lary diarrhea induced by enterotoxigenic strains of *E. coli*, and also for the elaboration of methods of prevention and treatment of colibacillosis. Mice of the BALB/c Sto genotype are the most sensitive model in this case.

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HEMOBACTERIAL AGGLUTINATION: A METHOD OF DETERMINING ANTIERYTHROCYTIC ANTIBODIES

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The development of a method of detecting antierythrocytic autoantibodies which is simple in use and more sensitive than the Coombs' test, now widely used in immunohematology, is important as a means of making the diagnosis of autoimmune hemolytic anemia (AIHA) more effective, and for use in isoserologic practice and related fields. A positive direct Coombs' test is observed in most patients with AIHA [10]. However, this test in some such patients is negative at the height of the disease because of destruction at this period of the erythrocytes on which most of the antibodies are fixed, whereas the quantity of antibodies on cells remaining undestroyed during the period of crisis is insufficient for detection by the Coombs' test [5]. The test may be negative in chronic cases of the disease, pursuing a sluggish course, when too few autoantibodies likewise are present on the surface of the erythrocytes [4]. A similar situation may arise in the case of incompatible blood transfusion, and also of pregnancy incompatible with respect to erythrocytic antigens.

Attempts to increase the sensitivity of determination of antierythrocytic antibodies has frequently been made [8, 9]. Among the suggested methods, one of the most sensitive is the aggregate-hemagglutination test [1]. However, it is relatively laborious and the agglutination obtained is fine-grained.

A test for antierythrocytic antibodies is suggested in this paper and its sensitivity is compared with that of the Coombs' test. Coagglutination of erythrocytes sensitized *in vivo*

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or *in vitro*, and of *Staphylococcus aureus* cells is used in this technique, and it is therefore called hemobacterial agglutination.

A protein, known as protein A, which is able to bind immunoglobulins through their Fc-fragments, is covalently bound with the cell wall of the staphylococcus. Since the active center of the antibodies remains accessible and preserves its specificity under these circumstances, addition of antigen to staphylococcal cells sensitized by antibodies induces their agglutination. This particular feature of the staphylococcus has often been used and, in particular, for serotyping bacteria (they are agglutinated in the presence of staphylococcus covered with antibacterial antibodies) [7], to detect antibodies against mycobacteria (erythrocytes loaded with myobacterial antigens are agglutinated in the presence of staphylococci sensitized with antimycobacterial serum) [6], and to determine phytoviruses in the virobacterial agglutination test (cells of *Staph. aureus* covered with antiviral serum are agglutinated in the presence of virus particles) [2, 3].

If cells, for example human erythrocytes to be tested for antierythrocytic antibodies, are sensitized with antibodies the Fc-fragment of these antibodies must be accessible for protein A present on the surface of the staphylococcal cell wall. This situation was utilized when developing the suggested method of detection of antierythrocytic antibodies. Staphylococci not sensitized by antibodies were used in the work.

For this reason it was possible to avoid nonspecific agglutination — the main handicap characteristic of techniques in which staphylococci are brought into contact with whole sera or sera taken in low dilutions.

Staph. aureus strain Cowan I, carrying large quantities of protein A on the cell wall, was grown in Petri dishes on agarized Hottinger's medium (produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology) until a continuous lawn of growth of the culture was obtained after 15-18 h at 37°C. The biomass was washed off with buffered physiological saline (BPS): 0.14 M NaCl in 0.008 M phsophate buffer, pH 7.2-7.4, which was used at all subsequent stages of the work. The staphylococcal cells were washed 3 times in BPS at room temperature for 5 min each time at 5000 rpm; a 5-10% suspension was prepared in BPS with 0.05% merthiolate, and it was used in the freshly prepared form or kept at 4°C in the freeze-dried form. For freeze drying a 5% bacterial suspension was prepared in 10% sucrose solution as cryoprotector, with 0.05% merthiolate in distilled water. The suspension was frozen to −50°C for 3 h. Freeze-drying was carried out for 22-24 h on an OE-950 apparatus (Hungary). Immediately before use the preparation was centrifuged twice or 3 times under the same conditions with 6-10 volumes of BPS relative to the original volume of suspension, to remove the cryoprotector. The bacterial suspension, both freshly prepared and resuspended in BPS after freeze-drying to 5% concentration, must be completely homogeneous. Heterogeneity of the conditions of culture or freeze-drying and indicates that the preparation is unsuitable for diagnostic purposes.

Rabbit antisera against total human globulins (titer not below 1:16 in the agar precipitation test against antigen in a concentration of 1 mg/ml) were incubated to remove heteroagglutinins for 1 h at room temperature and 1 h at 4°C with erythrocytes of groups O(I), A(II),and B(III) (1 ml of serum to 0.3 ml of packed erythrocytes of each group).

Human antirhesus sera were obtained from volunteers immunized with O(I) Rh^+ erythrocytes at the Central Blood Transfusion Station of the Ministry of Communications. Freshly prepared erythrocytes also were obtained there from donors and used in model experiments and to exhaust antisera from heteroantibodies.

The following conditions were chosen for the hemobacterial agglutination reaction in which erythrocytes coated with antierythrocytic antibodies were investigated: erythrocytes of a patient or erythrocytes from a donor, sensitized with antibodies in the model experiments (see below). The erythrocytes were washed 3 times with BPS to remove unbound protein, and incubated for 30 min at 37°C with rabbit antiglobulin serum (in a dilution giving the greatest sensitivity and clarity of agglutination in the Coombs' test) in the ratio of 1:3 by volume. After incubation the erythrocytes were washed 3 times with BPS. Equal volumes of a 20% suspension of erythrocytes and a 5% bacterial suspension were mixed on a smooth white fat-free tile (1 drop of bacterial suspension was added to 1 drop of erythrocytes and the material mixed with a glass rod). After incubation for 3-5 min at room temperature the reaction mixture must be added up to a concentration suitable for visual reading of agglutination (for example, 5 drops of BPS must be added to 2 drops of the mixture for this purpose). The result was read

visually in the course of 10 min, depending on the presence or absence of agglutination. Distinct agglutination in large granules is taken as a positive result.

The following controls were used. The staphylococcal suspension was mixed with: 1) washed, unsensitized erythrocytes of all groups; 2) erythrocytes of all groups, both Rh^- and Rh^+, incubated with antiglobulin serum, and then washed to remove unbound protein.

The comparative sensitivity of the method was tested in model experiments. For this purpose a series of specimens of erythrocytes from a group O(I) Rh^+ donor was first sensitized with increasing double dilutions of antirhesus serum for 30 min at 37°C (in the ratio of 1:3), after which the erythrocytes were washed 3 times with BPS and used in the hemobacterial agglutination test according to the formula given above, with parallel determination of the titer of antirhesus sera in the Coombs' test and in the suggested test.

As model experiments showed, the sensitivity of the suggested method of hemobacterial agglutination is 16-32 times greater than the sensitivity of the Coombs' test (when a mixture of rabbit antiglobulin sera was used in a titer of 1:16). Agglutination was very clear, in the form of large clumps. In all the controls, the test was negative.

Incidentally, the use of different batches of antiglobulin sera of the same titers does not guarantee identical sensitivity. The reason for this is the unequal affinity of Fc-fragments of different classes and subclasses of immunoglobulins for protein A, and for that reason, of a series of antiglobulin rabbit sera it is evidently that in which antibodies are present mainly in a class with high affinity for protein A will give maximal sensitivity, i.e., IgG and not IgM. That is why, if different rabbit antiglobulin sera are available, it is best to select the one which gives maximal sensitivity of determination of antirhesus antibodies in the model experiment (see above). In this way sensitivity significantly higher than that indicated above may be obtained.

It was shown that the Coombs' antiglobulin method can be replaced by a "nonglobulin" version of the suggested method. Antierythrocytic antibodies to patients' erythrocytes in the overwhelming majority of cases are of the IgG class, and for that reason addition of staphylococcal cells to such erythrocytes causes them to agglutinate. The technique of this version is similar to that of the "globulin" version described above, the only difference being that the stage of sensitization of erythrocytes with rabbit antiglobulin serum is omitted in it. In the model experiments, clear specific agglutination took place during the first minute. The sensitivity of the nonglobulin version was the same as the sensitivity of the Coombs' test.

The specificity of the two versions of the hemobacterial agglutination method was tested by investigating erythrocytes from 78 blood donors and 23 patients with various forms of anemias of nonimmune genesis (agglutination absent). The method gave positive results with 103 patients with AIHA in whom the Coombs' test was negative. The diagnosis thus made was confirmed clinically. The possibility of early diagnosis of hemolytic anemia in cases of incompatiblity of fetus and mother for Rhesus-factor also was demonstrated.

The results of the present investigation show that hemobacterial agglutination is a promising method for use in immunohematology. The method is quick and sensitive, gives distinct and massive agglutination, it is asy to perform and does not require the use of hyperimmune sera.

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