

## A METHOD FOR THE RAPID PREPARATION OF ANTI-MENINGITIS SERUM.

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The European war has brought about a greatly increased demand for antimeningococcic serum. Epidemic meningitis is known to be one of the attendants of armies in barracks and in the field, and the present war has proven no exception to this rule. Information is at hand indicating that at least the principal, if not all the belligerent countries have suffered from epidemics of meningitis to a greater or less extent. Now that we have knowledge of the manner in which epidemic meningitis is conveyed, namely, by means of meningococcus carriers who harbor that microorganism in the nasopharynx, the appearance of meningitis in widely separated countries and places is not at all remarkable. Meningococcic meningitis has prevailed either in epidemic form or sporadically in many European countries during the past 10 or more years. Hence the bringing together of recruits has had the effect of introducing carriers of the infection into the midst of the European armies. Not until the European war is over and the medical data of the war have been collected and analyzed shall we know whether the epidemics have been of equal severity throughout or whether some have been of greater severity than others. But the interim observations and studies have already yielded certain definite information of great importance in respect to the combating of the disease through the employment of the specific anti-meningococcus serum.

### *Types of Meningococcus.*

Biological studies of meningococci have led to their separation into two main groups, called respectively meningococci, or normal

meningococci,<sup>1</sup> and parameningococci (Dopter<sup>2</sup>). The two main groups are distinguished not by their cultural properties, but by their immunity reactions. This divergence in immunity reaction has proven of the greatest importance in perfecting the antimeningitis serum, since its action is specific and it affects those types of meningococci only for which its antibodies possess affinity.

At the outset, and before the two main groups of meningococci were clearly differentiated, the antimeningitis serum was prepared by employing a number of strains of meningococci in the immunizing process. The object, of course, was to cover ordinary variation in immunity properties of the different cultures; but with the discovery of the two types of meningococci this haphazard method no longer sufficed and it became necessary to use for purposes of immunization representatives of the two groups now designated meningococci and parameningococci. The sera now being prepared are for the most part produced by immunizing horses either with mixtures of these types of meningococci or with alternate injections of the two types.

Our recent studies indicate, however, that it does not suffice merely to employ representative normal and representative parameningococcus strains for the purpose of immunization, for while the two groups are, immunologically considered, fairly homogeneous, yet within each group there exist organisms which react weakly to the specific immune bodies produced by other strains of the same type. On the other hand, when these weakly reacting strains are themselves inoculated they evoke the formation of specific immune bodies to which they react strongly. Hence it is desirable to employ for immunization not merely one, but rather several strains of each group; and it is further desirable to change or alternate the strains as new ones are obtained which show an imperfect response to the immunity bodies already present.

The existence of different types of meningococcus was indicated early in the course of the serum treatment of meningitis through the observation that in certain cases the meningococci when brought under the influence of the antiserum failed to be affected by it. Since this was the exception, it was assumed that certain strains of meningo-

<sup>1</sup> Wollstein, M., *Jour. Exper. Med.*, 1914, xx, 201.

<sup>2</sup> Dopter, C., *Compt. rend. Soc. de biol.*, 1909, lxxvii, 74.

cocci were resistant, or fast, to the antimeningitis serum. Further studies, as has been mentioned, established the fact that two great groups of meningococci could be distinguished; and our later studies indicated that even within these groups variants occur which are less subject to the action of a polyvalent antimeningitis serum than the majority of strains.

We are at present only imperfectly informed as to the relative prevalence of the different types of meningococci in given foci of epidemic meningitis. The American experiences and apparently the experiences previous to recent studies in the war zone in France and England have indicated that normal meningococci greatly preponderate in cases of epidemic meningitis. It now appears that, in certain localities at least in which epidemic meningitis prevails in France and England among the army, the proportion of cases caused by normal meningococci as contrasted with cases caused by parameningococci may be no higher than 6 : 4 (Ellis,<sup>3</sup> Arkwright<sup>4</sup>).

This consideration has definite bearing on the preparation of antimeningitis serum and emphasizes the importance of proceeding in its preparation in such a manner as to produce quantitative results in which the antibodies for the parameningococci about equal in amount those for the normal strains.

#### *Mode of Preparation of the Serum.*

At the first appearance of the present outbreak of epidemic meningitis in Great Britain, The Rockefeller Institute was no longer engaged in the preparation of the antimeningitis serum. Two circumstances led to the resumption of its manufacture. The first was the probability that the epidemics abroad would extend and the demand for the serum would exceed that available from ordinary sources of supply. Moreover, the identification of The Rockefeller Institute with the original production of the serum brought to it urgent requests for serum from several of the countries at war, with which it seemed imperative to comply. Through the assistance rendered by The Rockefeller Foundation, which has been engaged extensively in war

<sup>3</sup> Ellis, A. W. M., *Brit. Med. Jour.*, 1915, ii, 881.

<sup>4</sup> Arkwright, J. A., *Brit. Med. Jour.*, 1915, ii, 885.

relief, funds were placed at the disposal of the Institute covering the cost of production of the serum, so that it could be supplied gratis to those countries from which the demand came.

But there was a second important reason which led to the resumption of the manufacture of the serum. At the time and after the appearance of epidemic meningitis, particularly among the British recruits, the supply of serum available in England was chiefly that prepared commercially. Its use was distinctly disappointing. Realizing that in all probability the failure lay with the samples of serum available, which by reason of some fault of production or preservation were inactive, it seemed desirable to produce a serum the activity of which could be relied upon (Osler, Rolleston<sup>5</sup>).

In a previous communication<sup>6</sup> from the laboratories of the Institute a method was described by means of which the preparation of the antidysenteric serum was greatly abbreviated. The method consists in making injections into the horse of cultures or extracts of dysentery bacilli on 3 successive days, after which a period of rest is permitted. It was ascertained that the immunity response to the bacteria or bacterial products thus injected was far greater than when they were introduced at periods separated from each other by the ordinary intervals of time. Moreover, it was determined that by this rapid method an efficient polyvalent antidysenteric serum could be produced, representing both the non-acid (Shiga) and the acid (Flexner) groups of bacilli. Instead of a period of 8 or 12 months required by the usual method of immunization, an equally strong serum was produced in the short period of 8 or 12 weeks.

The problem presented, therefore, in respect to the antimeningitis serum seemed essentially similar to that encountered with respect to the antidysenteric serum. In the case of the latter, two groups of the bacilli are dealt with: first, a fixed or Shiga group, which yields a soluble toxin; and second, a fluctuating group made up of very slightly divergent types of bacilli which do not yield a soluble toxin. In the case of the meningococci there are also two groups or types, neither being perhaps altogether fixed: the ordinary or normal meningococci

<sup>5</sup> Discussion on the treatment of cerebrospinal meningitis, *Proc. Roy. Soc. Med., Therap. and Pharmacol. Sec.*, 1915, ix, 1.

<sup>6</sup> Flexner, S., and Amoss, H. L., *Jour. Exper. Med.*, 1915, xxi, 515.

which readily undergo autolysis, and the parameningococci undergoing less perfect autolysis, both yielding, however, a toxic product. Hence it could be assumed that by employing a method similar to that worked out for the antidysenteric serum, horses might be rendered immune and made to yield an efficient polyvalent antimeningococcic serum in a period of time far shorter than is required by the usual method of subcutaneous inoculation. The substances employed in both instances are the same; namely, living cultures and the extract, or autolysate, inoculated alternately. By the old method from 6 to 12 months were required to produce a meningococcic serum of high immunity value. By the new method a similar result has been achieved in from 8 to 12 weeks.

In following out this plan, a difficulty was early encountered, and one which was indeed foreseen. Two general methods of immunization of horses have been followed for the production of antimeningitis serum. In one the cultures of meningococci or the autolysate is injected subcutaneously (Flexner and Jobling<sup>7</sup>); in the other, the injections are made intravenously (Dopter<sup>8</sup>). In the first instance, no ill effects arise, aside from the occasional production of sterile abscesses. In the other, after several inoculations have been made, the horses become extremely sensitive, so that sudden death has been known to follow an injection of the culture or autolysate. This danger can, however, be prevented, as has been shown by Dopter,<sup>9</sup> by employing a desensitizing injection of the culture before making the full inoculation.

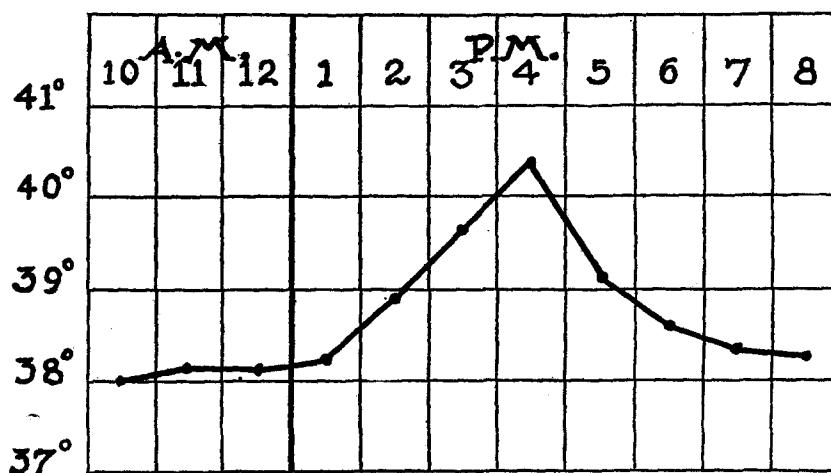
Hence the method adopted for the rapid production of the antimeningitis serum follows closely, but does not exactly reproduce, that already described for the rapid production of the antidysenteric serum. It consists in beginning with small doses of living meningococci injected daily for 3 days, followed by a period of rest of 7 days, when another series of injections is made. Thus for a 1,300 pound horse, one-twentieth of a 24 hour culture on slanted plain agar is injected on the 1st day. The material for injection is made up as follows: 2 cc. of physiological salt solution are added to a 24 hour agar slant

<sup>7</sup> Flexner, S., and Jobling, J. W., *Jour. Exper. Med.*, 1908, x, 141.

<sup>8</sup> Dopter, C., *Ann. de l'Inst. Pasteur*, 1910, xxiv, 96.

<sup>9</sup> Briot and Dopter, *Compt. rend. Soc. de biol.*, 1910, lxi, 174.

of meningococcus culture and the growth is suspended in it. Then 0.1 cc. of the suspension is transferred to 15 cc. of physiological salt solution and injected intravenously very slowly. The temperatures are taken hourly, beginning with the 4th hour after the injection, and continued until the temperature has reached its highest and begun to decline. 24 hours later 0.2 cc. of the suspension, and on the 3rd day 0.3 cc., that is about one-seventh of the agar slant, is used. After the lapse of 7 days, the dose given on the first of the 3 days corresponds with that given at the end of the last series; namely, in the instance in question, 0.3 cc. The temperature is again taken, and



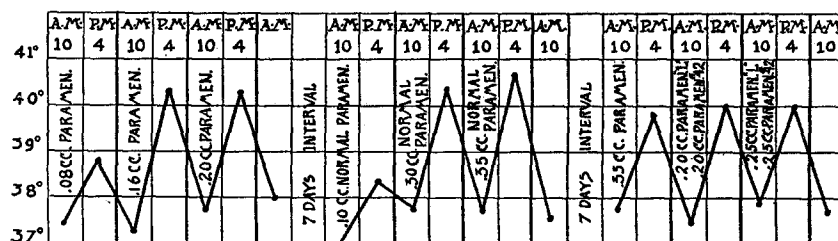
TEXT-FIG. 1. Typical febrile rise and fall following each single injection of meningococci.

if the rise does not equal 2.5–3°C. the conclusion drawn is that the dose has been too small. It is increased, therefore, for the injection 24 hours later above the usual rate of increase of 0.1 cc. according to the degree of rise of temperature. If the temperature does not fall to normal within 18 to 24 hours, the conclusion is drawn that the dose given has been too large (Text-fig. 1).

Following this plan, doses may be regulated with nicety and a maximum of reaction be obtained, we believe, with a minimum of danger. No serious effect is produced, although chills may attend the severer reactions. The greatest reaction, as a rule, is that pro-

duced by the first injection, whereas the succeeding injections on the 2nd and 3rd day tend to produce less severe reactions. Hence the increase between the second and the third injection may be larger than that between the first and second. In the second series of injections, the maximum doses have been from 0.4 to 0.45 cc., and in the third series from 0.5 to 0.6 cc. on the 3rd day. Since individual horses vary in their susceptibility, the first doses are small and the temperature curve forms the basis for adjusting the other doses (Text-fig. 2).

In Horse N only two series of injections were necessary before the typical and desired temperature curve was attained. The succeeding curves for any series agree closely with the third series in the chart.



TEXT-FIG. 2. Chart of Horse N showing febrile reactions and adjustment of doses in the first three series of injections.

In the succeeding series increase in the amounts injected is accomplished by the addition of new strains from time to time. For example, when the total dose is 0.6 cc. (*i.e.*, slightly more than one-third of the agar slant) it may consist of 0.2 cc. each of three strains. The largest amount of any single injection in a 1,300 pound horse has been one-fourth of each slant from seventeen different strains.

In preparing a polyvalent serum two slightly different procedures may be followed. According to one, the normal and the parameningococcus strains are inoculated within what may be designated as periods of two series; that is, the normal and the parameningococcus strains are alternated. According to the other, in which the autolysate is also employed, the period includes three series, one for the normal meningococcus, one for the parameningococcus strains, and one for the autolysate. The autolysate, in turn, is made up of equal parts

of a typical normal meningococcus and of a typical parameningococcus strain. Evidence is at hand, to be referred to below, which shows that the autolysate, at least in the sheep, excites little agglutinin formation while producing other protective principles.

*Desensitization.*

As stated, the horse becomes hypersensitive to the intravenous injections of the meningococci or its products apparently after the third or fourth series of injections. This reaction tends to be most severe after the first dose in each series, as might have been predicted. It was found that, acting upon the suggestion of Dopter,<sup>9</sup> desensitization may be effected apparently readily and certainly. Hence on the 1st day of each series about one-twentieth (later one-tenth) of a 24 hour slant of culture is injected intravenously, and 2 hours later the remainder of the dose is given. This desensitization suffices and need not be repeated on the 2nd and 3rd days, when the next doses are injected.

The danger from both brain and lung emboli must not be overlooked. Bull<sup>10</sup> has observed that suspensions of bacteria, introduced into the blood stream of an animal immune to the same organism, are clumped and deposited in the blood vessels of the brain, lungs, and in the spleen. If the bacteria are present in any considerable number, the clumps may occlude the cerebral and pulmonary capillaries and produce sudden death of the animal. We have found this to be true in the injection of meningococcus into normal and immune rabbits. It is possible that a part of the so called anaphylactic phenomena observed in immunization with meningococci can be explained by these facts, since the symptoms observed are similar if not identical in these two instances.

In order to avoid such a possible danger the suspensions of living meningococci are made up to a relatively larger volume, 15 to 20 cc., and introduced very slowly into the circulation.

<sup>10</sup> Bull, C. G., *Jour. Exper. Med.*, 1915, xxii, 466.



*Strains Employed in the Immunizing Process.*

In the beginning a group of five representative normal meningococcus strains and a group consisting of an equal number of parameningococcus strains were used alternately in the series of injections. The progress of the formation of immune bodies in the serum was followed by studying the agglutination, opsonization, and complement deviation reactions, with as many strains of meningococci as possible. In this manner strains not employed in the original series of inoculations may be divided into temporary lots according to their reaction with the serum. If the reactions are not within or near the zone of reaction of the strains already being used for immunization, representatives of such lots are selected and placed in the immunizing group. The remaining members of these lots are again tested later when immune bodies against the representative of the group have appeared in the serum. Should the serum now contain antibodies effective against all members of the group, it will, of course, not be necessary to include them all. Agglutination serves as the most specific index of immunological reaction except in the case of inagglutinable strains, which, in our experience, are infrequently encountered.

By the above procedure we have studied sixty-four strains of meningococci, and from this number twenty-two were selected to be added to the two original immunizing groups which consisted respectively of five normal meningococcus strains and five parameningococcus strains. For convenience in regulating the doses and for injecting we have added equally to each group.

We selected for addition to the normal group eleven of those which showed the least variation from the normal meningococcus; and the remaining eleven, exhibiting wider variations, were placed in the second group which originally consisted only of parameningococci. The strains used at present for immunizing are thirty-two, and have for convenience been divided into two lots. Lot A consisted originally of five normal and irregular strains of meningococcus, and to these have been added eleven normal and irregular strains. Lot B consisted originally of five parameningococcus strains (two from the Pasteur Institute, Paris, and three isolated in America) and to these have been added eleven parameningococcus and irregular strains exhibiting wider variation from the normal. One lot is used for three

successive injections and after a rest of 7 days the other lot is used in like manner. If care is not taken to balance the two lots, the immune bodies against one group may be developed to a greater extent than those against the other. To avoid this, the serum is tested immunologically after every second series if living organisms are injected, and after every third series if autolysate is also given. The relative doses of the two groups are regulated accordingly.

Since we have evidence that both normal meningococci and parameningococci differ among themselves in antigenic power, it is desirable to study all possible strains in the course of an epidemic in order that strains which are at variance with those being used in the production of an immune serum may be included in the immunizing groups.

This point is illustrated by the following experience with two strains. Strain NO was tested against the polyvalent serum of a horse highly immune against at least twenty normal and parameningococcus strains and found to be agglutinated in a dilution of 1 : 10. This strain, which was later found to be parameningococcus, was included in the immunizing group, and after two inoculations the agglutinins rose to 1 : 100, and after four injections to 1 : 200. When fewer strains are being used, the immune bodies may be developed more quickly. Strain Andrews, brought from England by Dr. A. Gardner Robb, when tested against a potent serum effective against ten normal meningococcus and parameningococcus strains, agglutinated in a dilution of 1 : 10. It was included in the immunizing group and after two series of injections, the agglutinating power of the serum for this strain rose to 1 : 200. We consider that the larger doses of Andrews which it was possible to employ caused the more rapid rate in the production of the agglutinins. Table I illustrates the development of agglutinins for strains relatively inagglutinable before their inclusion in the inoculation group.

TABLE I.

Strains.	Before injection.	After 1 injection.	After 2 injections.	After 4 injections.	After 5 injections.
Andrews. . . . .	1 : 10	1 : 100	1 : 200	.....	1 : 2,000
NO.....	1 : 10	.....	1 : 100	1 : 200	.....

*Autolysate.*

Equal parts of toluene autolysate<sup>11</sup> from normal meningococcus and from parameningococcus may be injected intravenously on 3 successive days forming one series, alternating with the two series of living meningococci. The serum of horses receiving the autolysate series develops agglutinins, opsonins, or power to deviate complement less quickly than that of animals receiving only the living organisms, though power to neutralize the toxin contained in the autolysate is developed.

TABLE II.

Serum.	Agglutinins.		Opsonins.		Complement deviation.	
	Normal meningococcus.	Parameningococcus.	Normal meningococcus.	Parameningococcus.	Antigen from normal meningococcus.	Antigen from parameningococcus.
Sheep B (autolysate).....	1 : 100	1 : 50	1 : 50	1 : 1,000	1 : 200	1 : 100
Horse L (living cultures and autolysate).....	1 : 5,000	1 : 2,000	1 : 2,000	1 : 5,000	1 : 10,000	1 : 5,000

A sheep received intravenously maximum doses of autolysate in series of 3 successive days and a rest of 7 days over a period of 9 months. Doses sufficient in size to cause marked febrile reaction and sometimes diarrhea, were administered. At the end of 9 months the serum exhibited only slight power of agglutination, opsonization, and complement deviation.

Table II shows the titer of the sheep serum compared with that of serum obtained from a horse receiving autolysate and also living meningococci.

The sheep serum when incubated with living meningococci and injected intraperitoneally into small guinea pigs was found to possess low anti-infectious value.

<sup>11</sup> Flexner, S., *Jour. Exper. Med.*, 1907, xx, 105.

*Immunity Value of the Antiserum.*

The immune bodies of the horse serum were estimated by testing its agglutinating and opsonizing power with normal and parameningococcus strains and by determining its power to fix complement in the presence of antigens made from these strains. Its anti-infectious power was determined by incubating varying amounts with one minimum lethal dose of living meningococci for 1 hour at 37°C., and injecting the mixture intraperitoneally into young guinea pigs weighing not less than 90 or more than 110 gm.

*Agglutination.*—Representative normal meningococci and parameningococci were selected for following the development of agglutinins.

TABLE III.  
*Horse L Serum.*

Period.	Before injection.	1st.	2nd.	4th.	7th.*	After 6 wks. of autolysate injections.†	10th.	11th.‡	13th.	14th.
Meningococcus.....	1:30	1:60	1:80	1:1,000	1:5,000	1:2,000	1:2,000	1:500	1:1,000	1:2,000
Parameningococcus..	1:10	.....	1:80	1:200	1:2,000	1:500	1:1,000	1:1,000	1:1,000	1:2,000

\* 7 periods extend over 10 weeks. 6 liters of blood were taken from the horse at this time.

† For 6 weeks after the 9th period the horse received autolysate only.

‡ Twelve new strains added. 1st period after bleeding.

*Horse M Serum.*

Period.	Before injection.	2nd.	4th.	7th.	10th.
Meningococcus.....	1:20	1:500	1:500	1:1,000	1:2,000
Parameningococcus.....	1:10	1:50*	1:1,000	1:1,000	1:2,000

10 periods extend over 12½ weeks.

\* This low figure shows that the injections had not been properly balanced. Too few parameningococci had been injected; accordingly larger doses were given with the result that the agglutinins increased greatly during the next periods. The highest dilution at which any of our sera agglutinated was 1:5,000 for both normal and parameningococci.

The reactions were made at 55°C. and read after 24 hours. Table III shows the increase of agglutinins by periods.

*Opsonins.*—The opsonins were estimated by the Neufeld technique. Table IV shows their development by periods.

TABLE IV.  
*Horse L Serum.*

Period.	5th.	7th.	9th.	Interval of 6 wks. Injected with auto- lysate only.	10th.	13th.	15th.
Meningococcus.	1 : 500	1 : 1,000	1 : 500*		1 : 500	1 : 1,000	1 : 2,000
Parameningo- coccus . . . . .	1 : 200	1 : 5,000	1 : 2,000		1 : 1,000	1 : 1,000	1 : 2,000

\* Bled 6 liters just before this period.

*Horse M Serum.*

Period.	2nd.	7th.	11th.
Meningococcus . . . . .	1 : 500	1 : 200	1 : 2,000
Parameningococcus . . . . .	1 : 200	1 : 1,000	1 : 2,000

*Complement Fixation.*—Tests for complement-binding bodies in the immune horse and sheep sera were made with antigens of regular normal meningococci as well as with those of irregular normal meningococcus and parameningococcus strains. The results are shown in Table V. It appears that in the serum from Horse L the power to bind complement ran fairly parallel with the power to agglutinate the meningococci.

TABLE V.  
*Complement Deviation by Serum L.*

Period.	4th.	7th.	8th.	9th.	Interval of 6 wks. Injected with auto- lysate only.	10th.	11th.	12th.	13th.	14th.
Meningo- coccus . . . . .	1 : 200	1 : 1,000	1 : 1,000	1 : 2,000		1 : 1,000	1 : 500	1 : 1,000	1 : 1,000	1 : 1,000
Paramenin- gococcus . . . . .	1 : 100	1 : 500	1 : 1,000	1 : 2,000		1 : 500	1 : 500	1 : 500	1 : 2,000	1 : 5,000

*Complement Deviation by Serum M.*

Period.	2nd.	4th.	5th.	6th.	9th.	12th.
Meningococcus . . . . .	1 : 200	1 : 2,000	1 : 5,000	1 : 2,000	1 : 2,000	1 : 1,000
Parameningococcus . . . . .	1 : 200	1 : 1,000	1 : 2,000	1 : 1,000	1 : 1,000	1 : 5,000

*Protective Value.*—Flexner recommended the use of small guinea pigs in determining the anti-infectious power of antimeningitis serum. If young guinea pigs weighing between 90 and 110 gm. are used and the experiment is run in quadruplicate, some measure of the protective power is obtained. One minimum lethal dose of the living meningococcus is incubated with varying amounts of immune serum for 1 hour at 37°C. and then injected intraperitoneally.

Table VI shows the anti-infectious power of serum L compared with normal horse serum.

TABLE VI.

*Anti-Infectious Power of Polyvalent Antimeningitis Serum.*

Strain.	2 cc. of normal horse serum.	0.2 cc. of polyvalent immune serum.	0.3 cc. of polyvalent immune serum.	0.4 cc. of polyvalent immune serum.
1 m. l. d. of living meningococci.	No protection.	Protection in 1 out of 4.	Protection in 3 out of 4.	Protection in 4.
1 m. l. d. of living parameningococci.	No protection.	Protection in 3 out of 4.	Protection in 3 out of 4.	Protection in 4.

If two minimum lethal doses are used, there is little protection after 48 hours. For example, in one series the control guinea pig receiving one minimum lethal dose and those receiving two minimum lethal doses plus 0.5 cc. of serum died in 12 to 18 hours. Three out of four guinea pigs receiving two minimum lethal doses plus 0.6 cc. of serum lived between 47 and 48 hours.

Normal horse serum exerts practically no anti-infectious action either with meningococcus or with parameningococcus. Immune serum produced by the rapid method possesses a considerable degree

of anti-infectious power. About 0.4 cc. of this polyvalent serum is capable of neutralizing the infecting power of one minimum lethal dose of the living meningococcus or parameningococcus.

#### SUMMARY.

Potent antimeningitis serum can be safely produced in the horse by the method of three successive intravenous inoculations of living meningococci and parameningococci repeated at stated intervals.

Sudden and alarming symptoms and sudden death are avoided by employing first a desensitizing injection and then by adjusting the doses according to the febrile reaction and by making the highly diluted injections slowly.

Horses undergoing this process of immunization remain in good condition and may even gain in weight.

Specific immune bodies appear in the serum early and rise rapidly.

By inoculating alternately several strains of living meningococci and parameningococci, and the autolyzed products of each, a polyvalent serum of high titer can be produced in 8 to 12 weeks instead of in the 10 months required by the subcutaneous method.

The serum produced by this rapid method has been employed therapeutically in America, England, France, and some other countries.

It is highly desirable to isolate meningococci from many sources and test the strains against the polyvalent serum. Strains which are not agglutinated in high dilution in such a serum should be included subsequently in the lot of strains used for immunization.