# Increase in the Haemolytic Activity of Anti-Erythrocyte Antibodies by Anti-Gamma-Globulin Serum

I. ŘÍHA

Department of Immunology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague 4

With technical assistance of L. Jiroutová

*Received May 18, 1964*

ABSTRACT

It appears that anti-gamma-globulin sera not only affect the haemagglutinating reaction but can also be employed for detecting antibodies at the erythrocyte surface through the haemolytic reaction. The observed increase in the haemagglutinating as well as haemolytic reaction brought about by anti-gamma-globulin sera is due mainly to the effect on the reaction of 7 S anti-erythrocyte antibodies, the reaction of macroglobulin antibodies being little affected.

---

Serological practice uses the so-called Coombs test for detecting incomplete antibodies. It consists in applying the corresponding anti-gamma-globulin serum which reacts with substances bound at the erythrocyte surface (by virtue of a specific bond with antigens located there) this bringing about agglutination of the erythrocytes (Kabat 1956). In the experiments described here we have modified the conventional Coombs test so that the reaction of the anti-gamma-globulin serum with antibodies bound at erythrocyte surface was detected by the haemolytic reaction in the presence of complement. It was shown that the addition of anti-gamma-globulin serum and of complement to a system of anti-erythrocyte serum and erythrocytes increases the haemolytic titre of these anti-erythrocyte sera. It was therefore attempted to establish the nature of the antibodies responsible for the increase of the haemolytic effect.

## MATERIALS AND METHODS

Rabbit hyperimmune serum against sheep erythrocytes was separated on Sephadex G-200 into a fraction containing macroglobulin antibodies, a fraction containing 7S antibodies and finally, a transitional fraction containing both types. The anti-gamma-globulin sera were obtained by immunizing pigs with rabbit gamma-globulin; in the experiments a serum containing only anti-gamma-globulin antibodies and another with a high titre of anti-gamma$_{1M}$-globulin antibodies were used. This last serum was partially absorbed by a mixture of serum proteins without gamma$_{1M}$-globulin and thus the level of antibodies against 7S gamma-globulin was considerably decreased.

Haemagglutination and haemolysis were carried out in test-tubes. The anti-erythrocyte serum and fractions tested were diluted in ascending series, mixed with a suspension of erythrocytes and incubated for 2 hours at 37° C. The erythrocytes were then centrifuged, washed and anti-gamma-globulin serum added. The anti-globulin sera were diluted 1 : 50 and added in 0.5 ml. volumes to washed erythrocytes. During the haemagglutination reaction the erythrocytes were kept in a refrigerator and haemagglutination values read after 20 hours. For the haemolytic reaction sensitized erythro-

cytes were incubated with anti-gamma-globulin serum for 2 hours at 37° C, complement added and the values of haemolysis read after 60 min.

## RESULTS AND DISCUSSION

As may be seen in Fig. 1 the addition of either of the anti-gamma-globulin sera to a system of erythrocytes sensitized

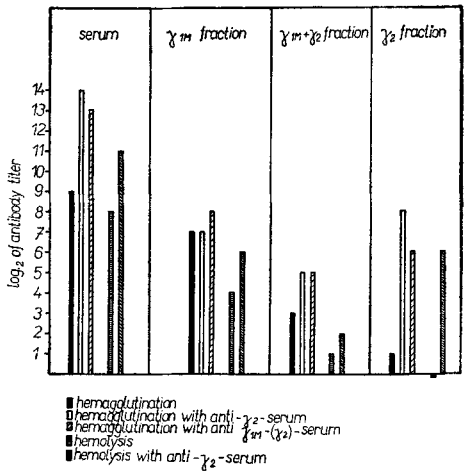


Fig. 1. Increase of haemagglutinating and haemolytic activity of antisheep erythrocyte serum and its $\gamma_{1M}$ and $\gamma_2$ fractions by anti-gamma-globulin sera.

with anti-erythrocyte serum was reflected in an increase of the haemagglutination titre (16 times with the anti-7S serum) and the addition of the anti-7S serum increases the haemolytic activity 8 times as compared with the control. In investigating the fractions it was shown that antibodies of the macroglobulin type are little influenced by adding anti-gamma-globulin sera either during the haemagglutinating or haemolytic reaction. Unfortunately the anti-gamma$_{1M}$ serum could not be used for the haemolytic reaction on account of its very high anti-complementary effect. The transitional fraction containing both types of antibodies yielded results similar to those with macroglobulin antibodies indicating that mainly gamma$_{1M}$ antibodies were present. The most pronounced effect was found with anti-erythrocyte antibodies of the 7S type where both sera increased the haemagglutinating activity, the anti-7S serum being more effective. The activity of the anti-gamma$_{1M}$ serum was apparently due to residues of nonabsorbed anti-7S antibodies. In testing the effect of the anti-gamma 7S serum on the haemolytic activity of this fraction a very substantial influence was observed, the haemolytic activity having been raised more than 64 times as compared with the control.

The lower efficiency of the anti-gamma$_{1M}$ antibodies against erythrocytes sensitized with gamma$_{1M}$ antibodies is most probably due to the fact that the macroglobulin antibodies possess a very high serological activity so that, for example, for haemagglutinating the erythrocytes only a few dozen molecules of the antibody per erythrocyte are required while with the 7S antibodies a value of 20,000 molecules per erythrocyte was found (Greenbury *et al.* 1963). For this reason it is not to be expected that the binding of the 7S molecules of anti-gamma-globulin antibodies could substantially affect this reaction of macroglobulin anti-erythrocyte antibodies.

## References

Kabat, A. E.: *Blood group substances.* Acad. Press, N. Y. 1956.

Greenbury, C. L., Moore, D. H.: Nuun, L. A. C.: *Reaction of 7S and 19 S components of immune rabbit antisera with human group A and AB red cells.* Immunology 6 : 421, 1963.

## ПОВЫШЕНИЕ ГЕМОЛИТИЧЕСКОГО ДЕЙСТВИЯ АНТИ-ЭРИТРОЦИТАРНЫХ АНТИТЕЛ С ПОМОЩЬЮ АНТИ-ГАММА-ГЛОБУЛИНОВОЙ СЫВОРОТКИ

*И. Ржига*

По данным поставленных опытов, анти-гаммаглобулиновые сыворотки не

только оказывают влияние на реакцию гемагглютинации, но и могут быть использованы для обнаружения антител на поверхности эритроцитов на основании реакции гемолиза. Это усиление реакции гемагглютинации и гемолитической реакции под влиянием антигаммаглобулиновых сывороток вызывается их действием на реакцию 7S антиэритроцитарных антител. На реакцию макроглобулиновых антител они не оказывают действия.