The Turkish Journal of Pediatrics
Volume 24, No. 3, July - September 1982

SPLEEN AND COAGULATION FACTORS*

*Gönül Hiçsönmez MD**, *Şinasi Özsoylu MD***

Key words: coagulation, factors VIII, II, VII/X, X, V, spleen function

The site of synthesis of antihemophilic factor (AHF, F VIII) has been the subject of numerous studies. The liver[1], spleen[2], kidneys[3], reticuloendothelial cells (REC) [4], leukocytes[5,6] and lymphocytes[7,8] have all been implicated in the synthesis of F VIII. In recent years the synthesis of the high molecular weight subcomponent of F VIII (F VIII R:AG) has been shown to be localized on the vascular endothelial cells. However, the site of low molecular weight subcomponent (F VIII:C) synthesis is not yet known[9,10]. Several studies on laboratory animals and human beings have suggested the spleen plays an important role in F VIII synthesis or in its storage[2,11-13]. The primary purpose of the present study was to elucidate the relationship between the spleen and F VIII. Other coagulation factors were also determined to see whether changes in these factors correlated with changes in AHF activity.

Material and Methods

The studies were carried out on samples of splenic arterial and venous blood from twelve patients undergoing splenectomy and 20 dogs.

The ages of the patients ranged between 3 and 29 years. Three patients had idiopathic thrombocytopenic purpura (ITP), 2 Cooley's anemia, 1 congenital spherocytosis, 3 cirrhosis, 1 hydatid cyst of the spleen, 1 Gaucher's disease and 1 hypersplenism of unknown etiology.

In this study peripheral venous blood was obtained before and during fluothane anesthesia and after laparotomy while the patients were under anesthesia. Splenic arterial and venous blood samples were also obtained from the patients who underwent therapeutic splenectomy.

Mongrel dogs weighing between 14 and 25 kg were employed for sham operations. Blood samples were obtained from every animal under the same conditions as described above for the patients, except that nembutal anesthesia (30 mg/kg) was used. In 10 of 20 animals, after blood samples were obtained as described above,

* From the Hematology Unit, Hacettepe University Institute of Child Health, Ankara.
** Professor of Pediatrics, Hematologist, Hacettepe University Institute of Child Health.

0.014 mg/kg adrenaline (6.6 mcg/ml) was injected into the splenic artery, and blood samples were obtained from the splenic vein within 3 and 7 minutes and from the splenic artery 5 minutes following the adrenaline injection. The values for procoagulant activity of F VIII and other coagulation factors obtained from splenic venous blood at 3 and 7 minutes did not show any statistically significant differences. For the statistical analysis the values obtained at 3 minutes were used.

For the human coagulation studies 9 volumes of blood were added to one volume of 0.1 M sodium oxalate. After separation of plasma, factor I, II, VIII, V, VII/X, and X levels were assayed[14-16] and factor activities expressed as percent of controls. One unit of factor activity is defined as the amount of factor present in one milliliter of normal mixture human plasma. The same method was employed for the animal studies except that the test plasma used in determination of mixtures of F II, F V, F VII/X was obtained from four normal dogs. For determination of F VIII concentration in dogs, F VIII-deficient plasma and substrate plasma obtained from human beings were used as indicated in previous studies[17]. For the statistical analysis Student's t test was used.

Results

Human Studies.

As seen in Table I there were no significant differences between factor VIII levels in the splenic artery and vein or between these and peripheral blood, either before or during anesthesia. Nor were there significant differences between the levels of the other coagulation factors.

TABLE I

Comparisons of Coagulation Factors of Splenic Arterial and Venous Blood and Peripheral Blood Before and During Anesthesia in Humans ($\bar{X} \pm SD$)

Coagulation factors %	Peripheral blood before anesthesia	Peripheral blood during anesthesia	Splenic arterial blood	Splenic venous blood
F VIII	102 ± 58	88 ± 41	109 ± 34	103 ± 45
F I (mg/dl)	293 ± 72	295 ±74	283 ± 87	281 ± 62
F II	77 ± 23	70 ± 24	76 ± 22	77 ± 20
F V	106 ±.20	91 ±26	99 ± 17	105 ± 25
F VII/X	73 ± 18	71 ± 17	70 ± 16	70 ± 20
F X	65 ± 19	70 ±17	75 ± 22	75 ± 21

No statistically significant difference was found between samples for any of the above factors ($p > 0.05$).

Animal Studies.

As in the human study, factor VII levels in the splenic artery and vein in the peripheral blood in dogs before and during anesthesia were not significantly different ($p > 0.05$) (Table II). Levels of the other coagulation factor values in the splenic artery and vein and the peripheral blood likewise were not significantly different from each other ($p > 0.05$).

TABLE II

Comparison of Coagulation Factors of Splenic Arterial and Venous Blood and Peripheral Blood Before and During Anesthesia in Dogs ($\bar{X} \pm SD$)

Coagulation factors %	Peripheral blood before anesthesia	Peripheral blood during anesthesia	Splenic arterial blood	Splenic venous blood
F VIII	119 ± 37	109 ± 35	126 ± 32	112 ± 25
F I (mg/dl)	415 ± 111	404 ±82	390 ± 94	396 ± 0
F II	85 ± 23	81 ± 15	83 ± 23	82 ± 20
F V	83 ± 13	83 ±14	85 ± 12	85 ± 15
F VII/X	108 ± 18	109 ± 19	111 ± 26	107 ± 22
F X	77 ± 20	69 ±17	75 ± 17	75 ± 15

No statistically significant difference was found between the values of any of the above factors ($p > 0.05$).

As seen in Table III, statistically significant increases in F VIII levels were observed in the splenic vein and peripheral blood following adrenaline injection into the splenic artery in dogs ($p < 0.02$). Although F VIII levels in the splenic artery were also found to be slightly elevated after adrenaline injection, the difference was not statistically significant ($p > 0.05$). Factor VIII activities of peripheral blood, splenic artery and veins following adrenaline injection did not differ significantly from each other.

Values obtained for other coagulation factors in the venous blood of the spleen 3 minutes after adrenaline injection and in the arterial blood of the spleen 5 minutes after adrenaline injection were not significantly different for any of the factors studied ($p > 0.05$). After injection of adrenaline a significant increase was seen in the splenic artery only for the factor VII/X complex ($p < 0.01$) (Table IV). Significant increases of factors II, V, VII/X and X were observed in the splenic vein after adrenaline ($p < 0.02$). Factor X values also increased in peripheral venous blood after adrenaline injection.

Discussion

The site of synthesis and storage of factor VIII has provoked a variety of opinions and circumstantial evidence. The spleen has been thought to be a major site of fac-

TABLE III

Factor VIII Levels in the Samples from 10 Dogs Before and After Adrenaline Injection

($\bar{X} \pm SD$)

	Peripheral venous blood	Splenic arterial blood	Splenic venous blood
F VIII % (before adrenaline)	109 ± 35	126 ± 32	112 ± 25
F VIII % (after adrenaline)	158 ± 31	150 ± 35	149 ±38
p	< 0.02	> 0.05	< 0.02

tor VIII synthesis by various authors[2,11-13,18]. Although F VIII synthesis has also been thought to occur in the liver[1], kidneys[2], leukocytes[5,6] and lymphocytes[7,8], other studies have cast doubt on the likelihood of synthesis in these organs[19,20].

In the present study the synthesis and storage functions of the spleen have been investigated. Factor VIII levels in the splenic artery and vein in both patients and dogs showed no significant difference (Tables I, II). These findings suggest that the spleen cannot be the organ of synthesis of factor VIII.

Structural malformations of the spleen have been reported in all the patients except those with ITP. It is also known that increase in plasma volume parallels increase in size of the spleen[21]. Since our aim was to compare the levels of coagulation factors in the arterial and venous blood of the spleen, dilution of these factors, which may have some relation to the size of the spleen would not affect the results in any way, and similarly, the effect of the normally or abnormally functioning spleen on the concentration of the coagulation factors would not change the results. In all of the patients, except the ITP cases, blood transfusions were given prior to obtaining blood samples for coagulation studies. Since it affects the arterial and venous blood of the spleen in the same way, hemodilution does not change the results. The animals studied had normal spleens and received no blood transfusions.

Coagulation factors were determined in the peripheral blood before and during anesthesia to determine the effect of anesthesia and light anoxia and to compare the peripheral blood values with those of splenic arterial and venous blood. It was observed that anesthesia and questionable oxygenation changes had no significant effect on the levels of coagulation factors (Tables I, II).

In recent years, the effect of adrenaline on factor VIII levels has been investigated in human beings and animals. Thus adrenaline has entered the group of factors report-

TABLE IV

Coagulation Factor Levels in Samples Obtained from 10 Dogs Under Anesthesia Before and After Adrenaline Injection
($\bar{X} \pm SD$)

Coagulation Factors %	Peripheral blood		Splenic arterial blood		Splenic venous blood	
	before adrenaline	after adrenaline	before adrenaline	after adrenaline	before adrenaline	after adrenaline
F I (Mg/dl)	385 ±84	368 ± 97	383 ±117	489 ± 181	438 ± 91	475 ± 100
F II	85 ± 12	98 ± 22	86 ± 13	99 ± 9	82 ± 9	106 ± 23*
F V	85 ± 11	100 ± 18	86 ± 11	97 ± 0	89 ± 13	106 ± 14*
F VII/X	99 ± 7.0	106 ± 11	94 ± 12	108 ± 14*	92 ± 9	115 ± 15*
F X	75 ± 12	90 ± 15*	79 ± 14	93 ± 15	79 ± 10	101 ± 21*

* The difference in values obtained before adrenaline injection is statistically significant ($p > 0.02$).

ed to have increased the factor VIII level[22-24]. Forwell and Ingram indicated that adrenaline was effective during the early stages of blood coagulation[25]. Later Ingram showed adrenaline to be the cause of the increase in factor VIII level[26]. Although the mechanism of the increase of factor VIII by adrenaline is not understood very well, it is probably related with the spleen. In splenectomized animals and human beings increase of the factor VIII level following intravenous adrenaline has not been observed[12,27].

In the present study, no significant difference was observed in the factor VIII level between arterial and venous blood of the spleen following the injection of adrenaline (Table II). The difference between the factor VIII levels of venous blood of the spleen, before and after adrenaline injection, was found to be significant (Table III). These findings suggest that the spleen functions as a storage organ for this factor. As indicated previously, the difference between factor VIII levels in the peripheral venous blood, before and after injection of adrenaline, was statistically significant (Table III). Investigation of the causes of the increase in factor VIII level of the peripheral blood, despite the lack of a significant increase in the artery of the spleen, will enhance our knowledge in this area.

In our study, when the synthesis functions of the other coagulation factors (I, II, V, VII/X, X) in the spleen were investigated in humans and animals, no evidence of splenic synthesis was found (Tables I, II). However, the values of factors II, V, VII/X, X in the blood of the spleen taken from animals before and after adrenaline injection indicated a significant increase (Table IV). These findings suggest a storage function of the spleen for those factors.

Summary

The relationship between coagulation factors and the spleen was investigated. The data suggest that factor VIII and other coagulation factors (F I, II, V, VII/X, X) are not synthesized in the spleen. However the spleen has a storage function for F VIII and some other factors such as F II, V, VII/X, X. It has also been found that anesthesia has no effect on the levels of the coagulation factors.

Acknowledgment

The authors wish to thank Y. Erk and E. Ercis for providing the blood. We are also grateful for the editorial assistance of V. Türker.

REFERENCES

1. Gardikas C, Bakaloudis P, Hatzioannou J, Kokkinos D. The factor-VIII concentration of the hepatic venous blood. *Br J Haematol* 11:380, 1965.
2. Webster WP, Reedick RL, Roberts HR. Release of factor-VIII (antihaemophilic factor) from perfused organs and tissues. *Nature* 213:1146, 1967.
3. Barrow EM, Graham JB. Kidney antihemophilic factor. Partial purification and some properties. *Biochemistry* 7:3917, 1968.
4. Gaynor E, Spaet TH. Failure of methyl palmitate induced reticuloendothelial blockade to affect plasma levels of factor-VIII. *Blood* 28:595, 1966.
5. Nadell J, Johnson NL, Pool JG, Greger WP. Thromboplastic activity in cultured blood leukocytes. *Clin Res* 14:169, 1966.
6. Kociba GJ, Loeb WF, Wall RL. Development of procoagulant (tissue thromboplastin) activity in cultured leukocytes. *J Lab Clin Med* 79:778, 1972.
7. Bouhasin JD, Monteleone P, Altay Ç. Role of the lymphocyte in antihemophilic globulin production: a rise in antihemophilic globulin levels in a hemophilic subject with acute lymphoblastic leukemia. *J Lab Clin Med* 78:122, 1971.
8. Rickles FR, Hardin JA, Pitlick FA, et al. Tissue factor activity in lymphocyte cultures from normal individuals and patients with hemophilia A. *J Clin Invest* 52:1427, 1973.
9. Bloom AL, Giddings JC, Wilks CJ. Factor-VIII on the vascular intima: possible importance in haemostasis and thrombosis. *Nature (New Biol)* 241:217, 1973.
10. Rand JH, Sussman II, Gordon RE, et al. Localization of factor-VIII related antigen in human vascular subendothelium. *Blood* 55:752, 1980.
11. Pool JG. Antihemophilic globulin (AHF, Factor VIII) activity in the spleen (Abst). *Clin Res* 14: 170, 1966.
12. Pudlak P, Farska I, Deimlova E. Changes in factors V and VIII after administration of adrenaline to normal and splenectomized rats. *Br J Haematol* 16:373, 1969.
13. Weaver RA, Price RE, Langdell RD. Antihemophilic factor in cross-circulated normal and hemophilic dogs. *Am J Physiol* 206:335, 1964.
14. McMillan CW, Diamond LK, Surgenor DM. Treatment of classic hemophilia: the use of fibrinogen rich in factor VIII for hemorrhage and for surgery. *N Engl J Med* 265:224, 1961.
15. Ratnoff OD, Menzie C. New method for determination of fibrinogen in small samples of plasma. *J Lab Clin Med* 37:316, 1951.
16. Owren PA. New clotting factors. Blood clotting and allied problems. Trans. 5th Conf. Josiah Macy Jr Found, 1952, p. 92.
17. Graham JB, Collins DL, Godwin ID, Brinkhous KM. Assay of plasma antihemophilic activity in normal, heterozygous (hemophilia) and prothrombinopenic dogs. *Proc Soc Exp Biol Med 77*: 294, 1951.
18. Desai RG, Choto R, Inami K, et al. Spleen infusion in the treatment of hemophilia and allied disorders. *Clin Res* 17:115, 1969.
19. Langdell RD, Bowersox LW, Weaver RA, Gibson WS. Coagulation properties of canine thoracic-duct lymph. *Am J Physiol* 199:626, 1960.
20. Waaler BA, Gustafsson BE, Hauge A, et al. Plasma levels of various blood clotting factors in germ-free rats. *Proc Soc Exp Biol Med* 117:444, 1964.

21. Blendis LM, Clarke MB, Williams R. Effect of splenoctomy on the haemodilutional anaemia of splenomegaly. *Lancet* 1:795, 1969.

22. Özsoylu Ş, Strauss HS, Diamond LK. Effects of corticosteroids on coagulation of the blood. *Nature* 195:1214, 1962.

23. Egeberg O. Changes in the activity of antihemophilic A factor (F.VIII) and in the bleeding time associated with muscular exercise and adrenaline infusion. *Scand J Clin Lab Invest* 15:539, 1963.

24. Rizza CR. Effect of exercise on the level of antihaemophilic globulin in human blood. *J Physiol* 156:128, 1961.

25. Forwell DG, Ingram GIC. The effect of adrenaline infusion on human blood coagulation. *J Physiol* 135:371, 1957.

26. Ingram GI. Increase in antihaemophilic globulin activity following infusion of adrenaline. *J Physiol* 156:217, 1961.

27. Libre EP, Cowan DH, Watkins SP Jr, Schulman NR. Relationships between spleen, platelets and factor VIII levels. *Blood* 31:358, 1968.