INHIBITION OF ANTIBODY ACTIVITY DURING REPEATED BLEEDINGS OF A PHYSIOLOGICAL ORDER OF MAGNITUDE

M. V. Zemskov and N. V. Zhuravleva

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Experiments on rabbits and albino mice showed that a factor suppressing the activity of natural and postvaccinal macromolecular antibodies is present in the body, and that its formation is induced by hyperproduction of γ-macroglobulins. The inhibiting factor is connected with the albumin fraction of the serum proteins and it is detected in liver extracts and the blood serum at the end of a cycle of five consecutive bleedings at intervals of five days. No increase in the fibrinolytic activity of the blood was observed under these conditions.

Bleedings to the extent of 0.5-0.7% of the body weight has a twofold action on the agglutinating activity of the blood serum. Five successive bleedings at intervals of 5 days initially increased the level of the serum agglutinins (after two blood losses), but later depress it (after 4-5 blood losses), and, finally, their level is raised again (36-42 days after the beginning of the experiments) [5]. The first increase in the level of natural dysentery agglutinins (against Shigella flexneri) in the blood serum was found to correlate with the principal features of antibody formation [4]. However, the mechanism of the decrease in antibody titers in the blood serum before their second increase remained unexplained.

The object of the investigation described below was to study the connection between this phenomenon and the basic indices of antibody formation and to examine the inhibiting properties of blood serum.

EXPERIMENTAL METHOD

Experiments were carried out on 108 noninbred rabbits weighing 2.5-3.5 kg and on 353 albino mice. The cycles of bleeding consisted of five withdrawals of blood from the marginal vein in a dose of 0.5% of the body weight at intervals of 5 days. The level of natural dysentry agglutinins (against S. flexneri), the blood serum protein fractions (by electrophoresis on paper; six fractions), the plasma-cell reaction of the lymph glands and spleen [3], and the content of antibodies in extracts of these organs were investigated in these animals. The preventive properties of the blood sera, the resistance of the animals to infection, the immunological response to injection of antigen, and the reaction to passive immunization with diagnostic rabbit unadsorbed antibacterial sera (Shigella flexneri, Salmonella typhi, Brucella), and also the effect of the sera of the experimental animals on the agglutinating properties of the above-mentioned antibacterial sera, were also investigated. The molecular properties of the antibodies of the experimental animals were studied by the test with cysteine [6, 8, 10] and by gel-filtration through Sephadex G-200, followed by immunological investigation of the resulting fractions (by the agglutination test). The numerical results were analyzed statistically by the method of confidence intervals, and the significance of the difference was determined from Student's tables and by means of the χ^2 criterion [2].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that an increase in the antibody concentration by the 11th day of the experiment (after two blood losses) was followed by a sharp decrease in their titers by the 21st day (the 5th

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TABLE 1. Indices of Antibody Formation during Bleeding

Day of investigation after beginning of expt.	Titer of agglutinins	γ-globulins (in g %)	Plasma blasts / plasma cells	Titer of agglutins in lymph gland glands	Preventive properties of blood serum (χ^2)
1st	1:98 (183–53)	0,68±0,16	68±39 / 13±9	1:105 (214–52)	—
11th	1:349 (621–196)	0,92±0,2	321±114 / 52±44	1:135 (402–45)	11,8
P	0,01	0,05	0,001	0,1	0,001
21st	1:74 (89–62)	1,03±0,2	237±138 / 63±33	1:297 (695–127)	0,4
P	0,1	0,01	0,002	0,05	0,5
37th	1:579 (1148–292)	0,73±0,16	133±54 / 44±18	1:160 (395–65)	7,4
P	0,001	0,5	0,05		0,01

blood loss). Nevertheless, the level of γ-globulin continued to rise, and the number of plasma cells and the concentration of antibodies in the lymph glands of the animals were high. The preventive properties of the blood sera were reduced by comparison with the previous period of the investigation and were almost indistinguishable from the control (before the beginning of the experiments). The same features were also characteristic of the resistance of the albino mice to infection with Shigella flexneri at different stages of the bleeding by the scheme described above.

Injection of dysentery monovaccine into 8 rabbits and 60 albino mice at the end of the cycle of bleeding caused no marked increase in the serum agglutinins compared with the control, although the concentration of γ-globulins in the experimental animals rose. The index of increase in the antibodies was 3.4 in the experimental seriex and 27 in the control (the ratio between the titers on the 5th day after immunization and their initial levels). The index of increase in γ-globulins was 1.4 in the experimental series and 1.1 in the control.

Passive immunization of the experimental animals on the 21st day after the beginning of bleeding did not cause an increase in the agglutinating titers of their blood sera, whereas in the control group of animals the level of specific antibodies rose sharply within 2 h after intravenous injection, and remained high subsequently ($P = 0.002$) for 48 h.

In the experiments in vitro to study the effect of the blood sera of the experimental animals on the agglutinating activity of antibacterial unadsorbed diagnostic sera the results were as follows. Preliminary contact between the antibacterial sera and the blood sera of the experimental animals obtained at the end of the cycle of bleeding sharply reduced the titer of the agglutination test with the specific diagnostic serum ($P = 0.001$) and the intensity of agglutination determined by Sergiev's method ($P = 0.01$). The blood sera of the control and experimental animals, taken at different times, has no such effect on suppression of agglutination. Investigations of the fibrinolytic activity of the blood of 30 rabbits by the methods of Copley [9] and Adel'son [1] showed that the reduced avidity of the antibodies thus demonstrated was not related to the fibrinolytic properties of the blood: no increase in fibrinolytic activity was observed at these times of the experiment.

Fractionation of the blood sera of the experimental and control animals by electrophoresis on paper and subsequent elution of the fractions by the methods of Ravich-Scherbo and Prokopenko [7] revealed inhibitory properties only in the albumin fractions from the experimental rabbits. The α_1- and α_2-globulin fractions were inactive. The β- and γ-globulin fractions, on the other hand, intensified the agglutination reaction of the antibacterial serum with the specific diagnostic serum: the titer up to 145%, and the intensity with a level of significance of $P = 0.02$, which can probably be explained by summation of the natural antibodies of the fraction and of the diagnostic serum. It was also shown that only extracts from the liver of the experimental animals killed at the end of the bleeding cycle, but not from the controls, could suppress the avidity of the antibodies. Extracts from the lymphoid organs, kidneys, and lungs either caused no change in or increased the agglutinating properties of the diagnostic sera. Investigation of the molecular properties of the antibodies by the cysteine method and also by gel-filtration through Sephadex G-200 showed that only macromolecular antibodies were sensitive to this inhibitory effect.

It can be concluded from these results that bleeding stimulates the formation of macroglobulin antibodies, the avidity of which is largely dependent on the state of the blood serum. The character of this state is determined by the inhibitory properties of the albumin fraction of the serum proteins, induced by an increase in the concentration of serum γ-macroglobulin. The agglutinating properties and preventive properties of the blood serum meanwhile are reduced. When the concentrations of these proteins in the blood serum are reduced (Table 1), the avidity of the natural antibodies is manifested fully and the blood sera lose their inhibitory qualities. The titers and intensity of the agglutination reaction in the blood serum and also its preventive properties are once again increased.

The correlation between inhibition of antibodies and the change in level of the serum M immunoglobulins, the plasma-cell reaction of the lymphoid system, and the concentration of antibodies in extracts of the lymphoid organs all suggest a definite role of the inhibitory factor in homeostasis. This appears all the more likely because the effect of inhibition of 19S-antibodies is seen not only after repeated measured blood losses, but also, as the writers' subsequent experiments showed, during immunization by repeated small doses of bacterial antigen.

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