A REPORT ON ANTIMENINGITIS VACCINATION AND OBSERVATIONS ON AGGLUTININS IN THE BLOOD OF CHRONIC MENINGOCOCCUS CARRIERS.

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Plates 47 and 48.

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Following an outbreak of epidemic meningitis at Camp Funston, Kansas, in October and November, 1917, a series of antimeningitis vaccinations was undertaken on volunteer subjects from the camp. Major E. H. Schorer, Chief of the Laboratory Section at the adjacent Base Hospital at Fort Riley, offered every facility at his command and cooperated in the laboratory work connected with the vaccinations. In the camp, under the direction of the Division Surgeon, Lieutenant Colonel J. L. Shepard, a preliminary series of vaccinations on a relatively small number of volunteers served to determine the appropriate doses and the resultant local and general reactions. Following this series, the vaccine was offered by the Division Surgeon to the camp at large, and given by the regimental surgeons to all who wished to take it.

Preliminary Series.

The preliminary series of vaccinations was carried out in the 342nd Field Artillery Regiment through the courtesy of Colonel Nugent and Major Czar C. Johnson, surgeon of the regiment. This organization volunteered *en masse* in response to the call issued by the Division Surgeon and offered a most promising opportunity for an extended series of observations. Moreover, only one case of meningitis had developed in the 342nd Field Artillery and the regiment had recently been covered in the search for meningococcus carriers. Dur-

ing the first experience the vaccination of known carriers was avoided and this regiment appeared to be free from them.

Choice and Preparation of Vaccine.—That at least two serologically distinct disease-producing types of meningococci exist has been known since Dopter[1] in 1909 described the "parameningococcus" obtained first from the nasopharynx and then from the blood and spinal fluid of active cases of epidemic meningitis. Following Dopter's discovery Wollstein[2] confirmed the serological distinction between normal or regular meningococci and parameningococci by a comparative study of agglutination, opsonization, and complement fixation of the two types, concluding that:

"Because of the variations and irregularities of serum reactions existing among otherwise normal strains of meningococci it does not seem either possible or desirable to separate the parameningococci into a strictly definite class. It appears desirable to consider them as constituting a special strain among meningococci not, however, wholly consistent in itself."

In a more recent study based on absorption of agglutinin and agglutination tests Gordon[3] has distinguished four groups of meningococci which he calls Types I, II, III, and IV. Groups I and II are distinct and correspond to the known types. They are responsible for 75 to 85 per cent of the cases of epidemic meningitis. Other meningococci he separates into Types III and IV. Tulloch[4] finds, however, that definite group relationships exist between Types I and III, and II and IV, so that a I–III group type and a II–IV group type can be distinguished. These and certain other strains can be classified only by the absorption of agglutinin test, since they agglutinate in two or more of the type sera. Type II also ' appears to include a complex subgroup and shows considerable variation among the cocci comprised therein." Nicolle, Debains, and Jouan,[5] employing a rapid method of agglutination or flocculation (without incubation) in low dilu-

[1] Dopter, C., Étude de quelques germes isolés du rhino-pharynx, voisins du méningocoque (paraméningocoques), *Compt. rend. Soc. biol.*, 1909, lxvii, 74.

[2] Wollstein, M., Parameningococcus and its antiserum, *J. Exp. Med.*, 1914, xx, 201.

[3] Gordon, M. H., The definition of the meningococcus, *National Health Insurance, Med. Research Committee, Special Rep. Series, No. 3*, London, 1917, 10.

[4] Tulloch, W. J., A study of the mechanism of the agglutination and absorption of agglutinin reaction, together with an examination of the efficacy of these tests for identifying specimens of the meningococcus isolated from 354 cases of cerebrospinal fever, *J. Roy. Army Med. Corps*, 1918, xxx, 115.

[5] Nicolle, M., Debains, E., and Jouan, C., Sur les méningocoques et les sérums antiméningococciques, *Bull. et mém. Soc. méd. hôp. Paris*, 1917, xli, 878; *Ann. Inst. Pasteur*, 1918, xxxii, 150.

tions of immune horse serum, have also reported four types of the meningococcus which they designate A (Gordon I and III, regular meningococcus), B (Gordon II and IV, parameningococcus), C, and D. Types A and B are common, Types C and D relatively rare. They find that certain meningococci are related to two or more of their specific types and must therefore be classed as "indeterminates."

In a recent article Mathers and Herrold[6] readily distinguish the two main types by agglutination, and group around them most of their atypical strains. The strains which fail to agglutinate in type sera have nevertheless similar antigenic properties, since "monovalent serum prepared from these strains agglutinates in a specific way meningococci belonging to one or the other of the large biologic groups."

It appears, therefore, that while two distinct types or groups of meningococci may be clearly differentiated, most of the other organisms that do not fall strictly within these two groups are either intermediates or irregular variants of one or the other. Meningococci of other types in as far as they can be distinguished are only occasionally responsible for a case of epidemic meningitis.

In order to cover the two pathogenic types of meningococci and variants of them, it has become the practice in this country, following the methods of Flexner and Jobling[7] and Amoss and Wollstein,[8] to prepare highly polyvalent therapeutic sera by the repeated injection of a number of representative strains with the object of producing a serum which would protect against any pathogenic strain that might be encountered. For protective inoculation high polyvalency would seem to be equally desirable, but the choice of strains for human vaccination is limited by the fact that only a few injections are practicable, whereas the production of therapeutic serum requires a long series of inoculations in which immunity is gradually built up by the repeated injection of small doses of each antigen. The inclusion of any considerable number of strains in a vaccine for human use might defeat the purpose of the vaccination by the introduction of toxic amounts of bacterial protein without a sufficient quantity of any one specific

[6] Mathers, G., and Herrold, R. D., Observations on meningococcus carriers and on the bacteriology of epidemic meningitis, *J. Infect. Dis.*, 1918, xxii, 523.

[7] Flexner, S., and Jobling, J. W., Serum treatment of epidemic cerebro-spinal meningitis, *J. Exp. Med.*, 1908, x, 141.

[8] Amoss, H. L., and Wollstein, M., A method for the rapid preparation of anti-meningitis serum, *J. Exp. Med.*, 1916, xxiii, 403.

antigen to give rise to protection against its given strain. But since 70 to 85 per cent of the cases of epidemic meningitis are caused by meningococci of the two main types, the limitation of the strains in a vaccine to representatives of these types would seem to be the rational procedure, holding out hope of protection against a large proportion of disease-producing strains.

Therefore, for the vaccine used at Camp Funston only three organisms were chosen from the stock of The Rockefeller Institute, but the vaccine may be regarded as having represented the two main types of the meningococcus. It was also anticipated that the epidemic at Camp Funston might furnish some strain or strains of meningococci not closely related to the main types and which ought therefore to be included in the vaccine, but a type study at Fort Riley showed that the sixteen strains recovered from active cases in December and January would be covered by a vaccine containing normal and para strains.

Method of Preparing Vaccine.—The vaccine used was made in the laboratory of The Rockefeller Institute. 16 hour growths on 1 per cent glucose agar in Blake bottles were washed off with isotonic salt solution, like strains pooled, and the concentrated suspensions immediately heated to 65°C. for 30 minutes to kill the cocci and inactivate the autolytic ferment. Experiments have shown that this temperature does not impair the antigenic properties of the organism and the intact cocci are less toxic than their autolyzed products.

Following the usual tests for purity and sterility the suspensions were standardized, diluted in 0.85 per cent salt solution, mixed in equal proportions of the three strains in concentrations of 1,000 million, 2,000 million, 4,000 million, and 8,000 million cocci per cc. and preserved with 0.35 per cent tricresol.

All the injections in the preliminary series were made strictly subcutaneously with a fine needle (No. 25) at the insertion of the deltoid muscle, usually in the left arm unless a recent vaccina "take" had occurred. Then the right arm was used. The tincture of iodine used as antiseptic was then sponged off with alcohol.

For the determination of dosage and the study of reactions and antibody formation six groups of about 50 men each were chosen from the various companies in the regiment. Successive groups received

increasing doses of vaccine in a series of three injections at 4 to 10 day intervals according to the schedule in Table I.

About 25 men of Groups I, II, III, V, and VI gave preliminary (control) blood samples for the study of immunity reactions on the day of the first injection and a later specimen was obtained from as many vaccinated men as were available on the 8th to 10th day after the third injection.

At Major Johnson's direction First Lieutenant Serge Androp, Medical Corps, obtained reports from the men at inspection the morning following the vaccinations in regard to the resulting local and general reactions.

TABLE I.

Vaccination Schedule, Preliminary Series.

Group No.	1st injection.	Interval.	2nd injection.	Interval.	3rd injection.	Interval. Blood samples taken.
	millions	*days*	*millions*	*days*	*millions*	*days*
I	500	4	1,000	4	2,000	8
II	750	9	1,500	10	3,000	9
III	1,000	9	2,000	10	4,000	9
IV	1,500	8	3,000	8	8,000	10
V	2,000	7	4,000	7	8,000	10
VI	2,000	6	5-6,000	6	10,000	9
VII	2,000	7	4,000	8	8,000	17

Dosage and Reaction.—The determination of the dosage of vaccine for subsequent groups followed from the reports of the reactions produced by the given doses. It was considered important to increase the doses gradually in order to locate closely the zone of mild reactions and to avoid unexpectedly severe results. Accordingly, the vaccinations were begun with the injection of 500 million cocci, and this initial dose was increased in successive groups by 250 or 500 million until it had reached 2,000 million. For the second and third doses in each group, the first dose was usually multiplied by two and by four. Thus it usually happened that a given dose had already been used as a second or third in one group before it was tried as the first dose in a later series.

Only two reactions of any consequence were reported from doses of less than 2,000 million cocci. An officer in Group II who explained

that he had long been hypersensitive to foreign protein and had reacted severely to the typhoid and paratyphoid vaccinations developed a severe local and general reaction, with headache and malaise, after the first injection of 750 million cocci. A second officer in Group II was similarly affected after a second injection of 1,500 million cocci and was confined to bed part of the following day. The two reactions after these small doses were merely transient, but they demonstrate a factor of individual susceptibility which was found to be of considerable importance in the determination of proper doses of vaccine.

When an initial dose of 2,000 million was reached (Group V) general reactions began to appear with greater frequency. None of the men in this group felt ill enough to report at sick call the following morning, but nine men stated upon question that they had felt feverish the preceding night. Four of them had had headache also and one reported a chill. Arms were moderately sore at the site of injection, but not sore enough to interfere with routine duties.

From this time on, a small number of the men in each group reported some local or general discomfort following the vaccination. The symptom most frequently mentioned was a "feverish sensation" often accompanied by headache, which was sometimes severe enough to cause loss of sleep. Morning temperatures, when taken, were reported normal. In a few instances there was transient nausea, malaise, or aching joint pains, and three reactions were initiated with a chill. Eight men had general reactions after the first and second doses, or after the second and third, and three complained of discomfort after all three injections.

On the whole the reactions produced by the vaccine in the preliminary series were mild as compared with those that occasionally follow injections of typhoid or paratyphoid prophylactic and there was little complaint among the men. The occurrence of an occasional reaction of greater severity even with the smaller doses, and increasing local tenderness after the injection of the larger doses of vaccine led to the choice of relatively lower doses for the general series throughout the camp rather than the attempt to push the dosage up to the limit of endurance. Later experience fully justified this decision. Doses of 2,000, 4,000, and 8,000 million had already been decided upon when Group VI was given a second injection of 5,000 or 6,000

million and a third injection of 10,000 to determine the relative security of the chosen doses. The injection of 10,000 million cocci caused general reactions in eleven men, but none of them was confined to bed or relieved from duty following the injection.

Finally, a seventh group of 99 men received the chosen doses of vaccine at weekly intervals about a week before the corresponding injections were given in the camp at large and served as a final check on the dosage. On giving the third injections to Group VII it was noted that many of the men still had a small painless area of subcutaneous induration at the site of the former injections. This persistent induration has been personally experienced after injection of meningococcus vaccine. It disappears gradually, leaving no trace. A more general discussion of reactions is reserved for the report on the larger vaccination series.

Immunity Reactions.—Agglutination is the reaction of choice for the study of antibody production with the meningococcus. It is the most delicate as well as the most specific, and is most easily made on a large number of sera and in multiple dilutions. In studying agglutination, however, several factors have to be taken into account aside from the obvious requirement of a carefully standardized technique. One of the most important is the relative agglutinability of various strains of meningococci and their response to one or both of the type (normal and para) immune bodies. Meningococci vary also in antigenic power and not always in consonance with their agglutinability, so that it is sometimes profitable to use certain type strains as antigens and other strains to test antibody production.

Most of the control sera taken in advance of vaccination from men of Groups I, II, III, V, and VI were used up in agglutination tests against the vaccine strains in a dilution of 1:10. Except in one instance in which the para strain was partially agglutinated, the results of these tests were uniformly negative. When the first of the serum specimens, taken on the 8th and 10th days after vaccination and tested in 1:10 dilution against the vaccine strains, also gave negative results, it was decided to collect all the available sera for later study in lower dilutions and against more easily agglutinable strains. This study was subsequently made at The Rockefeller Institute. A certain number of sera from each group was examined by macroscopic

agglutination with a modification of the Wright method in dilutions of 1:2 and higher against the following strains from the stock of The Rockefeller Institute:

No. 8. A "regular" which is also agglutinated in low dilutions of para serum.
No. 10. An intermediate which agglutinates in both "regular" and para serum.
No. 60. One of Dopter's paras which is relatively easily agglutinable and was represented in the vaccine.

On account of the limited amounts of serum available and the low dilutions required, the following method of agglutination was employed:

Capillary glass tubing of an internal diameter of 2 mm. is drawn out into capsules 8 to 10 cm. long (Fig. 1). For use the ends are snapped off and a nipple is slipped on one end and folded double, giving accurate control of the aspirated fluids. A file mark measures equal volumes of serum and salt solution for successive dilutions, which are made in the capsule and deposited in a row on a plate of solid paraffin in a Petri dish. The paraffin plate is conveniently cleaned by melting off a thin layer in hot water. Equal amounts of a serum dilution and a meningococcus suspension are measured in the capsule, mixed on the paraffin plate, and drawn up to form a column about 1 to 1.5 cm. long. Four or five such specimens, separated by air bubbles, are sealed in a capsule for incubation. Only one dilution of a serum should be used in a single capsule, but several meningococcus suspensions (4 billion per cc.) may be tested against it, as the admixture of specimens in the capsule is inappreciable. The capsules are woven through holes in a card which designates their contents, and incubated in a horizontal position for 24 hours at 55°C. Complete agglutination is indicated by a widespread flocculated sediment of organisms (Fig. 2). The flocculi are distributed through the clear fluid on rolling the tube briskly between the palms (Fig. 3). Absence of agglutination corresponds to a smooth line of sediment, which goes into even suspension on whirling. Partial agglutination is easily recognized in a combination of the two types of sediment. Results are read with the unaided eye or under a small hand lens.

As stated above, most of the control sera had been exhausted before this more comprehensive series of tests was undertaken. There remained fourteen normal control sera from Group VI, and it happened that the practically negative results obtained with the sera of Groups I, II, and III admit these specimens as controls, since the small doses of vaccine used in these groups did not give rise to demonstrable antibody formation. To these may be added twelve sera from supposed non-contacts obtained in New York City.

TABLE II.

Agglutination Tests with Sera of Vaccinated Men.
Dilution 1:2.

Controls, not vaccinated.	Strains.			Group I.	Strains.			Group II.	Strains.		
	8	10	60		8	10	60		8	10	60
N 1	−	−	−	5	++	++	++	71	−	−	−
N 2	−	−	−	8	−	−	−	73	−	−	−
N 3	−	−	−	11	−	−	−	77	++	++	−
N 4	−	−	+	13	−	−	−	79	−	−	−
N 5	−	−	−	14	−	+	−	81	−	−	−
N 6	−	−	−	15	−	+	−	88	−	−	−
N 7	−	−	−	20	−	−	−	94	−	−	−
N 8	−	−	−	21	+	−	−	100	−	−	−
N 9	−	−	−	24	−	++	−	112	−	−	−
N 10	−	−	−	25	−	−	−	113	−	−	−
N 11	+	−	−					114	−	+	−
N 12	−	−	−					115	−	−	−
Group III.				Group IV.				Group V.			
122	−	−	−	173	+	+	+	227	−	++	+
125	−	−	−	179	−	+	−	233	++	++	+
126	−	−	−	180	−	+	−	234	++	++	−
127	−	−	−	183	−	+	−	237	+	++	+
134	−	−	−	185	−	++	−	239	+	+	+
135	−	−	−	186	−	−	−	240	++	++	−
140	−	−	−	188	−	−	−	246	−	+	−
142	−	−	−	192	−	+	−	250	−	++	+
147	−	−	−	195	−	+	−	251	−	++	++
152	−	−	−	197	−	−	−	252	++	++	++
155	−	−	−	202	−	++	−	253	−	++	+
160	−	−	−	206	−	+	−	254	−	++	+
				210	−	+	−	255	−	++	+
				212	−	++	−	256	++	++	+
				215	−	+	−	257	−	++	++
								259	−	+	−
								261	++	++	+
								262	+	++	++
								263	++	++	++
								264	++	++	++
								267	−	++	++
								268	−	++	++

++ indicates complete agglutination, + partial agglutination, − no agglutination.

TABLE II—*Concluded.*

Group VI.	Before vaccination (controls). Strains.			After vaccination. Strains.			Group VII.	Strains.		
	8	10	60	8	10	60		8	10	60
269				−	−	−	322	−	++	++
270				−	+	+	323	−	++	++
271	−	+	+	−	+	−	324	−	+	++
273	+	+	−	−	++	++	326	−	++	++
274				+	++	−	331	−	+	+
278	−	−	−	+	++	−	334	−	++	++
284	−	−	−	−	+	−	335	−	++	++
286	−	−	−	+	++	+	354	−	++	++
287	−	−	−	−	++	−	380	−	++	++
288	−	−	−	−	+	−	395	−	+	+
289	−	−	−	−	+	−	397	−	++	++
290	+	+	−	+	++	+	399	−	++	+
291	−	−	−	+	+	−	406	−	++	+
292	−	−	−	−	++	−	409	−	++	++
294	−	−	−	−	+	−	415	+	++	++
296	−	−	−	+	++	++				
299				−	+	+				
310				−	+	+				
317				−	+	−				

Table II shows the absence of agglutination in Groups I, II, and III with few exceptions. In three cases only was more than a partial agglutination of one strain in the 1:2 dilution observed. The titers in these cases ran as follows:

Group.	Serum.	Strain.		
		8	10	60
I	5	1:32	1:8	1:8
	24	0	1:32	0
II	77	1:8	1:32	0

It may be assumed, therefore, that the number of unvaccinated men whose sera would agglutinate any of these chosen strains of meningococci is small, and on the basis of this assumption the observation that twelve of fifteen sera from Group IV agglutinate Strain

10 partially or completely is unmistakable evidence of antibody formation through the agency of the vaccine. Group V, in which the dosage reached that chosen for the camp at large, confirms this evidence of reaction, for all the twenty-two sera studied contained antibodies for the meningococcus. In some instances the more easily agglutinable strains were agglutinated in a dilution of 1:32 or 1:64 (Table III).

TABLE III.

Agglutinin Titers of Sera from Group V.

Serum.	Strains.		
	8	10	60
227	0	1:32	1:32
233	1:2	1:32	1:8
234	1:2	1:4	0
237	1:2	1:16	1:4
239	1:2	1:16	1:2
240	1:2	1:64	0
246	0	1:2	0
250	0	1:128	1:8
251	0	1:32	1:32
252	1:32	1:64	1:8
253	0	1:32	1:8
254	0	1:64	1:8
255	0	1:32	1:2
256	1:2	1:64	1:2
257	0	1:64	1:16
259	0	1:2	0
261	1:2	1:32	1:2
262	1:2	1:32	1:32
263	1:4	1:32	1:32
264	1:2	1:64	1:64
267	0	1:8	1:8
268	0	1:64	1:8

The final evidence of antibody production is furnished by a direct comparison of sera from Group VI taken before the first vaccination with sera taken 9 days after the third injection of vaccine, in which the appearance or increase in agglutinins is observable in all but two instances. The presence of agglutinins in the sera from Group VII merely confirms the previous findings. *It may, therefore, be stated*

that the injection of well tolerated doses of meningococcus vaccine is followed by specific antibody formation in the human body.

The preliminary series of vaccinations, therefore, served to establish the method of injection, the proper dosage for extended vaccination, the reactions which might be expected to follow the chosen doses, and the production of immune bodies in the serum of vaccinated men. On the basis of these findings the vaccine was offered to the camp at large.

General Series.

The vaccine used in the general series of inoculations in the camp was made by Lieutenant Peter K. Olitsky at The Rockefeller Institute by the methods already described. It was shipped to Fort Riley in bulk and was diluted in isotonic salt solution to standard concentration, preservedwith 0.3 per cent cresol and distributed in 50 cc. bottles to the regimental surgeons under Major Schorer's direction. The use of two suspensions, one of 4,000 million cocci per cc. (Vaccine A and B), and the other of 8,000 million (Vaccine C), adjusted the injection volumes to 0.5 and 1 cc. of Vaccine A and B and then 1 cc. of Vaccine C, volumes similar to those for the typhoid prophylactic, with doses of 2,000 million, 4,000 million, and 8,000 million cocci to be given at weekly intervals.

At the direction of the Division Surgeon, the Division Training Officer, Captain Albert Bower, to whom especial thanks is due for his mediation between the regimental surgeons and the laboratory, called a meeting of the surgeons at which the method of giving the vaccine and the results to be expected and observed were fully described and discussed. The surgeons were thus informed of the procedure and object of the vaccination and their cooperation was enlisted, without which little could have been accomplished. The response of the men when the vaccine was offered to them was due in large measure to the interest and example of the regimental surgeons.

According to the statistics of the division headquarters, the total strength of the 89th Division at this time was approximately 25,000 officers and men. Of these, 4,792 (19 per cent) took the first injection, 4,257 (17 per cent) the second also, and 3,702 (15 per cent) completed the series.

Part of the men received the full dosage as planned. About half of those vaccinated, whose third injection was due after February 4, 1918, were given a final injection of 4,000 million, on account of the occurrence of several fairly severe reactions from the larger dose among medical officers at Fort Riley. In some regiments the vaccinations had been completed before February 5.

Reactions.—After the first injections had been given, the opinion was almost universal in the camp that the vaccine caused less general and local reaction than the typhoid prophylactic. In very few regiments was a man excused from duty the following day on account of the reaction from the vaccination. The general feeling was that the second dose caused less reaction than the first, but there were a few men in almost every organization who had reactions of moderate severity, sometimes being confined to bed for the day with headache, joint pains, and nausea. Several cases of looseness of the bowels or transient diarrhea were noted. This symptom had not been encountered before. Careful inquiry in individual cases often elicited the information that men who complained of the effects of vaccination were suffering from mild coryza, bronchitis, etc., at the time of injection.

Among the units who took the third injections before the dosage was reduced, and so received a third dose of 8,000 million meningococci, there were several instances of fairly severe reactions, general and local, which necessitated relief from duty the following day. The reactions were not more severe than those that occasionally follow paratyphoid prophylactic and no untoward results were reported. The large majority of the men seem to have suffered no appreciable reaction whatever. The smaller doses of 4,000 million cocci caused even fewer reactions.

As in the preliminary series, the factor of individual susceptibility was prominent, a few officers and men suffering severely from doses which caused no general discomfort in the great majority of the men. In general, the more severe reactions occurred among the commissioned officers, and especially among the medical officers at the Base Hospital and in the Medical Officers' Training Camp at Fort Riley, due in part perhaps to more confining occupations, higher nervous tension, and more introspection than was common among the enlisted

men. In one regiment, through a misunderstanding, four men were given an initial dose of 8,000 million cocci, which was well tolerated. The surgeon reported:

"One had chill for 30 minutes, headache for 1 hour, slight local reaction.
One had slight headache, slight local reaction.
One had severe local reaction and headache for 24 hours.
One had slight local reaction, headache for 12 hours."

A survey of the reports of the regimental surgeons and of the observations in the preliminary series shows that headache was the most frequent symptom following injection, and accompanied most of the other symptoms encountered. Sometimes the reaction was initiated by a chill or chilly sensation, and a number of men complained of fever or feverish sensations during the following night. Next in frequency came nausea (occasionally vomiting), dizziness, and general "aches and pains" in the joints and muscles, which in a few instances were especially localized in the neck or lumbar region, causing stiff neck or stiff back. A few injections were followed by diarrhea. The reactions, therefore, occasionally simulated the onset of epidemic meningitis and several vaccinated men were sent as suspects to the Base Hospital for diagnosis.

Such transient reactions are illustrated by the following brief protocols:

Individual 1.—C. D., Private, Battery D, 342nd Field Artillery.

Jan. 15, 1918. 1st injection 2,000 million. "Sore arm."

Jan. 22. 2nd injection 4,000 million. "Sore arm, headache."

Jan. 29. 3rd injection 8,000 million. "Began to feel badly about 15 minutes later and had a chill. 3 hours later was sent to the Base Hospital complaining of headache, lumbar pain, stiff neck, and fever, 103°F. No nausea. Went to sleep about 2 hours later and slept well. Had entirely recovered the next morning."

Individual 2.—L. N., Private, Field Hospital Company 354, 314th Sanitary Train.

Jan. 21, 1918. 1st injection 2,000 million. "Slight local soreness."

Jan. 28. 2nd injection 4,000 million. "Slight local soreness."

Feb. 4. 3rd injection 8,000 million. "About an hour and a half after injection, was taken with a severe chill and a subnormal temperature. About 20 minutes later his temperature rose to 103.6°F. and then fell within the next 2 hours to 101°F. Temperature normal the following morning. He complained of no other symptoms."

The most severe illness immediately following vaccination is described by the officer himself as follows:

Individual 3.—J. M. K., First Lieutenant, 314th Sanitary Train.

Jan. 21, 1918. 1st injection 2,000 million. 4.30 p.m. Soreness at site of injection in evening. Slept well.

Jan. 22. Awakened feeling drowsy and listless. Local soreness worse. Pains in back of neck, calves of legs, thighs, lumbar region, and both arms. No appetite. Symptoms grew worse after holding sick call at the Infirmary. Perspired on slight exertion. Felt chilly and warm by turn. Went to quarters and lay in bunk. Soon felt feverish. Developed diarrhea about 11 a.m. Eight movements in 5 hours. Cup of hot chocolate at 1 p.m. Felt nauseated. Vomited six times in next 3 hours. Took sodium bicarbonate, 2 gm. in glass of warm water, promptly vomited. Retained 15 minutes later, and then began to feel better. Felt well next morning but had no appetite. Bowels still loose. Returned to duty. Appetite returned the evening of Jan. 24. Local soreness persisted 3 days.

		Temperature. °F.	Pulse.
Jan. 22,	9.45 a. m.	98.4	86
	1.15 p. m.	99.8	82
	3.45 p. m.	101.0	92
	7.30 p. m.	101.8	108
	10.00 p. m.	100.0	98
Jan. 23,	7.25 a. m.	97.8	98

Jan. 28. 2nd injection 4,000 million. Local reaction severe. Duration 3 days. General reaction, malaise of 24 hours duration.

Feb. 4. 3rd injection 8,000 million. Local reaction severe. Duration 4 days. General reaction, nausea, looseness of bowels (not diarrhea), chilliness, but no fever. Muscle soreness severe and general. Appetite absent for 2 days.

The occurrence of such reactions as those described above was rare but they illustrate the importance of the factor of individual susceptibility. The great majority of the men vaccinated were reported to have had no general symptoms, and very few men were confined to quarters after any dose of the vaccine.

Occurrence of Meningitis after Vaccination.—The records of cases of epidemic meningitis in Camp Funston have been followed up to June 4, 1918, thus covering the period that the 89th Division remained in camp. In the interval between January 21 when the vaccination was started in the camp and June 4, 46 cases of meningitis are reported to have entered the Base Hospital at Fort Riley. Of these patients, three had received one, two, or three injections of antimeningitis vaccine. The following data have been collected in regard to these cases.

Case 1.—L. T., Second Lieutenant, 314th Engineers.

Jan. 21, 1918. 1st injection 2,000 million. Next day neck was rather stiff. slight headache. Well for next 2 days, but working very hard on double duty.

Jan. 25. Fainted at Officers' Call. Felt "under the weather" with indefinite symptoms the next 2 days but remained on duty.

Jan. 28. 2nd injection 4,000 million. This made him feel so much better that he remarked to his friends that he "was getting to be a dope fiend in the stuff and couldn't get along without it."

Jan. 29. Felt worse again, headache, "no ambition." Following days he remained on duty, but tired easily and often felt chilly and feverish, had stiff neck and joint pains.

Feb. 2. Remained in bed. Throbbing headache, became nauseated and vomited twice. Sent to Base Hospital. Lumbar puncture on arrival showed a turbid fluid with many leukocytes and moderate numbers of intracellular Gram-negative diplococci.

Lieutenant T. had a very mild case of meningitis which yielded promptly to serum treatment.

Case 2.—E. H., Corporal, Company C, 354th Infantry.

Jan. 21, 1918. 1st injection 2,000 million. "Never felt anything. Arm not very sore."

Jan. 26. Went on 5 days leave, returning Jan. 31, and so missed second injection.

Feb. 1. "Felt sore all over." Had chills. Frontal headache.

Feb. 2. Headache continued, stiff joints and aching pains. Sent to Base Hospital. Diagnosis, epidemic meningitis.

Corporal H. had a severe case of meningitis but recovered.

Case 3.—J. C. N., Private, Company B, 340th Machine Gun Battalion.

Jan. 24, 1918. 1st injection 2,000 million.

Jan. 31. 2nd injection 4,000 million.

Feb. 8. 3rd injection 4,000 million. Reactions not reported.

Mar. 31. Acute otitis media and right frontal sinusitis.

Apr. 16. Epidemic cerebrospinal meningitis.

Apr. 24. Cerebrospinal fluid found positive for meningococcus for the last time.

Apr. 27. Ulcer of right cornea followed by slight opacity with slight impairment of vision.

May 5. Right facial paralysis which has since improved considerably.

Private N. will be ready for assignment to domestic military service about July 1, 1918.

The examples of Lieutenant T and Corporal H are instructive, since they may well be, and probably are, instances of meningococcus inoculation in individuals in the incubation period of a meningococcus meningitis. Assuming this to be the fact, the indication is that no

harm was done by the procedure. But whether this is the precise fact or not, the cases have no real bearing on the value of antimeningitis vaccination, because the interval between the first inoculation and

TABLE IV.

Agglutination of Meningococci in Sera of Vaccinated Men. Dilution 1: 2.

314th Engineers.	After 3rd injection.	Strains.			355 Infantry.	After 3rd injection.	Strains.			314th Sanitary Train.	After 3rd injection.	Strains.		
		8	10	60			8	10	60			8	10	60
	days					*days*					*days*			
501	17	−	+	−	535	15	−	−	−	585	17	−	++	+
502	17	−	+	−	536	15	−	++	+	586	17	−	+	+
503	17	−	+	+	537	15	+	++	++	587	17	−	−	−
504	17	−	−	−	538	15	−	++	+	590	17	+	−	−
506*	10	−	++	++	539	15	−	+	−	591	17	−	−	−
509*	10	−	−	−	541	15	−	++	+	593	17	−	+	+
510*	10	−	−	−	544	15	−	+	−	595	17	−	++	++
511*	10	−	++	++	545	15	−	++	+	598	17	+	++	++
512*	10	−	++	+	547	15	+	++	++	600	17	−	+	+
513*	10	−	++	−	549	15	+	+	+	606	17	++	++	++
514*	10	−	+	+	553	15	++	++	++	609	17	−	++	++
515*	10	−	+	−	558	15	+	++	+	610	17	++	++	++
516*	10	−	+	++	560	15	−	−	−	611	17	+	++	+
517*	10	−	−	−	562	15	+	+	−	612	17	−	++	++
518*	10	−	−	−	565	15	−	+	−	613	17	++	++	++
520*	10	+	++	++	566	15	−	−	−	614	17	−	++	++
528	18	−	−	−	568	15	−	−	−	615	17	−	−	−
531*	10	−	+	+	569	15	−	++	+	618	17	+	++	++
532	18	−	+	−	570	15	−	−	−	619	17	−	++	++
533	18	−	−	−	571	15	−	+	−	622	17	−	+	+
534	18	−	−	−	574	15	−	++	+					
					576	15	−	++	++					
					578	15	+	++	+					
					581	15	−	++	+					
					583	15	−	+	+					

* 3rd dose 4,000 million cocci.

the diagnosis of the meningitis was too brief to permit immunization to be effected.

It is clear that Private N developed meningitis at a period at which protection following vaccination should have been present. In his case the vaccination must be considered as having failed to afford

protection. A study of the type of the infecting meningococcus would conceivably have thrown light on the incident, or the incident may merely indicate that occasional individuals fail to be adequately immunized, just as in the analogous procedure of antityphoid inoculation.

Immunity Reactions, General Series.—In addition to the blood samples taken for study in the preliminary series of vaccinations, similar samples were obtained from a group of men in each of three regiments who had completed the vaccination series. The serum specimens were taken at an interval of from 10 to 18 days after the third injections had been given, and were examined for agglutinins by the method already described. The results of these tests are shown in Table IV. The sera of 50 men showed some content of agglutinins; the sera of 16 others failed to do so.

Examination for Agglutinins of the Blood Serum of Chronic Carriers.

While every case of epidemic meningitis is presumed to develop from the carrier state, many observers have noted the rarity of the disease among chronic carriers. The presence of the meningococcus in the nasopharynx is but one of the factors in the accident which results in the bacterial invasion of the body, and the relatively low infectivity of the meningococcus is to be credited to causes of resistance on the part of the carrier host among which may well be the appearance of immune bodies in the blood as the result of the multiplication of meningococci in the nasopharynx.

If the presence of agglutinins for the meningococcus could be demonstrated in the blood of chronic carriers, light would thereby be thrown on the mechanism of this resistance. With the object of detecting the possible existence of meningococcus agglutinins in the blood serum of carriers, a number of such sera have been studied in low dilutions by the capsule method described in an earlier section of this report.

Through the courtesy of Passed Assistant Surgeon J. L. Waterman, United States Naval Hospital, Brooklyn Navy Yard, and Dr. F. S. Westmoreland, Riverside Hospital, New York, opportunity was given to obtain blood samples from a number of chronic carriers of the meningococcus, and the corresponding carrier strains were kindly furnished

by Dr. A. W. Williams of the Department of Health, of the City of New York, or obtained directly from the men themselves. The men studied were known to have harbored meningococci for from 4 to 16 weeks.

20 carrier strains of the meningococcus have been subjected to the agglutination tests in low dilutions with the sera of the hosts from whom they were obtained and the carrier sera were tested against the stock strains of spinal origin that had been studied in the sera of vaccinated men from Camp Funston. 18 of the carrier strains were also

TABLE V.

Control Agglutination Tests with Normal Sera against Carrier Strains. Dilution 1: 2

Normal control serum.	Carrier Strains.																	
	124	125	127	128	129	133	134	136	139	141	147	150	151	153	156	160	161	162
1	−	−	−	−	−	−	−	−	−	++	+	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	+	−	++	++	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−
5	+	−	+	−	−	−	−	−	−	++	+	−	−	−	−	−	−	−
6	−	−	−	−	+	−	−	−	−	+	+	−	−	−	+	−	+	+
7	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−
8	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	+	−	−
9	++	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−
10	+	+	+	+	+	+	−	−	−	++	++	−	−	−	++	−	+	+
11	+	−	−	−	−	−	+	−	−	+	+	−	−	−	−	−	−	−
12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* No test.

tested with 12 normal sera from supposed non-contacts as controls. The results of these tests are recorded in Tables V to IX. The trials with non-contact sera (Table V) show that with two exceptions the carrier strains are not usually agglutinated in normal sera in a 1: 2 dilution. One of the exceptional strains was partially or completely agglutinated by 9 and the other by 8 of the 12 sera. The other cultures were only occasionally agglutinated in this low dilution of normal serum. One supposedly normal serum agglutinated 11 of the 18 carrier strains. In the presence of sera from their hosts, however,

all but 4 of these carrier strains were agglutinated (Table VI) and the sera in most instances showed agglutinins for stock spinal strains of the meningococcus. Subsequently the sera were reexamined in multiple dilutions, and at this time 2 of those previously found negative agglutinated their strains up to 1:4 and 1:8 (Table VII). The titers of the other sera ran from 1:4 to 1:64.

TABLE VI.

Agglutination of Homologous Nasal Strains and Stock Spinal Strains in Sera of Carriers.

Serum of.	Homologous nasal strains.	Spinal Strains.		
		8	10	60
Carrier 2	++	++	++	++
" 3	−	−	−	−
" 6	+	+	++	++
" 9	++	++	++	+
" 11	+	++	++	−
" 13	++	++	++	++
" 16	++	++	++	++
" 18	+	++	++	+
" 25	+	++	++	+
" 27	++	++	++	++
" 28	++	++	++	++
" 29	++	++	++	++
" 30	+	+	++	+
" 36	+	−	−	−
" 39	++	++	+	−
" 40	+	+	+	−
" 41	−	+	−	−
" 43	−	+	−	−
" 48	++	++	++	−
" 50	−	−	−	−

Appearance of Agglutinins in Sera after Storage.—On numerous occasions we have observed an increase in agglutinating power in sera which have stood at ice box temperature for some time. The fact was reported in a recent paper[9] as having occurred with typhoid agglutinins in guinea pig sera, and Tulloch[4] records an instance in

[9] Gates, F.L., Antibody production after partial adrenalectomy in guinea pigs, *J. Exp. Med.*, 1918, xxvii, 725.

TABLE VII.

Agglutination of Carrier Strains in Homologous Sera.

Serum of.	Homologous Strain.	Agglutination titers.					
		1:2	1:4	1:8	1:16	1:32	1:64
Carrier 2	124	++	+	+	−	−	−
" 3	125	+	+	+	−	−	−
" 6	127	+	++	+	++	−	−
" 9	128	++	++	++	++	++	+
" 11	129	+	++	++	++	++	++
" 13	133	++	+	−	−	−	−
" 16	134	++	++	++	+	−	−
" 18	136	++	++	+	+	−	−
" 27	139	++	++	+	+	−	−
" 28	141	++	++	+	+	−	−
" 36	147	++	+	+	+	++	++
" 39	150	++	++	++	++	+	+
" 40	151	−	+	+	+	−	+
" 41	153	++	*	+	−	−	−
" 43	156	−	−	−	−	−	−
" 46	160	++	++	++	++	++	++
" 48	161	++	++	++	−	−	−
" 50	162	++	+	−	−	−	−

* No specimen.

TABLE VIII.

Appearance of Agglutinins in Carrier Sera after Storage.
Dilution 1: 2.

Serum drawn May 24, 1918.	Examination of May 29.				Examination of June 12.				Examination of June 24.			
	Homologous Strain.	Spinal Strains.			Homologous Strain.	Spinal Strains.			Homologous Strain.	Spinal Strains.		
		8	10	60		8	10	60		8	10	60
16	−	−	−	−	+	++	++	+	++	No test.		
36	−	−	−	−	+	−	−	−	++	−	+	+
39	−	−	−	−	++	++	+	−	++	−	++	++
40	−	−	−	−	+	+	+	−	−*	−	−*	−
41	−	−	−	−	−	+	−	−	++	−	++	++
43	−	−	−	−	−	+	−	−	−	−	++	++
46	−	−	−	−	No test.				++	−	−	++
50	−	−	−	−	−	−	−	−	++	−	+	+

* + in higher dilutions.

rabbit serum during immunization to the meningococcus. This he explains as due to the presence of constituents of the serum or of the organisms which do not take part in the mechanism of agglutination, but which may be present "in such quantity or in such a physical state that they protect the united antibody-antigen complex from the flocculating action of the electrolytes."

Table VIII shows the appearance of agglutinins in some of the sera under discussion after they had stood, sealed, at 4°C. for some time. The other sera were not examined so early after bleeding. The

TABLE IX.

Agglutination of Stock Strains in Sera of Carriers.

Serum of.	Agglutination titers of homologous strains in rabbit serum.		Agglutination titers of stock spinal strains in carrier sera.		
	Normal.	Para.	No. 8	No. 10	No. 60
Carrier 27	1:400+	1:400	1:2	1 16	1:16
" 28	1:400+	1:100	0	1:32	1:4
" 36	1:400+	1:50	0	1:64	1:4
" 39	1:800	1:100	0	1:32	1:64
" 40	1:400+	1:50	0	1:64	0
" 41	1:400+	1:100	0	1:32	1:8
" 43	1:50	1:100	0	1:32	1:8
" 46	1:800	1:200	0	0	1:16
" 48	1:50	1:100	0	1:32	1:8
" 50	1:400+	0	0	1:8	1:2

inconstancy of the phenomenon has already been described[9] and is implied by Tulloch in his reference to "this particular serum."

A variation of a different order was encountered when ten of the sera studied on May 29 or June 12 were examined on June 24 in multiple dilutions with the stock spinal strains (Table IX). Six of the ten had agglutinated Strain 8 in the 1: 2 dilution. Now all but one serum failed to do so. Strains 10 and 60, however, were agglutinated in various serum dilutions by practically all the specimens examined, including No. 43, which had failed to agglutinate its homologous strain. Agglutinins for these spinal strains are only rarely found in normal sera (Table II).

DISCUSSION.

Heretofore meningococcus vaccines have not been extensively employed for prophylactic immunization, and only a few references are to be found in the literature that relate vaccination experiences. Davis,[10] in 1907, describing animal experiments and the therapeutic use of an autogenous vaccine reported a personal experience in which he suffered a very severe reaction following the subcutaneous injection of a 24 hour slant culture of a meningococcus heated to 65°C. for 30 minutes. Shortly after inoculation, nausea and vomiting were followed by a severe chill, lasting half an hour, and then intense headache, muscular pain, purging, and vomiting of bile. His temperature rose to 103°F., and during the remainder of the day, and in the night following, nausea and vomiting continued, with headache, thirst, and marked prostration. Later symptoms included a diffuse rash, herpes, and in the urine granular, hyaline, and epithelial casts. The reaction subsided gradually. The leukocytes rose to 44,050 on the 3rd day, and the opsonic index reached 2.3 on the 2nd day, returning to normal by the 5th day.

Sophian and Black[11] studied agglutination and complement fixation in serum specimens from ten students who had been vaccinated with two or three doses of a monovalent vaccine. The doses given were 500 or 1,000 million, 1,000 or 2,000 million, and 2,000 million cocci, at 7 day intervals. Their vaccine had been heated to 50°C. for 1 hour. Following the vaccinations they noted malaise, frontal headache, and slight fever with occasionally more severe symptoms; intense frontal or vertical headache, general bodily pain, nausea, vomiting, and a rise of temperature to 102–104°F. Labial herpes was seen. Using a readily agglutinable organism, they found the agglutinin titers of the sera of their vaccinated subjects to range from 1 : 200 to 1: 1,500. Complement was fixed in serum dilutions up to 1: 250.[12] Complement-fixing antibodies were found in low dilutions of the serum of seven of these men after an interval of 2 years.

Sophian and Black refer to Hall's experience in Kansas City in the vaccination of about 280 persons in families in which meningitis had occurred. A number of doctors and nurses were likewise inoculated, and in no instance did the disease occur subsequent to vaccination. About 100 persons in Dallas were vaccinated, but most of them did not complete the vaccination series. Two nurses developed epidemic meningitis some weeks after a series of two inoculations; both recovered.

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[10] Davis, D. J., Studies in meningococcus infections, *J. Infect. Dis.*, 1907, iv, 558.

[11] Sophian, A., and Black, J., Prophylactic vaccination against epidemic meningitis, *J. Am. Med. Assn.*, 1912, lix, 527. Black, J. H., Prophylactic vaccination against epidemic meningitis, *J. Am. Med. Assn.*, 1914, lxiii, 2126.

[12] Such figures must be accepted with reserve. They are far higher than laboratory experience would lead one to expect after such prophylactic doses of a meningococcus.

Recently Whitmore, Fennel, and Petersen[13] have reported an experience with a polyvalent lipovaccine in which total doses of 40,000 million or 80,000 million cocci were given subcutaneously in one or two injections. 55 men in all were vaccinated. 40,000 million cocci in one dose did not cause any general reaction. Two doses of 40,000 million cocci each at a 3 day interval were followed by two instances of constitutional reaction among 25 men. 5 men received 80,000 million cocci in a single injection which was followed after 24 hours by a moderate general reaction. In the first days after vaccination with the larger doses agglutinin formation was observed against three of the vaccine strains, especially those that respond to antibodies of both the normal and the para type.

These reports from the literature coincide with the present experience with meningococcus vaccine in their descriptions of the reactions that may be expected, and of the appearance of specific antibodies in the blood after vaccination. As Sophian and Black pointed out, the general symptoms indicate some degree of meningeal irritation and occasionally they may simulate the onset of meningitis. The symptoms are not progressive, however, and even though severe, they clear up in a few hours. In one instance in which a lumbar puncture was done on a suspect 3 days after a second dose of vaccine the spinal fluid was found normal. The illness described by Davis is instructive to show the severity of the symptoms which may follow an injection of meningococcus substance many times the proper dose. Whitmore, Fennel, and Petersen, by protecting their vaccine in oil, were able to give much larger doses in a single injection with only moderate constitutional effects.

Whatever may be the relation of agglutinins to specific protection against invasion the agglutination test is recognized as the most reliable indication of antibody formation due to the meningococcus, and is used generally in the standardization of therapeutic sera as an index of potency. With equal reason the presence of agglutinins may be taken as an index of active immunization after vaccination. We do not know the ratio of agglutinin formation to protective power, and can only discover by wide experience what agglutinin titers correspond to relatively complete immunity to meningococcus invasion. It is perhaps significant, however, that the agglutinin titers

[13] Whitmore, E. R., Fennel, E. A., and Petersen, W. F., An experimental investigation of lipovaccines: a preliminary note, *J. Am. Med. Assn.*, 1918, lxx, 427.

of the sera of vaccinated men are of the same order of serum dilutions, namely 1 : 4 to 1 : 64+, as those of chronic carriers of the meningococcus, who are usually refractory to the strains carried. A study of the blood of cases of meningitis which have recovered without serum treatment might be instructive in this connection.

Meningococcus agglutinins appear not to have been found previously in the blood of chronic carriers. Cathoire,[14] in a brief communication, reported that the agglutinin study of carrier sera led to no positive result, but with the Wright technique he was able to show a constant increase in opsonic power compared with sera from normal persons, and he therefore concluded that the relative immunity of carriers is to be explained by a specific change in the serum.

Herrick[15] thinks that the occurrence of relapses in some cases of epidemic meningitis "lends discouragement to vaccine prophylaxis and other measures for the production of immunity." It should be pointed out that relapses are probably caused by reinfection from small pockets in the meninges in which the meningococcus has been walled off, and so permitted to survive therapeutic measures otherwise effective. It may be mentioned in passing that the occurrence of relapses in typhoid fever has been no contraindication to the employment of typhoid prophylaxis. The object of prophylactic vaccination is to oppose the meningococcus at the threshold, and if a systemic invasion precedes the spinal infection, as recent observations tend to show, the building up of antibodies in the blood stream is the means by which a hematogenous incursion is to be combated.

SUMMARY.

1. A meningococcus vaccine suspended in salt solution has been given subcutaneously as a prophylactic to about 3,700 volunteers in three injections of 2,000 million, 4,000 million, and 4,000 or 8,000 million cocci at weekly intervals.

2. These doses rarely caused more than the mildest local and general reactions. Exceptionally a more severe reaction emphasized the

[14] Cathoire, E., Recherche du pouvoir opsonisant du sérum des porteurs sains de méningocoques, *Compt. rend. Soc. biol.*, 1910, lxix, 240.

[15] Herrick, W. W., The intravenous serum treatment of epidemic cerebrospinal meningitis, *Arch. Int. Med.*, 1918, xxi, 541.

presence of an unusual individual susceptibility to the vaccine. In such instances the symptoms were in part those of meningeal irritation and sometimes simulated the onset of meningitis.

3. Specific meningococcus agglutinins have been demonstrated in the blood serum of vaccinated men as compared with normal controls.

4. Moreover, agglutinins have been demonstrated in the blood serum of chronic carriers of the meningococcus. Evidence is thus brought forward that the relative immunity of chronic carriers to epidemic meningitis may be due to the presence of specific antibodies in the blood stream.

EXPLANATION OF PLATES.

PLATE 47.

FIG. 1. Capsule of glass tubing of 2 mm. internal diameter showing points at which it is snapped off for use, and file mark for measuring equal volumes of serum and meningococcus suspension.

FIG. 2. Appearance of agglutination specimens after incubation. (*a*) Complete agglutination. (*b*) No agglutination. (*c*) Partial agglutination.

PLATE 48.

FIG. 3. Appearance of completely agglutinated specimens after shaking.

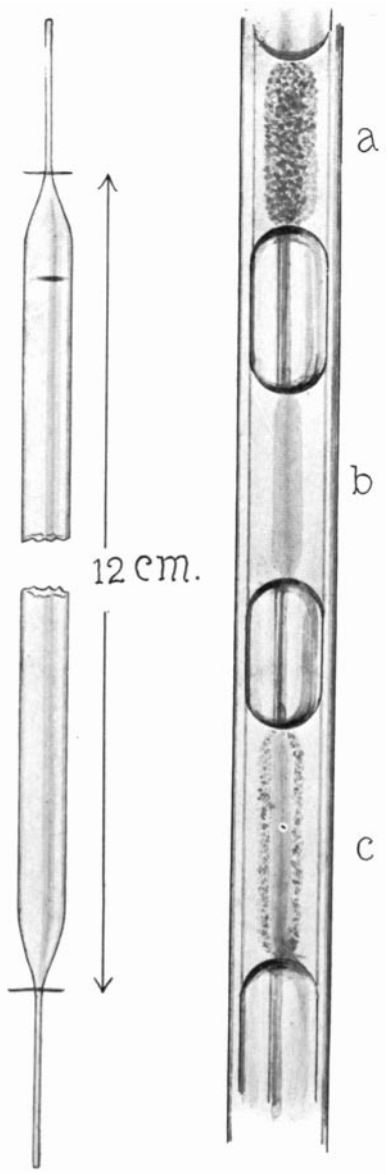

FIG. 1. FIG. 2.

(Gates: Antimeningitis vaccination.)

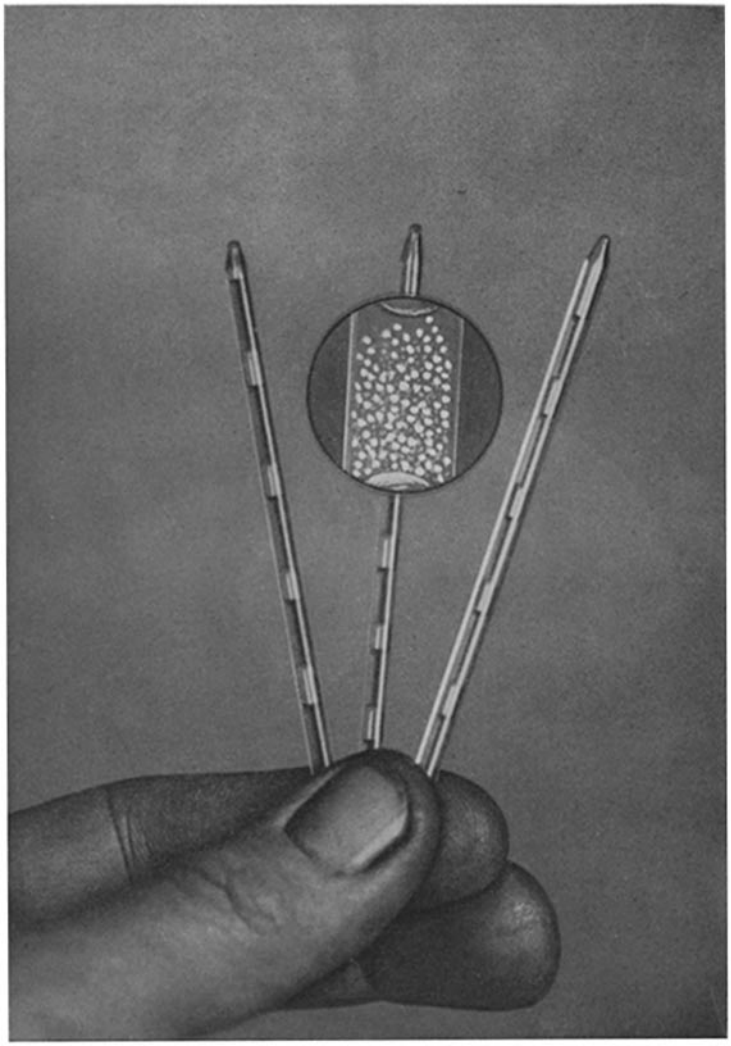

FIG. 3.

(Gates: Antimeningitis vaccination.)