Rabbit 4-28; 22 days convalescent. Entire abdominal area shaved, and varying amounts of culture, diluted to 0.2 cc. in broth, injected intradermally in various separated areas.

Area	Amount of broth culture	Reading at 24 hrs.
	<i>cc.</i>	
1	0.2	Local lesion with definite color and edema; no tendency to spread
2	0.04	As above
3	0.01	Local area of heightened color; no swelling
4	0.002	Trace of color at point of inoculation; no swelling
5	0.001	Very slightest trace of color at point of inoculation; no swelling

With amounts below those given, no sign of inflammation was observed

tion except that with the larger amounts the local lesion would amply safeguard the fact that virulent organisms had been injected. We have therefore used 0.2 cc. of undiluted 18-hour broth culture as a routine. This is injected, as in cases described in our previous paper, well up on the animal's side. Observations as to the character of the lesion and the animal's temperature are made at 24 hours. Blood cultures are not done unless the lesion is widespread, for in our experience the local type of lesion is associated with entirely negative blood cultures.

The resistance to infection has been studied by this method in over 50 cases. The results show some variation but each case falls dis-

tinctly into one of the divisions of a classification that has been devised and is shown in Table III. This classification is based on the type of immune reaction previously described for untreated convalescent cases and upon the assumption that the normal rabbit has no immunity against the pneumococcus.

The first class shown in Table III represents the normal rabbit, which has no immunity against intradermal pneumococcal infection and which after infection undergoes the type of disease that we have described in detail in the previous paper.

Classes 2 and 3 represent those cases which are spoken of as possessing partial immunity. The lesions in these cases are practically

TABLE III.
Classification of Results in Observations on Active Immunity.

Class	Designation of immunity	Readings at 24 hrs.		Course
		Area of lesion	Temperature	
1	—	Widespread	High	Typical febrile 5- to 7-day course
2	+	Widespread	Moderate (less than 105.0°)	Typical course except less severe
3	++	Widespread	Low (less than 104.0°)	Short and mild course
4	+++	Local	Little or no elevation of temperature	Lesion begins to subside in 36 to 48 hrs.
5	++++	No lesion or sign of lesion	No change in temperature	

indistinguishable from those in Class 1, but there is a significant lack of severity in the temperature reaction and other objective symptoms.

Class 4 has already been described. It represents the ordinary maximal immunity observed following an attack of the disease. It therefore probably is comparable to *absolute natural immunity*.

Into the fifth class fall those animals which have been immunized by a course of injections of dead pneumococci; that have built up a high antibody content and may be conveniently referred to as *hyper-immune*.

Active Immunity Following Recovery without Treatment.—Since only a small proportion of untreated cases survive an attack of the experi-

mental disease, it has not been possible to study some features of the acquired immunity in the greatest detail. It has been determined that a definite (+ + +) immunity is acquired and that this immunity persists at a high level for several months. In one case it persisted for 9 months but in two other instances the immunity was not maintained for this length of time. In one case in a series of ten, partial immunity only was observed at the end of the 2nd month. We hope to determine the limits of efficiency of this acquired immunity in future experiments. For the present no absolute conclusion is justified except that the immunity acquired seems to persist for several months but is not permanent.

Active Immunity in Treated Cases.—In repeated instances we have determined that if a rabbit subjected to dermal inoculation is treated within 24 hours with an effective dose of heterologous serum it promptly recovers but after the passive immunity has worn off, such an animal possesses no active immunity against subsequent infection. We have reinfected rabbits of this type at from 15 to 60 days following original infection and in no case have we observed more than a "one-plus" immunity. In several cases the immunity was entirely negative.

If treatment is delayed until the 48th or 50th hour it does not appear to interfere with the development of active immunity, for such cases show the characteristic "three-plus" immunity after recovery.

This same principle has been demonstrated in animals given a single vaccination of washed heat-killed pneumococci. No active immunity develops in such cases if they are given, within 24 hours after vaccination, an amount of heterologous immune serum corresponding to an effective dose for that hour.

It has been suggested that this arrest of developing immunity may be concerned with the lack of immunizing power of the substances resulting from the proteolytic digestion of the phagocytosed bacteria. It seems certain that the phagocytic activity of circulating leucocytes is increased by the agency of the immune serum and in the case of early treatment it might be reasoned that the bacteria or their antigenic fractions do not reach the tissues in form suitable for stimulating antibody production.

If cases are treated with heterologous immune serum in amounts insufficient to produce therapeutic effect (subeffective) a certain

number of animals recover spontaneously after a typical and severe course. In these instances the introduction of subeffective amounts of serum had not interfered with the development of immunity and these cases show the same immune character as do the untreated cases.

If an homologous antipneumococcic serum is used for arresting the course of the disease, and the rabbits are subsequently tested for immunity, the results differ from those obtained when heterologous serum is used. These animals, treated with the homologous serum, show a definite (+ + +) immunity following recovery. This immunity persists certainly for over a month at a high level and in one case a slight immunity (+) remained at 9 months. At this time there is no method by which it can be determined whether this result is due to an active immunity or to a prolonged and effective passive immunity. In this connection it is of interest to recall that Park (5) has reported a similar finding as regards diphtheria antitoxin, for he found that a guinea pig receiving 10 units of diphtheria antitoxin prepared in an immunized guinea pig remained immune for from 6 to 8 months, while a guinea pig receiving 10 units of antitoxin made in an immunized horse, remained immune for only 2 or 3 weeks.

Active Immunity and Agglutinins.—In twenty-eight cases of complete immunity to reinfection we have observed circulating agglutinins in about half. Partially immune animals have also been observed to show circulating agglutinins, even in as high a titer as 1-40. It is not easy to appraise the significance of these results, which however indicate quite clearly that the absence of circulating agglutinins does not signify the lack of immunity, and conversely it is also probable that the presence of circulating agglutinins does not indicate that the animal is completely resistant to infection.

Active Immunity and the Mouse-Protective Value of the Serum.—In a series of some twenty-five recovered rabbits we have correlated the immunity against infection with the protective value for mice of serum taken at that time. Animals whose sera are protective have always shown some degree of immunity to infection and in most instances this immunity is complete. Animals which are entirely non-resistant to infection show no protective substance but it is also true that some rabbits which are completely immune may possess serum with no protective value.

From the protective property alone, no conclusion is justified as to the immunity possessed by the animal. There remains only one method of testing the immunity of the experimental animal and that is to reinfect it.

Active Immunity after Vaccination with Killed Pneumococci.—It has been stated above that the "hyperimmunized" rabbits usually show a complete immunity (++++) against infection if such a test is carried out during the time that the antibody titer is high.

In our preceding paper we had occasion to use single vaccination as a means of studying the possible significance of the crisis. The knowledge that a single vaccination gave rise within 5 days to an immunity sufficient to protect an animal against infection has continued to interest us in its possible application to the disease in man. For this reason we have begun to study the immunity acquired in rabbits as a result of a single vaccination with heat-killed pneumococci. For routine intravenous vaccination we have used 10 cc. of a suspension, of washed heat-killed pneumococci, the density of which was 1.2 cm. (as measured by the method of Gates (6)). This is an extremely heavy suspension.

If the vaccinated rabbits are tested at 5 days by the usual method of intradermal inoculation of 0.2 cc. of undiluted broth culture, a three- or four-plus immunity is observed, in that respect being quite analogous to the convalescent case. In another sense the one-vaccination animal is unlike the convalescent for its acquired immunity wears off somewhat more quickly. In only one instance, of eight cases, did it persist in maximum degree for as long as 2 months.

The development of protective substances (for mice) in the rabbit has been quite thoroughly studied by Armstrong (7). In many of his experiments he observed a rise of protective substances within 3 to 5 days after a single injection of pneumococcus vaccine. We have confirmed that result. In Armstrong's series there was a gradual drop of circulating protective substances in from 8 days to several weeks; these intervals he held to be somewhat proportional to the amount of vaccine used. In our experiments this drop of protective substances has been irregular and apparently independent of the dosage.

The development of protective substances and immunity during a

period of 5 or 6 days following vaccination seems to be a characteristic of the pneumococcus rather than of the species of the animal used. We have found that mice develop an active immunity against infection within 4 or 5 days after a single injection of vaccine and this is possibly the fundamental reason that makes it unnecessary to observe mice in protection experiments for longer than that period of time. Through the kindness of Drs. White and Robinson of the Massachusetts Antitoxin and Vaccine Laboratory it has been possible to study the development of protective substances in horses after a single injection of heat-killed pneumococci. The sera of these horses were not examined on the 5th day but on the 6th protective substances were found in each of two cases. The crisis in human lobar pneumonia at 5 to 7 days must also be correlated with this characteristic period of time.

The Possible Application of These Findings.—In the foregoing paragraphs it has been established that, in rabbits, active immunity, whether acquired in disease or artificially by vaccination, is not of exceedingly long duration, and even at its height is unable in all cases effectively to overcome overwhelming doses of organisms immediately (the typical three-plus immunity). These facts are comparable to the present theories regarding active immunity in human lobar pneumonia.

There have been attempts, especially by Lister (8), and by Cecil and Austin (9), to use vaccination as a means of prophylaxis. Considering the difficulties in the preparation of a polyvalent vaccine and the apparently low antigenic properties of Type III, the results reported by these workers were very encouraging.

Although our experiments have been entirely with Type I and with experimental animals, it would seem that if vaccination could be applied in a systematic way to a susceptible population throughout the "pneumonia season" it should yield results. In such an application the two factors that must be especially considered are (a) the polyvalent character of the vaccine and (b) the shortness of the immune phase conferred by vaccination. It would seem possible to rule out the second point by the use of single subcutaneous injections of vaccine at 1- or 2-month intervals during the season of highest pneumonia morbidity.

SUMMARY.

1. The continuation of our experiments with intradermal Type I pneumococcus infection in rabbits has furnished further evidence of the marked analogies between this condition and that of human lobar pneumonia.

2. It has been found that the amount of antiserum necessary for successful therapy increases as the disease progresses, and that this progression has a definite mathematical character. Such a condition, it seems, can only be caused by a progressive accumulation of some toxic or antagonistic substance, the exact nature of which is not known.

3. Various lots of antipneumococcus sera have been tested for their therapeutic properties. The results from seven such sera show that this therapeutic value does not parallel the mouse-protective value. It is suggested that the rabbit technic may prove useful for the routine comparison and standardization of antipneumococcus sera since it represents a simple method for determining that property for which the serum is to be utilized.

4. The effect of non-specific therapy in this condition has been determined to be a transient disappearance from the blood stream of circulating organisms. This result was obtained with such heterologous materials as normal horse serum and typhoid vaccine but not with the homologous normal rabbit serum.

5. Rabbits recovering from the intradermal disease without treatment or with such inadequate treatment that the disease runs its normal course, were shown to have a definite though not permanent immunity. Cases in which the disease had been arrested at 24 hours by effective therapy with heterologous immune serum showed no immunity after the early disappearance of the passively administered elements. Cases which were brought to early recovery with immune homologous serum did show a definite immunity comparable to that which was developed in other animals as the result of an untreated course of the disease.

6. The immunity conferred by single and multiple vaccination is reported. The possibility of the application of such methods in the pneumonias of man is discussed and a method for such an application is suggested.

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