its content did not exceed 0.5-1% of the quantity of this antigen in the brain extract. A similar situation was observed by other workers when studying other neurospecific proteins (S-100, 14-32) [4, 6, 7]. The discovery of NSP in other organs and tissues can evidently be explained by the innervation of these organs.

Previously the writers noted species specificity of NSP 10-40-4: Antibodies obtained against NSP 10-40-4 from human brain give a distinct precipitation zone in agar gel with human and guenon brain extracts, a weaker zone with bovine, dog, and rabbit brain extracts, and a negative reaction with rat and guinea pig brain extracts [1].

The use of solid-phase immunoenzyme analysis enabled quantitative estimation of the degree of crossing of the antigenic determinants of NSP 10-40-4 for different species of animals. The percentage of crossing was determined from dilution curves of human and animal brain extracts during activation of the plate by antibodies against NSP 10-40-4 from human brain [3]. The results of assay are given in Table 2. Reactivity of human brain extract was taken at 100%. It will be clear from Table 2 that the reactivity of brain extracts of different species of mammals relative to antibodies against NSP 10-40-4 from human brain differs considerably.

A method of immunoenzyme assay of neurospecific antigen 10-40-4 has thus been developed, by means of which its concentration in human brain and other organs has been measured and the degree of crossing of the antigenic determinants of NSP 10-40-4 for different species of mammals has been determined.

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## DETERMINATION OF ANTIBODIES AGAINST STREPTOCOCCUS GROUP A POLYSACCHARIDE IN HUMAN SERUM BY AN IMMUNOENZYME METHOD


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Previous investigations revealed a high level of antibodies against *Streptococcus* group A polysaccharide (A polysaccharide) in acute rheumatic fever and other diseases caused by a streptococcus of this group [1, 6]. Antibodies against polysaccharide of streptococcus of group A-variant, containing a determinant common for different groups of polysaccharides were found in the same sera, but at a lower level, by radioimmunoassay [6]. A determinant common for A polysaccharide and polysaccharide of group L *Streptococcus* (L polysaccharide) also was found [3, 8]. Antibodies against this determinant could not be found in human sera by immunodiffusion in gel [1], but they were not studied by more sensitive method.

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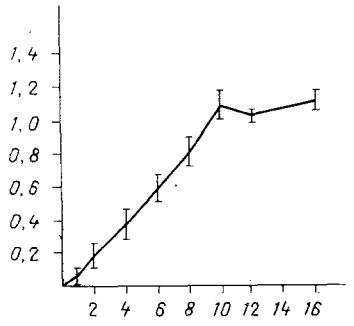


Fig. 1

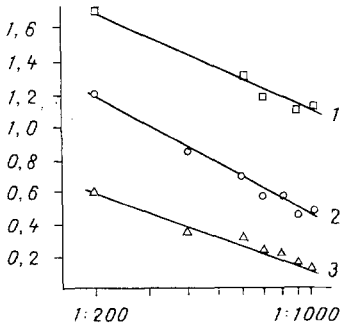


Fig. 2

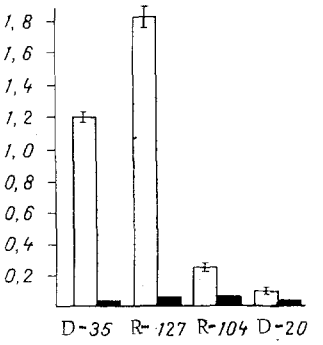


Fig. 3

Fig. 1. Determination of antibodies against A polysaccharide in serum D-35 (1:800) depending on concentration of polysaccharide applied to solid-phase carrier. Abscissa, concentration of A polysaccharide (in μg/ml); ordinate (here and in Figs. 2 and 3) optical density at 492 nm (in optical units).

Fig. 2. Determination of antibodies against A polysaccharide in human sera. Abscissa, dilutions of sera (log); 1) serum D-35, 2) serum D-15, 3) serum D-20.

Fig. 3. Determination of antibodies against A polysaccharide in human sera before and after inhibition with A polysaccharide. Abscissa, no. of sera; unshaded columns — reaction of sera before inhibition, black columns — reaction of sera after inhibition with A polysaccharide.

Discovery of a high level of antibodies against the specific determinant of a polysaccharide in human sera could be a reliable indicator of infection due to group A *Streptococcus*. Determination of antibodies against A polysaccharide is interesting also in connection with the existence of crossed reactions of A polysaccharide with antigen from epithelial cells of human and animal thymus and skin [4, 11].

The absence of a standard method of determination of antibodies against A polysaccharide in human sera makes it difficult to compare results obtained by different workers. A disadvantage of the highly specific immunodiffusion in gel method, when used for this purpose, is its relatively low sensitivity. The use of the passive hemagglutination test is complicated by the necessity for preliminary chemical modification of the A polysaccharide [7]. The quantitative and highly sensitive radioimmunoassay is complicated and for that reasons has not achieved widespread use.

The aim of this investigation was to develop a standard, highly sensitive and specific quantitative immunoenzyme method of determining antibodies against A polysaccharide in human blood serum.

Polystyrene panels containing 96 wells were obtained from the Moscow Experimental Factory, and horseradish peroxidase (HRP), with the designation Rz 3.0, was from Olaine, Latvia. The substrate for the enzyme was o-phenylenediamine (o-PDA) (Soyuzkhimreaktiv, USSR). Altogether 77 sera from patients with diseases of streptococcal etiology (erysipelas) and 53 sera from clinically healthy blood donors were used. The total immunoglobulin fraction was obtained from the sera of rabbits immunized with human IgG by precipitation with ammonium sulfate [9]. A conjugate of immunoglobulins with HRP was prepared by the periodate method [12]. A purified preparation of A polysaccharide was isolated from formamide extracts of whole microbial cells (strain J17A4) [5].

Into each well of the panel 200 μl of a solution of A polysaccharide in 0.01 M phosphate buffer, pH 7.4, made up in 0.15 M NaCl (PB) was added. After incubation for 18 h at 20°C the panels were rinsed with PB containing 0.1% Triton X-100 (PET). Next, 200 μl of the sera in dilutions made up in PBT was added to each well and the panel was incubated for 1 h at 37°C [13]. The wells were rinsed with PBT and filled with a solution of conjugate to human immunoglobulins in optimal concentrations, made up in PBT with 0.1% bovine serum albumin. Incubation was carried out for 1 h at 37°C, the wells were rinsed with PBT, and a solution of o-PDA in 0.1 M citrate-phosphate buffer, pH 5.6, with 0.02% $H_2O_2$ was added. The reaction was stopped after 30 min by addition of 50 μl of 8N $H_2SO_4$ and optical absorption was recorded on a "Titerteck" photometer.

The following controls were set up on each panel: 1) substrate control: A polysaccharide + o-PDA; 2) conjugate control: A polysaccharide + conjugate + o-PDA. The mean value of four parallel determinations made on one panel, with serum in a dilution of 1:800, was used as the index of optical density (D) at 492 nm for each serum. The mean value of the conjugate control was subtracted from the result. Control determination of antibodies for positively reacting standard serum (StS) was carried out on each panel. The content of antibodies against A polysaccharide in the sera was expressed in conventional optical units, obtained by multiplying the mean values of optical density obtained for each serum in optical units by a coefficient K: D (in conventional optical units) = D (in optical units) × K. The ratio of the constant value of 1.42 to the average index of optical density of the standard serum (DStS) on each panel, was taken as the conversion factor: $K = \frac{1.42}{D\ StS}$

In competitive inhibition experiments sera in a dilution of 1:800 were mixed with A polysaccharide in a concentration of 1.25 μg/ml and incubated for 1 h at 37°C, after which they were used for immunoenzyme assay of the antibodies parallel with uninhibited serum.

In the double immunodiffusion in gel test [2], to determine antibodies against A polysaccharide undiluted sera and A polysaccharide in concentrations of between 6.0 and 1.5 μg/ml were used.

All sera used for determination of antibodies by immunoenzyme assay were tested beforehand by double immunodiffusion in agar gel with A polysaccharide. Both active and inactive sera were found in this way. One of them, reacting in the precipitation test (PT) in gel with 60 μg/ml of A polysaccharide (D-35) was used for selecting the optimal concentration A polysaccharide required for binding with the solid-phase carrier in the immunoenzyme test. For this purpose antibodies against A polysaccharide were determined in this serum in dilutions of 1:200 to 1:6400, with concentrations of A polysaccharide from 1 to 16 μg/ml. The results of determination of antibodies against A polysaccharide in serum D-35 in a dilution of 1:800 with different doses of A polysaccharide are shown in Fig. 1. If the A polysaccharide concentration exceeded 10 μg/ml, there was no appreciable increase in the intensity of the reaction, and accordingly in subsequent experiments A polysaccharide was used in this concentration. In several experiments antibodies against A polysaccharide were determined by the immunoenzyme method in sera in dilutions of 1:200-1:1000. Plotting optical density against dilution of sera between semilogarithmic coordinates showed that the relationship between them was linear (Fig. 2). These results are evidence of a proportional change in the values of optical density depending on antibody concentration. This means that the antibody concentration in the sera can be estimated from the intensity of reactions observed at one of the above dilutions [13].

A dilution of 1:800, at which recordable values of optical density (not above 2.0) for sera with the highest concentration of antibodies against A polysaccharide were obtained, was chosen for testing. The level of antibodies in the selected sera was determined on the basis of positive results obtained in the immunodiffusion in gel test with high doses of A polysaccharide.

When the sera were tested by the immunoenzyme method four parallel determinations were made on each panel. Deviations from the mean value of optical density on one panel amounted to 0.06-0.25 optical unit for each serum. During analysis of the same sera on different panels, deviation from mean values of optical density in some cases exceeded 0.5 optical unit, which made it difficult to determine the antibody level in the sera. These deviations are evidently attributable to differences in the ability of the panels to bind antigen [10]. Subsequent investigations showed that reproducible results can be obtained by introducing a coefficient, calculated on the basis of the results of testing standard serum with a known content of antibodies against A polysaccharide (see above). Serum D-35, reacting in PT in gel with high doses of A polysaccharide, was chosen as the standard. On repeated assay of antibodies in 130 sera, with the use of the coefficient, results with a mean deviation of ±0.062 conventional optical unit were obtained in 97% of cases.

The specificity of the reactions observed was shown in experiments with competitive inhibition of positively reacting sera by A polysaccharide. Data on optical absorption of the sera when tested by the immunoenzyme method after inhibition by A polysaccharide demonstrate virtually total abolition of observable reactions (Fig. 3).

Comparison of activity of the sera tested by two methods showed correlation between positive reactions in the immunodiffusion in gel test and high values obtained during testing

by the immunoenzyme method. No serum with an antibody titer against A polysaccharide below 0.7 conventional optical unit reacted in the immunodiffusion in gel test. All sera forming a visible precipitate with A polysaccharide in PT had optical density values of between 0.7 and 1.8 conventional optical unit during antibody determination by the immunoenzyme method. Meanwhile 32% of sera with high optical density values gave negative results in the immunodiffusion test. Thus the immunoenzyme method enabled a lower level of antibodies against A polysaccharide to be determined, as well as, evidently, nonprecipitating antibodies, not detectable by the immunodiffusion test in gel.

Antibodies against A polysaccharide were found by the immunoenzyme method in virtually all human sera tested, including those from healthy blood donors. Considerable differences were observed in the level of detectable antibodies in different sera. The data given agree with those obtained previously by radioactive labeling [6] and are evidence of the high sensivity of the suggested method.

A highly sensitive, specific, and standard method of quantitative determination of antibodies against A polysaccharide in human sera has thus been developed. By means of this method, and also by inhibition with polysaccharides of streptococci of different groups, it will be possible in the future to discover what level of antibodies against the specific determinant of A polysaccharide is an indicator of infection caused by group A *Streptococcus*. If these investigations are undertaken, it will evidently be possible in future to recommend this method for the diagnosis of rheumatic fever and of other diseases connected with infection by group A *Streptococcus*.

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