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Neutralization of Human Serum Lysozyme by Sodium Polyanethol Sulfonate but Not by Sodium Amylosulfate

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Sodium polyanethol sulfonate (SPS) at 500 μg/ml, but not sodium amylosulfate (SAS) at 500 μg/ml, precipitated egg white lysozyme (1 mg and 50 μg of lysozyme per ml) as determined with the assay strain *Micrococcus lysodeikticus* ATCC 4698. Fresh and heat-inactivated (56°C, 30 min) human serum (80%, vol/vol) killed *M. lysodeikticus* (10^4 bacteria per ml at zero time) within 1 to 2 h after exposure. Addition of 250 to 500 μg of SPS per ml to fresh human serum protected *M. lysodeikticus* for 22 h as effectively as absorption of either fresh or heat-inactivated human serum with bentonite (10 mg/ml of serum, 10 min, 37°C); the latter procedure is known to remove serum lysozyme. In contrast, SAS at 250 and 500 μg/ml of serum retarded killing of the assay bacteria for periods of 4 h; after overnight (22 h) incubation, however, the number of *M. lysodeikticus* survivors had decreased significantly. The finding that SPS, but not SAS, at 250 to 500 μg/ml effectively neutralized serum lysozyme-mediated killing of a lysozyme-sensitive assay strain may be of relevance with respect to laboratory processing of human blood culture specimens.

For many years, sodium polyanethol sulfonate (SPS), a synthetic polyanionic anticoagulant, was used for the neutralization of complement-mediated bactericidal activity in human blood specimens and/or cultures (9, 18, 21, 27); most important, this polymer was shown to inhibit both the classical and the alternative pathways (10, 11) of human complement activation (25). Furthermore, this anticoagulant was shown to inhibit complement-dependent phagocytosis (1, 2). On the other hand, SPS failed to inhibit serum transferrin-mediated iron deprivation (25). It was shown also that SPS inhibited the growth of anaerobic streptococci, in particular *Streptococcus anaerobius* (12, 14, 28), an effect that was abrogated through addition of 1.2% gelatin to media (28). Similarly, the inhibitory effect of SPS against *Neisseria meningitidis* (7) was neutralized by supplementation of fluid and solid media with 1.2% gelatin (6). Recently, sodium amylosulfate (SAS), likewise a synthetic polyanionic anticoagulant, was introduced for use in human blood cultures (17). Apart from its neutralization of serum bactericidal activity, SAS supported the growth of anaerobic streptococci (15). Because a number of laboratories employed SAS as an anti-complementary additive (13, 16), this anticoagulant was compared with SPS with regard to its usefulness for this purpose. It was found that SPS was superior to SAS with respect to anti-complementary effects on a weight-for-weight basis (23). Like SPS, SAS failed to precipitate, i.e., antagonize, serum transferrin (Traub, unpublished data).

As part of our ongoing in vitro evaluation of SPS and SAS with regard to neutralization of various nonspecific antimicrobial systems in human serum, both anticoagulants were examined for their potential neutralization of serum lysozyme. Years ago, Cavallo et al. (3) discovered that SPS inhibited lysozyme. The manufacturer of SAS stated in an accompanying brochure that this latter anticoagulant likewise precipitated lysozyme. The experiments reported herein confirmed the finding of Cavallo et al. (3) for SPS; however, SAS failed to protect assay bacteria for extended periods of incubation.

MATERIALS AND METHODS

Bacteria. *Micrococcus lysodeikticus* strain ATCC 4698, a gift from W. Kersten, Institut für Physiologische Chemie, Erlangen, Germany, served for lysozyme and serum assays. The "promptly serum-sensitive" *Escherichia coli* strain C was used as a control in all serum assays (23-26).

Media. Tryptic soy broth (TSB) and agar (TSA) and brain heart infusion broth and agar were purchased from Difco Laboratories, Detroit, Mich. The bacteria were maintained on TSA slants at 4°C and in a mixture of 1 volume of brain heart infusion broth plus 1 volume of heat-inactivated bovine serum

(Grand Island Biological Co., Grand Island, N.Y.) at −65°C.

Reagents. Grade I egg white lysozyme (lot 75C-8483; activity, 40,800 U/mg of solid) was purchased from Sigma Chemical Co., St. Louis, Mo. Bentonite (lot 124C-0285) was obtained from Sigma Chemie München, Neubiberg, Germany. Two different batches of SPS were gifts from Hoffmann-La Roche AG, Basel, Switzerland (lot K 2710), and Hoffmann-La Roche, Inc., Nutley, N.J. (lot 120056; Ro 1-1521). Aqueous stock solutions of SPS (5,000 μg/ml [0.5%]) were sterilized by autoclaving at 121°C for 15 min and stored at 4°C. Two lots of SAS were obtained through the courtesy of G. D. Searle and Co., Chicago, Ill. (lots MH 4-24.A and MH4-24D SN-263). Aqueous stock solutions of SAS (5,000 μg/ml [0.5%]) were sterilized and stored like SPS. Ethylene glycol tetraacetic acid (EGTA; lot 47C-5013) was purchased from Sigma Chemical Co. Aqueous 0.1 M stock solutions of EGTA (pH 7.41) were prepared, membrane filter sterilized (0.2-μm membrane filter unit PS; Nalgene Labware Div., Nalge/Sybron Corp., Rochester, N.Y.) as previously described (23–25), and stored at 4°C. Certified ACS $MgCl_2 \cdot 6H_2O$ was purchased from Fisher Scientific Co., Fairlawn, N.J.; aqueous 0.5 M stock solutions of $MgCl_2$ were autoclaved for sterilization. Certified ACS disodium ethylenediaminetetraacetate (EDTA; lot 762132) was obtained from Fisher Scientific Co.; aqueous 0.1 M EDTA stock solutions (pH 7.43) were membrane filtered for sterilization and stored at 4°C. Phosphate-buffered saline (PBS; pH 7.5) was prepared by the formula of Schmidt (20).

Turbidimetric lysozyme assays. *M. lysodeikticus* was grown on a TSA slant at 35°C overnight, after which the growth was harvested with 5 ml of 0.154 M NaCl; a 0.2-ml portion of this cell suspension was glass spread onto each of two large TSA plates (14-cm diameter) which were incubated at 35°C overnight. Next morning, the growths were harvested with 10 ml of PBS each. The cell suspension was centrifuged at 12,062 × *g* and 4°C for 10 min. The cells were then washed three times with 10 ml of PBS at the same *g* force, after which the cells were taken up in 5 ml of PBS. From this cell suspension, 0.5 ml was transferred into 29 ml of PBS; the turbidity of this diluted cell suspension corresponded approximately to that of McFarland barium sulfate standard no. 4 (ca. 1.2×10^9 cells per ml). To 2.7-ml portions of this latter *M. lysodeikticus* suspension were added 0.3-ml portions of substrate (see below), including 0.3 ml of PBS (control). After incubation at 35°C for 30 min, the optical density was determined at 450 nm with a Zeiss PMQII spectrophotometer. The following substrates were examined: fresh and heat-inactivated (56°C, 30 min) human serum and solutions containing 1 mg and 50 μg of lysozyme per ml of PBS. In addition, 1.8-ml volumes of these four substrates received 0.2 ml of 5,000-μg/ml solutions of SPS and SAS. The tubes were incubated at 35°C for 30 min, after which the precipitates were separated by centrifugation at 27,713 × *g* and 4°C for 10 min. The carefully harvested supernatant fluids were assayed for residual lysozyme activity. The solutions containing 1 mg and 50 μg of egg white lysozyme per ml of PBS were exposed to three-times prewashed (PBS, 2,500 × *g* at 4°C for 10 min) bentonite (10 mg/ml) at 37°C for 10 min (5), after which the supernatant fluids (2,500 × *g* at 4°C for 10 min) were assayed against *M. lysodeikticus*. The activity of the solution containing 50 μg of egg white lysozyme per ml of PBS was titrated against *M. lysodeikticus* as follows: after the solution was diluted 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:16, and 1:32 in PBS, 0.3-ml volumes of each dilution were assayed for lysozyme activity as described above.

Serum assays. One healthy adult repeatedly served as blood donor (designated T-serum). In addition, six presumably healthy medical students donated blood; their sera were pooled (designated P-serum). The sera were processd and maintained in 5-ml portions at −65°C as previously described (22–26). Samples of fresh serum (designated T-C, P-C) were heat inactivated at 56°C in a water bath as required (designated T-56°, P-56°). The serum assays (final volume, 2 ml) were performed in sterile polycarbonate (C. A. Greine und Söhne, Nürtingen, Germany) and in polystyrene (Corning Glass Works, Corning, N.Y.) tissue culture tubes which were incubated stationary at 35°C. T-C or P-C (80%, vol/vol, unless specified otherwise) or 80% (vol/vol) T-C that had been chelated with 0.01 M EDTA or with 0.01 M MgC_2–0.01 M EGTA (4) were examined. T-C and P-C each received SPS and SAS at final concentrations of 500, 250, 125, and 63 μg/ml. Log-phase bacterial inocula (0.2 ml) of the assay strain *M. lysodeikticus* and of the control strain *E. coli* C were added to 1.8 ml of T-C or P-C, chemically modified T-C or P-C, T-56° or P-56°, or fresh and heat-inactivated human serum that had been absorbed with 10 mg of bentonite per ml at 37°C for 10 min by the method of Donaldson et al. (5). The bacterial inocula had been adjusted to yield approximately 1.5×10^4 colony-forming units (CFU) per ml at zero time. At 0, 0.3, 1, 2, 4, and 22 h after exposure, unless otherwise specified, samples were removed from the assay tubes and serially diluted 10-fold in TSB. For *M. lysodeikticus*, duplicate small TSA plates (10-cm diameter) received 0.1 ml of undiluted and 10-fold-diluted assay suspension; the inocula were glass spread. The TSA plates required at least 36 h of incubation at 35°C until the yellow-pigmented colonies of *M. lysodeikticus* had reached a diameter of 1.5 to 2 mm. In the case of control *E. coli* strain C, two brain heart infusion agar pour plates per 10-fold dilution served to determine the number of survivors (CFU/ml) after overnight incubation at 35°C (23–26).

RESULTS

Preliminary experiments served to determine whether both synthetic anticoagulants precipitated lysozyme from serum. Neither fresh nor heat-inactivated T-serum contained sufficient lysozyme to be detectable by the turbidimetric lysozyme assay method used (Table 1). Therefore, egg white lysozyme was employed to further investigate this aspect. Addition of SPS or SAS at 500 μg/ml to a solution containing 1 mg of lysozyme per ml of PBS resulted in an instant milky turbidity. No turbidity was noted upon addition of identical quantities of SPS and SAS

TABLE 1. *Precipitation of egg white lysozyme by SPS*

Substrate[a]	Absorbance at 450 nm
PBS control	1.060
T-C control	1.120
T-56° control	1.030
T-C + 500-μg/ml SPS supernatant fluid	1.270
T-C + 500-μg/ml SAS supernatant fluid	1.170
1-mg/ml lysozyme control solution	0.034
1-mg/ml lysozyme solution + 500-μg/ml SPS supernatant fluid	1.050
1-mg/ml lysozyme solution + 500-μg/ml SAS supernatant fluid	0.040
50-μg/ml lysozyme control solution	0.040
50-μg/ml lysozyme solution + 500-μg/ml SPS supernatant fluid	1.140
50-μg/ml lysozyme solution + 500-μg/ml SAS supernatant fluid	0.043
PBS control	1.440
1-mg/ml lysozyme control solution	0.044
1-mg/ml lysozyme solution + 10-mg/ml bentonite supernatant fluid	1.370
50-μg/ml lysozyme control solution	0.049
50-μg/ml lysozyme solution + 10-mg/ml bentonite supernatant fluid	1.100

[a] A 0.3-ml amount of substrate was added to 2.7 ml of *M. lysodeikticus* assay cell suspension.

to a solution containing 50 μg of lysozyme per ml. Upon centrifugation at high speed (27,713 × *g*), SPS-treated egg white lysozyme yielded a clear supernatant fluid and a clearly discernible precipitate on the bottom of the tubes, whereas SAS-treated lysozyme remained as turbid as before. As shown in Table 1, the supernatant fluids of SPS-treated lysozyme solutions (both 1 mg and 50 μg of lysozyme per ml) lacked enzymatic activity. Conversely, the respective supernatant fluids of SAS-treated egg white lysozyme solutions retained full lysozyme activity. Furthermore, absorption of solutions containing 1 mg and 50 μg of lysozyme per ml of PBS with 10 mg of bentonite per ml at 37°C for 10 min abolished lysozyme activity to an extent identical to that observed after treatment with SPS (Table 1).

The turbidimetric titration of the control solution containing 50 μg of lysozyme per ml of PBS against *M. lysodeikticus* (Table 2) yielded the following data: 10 to 12.5 μg of lysozyme per ml resulted in significant decreases in optical density at 450 nm. However, dilutions of the lysozyme solution as high as 1:8 to 1:32 (6.3 to 1.6 μg of lysozyme per ml) yielded optical density values that were comparable to those obtained for the PBS control.

Preliminary serum assays disclosed that 80% (vol/vol) T-C killed up to 10^4 CFU of *M. lysodeikticus* (Table 3). Furthermore, T-C at a dilution of 1:16 and T-56° at a dilution of 1:8 effectively killed small inocula of *M. lysodeikticus* within 1 h (Table 4); however, during overnight incubation, additional killing activity ensued. Chelation of T-C and T-56° with either 0.01 M EDTA or 0.01 M Mg-0.01 M EGTA failed to abrogate bactericidal activity against *M. lysodeikticus* (Table 5). In contrast, both chelators yielded appropriate results for control strain *E. coli* C (23-25), indicating that they were functional with regard to the inactivation of both pathways of complement activation (EDTA) and the classical pathway (Mg-EGTA) of the complement system.

The next series of serum assays established that added SPS at 500 μg/ml protected *M. lysodeikticus* as effectively as did absorption of fresh human serum with bentonite (Table 6). SAS at 500 μg/ml was effective for periods up to 4 h; however, the number of *M. lysodeikticus* survivors declined sharply during extended incubation. Comparable results were obtained with pooled fresh human serum (Table 7). Finally, it was ascertained that 250 to 500 μg of SPS per ml of fresh human serum protected *M. lysodeikticus* for up to 22 h of incubation. Again, SAS at 63 to 500 μg/ml of serum effected only transient protection (Table 8).

DISCUSSION

The finding that SPS completely precipitated egg white lysozyme (Table 1) confirmed the

TABLE 2. *Turbidimetric titration of egg white lysozyme against M. lysodeikticus*

Final concn of lysozyme in[a]:		Absorbance at 450 nm
μg/ml	U/ml	
50	2,000	0.032
25	1,000	0.049
16.6	666	0.115
12.5	500	0.292
10	400	0.370
8.3	333	0.489
7.1	300	0.615
6.3	250	0.795
3.2	125	0.892
1.6	63	0.950
PBS control		0.970

[a] A 0.3-ml amount of lysozyme was added to a 2.7-ml suspension of *M. lysodeikticus* to give the final concentrations listed.

TABLE 3. *Measurement of killing capacity of fresh human serum against M. lysodeikticus*

Sample time (h postexposure)	*M. lysodeikticus* survivors (CFU/ml) with:					
	T-C[a]	T-C	T-C	T-C	T-C	TSB control
0	4.5×10^3	4.5×10^4	4.5×10^5	4.5×10^6	4.5×10^7	4.5×10^3
4	0	0	1.0×10^1	7.0×10^2	4.4×10^3	—[b]
22	0	0	1.5×10^1	1.2×10^2	1.6×10^3	1.3×10^7

[a] T-C at 80% (vol/vol).
[b] —, Not done.

TABLE 4. *Titration of fresh and heat-inactivated human serum against M. lysodeikticus*

Sample time (h postexposure)	*M. lysodeikticus* survivors (CFU/ml) with[a]:											
	T-C diluted:						T-56° diluted:					TSB control
	1:2	1:4	1:8	1:16	1:32	1:64	1:2	1:4	1:8	1:16	1:32	
1	0	0	3.5×10^1	2.8×10^2	3.8×10^3	7.0×10^3	0	0	0	8.5×10^2	6.8×10^3	7.0×10^3
22	0	0	0	0	0	2.1×10^3	0	0	0	0	0	$>10^7$

[a] In all cases the zero time concentration of organisms was 5.7×10^3 CFU/ml.

earlier work of Cavallo et al. (3). Totally unexpected was the observation that SAS at 500 μg/ml produced an instant milky turbidity after addition to a solution containing 1 mg of lysozyme per ml of PBS, yet failed to reduce enzymatic activity at all, as judged by the turbidimetric assay method. Most likely, SAS interacted with a cationic compound that contaminated the hen's egg white lysozyme, even though the manufacturer stated that this enzyme preparation had been crystallized three times, dialyzed, and lyophilized. Control experiments established that bentonite completely absorbed egg white lysozyme at both enzyme concentrations employed (Table 1). Unfortunately, the concentration of lysozyme in T-serum was so low as to escape detection by our admittedly crude turbidimetric lysozyme assay method (Table 1). This method permitted assay of approximately 10 μg of lysozyme per ml only (Table 2), a concentration far above that reported for human plasma, namely 1 to 2 μg/ml (5). Nevertheless, bioassays of fresh and heat-inactivated human serum (Tables 3 and 4) against *M. lysodeikticus* strain ATCC 4698 were feasible. Chelation of fresh and heat-inactivated human serum with either 0.01 M EDTA or 0.01 M $MgCl_2$–0.01 M EGTA (Table 5) failed to modify serum lysozyme activity.

The serum experiments involving *M. lysodeikticus* and *E. coli* control strain C as assay organisms (Tables 6, 7, and 8) disclosed that SPS (two different batches) at 250 to 500 μg/ml effectively neutralized serum lysozyme activity, whereas SAS (two different batches), on an equal weight-for-weight basis, afforded only transient protection (up to 4 h) of *M. lysodeikticus* cell inocula.

As shown by Neu et al. (19), human serum lysozyme lysed only a limited number of species of gram-positive bacteria, i.e., *Bacillus subtilis* and *M. lysodeikticus*, but not test strains of *Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes,* and *Streptococcus faecalis*. On the other hand, Feingold et al. (8) demonstrated with human serum that complement plus antibodies attacked the lipopolysaccharide-phospholipid complex of the outer membrane and the cytoplasmic membrane of a rough assay strain of *E. coli* (strain K12 200P) and that lysozyme attacked the peptidoglycan layer. Most important, the primary effect of complement-antibody-mediated serum activity was not necessarily lethal, unless lysozyme attacked the murein layer of the outer membrane or unless secondary damage to the cell membrane occurred as well.

Donaldson et al. (5), who examined rabbit serum against *E. coli* strain B, noted that there ensued most efficient killing and lysis of cells when β-lysin, lysozyme, and complement-antibody-mediated bactericidal activity acted in concert. Elimination of lysozyme from rabbit serum (bentonite absorption) affected serum bactericidal activity the least; removal of β-lysin resulted in an intermediate effect. Heat inactivation of rabbit serum abolished serum bactericidal activity against *E. coli* strain B. And neither β-lysin or lysozyme alone affected the viability of this particular assay strain. Electron microscopic examination, including freeze-etch preparations, by these investigators permitted the following conclusions: the complement-antibody system damaged the lipolysaccharide layer of the outer membrane and the cytoplasmic membrane; lysozyme attacked the inner

TABLE 5. *Failure of chelators EDTA and Mg-EGTA to abolish bactericidal activity of fresh and heat-inactivated human serum against M. lysodeikticus*

Sample time (h post-exposure)	*M. lysodeikticus* survivors (CFU/ml) with[a]:							*E. coli* strain C survivors (CFU/ml) with[a]:				
	T-C	T-C-EDTA[b]	T-C-Mg/EGTA[c]	T-56°	T-56°-EDTA[b]	T-56°-Mg/EGTA[c]	TSB control	T-C	T-C-EDTA[b]	T-C-Mg/EGTA[c]	T-56°	TSB control
0.3								0	7.2×10^3	1.5×10^4	1.6×10^4	—[d]
1	0	0	6.0×10^1	0	0	6.0×10^1	—	0	3.6×10^3	6.5×10^3	1.9×10^4	—
2	0	0	0	0	0	0	—	0	6.5×10^3	0	4.8×10^4	—
22	0	0	0	0	0	0	$>10^7$	0	1.9×10^3	0	9.1×10^5	$>10^8$

[a] Zero time concentrations of organisms were 6.9×10^3 CFU/ml for *M. lysodeikticus* and 1.7×10^4 CFU/ml for *E. coli* strain C.

[b] T-C and T-56° at 80% (vol/vol) chelated with 0.01 M EDTA.

[c] T-C and T-56° at 80% (vol/vol) chelated with 0.01 M $MgCl_2$–0.01 M EGTA.

[d] —, Not done.

TABLE 6. *Effect of bentonite absorption, added SPS, and added SAS upon bactericidal activity of T-serum against M. lysodeikticus*

Sample time (h post-exposure)	*M. lysodeikticus* survivors (CFU/ml) with[a]:							*E. coli* strain C survivors (CFU/ml) with[a]:		
	T-C[b]	T-C + BENT-ABS[c]	T-C + SPS[d]	T-C + SAS[e]	T-56°[b]	T-56° + BENT-ABS[c]	TSB control	T-C	T-C + BENT-ABS[c]	T-56°
2	0	3.5×10^4	4.4×10^4	3.5×10^4	2.5×10^1	3.6×10^4	3.8×10^4	0	0	2.1×10^4
4	0	3.8×10^4	4.2×10^4	3.4×10^4	0	3.4×10^4	4.6×10^4	0	0	4.3×10^5
22	0	7.1×10^4	7.0×10^4	3.0×10^1	0	6.9×10^4	8.7×10^6	0	0	$>10^8$

[a] Zero time concentrations of organisms were 2.7×10^4 CFU/ml for *M. lysodeikticus* and 2.0×10^4 CFU/ml for *E. coli* strain C.

[b] T-C and T-56° at 80% (vol/vol).

[c] T-C and T-56° were absorbed with 10 mg of three-times prewashed bentonite per ml at 37°C for 10 min by the method of Donaldson et al. (5).

[d] SPS (lot K2710) was added to a final concentration of 500 μg/ml.

[e] SAS (lot M.H4-24.A) was added to a final concentration of 500 μg/ml.

TABLE 7. *Effect of bentonite absorption, added SPS, and added SAS upon bactericidal activity of pooled human serum against M. lysodeikticus*[a]

Sample time (h post-exposure)	*M. lysodeikticus* survivors (CFU/ml) with[b]:							*E. coli* strain C survivors (CFU/ml) with[b]:		
	P-C	P-C + BENT-ABS	P-C + SPS	P-C + SAS	P-56°	P-56° + BENT-ABS	TSB control	P-C	P-C + BENT-ABS	P-56°
1	2.5×10^1	1.3×10^4	1.4×10^4	1.4×10^4	2.0×10^3	1.3×10^4	1.3×10^4	0	0	2.0×10^4
3	0	1.4×10^4	2.3×10^4	1.4×10^3	1.5×10^1	1.4×10^4	1.5×10^4	—[c]	—	—
22	0	2.9×10^4	2.3×10^4	1.0×10^2	0	4.7×10^4	3.7×10^6	0	0	$>10^8$

[a] See Table 6 for explanatory footnotes.

[b] Zero time concentrations of organisms were 1.6×10^4 CFU/ml for *M. lysodeikticus* and 1.7×10^4 CFU/ml for *E. coli* strain C.

[c] —, Not done.

peptidoglycan layer of the outer membrane; and β-lysin damaged the cytoplasmic membrane.

With these observations at hand, it is most likely that serum lysozyme may enhance antibody-complement-mediated bactericidal activity against gram-negative bacteria both in vivo and in vitro. Thus, the synthetic anticoagulants used should effectively neutralize not only classically and alternatively activated human complement, but serum lysozyme as well. In both instances SPS proved superior to SAS (23, 25). In this context, Kocka et al. (16) and Hall et al. (13) observed no significant differences in the isolation frequencies of bacteria from SPS- and SAS-anticoagulated blood cultures, respectively; however, Hall et al. (13) observed that certain gram-negative bacteria were recovered less often from SAS-supplemented blood cultures. Con-

TABLE 8. *Quantitation of inhibitory effects of SPS and SAS upon serum lysozyme activity against M. lysodeikticus*[a]

Sample time (h post-exposure)	*M. lysodeikticus* survivors (CFU/ml) with[b]:										*E. coli* strain C survivors (CFU/ml) with[b]:	
	T-C + SPS[c] at 500 μg/ml	T-C + SPS at 250 μg/ml	T-C + SPS at 125 μg/ml	T-C SPS at 63 μg/ml	T-C + SAS[c] at 500 μg/ml	T-C + SAS at 250 μg/ml	T-C + SAS at 125 μg/ml	T-C + SAS at 63 μg/ml	T-C	TSB control	T-C	T-56°
1	1.1×10^4	9.4×10^3	9.2×10^3	2.2×10^3	1.0×10^4	9.2×10^3	9.9×10^3	1.6×10^3	0	8.8×10^3	0	1.6×10^4
2	9.8×10^3	9.7×10^3	9.3×10^3	5.0×10^0	9.9×10^3	9.6×10^3	3.5×10^3	7.5×10^1	0	9.0×10^3	0	4.8×10^4
22	1.7×10^5	2.0×10^5	2.0×10^1	0	0	0	0	0	0	$>10^7$	0	9.1×10^5

[a] See Table 6 for explanatory footnotes.

[b] Zero time concentrations of organisms were 8.2×10^3 CFU/ml for *M. lysodeikticus* and 1.7×10^4 CFU/ml for *E. coli* strain C.

[c] SPS (lot 120056, Ro 1-1521) and SAS (lot MH4-24D SN 263) were added to the indicated final concentrations before addition of bacterial inocula.

ceivably, this may have been due to either ineffective neutralization of complement or the failure of SAS to inactivate serum lysozyme for prolonged periods of incubation.

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