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PREPARATION AND STUDY OF THE ANTHRAX PROTECTIVE ANTIGEN. REPORT--ETC(U)
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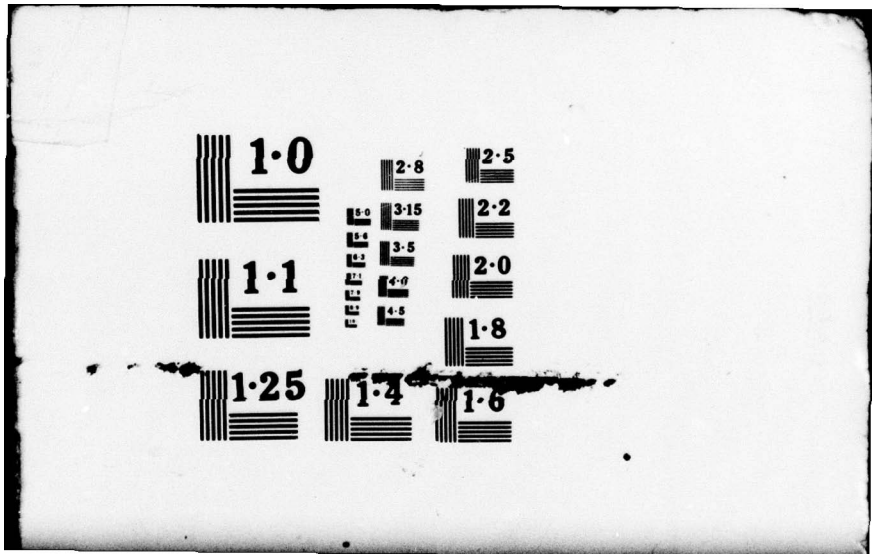
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PREPARATION AND STUDY OF THE ANTHRAX PROTECTIVE ANTIGEN. REPORT II. DEVELOPMENT OF TEST-PREPARATIONS FOR THE ASSESSMENT OF THE QUALITY OF ANTHRAX CHEMICAL VACCINES AND OF THE ANTIGENS OBTAINED AT VARIOUS STAGES OF ITS PREPARATION

(Submitted 11/II/1976)

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The first reports concerning the isolation and concentration of the anthrax protective antigen (PA) prepared in vitro appeared in 1954-1955 /7, 9/. Subsequently, Strang and Thorn /10/ isolated from filtrates of native Bac. anthracis cultures a highly purified PA which is homogeneous during ultracentrifuging and electrophoresis on paper and which possesses the qualities of a protein. When administered to rabbits the preparation evoked a specific immunity. In the diffusion precipitation reaction (DPR) according to Oukhtleroni, this antigen formed a single line with the horse serum obtained during the immunization of animals with Bac. anthracis spores of the Stern strain. Fish et al. /9/ using the method of salting-out with ammonium sulfate and subsequent chromatography on a column with polyacrylamide gel also obtained a serologically uniform PA which is an active immunogen. Vancurik /11/ obtained the most homogeneous PA preparations by saturating the filtrate of the cultural liquid with ammonium sulfate to 50%. When increasing the concentration of ammonium sulfate the PA became contaminated with extraneous antigens.

In the research by Yezepchuk /3,4/, Dunayev /2/, and Siromashkova /5/ PA preparations isolated by the acidic or the alcohol method from native cultures of the vaccine strain STI-1, according to DPR and immunoelectrophoretic data had in their composition from

3 to 7 antigenic components. However, this literature gives no information concerning the identification of a homogeneous PA.

Thus, today there is as yet no highly purified anthrax PA which can be viewed as being a test preparation.

The purpose of the present work was the preparation of a serologically homogeneous PA test-preparation and a serum, monospecific with respect to the test preparation, necessary for the rapid evaluation of the quality of the chemical vaccine and of the antigens obtained at various stages of its preparation.

Strain STI-1 was grown on a protein-less aminobenzoic acid medium of Wright et al. /12/ in 10 liter bottles equipped with special mixers. The nourishing medium had increased concentrations of glucose and sodium bicarbonate - up to 0.3 and 0.7%, respectively. Cultivation was carried out at 37° over 22-27 hours with continuous mixing by mixers rotating at 50 RPM. To isolate the antigen, the filtrate of the cultivated liquid was saturated with ammonium sulfate (50% saturation), the pH was established within the limits of 7.3 to 7.5 and left for 18 to 20 hours at 4 to 6°. The resulting precipitate was separated in a centrifuge, the sediment was then dissolved in distilled water taken in a volume of 1/40 of the initial volume of the filtrate.

Salting-out of the PA was carried out by gel filtration on a cooled column with a G-25 sephadex of the Farmacia fine chemicals company, balanced with 0.05 M succinate-borate buffer solution with a pH of 7.5. Fractions were taken in a volume of

4-4.5 ml with the aid of a cooled automatic Ultra-RAC fraction collector (made by LKB, Sweden). The elution profile for optical density at 280 nm was recorded automatically by a Uvicord-II flow absorption meter (LKB, Sweden) and a Recorder-2066 recorder.

The antigenic activity of the fractions of the preparation was found by DPR with a commercial anti-anthrax gamma-globulin diluted 8-fold. The protein was determined spectrophotometrically at 220 nm /6/ using an improved methodology[1] and an SF-16 instrument. Total nitrogen was determined according to Queldal.

To evaluate immunogeneity, the concentrated preparation was first sorbed with $Al(OH)_3$ at a ratio of 1:1. Five-fold dilutions were prepared and each dilution was administered intraperitoneally to 12 white mice weighing 15 to 18 g. 14 to 16 days after PA injection, the animals were infected with spores of the STI-1 strain in a dosage of $250 \cdot 10^6$. Immunogeneity was evaluated in LD_{50}, calculated according to Kerber in the modification by Ashmarin and Vorob'yev /1/.

During the analysis of the antigenic composition of the preparation the following were used: commercial anti-anthrax precipitating serum, anti-anthrax gamma-globulin and rabbit anti-serums prepared by us for the STI-1 strain and also for Bac. cereus, Bac. subtilis, Bac. mesentericus niger, Bac. pseudoanthacis, obtained from the Tarasevich State Scientific Research Institute for Standartization and Control of Medical Biological Preparations.

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1) The method of spectrophotometric determination was perfected by Rebrov and Zhdanovich.

TABLE 1.
Characteristics of Isolated PA Preparations (Average of 4 Experiments)

PA Preparation	Volume (ml)	Total Nitrogen (mg%)	Protein (mg%)	LD_{50}	
				in 1 ml	per 1 ml protein
Native	4000	10.1	Traces	--	--
Precipitated $(NH_4)_2SO_4$:					
Before salting-out	90	--	44.2	74	167
After salting-out	105	4.5	21.4	62	290

TABLE 2
Antigeneity of PA Preparations Obtained by Various Methods*

Method	Antigenic Properties			
	liquid preparation before lyophilization	Rehydrated Preparation		
		After Drying	After 6 Mos.	After 12 Months
Salt with Co-precipitator	1:16-1:32	1:16	1:16	1:8-1:16
Salt w/o co-precipitator (control)	1:8	0	--	--

* The antigenic properties of the preparations were expressed by the greatest dilution of PA which caused the appearance of a DPR line of precipitation with a specific anti-serum.

Immunodiffusionary analysis demonstrated that in the native PA filtrate there exist no fewer than 2 antigens. One of these was thermally stable, which may be evidence of its belonging to polysaccharide antigens. The other was thermolabile.

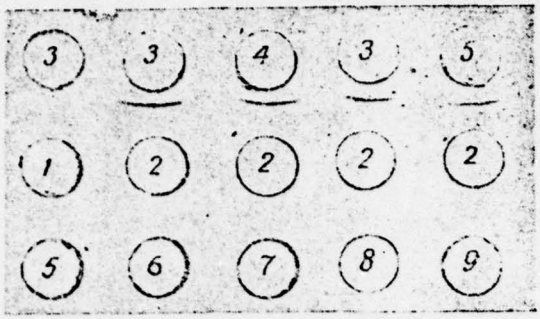

Figure 1. Immunodiffusionary Analysis of Purified Protective Antigen (PA) Preparation.

1) purified PA heated 30 min. @ 60°; 2) purified PA; 3) precipitating commercial antiserum; 4) experimental hyperimmune antiserum for STI-1; 5) anti-anthrax commercial gamma-globulin; 6),7),8),9) experimental antiserums for Bac. cereus, Bac. subtilis, Bac. mesentericus niger, Bac. pseudoanthracis, respectively.

During subsequent fractioning of the antigen with ammonium sulfate the preparation became purified as nonspecific nitrogen-containing components (Table 1) were eliminated from the thermally stable antigen (Figure 1). Thus, in purified concentrated PA preparations, the gel precipitation reaction yielded only one thermolabile antigen which was not type-specific and formed no precipitation lines with hyperimmune antiserums for closely related strains (Figure 1).

In the next experimental series the PA preparation was purified on a G-200 sephadex (Figure 2). The resulting antigen had a specific immunogenic activity of about 400 UD_{50} per 1 mg protein and contained from 8 to 10 mg% protein. After sorption

on $Al(OH)_3$ the preparation in increasing dosages was administered subcutaneously 10 times to rabbits at 5-day intervals. Blood was sampled 10 days after completion of the hyperimmunization cycle. Antiserum in DPR with native and purified (on G-25 and G-200 sephadexes) protective antigen formed just one precipitation line: i.e. it was monospecific. In subsequent experiments it therefore became possible to use it as a working standard for the antigenic activity of PA.

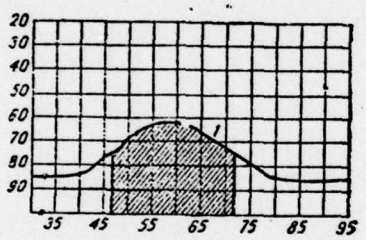

Figure 2. Fractioning Purified PA on a Column With G-200 Sephadex.

1) optical density: antigen-containing fractions located in shaded area of the chromatogram. Along abcissa axis - test tube numbers, along ordinate axis - penetration (T_{280} in %).

The use of purified PA as a test-preparation is of particular interest. However, observations have shown that it lost its antigenic activity while stored in liquid form for a period of 2 to 3 weeks. It was not possible to save the specific activity of PA preparations by means of lyophilization. Further research showed that the addition of cultural liquids to filtrates during salting-out of the antigen of the protein co-precipitator (approx. 5-7 mg% casein) makes possible the yield of more active

(by 2 to 4 times) liquid concentrated PA preparations which are stable during lyophilization and storage of dry PA test-preparation for 12 months (observation period) - Table 2.

Thus, the method we have used allows us to obtain a purified anthrax PA possessing a high specific immunogeneity, stability during drying-out and storage, and characterized by homogeneity of the antigenic composition.

The prepared hyperimmune anti-serum for the purified PA turned out to be monospecific. It may be used for quantitative evaluation of the antigenic nature of the preparations.

CONCLUSIONS

1. Using the salting-out method with the use of ammonium sulfate with subsequent gel-filtration on sephadexes there was obtained a specific, serologically homogeneous anthrax protective antigen (from strain STI-1).

2. A method is proposed to obtain lyophilized test-preparation of anthrax protective antigen which is capable of retaining its properties during 12 months (observation period). A hyperimmune monospecific anti-serum for this preparation was prepared.

3. It is suggested that the lyophilized preparation of anthrax protective antigen and its monospecific antiserum be used to evaluate the qualities of chemical vaccine and the antigens prepared at stages of its preparation.

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M. K. Kuzmich, M. I. Derbin, N. S. Garin, I. D. Kravets, I. V. Shentsev, V. S. Tarumov, A. A. Gorlanov, N. V. Sadovoy, N. V. Fedorova

A homogeneous anthrax protective antigen (from the ST-I strain) characterized by a high immunogenicity and stability was obtained. Hyperimmune, monospecific to this preparation antiserum was prepared. It is suggested that these preparations can be used for the assessment of the quality of the anthrax chemical vaccine and of the antigens obtained at various stages of its preparation.