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Research Article

THE ACTION OF HEPARIN IN THE PREVENTION OF PROTHROMBIN CONVERSION

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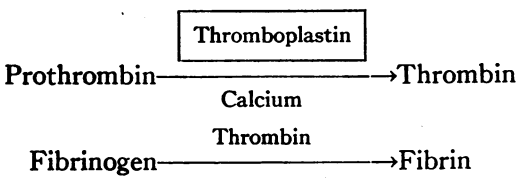
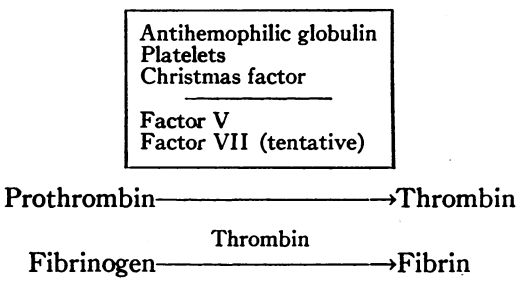
During physiological blood coagulation thrombin is formed from a precursor substance—prothrombin. According to the classical theory of blood coagulation as postulated by Morawitz in 1904, this conversion of prothrombin to thrombin is brought about by thromboplastin, acting in the presence of calcium (1). The thrombin then interacts with fibrinogen to produce fibrin. This theory may be expressed:



Thromboplastin is defined in this study as a substance which is directly responsible for the conversion of prothrombin to thrombin in the presence of calcium (2). The thromboplastin was thought originally to be provided by damaged tissue, but it is now realized that when blood is withdrawn by clean venipuncture without tissue contamination it coagulates under the action of its own thromboplastin. This blood thromboplastin is now known to be of great potency (3), and is certainly essential for physiological hemostasis. The relative significance of these two thromboplastin systems in hemostasis has not been defined.

Many factors have recently been postulated as participants in blood thromboplastin formation. It is accepted for the present that four and possibly five components are required to react to form blood thromboplastin. These are antihemophilic globulin, platelets, Christmas factor (P.T.C.), factor V (labile factor, proaccelerin, plasma accelerator globulin) and possibly factor VII (prothrombin conversion accelerator, S.P.C.A., proconvertin, co-thromboplastin) (2). Antihemophilic globulin is deficient in hemophilia, platelets in thrombocytopenia, the Christmas factor in Christmas disease and factor V as a rare congenital anomaly and in some cases of hepatic disease. Factor VII is the predominant deficiency in coumarin drug therapy and though it undoubtedly is required in the tissue thromboplastin system, the evidence for its participation in blood thromboplastin is incomplete. These components can be substituted for the thromboplastin of the classical theory and the theory of blood thromboplastin expressed as follows:



The order in which these components interact is uncertain but there is some evidence that antihemophilic globulin, platelets and the Christmas factor may interact to produce an intermediate product which is the equivalent of tissue extract, the other components subsequently entering the reactions involved in prothrombin conversion to thrombin (4). This concept of blood thromboplastin is in certain aspects analogous to the tissue mechanism, for in hemophilia, Christmas disease and thrombocytopenia the one-stage clotting time on the addition of tissue extract is normal, whereas deficiencies of factor V or VII cause a prolongation. This understanding of the components of blood thromboplastin may be incomplete as other factors have been suggested as being required, for example, P.T.A. (5), fourth thromboplastin component (6) and the Hageman factor (7).

With regard to the action of heparin in preventing coagulation, there is good experimental evidence that, while acting together with a cofactor in the albumin fraction of the plasma, it interferes with the thrombin-fibrinogen reaction (8, 9). It has also been suggested that heparin

interferes with the interactions resulting in thrombin formation (2, 10–12). Investigations on the action of heparin in preventing thrombin formation are difficult on account of the interference by the heparin with the thrombin-fibrinogen reaction. Thus, the components of blood thromboplastin can be incubated together with and without heparin and apparent interference with the production of thromboplastin demonstrated (3), but it can be argued that the results are merely a reflection of the heparin interfering with the thrombin-fibrinogen reaction.

The present investigation is concerned with the earlier stages of blood coagulation when subjected to the influence of heparin, using techniques where the heparin does not interfere with the final indicator system. It has been demonstrated that during blood coagulation under the influence of its own thromboplastin factor V, antihemophilic globulin and prothrombin are used up in the reaction (13–16). It is assumed that this consumption of factor V and antihemophilic globulin is due to their utilization in blood thromboplastin formation. Prothrombin consumption is the outcome of blood thromboplastin formation and the resulting conversion to thrombin.

METHODS

Preparation of reagents:

Prothrombin Fibrinogen Aluminum hydroxide (alumina) 3.8 per cent sodium citrate M/40 calcium chloride	As described by Biggs and Macfarlane (17)
Human brain extract	— As described by Brown and Douglas (18)

Centrifugation: All centrifugation was carried out at 4°C.

Glassware: All clotting times, whether of fibrinogen or plasma, were carried out in tubes of ½ inch diameter. The glassware was cleaned by standing in dichromate followed by rinsing in tap water.

Thrombin-fibrinogen dilution curve: As described by Biggs (19).

Measurement of prothrombin: As described by Douglas and Biggs (16). This technique is dependent on the separation of prothrombin from antithrombin and its activation thereafter by brain thromboplastin and calcium. The prothrombin was separated from the antithrombin by dilution and acidification to pH 5. The precipitate containing prothrombin was found to be freed not only from antithrombin, but also from heparin activity. There was, in consequence, no evidence of interference by heparin with the thrombin-fibrinogen reaction.

Measurement of factor V: As described by Douglas and Biggs (16), being a modification of the method of McClaughry and Seegers (20). Prothrombin is activated in an antithrombin free system by brain thromboplastin, calcium and factor V. The factor V is prepared from a normal and the test specimens. The test specimen is assayed by comparing the curve of activation with that of dilutions of the normal. The only difficulty in applying this technique to the present problem was the removal of the heparin from the appropriate test specimens and the prevention of its interference with the thrombin-fibrinogen reaction. It was found that treatment with alumina, which was employed in the preparation of the factor V from the specimens, removed the heparin by adsorption. This adsorption of heparin on alumina confirms the observations made independently by MacMillan and Brown (21).

Measurement of antihemophilic globulin: As described by Douglas and Biggs (16). This technique employs the thromboplastin generation test (22); it is dependent on the correction of thromboplastin generation of adsorbed hemophilic plasma by dilutions of adsorbed normal plasma. The curve of thromboplastin generation by mixtures of equal parts of adsorbed hemophilic and test specimens was compared with the dilutions of adsorbed normal in hemophilic plasma. A supply of hemophilic plasma was kept frozen at −20°C in a deep freeze. The heparin was removed by the process of adsorption on alumina.

Collection of specimens: The investigation was carried out on patients starting heparin therapy. Needles and syringes of identical size were used throughout. By venipuncture, using a wide bore needle (S.W.G. No 18), 30 ml. of blood was collected into a large syringe, care being taken to avoid frothing. This syringe was detached, the needle being left *in situ* and 10,000 units of heparin contained in another syringe, injected intravenously. Five minutes after the injection of heparin an identical specimen was collected by separate venipuncture from the other arm. A mixture was made immediately of 4.5 ml. of blood with 0.5 ml. of 3.8 per cent sodium citrate and further 4.5 ml. volumes of blood delivered into four identical graduated centrifuge tubes. These tubes were placed in a water bath at 37°C and at intervals of 15 minutes after collection 0.5 ml. of 3.8 per cent sodium citrate added and the contents of the tube mixed with a wooden applicator stick. In this way the process of clotting was arrested at fifteen-minute intervals after withdrawal of the blood. The tubes were left in the water bath for an hour to allow for the neutralization of any thrombin formed. After this the specimens were tested to determine the amounts of prothrombin, factor V and antihemophilic globulin present. In this way the pattern of utilization of these factors before and after the administration of heparin could be studied. The procedure described above was carried out on nine patients starting heparin therapy. In three of the observa-

TABLE I

The results of assays of prothrombin, factor V and antihemophilic globulin *

		Before heparin				After heparin			
		Time after venipuncture							
	Plasma	15 mins.	30 mins.	45 mins.	60 mins.	15 mins.	30 mins.	45 mins.	60 mins.
Prothrombin	100	79	51	10	0	100	100	100	100
	100	73	24	0	0	100	100	100	100
	100	74	10	0	0	100	100	100	100
Mean	100	75	28	3	0	100	100	100	100
Factor V	100	85	60	10	0	100	100	100	100
	100	70	40	0	0	100	100	100	100
	100	70	55	5	0	100	100	100	100
Mean	100	75	53	5	0	100	100	100	100
Antihemophilic globulin	100	100	15	0	0	100	100	100	100
	100	50	5	0	0	100	100	100	100
	100	60	5	0	0	100	100	100	100
Mean	100	70	8	0	0	100	100	100	100

* The mean of the three observations on each of these factors is also shown. The assays were made on plasma collected before giving heparin and on specimens citrated at 15, 30, 45, and 60 minutes following withdrawal of blood by venipuncture, both before and after the intravenous administration of heparin. The results are expressed as percentages of the amount of each of the factors present in the plasma before giving the heparin.

tions, prothrombin was studied, in three factor V and in the last three antihemophilic globulin.

RESULTS

The details of each of the assays is shown in Table I together with the mean of these results. It will be seen that after the administration of the heparin there was no utilization of any of these components up to one hour, whereas in the specimens collected before giving the heparin, all had been used up by the end of an hour.

DISCUSSION

Evidence has been produced to suggest that factor V and antihemophilic globulin are essential components of blood thromboplastin (2). During the clotting of blood under the influence of its own thromboplastin these factors disappear, presumably in consequence of their utilization in the formation of blood thromboplastin (16). In the presence of heparin, factor V and antihemophilic globulin are not used up. This can be interpreted as a consequence of interference with thromboplastin formation.

The investigation has shown that in the presence of heparin there is defective consumption of prothrombin. This is a further, non-specific, indication of failure in the blood thromboplastin system.

The techniques used in the investigation have employed methods for the removal of heparin from the incubation mixtures. There was in consequence no interference by heparin with the indicator system, the thrombin-fibrinogen reaction. The efficacy of the methods for the removal of heparin was confirmed by the demonstration that all the specimens collected after the administration of the heparin contained as much prothrombin, factor V and antihemophilic globulin as the plasma before administration of heparin. Any traces of heparin left in the incubation mixtures would have resulted in lower readings being obtained.

SUMMARY

1. Therapeutic doses of heparin have been shown to result in failure of utilization of antihemophilic globulin and factor V, believed to be essential components for blood thromboplastin formation. The consequent inability to convert prothrombin to thrombin has been demonstrated. The experimental results described are not the consequence of heparin acting on the indicator system.

2. This evidence of complete interference with prothrombin conversion and utilization of blood thromboplastin components may possibly indicate that this represents the main action of heparin rather than interference with the thrombin-fibrinogen reaction.

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