ROLE OF MENINGOCOCCAL ENDOTOXIN IN MENINGOCOCCAL PURPURA*

By CHARLES E. DAVIS and KAREN ARNOLD

(From the Department of Pathology, University of California, San Diego, California 92103)

(Received for publication 25 February 1974)

Injury of the skin by the meningococcus (MGC)[1] was described long before the discovery of the organism. Epidemics of meningococcal disease were once called "spotted fever" because most patients had hemorrhagic spots on the skin (1). In modern surveys petechial and purpuric eruptions occur in 50–65% of the cases (2–4). These lesions are histologically indistinguishable from the dermal Shwartzman reaction, which is produced experimentally by meningococcal endotoxin (5). Enteric bacilli also contain potent endotoxins, but purpura is uncommon in enteric bacteremia.

Greater systemic potency, greater skin potency, increased production, or accessibility of endotoxin could explain the greater frequency of skin lesions in meningococcemia. Because the yield of endotoxin from MGC is low and the organisms encapsulated, increased production or accessibility is probably not responsible.

To investigate these possibilities, we isolated and characterized highly purified endotoxins from meningococcal serogroups A, B, and C; the major causes of meningococcal disease. The biological potency of these preparations which were virtually free of contamination by protein, nucleic acid, and capsule was compared to that of endotoxins extracted from *Escherichia coli* 04 and 0:111 and *Salmonella typhimurium*. This comparison was based on their relative capacities to kill mice, and to elicit the dermal and generalized Shwartzman reactions.

Materials and Methods

Source of Microorganisms.—Strains of *Neisseria meningitis* from the collection of the Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, D. C., were generously supplied by M. S. Artenstein. Strains B_{11}, C_{11}, and A_1 are standard strains used in vaccine studies and were originally obtained from patients with meningitis or meningococcemia. The letter in the designation of each strain of MGC indicates its serotype; so that A_1 is serogroup A, B_{11} and 135B are serogroup B, and C_{11} is serogroup C. Strain 135B was obtained from the nasopharynx of a carrier and is hydrophobic, settling out of suspension in broth cultures. *E. coli* 04 and *S. typhimurium* were from human blood. The origin of *E. coli* 0:111 has

* Supported by U. S. Army contract MED/DADA 17-72-C-2040.

[1] *Abbreviations used in this paper:* LPS, lipopolysaccharide; MeTMSi, methyl ester trimethylsilyl ether derivatives; MGC, meningococcus; TSB, trypticase soy broth; TBA, thiobarbituric acid.

been described previously (6). Lyophilized organisms were rehydrated with trypticase soy broth (TSB) and grown on chocolate agar overnight under CO_2 at 37°C.

Endotoxins.—A single colony was transferred from chocolate agar to 20 ml of TSB. After 24–48 h of growth under CO_2, the culture was inoculated into 200 ml of TSB and incubated under CO_2 for 48 h. This culture was inoculated into a 12 liter carboy and incubated for 72 h. After the bacteria were killed with 1% formaldehyde, they were collected by continuous centrifugation, washed three times with 95% alcohol and twice with acetone before drying for 48 h in a vacuum oven at 40°C. This method gives about 150 mg of dry meningogoccal cells/liter. Endotoxins were extracted from each strain of enteric bacillus and MGC by the phenol-water method of Westphal (7) with the following modifications. Organisms were always grown for 3 days before the cells were harvested. The cultures were killed with a deproteinating terminal concentration of 1% formaldehyde. Two water extractions of the hot phenol were used to increase the yield of endotoxin but the layer between phenol and water was discarded. After dialysis to remove phenol, the endotoxin pellet was sedimented by ultracentrifugation once or twice at 100,000 *g* for 3 h. Precipitation with 70% ethanol was avoided because it increased RNA contamination. After resuspension of the pellet, the material was lyophilized, collected, and weighed. Percent yield was calculated from the weight of dry cells. Endotoxin from enteric bacilli was also extracted by the standard phenol-water method.

Chemical Analysis.—Dry weight chemical composition was determined after weighing and rehydrating the lyophilized material. Total protein was determined by the Folin-Ciocalteu method (8) with lysozyme as the standard. RNA was estimated by optical density at 280 and 260 nm. Sialic acid and Keto-deoxy-octulosonate were determined by the thiobarbituric acid method of Warren (9) before and after hydrolysis with 0.1 N H_2SO_4 at 80°C for 30 min. The presence of lipid A was confirmed by the method of Galanos et al. (10). All standards for chemical assays and gas-liquid chromatography were of the highest purity available from Sigma Chemical Co., St. Louis, Mo. or Calbiochem. San Diego, Calif.

Gas-Liquid Chromatography.—Trimethylsilyl derivatives of the sugars were prepared for gas chromatography as previously described (6). Sialic acid and amino sugars were determined separately from neutral sugars by methods developed by Sweeley and Walker (11) and modified by Marcus et al. (12). Briefly, neutral sugars were hydrolyzed overnight at 80°C in 0.5 N methanolic-HCl, hexane extracted, neutralized by ion exchange over CG4B (Mallinckrodt Chemical Works, St. Louis, Mo.) in the hydroxyl phase with methanol elution, dried in a Buchler flask evaporator (Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N. J.), removed from the evaporator flask quantitatively with methanol, redried under nitrogen, and converted to trimethylsilyl ethers with a mixture of pyridine, hexamethyldisilazane, and trimethylchlorosilane (10:4:1). Amino sugars and sialic acid derivatives were prepared by hydrolysis in 0.1 N methanolic-HCl because sialic acid standards were destroyed by 0.5 N HCl under these conditions (80°C overnight). After hydrolysis and hexane extraction, 150 mg of silver acetate were added to the hydrolysate and allowed to react at room temperature for 5 min. The hydrolysate was dried under nitrogen, the tubes rinsed with methanol, redried under nitrogen, desiccated over KOH for 15 min in vacuo, reacetylated with methanol-acetic anhydride (3:1), thoroughly mixed, and allowed to react under nitrogen for 24 h in the dark. The silver salts were removed by filtration through sintered glass of ultrafine porosity, dried again under nitrogen and over KOH, and ethers prepared by adding 0.2–0.5 ml of Bis-(trimethylsilyl) trifluoroacetamide. Gas chromatography was performed at 160°C for neutral sugars and amino sugars and at 200°C for sialic acid on a Hewlett-Packard 402 gas chromatograph fitted with a 6 foot, U-shaped, 3% SE-30 column (Hewlett-Packard Co., Avondale, Pa.). The ratio of galactose to glucose was determined with a Hewlett-Packard 3370A integrator. Peaks were identified by comparison with authentic standards and by mass spectrometry (LKB mass spectrophotometer-gas chromatograph unit, Departments of Biology and Medicine, University of California, San Diego, Calif.

Tests for Biologic Activity.—Pyrogenicity was measured in conditioned 2.0 kg white New Zealand rabbits. Mouse lethality was determined in groups of 6–8, 20–25 gm CF1 mice (Carworth Farms, Kalamazoo, Mich.) given 0.25-ml vol of serial two-fold dilutions of endotoxin. Deaths were tabulated for 96 h and the lethal dose (LD_{50}) calculated by the Reed-Muench method (13).

Skin sites for the dermal Shwartzman reaction were prepared in groups of 10–20 rabbits by intradermal injections of serial dilutions of 0.25-ml samples of endotoxin. The reaction was provoked 21 h later by intravenous injection of 0.5-ml samples. Any hemorrhage or necrosis of the skin after the provocative dose was recorded as a positive reaction. The experiment was terminated 24 h after the provocative dose and the 50% effective dose estimated graphically or by the Reed-Muench method (13). The generalized Shwartzman reaction was elicited in groups of 10–20 rabbits by two intravenous injections of serial twofold dilutions of 0.5-ml vol of endotoxin given 21 h apart. Positive reactions were tabulated 18 h after provocation. Animals that died before provocation were eliminated from the calculations. Kidneys were judged positive only if hemorrhage or necrosis of the cortex was found during careful gross inspection.

Clearance of Endotoxins from Skin.—1 kg rabbits were inoculated intradermally with 2 μg (0.25 ml) of either *E. coli* 04 or MGC C_{11} endotoxin labeled with $Na_2{}^{51}Cr$ 04 (New England Nuclear, Boston, Mass.) by a standard method (14). At the time of injection a circle twice the diameter of the intradermal bleb was drawn with indelible pencil around the site of injection. At 1, 4, and 21 h 10 animals from each group were sacrificed and the skin excised along this circle. The radioactivity of the excised skin was measured individually in a liquid-well scintillation counter and expressed as the percentage of administered dose of radioactivity.

RESULTS

Chemical Analysis.—Lipopolysaccharides (LPS) extracted from MGC and enteric bacilli by this modification of the phenol-water technique were equally pure and virtually free of protein and RNA. Occasionally it was necessary to centrifuge the endotoxins twice at 100,000 *g* in order to lower the RNA content to 1%. Thiobarbituric acid (TBA)-reactive material was determined before and after hydrolysis with H_2SO_4 (Table I). Even before gas-liquid chromatography showed that these endotoxins were free of measurable sialic acid, it was clear that the TBA-reactive material was not sialic acid because endotoxin from MGC A and *E. coli* 0:111 contained as much TBA-reactive material as MGC C and B strains. The capsules of MGC B and C consist primarily of acetylated sialic acid (15), but the capsule of MGC A is primarily acetylated D-mannosamine phosphate (16), which does not react in the TBA assay (9), and *E. coli* 0:111 does not contain sialic acid. The small concentrations of "free" TBA-reactive material in each of these endotoxins were most likely KDO, which reacts in the TBA assay (6), released by partial hydrolysis of the endotoxins by TBA. After hydrolysis with 0.1 N H_2SO_4, TBA-reactive material in meningococcal endotoxins rose from 2- to 4-fold and in *E. coli* 0:111 LPS, which has no sialic acid, more than 10-fold. This increase is accounted for by the release of KDO from the core region of the endotoxin. Particulate, acid-insoluble, lipid A was present in all LPS after 3 h of hydrolysis in 1% acetic acid or 30–60 min in 0.1–1 N HCl.

Gas-Liquid Chromatography.—Fig. 1 shows the chromatograms of the methyl

TABLE I

*Biochemical Comparison of Meningococcal and Enteric Endotoxins**

	Meningococcal				Enteric bacilli		
	A_1	135B	B_{11}	C_{11}	04	0:111	*S. typhimurium*
Yield (%)	1.2	1.6	1.0	1.3	—	.9	—
Protein (%)	.30	.25	1.25	.5	.75	.75	1.0
RNA (%)	<1	<1	<1	<1	<1	<1	<1
Galactose/glucose	2.0	0	.9	1.5	.32	.76	5.6‡
TBA reactive (prehydrolysis)	3.6	4.1	2.5	3.4	—	1.0	—
TBA reactive (posthydrolysis)	6.6	9.9	9.2	7.2	—	12	—

* All endotoxins were extracted by the modification of the phenol-water procedure described in the Materials and Methods section. Protein was determined by the Folin-Ciocalteu method, RNA by light absorption at 280 and 260 nm. TBA activity for keto-deoxy sugars was determined before and after hydrolysis with 0.1 N H_2SO_4 for 1 h at 75°C. Since the preparations have been shown to be free of capsular contamination, TBA reactivity represents keto-deoxy-octulosonate. The ratio of galactose to glucose was determined by gas-liquid chromatography and a computerized peak integrator.

‡ Galactose + mannose: glucose.

ester trimethylsilyl ether derivatives (MeTMSi) of the neutral sugars of C_{11} and 135B. The LPS from MGC A_1 and B_{11} were qualitatively and quantitatively similar to MGC C_{11}. All contained glucose, galactose, heptose, and glucosamine. A slight increase in the size of the first peak of the galactose triplicate (α-galactose) of MGC B_{11} suggested a small amount of mannose, not present in the other MGC LPS. Mannose has previously been shown to cochromatograph with α-galactose (6). The composition of LPS from 135B was quite different, containing only glucose and glucosamine. This is equivalent to the composition of the "rough" UDP-galactose-epimeraseless mutants of the Enterobacteriaceae (7).

Fig. 2 is a composite of chromatograms of the acetylated TMSi ethers of MGC B_{11} and a *N*-acetyl sialic acid standard showing the presence of *N*-acetyl-glucosamine and the absence of sialic acid. The chromatograms of the acetylated derivatives of the LPS from all MGC and enteric bacilli were identical. All contained *N*-acetyl-glucosamine, and none contained *N*-acetyl sialic acid. LPS from MGC A_1 was free of D-mannosamine phosphate and acetylated mannosamine. LPS from enteric bacilli were also free of surface substances or capsular material detectable by gas-liquid chromatography. Specifically, none contained sugars, amino sugars, or sugar acids except those known to be associated with the endotoxins. The ratios of galactose to glucose (Table I) varied among individual strains with no pattern distinguishing the two groups. Since higher concentrations of galactose indicate longer "O" side chains (17, 18), these

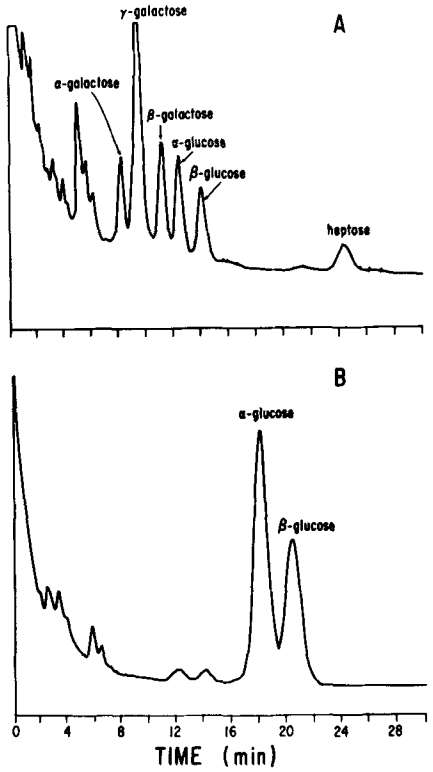

FIG. 1. MeTMSi derivatives of neutral sugars of meningococcal endotoxins chromatographed on 6 foot glass columns packed with 3% SE-30 on Chromosorb W. Carrier gas was nitrogen at 40 ml/min. Injector and detector temperatures were 210°C; column oven temperature was 160°C. (A) C_{11} endotoxin and (B) 135B endotoxin. Note absence of galactose and heptose. Chromatograms of A_1 and B_{11} are virtually identical to C_{11}.

results mean that the O side chains of the enteric endotoxins were not shorter than MGC endotoxins. MGC 135B is galactose deficient and autoagglutinable, a property associated with short or absent side chains in the Enterobacteriaceae.

Fever.—Fever curves produced by 0.1 μg of endotoxin from MGC A_1, 135B, and C_{11} are shown in Fig. 3. The fever curves produced by each endotoxin are plotted as the mean of two rabbits. These endotoxins are potent pyrogens. MGC A_1 and 135B produced classic biphasic curves at 1.0 and 0.1 μg doses. MGC C_{11} overwhelmed the animals, so that a huge single peak of greater than 5 h duration was produced by 1.0 and 0.1 μg doses.

Potency Comparisons of MGC and Enteric Bacilli.—Since the endotoxins were extracted by the same technique and were equally pure, they were suitable for comparisons of potency. Meningococcal LPS was no more potent as a

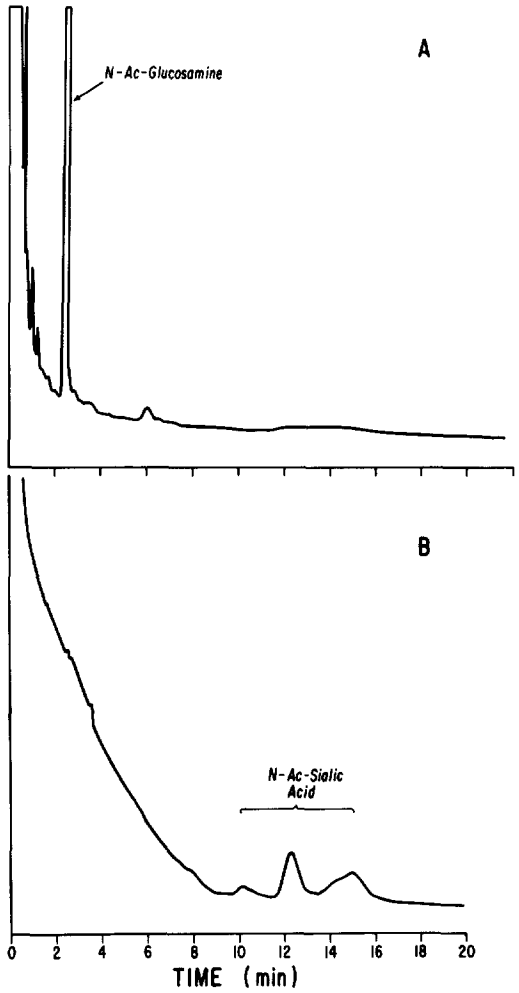

FIG. 2. Acetylated MeTMSi derivatives of (A) MGC B_{11} and (B) *N*-acetyl-sialic acid. Operating conditions and column were identical to Fig. 1 except that oven temperature was 200°C because *N*-acetyl-sialic acid is not eluted from the column at 160°C. Note presence of *N*-acetyl glucosamine and absence of sialic acid from MGC B_{11}. Chromatograms of 135B, C_{11}, and A_1 are identical to B_{11} shown in A.

lethal toxin in mice (Table II). Except for MGC C_{11}, the enterics were slightly more potent, but the two groups were not clearly different.

MGC and enteric LPS were both very potent in the induction of the generalized Shwartzman phenomenon. All preparations caused bilateral renal cortical necrosis or hemorrhage in more than 50% of the animals at doses of 6 μg (Table III) and differed little at 3 μg.

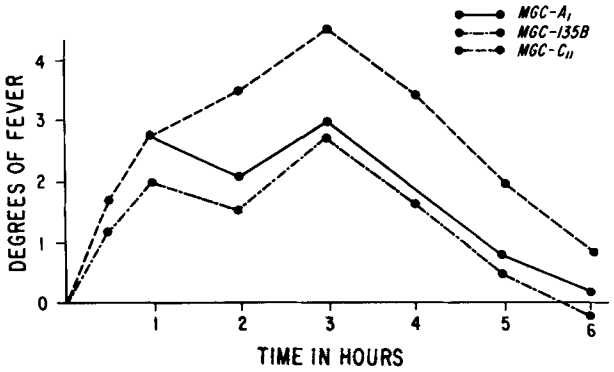

FIG. 3. Fever curves produced by the intravenous injection of 0.1 μg of MGC endotoxins from A_1, 135B, and C_{11} in 2 kg conditioned rabbits. The fever curves produced by each endotoxin are plotted as the mean of two rabbits. MGC A_1 and 135B produced classic biphasic curves at 1.0 and 0.1 μg. MGC C_{11} overwhelmed the animals at 1.0 and 0.1 μg producing a single huge peak of greater than 5 h duration.

TABLE II

*Comparison of Endotoxin Potency in Mouse Lethality Assay**

	Meningococci				Enteric bacilli		
	A_1	135B	B_{11}	C_{11}	04	0:111	*S. typhimurium*
LD_{50} in μg	370	420	540	290	290	—	250

* Groups of 6–8, 20–25 gm, CF1 mice were given 0.25-ml vol of serial twofold dilutions of endotoxin i.v. Deaths were tabulated for 96 h. All endotoxins were extracted by the modification of the phenol-water technique described in the Materials and Methods section.

The meningococcal endotoxins individually and as a group were remarkably more potent than enteric LPS for producing the dermal Shwartzman reaction (Table IV). The 50% effective dose was 1 μg for MGC A_1, less than 1 μg for MGC B_{11}, and 1.2 μg for MGC C_{11} and 135B. By contrast the 50% effective dose for the enteric endotoxins was 6.5–9.3 μg. This remarkable 5- to 10-fold difference in potency between the two groups was so consistent that all the meningococcal endotoxins elicited the dermal Shwartzman in 100% of the animals at 2 μg and none of the enteric preparations were 50% effective at 5 μg.

To be certain that the method of extraction did not affect the potency of enteric LPS we compared the purity and potency of enteric LPS extracted by the standard and modified phenol-water techniques (Table V). The two preparations were of equal purity and equal potency for mouse lethality, the dermal Shwartzman phenomenon, and the generalized Shwartzman phenomenon. The method of extraction also did not alter the ratios of galactose to glucose.

TABLE III

*Comparison of Potency for the Generalized Shwartzman Phenomenon**

Dose in μg		No. and percent positive						
		Meningococci				Enteric bacilli		
Prep	Prov	A_1	135B	B_{11}	C_{11}	04	0:111	*S. typhimurium*
12	12	—	—	—	—	13/19 68%	17/20 85%	—
6	6	12/20 60%	13/17 77%	11/20 55%	16/20 80%	11/19 58%	10/20 50%	16/19 84%
3	3	7/18 39%	9/16 56%	8/20 40%	12/20 60%	4/19 21%	6/20 30%	

* Two i.v. injections of 0.5 ml were given to 1 kg white New Zealand rabbits 21 h apart. Animals surviving beyond 6 h postprovocation were autopsied at 24 h and judged positive if gross hemorrhage or necrosis of the renal cortex was visible. All endotoxins were extracted by the modification of the phenol-water technique described in the Materials and Methods section.

TABLE IV

*Comparison of Endotoxin Potency for the Local Shwartzman Phenomenon**

Dose in μg		No. and percent positive						
		Meningococci				Enteric bacilli		
Prep	Prov	A_1	135B	B_{11}	C_{11}	04	0:111	*S. typhimurium*
10	10		—		—	17/20 85%	8/10 80%	17/20 85%
5	5	20/20 100%	20/20 100%	20/20 100%	20/20 100%	6/20 30%	2/10 20%	3/20 15%
3	3	20/20 100%	20/20 100%	—	20/20 100%	—	—	—
2	2	20/20 100%	20/20 100%	—	20/20 100%	—	—	—
1	1	5/10 50%	6/20 30%	15/20 75%	7/20 35%	—	0/20 0	—
LD_{50} (μg)‡		<1	1.2	<1	1.2	6.5	7.3	9.3

* Preparatory dose of 0.25 ml was given intradermally to 1 kg white New Zealand rabbits 21 h before the provocative i.v. dose of 0.5 ml. Animals were judged positive only if hemorrhage or necrosis of the skin occurred within 24 h of the provoking dose. All endotoxins were extracted by the modification of the phenol-water technique described in the Materials and Methods section.

‡ 50% effective dose.

Table VI shows that the preparatory or skin dose of meningococcal endotoxin is responsible for its unique potency for the dermal Shwartzman. When 2 μg doses of endotoxin were used, C_{11} produced dermal Shwartzmans in 90% of the animals but *E. coli* 04 in only 10%. If C_{11} was used as the preparatory dose, 94 was as effective as C_{11} for provoking the reaction. *E. coli* 04, however, could not prepare the skin effectively even if MGC C_{11} was used as the provocative injection.

TABLE V

*Comparison of Enteric Endotoxins Extracted by the Modified and Standard Phenol-Water Techniques**

	Modified technique			Standard technique		
	0:111	*S. typhimurium*	04	0:111	*S. typhimurium*	04
Protein (%)	.75	1.0	.75	.75	.75	.5
RNA (%)	<1	<1	<1	<1	<1	<1
Galactose/glucose	.76	5.6‡	—	.76	5.6‡	.60
Mouse LD_{50} (mg)	—	—	.29	—	.27	.29
Generalized Shwartzman (μg) 12/12 (Prep/Prov)	—	—	13/19 68%	—	—	10/19 53%
Local Shwartzman (μg) 10/10 (Prep/Prov)	—	11/20 55%	17/20 85%	—	14/20 70%	12/20 60%
5/5	—	3/20 15%	6/20 30%	—	3/20 15%	4/20 20%

* Endotoxin was extracted from each strain of enteric by the standard and modified phenol-water techniques. Chemical and biological assays were performed as described in the text and preceding tables.

‡ Galactose + mannose: glucose.

TABLE VI

*Role of Preparatory (Skin) Dose in Dermal Shwartzman**

Preparatory	Provocative	No. Positive
MGC C_{11}	MGC C_{11}	9/10
E. coli 04	*E. coli* 04	1/10
MGC C_{11}	*E. coli* 04	8/10
E. coli 04	MGC C_{11}	1/10

* All preparatory and provocative doses were 2 μg of either *E. coli* 04 or MGC C_{11} endotoxin.

^{51}Cr-labeled MGC C_{11} and *E. coli* 04 endotoxins were cleared equally rapidly from the skin. At 1, 4, and 21 h the percentage of administered radioactivity ± 2 SEM remaining in the skin for C_{11} was 82 ± 4.2, 67 ± 2.4, and 49 ± 3.6, and for *E. coli* 04, 72 ± 4, 64 ± 1.7, and 47 ± 3.2. Calculation of the amount of endotoxin corresponding to the percentage of remaining radioactivity emphasizes how little the clearance differs. At 1 h, when the difference in percent clearance was the greatest, 1.6 μg of C_{11} remained compared with 1.4 μg of 04.

DISCUSSION

We have shown in this study that meningococcal endotoxin is uniquely potent in its effect on the skin. This may explain the high frequency of purpuric skin lesions in meningococcemia and underscores the possible importance of endotoxin in the pathophysiology of this disease. Although shock and consumption coagulopathy are manifestations of endotoxemia (19) that occur frequently in meningococcemia (20), neither occurs more commonly in meningococcal than other types of gram-negative septicemia. Thus, it was significant that meningococcal LPS was more potent than enteric endotoxins only in the dermal Shwartzman.

There have been few studies of meningococcal endotoxin reported in the literature. This is surprising since considerable impressive evidence links the major manifestations of meningococcemia to endotoxemia (5, 20). The large capsule has probably discouraged investigators who also have not studied the endotoxins of other encapsulated medically important bacteria such as klebsiella. Mergenhagen (21) concluded that LPS from one strain of group C MGC contained 9% sialic acid, while Hackenthal (22) found that the sialic acid in meningococcal LPS was a capsular contaminant. This problem of capsular contamination was overcome by the modifications of the phenol-water technique described in this report. Endotoxin extracted from the meningococci of serogroup A and from *E. coli* 0:111 contain as much TBA-reactive material as that from meningococci of serogroups B and C (Table I) suggesting that KDO is totally responsible for this TBA reactivity.

Except for the apparent freedom from contamination with capsular sialic acid, the chemical composition of meningococcal endotoxin from representative organisms of serogroups A, B, and C agrees very closely with that found by Mergenhagen (21) in his analysis of a group C strain. The composition of the endotoxin from these meningococci is also identical to that described for many enteric bacilli. The endotoxin from MGC 135B may be "rough" since the organism is hydrophobic, settling to the bottom of broth cultures, and since the LPS contains no galactose and little or no heptose (Fig. 1). Rough strains of enteric bacilli lack the enzyme UDP-galactose-epimerase and are unable to incorporate galactose into the O side chains of their LPS. Because their deficient

O side chains are short, these strains are hydrophobic. Although there is considerable disagreement in the literature concerning the degree of reduction in biologic activity, endotoxin derived from these rough mutants generally is less toxic than that from smooth strains (18). This reduction in toxicity can be partly corrected if the LPS is made more soluble by the addition of triethylamine (18, 23). If MGC 135B is the meningococcal equivalent of a rough enteric bacillus, it is surprising that it is fully toxic without such treatment.

Since MGC and enteric LPS were extracted by the same technique and were equally pure (Table I), they were suitable for comparisons of potency. When these preparations were compared for lethality in mice (Table II), meningococcal LPS was no more potent than enteric endotoxins. Meningococcal and enteric endotoxins were also equally potent in the generalized Shwartzman reaction (Table III). The two groups of endotoxins were, however, consistently and remarkably different in potency for the dermal Shwartzman reaction (Table IV). All meningococcal endotoxins were effective at doses of 1 μg and were 5–10 times more effective than any of the enteric endotoxins studied.

The greater skin potency of meningococcal LPS was not explained by the polysaccharide composition of the endotoxin. The length of the side chains, determined indirectly by the ratio of galactose to glucose (Table I), was not correlated to potency, as predicted from the fact that the galactose-deficient serogroup B strain (135B) was fully potent in all the assays. It is also unlikely that the greater potency of meningococcal LPS for the skin is related to the composition of the polysaccharide side chains since no unique components or combinations of components were detected (Figs. 1 and 2).

Because the length of the O side chains varies inversely with the percent of lipid in LPS, total lipid content should not differ from enteric endotoxins. The fact that the preparatory or skin dose of meningococcal endotoxin was responsible for its greater potency in the dermal Shwartzman (Table VI) suggested that the fatty acid composition of meningococcal lipid A might differ in some manner that increased its affinity for the skin. If meningococcal lipid A had greater affinity for skin lipids or a configuration that promoted binding, more of the intradermal dose might remain and cause greater damage since severity is dose related in the dermal Shwartzman (Table IV). This was not the case, however, because radiolabeled meningococcal and enteric endotoxins were cleared from the skin equally. Further studies of lipid A content and composition and investigation of the interaction of meningococcal LPS and complement are in progress.

The greater skin potency of the LPS from MGC explains the prominence of purpuric skin lesions in meningococcemia and points out the importance of comparative studies of endotoxins. This separation of skin potency from mouse lethality suggests that comparisons of the properties of LPS may explain other differences in clinical syndromes caused by gram-negative bacteria.

SUMMARY

Enteric bacilli and meningococci (MGC) both contain potent endotoxins, but purpuric skin lesions indistinguishable from the experimental dermal Shwartzman reaction are much more common during meningococcal bacteremia than during bacteremia with enteric organisms. Highly purified lipopolysaccharides (LPS), virtually free of contamination by protein, RNA, and capsule, were extracted by a modification of the phenol-water technique from MGC (serogroups A, B, and C) and enteric bacilli (*Escherichia coli* 04 and 0:111, and *Salmonella typhimurium*). Polysaccharides in these LPS were similar by gas chromatography except for one galactose-deficient strain of MGC (135B).

LPS from MGC and enterics were equally potent for the general Shwartzman reaction and mouse lethality, but LPS from MGC was 5–10 times more potent in inducing the dermal Shwartzman reaction. The greater skin potency of LPS from MGC explains the prominence of purpura in meningococcemia. Comparison of the properties of LPS may explain other differences in clinical syndromes caused by gram-negative bacteria.

REFERENCES

1. Bell, W. E., and D. L. Silber. 1971. Meningococcal meningitis: past and present concepts. *Mil. Med.* **136**:601.
2. Carpenter, R. R., and R. G. Petersdorf. 1962. The clinical spectrum of bacterial meningitis. *Am. J. Med.* **33**:262.
3. Kaufman, B., H. Levy, and B. D. Zaleznak. 1951. Statistical analysis of 242 cases of meningococcal meningitis. *J. Pediatr.* **38**:705.
4. Swartz, M. N., and P. R. Dodge. 1965. Bacterial meningitis—a review of selected aspects. *N. Engl. J. Med.* **272**:725, 779, 842, 898, 954, 1003.
5. Margaretten, W., I. Csavossy, and D. G. McKay. 1967. An electron microscopic study of a case of meningococcemia in man. *Am. J. Dis. Child.* **114**:268.
6. Davis, C. E., S. D. Freedman, H. Douglas, and A. I. Braude. 1969. Analysis of sugars in bacterial endotoxins by gas-liquid chromatography. *Anal. Biochem.* **28**:243.
7. Westphal, O., O. Luderitz, and E. Bister. 1952. Uber die extraction von bacterien mot phenol/wasser. *Z. Naturforsch. Teil B.* **7**:148.
8. Folin, O., and V. Ciocalteu. 1927. Tyrosine and tryptophane determinations in proteins. *J. Biol. Chem.* **73**:627.
9. Warren, L. 1959. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.* **234**: 1971.
10. Galanos, C., O. Luderitz, and O. Westphal. 1971. Preparation and properties of antisera against the lipid-A component of bacterial lipopolysaccharides. *Eur. J. Biochem.* **24**:116.
11. Sweeley, C. C., and B. Walker. 1964. Determination of carbohydrates in glycolipids and gangliosides by gas chromatography. *Anal. Chem.* **36**: 1461.
12. Marcus, A. K., H. L. Ullman, and L. B. Safier. 1972. Studies on human platelet gangliosides. *J. Clin. Invest.* **51**:2602.

13. Reed, L. J., and H. Muench. 1938. A simple method for estimating endpoints. *Am. J. Hyg.* **27**:493.
14. Braude, A. I., F. J. Carey, D. Sutherland, and M. Zalesky. 1955. Studies with radioactive endotoxin. I. The use of ^{51}Cr to label endotoxin of *Escherichia coli*. *J. Clin. Invest.* **34**:850.
15. Gotschlich, E. C., T. Y. Liu, and M. S. Artenstein. 1969. Human immunity to the meningococcus. III. Preparation and immunochemical properties of the group A, group B, and group C meningococcal polysaccharides. *J. Exp. Med.* **129**:1349.
16. Liu, T. Y., E. C. Gotschlich, E. K. Jonssen, and J. R. Wysock. 1971. Studies on the meningococcal polysaccharides. Composition and chemical properties of the group A polysaccharide. *J. Biol. Chem.* **246**:2849.
17. Luderitz, O., A. M. Staub, and O. Westphal. 1966. Immunochemistry of O and R antigens of salmonella and related enterobacteriaceae. *Bacteriol. Rev.* **30**:192.
18. Ryan, J. L., A. I. Braude, and M. Turck. 1973. Galactose deficient endotoxin from urinary *E. coli*. *Infect. Immun.* **7**:476.
19. Braude, A. I., H. Douglas, and C. E. Davis. 1973. Treatment and prevention of intravascular coagulation with antiserum to endotoxin. *J. Infect. Dis.* **128**:157 (Suppl.)
20. Hoffman, T. A., and E. A. Edwards. 1972. Group-specific polysaccharide antigen and humoral antibody response in disease due to *Neisseria meningitis*. *J Infect. Dis.* **126**:636.
21. Mergenhagen, S. E., G. R. Martin, and E. Schiffman. 1963. Studies on an endotoxin of a group C *Neisseria meningitidis*. *J. Immunol.* **90**:312.
22. Hackenthal, E. 1969. Distribution of keto-deoxyoctonic acid and sialic acid in *N. meningitidis*. *J. Immunol.* **102**:1099.
23. Galanos, C., O. Luderitz, and O. Westphal. 1969. A new method for the extraction of R polysaccharides. *Eur. J. Biochem.* **9**:245.