CHEMICAL STUDIES ON BACTERIAL AGGLUTINATION

V. Agglutinin and Precipitin Content of Antisera to Haemophilus influenzae, Type B*

By HATTIE E. ALEXANDER, M.D., and MICHAEL HEIDELBERGER, Ph.D.

(From the Departments of Pediatrics and Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York)

(Received for publication, October 6, 1939)

Agglutinating and precipitating antisera to *Haemophilus influenzae*, type B, have been recorded in recent years by Pittman (1), and by Ward and Wright (2), and have also been studied by Pittman and Goodner (3), and Fothergill, Dingle, and Chandler (4). The titers of these sera were determined by the usual relative methods. Knowledge of the actual antibody content of anti-influenza type B sera has now become urgent because of indications that such sera may possess curative action in influenzal meningitis in children (5). The quantitative, absolute methods for the estimation of agglutinin nitrogen (6) and precipitin nitrogen (7) developed in this laboratory proved adaptable to the problem. The present paper deals with the agglutinin content of antisera obtained from the horse and rabbit as estimated with suspensions of influenza bacilli prepared in different ways and with different strains, and also discusses the precipitin content of such sera as determined with different preparations of the specific polysaccharide of the type B influenza bacillus. The methods used for obtaining antisera from rabbits are also described, since the analytical control has aided in the production of rabbit sera containing five to ten times as much antibody as the best available when this study was initiated.

1. Preparation of Haemophilus influenzae Type B Suspensions and Immunization of Rabbits.—The fundamental principles stressed in a previous report (5) have been adhered to in the production of the antigen used for immunization. Well encapsulated type B strains recently isolated from blood or spinal fluid cultures have been used. When the ability of these strains to produce well defined capsules diminished on transplanting, a mucin suspension of the culture was passed through mice. The first transplant after recovery from the peritoneum was used for seeding Levinthal (8) agar plates. 0.3 ml. of a 24 hour Levinthal broth culture was spread over each plate and incubated for 6

* The work reported in this communication was carried out in part under the Harkness Research Fund of the Presbyterian Hospital.

hours. The growth was then washed off with 5 ml. of 0.85 per cent sodium chloride containing 0.5 per cent of formalin. The resulting suspension was diluted to a standard turbidity of 3.5 by the Gates turbidometer. This type of antigen may be stored in the cold for at least a month and still retains definite capsules when tested by the swelling phenomenon (5). Since there is evidence that the type specific cells are very susceptible to temperature changes and mechanical influences, the stored antigen was checked frequently for presence of capsule. This standard suspension, averaging 1,800,000,000 per ml. by colony counts, was used according to the following schedule in the production of rabbit antisera. Immediately before injection the antigen was diluted with an equal quantity of saline. 24 New Zealand white male rabbits about 6 months of age were divided into 2 groups of 12 each.

TABLE I

Schedule of Injections of Rabbits with Haemophilus influenzae, Type B

Rabbit group 1-12	Vaccine			Agglutinin nitrogen in serum pool at end of course (from Table IV)
	Daily dose 4 times weekly	Number of injections	Total quantity	
	ml.		*ml.*	*mg. per ml.*
Course I	0.1–1.0	16	10.1	0.60
Course II	1.0	14	14.0	0.60
Course III	0.1–1.0	13	10.7	0.83
Course IV	0.5–1.0	13	12.0	1.08
Total			46.8	
13-24				
Course I	0.1–1.0	16	10.1	0.50
Course II	1.0–3.0	14	24.4	0.77
Course III	0.1–3.0	13	21.2	0.77
Course IV	0.5–3.0	13	28.0	0.73
Total			83.7	

During the first month of immunization rabbits 1 to 12 received the standard vaccine and rabbits 13 to 24 the standard vaccine washed once with 0.5 per cent formalinized saline. The same schedule and dosage were used for each group. With an initial dose of 0.1 ml. and with inoculations on 4 consecutive days out of each week the amount was increased by 0.1 ml. daily until 1.0 ml. was reached. This dose was repeated for the next 5 injections, after which a rest period of 6 days elapsed prior to sample bleedings.

Since analysis of these failed to show much difference between the antisera produced by the washed and unwashed vaccines (Tables I and IV), unwashed vaccine was used for all subsequent immunizations. In an attempt to determine the optimum dosage of antigen rabbits 1 to 12 were injected with a constant amount, 1.0 ml., and rabbits 13 to 23[1] were increased gradually to 3 ml. After large bleedings of 40 ml. per rabbit, 0.1 ml.

[1] During the first four courses of injections 3 rabbits died in each group.

of antigen was given the following day. The schedule used during the previous course was then resumed for each group of rabbits for an additional 2 months with 40 ml. bleedings per rabbit at monthly intervals. The analysis of the antibody content at these intervals showed that while the group 13 to 23, receiving up to 3 ml. of antigen daily, showed a more rapid rise, it became stationary sooner and never reached as high a peak. Although this may have been due to individual variations in the two groups, the dose per day for all rabbits was changed to 1.0 ml. after a 3 months' period of comparison of the dosages described. The immunization procedure is summarized in Table I and the data on the agglutinin content of the pooled bleedings from each series of rabbits are given in Table IV.

Suspensions for the quantitative agglutinin method were prepared in the same way as the vaccines for injection. The formalinized suspensions were allowed to stand in the cold for at least 48 hours prior to centrifugation and removal of the formaldehyde by three washings with chilled 0.9 per cent saline.[2] The cells were then taken up in chilled saline containing 1:10,000 merthiolate,[3] and were evenly suspended and filtered through a loose cotton plug in order to remove any lumps not visible to the naked eye. The suspensions were finally adjusted to a volume such that 1 ml. contained 0.4 to 0.6 mg. of nitrogen.

In several instances cells from broth cultures were used, as noted in the tables.

2. Determination of Agglutinin Nitrogen.—The method originally used for the estimation of agglutinin nitrogen in antipneumococcus sera (6) was found applicable with little modification other than the neutralization of the antisera to about pH 7 (phenol red) in order to avoid extraction of nitrogen from the bacillary suspension during the analysis. This precaution contributed greatly to the accuracy and reproducibility of the results. The method depends upon the addition of an accurately measured amount of a washed bacterial suspension to an accurately measured volume of antiserum, the relative amounts being so chosen that the bacteria are in excess. After agglutination is complete the difference in nitrogen between the agglutinated bacilli, suitably washed, and the nitrogen content of the same volume of unagglutinated bacilli, gives, in milligrams, the agglutinin nitrogen content of the volume of serum chosen. An analysis of the supernatant is also carried out in order to make certain that all antibody has been removed. Details of the method are given in (6). In its application to neutralized anti-influenza sera and plasmas it was found advisable, when maximum values for type specific agglutinin were desired, to use formalin-killed suspensions of a 6 hour growth of *H. influenzae*, type B. As noted in Table II, there seemed little difference in the rabbit antisera tested whether the bacilli were grown on agar or in broth. Heat-killed cultures, however, were found to have lost much capsular substance, so that repeated absorptions with suspensions prepared from them were necessary to remove an amount of agglutinin comparable with that taken out by a single absorption with formalin-killed cells.

Usually 1.5 ml. of bacillary suspension containing 0.6 to 0.9 mg. of nitrogen was added to 1.0 ml. of a weak serum, or to 1.0 ml. of a suitable dilution of a stronger serum. In measuring the suspension into the blank tubes and the sera great care must be taken to exclude bubbles from the pipette. If this is done a Krogh pipette (9) may be used

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[2] Using a refrigerating centrifuge manufactured by the International Equipment Co., Boston.

[3] Manufactured by Eli Lilly and Sons, Indianapolis.

to advantage, particularly when many analyses are to be run. This pipette may be set at the desired volume which need not even be accurately known, since the pipette delivers exactly the same volume into the blank tubes as into the analyses. Much labor and eye strain may be avoided in this way.

TABLE II

Agglutinin Nitrogen Carried Down from Anti-Influenza Type B Sera by Haemophilus influenzae Suspensions

Horse sera	Dilution	Volume of dilution used	Influenza bacillus suspension	N content of control suspension	N content of agglutinated suspension	Difference (agglutinin N)	Agglutinin N in supernatant on 2nd absorption	Total agglutinin N	Agglutinin N per ml. of undiluted serum
		ml.		*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
I 60	1:5	1.0	Type B, FK	0.760	0.898	0.138	0	0.14	0.70
"	"	1.0	Unclassified, MK	0.714	0.736	0.022	0	0.02	0.10
712	1:1	1.0	" "	0.714	0.808	0.094	0.003	0.10	0.20
"	"	0.50	Type B, FK	0.874	0.944	0.070	0.014	0.08	0.32
Rabbit sera									
Pool a*	Undil.	1.0	" " "	0.878	1.004	0.126	0.012	0.14	0.14
" "	"	1.0	" " " †	0.642	0.742	0.100	0.008	0.11	0.11
$13–23_1$	"	0.5	" " "	0.866	1.120	0.254	0	0.25	0.50
" "	"	0.5	" " " †	0.642	0.874	0.232	0.010	0.24	0.48
$13–23_3$	3:10	1.0	" " HK	0.552	0.652	0.100	0.094‡	>0.19	>0.63
" "	"	1.0	" " FK	0.606	0.818	0.212	0.020	0.23	0.77
$13–23_4$	"	1.0	" " "	0.760	0.964	0.204	0.018	0.22	0.73
" "	Undil.	1.0	R strain, "	0.760	0.818	0.058	0.026	0.08	0.08§

FK, HK, MK, denote organisms from plate cultures, killed by 0.5 per cent formalin, by heat, by 1:15,000 merthiolate, respectively.

Subnumerals after rabbit serum pool numbers indicate course numbers.

* Best commercial rabbit serum pool available at beginning of work.

† From broth culture.

‡ 2nd and 3rd absorptions; incomplete.

§ The supernatants from the R agglutinin estimation yielded 0.56 mg. per ml. type specific precipitin nitrogen.

In the case of an unknown serum preliminary tests may be made rapidly to establish the maximum amount that may be safely used with a given suspension. If an appreciable amount of agglutinin is present the bacilli, if not used in excessive quantity, clump rapidly, even at 0°, and the mixture may be centrifuged and the supernatant tested with more bacilli. Most of the analyses recorded in the tables were carried out at 0°, with

thorough mixing, and were allowed to stand in the ice box until the next day, when the tubes were centrifuged in the cold, carefully decanted as described in (6) and washed twice with chilled saline. In general the supernatants from duplicates were combined before addition of a second measured charge of bacilli, since the amount of nitrogen removed in this case was usually nil or very small, and much labor could be saved in this way. Indeed, unless the greatest accuracy is desired, analysis of the supernatants need not be completed if, after 48 hours in the cold, with occasional mixing, the tubes show no greater sedimentation than the controls. Not more than a few hundredths of a milligram of antibody nitrogen can be present in the supernatant without causing visible agglutination (*cf.* column 8, Table II).

Especially analyzing supernatants it was often impossible to decant from the centrifuged bacilli without loss into the new supernatants, and it was then necessary to centrifuge these separately and wash the small amount of sedimented bacilli with the supernatants from the first and second washings of the main portions, after which the two portions of bacilli could be combined. The blanks, containing only bacilli and saline, could seldom be decanted without loss even though the last few drops of liquid were left in the tubes, and all supernatants and washings were therefore centrifuged again. The quantitative agglutinin method proved applicable to rabbit plasmas as well as to sera.

In Table II are given analyses of two different horse sera with a type B strain and an unclassified non-specific strain of influenza bacillus. Serum I 60, prepared by the Massachusetts State Serum Laboratories and obtained through the courtesy of Dr. Leroy D. Fothergill of the Harvard University Medical School, contained mostly type-specific antibody, while serum 712, prepared from old laboratory strains by the New York City Department of Health, contained about one-half as much anti-type B agglutinin and twice as much non-specific agglutinin. The remaining analyses in the table illustrate the behavior of various pools of antisera from rabbits, one of which represents the best available commercially at the inception of this work. As these studies progressed it was found possible to equal in rabbit sera the agglutinin content of the better of the two horse sera, and even higher titers were obtained in later bleedings, as will be noted below. Results with an R strain are given in the last line of the table, showing that relatively little of the antibody in the serum pool tested was non-specific.

3. Preparation of Crude Specific Polysaccharide of Type B Influenza Bacillus.—The existence of a type specific polysaccharide was noted by Pittman (1), and Dingle and Fothergill (10) have recently prepared the carbohydrate from peptone-free casein hydrolysate cultures and described its properties. We are greatly indebted to Dr. Dingle for a sample of this product. Since three times as much of our own polysaccharide, prepared from agar washings of type B culture, was required to precipitate a given amount of antibody N it is concluded that our product consisted of at least two-thirds soluble agar derivatives. We shall therefore omit a detailed description of the various batches and give only the method of preparation, since the crude polysaccharide may be obtained fairly easily and is a convenient reagent for the estimation of type specific precipitin in both horse and rabbit anti-influenza B sera.

8 to 12 hour growths of type B influenza bacillus on Levinthal agar plates were washed off with the minimum amount of saline and killed with 1 per cent phenol or 0.5 per cent formaldehyde. After several days at room temperature the mixture was centrifuged and the yellowish supernatant treated with acetic acid to maximum turbidity. The

resulting precipitate was centrifuged off in the cold, after which about 10 gm. of sodium acetate per 100 ml. was dissolved in the supernatant. The solution was treated with 5 to 10 volumes of alcohol and allowed to stand until the resulting fine precipitate had settled. The sedimented material was packed in a centrifuge tube, washed with acetone, and dried *in vacuo*. When the residue was taken up in water considerable insoluble material remained, and this was centrifuged off. The solution was reprecipitated and dried as above until little but water-soluble material remained. At this point one or two shakings with chloroform and butyl alcohol (11) proved helpful in case the biuret reaction was still positive. The water-soluble fraction was taken up in the minimum amount of

TABLE III

Anticarbohydrate (Precipitin) Nitrogen of Anti-Influenza Type B Sera

Horse sera	Volume used	Polysaccharide	Amount used	Antibody N precipitated	Antibody N precipitated per ml. of serum	$\frac{\text{Precipitin N}}{\text{Agglutinin N}} \times 100$
	ml.		*mg.*	*mg.*	*mg.*	*per cent*
I 60	2.0 (1:5)	Type B, broth	0.5*	0.30	0.75	107
"	" "	" " agar	0.5	0.39	0.98	140
"	" "	Agar (1:1000)	0.3	0.15†	0.38	54
I 60, agar supernatant		Type B, agar	0.5	0.24	0.60	86
Rabbit sera						
$13–23_1$	1.0	" " "	0.5	0.33	0.33	66
" "	1.0	" " broth	0.15	0.26	0.26	52
$13–23_3$	2.0 (3:10)	" " agar	0.5	0.34	0.57	74
$13–23_4$	0.5	" " broth	0.05	0.30	0.60	82
" " supernatant from R agglutinations		" " "	0.18	1.12	0.56	77
$1–11_4$	0.5	" " "	0.065	0.53	1.06	98

Subnumerals indicate course numbers.

* Unnecessarily large excess.

† Deducting blank for N precipitated under similar conditions from non-bacillary horse serum.

water and precipitated twice with glacial acetic acid. This removed the phosphate which usually accompanied the polysaccharide up to this point. After reprecipitation with redistilled alcohol and washing with redistilled acetone the product was dried *in vacuo*. Stock solutions of 1 mg. per ml. were made up for the analyses.

4. Quantitative Estimation of Type Specific Anticarbohydrate Precipitin in Type B Anti-Influenza Bacillus Sera.—Analyses for anticarbohydrate precipitin were made according to (7) by addition of a slight excess of polysaccharide solution to an accurately measured volume of diluted serum and estimation of the amount of nitrogen in the washed precipitate after 48 hours at 0°C. In the case of the horse sera tested a considerable excess of polysaccharide could be used without causing inhibition of the precipitin

reaction, but the rabbit sera were much more sensitive, especially when the purer carbohydrate from the broth cultures was used. It was therefore necessary to run preliminary experiments on 0.5 ml. portions of rabbit serum to determine the amount of polysaccharide necessary to leave a small excess. As precipitation in all but the weakest sera was quite rapid at room temperature these tests consumed little time and little serum. The quantitative precipitin method could be used for rabbit plasmas as well as for sera.

The data obtained are summarized in Table III. It will be noted that in the rabbit antisera the broth culture polysaccharide precipitated nearly as much antibody as did the agar product, and in one instance (Table III, $1–11_4$) the precipitin content equalled the agglutinin content (*cf.* Table II). In the other sera the larger agglutinin content is presumably due to antiprotein which would also be removed by the bacilli. The horse sera, however, showed an entirely different behavior, and this is illustrated by the data on serum I 60 in the tables. It is seen that the polysaccharide from the broth culture

TABLE IV

Agglutinin Nitrogen Content of Sera of Rabbits after Successive Courses of Haemophilus influenzae, Type B Injections

Rabbit sera	Dilution	Volume of dilution used	N content of control suspension	N content of agglutinated suspension	Difference (agglutinin N)	Agglutinin N in supernatant on 2nd absorption	Total agglutinin N	Agglutinin N per ml. of undiluted serum
		ml.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
$1–12_1$	Undil.	0.5	0.866	1.158	0.292	0.006	0.30	0.60
$1–11_2$	3:10	1.0	0.690	0.856	0.166	0.013	0.18	0.60
$1–11_3$	" "	1.0	0.606	0.838	0.232	0.014	0.25	0.83
$1–11_4$	1:3	0.5	0.760	0.932	0.172	0.007	0.18	1.08
$13–23_1$	Undil.	0.5	0.866	1.120	0.254	0	0.25	0.50
$13–23_2$	3:10	1.0	0.690	0.900	0.210	0.018	0.23	0.77
$13–23_3$	" "	1.0	0.606	0.818	0.212	0.020	0.23	0.77
$13–23_4$	" "	1.0	0.760	0.964	0.204	0.018	0.22	0.73

Subnumerals indicate course numbers.

precipitated an amount of antibody roughly equal to that found as agglutinin either with broth- or agar-grown suspensions. The polysaccharide from agar cultures, however, precipitated an amount of anticarbohydrate greatly in excess of the agglutinin nitrogen. Parallel experiments with agar itself showed that horse serum I 60 contained 0.38 mg. per ml. of protein nitrogen precipitable by agar and presumably formed in response to the antigenic stimulus of the traces of agar remaining on the agar-grown bacilli used for the immunization (*cf.* also Sordelli and Mayer (12)). The supernatant from the agar precipitate in serum I 60 was treated with influenza B polysaccharide, giving an additional 0.60 mg. per ml. of anticarbohydrate. This, added to the amount precipitated by agar alone, equaled 0.98 mg. per ml., the same quantity of nitrogen as was precipitated by the crude polysaccharide from the agar cultures. Influenza B polysaccharide prepared from such cultures therefore contains much soluble agar, and this is also borne out by the relatively large amounts required for complete precipitation of the type specific antibody.

5. Purification of Antibody in Type B Anti-Influenza Rabbit Sera.—In Table V are given data on the purification of rabbit antibody to *H. influenzae*, type B, by the method recently published from this laboratory for antipneumococcus sera (13). The sera are allowed to stand in the cold, centrifuged, and diluted with several volumes of water. The total globulin is then precipitated by addition of an equal volume of sodium sulfate solution, saturated at 35–38°C. All water, materials, etc., are specially sterilized for intravenous injection and the precipitated globulin is collected sterilely in a Sharples supercentrifuge, dissolved in water and saline, centrifuged from any remaining particles, and filtered through a Chamberland L2 filter. The solution is finally warmed to 60° for one-half hour as was recommended by Goodner, Horsfall, and Dubos for processed antipneumococcus rabbit sera (14). From the agglutinin content of the original serum pools and the resulting globulin solutions it is evident that substantially all of the antibody present is recovered in this method. Since any analytical error is multiplied several hundredfold in calculating the total antibody content in several hundred milli-

TABLE V

Data on Antibody Globulin Prepared for Therapeutic Use from Anti-Haemophilus influenzae, Type B Rabbit Sera

Preparation	Volume of rabbit serum pool used	Agglutinin N content of rabbit serum pool used	Precipitin N content of rabbit serum pool used	Recovered antibody globulin				Volume of recovered globulin	Agglutinin recovery	Precipitin recovery
				Total N	Agglutinin N	Precipitin N	Agglutinin N / Total N × 100			
	ml.	*mg.*	*mg.*	*mg. per ml.*	*mg. per ml.*	*mg. per ml.*	*per cent*	*ml.*	*per cent*	*per cent*
C	170	31	17		0.20	0.08		135	87	65
D	400	252		2.92	0.48	0.30	16	510	97	
E	380	137		3.38	0.48		14	305	107	

liters of serum, and, in addition, the total serum and antibody solution volumes were only approximately measured, it is clear that the figures might fluctuate rather widely around 100 per cent.

DISCUSSION

The data summarized in the tables illustrate the application of quantitative, absolute methods of agglutinin and precipitin analysis to horse and rabbit antisera to *H. influenzae*, type B. Table I presents in outline an immunization procedure that has led to the production of sera of greatly improved antibody content. In Table II it is shown how total agglutinin may be determined with formalin-killed suspensions of the homologous strain in the mucoid phase and how group agglutinins may be estimated with heterologous or R strains. It is also shown in this table and in Table IV how, once the actual antibody content of the rabbit sera originally on hand was known, it was possible to increase the agglutinin and precipitin

content five to ten times by improved methods of immunization and frequent analytical control. The sera now being obtained with a content of 1 mg. of antibody nitrogen per ml. compare quite favorably with average rabbit antipneumococcus sera. In the anti-influenza sera, also, the principal antibody present is anticarbohydrate, just as in the type specific antipneumococcus sera. Whether or not the chief protective antibody is anticarbohydrate is less clear than in the case of the antipneumococcus sera, but it is hoped that studies now in progress will answer this question.

Of the various type B (M, Dawson (15)) suspensions used the formalin-killed proved most satisfactory, and these absorbed approximately the same amount of antibody from the sera whether the bacilli originated from broth or agar cultures. Optimal conditions for capsule formation (6 hours' growth on Levinthal agar) favored the removal of anticarbohydrate from the sera. Microscopic examination showed that much of the capsular material was lost when young cultures were killed by heat, and such suspensions failed to remove agglutinins completely when added to sera in amounts comparable to those of the formalin-killed suspensions which absorbed the sera completely in a single agglutination.

Table III summarizes the precipitin content of a single horse serum and of a number of rabbit sera. Very much less specific polysaccharide prepared from broth cultures was required to precipitate the antibody than was needed of samples prepared from agar cultures, so that it is evident that the latter preparations were grossly contaminated with soluble agar derivatives.[4] The total amount of antibody precipitable with the preparations from the two sources was almost the same in the case of the rabbit antisera, but in a horse serum (Table III) the antibody precipitated by the broth polysaccharide agreed quite well with the agglutinin value, while far more nitrogen was precipitated by the carbohydrate from the agar cultures. That the excess consisted of anti-agar, as noted in other instances by Sordelli and Mayer (12), was shown by the far larger amount of nitrogen precipitated from the serum by dilute agar solution than was thrown down by the same agar solution from the serum of another horse which had been injected solely with a protein. After absorption with agar the anti-influenza horse serum still showed an almost undiminished content of type B anticarbohydrate nitrogen. Possibly the slight excess of precipitin over agglutinin shown by the broth polysaccharide might be traced to antigenic broth components remaining in the preparation.

[4] The complicated procedures necessary for the elimination of such impurities are well illustrated in a recent paper by Miles and Pirie (16) on brucella antigens.

While the anti-influenza type B rabbit sera contained little anti-agar the proportion of anticarbohydrate precipitin to total antibody (agglutinin) appeared to vary rather widely. In general at least three-quarters of the antibody was accounted for as anticarbohydrate, and presumably the remainder consisted of antibodies to the somatic components of the bacillus.

Table IV shows the results of serial courses of immunization of two groups of rabbits (12 each, at the start). The details of the immunization procedure are given in section 1 of the experimental part and in Table I. Since most of the serum obtained was needed for therapeutic purposes the sera of individual animals were not analyzed. It appears, however, from the successive values obtained in the two groups, that the injection of massive amounts of type B organisms in the M phase offers little advantage over the use of more moderate amounts, as practised in the case of rabbits 1 to 12. However, the experiments must be repeated before a definite conclusion may be drawn.

From Table V it will be noted that substantially all the antibody in the rabbit sera may be recovered in the total globulin according to the simple procedure given in reference 13. Antibody prepared in this way has been repeatedly administered intravenously with no disturbing reactions. It would, however, doubtless be advisable to remove the sodium sulfate present by dialysis before intrathecal injections were attempted.

SUMMARY

1. The quantitative, absolute methods of agglutinin and precipitin analysis previously developed for antipneumococcus sera have been shown to be applicable to horse and rabbit anti-influenza type B sera and plasmas.

2. With the aid of these methods and improved immunization schedules the antibody content of the rabbit sera has been increased five to ten times.

3. The method recommended for the purification of rabbit antipneumococcus antibody has also been found applicable to rabbit anti-influenza type B sera.

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