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Independence of Protamine Titration and Platelet Level in Certain Hemorrhagic Diseases.

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Following the addition of a standard quantity of heparin to normal blood, the amount of protamine sulfate required to clot this blood within one hour is remarkably constant.[1] Although species differences do exist the amount of protamine required for members of the same species varies to only a slight degree.

This protamine requirement as determined by the protamine titration[2] may be greatly increased in certain pathologic states associated with hemorrhage. Increased protamine requirements in whole blood of patients have been observed in association with acute leukemia, chronic leukemia, some cases of idiopathic thrombocytopenia, secondary thrombocytopenia, aplasia of the bone marrow, and in the toxic states associated with total body X-radiation, nitrogen mustards and aminopterin therapy. All of these conditions are frequently associated with thrombocytopenia. The hemorrhagic complications of these disorders are often temporarily improved or controlled and the protamine titration returned to normal by the administration of toluidine blue, even though the platelet count is unchanged. An increased protamine titration has also been found in bleeding patients with normal platelet counts and normal prothrombin activity.[2] These patients likewise respond to toluidine blue and protamine therapy, and the protamine titrations return to or toward normal.

These and other observations suggest that the increased protamine titration may be due to a defect in the clotting mechanism similar although not identical with that produced by the intravenous injection of commercial heparin. We have been able to demonstrate certain differences between this heparinoid defect and that produced by the intravenous injection of commercial beef heparin in both man and dog.[3] The principal difference appears to be in anticoagulant potency. The bloods of patients and dogs with these disorders rarely inhibited or delayed coagulation of normal bloods. Furthermore, the protamine titration was increased in many of our patients and experimental animals to such an extent that had the increased protamine titration resulted from heparinemia identical with that produced by an injection of commercial beef heparin (Abbott), the blood would invariably have been incoagulable. Incoagulable blood was found in only a few instances. Generally, the clotting time was prolonged only 2 to 4 times the normal values.

Recently Conley, Hartmann and Lalley[4] have observed that normal human plasma could be made more sensitive to the effects of added heparin by removing most of the platelets by centrifugation. They concluded that these plasma data were applicable to the protamine titration and stated that the protamine titration "can be explained by the variation in the platelet concentration alone." The protamine titration, however, is performed on whole blood.

Observations of the type reported by the Hopkins group[4] were investigated by us in

1 Allen, J. G., Moulder, P. V., Elghammer, R. M., Grossman, B. J., McKeen, C. L., Sanderson, M. H., Egner, W. M., and Crosbie, J., *J. Lab. and Clin. Med.*, 1949, **34**, 473.

2 Allen, J. G., Grossman, B. J., Elghammer, R. M., Moulder, P. V., McKeen, C. L., Jacobson, L. O., Pierce, M., Smith, T. R., and Crosbie, J., *S.G.O.*, in press.

3 To be published.

4 Conley, C. L., Hartman, R. C., and Talley, J. S., Proc. Soc. Exp. Biol. and Med., 1948, **69**, 284.

the course of development of the protamine titration. It was found that prothrombin deficient and thrombocytopenic bloods were more sensitive than normal bloods to the effects of added heparin, both *in vitro* and *in vivo*. In this connection the observations of Howell are of interest.[5]

He observed that the effects of amounts of heparin just sufficient to prevent clotting could be overcome by cephalin obtained from brain tissue, recognizing this as a substance also found in platelets. It is possible that other factors may also influence both *in vitro* and *in vivo* heparin sensitivity.

It is doubtful if the increases in the protamine titration can be explained by thrombocytopenia alone. The protamine titration may be normal in severe thrombocytopenia and may be markedly increased in patients with normal platelet counts and prothrombin times.

In order to produce an increase of 0.020 mg in the protamine titration (the smallest increment of increase used) 0.018 mg of liquid beef heparin (Abbott) per ml of human blood was required. This was 2 to 5 times the amount of heparin required to increase the clotting time of whole blood or plasma *in vitro*, and was 25- to 50-fold greater than that required for "platelet-free" plasma.[4] These comparisons were made on normal human blood and plasma and do not necessarily apply to bleeding disorders. Moreover, it has been observed that animals on aminopterin develop an increased protamine titration without thrombocytopenia or an increased *in vitro* heparin sensitivity.[7]

The protamine titration, however, is affected by several factors, most of which influence fibrin formation. Among these factors are: heparin or heparin-like substances, prothrombin deficiency, impairment of the conversion of prothrombin to thrombin, hemophilia, and the absence of fibrinogen. It is possible to set up a very sensitive protamine titration over a narrow range of protamine concentration which can be altered by changes in the platelet count but such a titration would lose its clinical value. The problem of the effect of thrombocytopenia appears to be sufficiently controlled that the results of the protamine titration[1] must be explained on some basis other than platelet deficiency alone.

Twenty-one patients, representing a variety of malignant and benign disorders, had normal platelet counts ranging from 180,000 to 324,000 per cmm but showed mild to marked increase in the protamine titration (0.16 mg to 0.40 mg). On the other hand, eighteen patients with similar bleeding disorders had moderate to marked thrombocytopenia (20,000 to 108,000 platelets per cmm). In these the protamine titration was normal. These observations were repeated 6 to 9 times on some patients. Most patients who had an increased protamine titration also had thrombocytopenia of some degree. In those who responded to toluidine blue the platelet count, before and after partial or complete control of bleeding occurred, was not appreciably altered, Table I.

In Werlhof's syndrome three patients had platelet counts ranging from 2,000 to 60,000 and normal protamine titrations. These did not respond to toluidine blue. Three other patients with platelet counts from 23,000 to 60,000 per cmm and increased protamine titrations (0.16 to 0.20 mg), showed some decrease in bleeding as their titrations returned to normal, but all 3 required splenectomy for complete control of bleeding. In only one of these was the response to toluidine blue impressive. The detailed studies on these and other patients are presented elsewhere.[2]

Comment. These data indicate that the protamine titration may be increased in the presence of normal platelet counts as well as in severe thrombocytopenia. Moreover, the protamine titration can be returned to normal by the administration of adequate toluidine blue without materially altering the platelet count. In addition, thrombocytopenia may

[5] Howell, W. H., *The Harvey Lectures*, 1917, **12**, 272.

[6] Allen, J. G., Sanderson, M. H., Milham, M., Kirschon, A., and Jacobson, L. O., *J. Exp. Med.*, 1948, **87**, 71.

[7] Grossman, B. J., Sanderson, M. H., and Allen, J. G., to be published.

TABLE I.
Patients with Increased Protamine Titrations and Low Platelet Counts Before and After Receiving Toluidine Blue.

Patient	Diagnosis	Platelets		Protamine titration*	
		Before toluidine blue, 1000 per cmm	After toluidine blue, 1000 per cmm	Before toluidine blue, mg protamine sulfate	After toluidine blue, mg protamine sulfate
453730	Acute leukemia	190	17	.18	.14
K-a234	" "	20	80	.18	.16
413730	" "	52	24	.18	.16
436363	Acute leukemia Aminopterin Rx	140	24	.18	.16
341183	Hodgkin's Disease Nitrogen Mustard Rx	124	37	.20	.16
395060	" "	83	85	.20	.14
409460	" "	64	37	.20	.14
435361	" "	81	75	.16	.14
453179	" "	19	70	.16	.14
M-a186	Chronic leukemia Spray irradiation	10	16	.18	.16
423054	Cancer Breast P^{32} therapy	100	93	.18	.16
T-a229	Toxic Pancytopenia	7	16	.18	.14
15517	Toxic depression of bone marrow	85	15	.18	.14
H-b32	Menorrhagia	66	72	.16	.14

* Following the addition of a standard concentration of heparin to normal blood a remarkably constant amount of protamine is required to restore clotting. (Laboratory normal is 0.140 mg protamine sulfate (Lilly) per ml blood containing 0.091 mg heparin (Abbott)).

occur without increasing the protamine titration.

The protamine titration is probably increased by factors which directly or indirectly delay or inhibit fibrin formation. Abnormal values in the protamine titration can not be interpreted unless all known clotting factors can be evaluated. The titration can be increased when large quantities of heparin or heparin-like substances are present, but it does not necessarily follow that the factor(s) responsible for the increased protamine titration in these patients was actually heparin or even heparin-like. Some endogenous compounds, not of the heparin family, were found to be mild anticoagulants and were inhibited by both toluidine blue and protamine sulfate.[3] The exact nature of the factor(s) responsible for the defect observed in these patients remains unknown.

Summary. 1. The platelet concentration influences the sensitivity of bloods and plasmas to the effect of added heparin. The anticoagulant potency of heparin is also enhanced by prothrombin deficiency. The potentiated effect of heparin in thrombocytopenic bloods may be due to reduced thromboplastin (cephalin) activity.

2. The protamine titration may be increased when the platelet count is normal and it may be normal in the presence of thrombocytopenia. It is influenced by heparin, heparinoid substances, prothrombin deficiency, hemophilia, and possibly other factors.

3. Increased protamine titrations may occur in bleeding patients who have no other apparent clotting defects, including the whole blood clotting time. Many of these patients cease bleeding when given intravenous toluidine blue. The protamine titration under these conditions can be returned to or toward normal regardless of the platelet level.