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THE BIOCHEMISTRY OF THROMBOPLASTIN, ITS FORMATION AND ACTION

By P. A. Owren, S. I. Rapaport, P. Hjort and K. Aas.

Biggs and Macfarlane in their book [1] wisely warn the coagulation worker : " Always, it seems, the next will be the winning combination revealing the one clue that has been missed by everyone else, the flash of illumination that will show the long sought, simple explanation of blood coagulation. Undaunted by the perpetual postponement of this revelation, the investigator enters the last stage of his evolution as a coagulation worker, and becomes a maker of theories. " With this in mind we hesitate to offer the coagulation theory illustrated in figure 1. We recognize it as incomplete. Clotting factors which are still insufficiently characterized, such as the platelet accelerators and possible new plasma thromboplastin factors, have been omitted ; so has the whole system of clotting inhibitors. The theory will be changed whenewer it fails to explain the available facts. It is useful primarily for orientation : to explain how we think the various clotting factors interact and to stimulate tomorrow's experiments.

As figure 1 shows, the role of thromboplastin in this scheme is that of an intermediate activity which arises during clotting from the interaction of a platelet factor and af least two plasma factors, which is equivalent to the clotting activity of aqueous tissue extracts, and which does not directly convert prothrombin to thrombin. This is considerably different from Morawitz' (1904) concept of thrombokinase as an activity which is liberated fully formed either from injured platelets or from damaged tissue and which needs only calcium ions to activate prothrombin to thrombin. Howell (1912) [2] objected to the concept of thrombokinase as the direct and only activator of prothrombin and chose Nolf's term *thromboplastin* for " the extractible substance or substances in the tissues which facilitates the process of clotting ". Howell also showed that aqueous and ether extracts of tissues differ in that the former consist of a lipoid combined with a protein, while

the latter contains only the lipoid factor. Most coagulation workers today use the terms tissue thromplastin and tissue thrombokinase interchangeablely for the clotting activity of the more powerful aqueous

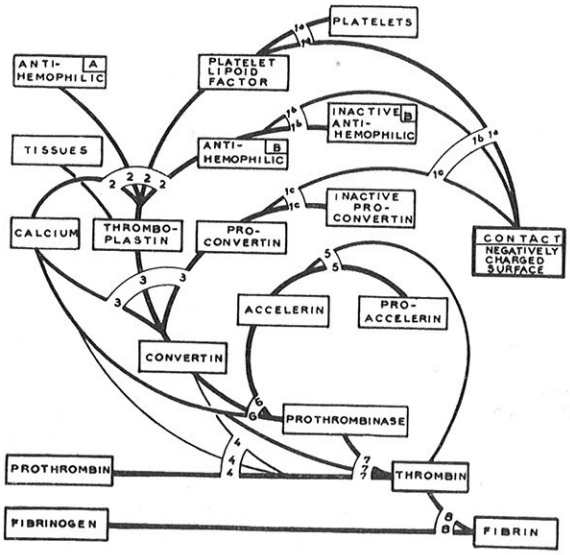

FIG. 1. — *Blood coagulation theory of* P. A. OWREN.

1. Contact initiates blood coagulation by *a)* disintegration of platelet and release of the platelet lipoid thromboplastin factor ; *b)* activation of the anti-hemophilic B factor (plasma thromboplastin component or PTC, CHRISTMAS factor) and *c)* activation of proconvertin (co-thromboplastin, SPCA, factor VII, the stable factor).

2. Tissue injury yields thromboplastin directly. In the blood the platelet lipoid factor, the anti-hemophilic A factor and the activated anti-hemophilic B factor interact in the presence of calcium to form thromboplastin.

3. Thromboplastin and proconvertin combine in the presence of calcium to form convertin.

4. Convertin together with calcium brings about a minimal conversion of prothrombin to thrombin.

5. This initially formed thrombin starts the accelerator system, i.e., the conversion of proaccelerin to accelerin.

6. Convertin and accelerin interact in the presence of calcium to form prothrombinase.

7. Prothrombinase in the presence of calcium produces accelerated conversion of prothrombin to thrombin.

8. Thrombin is now in sufficient quantity to convert fibrinogen to fibrin.
The system of inhibitors is not included in the scheme. The antihemophilic B factor and proconvertin are inactive in their native state probably because of binding to an inhibitor. Plasma also contains substances which inactivates thromboplastin, convertin, accelerin, prothombinase and thrombin.

extracts. Expressions such as " partial or incomplete thromboplastin " [3] have been suggested for the less active préparation such as the ether extract.

The effect of tissue thromboplastin upon blood coagulation has been extensively studied. Aqueous tissue extracts appear to provide the equivalent of the combination of the platelet lipoid factor (platelet thromboplastic factor, platelet factor III) the anti-hemophilic A factor

(anti-hemophilic globulin or AHG), and the anti-hemophilic B factor (plasma thromboplastin component or PTC). Tissue thromboplastin may also provide the equivalent of less well known thromboplastic factors recently described by Rosenthal, Dreskin and Rosenthal [4] (plasma thromboplastin antecedent or PTA), by Koller [5] (factor X) and by Spaet, Aggeler and Kinsell [6] (the IV thromboplastic factor). Whether these are identical or different factors is still to be determined.

Less is known about the chemistry of tissue thromboplastin. Chargaff and co-workers (1944) [7] have isolated a purified lipoprotein with thromplastic activity. Feissly (1945) [8] has also confirmed that thromboplastin is a lipoprotein. However, neither the protein nor the lipoid component has been well characterized. Most workers have considered the lipoid to be cephalin but Fisher and Hecht (1934) [9] found that purification of cephalin resulted in a progressively decreasing activity.

Clotting in the absence of tissue thromboplastin is initiated by contact with a foreign surface. It is an assumption which remains to be proved, that one step in the process is the combination to form one activity of those clotting factors which are circumvented by tissue thromboplastin (platelet lipoid factor and the anti-hemophilic A and B factors). This hypothetical intermediate activity has been called plasma thromboplastin in figure 1 because it is equivalent in effect to tissue thromboplastin and, like tissue thromboplastin, must enter into secondary reactions with proconvertin and proaccelerin to form the final prothrombin converting principle. In contrast to this definition, Biggs and Macfarlane (1953) have given the name plasma thromplastin to the over all prothrombin converting activity which arises during clotting, including the effect produced by proaccelerin and proconvertin. Plasma thromplastin activity in this sense is what we have termed in figure 1 prothrombinase.

THE EFFECT OF SURFACE CONTACT UPON CLOTTING

It has been known for a long time that contact with a glass surface not only liberates platelet clotting factors but also changes the activity of plasma clotting factors. A knowledge of these plasma changes is essential to understand plasma thromboplastin formation and action.

Figure 2 illustrates an experiment which confirms that a foreign surface is needed for rapid prothrombin conversion despite an excess of platelet thromboplastin factor. Exposure of a platelet poor plasma to glass is seen to make the plasma more sensitive to the effect of platelet extract. The main effect is a shortening of the latent period

before the phase of rapid thrombin formation begins. The velocity of thrombin formation is less influenced for this depends upon the activity of the proaccelerin-accelerin system. The sudden small drop in prothrombin concentration in figure 2 which occurs immediately after recalcification is caused by an adsorption of prothrombin onto the freshly formed calcium oxalate (Aas, 1952 [10]), and does not occur if citrate is used as the anticoagulant.

The specific effect on plasma produced by glass contact has been

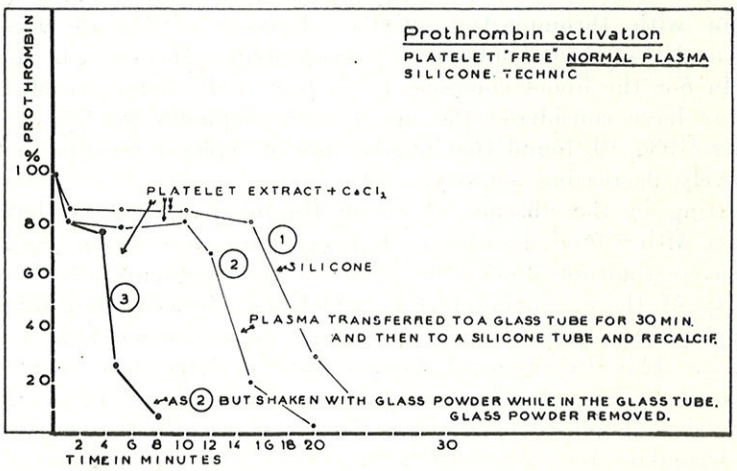

Fig. 2.

1. Prothrombin conversion is delayed in recalcified plasma added platelet extract if contact is minimal.

2. Contact with a glass surface for 30 min. increases the reactivity of platelet free plasma to platelet extract.

3. Prior exposure to quartz glass powder greatly increases the reactivity of a platelet " free " plasma to platelet extract.

variously ascribed to the removal of an antithromboplastin (Tocantins, 1945 [11]), to the removal of an inhibitor of prothrombin (Fiala, 1951 [12]), to the activation of a hypothetical precursor of prothrombin, " prothrombinogen " (Quick and Stefanini, 1949 [13]), to the activation of " thromboplastinogen " (Quick and Epstein, 1952 [14]), and to the activation of the " Christmas factor " (anti-hemophilic B factor) (Biggs, Douglas and Macfarlane, 1953 [15]).

Rapaport et al. (1954) [16] reinvestigated the effect of glass contact upon the various plasma clotting factors. Both the anti-hemophilic B factor and proconvertin were shown to be activated by glass contact, and are therefore assumed to exist in the circulation in a less active

form. Glass contact did not influence anti-hemophilic A factor, prothrombin or proaccelerin activity.

The effect of glass is shown by the experiment summarized in table I. A sample of platelet poor plasma was divided into two portions, one of which was exposed only to silicone coated surfaces and the other portion of which was shaken with 1/4 volume of fine quartz glass powder for 15 minutes and the powder then removed by centrifugation. The two portions were then tested in one stage assay systems for the known plasma clotting factors.

TABLE I. — *Activation of proconvertin, and antihemophilic B factor by glass.*

TEST SYSTEM	CLOTTING TIMES: " Silicone " plasma sec.	CLOTTING TIMES: " Glass " plasma sec.
1. QUICK's ,' prothrombin time " (1)	13.9	10.4
2. P. and P. method (OWREN and AAS, 1951 [31]) (2).....	30.5	24.2
3. Specific method for proconvertin (OWREN and AAS, 1951 [31])..	*36.3*	*28.2*
4. Specific method for prothrombin (OWREN and AAS, 1951 [31])..	31.7	31.8
5. Specific method for proaccelerin.. (OWREN, 1947 [19])	29.0	29.7
6. Specific method for antihemophilic A factor (RAPAPORT and al., 1954, [17])	109.2	104.0
7. Specific method for antihemophilic B factor (RAPAPORT and al., 1954 [17])	*116.0*	*76.0*

(1) QUICK's method. The clotting time depends upon the combined activity of prothrombin, proconvertin and proaccelerin.

(2) P. and P. method (Prothrombin and proconvertin method). The clotting time depends upon the combined activity of prothrombin and proconvertin.

Table I shows that the increase in proconvertin activity is reflected in every system which is sensitive to proconvertin, i.e. the specific proconvertin system, the prothrombin and proconvertin system (P. and P). system and QUICK's " prothrombin time " test. No change is seen in the prothrombin and proaccelerin systems because in both of these systems any change in proconvertin activity of the test sample will be masked by an excess of proconvertin in the other reagents used,

and the change in activity of the antihemophilic B factor is masked by the tissue thromboplastin reagent. The anti-hemophilic A and B factor activities were measured in test systems which took into account the associated increase in proconvertin activity.

TABLE II. — *Activation of proconvertin by glass.*

	PROCONVERTIN TIME	
	" Silicone " plasma	" Glass activated " plasma
Normal plasma	36.3	28.2
Hemophilia A plasma	34.5	26.5
Hemophilia B plasma	31.5	26.5
Parahemophilia plasma	31.5 (1)	26.5
Hypoproconvertinemia plasma (2 % proconvertin)	107.0	79.5

(1) Plasma stored in glass.

The increase in proconvertin activity produced by glass was confirmed by finding the same results when hemophilia A, hemophilia B and parahemophilia plasmas were tested (see table II). The very long proconvertin time of congenital hypoproconvertinemia plasma was moderately reduced because of the activation of the small amount of

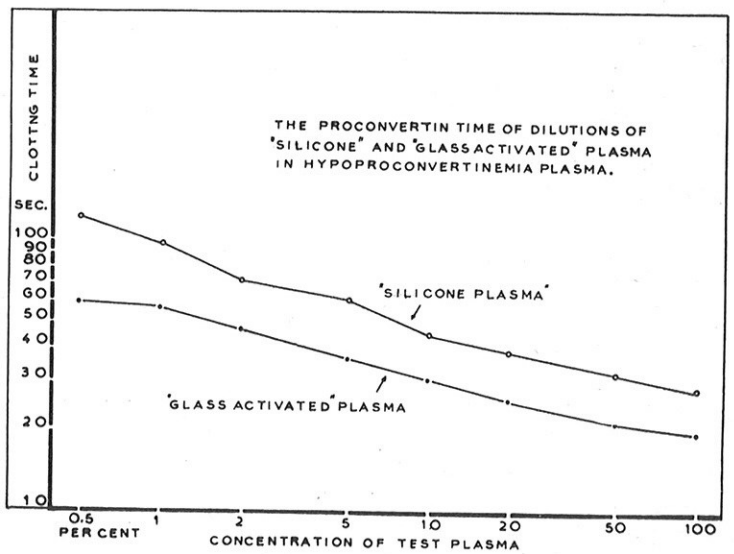

FIG. 3. — The difference in proconvertin time betwen " silicone " plasma and " glass activated " plasma persists on variation of the proconvertin concentration.

proconvertin which this plasma contains. Furthermore, when normal " silicone " plasma and normal " glass activated " plasma were diluted in congenital hypoproconvertinemia plasma the difference between the two plasmas persisted (see figure 3). Such dilution provides an excess of every known plasma factor except proconvertin.

The activation of proconvertin explains why Alexander et al., (1949) [18] found the development of SPCA activity during the storage of plasma. By exposure to a large glass surface, as in the above experiments, the same activation is accomplished in a few minutes. Our findings definitely contradict the theory of Quick and Stefanini who ascribe the shortening on storage to an activation of prothrombinogen to prothrombin, a theory also advocated by Lewis and Ware (1954) [19].

THE INTERACTION OF THROMBOPLASTIN AND PROCONVERTIN TO FORM CONVERTIN

There is much evidence that neither tissue nor plasma thromboplastin acts directly upon prothrombin but first interacts with proconvertin to form a reaction product which has been called convertin. This evidence is reviewed below because some coagulation workers still do not accept the existence of either proconvertin or convertin.

During early investigations in our laboratory upon the formation of the final prothrombin converting principle, factor VI or prothrombinase [20], the formation of prothrombinase was found to require two plasma factors besides thromboplastin and calcium. These factors were provisionally named factor V and co-factor V and later proaccelerin and proconvertin (Owren, 1947 [20,21]). When proconvertin was separated from prothrombin (Owren and Bjerkelund, 1949) [22]) proconvertin's effect upon thrombin formation was further analysed. The interaction of thromboplastin and proconvertin to form convertin was then confirmed by experiments upon parahemophilia plasma where there is no disturbance caused by the proaccelerin-accelerin system (Owren, 1950 [23]). The formation of convertin and its effect upon prothrombin conversion has since been further studied in blood and plasma and by the use of isolated factors (Aas, 1952 [10], Owren, 1952 [24]).

Findings from several other laboratories also indicated the existence of proconvertin and its reaction with thromboplastin Jacox (1949) [25] demonstrated an increase in the activity of brain thromboplastin after incubation with serum, a finding suggestive of the presence of a thromboplastin co-factor in serum. Alexander and co-workers (1948, 1949, 1950) [26, 18, 27] in more extensive studies demonstrated the presence of a clotting factor in serum which was termed the serum

prothrombin conversion accelerator or SPCA, the activity of which is greatly increased by incubation with thromboplastin. MANN and co-workers (1949, 1951) [28, 29] also demonstrated that preliminary mixing of tissue thromboplastin with serum potentiates its effect on the convertion of prothrombin to thrombin. The serum factor which increased the activity of thromboplastin was found to be reduced in dicumarol plasma. MANN regarded it as part of the thromboplastin complex and named it " co-thromboplastin activity ".

Part of the confusion which exists about proconvertin and its interaction with thromplastin stems from an attempt to distinguish a single plasma form and a single serum form of this activity. Thus, ALEXANDER refers to a plasma precursor of SPCA and SPCA, and KOLLER to a plasma precursor of factor VII and factor VII. Furthermore, the term proconvertin has been misunderstood by some to mean only the activity in plasma while the term convertin has been confused with proconvertin as it exists in serum.

We have found it useful to refer to proconvertin activity in the following way. As shown above, proconvertin exists in the circulating blood in a less active form, probably because it is combined with an inhibitor. This form was call " *inactive proconvertin* ". Plasma prepared by silicone technique should contain only " *inactive proconvertin* ". When plasma is exposed to glass there is a gradual increase in proconvertin activity probably caused by the adsorption of the inhibitor onto the glass. Plasma after contact with glass, therefore, contains a mixture of " *inactive proconvertin* " and what we call " *active proconvertin* ". The content of the latter increases as the plasma stands and this explains the shortening of the QUICK " prothrombin time " during the first hours plasma is stored.

The proconvertin activity of serum will depend upon how the blood is clotted and how long after clotting the serum is examined. When blood clots spontaneously in silicone coated tubes the serum will contain mainly " *inactive proconvertin* ". When blood clots in contact with glass the serum will contain a mixture of " *inactive proconvertin* " and " *active proconvertin* ". However, if blood is clotted by the addition of tissue thromboplastin the resultant serum will contain only " *active proconvertin* " because the excess thromboplastin in the presence of calcium completely activates proconvertin.

Furthermore, fresh serum will contain not only proconvertin in either or both of its forms but also convertin, a qualitatively different activity formed by the combination of thromboplastin with " *active proconvertin* " in the presence of calcium. The amount of convertin in fresh serum varies with the amount of thromboplastin formed during clotting or added to the plasma. As the serum stands the convertin is inactivated ans this will tend to lessen the accelerator effect of the serum. How-

ever, at the same time, any inactive proconvertin in the serum is being activated by contact with glass and this will tend to increase the serum activity. Thus, it is apparent that one can not generalize about serum activity. Rather it is necessary to analyze the effect of a given serum in the light of the knowledge of when and how it was prepared.

TABLE III. — *The formation of convertin by incubation of thromboplastin and proconvertin in the presence of calcium.*

Incubation time (37° C)	CLOTTING TIMES produced by incubation of: Thromboplastin + Proconvertin with calcium	Thromboplastin + Proconvertin without calcium
0 sec.	22.4 sec.	22.4 sec.
45 —	12.2 —	22.4 —
90 —	10.8 —	21.5 —
180 —	8.1 —	20.0 —
270 —	8.1 —	21.0 —
360 —	8.0 —	22.8 —

Test system: 0.2 ml proconvertin deficient plasma;
0.2 — adsorbed ox plasma;
0.2 — incubation mixture;
0.2 — $CaCl_2$.

Table III summarizes an experiment which illustrates the formation of convertin on incubating tissue thromboplastin, proconvertin and calcium. Human brain thromboplastin was prepared by a technique previously described (OWREN, 1947, 1949 [20, 31]). The proconvertin reagent was prepared from serum obtained by adding a small amount of tissue thromboplastin to normal blood, followed by high speed centrifugation and storage for 8 days at room temperature. The proconvertin was adsorbed from the serum by barium sulfate, eluted with 0.2 M sodium citrate and dialysed against saline. This preparation was free of prothrombin and accelerin.

The proconvertin-convertin activity was tested by a modification of the specific proconvertin assay of OWREN and AAS (1949) [32]. The system consisted of 0.2 ml plasma from a patient with congenital hypoproconvertinemia (supplied prothrombin in excess), 0.2 ml of the mixture to be tested in an appropriate dilution,0.2 ml of adsorbed ox plasma (stabilizes the proaccelerin concentration) and 0.2 ml of calcium chloride. The clotting time obtained in this system will measure only convertin activity if the mixture to be tested is free of thromboplastin. If 0.2 ml of brain thromboplastin is added to the test system, or if thromboplastin is added along with the test mixture, the clotting time obtained will reflect the activity of both proconvertin and convertin.

Table 3 shows that incubation of thromboplastin and proconvertin in the presence of calcium produces a powerful clotting activity far greater than that of a mixture of thromboplastin and proconvertin in the absence of calcium. The generation of this activity is time consuming. It is this new activity which is called convertin.

TABLE IV. — *Sedimentation of convertin by high speed centrifugation of an incubation mixture of thromboplastin, proconvertin and calcium.*

	CLOTTING TIMES in the proconvertin-convertin test system	
	Incub. with Ca.	Incub. without Ca.
Incubation mixture	12.0	22.8
Sediment (washed twice)	*14.0*	*65.5*
Supernatant	29.5	96.5

Table 4 demonstrates that convertin can be sedimented by high speed centrifugation. The sediment from the incubation mixture with calcium, after being washed twice and resuspend to its original volume, exhibited only a slightly reduced convertin activity. In contrast, the sediment in the control experiment without calcium consisted only of thromboplastin as shown by the clotting time of 65 seconds on its addition to proconvertin deficient plasma.

Convertin can be shown to be different from a simple mixture of thromboplastin and proconvertin in another way. RAPAPORT et al. (1954) [33] have found that the lipid inhibitor described by TOCANTINS blocks the reaction between tissue thromboplastin and proconvertin to form convertin, but is completely ineffective in the presence of preformed convertin. Thus, in table V, convertin, both before and after sedimentation, is seen to be resistant to the effect of the lipid inhibitor. The use of the lipid inhibitor provides a simple method to distinguish between proconvertin and convertin activity.

TABLE V. — *The immunity of convertin to the lipid inhibitor of brain.*

	CLOTTING TIME in the proconvertin-convertin system
Thromboplastin + proconvertin + Ca. incubated for 6 min.	12 sec.
Lipid inhibitor of brain: Added before incubation	58.5 —
Added after incubation	11.5 —
Convertin, sedimented, washed and resuspended	13.5 —
Convertin + lipid inhibitor	14.0 —

Convertin in saline solution is stable for at least 5 hours at 37° C and at least 2 days at 6° C. However, if convertin is added to citrated normal plasma there is a reduction to less than 50 % of the original activity in one hour. Convertin differs from thromboplastin and proconvertin in its lability to heat. Convertin is almost completely destroyed by heating for 1 hour at 56° C, whereas proconvertin in saline solution loses only about 50 % of its activity. Brain thromboplastin, as measured by its activity in one stage clotting systems, is unchanged after such heating.

In the past our use of the terms proconvertin and convertin has been misunderstood by some to mean that proconvertin is activated to convertin by an effect of thromboplastin without the latter entering as an integral part of the convertin complex. The sedimentation experiments (see table IV) strongly suggest the opposite, i.e., that convertin is a particulate substance formed by the specific binding of proconvertin onto the surface of the thromboplastin particle by calcium. This mechanism of convertin formation explains the quantitative interrelationship between thromboplastin, proconvertin and convertin previously demonstrated by Aas (1952) [10] and Owren (1952) [24].

In contrast, no specific time consuming reaction has been demonstrated between thromboplastin and proaccelerin or between thromboplastin and accelerin. However, when tissue thromboplastin is sedimented by high speed centrifugation it can carry down with it a little proaccelerin or accelerin from purified solutions, or from plasma or defibrinated plasma. This is probably an unspecific adsorption for the proaccelerin or accelerin can be largely removed by repeated washings.

The initial phase of slow thrombin formation by convertin and calcium

Convertin and calcium can convert prothrombin to thrombin slowly in the absence of proaccelerin or accelerin. This can be demonstrated with our particular parahemophilia plasma which is completely free of proaccelerin. (Thus the addition of thrombin to this plasma does not increase its activity as would occur if even a trace of proaccelerin were activated to accelerin. Storage of the plasma does not lengthen its one stage " prothrombin time " as would be expected if any proaccelerin were inactivated by the storage. Johnson and Seegers (1953) [34] could find no Ac-globulin in this plasma by their method.)

This plasma was prepared platelet " free " by silicone technique and prolonged high speed centrifugation (40,000 rpm for 1 hour) to remove any possible influence of a platelet accelerator. When a 0,11 gm % suspension of crude cephalin which had been boiled for 5 minutes was

added to this plasma clotting occurred about 8 minutes after recalcification. When brain thromboplastin which had been boiled for 5 minutes was added to the plasma clotting occurred about 100 seconds after recalcification. Furthermore, prothrombin prepared completely free of proaccelerin will slowly form thrombin although the rate of thrombin is greatly increased by even small amounts of added accelerin (Aas, 1952 [10]).

THE INTERACTION OF CONVERTIN AND ACCELERIN TO FORM PROTHROMBINASE

Convertin and accelerin can combine to form an activity which can be isolated by high speed centrifugation and which can rapidly clot plasma deficient in any of the known clotting factors except prothrombin and fibrinogen. Table VI summarizes an experiment which illustrates the action of prothrombinase. Convertin was prepared by the incubation of tissue thromboplastin and proconvertin with calcium as described above. Accelerin was made by the addition of thrombin to adsorbed prothrombin and proconvertin free ox plasma. The accelerin was then precipitated by dilution and acidification. Solutions of convertin and accelerin were mixed in the presence of calcium and then submitted to high speed centrifugation. The resultant sediment was washed 4 times and a suspension of the washed sediment was added with calcium to different plasmas defective in the various

Table VI. — *Comparison of the clotting promoting effect of brain thromboplastin, convertin, Russel viper venom and prothrombinase on various pathological plasmas.*

	Plasmas recalcified without thromboplastin					
	Normal	Hemophilia A	Hemophilia B	Proconvertin deficient	Proaccelerin deficient	Fibrinogen
Buffer	Ca. 1½ min.	Ca. 10 min.	Ca. 10 min.	1½-2 min.	Ca. 8 min.	> 8 min.
Thromboplastin	15.0 sec.	15. sec.	14.9 sec.	65.0 sec.	70.0 sec.	> 8 min.
Convertin sedimented, washed and resuspended)	11.5 sec.	11.4 sec.	11.7 sec.	11.9 sec.	40.5 sec.	> 8 min.
Russel viper venom + crude cephalin.	5.0 sec.	5.1 sec.	5.3 sec.	5.2 sec.	40 sec.	> 8 min.
Prothrombinase (sedimented, washed abd resuspended).	7.2 sec.	7.6 sec.	7.2 sec.	7.9 sec.	8.0 sec.	> 8 min.

clotting factors. As table VI shows, prothrombinase produced rapid prothrombin conversion in every plasma. Prothrombinase activity is

independent of the thromboplastin, proconvertin or proaccelerin content of the plasma. The clotting activity of thromboplastin, convertin and Russell viper venom plus crude cephalin have also been included in table VI to show the relation of each to prothrombinase.

Prothrombinase is relatively stable in saline solution but is inactivated when added to plasma or serum.

CONCLUSIONS

1. Tissue thromboplastin has been defined as the clotting activity present in aqueous tissue extracts. Plasma thromboplastin has been defined as a clotting activity which may arise during spontaneous clotting from a platelet factor and at least 2 plasma factors, which is equivalent in activity to tissue thromboplastin, and which does not directly convert prothrombin to thrombin.

2. The place of thromboplastin in the clotting scheme presented in figure 1 has been discussed in detail. The relation of thromboplastin activity to the changes produced by contact of plasma with a foreign surface such as glass has been analyzed.

3. Proconvertin activity has been redefined. The reaction between thromboplastin and proconvertin to form convertin has been shown to involve the binding of proconvertin onto thromboplastin by calcium.

4. Convertin and accelerin have been found to combine to form a sedimentable activity called prothrombinase which in the presence of calcium can rapidly clot plasma in the absence of thromboplastin, or its precursors, proconvertin or proaccelerin.

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REFERENCES

[1] Biggs (R.) and Macfarlane (R. S.). *Blood Coagulation.* Blackwell Scientif. Publ. Oxford, 1953.
[2] Howell (W. H.). *Am. J. Physiol.*, **31**, 1 (1912).
[3] Langdell (R. D.), Wagner (R. H.) and Brinkhous (K. M.). *J. Lab. and Clin. Med.*, **41**, 637 (1953).
[4] Rosenthal (R. L.), Dreskin (O. H.) and Rosenthal (N.). *Proc. Soc. Exp. Biol. and Med.*, **82**, 171 (1953).
[5] Koller (F.). *Blood*, **9**, 286 (1954).
[6] Spaet (F. H.), Aggeler (P. M.) and Kinsell (B. G.), cit. : Aggeler (P. M.), White (S. G.) and Spaet (T. H.). *Blood*, **9**, 246 (1954).
[7] Chargaff (E.), Bendich (A.) and Cohen (S. S.). *J. Biol. Chem.*, **156**, 161 (1944).
[8] Feissly (R.). *Helv. Med. Acta*, **12**, 215 (1945).
[9] Fischer (A.) and Hecht (E.). *Biochem. Z.*, **269**, 115 (1934).

[10] Aas (K.). *Prokonvertin og Konvertin.* Akademisk Trykningscentral, Oslo, 1952.
[11] Tocantins (L. M.). *Am. J. Physiol.*, **143**, 67 (1945).
[12] Fiala (S.). *Nature*, **167**, 279 (1951).
[13] Quick (A. J.) and Stefanini (M.). *J. Lab. and Clin. Med.*, **34**, 1203 (1949).
[14] Quick (A. J.) and Epstein (E.). *J. Applied Physiol.*, **4**, 840 (1952).
[15] Biggs (R.), Douglas (A. S.) and Macfarlane (R. G.). *J. Physiol.*, **122**, 538 (1953).
[16] Rapaport (S. I.), Aas (K.) and Owren (P. A.). *Scand. J. Lab. Clin. Inv.*, **6**, 82 (1954).
[17] Rapaport (S. I.), Aas (K.) and Owren (P. A.). (To be published.)
[18] Alexander (B.), De Vries (A.) and Goldstein (R.). *Blood*, **4**, 739 (1949).
[19] Lewis (H. L.) and Ware (A. G.). *Blood*, **9**, 520 (1954).
[20] Owren (P. A.). The coagulation of blood, *Acta Med. Scandinav.*, suppl. 194 (1947).
[21] Owren (P. A.). *Bull. Acad. Suisse d. Sc. Méd.*, **3**, 163 (1947).
[22] Owren (P. A.) and Bjerkelund (C.). *Scandinav. J. Clin. and Lab. Invest.*, **1**, 162 (1949).
[23] Owren (P. A.). *Proc. of the third Internat. Congress of the International Society of Hematology* at Cambridge, England, 1950, p. 22, 379-390, 475. New-York, 1951. Grune and Stratton.
[24] Owren (P. A.). *Tr. 5th Conf. on Blood Clotting and Allied Problems.* Josiah Macy Foundation, 1952, p. 92.
[25] Jacox (R. F.). *J. Clin. Investig.*, **28**, 493 (1949).
[26] Alexander (B), De Vries (A.) and Goldstein (R.). *J. Clin. Investigation*, **27**, 523 (1948).
[27] Alexander (B.), Goldstein (R.). and Landevehr (G.). *J. Clin. Investigation*, **29**, 881 (1950).
[28] Mann (F. D.). *Am. J. Clin. Path.*, **19**, 861 (1949).
[29] Mann (F. D.) and Hurn (M.). *Am. J. Physiol.*, **164**, 105 (1951).
[30] Koller (F.), Loeliger (A.) and Duckert (F.). *Acta Haemat.*, **6**, 1 (1951).
[31] Owren (P. A.). *Scand. J. Clin. Lab. Inv.*, **1**, 81 (1949).
[32] Owren (P. A.) and Aas (K.). *Scand. J. Clin. Lab. Inv.*, **3**, 201 (1951).
[33] Rapaport (S. I.), Aas (K.) and Owren (P. A.). *J. Lab. and Clin. Med.* (In print.).
[34] Johnson (J. F.) and Seegers (W. H.). *Michigan State M. J.*, **52**, 537 (1953).

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