THE DIRECT COOMBS TEST AFTER BLOOD LOSS IN DOGS

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Ya. G. Uzhanskii [2,3] showed experimentally that the decrease in the erythrocyte count in the posthemorrhagic period does not correspond to the degree of dilution of the blood by the tissue fluid. This fact was interpreted as evidence of stimulation of erythrodiaeresis after blood loss.

The authors' earlier investigations on dogs [5] revealed a shortening of the half-life of the animals' own erythrocytes, labeled with radioactive chromium, after an unreplaced acute blood loss.

The mechanism of the posthemorrhagic fragmentation of the red blood cells after acute blood loss has not been studied. Data in the literature show that immunological mechanisms may be concerned in posthemorrhagic erythrodiaeresis. For instance, when the direct and indirect Coombs tests are used, positive results are found within the first hours after blood loss [4]. In other investigations [6], a positive result in the direct Coombs test was not obtained until the 3rd day after acute blood loss. In these experiments, the authors used a layer of blood cells rich in reticulocytes in the reaction.

The object of the present investigation was to study the agglutinating properties of the blood cells of dogs after severe and unreplaced blood loss.

EXPERIMENTAL METHOD

Experiments were conducted on dogs weighing from 8 to 22 kg. Acute blood loss was produced by withdrawing blood rapidly from the femoral artery in a volume of 35-40 ml/kg body weight (40-45% of the circulating blood volume). Blood samples for investigation were taken before bleeding, and 2 h and 1, 3, 5, 7, 10, 14, 17, 19, 21, 24, and 27-32 days after taking the blood. One of the dogs (No. 6) was bled repeatedly at intervals of 7 days (between the 1st and 2nd bleedings) and 14 days (between the 2nd and 3rd). The direct and indirect Coombs tests were used in the investigations. Antiglobulin was obtained from rabbits immunized with dogs' serum. Before use, in order to remove heteroagglutinins, the immune sera were absorbed by a mixture of dogs' erythrocytes which had been washed ten times. The heteroimmune sera thus obtained contained precipitins in a titer of 1:2000. In the indirect Coombs test their activity in relation to two isoimmune sera obtained by repeated blood transfusions corresponded to a titer of 1:64. The isoimmune sera were prepared by cross transfusion, using 20 ml of whole, unstabilized blood, at intervals of 14 days for 4 months. Two weeks after the last transfusion, blood was taken for obtaining the isoimmune serum. The indirect Coombs test was performed by incubating the erythrocytes with the test serum for 1 h at 37°. Next, to a drop of a 5% suspension of the erythrocytes on a flat surface, a drop of antiglobulin serum was added. The results were read macro- and microscopically.

During the experiments with the animals' blood after acute blood loss it was noticed that, after remaining for a short time at room temperature, the samples separated into three clearly defined layers. Because of this, in the subsequent investigations the direct test was performed with the cells of these layers. The top layer, a mixture of platelets and leukocytes, proved to be unsuitable for investigations of this type because of the ability of the cells to undergo nonspecific aggregation.

In the direct Coombs test tubes containing the blood samples for investigation, diluted in 3.5% sodium citrate solution in the proportion of 3 ml stabilizer to 7 ml blood, were kept at room temperature for 1 h. The top layer, consisting of a suspension of platelets and leukocytes, was drawn off and discarded. The suspension of erythrocytes and reticulocytes (the 2nd layer), and also the residue, consisting mainly of erythrocytes, were used in the direct test. Blood cells were washed three times before being used in the test. The controls were blood samples from dogs Nos. 1, 3, 11, 12, and 15, which were not bled.

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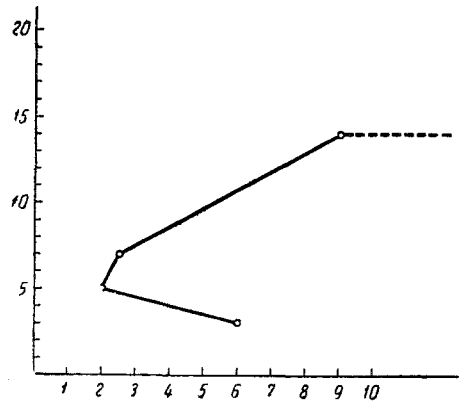

Dynamics of results of the direct Coombs test on dog No. 17. Along the axis of abscissas – time of appearance of agglutination after addition of antiglobulin (in min); along the axis of ordinates – days after bleeding.

Results of the Direct Coombs Test on Dogs at Various Times after Blood Loss

Dog No.	Before blood loss	Time after blood loss: hours 2	days 1	3	5	7	10	14	17	19	21	22	27–32
6	—	+	+	+	+	+	—						
8	—			+	+		+		+	+		+	—
9	—			+	+		+		+	+		+	—
10	—			+	+		+		—	—		—	—
13	—	—	+	+	+	+	+	+	—		—		
14	—	—	+	+	+	+	+	+	+	—	+		—
16	—	—	—	+	+	+		+	+		—		
17	—	—	—	+	+	+		+	—		—		

Note: The blood samples from dog No. 6 were investigated after repeated blood loss (at an interval of 7 days).

EXPERIMENTAL RESULTS

At all the time intervals after acute blood loss that investigations were made, the serum of the dogs gave negative results in the indirect Coombs test both with the erythrocytes of the same animal and with those of the healthy control dogs. Different results were obtained in the direct Coombs test. In all the experimental dogs the blood cells of the second layer, obtained after separation of the suspension into layers, gave agglutination on the addition of the antiglobulin serum. The results of the control investigations showed that these cells were incapable of agglutinating in the presence of normal rabbit serum (antiglobulin control) and also of physiological saline. These results showed that agglutination was due to the presence of a protein, which reacted with antiglobulin, on the surface of the active layer of the cells.

The results of the investigations are given in the table.

It is interesting that positive results in the direct Coombs test were observed with the dogs 1-3 days after bleeding. In dog No. 6, a positive result in the direct test was observed 2 h after bleeding. This was evidently due to repetition of the bleeding 7 days later, during which period the direct Coombs test still remained positive.

The results of the direct Coombs test on the dogs remained positive for almost 2-4 weeks. The intensity of the positive results of this test did not reach a maximum at once (see figure). When the result of the direct Coombs test subsequently became positive, during the first days it was not clearly defined. It reached its maximal intensity at the end of the first week (5th-7th day) after bleeding. An equally slow decrease in the degree of agglutinability of the cells was observed as the interval of time after bleeding grew longer. Concurrently with the disappearance of the positive Coombs test, the blood lost its property of separating into cell layers.

The results described above show that acute blood loss leads to positive results of the direct Coombs test in experimental animals. The mechanism of origin of this phenomenon after acute, severe blood loss is uncertain.

The results of the indirect Coombs test showed that in all the experimental animals, at all the time intervals investigated, freely circulating antibodies were absent. This conclusion was confirmed by the results of the direct Coombs test carried out on the blood samples. It is difficult to accept that freely circulating antibodies were responsible for the positive results of the direct Coombs test, for in that case all the blood cells of the experimental animal would be free to undergo agglutination in the presence of antiglobulin serum. In the authors' opinion, the ability of some of the cells to agglutinate under the influence of antiglobulin serum may be explained in two ways. Possibly these cells were loaded with antibodies while they were still in the stage of maturation in the hemopoietic tissue. In this case, sensitization may be regarded as a distinctive type of morphogenetic autosensitization [1]. Another possible explanation is that some cells, especially if young, distributed by the body in large numbers after acute blood loss, may contain on their surface or may adsorb a certain protein, which behaves like a plasma antigen and can be agglutinated by antiglobulin.

In future investigations, it is proposed to examine the mechanism of the increased agglutinability of some of the blood cells appearing in animals after acute blood loss.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
