Symposium on Hemophilia and Similar Disorders*

Blood Coagulation Disorders in Hemophilia

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Hemophilia is a wellknown hereditary disease with sex-linked recessive transmission, but it was only a few years ago that the coagulation disorders of hemophilia were proved to be caused by a disturbance of blood thromboplastin formation.

The subjects of this paper are to present the mechanism of blood thromboplastin formation and the results of recent laboratory investigations on about 12 cases of hemophilia encountered recently in our clinic. According to the laboratory findings the author should like to express how to designate hemophilia and hemophiloid state.

Mechanisms of blood thromboplastin formation

Platlet factor 3 was obtained by the method of Bell and Alton⁽¹⁾ from rabbit brain. Factor VIII was prepared by the method of Bidwell⁽²⁾ from bovine plasma, factor IX by the method of White and Aggeler⁽³⁾ from human plasma. Factor X was prepared by the method of Hougie⁽⁴⁾. Factor XI (PTA) was prepared from normal serum which was absorbed with BaSO₄ and decalcified with sodium oxalat. Factor V was prepared by the method of Pool⁽⁵⁾. M/40 CaCl₂ solution was used as calcium.

When thromboplastin generation test was carried out with all these factors, complete thromboplastin was generated after incubation of 3 or 4 minutes. When, however, any one of these factors was deficient, in no instance the amount of thromboplastin gener-

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ated exceeded 10 to 15 per cent. Only when factor XI was deficient, 85 per cent of thromboplastin was generated. So investigations of the relation between Hageman factor⁶⁾, factor XI and factor IX in the formation of blood thromboplastin were carried out. Intact plasma and serum were prepared as previously reported⁷⁾. Exhausted plasma and serum of Hageman factor were prepared using celite as an activating and absorbing agent⁸⁾. The results were shown in Fig. 1. When exhausted serum and exhausted plasma were used, it

Fig. 1. Influence of absorbed celite on the thromboplastin formation of exhausted materials.

showed evidently a defect in the formation of blood thromboplastin (curve D). When 0.1 ml. of the absorbed celite suspension from normal intact serum was added to the thromboplastin formation mixture in which both exhausted plasma and exhausted serum were incubated, the defect of thromboplastin formation was remarkably corrected (curve C). The celite which was used after treatment of BaSO_4 -absorbed normal serum or PTC deficient serum, showed the same correcting effect (curve B and A).

These results suggest that Hageman factor might activate factor XI and factor IX.

From above results blood thromboplastin formation is expressed by the following formula.

Coagulation disorders in hemophilia

1. Whole blood clotting time and bleeding time

Whole blood clotting time was remarkably prolonged. In two cases it was over 6 hours, but in 4 cases almost normal clotting time was observed. These cases have been attracted attentions recently as mild hemophilia.

Bleeding time, however, was normal in all cases. This is the characteristic of hemophilia. It may be supposed that the bleeding time was principally controlled by the function of both platelets and capillaries and clotting factors play only a secondary role.

2. One stage prothrombin time

One stage prothrombin time was normal in all cases. This is the important findings in order to differentiate hemophilia from other hemorrhagic diseases caused by a disturbance of the second phase of coagulation (conversion of prothrombin to thrombin).

3. Prothrombin consumption during blood coagulation

As shown in Fig. 2, in normal subjects prothrombin was consumed over 85 per cent by the end of 2 hours after blood collection. In hemophilia, however, prothrombin was not consumed after 30 minutes or one hour. In only mild hemophilia 50 per cent consumption of prothrombin was observed after two hours.

4. Thromboplastin generation test

The clotting time and prothrombin consumption test do not provide any distinction between the different defects of thromboplastin formation. The thromboplastin generation test permits the detection of slight degrees of abnormalities and, more important,

Fig. 2. Prothrombin consumption during coagulation in hemophilia.

differentiates one type of abnormality from another.

When the thromboplastin generation test is carried out in cases of varying clinical severity, the amount of thromboplastin formed in this test bears some relation to clinical severity.

The principles of thromboplastin generation test are expressed by the following schema.

If thromboplastin formation in the mixture of patient's BaSO_4 -absorbed plasma and normal serum gave abnormal results, patient's plasma indicates a defect of factor VIII. Patient will be designated as hemophilia A. On the other hand, if thromboplastin formation in the mixture of patient's serum and normal BaSO_4 -absorbed plasma gave abnormal results, patient's serum indicates a defect of factor IX. Patient will be designated as hemophilia B.

Hemophilia A

Factor VIII levels of hemophilia A estimated by the thromboplastin generation test gave the following results. In mildly affected cases who showed normal clotting time factor VIII levels were 12

to 30 per cent, but were 4 to 6 per cent in severe cases. These levels were correlated intimately with clinical features.

Hemophilia B

Two patients of brothers aged 21 years and 9 years with familial PTC deficiency have been recently encountered. They had a markedly prolonged clotting time of 6 and 8 hours, respectively. Factor IX level estimated by the thromboplastin generation test gave 5 to 6 per cent.

Combined hemophilia A and B

One patient with combined deficiency of factor VIII and factor IX has been encountered. He was a male aged 31 years with no family history. He showed a markedly prolonged clotting time of 140 to 300 minutes. Factor VIII and factor IX levels estimated by the thromboplastin generation test gave 8 and 20 per cent, respectively.

Factor VIII consumption during blood coagulation

Factor VIII consumption has been studied in normal and hemophilic blood maintained at 37°C for one hour after collection, using assay method⁹⁾ of factor VIII. The results were shown in Fig. 3.

Fig. 3. Factor VIII consumption during blood coagulation in hemophilia.

In normal subjects factor VIII was completely utilized by the end of one hour. In hemophilia A of both mild and severe types and combined hemophilia factor VIII consumption was completely defective.

Activity of platelet factor 3

Activity of platelet factor 3 has been estimated by the method of thromboplastin generation test using normal plasma, normal serum and patient's platelets. In hemophilia A, hemophilia B and combined hemophilia 85 to 100 per cent of thromboplastin was generated. These results indicate that platelet factor 3 activity is not defective in hemophilia.

As above described the difference of hemophilia A and hemophilia B was characterized only by a deficiency of factor VIII and/or IX. They could not be differentiated by other clinical features and inheritance. It is reasonable at present to designate hemophilia A and B as hemophilia.

Coagulation disorders of PTA deficiency

Two patients with PTA deficiency who have no family history have been encountered. One was a male aged 20 years, who had epistaxis since the age of 12. The other was a female aged 18 years who also had epistaxis since the age of 6.

In the laboratory findings, bleeding time, platelet count, prothrombin, factor V, factor VII and fibrinogen gave negative results. Only whole blood clotting time was slightly prolonged. The results of the thromboplastin generation test were shown in Fig. 4 and 5. The amount of thromboplastin generated in the mixture of patient's BaSO_4 -absorbed plasma and normal serum was 95 or 85 per cent. Mixture of patient's serum and normal BaSO_4 -absorbed plasma gave 85 or 70 per cent of thromboplastin. On the other hand, mixture of patient's BaSO_4 -absorbed plasma and patient's serum gave only 42 or 45 per cent of thromboplastin.

From these results these two patients were thought to be a deficiency of PTA¹⁰⁾.

Fig. 4. Thromboplastin generation test of PTA deficiency (1).

Fig. 5. Thromboplastin generation test of PTA deficiency (2).

Findings by use of thrombelastography

Thrombelastography is an important method in order to estimate intrinsic blood clotting system. Thrombelastographic analysis in various types of hemophilia was carried out. The results were shown in Fig. 6. As can be seen in hemophilia and PTA deficiency the thrombelastogram is typically characterized by:

- 1) markedly prolonged reaction time r
- 2) markedly prolonged k value
- 3) normal ma value.

The so-called postmaximal relaxation of clot is found only slightly in 3 mild cases.

Fig. 6. Thrombelastograms in hemophilia and PTC deficiency.

The prolonged r and k represent the thrombelastographic equivalent of the slowed formation of thromboplastin, thrombin and fibrin. Due to the similarity in AHG (F. VIII), PTC (F. IX) and PTA (F. XI) deficiencies thrombelastography is not able to distinguish these conditions. This characteristic, together with the presence of a normal ma, represents a basis for the differential diagnosis of hemophilic syndromes from other conditions.

Grade of severity of hemophilia in a given family

By the method of thromboplastin generation test or factor VIII assay method⁹⁾, estimation of factor VIII or factor IX level of one familial mild hemophilia A, one familial severe hemophilia A and one familial hemophilia B was carried out.

As seen in Table 1, factor VIII or factor IX level was below 3 to 4 per cent in severe hemophilia A, 10 to 30 per cent in mild hemophilia A and below 5 per cent in severe hemophilia B.

Table 1. Grade of Severity of Hemophilia in a Given Family

Type of Disease	Familial relation	Age	Factor VIII or Factor IX	
			T. G. T. (%)	Assay method (%)
Severe Hemophilia A (■■■■)	1st brother	40	3	below 1
	2nd brother	27	4	below 3
Mild Hemophilia A (■■■■)	1st brother	28	30	10
	2nd brother	26	14	15
	3rd brother	22	12	10
Severe Hemophilia B (■■■■)	1st brother	21	5	
	2nd brother	9	4.5	

These results lead to the assumption that the decrease of factor VIII or factor IX and the grade of severity of disease is relatively constant in a given family, so that familial types of the disease are thereby created.

Factor VIII level of known carriers

It has been impossible to find out hemophilic carriers, since they do not reveal clinical symptoms. It is, however, very important and interesting clinically if it is possible to find out them by use of any new method.

By use of thromboplastin generation test, estimation of factor VIII level of two mothers and two sisters in two hemophilia families was carried out. The findings, as listed in Table 2, showed a decrease of factor VIII level to 38 or 42 per cent in two mothers.

Table 2. Factor VIII Level of Known Carriers

Type of Disease	Familial relation	Age	Factor VIII
Hemophilia A (████)	mother	71	38%
	sister	30	73%
Mild Hemophilia A (████)	mother	50	42%
	sister	18	90%

This is suggestive that it may be possible to find out carriers, but it is necessary for us to make further investigations in order to give conclusion.

Summary

There are various types of hemorrhagic diseases caused by the deficiency of different factors involved in the blood thromboplastin formation. Their differentiation is only possible by evaluating the coagulation defect with exact laboratory method such as thromboplastin generation test.

The designation hemophilia may only be applied to these which are caused by a deficiency of factor VIII and/or IX. A deficiency of factor VIII together with factor IX in a given patient is not called concomitant hemophilia but called combined hemophilia. Other conditions must be involved in the designation of hemophiloid state.

The degree of the diminution of factor VIII or factor IX is inherited and constant in a given family. Familial types of the

disease are thereby created. It is suggestive to find out carriers by use of thromboplastin generation test.

It is notable that 4 cases of mild hemophilia were found recently in our clinic.

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