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SIALIC ACID IN THE 'F. TULARENSIS' AND 'F. NOVICIDA' ANTIGENS,(U)
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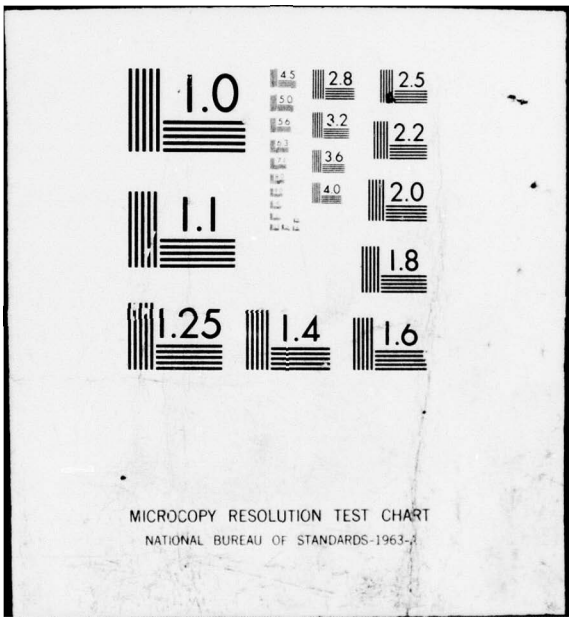
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Rodionova, I.V.

SIALIC ACID IN THE F. TULARENSIS AND F. NOVICIDA ANTIGENS

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Polysaccharides play an important role in determining the antigenic structure of a tularemia microbe. There is an established dependence between the composition of the hydrocarbon component of the specific lipo-polysaccharide-protein complex of F. tularensis and the virulent and immunogenic properties of the strains /4, 6, 7/. However, the nature of the hydrocarbon components in the antigenic complexes of the tularemia microbe, judging by modern data, is insufficiently known. Therefore, further information on the structure of the specific polysaccharide of this causative agent may be of certain interest.

As for F. novicida, in the only strain of this species similar to the causative agent of tularemia there was found to be the same somatic antigen complex as in the corresponding complex in F. tularensis /3/, but there are no data concerning its chemical composition.

The purpose of the present paper was to find sialic acid[1)] in antigens isolated using the Bouaven method from

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1) Group name for various N-acetylizogenic derivatives of nine-carbon monosaccharide -- neuraminic acid.

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strains of F. tularensis and F. novicida of different virulence, and the determination of its role in specific properties manifested by the antigens.

In this work were utilized 2 highly virulent strains of the tularemia microbe -- No. 503/830 and Schu, belonging to the holarctic and nonarctic races, respectively. The minimum lethal dose of these cultures for white mice when introduced subcutaneously was 1 microbe cell, measured by the standard of the Tarasevich Institute. In addition, strains were taken of Schu-attenuated and vaccine No.15-reduced, which possess residual virulence for laboratory animals and also immunogenic properties, as well as 3 holarctic avirulent cultures of strains No. 503-attenuated, No. 15-attenuated and 21/400, not pathogenic for white mice in a dose of 1 billion microbe cells and non-immunogenic. The weakened variants were obtained from the respective virulent and vaccine strains by attenuation under laboratory conditions.

The F. novicida strain, studied in detail by Olsuf'ev et al. /3/ was somewhat less pathogenic for laboratory animals (was not fatal to all experimental animals) than the virulent strains of the tularemia microbe.

Using Bouaven's method in Sipitsina's modification /5/ antigens were prepared from dry microbe mass of 72-hour cultures of these strains, degreased by increasing concentrations of acetone, on a medium of hydrolyzate of fresh fish with cystene and glucose. The obtained antigens were

reciprocated with alcohol from 0.1 n. acetic acid and dried in a vacuum extractor. All the preparations were, according to immunoelectrophoresis data, serologically homogeneous substances -- that is, they contained 1 positively charged component and formed 1 line of precipitation with homologous antiserums.

Sialic acid was revealed by Warren's thiobarbiturate method /11/ following hydrolysis of the antigen in 0.1 n. H_2SO_4 at 80^o for 1 hour. Spectrophotometry was carried out at 549 nm on an SF-4 instrument against unhydrolyzed antigen tests. The acid content was calculated using the standard curve for N-acetylneuraminic acid (produced by the Light Co.) and expressed as percentages of air-dried antigen preparation. To determine 2-desoxypentose the thiobarbiturate method was used with a reading at 532 nm, also the Dishe method in a modification suggested for DNA /1/, and calculated along the respective standard curves for 2-desoxyribose of the Reanal Co. The antibody neutralization reaction was used by us in the manner described by Meshcheryakova and Yemel'yanova /2/, with erythrocytary tularemia diagnosticum, Series No. 38.

It is known that during determination of Sialic acid with the Warren method it is possible to obtain a second absorption maximum at 532 nm at the expense of the 2-desoxyribose. In this case a correction must be made,

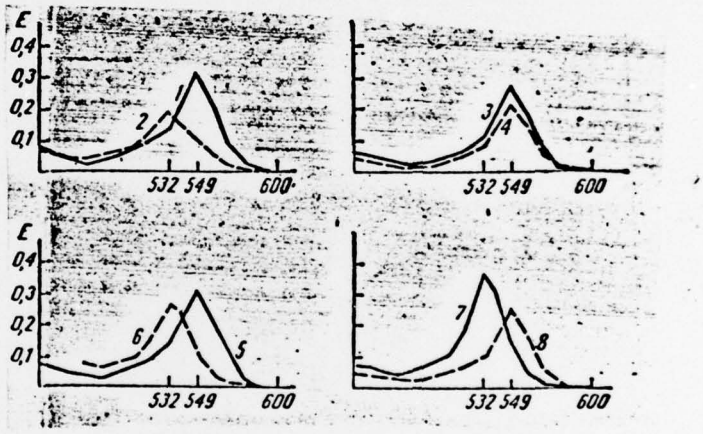
since the light absorption by this substance at 549 nm is significant.

The results obtained from spectral analysis of chromophores after processing antigen hydrolyzates with the Warren method, permitted the determination of a substantial difference between the virulent and vaccine strains of F. tularensis and F. novicida from the fully attenuated variants (Figure 1). The character of the absorption curves with distinct peak at 549 nm of the antigens of virulent strains of tularemia microbe No. 503/830 and Schu, vaccine No. 15-reduced and weakly attenuated Schu, and also F. novicida provided evidence of the presence in them of a quantity of Sialic acid. On the contrary, in antigens of fully avirulent strains of No. 503-attenuated, No. 15-attenuated and 21/400 there was demonstrated an absorption maximum at 532 nm, characteristic of 2-desoxyribose.

Since Warren's method is nonspecific with respect to 2-desoxyribose, it was decided to check these data by parallel determination in the antigens of 2-desoxypentose with diphenylamine, according to Dische. The mean arithmetic values of several determinations from 2 hydrolyzates of each antigen demonstrated (Table 1) that regardless of the method used, 2-desoxyribose was found only in the antigens of avirulent strains of the causative agent of tularemia, and its content did not exceed 0.5%.

Figure 1. Absorption spectra of sialic acid in Bouaven antigens of various strains of F. tularensis and F. novicida during determination with Warren's thiobarbiturate method.

1 - strain No.503 (500 mkg antigen); 2 - strain No.503-attenuated (1500 mkg antigen); 3 - strain Schu (500 mkg antigen); 4 - Schu-attenuated strain (1500 mkg antigen); 5 - strain No. 15-reduced (500 mkg antigen); 6 - strain No.15-attenuated (1500 mkg antigen); 7 - strain 21/400 (1500 mkg antigen); 8 - strain F. novicida (500 mkg antigen). Along the abscissa -- wave length in nm.



Since 2-desoxypentose and their compounds, aside from DNA, are met with fairly infrequently in nature, the data obtained indicate the presence of nucleic substances in the antigens. This conclusion was supported totally by the study of absorption spectra in the UV range of antigen

Table 1. 2-desoxyribose content in Bouaven antigens of F. tularensis and F. novicida (in % of air-dried preparatinns).

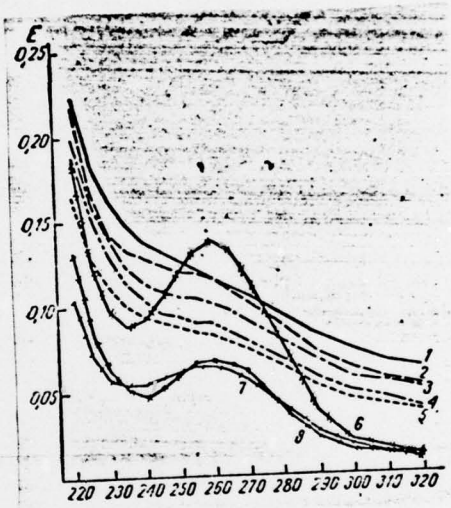
Type	Strain	2-desoxyribose content	
		Warren	Dische
F. tula-rensis	No.503/830	--	0
	Schu	--	0
	Schu-attenuated	--	0
	No.15-reduced	--	0
	No.503-attenuated	0.19 0.29	0.17 0.41
	No.21/400	0.40	0.27
F. novicida		--	0

NOTE: Method is not usable due to the absence of the absorptive maximum at 532 nm.

Table 2. Sialic acid content (in %) in Bouaven antigens of F. tularensis and F. novicida, recalculated to N-acetylneuraminic acid.

Type	Strain	N-acetylneuraminic acid content
F. tula-rensis	No.503/830	4.10
	Schu	3.41
	Schu-attenuated	2.63
	No.15-reduced	3.85
F. novicida		3.05

Figure 2. Absorption spectra of Bouaven antigens isolated from various strains of F. tularensis and F. novicida: Schu (1), novicida (2), No.15-reduced (3), No.503/830 (4), Schu-attenuated (5), No.15-attenuated (6), No. 21/400 (7) and No. 503-attenuated. Along the abscissa -- wave length in nm.



solutions (Figure 2). Specific complexes of avirulent strains No. 503-attenuated, No. 15-attenuated and 21/400, in contrast to other strains of F. tularensis and F. novicida possessed a distinctly expressed maximum at 255 to 265 nm, which is characteristic for nucleic acids. Our data are in complete accord with previously determined regular increase in the summary quantity of nucleic acids in the culture and antigen of avirulent strain No. 21/400, as compared to the virulent and vaccine strains of the causative agent of tularemia./6/.

On the basis of the research carried out, we were able to diminate Warren's recommended correction for 2-desoxyribose in subsequent quantitative determinations of Sialic acid in the antigens of virulent and vaccine strains of the tularemia microbe and F. novicida.

In addition to 2-desoxyribose, alpha-fucose and lipids serve to alter optical density at 532 and 549 nm. Of these, alpha-fucose was not found in antigens of the tularemia microbe, while the lipid content exceeded 40% in virulent strains, decreasing to 0.4% in the avirulent /4-6/.

In the literature we found no data on the composition of the lipid component of specific substances of the causative agent. However, taking into consideration that unsaturated fatty acids may affect substantially the result of calculating for Sialic acid, we utilized the processing of antigens from strains No. 503/830 and No. 21/400 with bromide, which Diringer /9/ recommends for materials with high lipid content. 5-10 mg antigen suspensions were dissolved in 1 ml chloroform to which were added drops of a 1% bromide solution in chloroform until a light yellow color was obtained. Then the samples were dried under a fan, re-dissolved in 1 ml chloroform and dried again, in order to eliminate excess bromide. At the same time this procedure was used with control suspensions of the antigen, to which bromide was not added. The dried

residue was dissolved in 1 ml 0.1 n. H_2SO_4, and Warren's method was used to reveal Sialic acid and 2-desoxyribose.

The processing of the preparations with bromide cause only an insignificant decrease in the determined amount of the indicated monosugars, as compared to the control samples: 4.06% as against 4.10% Sialic acid in the antigen from strain No.503/830, and 0.39% as against 0.40% 2-desoxyribose in the antigen from strain No. 21/400. On this basis we came to the conclusion that lipids of the tularemia microbe do not form a chromophore with the thiobarbiturate acid, while the slight decrease in monosugar output may be caused by incomplete elimination of bromide prior to hydrolysis. This experience permitted us to reject the correction for unsaturates fatty acids when determining Sialic acid.

The mean arithmetic data obtained during determination in 3-4 hydrolyzates of each antigen demonstrated (Table 2) that the Sialic acid content in the antigenic complexes of virulent and vaccine strains of F. tularensis and F. novicida varied within 2.63 - 4.10% and, evidently, depended to some extent on the virulence of the culture. This dependence is followed through when comparing genetically related strains. The virulent culture of the Schu strain showed 3.41% Sialic acid in the antigen, while an insignificant decrease in the virulence of the Schu-attenuated

strainled to a decrease of this component in polysaccharide to 2.63%. In the antigen of the highly attenuated variant of strain No. 503 Sialic acid was totally absent.

Despite the fact that the various derivatives of neuraminic acid were found in the composition of a number of heteropolysaccharides, there is no complete proof of their role as a specific part of somatic antigens. Thus, it was found that with the formation of antigenic factor 48 in Salmonella there is a close connection to biosynthesis of colomine (polyneuraminic) acid /12/. Similar relationships are found for the strain Citrobacter 5396/38 in chiasmic reaction with the O-antigen of Salmonella group 48 and in Arizona No.29/200 /10/. However, it is not impossible that neuraminic acid may derive from the admixed mucopolysaccharides that are found in these microorganisms /8/.

To determine the role of Sialic acid in the antigen of F. tularensis we staged a neutralization reaction of antibodies with the antigen of strain 503/830 in native state and after removing the Sialic acid from it as the result of hydrolysis of the preparation at 80° during 1 hour in 0.1 n. H_2SO_4 and subsequent dialysis against the physiological solution. The reaction yielded native antigen with a minimum quantity of 0.08 mkg/ml. As a result of the hydrolysis the serological activity of the antigen decreased, as manifested in a substantial increase of the minimum neutralizing dose (1.25 mkg/ml). However, this change in the sero-

logical activity of the preparation, evidently, cannot be connected only to the yield of Sialic acid.

Thus, increasing hydrolysis temperature to 100° caused an insignificant decrease in Sialic acid yield, as compared to that at 80° (3.56% as opposed to 4.10%). At the same time, this was accompanied by a sharp decrease in the activity of antigen hydrolizate, determined in the antibody neutralization reaction only at a concentration of 62.5 mkg/ml.

Based on the research carried out it may be possible that there exists a chemical bond between Sialic acid and other components in the polysaccharide of virulent and vaccine strains of the tularemia microbe, but this acid plays no significant role in the manifestation of specific properties of the antigens.

CONCLUSION

In the antigens of virulent and vaccine strains of F. tularensis 503/830, Schu, Schu-attenuated and No. 15-reduced, and also F. novicida, isolated with Bouaven's method, Sialic acid was found. In antigens of avirulent strains of No. 503-attenuated, No. 15-attenuates and No. 21/400 F. tularensis Sialic acid was absent, but in contrast to virulent strains they exposed 2-desoxyribose, at the expense of the presence of DNA.

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SIALIC ACID IN THE F. TULARENSIS AND F. NOVICIDA ANTIGENS

I. V. Rodionova

Sialic acid was revealed in the antigens of virulent (503/830 and Schu) and vaccine (Schu-attenuated and 15-reduced) strains of F. tularensis and also in the antigens of F. novicida isolated by the treatment with trichloracetic acid, by the thiobarbiturate method of Warren. Its content depended on the culture virulence. Sialic acid was absent in the antigens of avirulent strains of the causative agent of tularemia (503-attenuated, 15-attenuated and 21/400), but, in difference from the virulent strains, there was revealed 2-desoxyribose on account of the presence of DNA. Serological activity of F. tularensis antigen decreased only 15 times in the antibody neutralization reaction after a complete release of sialic acid as a result of hydrolysis of the preparation in 0.1 N, H_2SO_4 at 80° C for one hour, i. e. it persisted at a sufficiently high level. On the basis of investigations carried out it can be admitted that there existed a chemical association between the sialic acid and other composites in the polysaccharide of the virulent and the vaccine strains of F. tularensis, but that this acid played no significant role in the manifestation of specific properties by the antigen.