Deficiency of Plasma Prothrombin Conversion Accelerators in the Postoperative State with a Description of a Simple Method of Assay

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A PRACTICAL CLINICAL METHOD for determining the activity in plasma of the accelerators of prothrombin conversion has not been so rapidly forthcoming as have been descriptions of their properties and reactions. Those methods which have been described have employed two general principles. The first of these is the ability of the unknown plasma to restore the total prothrombin activity of a mixture from which prothrombin accelerators have been totally or partially removed; the second the comparison of the total prothrombin activity of a given unknown done by a standard method with that done similarly but in the presence of an excess of prothrombin conversion accelerators.

In the following report "prothrombin conversion accelerator" is used as an inclusive term to cover the various substances in plasma designated by their respective describers as "factor V," "labile factor," "accelerator globulin," "plasmatic co-factor of thromboplastin." Although the exact relationship of these substances to each other has not yet been made entirely clear, it is probable that it is a close one and that such a group is justified. The term "prothrombin" is used to designate that portion of the prothrombin complex which remains after accelerators have been removed.

The first principle has been employed by Owren,[1] who by incubation of plasma rendered it free of factor V and measured its total prothrombin activity before and after the addition of the test plasma. De Vries, Alexander and Goldstein[2] determined the "serum prothrombin conversion accelerator" content of serum by adding it to a prothrombin-poor mixture of normal plasma and plasma adsorbed with barium sulfate. Ware and Seegers[3] added the unknown plasma or serum to a mixture of purified prothrombin,[4] thromboplastin, and calcium and tested for Ac-globulin by measuring its ability to increase the coagulability of that mixture. Quick[5] and Stefanini[6] have employed the same principle for testing for labile factor using stored plasma as a prothrombin-poor reagent.

Because the above methods have entailed the possible addition of procoagulants other than prothrombin accelerators to the reaction mixture the second principle, that of determining prothrombin activity in the presence and in the absence of an added excess of accelerators has been employed. Ware and Seegers,[7] using the two-stage prothrombin method, Owren,[8] using a one-stage method and Olwin,[9] using both the one-stage and the two-stage methods, have done this. In all three instances beef serum was used as a potent source of accelerators.

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The two-stage method[10] of determining total prothrombin activity has been championed by its proponents as more accurate than the one-stage.[11] Whether or not this is so, the two-stage method does not by itself distinguish between standard prothrombin and its accelerators unless one of the above mentioned technics is used. Ware, Guest and Seegers[12] demonstrated that not only the speed of conversion of prothrombin but the yield of thrombin varied with the concentration of accelerators in the test plasma. Quick and Stefanini[13] have shown a greater consumption of prothrombin the greater the concentration of labile factor present. Owren[8] agrees and considers the comparatively low yield of thrombin which is obtained in the presence of a low concentration of accelerators to be due to the fact that slow thrombin formation affords a longer time for neutralization by the antithrombins of the plasma. The only coagulation factor, therefore, not controlled by the one-stage method which the two-stage test does control is fibrinogen. Alterations in plasma fibrinogen of a degree which interferes with coagulation are rare. We have accordingly adopted the more simple one-stage method in our determination of prothrombin conversion accelerators.

Our interest in the use of this test was stimulated by finding a decrease in total prothrombin activity in studies of alterations of coagulation factors in the postoperative state.[16] Further observations in this regard were made using the test and are recorded below.

In the test outlined the second principle, that of eliminating variations in accelerators by purposely adding an excess of them has been utilized. The method, although evolved independently, is similar to that of Owren.[8] The fact that in our method the excess of accelerators is added at the same time as the thromboplastin from a previously prepared mixture and that we use beef serum instead of plasma distinguish the methods. Beef serum is a satisfactory source of accelerators because of its availability and because it has been shown to be[14] three times as rich in accelerators as is human serum. It has been shown by Ware and Seegers to be stable at room temperature for over twelve hours, at 5 C. for two to three weeks, and at −10 C. to −20 C. for one year. In use it is diluted 600 times. It is treated with barium carbonate in order to remove any possible standard prothrombin present.

Method

Reagents

1. Rabbit brain thromboplastin extract provided by the Difco Laboratories, giving a normal prothrombin time of thirteen to fifteen seconds.

2. Sodium oxalate solution 0.10 M. Dissolve 1.34 Gm. of anhydrous reagent grade sodium oxalate in 100 ml. of distilled water.

3. Calcium chloride solution 0.02 M. Dissolve 0.222 Gm. of anhydrous reagent grade calcium chloride in 100 ml. of distilled water.

4. Serum Ac-globulin prepared from beef blood as described by Ware and Seegers.[7] This is transferred to small tight stoppered bottles and stored at −10 C. Only one vial is used at a time and that is kept frozen between periods of use.

5. Accelerator thromboplastin extract. To 3 ml. of rabbit thromboplastin extract is added 0.10 ml. serum Ac-globulin and 0.10 ml. sodium oxalate.

Procedure

Blood in the quantity of 4.5 ml. is obtained by venipuncture and mixed with 0.5 ml. of 0.10 M. sodium oxalate. The oxalated blood is then centrifuged for fifteen minutes at 1000 r.p.m. The plasma is then pipeted off into a clean test tube.

Using only the upper 0.1 ml. portion of a 0.2 ml. pipet, 0.1 ml. of plasma is pipeted into four small serologic tubes. Still using only the upper 0.1 ml. of the pipet, 0.1 ml. of modified thromboplastin extract is added to two of the tubes containing oxalated plasma. At the same time and in the same way 0.1 ml. of unmodified thromboplastin extract is added to the other two tubes containing plasma.

The four tubes are then placed in a water bath at 37 C. for a few seconds. The stop watch is started with the rapid addition of 0.1 ml. of calcium chloride and the end point determined. The first appearance of a fibrin web is the end point, the detection of which is facilitated by observation under a magnified reviewer. All determinations are done in duplicate.

Since all thromboplastin extracts are made daily a prothrombin curve for varying saline dilutions of plasma is constructed for each extract. Pooled normal human plasma is used for the curves.

Results

The method was used under varying conditions designed to test its validity.

Normal Blood

Fifty-two observations were made on normal plasmas procured by venipuncture for reasons other than those associated with deficiencies of blood coagulation. The mean value for the total prothrombin activity of these observations was 96 per cent of normal. The mean value for the prothrombin activity when the accelerator thromboplastin was used was 98 per cent of normal. Varying dilutions of normal plasma with 0.85 per cent sodium chloride solution as shown in figure 1 show a close correlation of prothrombin activity whether standard thromboplastin or accelerator thromboplastin was used. Thus the accelerator thromboplastin reagent affected very little the results procured on normal plasma.

The Use of Purified Accelerator Globulin in Lieu of Beef Serum Reagent

In order to demonstrate that beef serum actually was adding accelerators tests were made using an excess of Dr. Seegers' purified Ac-globulin* instead of the beef serum reagent. Essentially similar values for the activity of regular prothrombin were obtained in both instances (table 1).

Stored Blood

Since Quick has demonstrated[15] that the decrease in the prothrombin activity of stored plasma is due to a disappearance of labile factor from the prothrombin

* Procured by the kindness of Dr. William Harrington of the Boston City Hospital.

complex, the test was performed at intervals of from two to four days on five specimens of normal citrated blood allowed to stand at 4 C. for twenty-three days. Figure 2 demonstrates that the gradual decline in total prothrombin ac-

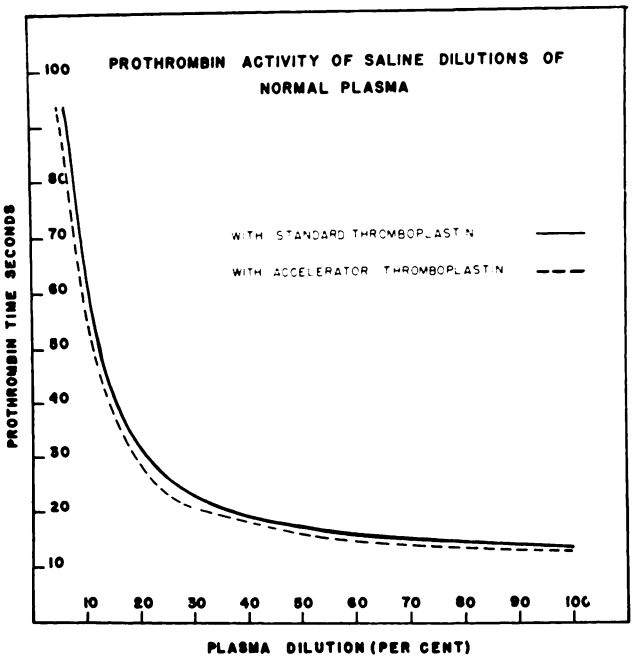

FIG. 1.—Normal blood. The failure of an excess of prothrombin conversion accelerator to enhance significantly the prothrombin activity of normal blood is demonstrated in varying dilutions.

TABLE 1.—*A Comparison of the Accelerator Activity of Beef Serum Reagent with that of Purified Ac-globulin*

	Total Prothrombin Activity	Prothrombin Activity with Accelerator Thromboplastin	Prothrombin Activity with Seegers' Purified Ac-globulin
	%	%	%
Fresh normal pooled plasma	100	100	100
Stored plasma, 9 days	63	100	100
Stored plasma, 61 days	1	81	76
Stored plasma, 69 days	2	78	82
Stored plasma, 76 days	4	92	90
Stored plasma, 84 days	5	84	83
(Dicumarol) plasma	43	45	45
Vitamin K deficient plasma	69	72	72

tivity was due to decreases in accelerators of prothrombin conversion which at the end of the twenty-three days reached a deficiency of about 30 per cent. At this time the activity of standard prothrombin had also begun to decrease.

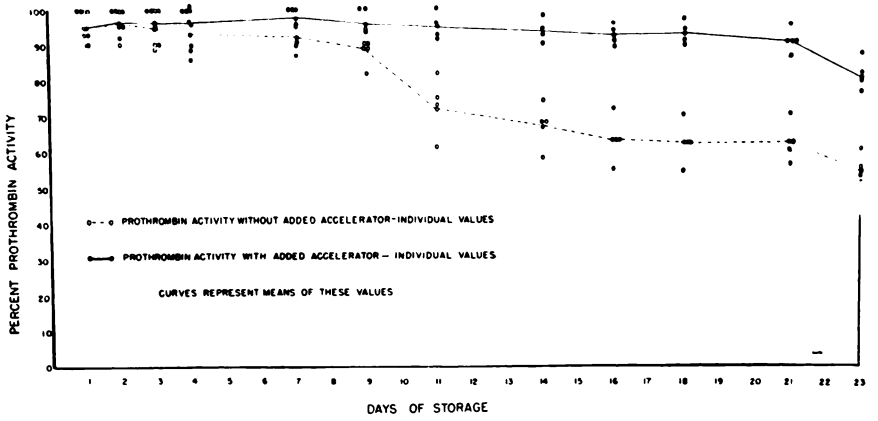

FIG. 2.—The disappearance of prothrombin conversion accelerator from stored blood.

TABLE 2.—*Prothrombin Activity of 25 Dicumarolized Plasmas*

Plasma No.	With Standard Thromboplastin	With Accelerator Thromboplastin
	%	%
1	15	18
2	16	16
3	17	18
4	17	18
5	17	18
6	20	19
7	20	23
8	20	23
9	23	22
10	23	24
11	25	23
12	27	26
13	27	26
14	30	33
15	33	32
16	33	34
17	34	32
18	35	33
19	35	34
20	35	34
21	35	38
22	38	36
23	38	44
24	39	42
25	60	63
Mean	28.4	29.2

Dicumarolized Blood

That the administration of dicumarol affects primarily the standard prothrombin of the blood rather than the accelerators of prothrombin conversion[6] is corroborated by this test. Twenty-five observations on patients who had

received varying doses of dicumarol revealed that the mean value for the observations using standard thromboplastin was 28.4 per cent, for those using the modified extract, 29.2 per cent (table 2), an insignificant difference.

Decrease in Prothrombin Accelerators in the Early Postoperative Period

It has been shown by several observers, including those in this laboratory,[16] that there is a decrease in the total prothrombin activity of the plasma in patients in the early days after major surgical operations, usually maximal about the third day. Figure 3 presents the results procured when the described test was

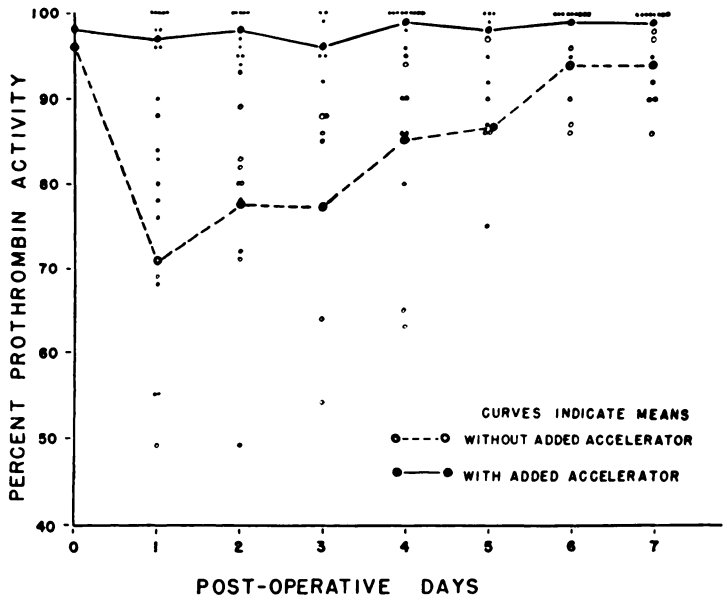

FIG. 3.—The diminution of prothrombin conversion accelerator in the early postoperative period.

used on postoperative patients and demonstrates by adding an excess of accelerators that no deficiency in standard prothrombin exists. The prothrombin deficiency, then, is due to a decrease in prothrombin accelerators during this period.

DISCUSSION

It has been confirmed that the beef serum reagent does not contain prothrombin. If to a solution of the beef serum is added an excess of thromboplastin, an optimal amount of calcium and an excess of bovine fibrinogen, no clot takes place.

The manner in which the results of the test are best expressed is a matter of some debate. What fraction of normal total prothrombin activity is contributed by accelerators and what fraction by standard prothrombin is not known. This being the case it seems more convenient to express the results of the test in percentage of accelerator deficiency, the percentage being calculated in reference to total prothrombin activity. Thus if the test shows 100 per cent prothrombin activity in the presence of an excess of accelerators and only 70 per cent without

added accelerators, then the deficiency of accelerators in that plasma is taken to be 30 per cent.

Theories as to the reasons for a deficiency of accelerators in the early days after major operations range from transitory liver damage to the possibility that of the prothrombin elements lost in the blood shed during operation the regular prothrombin is replaced by blood transfusion whereas the prothrombin accelerators presumably are inadequately replaced if the blood is more than a week old. These theories demand further exploration.

It is not yet known how wide a clinical application tests of this nature will have. To date a deficiency of accelerators as dissociated from one of prothrombin has been found only in stored blood and in individuals who have certain types of congenital hypoprothrombinemia or "para-hemophilia."

Since we have not yet used the test extensively in patients with liver disease we do not know which element of the prothrombin complex is first affected by failure of liver function. It has been stated that both prothrombin accelerators and prothrombin may be decreased.[6] If this is the case a distinction between the elements of the prothrombin complex which may be deficient in such conditions, as well as in postoperative states, will undoubtedly be of aid in determining possible responses to blood transfusion or vitamin K therapy on the one hand or to dicumarol therapy on the other.

The term prothrombin conversion accelerator is used in this report to designate the plasma factors with the full realization, as has been pointed out by Quick and Stefanini,[13] that they may not be true accelerators but merely accessory factors in the prothrombin complex. If this is so they are thereby clearly distinguished from Alexander's prothrombin conversion accelerator of serum,[2] and the use of beef serum as a reagent to test for them may be criticized. Alexander[17] has, however, shown that bovine serum contains both serum and plasma factors, and it is logical to suppose that a deficiency in plasma factors is actually being measured by the test.

Summary

1. A simple method for distinguishing between prothrombin and prothrombin conversion accelerators has been described.

2. A deficiency in prothrombin conversion accelerators in the early days after major surgical operations has been observed.

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