RED CELL STROMA IN DOGS

Stroma Protein and Stroma Lipides Vary in Different Types of Anemia*

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The first paper from this laboratory dealing with red cell stroma (5) was concerned largely with methods as applied to red cells in normal dogs and in anemic dogs. Various method difficulties and controls were recorded. *Simple anemia* due to blood loss caused an increase in red cell stroma protein. As the anemia decreased and the red cell hematocrit values rose to normal levels, the stroma protein fell to normal values.

This paper deals with a variety of anemias—hemorrhage anemia (by blood depletion), hemolytic anemia, and anemia with hypoproteinemia. The hemolytic anemia showed an unexpected large increase in stroma protein. Anemia with hypoproteinemia seemed not to modify the expected anemic response—a rise in stroma protein. Multiple abscesses produced by turpentine injection sometimes decreased the stroma protein levels associated with anemia.

Methods

The isolation, preparation, and fractionation of red cell stroma have been described in detail elsewhere (5) as also the method for hemoglobin analysis (2). Plasma protein analyses were made by the microKjeldahl technique.

In brief: stroma is prepared from washed red cells by laking and agglutination of stroma with acetate buffer. The stroma suspension, following standing in the ice box for 24 hours, is washed repeatedly in the centrifuge at ice box temperatures. Lipide determination involves several extractions with alcohol-ether mixture by refluxing in hot water. The final extraction employs a mixture of methanol and chloroform. The extracted residue is used for total protein analysis. Total phospholipids were estimated by the method of Fiske and Subbarow (1).

The dogs referred to in all experiments belong to the anemia colony and represent a mixture of Bull Terrier and Dalmatian. All were kept under uniform conditions and, save for the induced experimental states appeared normal. The *hemorrhage anemia* was produced by frequent and long continued blood removal (12 to 15 weeks.) This procedure exhausted the reserve stores of hemoglobin precursors. The desired hemoglobin level of approximately 6 to 7 gm. per cent was maintained by further smaller bleedings. A basal diet of salmon bread was found to aid in maintaining this anemia level (2).

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Diets.—In all experiments other than those with hypoproteinemia, the basal ration consisted of salmon bread, a small amount of skim milk (Klim), and canned salmon (2). The diet of the hypoproteinemic animals consisted of a protein-free biscuit prepared with corn starch, dextrin, sugar, minerals, vitamins, and fat (3).

Turpentine abscesses (Table IV) were produced by subcutaneous injection of 1 cc. turpentine on back and shoulders. The abscess formed within 3 days and maximum pus accumulation to the amount of about 50 to 60 cc. usually occurred on the 4th day. If the abscess did not drain spontaneously, a small stab wound was made to permit proper drainage. On the day of drainage, a second turpentine injection was given and after the resulting abscess had run a similar course two more were produced in succession.

Anemia and hypoproteinemia (Table II) were produced in some dogs by superimposing a hypoproteinemia upon the already existing hemorrhage anemia by feeding an almost protein-free diet. In other experiments both conditions were produced by heavy bleeding plus a very low protein diet from the start of the experiment.

Phenylhydrazine[1] *anemia* was produced by daily subcutaneous injections of "Acetphenylhydrazide" in doses beginning with 25 mg. and increasing to 150 to 200 mg. The phenylhydrazine was dissolved in warm salt solution. Frequent sampling determined the hemoglobin level. No unfavorable reactions other than red cell destruction resulted from the injections.

EXPERIMENTAL OBSERVATIONS

Most of the experimental data are given in the form of tables. Except where note is made to the contrary, all dogs in the anemia series had a clinically normal aspect; appetite was good and there was no significant weight loss.

Table I shows the normal *control* figures for the stroma fractions, hemoglobin levels, and plasma protein per cent in circulation. These figures are reasonably uniform and establish the physiological variations in these standard dogs. In *anemia by blood depletion* the increase in stroma protein is obvious and represents an average rise of 75 per cent above normal. There are small increases in total lipide and phospholipide fractions.

When a fairly severe grade of *hypoproteinemia* was added to the hemorrhage anemia we noted only insignificant changes in the stroma protein (Table II). In the last 2 experiments (Table II, dogs 46-9 and 49-2) when the anemia and hypoproteinemia were *produced together* in the normal dog, we recorded significant increases in the total stroma lipide, when the plasma protein levels showed a severe hypoproteinemia. It was difficult to hold a plasma protein level below 4 gm. per cent for long periods as appetite was impaired and the clinical condition deteriorated. The explanation for this rise in total lipide is not at hand and merits further study.

Weight loss was observed in all experiments involving hypoproteinemia. This was due at least in part to the very low protein diet. In certain experiments (Table II, dogs 50-62 and 47-26) there was extreme weight loss which was due in part to low food consumption. These 2 dogs had been bled so heavily that in spite of transfusions they did not recover. No visceral abnormalities were found at autopsy.

[1] Phenylhydrazine was obtained from Eastman Kodak. $CH_3CONHNHC_6H_5$. Molecular weight 150.18.

Hemolytic anemia (phenylhydrazine) showed some striking changes in the stroma proteins. From normal levels of 8 to 9 mg. per cc. red cells the increase went to levels of 26 to 38 mg. per cc. red cells—a three- or fourfold increase.

TABLE I

Stroma Fractions

Normal and Anemic (Blood Removal)

Normal stroma fractions—mg. per 1 cc. red cells				Hemoglobin	Plasma protein
Dog No.	Total protein	Total lipide	Total phospholipide		
				gm. per cent	*gm. per cent*
46-7	7.9	5.7	2.2	14.1	6.4
46-7	8.7	6.3	2.6	17.4	6.4
46-10	8.9	6.9	2.4	—	—
46-5	8.8	4.6	2.4	17.8	6.4
46-12	8.5	4.6	2.7	14.1	6.8
46-7	8.2	5.9	2.4	14.2	6.5
46-9	8.8	5.3	2.3	13.8	6.0
46-10	9.2	6.6	2.3	14.3	6.6
Average..........	8.6	5.7	2.4		
Stroma fractions during anemia—mg. per 1 cc. red cells				**Hemoglobin**	**Plasma protein**
Dog No.	**Total protein**	**Total lipide**	**Total phospholipide**		
				gm. per cent	*gm. per cent*
46-7	13.2	7.3	3.4	6.9	6.6
46-7	14.5	6.6	3.2	5.1	6.1
46-7	16.1	7.3	3.5	4.8	6.1
46-12	16.6	6.5	3.5	6.2	5.7
46-5	14.1	8.0	3.7	6.5	6.8
46-10	17.6	8.9	2.8	6.1	6.1
46-10	16.2	8.5	2.8	6.6	6.6
50-62	16.3	7.2	3.7	4.3	6.5
50-62	14.8	7.8	3.6	6.6	6.7
45-2	12.8	6.8	3.6	6.5	5.0
46-5	15.0	7.9	3.9	6.9	6.8
Average..........	15.2	7.5	3.4		

In general, the more severe the anemia, the higher were the stroma protein levels (Table III).

The total lipide of the stroma sometimes showed increases with the increase in the hemolysis (dogs 45-2 and 50-73), but the reaction was not uniform, nor was it observed in the first 2 experiments in Table III.

Turpentine *abscesses* when added to the hemorrhage anemia seem to cause a slight decrease in the stroma protein as compared to the simple anemia levels

TABLE II

Stroma Fractions

Anemia and Hypoproteinemia

Dog No.	Anemia and hypoproteinemia		Stroma fractions mg. per 1 cc. red cells			Hemoglobin	Plasma protein	Weight
		Wks.	Total protein	Total lipide	Total Phospholipide			
						gm. per cent	*gm. per cent*	*kg.*
50-62	Anemia, hemorrhage	0	15.6	7.5	3.7	5.5	6.6	20.7
	Anemia and hypoproteinemia	18	17.0	7.5	3.7	6.2	4.4	13.7
	Anemia and hypoproteinemia	24	17.2	6.9	4.1	4.4	3.8	14.4
47-26	Anemia and hypoproteinemia	9	9.8	6.1	2.9	9.4	4.9	18.8
	Anemia and hypoproteinemia	20	14.5	7.0	3.8	6.1	4.7	15.7
	Anemia and hypoproteinemia	22	14.8	8.9	4.3	6.3	4.3	12.5
45-2	Anemia, hemorrhage	0	12.8	6.8	3.6	6.5	5.0	17.6
	Anemia and hypoproteinemia	1	13.1	8.5	2.6	7.9	4.0	16.5
	Anemia and hypoproteinemia	2½	12.6	9.3	2.8	8.0	4.6	16.1
46-9	*Normal* control	0	8.8	5.3	1.9	19.3	5.8	19.4
	Anemia and hypoproteniemia	23	13.8	10.7	2.3	7.0	4.6	18.0
	Anemia and hypoproteinemia	28	16.6	11.2	2.3	4.3	3.8	15.4
49-42	*Normal* control	0	9.5	10.6	2.1	12.4	7.6	19.8
	Anemia and hypoproteinemia	7	13.5	9.6	2.2	8.3	5.6	17.2
	Anemia and hypoproteinemia	18	16.6	12.9	2.5	4.8	3.6	15.1

TABLE III

Stroma Fractions

Phenylhydrazine Anemia

Dog No.	Anemia		Stroma fractions mg. per 1 cc. red cells			Hemoglobin	Plasma protein
		Wks.	Total protein	Total lipide	Total phospholipide		
						gm. per cent	*gm. per cent*
46-9	Normal average	0	8.7	5.4	1.9	14.4	5.9
	Anemia progressing	6	26.3	5.7	2.5	9.1	6.3
	Anemia level	11	26.3	4.6	2.4	6.6	7.0
46-7	Normal average	0	9.1	5.1	2.2	14.1	6.5
	Anemia progressing	3	18.5	5.0	2.5	10.8	6.3
	Anemia level	5	19.0	9.4	2.7	7.4	6.4
	Anemia level	6	22.8	6.2	2.8	5.4	7.0
	Anemia level	10	29.0	6.3	3.1	5.4	6.6
45-2	Normal average	0	8.7	5.5	2.4	19.7	7.0
	Anemia progressing	4	16.3	11.5	2.4	10.9	7.2
	Anemia level	13	21.0	11.1	2.4	6.9	6.5
	Severe anemia level	17	33.7	13.4	2.8	3.6	6.3
50-73	Normal average	0	8.1	8.2	2.1	17.5	6.5
	Anemia progressing	12	22.2	11.9	2.7	9.0	5.6
	Anemia level	13	38.0	12.7	3.4	5.2	6.5

(Table IV). The reaction to these abscesses was uniform—fever, leucocytosis, localized abscess with abundant pus formation. Release of the pus returned these abnormalities promptly to normal. Then a second abscess was produced. The total period of 16 to 17 days (4 successive abscesses in each dog) did

TABLE IV

Stroma Fractions

Turpentine Abscess—Anemia, Hemorrhage

Dog No.	Normal and anemia	stroma fractions mg. mg. per 1 cc. red cells			Hemoglobin	Plasma protein	Weight
		Total protein	Total lipide	total Phospholipide			
					gm. per cent	*gm. per cent*	*kg.*
46-5	*Normal* control	8.8	4.6	2.4	17.8	6.4	20.0
	4th abscess draining	9.1	5.5	2.4	12.4	6.8	19.8
	2 wks. after complete healing	8.6	5.5	2.5	13.7	6.3	19.7
	Anemic	14.5	8.0	3.0	6.6	6.2	19.7
	4th abscess draining	9.6	7.1	2.9	7.4	5.5	19.5
	2 wks. after complete healing	10.9	5.8	3.1	6.1	7.0	20.0
46-10	*Normal* control	8.9	6.9	2.4	15.7	7.0	19.8
	4th abscess draining	9.0	5.9	2.6	12.6	6.0	19.3
	2 wks. after complete healing	9.2	6.6	2.3	14.4	6.6	19.9
	Anemic	16.2	8.5	2.8	6.6	6.6	19.7
	4th abscess draining	12.6	5.1	2.4	8.1	6.6	18.9
	2 wks. after complete healing	13.2	6.1	2.9	7.6	6.3	18.3
46-12	*Normal* control	8.5	4.6	2.7	14.1	6.8	19.3
	4th abscess draining	8.6	5.8	2.3	14.4	6.4	17.8
	2 wks. after complete healing	8.5	5.0	2.3	17.8	6.3	18.9
	Anemic	16.6	6.5	3.5	6.2	5.7	17.6
	4th abscess draining	15.9	7.7	3.8	4.4	6.4	18.6
	2 wks. after complete healing	17.3	6.5	2.9	5.5	6.7	18.4

not make the dog clinically very ill, but did accelerate the nitrogen metabolism, with large increase in urinary nitrogen, and sometimes caused slight weight loss. Infection did impair the production of hemoglobin and red cells (4) and this reaction was probably responsible for the slight decrease in the stroma protein. The lipides showed no response to the abscesses.

Chloroform poisoning in dogs has been much studied and it may cause a good deal of disturbance in the levels of plasma globulins, especially fibrinogen and various other factors in coagulation. The essential lesion in chloroform poisoning in dogs is liver injury which may be so extensive as to cause death owing to lack of liver function.

Occasional observations on red cell stroma fractions have been made in severe and mild chloroform poisoning. No significant changes in stroma protein or stroma lipides have been noted in the affected dogs.

DISCUSSION

These experiments show clearly that the production of *new red cell stroma and of hemoglobin* has a high priority in the total body demands upon reserve protein stores. When these protein stores are depleted by continuing hypoproteinemia plus anemia and the dog regenerates great numbers of red cells, stroma protein and hemoglobin are no less abundant than in simple anemia without hypoproteinemia. There may be some increase in total *lipide* of the stroma (Table II) in very severe hypoproteinemia, but the cause of this change is not understood. Likewise an increase in total lipide in the stroma may be associated with severe hemolytic anemia (phenylhydrazine). This response is found in some dogs with great *surplus* of red cell material derived from continuing hemolysis. With hypoproteinemia on the contrary, the *reserve* of stroma and hemoglobin building material is greatly *decreased*, yet high values for total stroma lipides are observed. This seems a contradiction but probably means that the reserve stores are not the important factor.

Perhaps it is surprising that multiple *abscesses* cause so little change in stroma protein and hemoglobin production. Certainly the nitrogen metabolism is greatly disturbed and a large excess of nitrogen appears in the urine. Also the fever and leucocytosis are obvious. Abscesses and related tissue injury may slow up the production of red cells and hemoglobin (4). All of this disturbance, however, causes only a slight decrease in the stroma protein anemic level (Table IV). There is no significant change in the lipides of the stroma.

SUMMARY

Normal red blood cells in dogs contain *stroma* in fairly uniform amounts. This red cell stroma is rich in proteins and lipides.

Anemia due to blood loss causes an increase in stroma protein. The highest levels of stroma protein are found in the severe anemias. As the anemia is corrected by red cell regeneration, the stroma protein level falls to normal.

Anemia due to blood destruction (phenylhydrazine) presents *very high levels* of *stroma protein*—almost double the increase noted in anemia due to blood loss.

Hypoproteinemia added to anemia due to blood loss causes no significant change on the stroma protein level.

Abscesses due to the subcutaneous injection of turpentine during the anemia cause slight decreases in the stroma protein levels. Chloroform poisoning has no effect on the stroma protein levels.

The total lipides of the stroma are rather stable and are little influenced by anemia. In certain experiments with hemolytic anemia and with hypoproteinemia, there is a significant rise in total lipide figures.

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