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THE ACTION OF THROMBOPLASTIC SUBSTANCES

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Studies of the initial stages of blood coagulation have shown that, following contact with a foreign surface, a potent coagulant activity is produced by a reaction involving at least five factors contained within the blood itself (Biggs, Douglas & Macfarlane, 1953*a*, *b*). This activity has been referred to throughout these studies as 'thromboplastic', and it is implied that it is due to the formation of an actual, though labile, substance termed 'blood thromboplastin'. It is now necessary to consider more precisely what is usually meant by this term 'thromboplastin', the substances and activities to which it has been applied, and the relationship of these to the newly recognized activity developed in blood.

According to the classical theory of coagulation derived from the work of Schmidt (1892), Morawitz (1905) and Mellanby (1909), the conversion of prothrombin to thrombin is brought about by a factor called 'thromboplastin' or 'thrombokinase', thought to be provided by damaged tissues and possibly by the platelets. Conventional views on the action of this factor, as summarized by Howell (1912), 'assume that the thromboplastic substance is of the nature of an activator or co-ferment essential to the conversion of prothrombin' and that thromboplastic substances 'cause the formation of thrombin from prothrombin by a process of splitting, or as we say now activation'. Other views, including Howell's own, diverged from this concept of thromboplastin as the direct and essential activator of prothrombin, but most modern work has been based on it.

Unfortunately, the role of thromboplastin as the direct activator of prothrombin is difficult to substantiate, because it is difficult to prepare prothrombin free from other factors which might participate in its activation. In practice, therefore, thromboplastic activity is usually judged by the ability of a given preparation to accelerate the clotting of recalcified citrated or oxalated blood or plasma, it being shown that the preparation does not contain thrombin. By this criterion a variety of substances have been regarded as thromboplastic. These include extracts of many tissues, particularly brain and

lung, and various lipoid or lipo-protein fractions derived from them; certain venoms, particularly Russell's viper venom, and certain proteases, particularly trypsin. These substances, under suitable conditions, will clot normal recalcified plasma in 10–30 sec where the clotting time of the recalcified plasma alone is 2–3 min. It was supposed that, despite their diversity, they had a common ability to activate prothrombin.

The use of recalcified plasma to detect thromboplastic activity can now be seen to involve serious difficulties of interpretation. The relatively long clotting time of such plasma is not due to a simple deficiency of thromboplastin as originally assumed; it is due to the relatively slow operation of a system which finally produces a thromboplastin more potent as a coagulant than any of the 'thromboplastic' substances already mentioned. Acceleration of the clotting of recalcified plasma therefore may be produced in different ways. The effect of an added preparation may, in fact, be due to its direct activation of prothrombin, that is, to a 'classical' thromboplastic action. But a similar accelerating effect may also be produced by accelerating in some way the formation of the intrinsic thromboplastin. Such an indirect effect must be clearly distinguished from a direct action on prothrombin if the mode of action of the so-called thromboplastic substances is to be understood.

Evidence already exists that tissue extracts and Russell's viper venom require the co-operation of other factors in order to promote the conversion of prothrombin. Brain extract, widely used as 'thromboplastin' in the one-stage prothrombin time test, will not cause its usual rapid coagulation of plasma if this plasma lacks factor V or factor VII. In attempts to reconcile this finding with the current conviction that brain extract is a typical thromboplastin, factors V (Owren, 1947) and VII (Koller, Loeliger & Duckert, 1951) have been supposed to be necessary accelerators of a direct reaction between brain thromboplastin and prothrombin. But the recent observation that factors V and VII are concerned in blood thromboplastin generation suggests that this previous supposition may not be correct; the brain extract may require to be activated by factors V and VII before it is capable of converting prothrombin to thrombin. Russell's viper venom, which also has been used widely as a thromboplastin in prothrombin determinations, requires the presence of platelets or a lipoid such as lecithin in order to accelerate coagulation, and is incapable of clotting recalcified plasma from which platelets and lipoid have been removed (Macfarlane, Trevan & Attwood, 1941). It is unlikely therefore to have a direct effect on prothrombin.

It is clear that the recognition of the thromboplastin-generating system of the blood has made necessary a revision of existing views on the mode of action of so-called thromboplastic substances. In this communication evidence is presented which may clarify the ways in which these substances accelerate coagulation, and indicate their relationship to, or dependence on, the factors

involved in blood thromboplastin formation. The substances investigated are brain extract, Russell's viper venom and trypsin, which were thought likely to be representative thromboplastic agents. Since information on the action of such factors can often be obtained by examining the conditions under which they are inhibited, an investigation of soya-bean trypsin inhibitor and of heparin, both of which are known to antagonize so-called thromboplastic activity, have been included.

METHODS

Human brain extract was prepared as described by Biggs & Macfarlane (1953).

Russell's viper venom. The preparation 'Stypven' of Burroughs and Wellcome was used.

Trypsin. Armour crystalline trypsin was used.

Soya-bean inhibitor was kindly provided by Dr M. Kunitz.

Heparin made by Abbott Laboratories was used.

Factor V, prothrombin and fibrinogen were prepared as described by Biggs & Macfarlane (1953).

Serum was collected as described by Biggs *et al.* (1953*a*).

The generation of thromboplastin was tested essentially as described by Biggs *et al.* (1953*b*). In various tests components of the thromboplastin system were replaced wholly or in part by various substances. The amount of thromboplastic activity developed in these experiments was assessed in terms of the blood thromboplastin system using the dilution curve prepared by Biggs *et al.* (1953*a*). The modifications in technique required by the different experiments are indicated in the appropriate sections.

RESULTS

Brain extracts

If brain extract undergoes a reaction with factors V and VII before the product reacts with prothrombin then the incubation of brain extracts with these factors will enhance the activity of the extract. In these experiments normal serum was used as a source of factor VII; although normal serum also contains the Christmas factor this will not influence the results because there is evidence that the Christmas factor is not required for the action of brain extract. The increased activity of the brain can be tested by demonstrating the ability of the mixture to clot either recalcified citrated plasma or a mixture of prothrombin and fibrinogen.

Brain extract and the coagulation of plasma. When a relatively concentrated preparation of brain extract which had been incubated with serum and factor V and $CaCl_2$ was added to normal recalcified plasma it caused clotting in 5 sec. The brain extract alone diluted with 0·85% NaCl and M/40-$CaCl_2$ instead of serum and factor V and $CaCl_2$ caused clotting in 20 sec. It is clear that pre-incubation with factors V and VII greatly enhanced the activity of the brain extract. Since such very short clotting times are difficult to record reliably, brain extracts diluted 1/50 were used in the majority of experiments. When such diluted brain extracts were incubated with factor V and normal serum for 5 min there was an increase in activity of about 12-fold. At this dilution the brain extract clotted normal plasma in the presence of $CaCl_2$ in

44 sec. The mixture containing factor V and serum caused clotting in 14–15 sec. The results of this experiment are shown in Table 1 (top line). Serum or factor V alone added to brain caused a two- to fourfold increase in activity, the results being rather variable and presumably depending on the amount of factor VII contaminating the factor V preparation and the amount of factor V

TABLE 1. 0·1 ml. of 'thromboplastic' substance was mixed with 0·4 ml. amounts of various mixtures. The completed mixtures were then incubated for 5 min at 37° C and 0·1 ml. amounts were withdrawn and added to 0·1 ml. of platelet-poor citrated plasma from a normal subject together with 0·1 ml. amounts of $CaCl_2$-M/40 and the clotting times recorded in seconds. The figures represent the average results of four independent experiments.

	Mixture added to 'thromboplastic' substance				
'Thromboplastic' substance used	Saline, 0·3 ml. $CaCl_2$, 0·1 ml.	Saline, 0·1 ml. Factor V, 0·1 ml. Serum, 0·1 ml. $CaCl_2$, 0·1 ml.	Saline, 0·1 ml. Factor V, 0·1 ml. Platelets, 0·1 ml. $CaCl_2$, 0·1 ml.	Saline, 0·1 ml. Platelets, 0·1 ml. Serum, 0·1 ml. $CaCl_2$, 0·1 ml.	Factor V, 0·1 ml. Serum, 0·1 ml. Platelets, 0·1 ml. $CaCl_2$, 0·1 ml.
Brain extract, 0·1 ml.	44	15	28	17	9
Russell's viper venom, 0·1 ml.	50	25	15	13	10
Trypsin, 0·1 ml.	60	51	29	21	22

The brain extract used was prepared by suspending 0·5 g of dried human brain in 10 ml. of 0·85% NaCl and diluting the supernatant emulsion 1/50.

The Russell's viper venom was a 1/50,000 dilution of the Burroughs and Wellcome preparation Stypven.

The trypsin was a solution of the Armour crystalline product at a concentration of 1 mg/ml. in 0·85% NaCl.

Factor V was isolated from normal plasma and used at a dilution of 1/5 of that present in normal plasma.

Normal serum was used at a dilution of 1/5 with 0·85% NaCl.

The platelets were prepared from normal plasma and used at a concentration of 3 times that of normal plasma.

in the serum. Platelets and serum used together caused a considerable improvement in activity which may have been due partly to the factor V provided by platelet suspensions (Ware, Fahey & Seegers, 1948; Biggs & Macfarlane, 1953). When both serum and factor V were present the addition of platelets led to a further increase in activity.

In other experiments diluted brain extracts were incubated with various mixtures of known blood thromboplastin components and the activity assessed at intervals (Fig. 1). It will be seen that as the time of incubation lengthens the thromboplastic activity of the mixtures increases, suggesting a reaction between the brain extract and the blood components.

These results show that the clot-accelerating activity of brain extracts for normal plasma can be greatly increased by factor V and a substance present in serum. When platelets, normal serum and factor V are incubated with

diluted brain extract, the mixture then contains all the components necessary for blood thromboplastin formation except antihaemophilic globulin. It may therefore be suggested that this substance, or something which will replace it, is present in high concentration in brain extract. The main conditions which limit the activity of brain extract are the absence of factors V and VII.

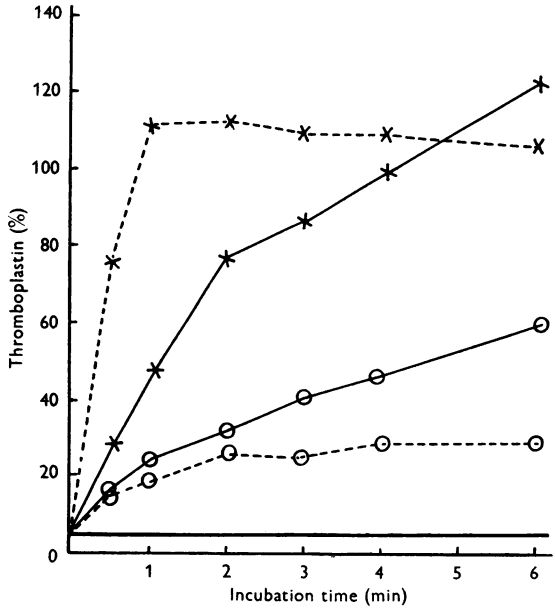

Fig. 1. Curves demonstrating the enhanced thromboplastic activity of brain extract (continuous lines) and Russell's viper venom (broken lines) on incubation with various blood thromboplastin components. ×——×, brain extract, platelets, factor V and normal serum; O——O, brain extract, factor V and normal serum; × - - - - ×, Russell's viper venom, platelets, factor V and serum; O - - - -O, Russell's viper venom, factor V and serum.

In this and in subsequent illustrations the thromboplastic activity was measured by removing 0·1 ml. samples at intervals and adding these to 0·1 ml. amounts of citrated normal plasma which were recalcified with 0·1 ml. of M/40-$CaCl_2$ and the clotting times were observed. The clotting times were interpreted in terms of thromboplastin concentrations using a dilution curve (Biggs *et al.* 1953*a*).

Brain extract and the coagulation of a mixture of prothrombin and fibrinogen. The fact that brain extract reacts with factors V and VII before the conversion of prothrombin to thrombin occurs can be demonstrated rather more clearly using prothrombin and fibrinogen separated from plasma. Brain extract alone has little power to clot a mixture of prothrombin and fibrinogen (Table 2). If serum and factor V are added to the brain extract and the mixture incubated with $CaCl_2$ there is a progressive and marked improvement in the ability of the mixture to clot prothrombin and fibrinogen in the presence of $CaCl_2$. The addition of factor V and serum to the substrate prothrombin and fibrinogen

has some effect, but this is not nearly so striking as when the reagents are incubated with the brain. The inclusion of either factor V or serum in the brain mixture has little effect.

These results suggest that the brain extract first reacts with factors V and VII and that the product of this reaction converts prothrombin to thrombin.

TABLE 2. In different experiments brain extract was incubated at 37° C with the substances indicated in column 1. At intervals of 1 min 0·1 ml. was removed from the incubation mixture and added, together with 0·1 ml. of M/40-$CaCl_2$ to the substrate indicated for each experiment in column 2. The clotting times of the substrate were recorded in seconds.

		Incubation time in minutes					
Incubation mixture	Substrate	1	2	3	4	5	6
Brain, 0·2 ml. 0·85% NaCl, 0·4 ml. $CaCl_2$-M/40, 0·2 ml.	Prothrombin, 0·1 ml. Fibrinogen, 0·1 ml. 0·85% NaCl, 0·2 ml.	85	85	84	88	103	73
Brain, 0·2 ml. 0·85% NaCl, 0·4 ml. $CaCl_2$-M/40, 0·2 ml.	Prothrombin, 0·1 ml. Fibrinogen, 0·1 ml. Factor V, 0·1 ml. Serum, 0·1 ml.	37	36	33	35	32	27
Brain, 0·2 ml. Factor V, 0·2 ml. Serum, 0·2 ml. $CaCl_2$-M/40, 0·2 ml.	Prothrombin, 0·1 ml. Fibrinogen, 0·1 ml. 0·85% NaCl, 0·2 ml.	40	25	20	18	16	15
Brain, 0·2 ml. Factor V, 0·2 ml. 0·85% NaCl, 0·2 ml. $CaCl_2$-M/40, 0·2 ml.	Prothrombin, 0·1 ml. Fibrinogen, 0·1 ml. Serum, 0·1 ml. 0·85% NaCl, 0·1 ml.	55	46	40	49	41	36
Brain, 0·2 ml. Serum, 0·2 ml. 0·85% NaCl, 0·2 ml. $CaCl_2$-M/40, 0·2 ml.	Prothrombin, 0·1 ml. Fibrinogen, 0·1 ml. Factor V, 0·1 ml. 0·85% NaCl, 0·1 ml.	40	35	32	33	32	27

The brain extract used was prepared by suspending 0·5 g of dried human brain in 10 ml. of 0·85% NaCl.

The factor V was used at a concentration of 1/5 of that in whole plasma.

The normal serum was used at a dilution of 1/5 in 0·85% NaCl.

The prothrombin was used at a concentration of about 500 units/ml. (50 units in each substrate test). One unit of prothrombin is the amount converted to 1 unit of thrombin, the thrombin unit being approximately the same as the National Institute of Health unit.

The fibrinogen was used at a concentration of approximately 0·6% (a concentration of 0·1% in the substrate mixture).

Russell's viper venom

Like brain extract Russell's viper venom is not a complete thromboplastin; it is known to require a lipoid for its activity (Macfarlane *et al.* 1941). In experiments in which Russell's viper venom was incubated with various thromboplastin factors it is clear that Russell's viper venom requires factor V, serum and platelets for its optimum activity (Table 1, middle line). The increase in activity which follows the incubation develops more rapidly than

in similar experiments using brain extracts (Fig. 1). Russell's viper venom is much more dependent on the presence of lipoid than is brain thromboplastin (Table 1 and Fig. 1).

Trypsin

Similar experiments were carried out with a solution of Armour crystalline trypsin at a concentration of 0·2 mg/ml. in the mixtures. From Table 1 (bottom line) it is clear that the activity of trypsin is increased by incubation with serum and platelets or serum, factor V and platelets. The increased activity can be demonstrated immediately after mixing the appropriate reagents. The action of trypsin appears to be less dependent on the presence of factor V than are the other thromboplastin preparations.

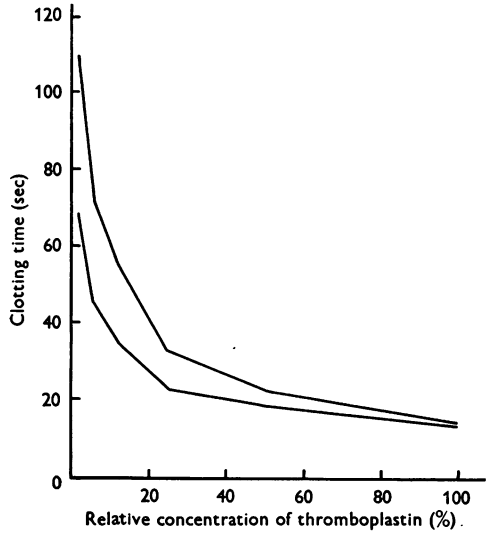

Fig. 2. Curves demonstrating the clotting time of normal plasma on recalcification in the presence of various concentrations of blood thromboplastin.

The results illustrated by the lower curve were obtained by adding 0·1 ml. of blood thromboplastin and 0·1 ml. of M/40-$CaCl_2$ to a mixture of 0·1 ml. of citrated normal plasma and 0·1 ml. of 0·85% NaCl. The results illustrated in the upper curve were obtained by adding 0·1 ml. of the same blood thromboplastin and 0·1 ml. of M/40-$CaCl_2$ to a mixture of 0·1 ml. of citrated normal plasma and 0·1 ml. of a solution of soya-bean inhibitor in 0·85% NaCl at a concentration of 12·5 mg/100 ml.

Soya-bean inhibitor

Soya-bean inhibitor and the reaction of thromboplastin with prothrombin. When dilutions of blood thromboplastin are added with $CaCl_2$ to normal plasma a curve relating clotting time and relative concentration of thromboplastin may be drawn (Fig. 2). The addition of a high concentration of soya-bean inhibitor to the substrate causes little change in clotting time when thromboplastin and $CaCl_2$ are added (Fig. 2). These results suggest that the

inhibitor does not interfere with the reaction between thromboplastin and prothrombin.

Soya-bean and the destruction of formed thromboplastin. When soya-bean inhibitor is added to formed blood thromboplastin at a concentration 1/10 of that used in the previous experiment the rate of destruction of thromboplastin is very greatly increased (Fig. 3). If different concentrations of inhibitor are

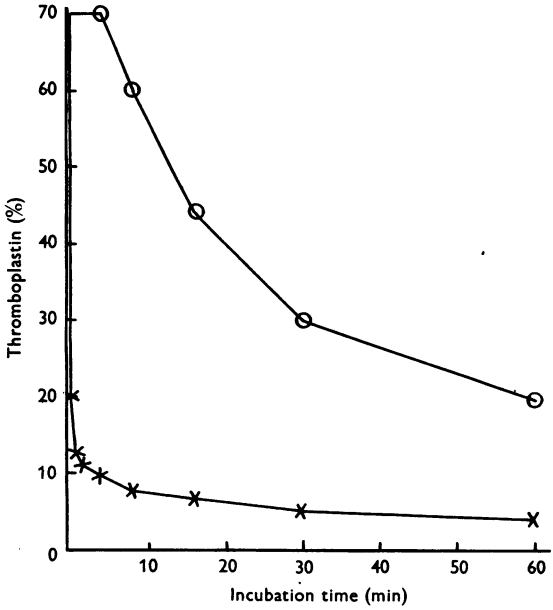

Fig. 3. Curves demonstrating the reduction of thromboplastic activity which occurs when blood thromboplastin is incubated at 37° C for 1 hr in the presence or absence of soya-bean inhibitor. The results illustrated in the upper curve were obtained by incubating 0·9 ml. of blood thromboplastin with 0·1 ml. of 0·85% NaCl. The results illustrated in the lower curve were obtained by incubating 0·9 ml. of the same blood thromboplastin preparation with 0·1 ml. of a solution of soya-bean inhibitor at a concentration of 12·5 mg/100 ml. to give a final concentration in the mixture of 1·25 mg/100 ml.

added to the reagents required for thromboplastin generation the general pattern of the resulting curves suggests that the main effect of the inhibitor is an increased speed of destruction of thromboplastin (Fig. 4). There is no change in the speed of thromboplastin formation.

Soya-bean inhibitor and thromboplastin formation. When soya-bean inhibitor is incubated with various combinations of the thromboplastin-generating components, and after incubation the mixtures are completed to contain all of the necessary factors, thromboplastin formation occurs though the speed of thromboplastin destruction is greatly increased (Fig. 5). These findings suggest that the inhibitor does not destroy any of the separate thromboplastin

constituents nor does it prevent any preliminary reactions of thromboplastin formation if these occur.

Soya-bean inhibitor and brain extract. When added to the substrate in testing the ability of brain extracts to accelerate the clotting of recalcified plasma, the soya-bean inhibitor must be present in high concentration to cause any recordable inhibition. It appears, therefore, that the inhibitor does not interfere with the formation of complete thromboplastin from brain and factors V and VII; neither can the inhibitor prevent the reaction of this formed thromboplastin with prothrombin. If, on the other hand, brain extract is first incubated

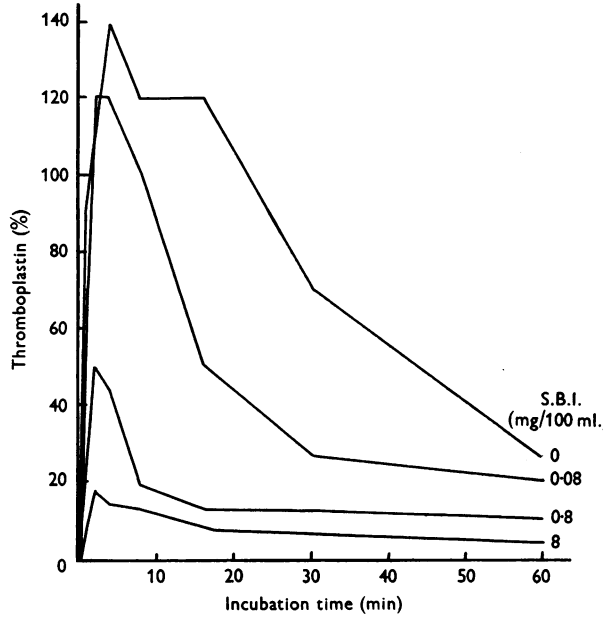

Fig. 4. Curves demonstrating the rate of formation of thromboplastin from all of the necessary blood constituents (factor V, antihaemophilic globulin, platelets and normal serum, and $CaCl_2$) and its disappearance in the presence of various concentrations of soya-bean inhibitor (S.B.I.). The concentrations of soya-bean inhibitor are calculated as mg/100 ml. in the mixture forming thromboplastin.

with factor V, serum and $CaCl_2$ and soya-bean inhibitor is added to the product of this reaction the activity disappears very rapidly (Fig. 6). The inhibitor therefore does not appear to prevent the formation of thromboplastin from brain extract but it destroys the formed thromboplastin rapidly.

Heparin

Heparin in association with a plasma co-factor is known to inhibit the thrombin–fibrinogen reaction; that it also probably interferes with thromboplastin formation is suggested by the fact that prothrombin is not converted to thrombin in the presence of an excess of heparin.

Heparin and the destruction of formed thromboplastin. In the presence of concentrations of heparin of $\frac{1}{2}$ or $\frac{1}{4}$ unit/ml., formed thromboplastin is rapidly destroyed. With concentrations less than these the effect is more gradual, being less marked in the first few minutes (Fig. 7).

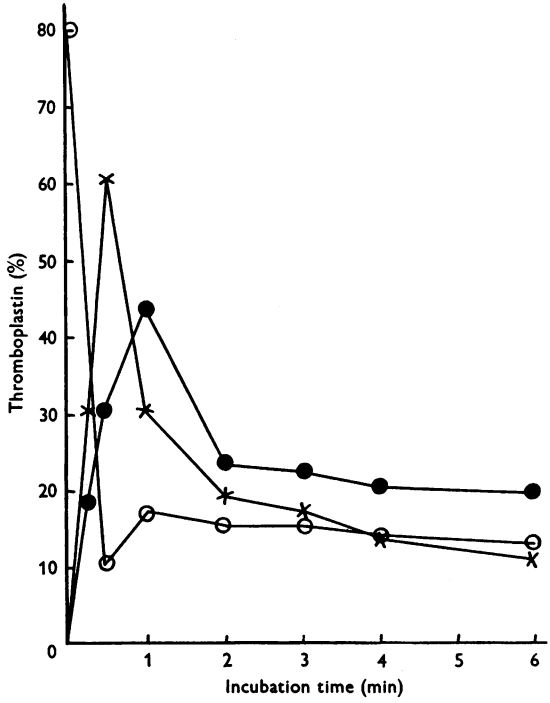

Fig. 5. Curves to show the formation of thromboplastin from reagents previously incubated with soya-bean inhibitor. The results represented × —— × were obtained by incubating factor V, platelets, normal serum and M/40-$CaCl_2$ with soya-bean inhibitor at a concentration of 1·5 mg/100 ml.; after 5 min antihaemophilic globulin was added and the formation of thromboplastin in the mixture was tested at various intervals. The results represented ●——● were obtained by incubating antihaemophilic globulin, normal serum and M/40-$CaCl_2$ with the same soya-bean inhibitor concentration; after 5 min factor V and platelets were added and the formation of thromboplastin was tested as before. The results represented ○——○ show the destruction of formed thromboplastin in the presence of the same concentration of soya-bean inhibitor.

Heparin and the formation of thromboplastin. In the presence of heparin, at a concentration as low as 1/30 unit/ml. in the mixture, the formation of thromboplastin is markedly depressed (Fig. 8). The formation of thromboplastin is inhibited by levels of heparin which have little effect on formed thromboplastin (see Fig. 7).

It therefore appears that heparin differs in its mode of action from soya-bean inhibitor. Heparin, in very low concentrations, prevents the formation of thromboplastin.

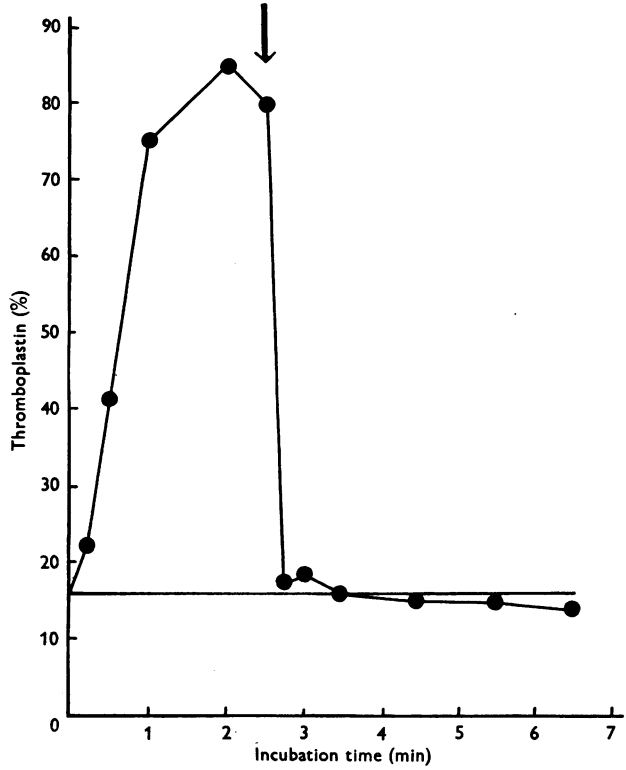

Fig. 6. The curve represents the results of incubating brain extract with factor V, normal serum and $CaCl_2$. When the maximum activity had developed, soya-bean inhibitor at a concentration of 5 mg/100 ml. was added (indicated by arrow) and the thromboplastic activity disappeared rapidly. The horizontal line represents the activity of the brain extract alone. In this experiment the clotting times have been interpreted in terms of thromboplastin concentration using a dilution curve.

DISCUSSION

If thromboplastin is defined as the direct activator of prothrombin then brain extract, Russell's viper venom and trypsin, the substances investigated here, are not complete thromboplastins. Brain extract will not activate prothrombin effectively in the absence of factors V or VII. Thrombin is formed rapidly by a mixture of prothrombin, factors V and VII, and brain extract, but a far more rapid generation of thrombin occurs if a mixture of brain extract, and factors V and VII is pre-incubated and then added to prothrombin. It is therefore probable that factors V and VII react with the brain preparation to form complete thromboplastin, and do not merely accelerate a direct reaction between brain extract and prothrombin as supposed by Owren (1947) and Koller *et al.* (1951). Since brain extract appears to react with two factors already shown to be required for the formation of blood thromboplastin, it

seems probable that brain and perhaps other tissue preparations appear to be 'thromboplastic' because they accelerate or by-pass one or more stages of blood thromboplastin generation. If this view is correct then brain extract is equivalent to a mixture of platelets, antihaemophilic globulin and Christmas factor, being able to operate in the absence of any or all of these factors. But thromboplastic activity is generated much more rapidly by a mixture of brain extract and factors V and VII than by the five components of the blood thromboplastin-forming system. It is therefore likely that the active principle

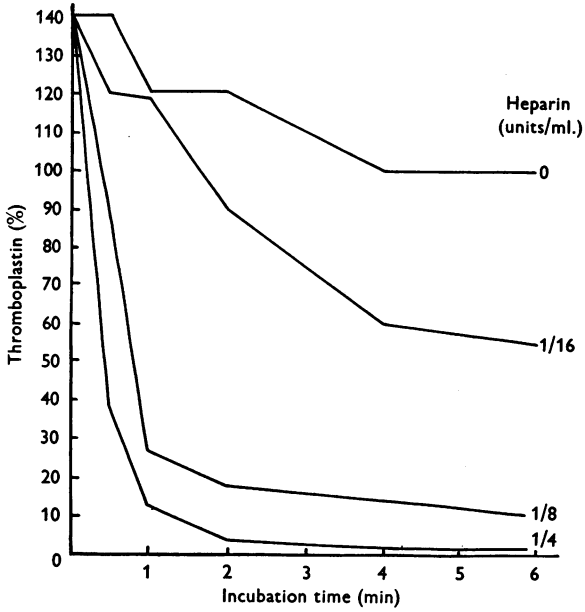

Fig. 7. Curves demonstrating the reduction in thromboplastic activity when formed (blood) thromboplastin is incubated at 37° C with various concentrations of heparin. In each experiment 0·9 ml. of thromboplastin was incubated with 0·1 ml. of saline or heparin solution. The concentrations of heparin indicated are the final concentrations in the mixtures.

of the brain extract is similar to, or may actually be, the product of a reaction between platelets, antihaemophilic globulin and Christmas factor. If the formation of this product is a relatively slow but necessary stage of the formation of thromboplastin in blood, the addition of the pre-formed product will greatly accelerate coagulation. Thus the clotting time of recalcified plasma is usually about 3 min, most of this time being occupied by thromboplastin formation. The clotting time of the plasma with added brain extract is about 15 sec, the shortening being due to the by-passing of the slow formation of an intermediate product. The clotting time of the plasma, to which has been added a pre-incubated mixture of brain extract, factors V and VII, may be as short

as 5 sec, this reduction being due to the elimination of the relatively fast reaction between brain and these two factors. The final 5 sec is presumably occupied by the reaction times of thromboplastin with prothrombin and of thrombin with fibrinogen.

The practical importance of brain extract lies in its use in the 'prothrombin-time' test, which is mainly applied in the control of anticoagulant therapy, and in the recognition of vitamin K deficiency. It is precisely because brain extract is not a complete thromboplastin that it is a valuable reagent in this

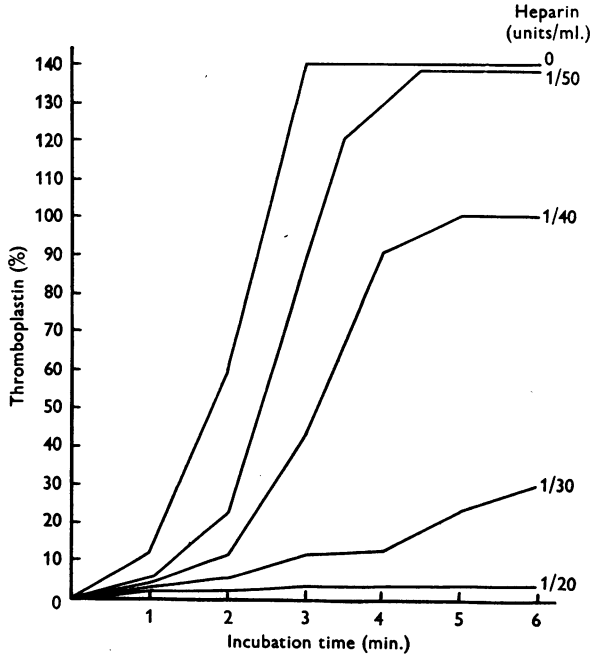

Fig. 8. Curves to show thromboplastin formation in the presence of various concentrations of heparin. The upper curve shows thromboplastin formation with no added heparin. The lower curves show the effects of different concentrations of heparin; the heparin concentrations are calculated per ml. of the mixture.

test. Being sensitive to a deficiency of factors VII and V, it reveals the response to anticoagulant therapy, the main effect of which is to reduce factor VII, and allows the recognition of factor V deficiency. If it were a complete thromboplastin, its use would be largely limited to demonstrating prothrombin deficiency, a rare and relatively unimportant condition.

The nature of the active principle of brain and other tissue extracts may well provide a clue to the nature of complete thromboplastin. Unfortunately, previous work which has sought to identify this active principle has been hampered by the lack of understanding of the action of so-called 'thrombo-

plastic' substances. Many workers, notably Howell (1912) and Chargaff, Bendich & Cohen (1944) have ascribed thromboplastic activity to various lipoid or lipo-protein fractions prepared from tissues or blood. It is probable that these preparations are not thromboplastin in the classical sense, but substitutes for one or other of the components required for thromboplastin formation, the accelerating effect of such substitutions being interpreted as thromboplastic activity. Thus a suitable lipoid or serum preparation, will accelerate the coagulation of a platelet-deficient, or factor VII-deficient system respectively, the apparent 'thromboplastic' action being conditioned by the unappreciated deficiencies of the test system used. With new knowledge available fresh investigations of the activity of relatively pure tissue and blood derivatives are likely to be rewarding.

Though both Russell's viper venom and trypsin have been regarded as thromboplastins in that they were thought to promote coagulation by activating prothrombin, they cannot be considered 'physiological' coagulants and their action may have no close parallel in the natural process of clotting. Russell's viper venom is remarkable for an extreme activity which can be demonstrated in dilutions of one in many millions. Like brain extract, the venom requires both factors V and VII for effective activation of prothrombin. Unlike brain extract the venom requires in addition the presence of platelets or a suitable lipoid substitute such as lecithin. It is interesting that lecithin will not act as a substitute for platelets in blood thromboplastin generation, but will form an intensely active thromboplastin with venom together with factors V and VII. The venom, in the presence of lipoid, appears to react more rapidly with factors V and VII than does brain, thus the clotting time of plasma with venom and lecithin is considerably shorter than with brain extract. Trypsin is also dependent on the presence of platelets, and factors V and VII, for the promotion of thromboplastin generation, and it also reacts extremely rapidly with these factors. Trypsin thus seems to resemble Russell's viper venom in its mode of action, but it is less critically dependent on the amount of factor V. In view of many suggestions that thromboplastic activity involves proteolytic action, it should be noted that Russell's viper venom, unlike trypsin, produces no obvious proteolysis.

The apparent antithromboplastic actions of heparin and soya-bean trypsin inhibitor are of interest. Heparin in relatively low concentrations produces a considerable delay in blood thromboplastin formation. It is much less effective as an inhibitor of pre-formed thromboplastin, though in high concentrations it will produce some destruction of this activity. Studies of the exact mode of action of heparin are complicated by its activity as an antithrombin, and by the fact that thrombin itself seems to catalyse thromboplastin generation (Biggs & Macfarlane, 1953). Further study is thus required. Soya-bean trypsin inhibitor is less difficult to investigate since it has little or

no inhibitory effect on thrombin. The anticoagulant action of this substance seems to be entirely due to its inactivation of formed thromboplastin. Thromboplastin generation is not delayed by the presence of the inhibitor, nor are the individual components of the reaction destroyed by it. The amount of thromboplastin demonstrated, however, is reduced in proportion to the amount of inhibitor present and the addition of the inhibitor to pre-formed thromboplastin destroys the activity whether this is generated solely from blood components or from mixtures including brain extracts or Russell's viper venom. This apparently specific inhibition of the activator of prothrombin in the absence of an effect on prothrombin itself (Macfarlane, 1947) strongly supports the view that complete thromboplastin is not a mixture of factors but an actual, if labile, substance which directly activates prothrombin.

SUMMARY

1. Thromboplastin is defined in this paper as a substance which is directly responsible for the conversion of prothrombin to thrombin in the presence of $CaCl_2$.

2. Experiments with brain extracts have suggested that this extract reacts with factors V and VII before it is capable of converting prothrombin to thrombin. Brain extract is therefore not itself thromboplastic but can be converted to thromboplastin after reaction with factors V and VII.

3. Experiments with trypsin and Russell's viper venom suggest that the action of these two substances is similar to that of brain but the reaction with factors V and VII is more rapid.

4. Soya-bean trypsin inhibitor, which is known to inhibit coagulation, appears to act by destroying formed thromboplastin.

5. Heparin in low concentrations prevents the formation of blood thromboplastin from its precursors.

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REFERENCES

Biggs, R., Douglas, A. S. & Macfarlane, R. G. (1953*a*). The formation of thromboplastin in human blood. *J. Physiol.* **119**, 89–101.

Biggs, R., Douglas, A. S. & Macfarlane, R. G. (1953*b*). The initial stages of blood coagulation. *J. Physiol.* **122**, 538–553.

Biggs, R. & Macfarlane, R. G. (1953). *Human Blood Coagulation and its Disorders.* Oxford: Blackwell's Scientific Publications.

Chargaff, E., Bendich, A. & Cohen, S. S. (1944). The thromboplastic protein: Structure, properties and disintegration. *J. biol. Chem.* **156**, 161–178.

Howell, W. H. (1912). The nature and action of the thromboplastic (zymoplastic) substance of the tissues. *Amer. J. Physiol.* **31**, 1–21.

Koller, F., Loeliger, A. & Duckert, F. (1951). Experiments on a new clotting factor (factor VII). *Acta haemat.* **6**, 1–18.

MACFARLANE, R. G. (1947). The action of soya-bean trypsin inhibitor as an antithromboplastin in blood coagulation. *J. Physiol.* **106**, 104–111.

MACFARLANE, R. G., TREVAN, J. W. & ATTWOOD, A. M. P. (1941). Participation of a fat soluble substance in the coagulation of the blood. *J. Physiol.* **99**, 7–8*P*.

MELLANBY, J. (1909). The coagulation of the blood. *J. Physiol.* **38**, 28–112, 441–503.

MORAWITZ, P. (1905). Die Chemie der Blutgerinnung. *Ergebn. Physiol.* **4**, 307–422.

OWREN, P. A. (1947). The coagulation of blood. *Acta med. scand.* Suppl. 194.

SCHMIDT, A. (1892). *Zur Blutlehre.* Leipzig. Cited by MORAWITZ, P. in *Ergebn. Physiol.* 1905, **4**, 307–503.

WARE, A. G., FAHEY, J. L. & SEEGERS, W. H. (1948). Platelet extracts, fibrin formation and interaction of purified prothrombin and thromboplastin. *Amer. J. Physiol.* **154**, 140–147.