STUDIES ON THE SHWARTZMAN PHENOMENON

I. Detoxification of Meningococcus Culture Filtrates

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The conversion of toxin into toxoid has been studied by Ehrlich (1) and Ramon (2–4) for diphtheria toxin, by Behring and Ransom (5), Lowenstein (6), and von Eisler and Lowenstein (7) for tetanus toxin, by Weinberg and Goy (8) for the toxins of the *B. botulinus* and of the gas gangrene anaerobes (*i.e.*, *B. oedematiens*, etc.). Ramon (9) has also converted abrin and cobra venom into toxoids. The change was observed to occur spontaneously (Ehrlich (1), Behring and Ransom (5)), and also could be induced artificially by means of various physical and chemical agents; namely, for tetanus toxin iodine trichloride (Behring and Ransom (5)), formalin (0.1 to 0.2 per cent) and exposure to a Nernst lamp (Lowenstein (6)), formalin (0.1 to 0.2 per cent) and slight heat (30°C.) (von Eisler and Lowenstein (7)); and for diphtheria toxin formalin (0.3 to 0.4 per cent) and heat (40–42°C.) (Ramon (4)). By similar procedures (formalin and heat) abrin, cobra venom (Ramon (9)), and toxins of the *B. botulinus* and the gas gangrene anaerobes (Weinberg and Goy (8)) have also been converted into toxoid states.

These studies elaborated the concept of a toxoid first announced by Ehrlich. In its completed form, the definition of a toxoid is that it is a toxin so altered that the toxicity is decreased whereas the antibody-combining capacity and the antigenicity are essentially undiminished. It has been observed (Lowenstein (6), Ramon (4)) that the antigenicity of tetanus and diphtheria toxoids varies with the potency of the mother filtrates. It can be estimated by the antitoxin-combining capacity, to which it is directly proportional.

By means of the phenomenon of local skin reactivity to bacterial filtrates, Shwartzman (10–13) has demonstrated the existence of a new

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category of bacterial exotoxins. They are filterable and best obtained under conditions of insignificant cell autolysis. The potency of a filtrate does not depend solely on the number of bacterial cells in the culture from which it is obtained. The toxins are highly antigenic. The potency of the factors necessary for the phenomenon can be accurately titrated. They are neutralizable in multiple proportions by specific antisera. Within the same bacterial species, there may be considerable variation in the potency of filtrates obtained from cultures of various strains. In a given filtrate, the potency seems to parallel the lethal effect on rabbits.

The demonstration of exotoxins of a new type in a large number of unrelated organisms as the members of the *coli*-typhoid-dysentery group, the meningococcus, the Pneumococcus Type III, the *Streptococcus haemolyticus-scarlatinae* (Shwartzman (12)), the gonococcus (Shwartzman (14)), the *Bacillus influenzae* (*Haemophilus influenzae*) (Frisch (15)), the *Bacillus pertussis* (Gross (16)), and the tubercle bacillus (Bieling (17)) was of great importance.

It was deemed of interest to determine whether this type of bacterial toxin could be converted into a toxoid state. With this aim in view, the following experiments were undertaken.

Methods and Materials

These experiments were performed on rabbits which had been employed once within the previous week for the elicitation of the Shwartzman phenomenon, receiving for this purpose a variety of materials. Only those rabbits were selected in which the phenomenon had failed to occur. Unpublished experiments suggest that such rabbits may be more sensitive to the Shwartzman phenomenon than stock rabbits. Further experiments upon this point are now under way.

Toxins.—(*a*) Meningococcus "agar washings" toxic filtrates were prepared and titrated as described by Shwartzman (18, 19). Filtrate 1409 was prepared from meningococcus Strain 123, Group I; Filtrate 1675 from meningococcus Strain 383, Group II; Filtrate A52 was pooled from Filtrates 1233A, 1233B, 1233C, and 1233D, prepared from meningococcus Strains 44B Serum 8, 44B Serum 13, 44B Serum 15, and 44B Serum 16, respectively, all Group III (Shwartzman (20)). Meningococcus Filtrate 1675 was kept in the refrigerator at 6°C. from June 16, 1931, to Dec. 18, 1931 (time of retitration). The titers of the preparations are indicated in Table I.

(*b*) 1. 147 cc. of meningococcus "agar washings" toxic Filtrate 1409 plus 3 cc. of 20 per cent formalin to make a total volume of 150 cc. with a final concentration of 0.4 per cent formalin were placed in a ground glass-stoppered bottle, sealed

with paraffin, and kept in an incubator at 37°C. for 35 days. The resulting preparation is referred to as meningococcus Toxoid 1409A.

2. 2 cc. of 40 per cent formalin were added to 95 cc. of meningococcus Toxoid 1409A, thus making a final total concentration of 1.2 per cent formalin. This mixture was placed in a ground glass-stoppered bottle, sealed with paraffin, and kept in an incubator at 37°C. for 3 weeks (meningococcus Toxoid 1409B).

3. 110 cc. of meningococcus "agar washings" toxic Filtrate A52 plus 2.75 cc. of 40 per cent formalin (to make a final concentration of 1 per cent formalin) were placed in a ground glass-stoppered bottle, sealed with paraffin, and kept in an incubator at 37°C. for 23 days (meningococcus Toxoid A52).

Sera.—1. Antimeningococcus Serum H251 was obtained from Horse 8.

Immunization was started Oct. 13, 1930. The details of a similar course of immunization have been described elsewhere (Shwartzman (21)). In general, this consisted of the following: For 6 weeks, the horse received only weekly, subcutaneous injections of *B. coli* toxin. Thereafter, an intravenous injection of heat-killed *B. coli* vaccine was made with each subcutaneous injection of *B. coli* toxin, the dosage of both gradually increasing. After 6 weeks, polyvalent meningococcus toxin was added in the material for subcutaneous injection and meningococcus vaccine in that for intravenous injection. Finally the animal received intravenously incubated mixtures of meningococcus toxin and anticoli horse serum. Bleeding H251 was performed Mar. 10, 1931.

2. Serum H230 was obtained from Horse 60 which was immunized in order to obtain the auxiliary antibody (Shwartzman (21)).

Immunization was begun June 27, 1930. The animal received weekly subcutaneous injections of polyvalent *B. coli* toxin, of gradually increasing dosage. Intravenous injections were started Sept. 12, 1930. They consisted of increasing amounts of polyvalent heat-killed *B. coli* vaccine. In the last 5 weeks of immunization, 3 intravenous injections were made of incubated mixtures of polyvalent *B. coli* toxin and anticoli horse serum. The course of immunization lasted 8 months with one period of rest for 3 weeks. Bleeding H230 was performed Feb. 11, 1931.

3. Serum H290 was a later bleeding from Horse 60. After 2 weeks' rest, immunization was continued. The animal received 4 subcutaneous injections of polyvalent *B. coli* toxin, 2 intravenous injections of *B. coli* vaccine, and 2 intravenous injections of incubated mixtures of *B. coli* toxin and Serum H230. Bleeding H290 was performed Apr. 28, 1931.

Neutralizations.—Neutralizations of the toxins and toxoids by the horse sera were carried out as described by Shwartzman (19).

Agglutinations.—Agglutinations were carried out with standard suspensions of live 24 hour cultures of meningococci. Mixtures of 0.25 cc. of bacteria and 0.25 cc. of serum were kept in a water bath at 37°C. for 18 hours and then read promptly.

Precipitations.—Mixtures were made of 0.2 cc. serum and 0.2 cc. toxoid, 0.2 cc. serum and 0.05 cc. toxoid, 0.05 cc. serum and 0.05 cc. toxoid, and 0.05 cc. serum and 0.2 cc. toxoid. The mixtures were similarly incubated. Precipitations were also carried out using toxin as precipitinogen.

Titration of Meningococcus Skin-Preparatory and Reacting Factors

It has been demonstrated by Shwartzman (13) that there exists a quantitative reciprocal relationship between the skin-preparatory and reacting factors of unrelated potent toxins. Therefore, with the use of meningococcus Toxin 1409 skin-preparatory factors it was possible to test meningococcus Toxoids 1409A and B for reacting factors and with meningococcus Toxin 1409 reacting factors to test meningococcus Toxoids 1409A and B for skin-preparatory factors. The results are summarized in Table I.

As is seen from Table I (Groups 1 and 2), meningococcus Toxin 1409 had a titer of 1400 reacting units. Meningococcus Toxoid 1409A used for preliminary experiments was not titrated to the end-point. However, it is seen from Table I (Groups 3 and 4), that there was a considerable reduction in skin-preparatory and reacting potency. Thus, of 3 rabbits which received 0.25 cc. of undiluted meningococcus Toxoid 1409A intradermally, and 24 hours later an intravenous injection of 64 reacting units of meningococcus Toxin 1409 per kilo of body weight, 1 rabbit developed a strong and 1 a weak reaction. Of 3 rabbits which received 0.25 cc. of undiluted meningococcus Toxin 1409 intradermally and 24 hours later an intravenous injection of 1 cc. of meningococcus Toxoid 1409A diluted 1:22 (a dilution corresponding to 64 reacting units of meningococcus Toxin 1409—the mother filtrate) per kilo of body weight, only 1 developed a positive reaction and that of moderate intensity. After an intravenous injection of 64 reacting units of meningococcus Toxin 1409 at least 2 of 3 rabbits show strongly positive reactions.

Meningococcus Toxoid 1409B had no skin-preparatory factors for 64 and 128 reacting units of meningococcus Toxin 1409 per kilo of body weight (Table I, Groups 5 and 6). Using meningococcus Toxin 1409 skin-preparatory factors, meningococcus Toxoid 1409B revealed only about 17 reacting units per cc. (Table I, Groups 7 and 8). Meningococcus Toxoid 1409B, therefore possessed no skin-preparatory factors and only 1.2 per cent of the reacting factors present in the mother filtrate.

Incidentally, it is of interest to note that following the intradermal injection of a toxoid preparation, there developed at the injected skin site an area of blanching 10 to 15 mm. in diameter surrounded by a

TABLE I

Titration of Potency of Skin-Preparatory and Reacting Factors of Filtrates Studied

Group No.	Skin-preparatory factor (0.25 cc. of undiluted filtrate)	Dose per kwt.*	No. of reacting units	No. of rabbits	No. of rabbits showing reactions	Titer in reacting units per cc.
1	mngo† T‡ 1409	1 cc. mngo T 1409 diluted 1:1200		3	2	1400
2	" " 1409	1 " " " 1409 " 1:1600		3	0	
3	" Toxoid 1409A	1 " " " 1409 " 1:22	64	3	2 (1 weak)	
4	" T 1409	1 " " Toxoid 1409A " 1:22	?§	3	1 (weak)	
5	" Toxoid 1409B	1 " " T 1409 " 1:11	64	3	0	
6	" " 1409B	1 " " " 1409 " 1:22	128	3	0	
7	" T 1409	1 " " Toxoid 1409B " 1:11		3	1	17
8	" " 1409	1 " " " 1409B " 1:22		3	0	
9	" T A52	1 " " T A52 " 1:500		3	1	750
10	" " A52	1 " " " A52 " 1:1000		3	0	
11	" " 1409	1 " " Toxoid A52 " 1:15		3	1	23
12	" " 1409	1 " " " A52 " 1:30		3	0	
13	" " 1675 (July)	1 " " T 1675 " 1:1100		3	1	1300
14	" " 1675 "	1 " " " 1675 " 1:1500		3	0	
15	" " 1675 (Dec.)	1 " " " 1675 " 1:100		3	1	150
16	" " 1675 "	1 " " " 1675 " 1:200		3	0	

* kwt. = kilo of body weight.

† mngo = meningococcus.

‡ T = toxin.

§ ? = Toxoid 1409A not titrated to the end-point.

purpuric zone 1 to 2 mm. in width. A similar observation has been made by Burnet (22) who suggested that spontaneous local desensitization might be taking place in the center of the skin sites injected with toxoid preparations. However, control injections of 0.25 cc. of 1 per cent formalin produce the same effect. Pituitrin (Klein (23)) and adrenalin have a similar action on the rabbit's skin. This circinate distribution of the purpuric reaction at the prepared skin site must therefore be considered as a non-specific manifestation of primary local toxicity of the injected substances.

A Besredka (24) disintegrate of a *B. dysenteriae* Flexner strain to which 0.2 per cent formalin had been added and which had been kept at 37°C. for 1 month was referred to by Burnet as toxoid. His toxoid preparation suffered a loss only in skin-preparatory, but not in reacting potency (no titrations to the end-point), as compared with the mother filtrate. Studies of the antibody-combining capacity or antigenicity are not mentioned.

Titration of Antibody-Combining Capacity of Meningococcus Toxoids

Neutralized mixtures of meningococcus Toxin 1409 and meningococcus Toxoid 1409B with antimeningococcus horse Serum H251 and auxiliary antibody Serum H230 were titrated as described by Shwartzman (21). The results are summarized in Table II (Groups 1 to 5).

As is seen from Group 1 of Table II, consistent neutralization was obtained with 100 units of meningococcus Toxin 1409 (1 cc. of a 1:14 dilution of meningococcus Toxin 1409). With the use of an equal amount of serum, consistent neutralization was obtained with only 1.7 units of meningococcus Toxoid 1409B (1 cc. of a 1:10 dilution of meningococcus Toxoid 1409B) (Table II, Group 3). The ratio of the antibody-combining capacity of meningococcus Toxoid 1409B to that of meningococcus Toxin 1409 is, therefore, that of 10:14, thus showing in the toxoid a loss of only 28.7 per cent of the antibody-combining capacity associated with a 98.8 per cent loss of toxicity as compared to the mother filtrate.

It remained to determine whether 1.7 reacting units of meningococcus Toxoid 1409B actually held in combination all of the neutralizing antibody in the serum used for the neutralization titrations (0.9 cc. of Serum H251 plus 0.1 cc. of Serum H230). If the mixture contained an excess, *i.e.* unbound antibody, then consistent neutralization

TABLE II

Titration of Antibody-Combining Capacity of Filtrates Studied

Group No.	Preparation	Skin-preparatory factors	Titer of reacting factors in units per cc.	Intravenous injection: 1 cc. of following dilution per kwt.	Sera used for neutralization in amounts per kwt.	Results of neutralization titrations		
						CN*	IN†	NN‡
1	mngo T 1409	+§	1400	1:14	0.9 cc. Serum H251 +0.1 cc. Serum H230	100		
2	" " 1409	+	1400	1:10	" "		140	
3	" Toxoid 1409B	−‖	17	1:10	" "	1.7		
4	" " 1409B	−	17	1:7	" "		2.4	
5	" " 1409B	−	17	Undiluted	" "		17	
6	" T 1675 (July)	+	1300	1:10	+0.9 cc. Serum H251 +0.1 cc. Serum H290	130		
7	" 1675 "	+	1300	1:7.5	" "			175
8	" " 1675 (Dec.)	+	1500	1:13.6	" "	11		
9	" " 1675 "	+	1500	1:10	" "			15

* CN = consistent neutralization.

† IN = irregular neutralization.

‡ NN = no neutralization.

§ + = 0.25 cc. of undiluted filtrate was able to prepare the skin of a rabbit for the phenomenon.

‖ − = no skin-preparatory potency.

should still be obtained after the addition of a small amount of toxin to the mixture. Accordingly the following experiment was performed.

Each of 4 rabbits received an intradermal injection of 0.25 cc. of undiluted meningococcus Toxin 1409 followed 24 hours later by an intravenous injection of a mixture of 1 cc. of meningococcus Toxoid 1409B diluted 1:10 (1.7 reacting units) plus 0.9 cc. of Serum H251 plus 0.1 cc. of Serum H230 plus 0.5 cc. of meningococcus Toxin 1409 diluted 1:70 (10 reacting units) per kilo of body weight. Reactions were elicited in 3 of the 4 rabbits (no neutralization).

This demonstrates conclusively that 1.7 reacting units of meningococcus Toxoid 1409B actually combined with all of the neutralizing antibody in 0.9 cc. of Serum H251 plus 0.1 cc. of Serum H230.

Spontaneous Meningococcus Toxoid Formation

The titer of reacting units of a bacterial filtrate potent in eliciting the phenomenon often diminishes on standing (Shwartzman (13)). It was observed by Ehrlich (1) that, on standing, diphtheria toxin often suffered a decrease in the titer of direct toxicity whilst the antitoxin-combining power remained essentially unaltered. It was, therefore, of interest to determine whether the decrease in the titer of reacting units of a bacterial filtrate which occurred on standing was associated with a diminution of the antibody-combining capacity.

For this purpose the following was done.

The preparation of meningococcus "agar washings" toxic Filtrate 1675 was completed on June 16, 1931. In the period, July 15 to 17, 1931, the titer of reacting units and the antibody-combining capacity were determined. These determinations were performed on stock rabbits. Antimeningococcus horse Serum H251 and anticoli horse Serum H290 were used for the neutralization experiments. In the period Dec. 18 to 23, 1931, these determinations were repeated on used rabbits. The results are summarized in Table I (Groups 13 to 16) and Table II (Groups 6 to 9).

As is seen from Table I, despite the possibly increased sensitivity of used rabbits, the titer of reacting units dropped from 1300 to 150 units. However, the same amount of serum (0.9 cc. of Serum H251 plus 0.1 cc. of Serum H290) which consistently neutralized 1 cc. of a 1:10 dilution of meningococcus Toxin 1675 in July was necessary for consistent neutralization of 1 cc. of a 1:13.6 dilution of meningococcus Toxin 1675 in December (Table II, Groups 6 to 9). Therefore, in

December, the toxin retained but 11.5 per cent of the July reacting titer. In contrast with this, in December, it retained 73.5 per cent of the antibody-combining capacity present in July. The relative preservation of the antibody-combining capacity, despite the decrease in titer of reacting units which takes place in meningococcus toxin on standing, resembles closely, therefore, the transformation of diphtheria toxin into toxoid under similar conditions.

Lethal Effect of Meningococcus Toxoid

An intravenous injection of an "agar washings" toxic filtrate produces a considerable mortality in rabbits. There appears to be a definite parallelism between the lethal effect and the titer of reacting

TABLE III

Lethal Effect of Filtrates Studied

Group No.	Intravenous injection per kwt.	No. of reacting units	No. of rabbits	Result
1	1 cc. mngo T A52 diluted 1:5	150	3	1 died after 2 hrs. 2 survived
2	1 " " Toxoid A52 undiluted	23	3	All survived
3	2 " " " A52 "	46	3	" "

units of a given filtrate (Shwartzman (13)). It was of interest, therefore, to determine whether the decrease brought about in the titer of reacting units in meningococcus toxoid preparations, was associated with a parallel decrease in lethal effect.

From Table I (Groups 9 and 10), it can be seen that meningococcus Toxin A52 possessed a titer of 750 reacting units. In contrast to this meningococcus Toxoid A52 possessed a titer of only about 23 reacting units (Table I, Groups 11 and 12). The lethal effect of both of these filtrates was studied. The results are summarized in Table III.

Of 3 rabbits which received intravenously 1 cc. of meningococcus Toxin A52 diluted 1:5 (150 units) per kilo of body weight, 1 died 2 hours after the injection, and the others survived (Table III, Group 1). Two groups, each of 3 rabbits, received intravenous injections of 1 cc. and 2 cc., respectively, of undiluted meningococcus Toxoid A52 per kilo of body weight; all survived (Table III, Groups 2 and 3).

It can be stated that death following an intravenous injection of a meningococcus filtrate generally occurs within 4 hours. In these experiments, the surviving rabbits were observed for 3 days after the injection and showed no signs of intoxication.

Occasionally a death occurs after an intravenous injection of 10 to 25 units per kilo of body weight. An injection of from 50 to 100 units is frequently followed by death. It appears that an injection of from 100 to 200 units is regularly associated with mortality, the percentage being proportional to the dosage. From Table III (Group 1), it can be seen that an injection of 150 units of meningococcus Toxin A52 was followed by the death of 1 of 3 rabbits. From Groups 2 and 3, it can be seen that the injection of 1 and 2 cc. of meningococcus Toxoid A52 representing the volume of 750 and 1500 units of the mother filtrate (meningococcus Toxin A52) per kilo of body weight, was tolerated perfectly well by the 6 animals injected. This demonstrates a striking diminution in the lethal effect of the toxoid as compared to the mother filtrate.

Antigenicity of Meningococcus Toxoid

It remained to ascertain the antigenicity of a meningococcus toxoid preparation; *e.g.*, meningococcus Toxoid 1409B. Lowenstein (6) had noted that the antigenicity of a tetanus toxoid was directly proportional to its antitoxin-combining capacity. In view of the fact that meningococcus Toxoid 1409B possessed 71.3 per cent of the antibody-combining capacity of the mother filtrate (meningococcus Toxin 1409), it was expected that the toxoid would still be antigenic.

6 rabbits were immunized, each by intradermal injections of 0.5 cc. of undiluted meningococcus Toxoid 1409B on Jan. 30, Feb. 5, 10, 17, and 24, and by intravenous injection on Feb. 6, 11, 18, and 25 of 1 cc. of meningococcus Toxoid 1409B diluted 1:20, 1:10, 1:5, and undiluted, respectively, per kilo of body weight. 3 of the rabbits survived until Feb. 29, when they were bled. A standard suspension of a 24 hour culture of meningococcus Strain 123, Group 1 (the strain which furnished meningococcus Toxin 1409), was agglutinated with the sera of these animals. The results are set down in Table IV. For comparison, the same suspension of meningococci was agglutinated with Serum R332, a stock, Group 1, meningococcus, rabbit agglutinating serum.

As can be seen from Table IV, the agglutinating titers of the sera produced by immunization with meningococcus Toxoid 1409B varied

TABLE IV

Agglutination Titration of Meningococcus Toxoid 1409B Antisera

Serum employed	Antigen employed for preparation of serum	Serum dilution									
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2580	1:5120
Serum Rabbit 9-50	Meningococcus Toxoid 1409B	++	+++	++++	++	++	++	0	0	0	0
Serum Rabbit 9-40	" "	+++	+++	++++	+++	++	+	+	+	±	0
Serum Rabbit 4-10	" "	+	+	++++	++++	+++	++	++	0	0	0
Serum Rabbit 3-32	Group 1 meningococcus vaccine	++++	++++	±*	+++	+	±	0	0	0	0

++++ = complete agglutination.
+++ = almost complete agglutination.
++ = partial agglutination.
+ = slight agglutination.
0 = no agglutination.
* Interzone(?).

from 320 to 1280, whereas the control serum had a titer of only 160. Sera obtained from trial bleedings of the rabbits performed January 28, 1932, were free of spontaneous agglutinins for meningococci. The sera contained no precipitins either for meningococcus Toxin 1409 or meningococcus Toxoid 1409B. The sera were unable to neutralize meningococcus Toxin 1409 reacting factors (1 cc. of serum mixed with 3 units of toxin). However, rabbits do not readily form precipitins for bacterial filtrates nor are they good antitoxin producers. Nevertheless, further studies are under way with the aim of producing precipitating and antitoxic rabbit sera.

DISCUSSION

The experiments cited demonstrate that meningococcus culture filtrates treated with formalin and heat can be modified similarly to diphtheria toxin. The toxicity, as measured by the titer of reacting units, and the lethal effect are markedly decreased. In contrast, the antigenicity and the antibody-combining capacity are essentially unimpaired.

Furthermore, no comparable loss in antibody-combining capacity is associated with the decrease in toxicity occurring on standing. Ramon (4) by precipitation tests has found that diphtheria toxoid flocculates with diphtheria antitoxin to the same titer as the mother filtrate (toxin).

It was the detoxifying alteration of the sort undergone by diphtheria toxin that led Ehrlich to the description of diphtheria toxoid. Since meningococcus culture filtrates can spontaneously or artificially be modified in a similar manner, it appears justifiable to consider such a modified meningococcus culture filtrate as meningococcus toxoid.

As indicated in the introductory section, all of the classical toxins can be converted into toxoid states. The production of a meningococcus toxoid is further evidence of the similarity in properties between the bacterial exotoxins studied by Shwartzman and the classical bacterial exotoxins, *e.g.*, diphtheria and tetanus.

The demonstration of a meningococcus toxoid has a considerable practical importance. It is common experience that animals used for the production of therapeutic sera eventually succumb to the toxic effects of the injected antigens. Since toxoids show little lethal effect,

it is suggested that they be employed in the production of therapeutic sera.

As yet unpublished experiments suggest that serum from certain human beings may possess the capacity to neutralize meningococcus reacting units. Since meningococcus toxoid is antigenic and has a very low titer in reacting units (relatively non-toxic), it may possibly be of use in the active immunization of man against meningococcus infections.

It is still common practice to evaluate therapeutic meningococcus sera by their agglutinin titers. However, this criterion of evaluation reveals a lack of correlation between the agglutinin titer and the therapeutic efficacy of a serum. Recently, Shwartzman (20, 25) has suggested that therapeutic meningococcus sera be graded by their capacity to neutralize meningococcus reacting factors. However, the demonstration of toxoid formation should be taken into consideration inasmuch as, in filtrates with different degrees of toxoid formation, the same number of reacting units may represent varying antibody-combining capacities.

It would seem advisable to keep as a standard an immune serum which had been titrated against freshly prepared meningococcus toxin, in which only insignificant, if any, toxoid formation had occurred. Other sera could then be compared to this by their capacity to neutralize any given meningococcus filtrate.

SUMMARY AND CONCLUSIONS

Formalin induces a considerable change in meningococcus culture filtrates. This consists of a marked decrease in toxicity as concerns both the Shwartzman phenomenon and the lethal effect, with relative preservation of the antibody-combining capacity and antigenicity.

A similar modification occurs spontaneously in meningococcus culture filtrates on standing.

Inasmuch as these changes parallel those occurring in the conversion of diphtheria toxin into toxoid, it is justifiable to consider such altered meningococcus toxin as meningococcus toxoid.

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