CHANGES IN SUBMICROSCOPIC ORGANIZATION OF ERYTHROCYTES AFTER ACUTE BLOOD LOSS IN DOGS

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UDC 616-005.1-092.9-07: 616.155.1-076.4

After blood loss amounting to 35-40% of the circulating blood volume in dogs, changes (anisocytosis, poikilocytosis) were found in the ultrastructure of the erythrocytes in the circulating blood on the 1st day, while by the 5th-7th day the distribution of hemoglobin in the erthrocytes was disturbed and they developed a state of prehemolysis or hemolysis. Similar changes arise in normal erythrocytes when incubated with serum obtained from dogs after blood loss.

In the posthermorrhagic period breakdown of erythrocytes of the experimental animal is increased [6]. Investigations with labeled erythrocytes have shown an increase in accidental destruction of erythrocytes during the first 24 h, shortening of their half-life period, and an increase in hemolytic activity of the serum [2-4, 7, 8, 11, 14]. However, these changes are characteristic of severe, unreplaced blood losses (35-40% of the curculating blood volume – CBV). Loss of 10 and 20% of the CBV is followed by only a slight increase in accidental destruction of erythrocytes in the first 24 h without any decrease in their half-life period [3].

Changes in ultrastructure of the erythrocytes in dogs during the first ten days after blood losses of different extent were studied in this investigation.

EXPERIMENTAL METHOD

In nine experiments on 13 dogs acute blood loss was produced by rapid removal of blood from the femoral artery. The CBV was determined before bleeding by means of the dye T-1824. In one group of experiments the ultrastructure of erythrocytes of the peripheral blood was studied after blood loss amounting to 10, 20, and 40% of the CBV; in the other group, the ultrastructure of erythrocytes preliminarily incubated with posthemorrhagic serum (blood loss of 40% of the CBV) was investigated.

In the first case blood for investigation was taken in solution 7B (Central Institute of Hematology and and Blood Transfusion) before the experiment and 1, 5-6, 7, and 9-10 days after blood loss. The tube was placed in a vessel containing ice.

In the experiments in which dogs' erythrocytes were incubated, the dogs were bled, and their blood serum was obtained 1, 5, 7, and 9-10 days later. Erythrocytes of healthy dogs (0.3-0.5 ml) were placed in tubes with normal (control) and posthemorrhagic serum (2 ml) and left in a refrigerator at 4°C for 16-18 h.

Blood samples were fixed for 45-80 min in 1% osmium tetroxide solution, diluted with isotonic buffer (pH 7.2-7.4). The erythrocytes were washed, then dehydrated in alcohols, and embedded in a butyl methacrylate mixture. Benzoyl peroxide was used as catalyst. Polymerization took place at 55-60°. Ultrathin sections were cut with a glass knife on an LKB Ultratome. Sections were studied and erythrocytes counted (300-400) in the JEM-7 electron microscope with an acceleration voltage of 80 kV.

Laboratories of Cytology and Pathophysiology, Central Institute of Hematology and Blood Transfusion, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 9, pp. 106-109, September, 1970. Original article submitted February 9, 1970.

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TABLE 1. Destruction of Normal and Anemic Erythrocytes of Dogs Observed under the Electron Microscope

Dog. no.	Volume of blood loss (% of CBV)	Number (in %) of circulating blood erythrocytes with modified ultrastructure					Dog. No.	Volume of blood loss (% of CBV)	Number (in %) of erythrocytes with modified ultrastructure after incubation with serum				
		before blood loss	after blood loss (days)						normal	posthemorrhagic after blood loss (days)			
			1	5—6	7	9—10				1	5	7	9—10
1	16	4,0	5,0	5,5	4,0	4,5	7	42	4,0	3,0	13,0	32,0	9,0
2	15	4,0	5,0	7,0	4,0	4,5	8	33	3,0	4,0	10,0	6,0	4,0
3	25	3,0	3,0	9,0	5,0	3,0	9	35	3,0	2,0	9,0	27,0	12,0
4	40	2,8	6,8	21,8	4,2	3,0							
5	37	3,0	2,0	9,0	27,0	8,0							
6	38	2,0	3,0	8,0	34,0	10,0							

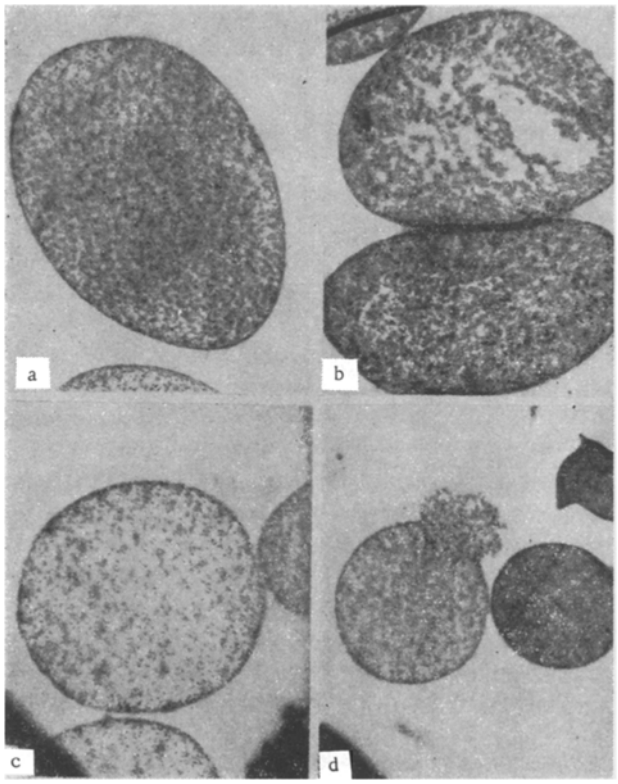

Fig. 1. Ultrathin sections of dogs' erythrocytes under normal conditions and after acute blood loss: a) normal erythrocyte (8500×); b) aggregation of single granules, coarsening of granular structure, thickening of cell membrane (9000×); c) cavities inside cell, commencing hemolysis (10,000×); d) rupture of membrane, contents of erythrocyte liberated into surrounding medium (hemolysis) (4900×).

EXPERIMENTAL RESULTS

Electron-microscopic investigation (Table 1, Fig. 1) showed that circulating blood erythrocytes of the dogs before the experiment in most cases (96-98%) were of uniform structure, with a high degree of osmiophilia. The contents of the cell, which can be identified with hemoglobin, were of a finely granular structure (Fig. 1a). Granules (50-80 Å in diameter) were distributed uniformly in the cell. The erythrocyte membrane could be distinguished as a dense band at the periphery of the cell, about 100-150 Å in thickness. Similar descriptions of the submicroscopic structure of erythrocytes are given in the literature [5, 11, 12, 13].

However, besides the cells described above there were other erythrocytes whose submicroscopic structure was slightly modified. The internal contents of these cells were less dense, and evidently because of aggregation of individual granules, coarsening of their granular structure was observed. The cell membrane also was thickened, reaching in some case 180-250 Å (Fig. 1b). Other erythrocytes were observed with cavities inside the cell, indicating commencing hemolysis (Fig. 1c). In a few cases the membrane was ruptured and the contents of the erythrocyte liberated into the surrounding medium (Fig. 1d).

The number of erythrocytes with the changes described above amounted to 4% in the control series (Table 1). Their presence can apparently be attributed to the "age" of the cell, i. e., to different stages of its ontogenetic development. A similar picture is observed in specimens of erythrocytes incubated with normal dog serum.

No special features were found in the submicroscopic structure of the erythrocytes 24 h after blood loss and also after incubation with posthermorrhagic serum obtained at this time. The number of modified cells did not exceed 2-6.8%. However, it must be emphasized that at these times (after a blood loss of 35-40% of the CBV) considerable anisocytosis and poikilocytosis were found in both groups of experiments, indicating commencing changes in the erythrocytes or in their surrounding medium.

Later (5th-10th days) in the posthemorrhagic period, in experiments with small and medium blood losses (dogs Nos. 1-3) no disturbances of the submicroscopic structure of the erythrocytes were found. Not until the 5th day was there a small increase (by 2-3 ×) in the percentage of modified cells.

In severe blood loss (dogs Nos. 4-6), on the 5th-7th day there was a marked increase in the number of modified erythrocytes: the percentage of those destroyed at these times reached 21-34. The uniform distribution of hemoglobin was clearly disturbed, many erythrocytes were in a prehemolytic state, and others were completely hemolyzed. Similar results were obtained with erythrocytes incubated with posthemorrhagic sera (dogs Nos. 7-9).

By the 9th day of severe blood loss (dogs Nos. 5 and 6) the percentage of modified erythrocytes was 3-5 times greater than in the control. The ultrastructure of most cells was still indistinguishable from that observed initially (before blood loss).

Analysis of the results of a study of the submicroscopic organization of erythrocytes in the course of the posthemorrhagic period suggests that during the first 24 h after severe, unreplaced blood loss changes develop in the erythron system. These changes are manifested by the appearance of anisocytosis and poikilocytosis. The absence of any marked destruction of erythrocytes at this time can evidently be explained by the depot phenomenon observed after blood loss [8] and by the increased ability of the spleen to selectively eliminate damaged erythrocytes [1, 9, 15, 16]. Destruction of erythrocytes reached a maximum on the 5th-7th day after blood loss.

The discovery of corresponding changes in normal erythrocytes when incubated in vitro with posthemorrhagic serum indicates that if such serum is taken starting from the 1st day after blood loss and until the 5th-7th day, it possesses hemolytic activity.

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