HEMOLYTIC FACTOR IN POSTHEMORRHAGIC ANEMIA IN DOGS

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In acute blood loss the blood serum, its dialysate, and acetone extracts of the spleen acquire hemolytic activity. Saline extracts of the spleen do not possess hemolytic activity.

After acute unreplaced blood loss in animals, some of the animal's own erythrocytes are destroyed [2, 6-10]. According to some workers, the storage of erythrocytes in the spleen or their passage through it contributes toward their more rapid destruction [11, 14]. The writer's investigations on splenectomized dogs confirmed the role of the spleen in posthemorrhagic erythrodieresis [4]. According to some reports, posthemorrhagic serum, 10 min after blood loss, acquires hemolytic properties, possibly because of the entry of hemolytically active substances from the spleen into the blood. After blood loss the extracts of many organs, especially the spleen, in fact possess hemolytic properties if they are incubated in vitro with erythrocytes [1, 3, 13].

The object of the present investigation was to study the hemolytic activity of normal and posthemorrhagic sera and also of extracts of the spleen following severe, unreplaced blood loss. Attempts were also made to obtain a dialysate of posthemorrhagic serum and to study its hemolytic properties.

EXPERIMENTAL METHOD

Experiments were carried out on 84 dogs. Serum for testing was taken before and also 2 h, and 1, 3, 5, 7, and 10 days after blood loss. Saline and acetone extracts of spleens were obtained by the method of Gostev and Petryashina [5]. Spleens of healthy dogs (control) and of dogs sacrificed 1, 3, 5, 7, and 10 days after blood loss were used in the experiments.

Dialysates of the spleens were obtained before the experiment and 1, 3, and 5 days after blood loss. Dialysis in cellophane was carried out against distilled water (25 ml serum and 125 ml water) at 4°C for 3 days. The dialysates were lyophilized at the Experimental Production Laboratory of Blood Preparations and Components and the Laboratory for Lyophilization of Biological Preparations, Central Institute of Hematology and Blood Transfusion. From the resulting powder a 1% solution was prepared. The solvent was 0.85% NaCl solution.

The hemolytic activity of the serum, extracts of spleens, and dialysates were studied by Dacie's method [12]. Erythrocytes of donor dogs, labeled with radioactive chromium [8], were incubated with normal and posthemorrhagic sera, extracts of spleens, and solutions of dialysates for 16-18 h at 4°C. Radioactivity was counted by means of a scintillation apparatus. The radioactivity of the mixture was taken as 100%, and the degree of hemolysis of the erythrocytes was calculated from the radioactivity of the supernatant as a percentage.

RESULTS

It is clear from Table 1 that following incubation with normal serum for 24 h only 1% of erythrocytes was destroyed. The hemolytic activity of the serum increased 24 h after blood loss and remained at about

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TABLE 1. Hemolytic Activity of Blood Serum, Its Dialysate, and Extracts of Spleens before and after Blood Loss in Dogs (M ± m)

Material tested	No. of expts.	Hemolytic activity, %					
		before blood loss	time after blood loss, days				
			1	3	5	6	10
Plasma	15	1,4±0,2	3,3±0,6	3,4±0,6	3,1±0,4	2,4±0,5	0,9±0,1
	P		<0,01	<0,01	<0,001	=0,05	>0,2
Dialysate	19	1,7±0,2	2,1±0,2	3,8±0,5	11,9±1,1	—	—
	P		>0,1	<0,001	<0,001		
Saline extract of spleen	46	1,3±0,2	1,3±0,2	1,3±0,3	1,0±0,3	1,0±0,2	—
	P		>0,5	>0,5	>0,2	>0,2	
Acetone extract of spleen	46	1,7±0,1	4,0±0,6	5,4±0,5	4,8±0,4	2,4±0,8	1,4±0,2
	P		<0,01	<0,001	<0,001	>0,2	>0,1

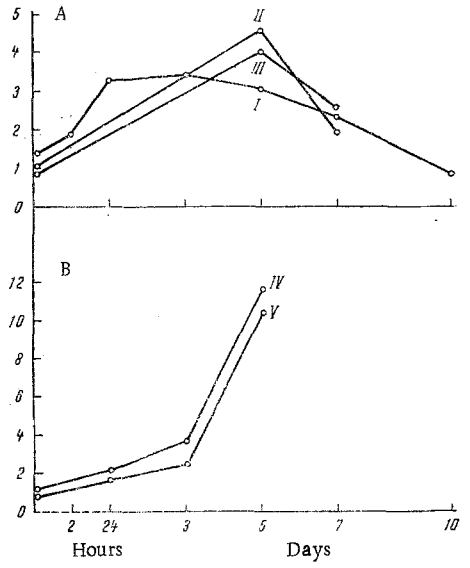

Fig. 1. Hemolytic activity of blood serum (A) and dialysate (B) of serum following blood loss in dogs. Abscissa, days and hours of observation; ordinate, hemolytic activity (in percent); A) hemolytic activity of serum; I-II) native serum; III) serum after heating; IV) native dialysate; V) dialysate after heating.

the same level for the next 3-5 days, returning to normal by the seventh day. The hemolytic properties of posthemorrhagic sera also were maintained after heating to 70° for 30 min (Fig. 1A); this suggests that the posthemorrhagic erythrodieresis is not immunologic in nature.

The study of dialysates of the sera showed that 3 days after blood loss the hemolytic activity of the dialysate had increased, and it reached a maximum by the fifth day. Boiling the dialysate did not reduce its activity (Fig. 1B). Hence, the hemolytically active substance is thermostable.

Posthemorrhagic saline extracts of the spleen did not destroy erythrocytes so intensively as extracts of normal spleen. In acetone extracts of the spleen, on the other hand, the hemolytic activity increased after blood loss, especially between the third and fifth days.

Hence, after blood loss hemolytically active substances enter the blood stream of dogs. The greatest activity of the serum and its dialysate is observed on the third-fifth days after blood loss. Meanwhile acetone extracts of posthemorrhagic spleens acquire hemolytic activity. Saline extracts do not possess such activity, and this may perhaps indicate the lipid nature of the hemolytic factor. The possibility is not ruled out that the hemolytic factors of the blood and spleen are identical. In the serum they are responsible for intravascular hemolysis, while in the spleen they have a local action on erythrocytes, some of which are stored there after blood loss.

LITERATURE CITED

1. V. N. Benevolenskii, Uspekhi Sovr. Biol., 50, 3 (6), 310 (1960).
2. A. A. Bogomolets, Selected Works [in Russian], Vol. 3, Kiev (1958), p. 143.
3. G. I. Bokov, Dokl. Akad. Nauk SSSR, 115, No. 2, 418 (1957).
4. N. A. Gorbunova, Pat. Fiziol., No. 5, 63 (1965).
5. V. S. Gostev and M. N. Petryashina, Biokhimiya, No. 1, 29 (1952).
6. Ya. G. Uzhanskii, Role of Destruction of Erythrocytes in the Mechanism of Blood Regeneration [in Russian], Leningrad (1949).
7. Ya. G. Uzhanskii, I. V. Pavlova, T. G. Mordovskii, et al., Pat. Fiziol., No. 4, 52 (1960).
8. N. A. Fedorov and N. A. Gorbunova, Pat. Fiziol., No. 6, 65 (1963).
9. N. I. Berlin and C. Gotz, Proc. Soc. Exp. Biol. (New York), 78, 788 (1951).

10. N. I. Berlin, T. A. Waldmann, and S. M. Weisman, Physiol. Rev. 39, 577 (1959).
11. A. J. Bowdler, Clin. Sci., 23, 181 (1962).
12. J. V. Dacie, J. Path. Bact., 52, 331 (1941).
13. J. I. Magalini, W. Blumenthal, and M. Stefanini, in: Proceedings of the Sixth International Congress of the International Society of Hematology, New York (1958), p. 451.
14. T. A. J. Prankerd, Quart. J. Med., 29, 199 (1960).