AD 676984

TRANSLATION NO. 99

DATE: Sept 1968

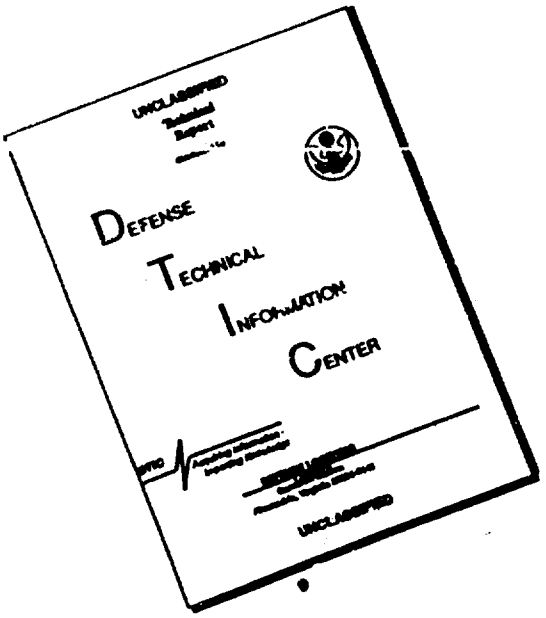
DDC AVAILABILITY NOTICE

This document has been approved for public release and sale; its distribution is unlimited.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Reproduced by the
CLEARINGHOUSE
for Federal Scientific & Technical
Information Springfield Va. 22151

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

#99

Trans. Acad. Sci., USSR., 1955, v. 105(2) Nov; 315-318

G. M. Shipitsina

On the chemical composition of the antigenic substances of the agent of tularemia.

In this report the virulent strain, No. 21, of the bacterium tularense, representing the S-form of microbe, was utilized. The cultures were cultivated for 3 days at 37 C. on a fish agar with cystine and glucose. The antigenic substances were extracted from a rinsed, fatless, dry microbic mass.

The antigenic substances of B. tularense were extracted by the following methods: I--extraction with 5% trychloracetic acid at 0 C. for 3 hours (Duaven method)(1); II--extraction by this same acid but for longer periods of time and at a higher temperature (modified Duaven method); III--extraction by phenol (A. P. Nonikov method)(1). The average outcome of the antigenic substances were: for I--3.84%; for II--5.95%; for III--3.24%. The extracted substances were rinsed many times by reprecipitation of them with alcohol from water solutions. Subsidiary workings of the obtained preparations with chloroform (2) indicated an absence of any mechanical tinge of albumin in them. The extracted substance possessed antigenic and allergenic properties, and also a serological activity, which gave a precipitating reaction in great dilutions on anti tularemia serums.

An attempt was made to decompose the antigens into separate components by means of heating for 4 hours in a boiling water bath, in the presence of 0.1N of acetic acid. Unfortunately the attempt was without results. Exchanging the hydrolysates with heated chloroform, we were able to extract an abundant residue from them, which dissolved well in

a mixture of equal parts of heated chloroform and ethyl alcohol. After removal of the chloroform extracted residue, the hydrolysate was worked with various quantities of ethyl alcohol.

As a result of the above working the antigens of the tularemia microbe were divided into 5 fractions: I-substances non-soluble in a mixture of alcohol-chloroform (6.0-7.3%); II-residue from 4 volumes of alcohol (23.3-33.2%); III-residue from 10 volumes of alcohol (13.4-15%); IV-substances extractable from the hydrolysate by chloroform and ether, plu substances which entered into a mixture of alcohol-chloroform during the obtaining of fraction I (33.9-37.8%); V- dry balance after removal of all the above fractions(11.8-18.2%). The antigens, extracted by verious conditions of trychloroacetic acid, were characterized by a similar content of separate fractions. The antigen obtained by the Konikova method differed from them somewhat.

A chemical analysis of the original antigens and products of their fractionation indicated that during hydrolysis with acetic acid the separation of the antigenic substances into chemically individual components (specific polysaccharides and albumin) does not take place (Table 1). Each fraction, except the lipid (fraction IV), evidently, represents an intricate mixture of various elements of the antigen, or complexes containing carbohydrate as well as nitrous elements. Nucleic acids were detected in the composition of fraction II.

The studying of the quantitative composition of the carbohydrate and albuminous components of the original antigen, and their separate fractions, was done by chromatography on paper.

The composition of the monosaccharides, obtained as a result of hydrolysis, was studied on single dimensional chromatograms, taking place in a mixture of n-butanol-acetic acid-water(40:10:50). As a

developer we used anilinphtalate in water-impregnated butanol. For the identification of the glucose and galactose we, besides this, used reactions with phenylhydrazine, having in mind that the osazones of these monose are slightly different.

It was proven that all the antigens, irregardless of the method used for their extraction, contain quantitatively similar specific polysaccharides. Results of the chromatographic analysis of the polysaccharides of the antigens isolated by the Duven method, and the fractions II and III obtained from them, indicate that the carbohydrate components of the whole antigen and its fraction II are qualitatively of the same composition. They contain the following monosaccharides: galactose, arabinose, xylose, uronic acids and hexosamine. In the composition of the carbohydrate component of fraction III there were other saccharides detected, and namely: glucose, mannose, xylose and hexosamine(analyticly).

It is necessary to note that chromatographic analysis of the composition of the polysaccharides of the original antigen disclosed no glucose or mannose. Judging by the intensiveness of the color of the stains on the chromatograms, the basic mass of the polysaccharides of the original antigen consists of galactose and arabinose. The intensively dyeing stains of these sugars on the chromatograms could have masked the weakly dyeing stains of the mannose and glucose.

Chromatographic study of the albuminous components of the antigenic complexes, extracted by various methods from a virulent strain of B. tularense, indicated that they have a qualitatively similar amino-acid content. Separation of the aminoacids of the hydrolysates being studied was done on one- and two-dimensional chromatograms. Water impregnated butanol was used in a mixture of n-butanol-acetic acid-water

(40:10:50) as a solvent during the two-dimensional chromatography. An 0.2% solution of ninhydrin was used in water-saturated butanole as developer. The aminoacidic composition of the albumin of the antigen, extracted by the Buaven method, is represented on the two-dimensional chromatogramm. 45 stains were located on the chromatogramm, of which one was unidentified. It is believed that it belongs to α,ε-diaminopymelic acid. Histidine and lysine are located on this chromatogramm in one stain(spot). Their presence was indicated on the single-dimensional chromatogramms. The histidine, besides this, was detected by the Paul reaction. The presence of proline was indicated on a separate chromatogramm during the use of isatin. Consequently, the albuminous components of the antigen contain more than 17 aminoacids. In regard to the aminoacidic composition of the albuminous component, fraction II and III did not differ from each other.

The conducted studies established that the antigenic complex of the tularemia microbe, by its chemical nature, significantly differs from the full antigens of Buaven, from the bacteria of the intestinal-typhus group, studied to this time. Two complicated complexes-fractions II and III- enter into the composition of the tularemia antigen.

It was established that the latter complexes are separate components, firmly linked together. Thus, fraction II represents a stable complex, consisting of polysaccharides, albumin and nucleic acid, and fraction III-stable polysaccharides, albuminous complex. The albuminous components of the said fractions contain at least 17 similar aminoacids. The presence of qualitatively different polysaccharides in these fractions indicates: either two antigens, or one with various determinant groups.

Great interest is shown in the detection of an exceptionally high content of the lipid fraction in the tularemia antigen(approx. 40% from the original antigen), sharply differing from the analogical fractions of the Duaven full antigens, by characteristics.

INST. EPID. MICROB. ,imene H. F. GAMALE. AMS., USSR.,
Entered for publication 14/mar/55.

one table-copied
two illustrations- not reproduced.

Literature

1. V. I. Tovarnitski, J.MI., 2, 37(1947); 10, 31 and 11, 82(1948). 2. M. G. Savage, D. B. Zackson, A. Z. Smolens. J. Biol. Chem., 124, 425, (1938). 3. A. N. Belozerski, N. I. Prochuryakov, Prac. Manuel for the Biochemistry of Plants, 1951. 4. S. M. Partridge, Nature, 164, 443 (49). 5. L. Elson, W. Morgan, Biochem. J., 27, 1824(1933).

le 1. Chemical characteristics of antigens and their separate fractions (data expressed in % from dry weight of prep.

mical indicators	Orig. antigen			I fraction			II fraction			III fraction			IV fraction			V fraction		
	B*	MB*	K*	B	MB	K	B	MB	K	B	MB	K	B	MB	K	B	MB	K
on nitrogen	7,1	7.4	5.9	8.6	8.5	5.7	8.7	8.5	8.5	9.5	9.5	10.1	2.5	2.6	2.0	7.7	7.5	7.5
mon Phosphorus	1.0	1.2	0.9	0.8	1.4	0.6	1.7	1.5	1.9	0.6	0.6	0.6	1.2	1.0	0.9	0.8	0.8	0.3
sphorus Nucne acid **							1.5	1.5										
oxyribonucc acid **							5.2	5.1										
onucleic acid ***							10.5	10.9										
ucing subnces	20.7	20.2	20.1	7.1	5.1	6.9	27.9	28.5	26.7	25.5	25.0	25.4	5.6	3.9	4.2	24.2	27.0	25.9
osamine	11.0	10.9	9.6	0	0	0	16.8	13.4	14.0	20.3	20.2	19.6	2.8	3.0	1.8	7.7	10.9	5.8
rogen hexsoe(by calculion)	0.9	0.9	0.8	0	0	0	1.3	1.1	1.1	1.6	1.6	1.5	0.2	0.2	0.1	0.6	0.9	0.5
rogen purine calculation)							1.7	1.8										
min(by calion)				53.6	53.1	55.4	35.5	35.9	--	49.2	49.7	53.3						

* Antigens obtained: B-Buaven, MB--Modified Buaven method, K-Konikov.
** Data of the substance were determined only in fraction II, Fractionation of the phosphorus combinations according to Shmidt and Tangauser(3).