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Action of Sulfated Polyanions Used in Blood Culture on Lysozyme, Complement and Antibiotics

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ABSTRACT

The modes of action of sulfated polyanions, sodium amylosulfate and sodium polyanethol sulfonate were studied. These agents inhibited the activity of lysozyme and formed insoluble complexes with this enzyme. The polyanions inactivated complement and lessened the effectiveness of antibiotics against bacteria.

Introduction

There is a growing interest in devising more effective methods of isolating organisms from blood and other body fluids. Sulfated polyanions, such as sodium amylosulfate (SAS) * and sodium polyanethol sulfonate (SPS) are known to neutralize the natural antibacterial factors of whole blood.[3,8] Moreover, SPS lessens the action of certain antibiotics that might be present.[7,9]

Little information is available concerning the mechanism whereby polyanions facilitate recovery of bacteria from blood. Part of the effect, however, is related to the fact that SPS and SAS are calcium chelating agents. They prevent coagulation and the physical entrapment of organisms in the blood clot. The agents are also suspected of interacting with antibody-complement systems, beta-lysin and lysozyme[4,8] in some manner so that bacterial growth is inhibited.

With a view toward elucidating the mode of action of polyanions on human blood, the effect of SPS and SAS on complement, lysozyme and antibiotics was studied.

Materials and Procedure

Organisms used from Searle Diagnostic Research Laboratory stock cultures included *Micrococcus lysodeikticus, Escherichia coli* 136-E, *Streptococcus fecalis* ATCC 10541, and *Staphylococcus aureus* LG. Trypticase Soy Broth (TSB) (Baltimore Biological Laboratory, Baltimore, MD) was employed for routine culture and dilution of organisms. Organisms were assayed for viability on Trypticase Soy Agar (TSA) pour plates.

Antibiotics studied were streptomycin sulfate, polymyxin B sulfate, penicillin G and tetracycline hydrochloride (Nutritional Biochemical Co., Cleveland, OH), kanamycin sulfate (Sigma Chemical Co., St. Louis, MO), neomycin and lincomycin (Grand Island Biochemical Co., Grand Island, NY), gentamycin sulfate (Schering Corp., Bloomfield, NJ), sodium cephalothin and vancomycin (Eli Lilly and Co., Indianapolis, IN). All antibiotics were prepared

* Available as TEKIT® S-A-S, Searle Diagnostic, Inc., Box 2440, Columbus, OH 43216.

TABLE I

EFFECT OF SODIUM AMYLOSULFATE (SAS) AND SODIUM POLYANETHOL SULFONATE (SPS) ON ANTIBIOTICS

Antibiotics	*Test Organisms**	*Highest Concentration (µg per ml) of Antibiotic Without Growth Inhibition†*		
		Control	*SAS*	*SPS*
Streptomycin sulfate	Escherichia coli	50	140	140
Polymyxin B sulfate	Escherichia coli	1.1	2.0	2.0
Tetracycline-HCl	Escherichia coli	2.0	2.0	2.0
Penicillin G (sodium)	Streptococcus fecalis	2.0	2.0	2.0
Kanamycin sulfate	Escherichia coli	50	65	65
Gentimicin sulfate	Escherichia coli	10	35	20
Cephalothin (sodium)	Escherichia coli	26	26	26
Vancomycin	Streptococcus fecalis	1.0	1.0	1.0
Neomycin	Escherichia coli	90	105	105
Lincomycin	Staphylococcus aureus	15	15	15

*All organisms were grown in trypticase soy broth.
†Each study was verified at least once.

in aqueous solution and membrane filtered.

Sodium amylosulfate (G. D. Searle & Co., Chicago, IL) was prepared in a 5 percent (w/v) aqueous solution and autoclaved. Sodium polyanethol sulfonate was purchased as a sterile aqueous solution (Grobax™, Roche Diagnostics, Nutley, NJ).

For lysozyme assay, *M. lysodeikticus* was grown to mid log phase and then incorporated into TSA plates at a concentration of about 10^7 organisms per ml. After the plates had solidified, they were spotted with 0.05 ml of a lysozyme solution (Nutritional Biochemical Co., Cleveland, OH) and incubated overnight at 37°C. The lysozyme solution was prepared at a concentration of 10 mg per ml and membrane filtered.

Fresh human serum was obtained from laboratory personnel. Rabbit complement, antisheep hemolysin and washed sheep cells were obtained from Grand Island Biochemical Co. (Grand Island, NY) and membrane filtered. Complement titers were determined by the technique of Evans.[2]

Polyacrylamide electrophoresis of serum lipoprotein was performed by the method of Frings et al.[6] Lysozyme was studied electrophoretically at pH 4.3 with reversed electrodes using a 7.5 percent polyacrylamide gel.

Results and Discussion

Each antibiotic was serially diluted in TSB containing final concentrations of 0.05 percent SAS or SPS. Then 0.1 ml of a growing culture was added to each tube and

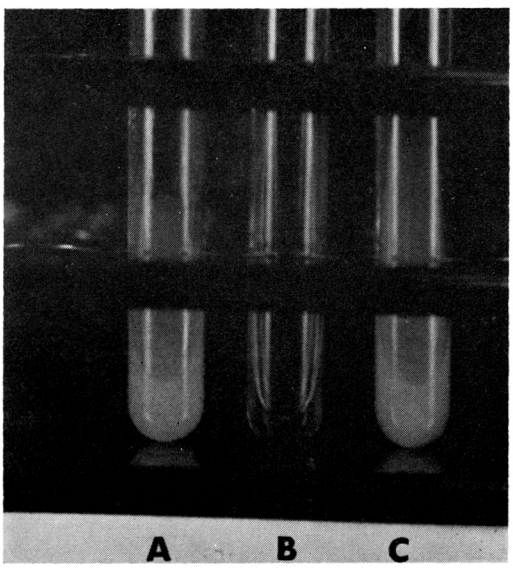

FIGURE 1. Precipitation of lysozyme by polyanions. From left: (A) SAS, (B) Control solution, (C) SPS.

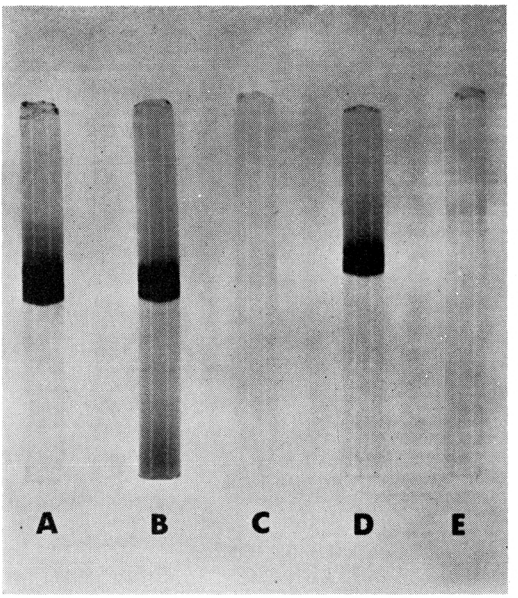

FIGURE 2. Polyacrylamide electrophoresis of lysozyme. From left: (A) control lysozyme, (B) SAS resolubilized precipitate, (C) SAS supernatant, (D) SPS resolubilized precipitate, and (E) SPS supernatant.

incubated overnight at 37°C. The amount of streptomycin, polymyxin B, kanamycin, gentamycin and neomycin required to kill test organisms *in vitro* was increased in the presence of SAS or SPS (table I). The activity of tetracycline, penicillin G, sodium cephalothin, vancomycin and lincomycin was not affected by the polyanionic additives.

TABLE II

EFFECT OF SODIUM AMYLOSULFATE AND SODIUM POLYANETHOL SULFONATE ON THE COMPLEMENT REACTION

Additive	*ml of Complement**				
	0.2	0.4	0.6	0.8	0.0
Saline	+§	+	+	+	-
SAS†	-	-	-	-	-
SPS†	-	-	-	-	-

*Complement was diluted 1/30 rabbit complement and hemolysin was diluted 1/100 in saline.
†SAS was sodium amylosulfate at 0.05 percent (w/v) and SPS was sodium polyanethol sulfonate at 0.05 percent (w/v).
§The + indicated lysis of sheep red blood cells.

TABLE III

EFFECT OF SODIUM AMYLOSULFATE AND SODIUM POLYANETHOL SULFONATE ON THE BACTERIODICAL ACTION OF COMPLEMENT*

Treatment	*Organisms per ml*	
	Trial 1	*Trial 2*
None	8.4×10^4	0
SAS†	1.0×10^7	2.5×10^5
SPS†	9.6×10^6	---
Control§	9.8×10^6	2.6×10^5

*Organism used was Escherichia coli. Rabbit complement was diluted 1/4 with Trypticase Soy Broth.
†SAS refers to sodium amylosulfate at 0.05 percent (w/v) and SPS refers to sodium polyanethol sulfonate.
§Control contained Trypticase Soy Broth in place of complement.

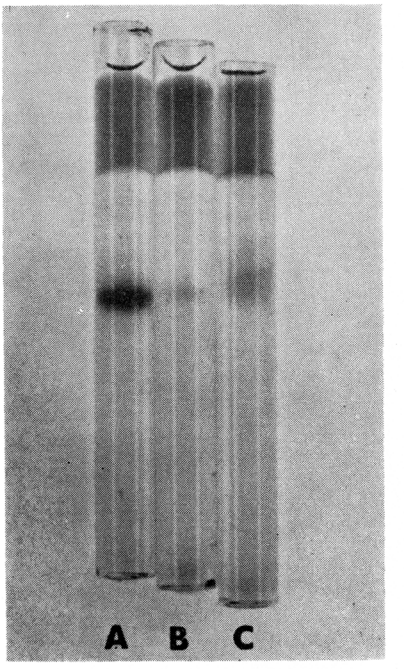

FIGURE 3. Polyacrylamide electrophoresis of serum lipoprotein. From left: (A) human serum, (B) human serum treated with SAS, (C) human serum treated with SPS.

Lysozyme (10 mg per ml) was precipitated from aqueous solution with SAS or SPS at 0.05 percent (w/v) (figure 1). The precipitate was washed with distilled water resolubilized with 0.01 N NaOH. Both precipitate and supernatant fractions were examined with polyacrylamide electrophoresis. The supernatant solutions showed no lysozyme band, whereas the mobility of the precipitates was similar to the control solution (figure 2).

The supernatant and precipitates were diluted in sterile distilled water and spotted on TSA plates containing *M. lysodeikticus*. There was no lytic activity in the undiluted supernatant fractions although the redissolved precipitates showed lytic activity in dilutions up to 10^6.

Human serum that had been treated with SAS and SPS and centrifuged was examined with polyacrylamide electrophoresis and found to have decreased bands of beta-lipoprotein (figure 3). The precipitate formed by SAS has been shown to contain the beta-lipoprotein.[10]

Commercial rabbit complement diluted 1/30 in cold saline was mixed with SAS or SPS to a final concentration of 0.05 percent. This complement was then used in a system with sensitized sheep cells and hemolysin. Results indicated that SAS or SPS completely inhibited the complement hemolysis reaction of sheep cells (table II).

Sterile rabbit complement diluted 1/4 with TSB either completely killed or considerably lessened the number of viable *E. coli* cells depending upon the initial concentration of the organisms. SAS or SPS prevented any decline in viability as a result of the complement treatments (table III).

These experiments suggest some of the possible modes of action of SAS on blood and other body fluids. Although SAS acted in a manner similar to SPS, there were quantitative differences in their actions. SAS tended to cause a more floccular precipitate than SPS and was found to be less toxic than SPS towards mycoplasma species.[1,5]

Since SAS inactivated some of the antibacterial factors in blood at least as well as SPS, it is presently being clinically tested as an inexpensive blood culture additive.

Conclusion

SAS and SPS were shown to precipitate lysozyme and inhibit the action of complement on erythrocytes and bacteria. The effectiveness of some antibiotics on sensitive bacteria was also lessened by these additives.

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