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Evidence that Platelet Accelerator (Platelet Factor 1) Is Adsorbed Plasma Proaccelerin

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THE CLASSIC COAGULATION THEORY of Morawitz[1] presumes that platelets contain a powerful preformed thromboplastin (thrombokinase). However, Quick reported in 1947[2] that platelets contain little, if any, preformed thromboplastin but supply an enzyme which reacts with a plasma thromboplastin precursor to form thromboplastin. Many workers now believe that platelets supply a thromboplastic factor equivalent in clotting activity to ether extracts of tissue, that is, to Howell and McLean's "cephalin."[3] Itself weak, this factor is thought to interact in an as yet unknown manner with at least two and possibly more plasma factors to produce a powerful clotting activity comparable to saline brain extract thromboplastin.

The possibility that the role of platelets in clotting extends beyond supplying a thromboplastic factor arose when Feissly[4] found both a heat stabile and a heat labile clotting factor in platelet preparations. In 1947, Mann, Hurn and Magath[5] reported that platelet extracts shorten the long thromboplastin time of stored plasma, that is, can substitute for proaccelerin (plasma Ac-globulin, labile factor) activity. Three platelet clotting factors were described by Ware, Fahey and Seegers.[6] They found, in addition to the thromboplastic factor, a platelet accelerator or platelet factor 1 that acts like serum Ac-globulin (accelerin) and a platelet factor 2 that speeds the conversion of fibrinogen to fibrin by thrombin. The existence of these factors has been confirmed[7, 8] and widely accepted.[9, 10, 11]

Ware and his co-workers reported that the platelet accelerator and serum Ac-globulin, although acting alike, are entirely different proteins. McClaughry and Seegers stated[12] that platelet accelerator is "an integral part of the platelets." However, Mann, Hurn and Barker[13] and also Biggs and Macfarlane[14] suggested, but offered no evidence for, an alternate possibility, that platelet accelerator might be adsorbed plasma proaccelerin.

The data to follow strongly support this adsorption hypothesis. Platelet accelerator is shown to behave like proaccelerin (plasma Ac-globulin) rather than accelerin (serum Ac-globulin). Naturally occurring and artificially prepared accelerator-free platelets are found to adsorb proaccelerin readily from citrated plasma. This adsorbed proaccelerin is not removed by washings, and acts like the platelet accelerator.

Reagents

Platelets. Human venous blood was obtained after stasis of less than one minute by a clean puncture with a 19 gauge, Arquard 2 C (Armour) coated needle. The first five ml. were discarded and then 27 ml. were drawn directly into a siliconized ice cooled tube containing three ml. of a 3.13 per cent (w/v) sodium citrate solution. This was centrifuged immediately

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for 30 minutes at 900 r.p.m. (ca. 247 *g*) in a refrigerated centrifuge. The plasma was removed and recentrifuged twice at 700 r.p.m. (ca. 150 *g*) for 15 minutes. This platelet-rich plasma was transferred to lusteroid tubes which were spun for 15 minutes at 15,000 r.p.m. (ca. 15,000 *g*) at 2 C. The resultant platelet-poor plasma was removed. The platelet sediment was broken up carefully with a thin, siliconized glass rod. The platelets were washed four times with three ml. aliquots of a fluid made up of two parts veronal buffer, two parts 25.6 mM sodium citrate solution and six parts 0.9 gm. per cent saline. The platelets were then resuspended in three ml. of the same fluid, that is, in a volume $\frac{1}{10}$ that of the original citrated blood. This homogeneous suspension contained about 750,000 platelets per cu. mm. and, on microscopic examination, very few platelet aggregates.

Normal platelet-poor citrated plasma. The platelet-poor plasma obtained during the procedure described above was centrifuged for 60 minutes more at 40,000 r.p.m. (ca. 105,000 *g*). The resultant plasma contained only 250–700 platelets per cu. mm.

Proaccelerin-deficient citrated plasma. This plasma was prepared from a patient with parahemophilia in whose plasma no proaccelerin, but normal amounts of the other clotting factors, could be demonstrated.[15]

Proconvertin-deficient citrated plasma was prepared from a patient with congenital hypoproconvertinemia. This plasma contains only about two per cent of proconvertin but normal amounts of all other clotting factors.[16, 17]

Proconvertin-free citrated ox plasma was prepared by filtration once through a 20 per cent and twice through a 50 per cent asbestos filter pad as described previously.[18] This plasma was used in the proconvertin assay to stabilize the proaccelerin concentration. Because it still contains a little prothrombin it cannot be used for the specific prothrombin assay with Russell's viper venom.[19] For this test *prothrombin-free oxalated ox plasma* was prepared by filtration once through a 20 per cent asbestos filter followed by adsorption with 75 mg. $BaSO_4$ (Baker) per ml. of plasma.

Thromboplastin. A saline-buffer suspension of human brain was prepared as described previously[20] and stored in the liquid frozen state at −20 C.

Russell's viper venom-"cephalin" reagent. Russell's viper venom (Stypven, The Burroughs Wellcome Co.) was diluted 1 to 40,000 in a 0.09 Gm. per cent suspension of crude "cephalin." The details of preparation have been described elsewhere.[19]

Thrombin. Hemoclaudan (Leo, Copenhagen) was dissolved in saline and dialyzed overnight against four changes of saline at 4 C.

Trypsin. Trypure (Novo) which contains 22–25 Anson units per gram was dissolved in veronal buffer.

Purified proaccelerin. This was prepared by the method of Owren.[15]

Veronal buffer was made by mixing sodium diethyl barbiturate 11.75 Gm., sodium chloride 14.67 Gm., 0.1 N HCl 430 ml. and distilled water to 2,000 ml. This buffer's pH is 7.35 and ionic strength is 0.154.

Calcium chloride dissolved in distilled water was made up to optimal strength (25–35 mM) for each clotting system.

Methods

The technic of the specific one-stage assays used in these experiments is summarized in table 1. These one-stage systems for proaccelerin, proconvertin and prothrombin provide optimal concentrations of all of the clotting factors except the one to be measured. The clotting times are translated to concentrations in per cent by reference to correlation graphs made from various dilutions of a standard normal plasma.

Platelet accelerator activity was measured in the proaccelerin assay system. (This system will also measure accelerin activity.) The dilution of the platelet suspension by the other reagents of the clotting mixture eliminated any effect of platelet factor 2 as shown by the absence of acceleration of thrombin conversion of fibrinogen to fibrin. Any effect of the platelet thromboplastic factor was masked by the powerful tissue thromboplastin used in the test.

All experiments were done at 37 C. and at least in duplicate.

TABLE 1.—*Technic of one-stage assays for proaccelerin, proconvertin and prothrombin*

Reagents	ml. added to the system for the assay of		
	Proaccelerin	Proconvertin	Prothrombin
Thromboplastin	0.2	0.2	—
Russell's viper venom in "cephalin"	—	—	0.2
Proaccelerin deficient plasma	0.2	—	—
Proconvertin deficient plasma	—	0.2	—
Prothrombin-proconvertin free ox plasma	—	0.2	0.2
Test material*	0.2	0.2	0.2
Calcium chloride	0.2	0.2	0.2

* Test material is diluted (without changing ionic strength or citrate concentration) sufficiently to give clotting times in the 20–60 second range.

RESULTS

1. *The proaccelerin, proconvertin and prothrombin activity of normal platelet suspensions*

Table 2 summarizes 10 experiments in which the proaccelerin, proconvertin and prothrombin activity of four times washed platelet suspensions were measured. As expected, these suspensions contained significant proaccelerin, but only traces of proconvertin and prothrombin activity. The wide range obtained for proaccelerin activity reflects its lability. Quick handling of the suspensions at low temperature gave higher and less variable values. These data, corrected to a platelet count of 250,000 per cu. mm. of plasma, indicate that platelets contain about six per cent of the proaccelerin activity of normal citrated plasma. This agrees with the value Ware, Fahey and Seegers found for bovine platelet extracts.[6]

Wash water activities were also measured. Proconvertin and prothrombin activity rapidly decreased to immeasurable values in successive wash waters (see table 3). These activities are either only loosely adsorbed onto the platelets or easily eluted by citrate-buffer-saline solution.

As table 4 shows, the proaccelerin activity of platelets and of their successive wash waters decreased very slowly. The failure of tiny platelet fragments to sediment with the centrifugal force used may explain the small loss of platelet suspension activity. The persistence of platelet accelerator activity after 10

TABLE 2.—*The average proaccelerin, proconvertin and prothrombin activities of ten suspensions of normal platelets (750,000 platelets per cu. mm.)*
Normal undiluted plasma activity is taken as 100 per cent

Platelet Activity	Per cent of normal undiluted plasma	
	Average	Range
Proaccelerin	18.7	10–25
Proconvertin	0.2	0.1–0.3
Prothrombin	<0.1	—

TABLE 3.—*Proconvertin and prothrombin activity in the wash waters*

The wash water volume, like that of the final platelet suspension, equals 1/10 the volume of the original citrated blood sample.

Wash water no.	Activity in per cent of normal undiluted plasma	
	Proconvertin	Prothrombin
1	3.6	3.0
2	0.4	>0.1
3	0.1	>0.1

TABLE 4.—*The proaccelerin activity of platelet suspensions and their successive wash waters*

Platelet concentration and wash water volume as in Tables 2 and 3

Assayed after washing no.	Proaccelerin activity as per cent of normal undiluted plasma	
	Platelet suspension	Wash water
1	—	30.0
2	—	8.2
3	18.2	7.0
4	17.0	5.0
5	21.0	4.5
6	20.0	5.0
7	16.0	4.4
8	15.4	3.5
9	16.0	3.1
10	16.2	3.2

washings confirms the findings of Biggs and Macfarlane.[14] It means that proaccelerin activity, if not an integral part of the platelets as we will show, is tightly bound to platelets by a mechanism independent of calcium ions.

2. *Thrombin activation of the platelet accelerator*

Accelerin is defined as the increased accelerator activity which rapidly develops when thrombin is added to proaccelerin. Ware and Seegers, who first described this reaction,[21] use the terms plasma Ac-globulin and serum Ac-globulin for proaccelerin and accelerin, respectively.

Ware, Fahey and Seegers[6] considered platelet accelerator the equivalent of serum Ac-globulin because the kinetics of prothrombin activation by platelet accelerator and serum Ac-globulin were identical in their two-stage system. If platelet accelerator were like serum Ac-globulin rather than like plasma Ac-globulin, adding thrombin to a platelet suspension should not increase its accelerator activity. Yet the experiments summarized in table 5 show that thrombin augments the accelerator activity of fresh platelet suspensions tenfold. Platelet suspensions stored overnight at 4 C. exhibited a four- to eight-fold thrombin induced increase in activity.

Thrombin can lyse platelets[22, 23] and therefore could increase platelet *suspension* activity by liberating more accelerator rather than by increasing the activity of that already available. However, when platelet *extracts*, prepared by

TABLE 5.—*Activation of the platelet accelerator by dilute thrombin*
(final concentration about 0.1 units/ml. measured by Quick's method).
Incubation mixture: 0.2 ml. platelet suspension (750,000/cu. mm.)
2.8 ml. citrate-buffer-saline
0.1 ml. thrombin (about 2.5 units/ml. measured by Quick's method)

Time after adding thrombin (seconds)	Platelet accelerator activity as per cent of normal undiluted plasma	
	Sample 1	Sample 2
0*	1.05	0.96
30	4.0	3.0
90	8.8	5.6
180	10.0	10.5
300	10.0	12.0
600	10.5	11.0

* The 0 second value is that obtained by adding 0.1 ml. saline to the incubation mixture in place of thrombin.

the method of Ware, Fahey, and Seegers,[6] were substituted the same degree of thrombin activation was obtained. Furthermore, the thrombin did not appear to alter either the number or the morphology of the platelets in our suspensions as examined with the light microscope. This may be because we used much weaker final thrombin concentrations (about 0.1 TU/ml. as measured by Quick's method,[24]) than those workers who observed platelet lysis. Our results agree with those of Desforges and Bigelow[25] who found no consistent clumping or disintegrating effect of thrombin upon platelets.

Therefore, we believe that thrombin activates the platelet accelerator itself. This in turn means that the platelet accelerator acts like *pro*accelerin (plasma Ac-globulin) rather than accelerin (serum Ac-globulin).

3. *The failure of platelets to shorten the thromboplastin time (Quick test)*

Some clotting theories[26] assign an important role to the platelet accelerator. These state that the platelet accelerator, precisely because it acts like accelerin instead of proaccelerin, catalyzes the initial formation of thrombin. If this were so, the thromboplastin time of platelet poor plasma should be longer than that of platelet rich plasma for the former would be deprived of this preformed accelerin activity. However, as the experiments summarized in table 6 reveal, the thromboplastin time of platelet poor and platelet rich samples of plasma are almost identical. This is further, though indirect, evidence that the platelet accelerator acts like proaccelerin rather than accelerin.

4. *The platelet accelerator deficiency of parahemophilic platelets and its correction by incubation with normal platelet-poor plasma*

As table 7 shows, platelet suspensions from our patient with parahemophilia (congenital plasma proaccelerin deficiency) possess only a trace of accelerator activity. However, as expected, adding thrombin definitely increased this trace activity (see table 8).

Two ml. of a suspension of parahemophilic platelets were added to nine ml.

TABLE 6.—*The failure of platelets to alter the thromboplastin time (Quick test)*

A comparison of platelet-rich and platelet-poor plasma exposed only to silicone surfaces.

Clotting system: 0.2 ml. thromboplastin
0.2 ml. plasma
0.2 ml. calcium chloride 25 mM.

Sample	Clotting time in seconds			
	Thromboplastin undiluted		Thromboplastin diluted 1/100	
	Platelet-rich plasma	Platelet-poor plasma	Platelet-rich plasma	Platelet-poor plasma
1	12.5	13.1	46.3	48.2
2	12.6	13.4	46.7	48.8
3	13.0	13.5	50.2	51.1
4	13.6	13.9	52.6	52.9

TABLE 7.—*The proaccelerin, proconvertin and prothrombin activities of parahemophilic platelets (750,000 per cu. mm.)*

Averages of four experiments

Platelet Activity	Per cent of normal undiluted plasma	
	Average	Range
Proaccelerin	0.22	0.18–0.30
Proconvertin	0.12	0.10–0.16
Prothrombin	0.1	—

TABLE 8.—*Activation of the trace of platelet accelerator on parahemophilic platelets by dilute thrombin*

Incubation mixture: 3.0 ml. parahemophilic platelet suspension (750,000 platelets per cu. mm.)
0.1 ml. thrombin (about 2.5 units per ml. measured by Quick's method)

Time after adding thrombin (seconds)	Platelet accelerator activity as per cent of normal undiluted plasma	
	Sample 1	Sample 2
0*	0.24	0.12
30	0.38	0.20
90	0.43	0.24
180	—	0.25
300	0.43	0.22
600	0.50	0.22

* The 0 second value as in Table 5.

of platelet-poor normal plasma and incubated for one hour at 4 C. The platelets were then separated by centrifugation, washed four times in citrate-buffer-saline solution and tested for accelerator activity. The result, given in table 9, shows that parahemophilic platelets readily adsorb proaccelerin from normal plasma. They acquire "platelet accelerator" activity that withstands repeated washings.

Proaccelerin adsorbed onto parahemophilic platelets could be activated by thrombin. However, only a two-fold activity increase was obtained. The failure

TABLE 9.—*The accelerator activity of parahemophilic platelets before and after incubation with normal platelet-poor plasma*

Incubation mixture: 2 ml. parahemophilic platelet suspension (750,000 per cu. mm.)
9 ml. normal platelet-poor plasma

Platelet suspension	Proaccelerin activity in per cent of normal undiluted plasma
Before incubation (washed 4 times)	0.3
After incubation (washed 4 times)	14.0

to get greater thrombin potentiation may reflect the experimental conditions which can greatly influence, as shown below, the quantitative result of this reaction.

5. *Production of accelerator deficiency of normal platelet suspensions by trypsin and its subsequent correction by normal platelet-poor plasma*

In preliminary experiments trypsin was found to destroy proaccelerin either in plasma or in purified solution. With weak trypsin preparations a definite activation of proaccelerin to accelerin preceded this destruction. Activation curves were obtained like those for the trypsin activation of prothrombin to thrombin.[27] With stronger trypsin preparations proaccelerin activity vanished very rapidly.

Two ml. of trypsin in veronal buffer (0.1 mg./ml.) were added to four ml. of normal platelet suspension and incubated for 30 minutes at 37 C. The platelets were then separated by centrifugation, washed four times and tested for accelerator activity. A greatly reduced activity was found without change in the number or appearance of the platelets. These trypsin-treated accelerator deficient platelets were then put back into their own platelet-poor plasma for 45 minutes at 4 C. Again they were separated, washed four times and tested for accelerator activity. As table 10 shows, the accelerator activity after re-exposure to plasma closely approached the pre-trypsin value.

6. *Some properties of the platelet accelerator*

(a). *Sedimentation by centrifugation.* An extract was made by triturating washed platelets with one drop of citrate-buffer-saline solution and then in-

TABLE 10.—*Destruction of platelet accelerator activity by trypsin and its restoration by incubation of trypsinized platelets with normal platelet-poor plasma*

Trypsin incubation: 4 ml. normal platelet suspension (750,000 per cu. mm.)
2 ml. trypsin solution (100 μg/ml.)
Plasma incubation: 2 ml. washed trypsinized platelets (750,000 per cu. mm.)
5 ml. normal platelet-poor plasma

Platelet suspension	Proaccelerin activity in per cent of normal undiluted plasma	
	Sample 1	Sample 2
Before trypsinization (washed 4 times)	20.0	22.0
After trypsinization (washed 4 times)	1.16	0.52
After trypsinization (washed 4 times) and incubation with normal plasma (washed 4 times again)	20.5	16.2

creasing the volume with the same fluid to $\frac{1}{10}$ that of the original citrated blood sample. When this extract was centrifuged for 30 minutes at 2,500 r.p.m. (ca 1,900 *g*) the supernatent remained cloudy and retained about 50 per cent of the accelerator activity. Further centrifugation of the supernatant at 22,500 r.p.m. (ca 32,000 *g*) converted it to a water clear solution with about 15 per cent of the original activity.

(b) *Thermo-lability.* A platelet suspension heated to 53 C. rapidly lost its accelerator activity. Only eight per cent of the original activity remained after 15 minutes and less than five per cent after 30 minutes.

(c) *Storage lability.* Figure 1 is a plot of the deterioration of platelet accelerator activity on standing at 37 C. and at 4 C. About 50 per cent of the activity is lost within two hours at 37 C. Accelerin forming capacity also disappears, that is, the proportional increase in accelerator activity produced by thrombin is less than with fresh suspensions. After 18 hours at 37 C. very little accelerator remained and its activity increased only two fold upon adding thrombin. This combined loss of accelerator and accelerin forming capacity may result from a slow conversion of proaccelerin to accelerin on standing with subsequent inactivation of the accelerin. (If the glassware is not scrupulously clean, a rapid activation to accelerin may occur, possibly due to thrombin adsorbed onto the glass and persisting after washing.[28])

The deterioration of accelerator activity at 4 C. agrees with the finding of Mann, Hurn and Magath[5] of a 90 per cent loss of "prothrombin converting activity" after 24 hours at icebox temperature. Accelerin forming capacity held up better than at 37 C. and a four to eight fold increase in activity could be produced in preparations that had stood for 18 hours. This means that the greater part of the activity remaining at 4 C. is still due to proaccelerin.

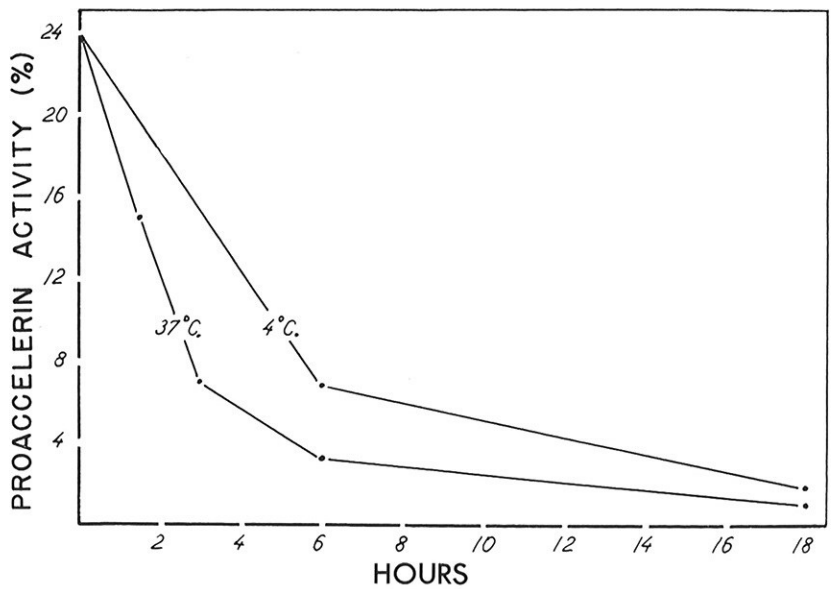

FIG. 1.—The deterioration of platelet accelerator activity on standing. Platelet accelerator activity is expressed as per cent of proaccelerin activity of normal undiluted plasma.

(d) *Freezing and thawing.* Freezing and thawing greatly reduced accelerator activity and accelerin forming capacity. Only about 10 per cent of the original activity persisted and it showed from zero to two fold activity increase with thrombin.

Discussion

Our data show that human platelets possess accelerator activity equal to about six per cent of the proaccelerin activity of plasma. This agrees with the five per cent value for accelerator activity of bovine platelets found by Ware, Fahey and Seegers.[6] The binding of accelerator activity to platelets may be independent of calcium ions for it withstands ten washings in citrate-buffer-saline solution. The absence of significant prothrombin or proconvertin activity in platelet suspensions confirms the report of Mann, Hurn and Barker[13] that platelets have little effect upon the thromboplastin time of "dicumarol plasma."

The evidence that platelet accelerator activity is adsorbed plasma proaccelerin and not an integral part of the platelets consists of the demonstration that (1) platelet accelerator activity acts like proaccelerin rather than accelerin, (2) platelets from a patient with parahemophilia lack significant accelerator activity, and (3) parahemophilic platelets and trypsin-treated accelerator deficient normal platelets readily adsorb proaccelerin from plasma.

The previous wide acceptance of the equivalence of platelet accelerator and accelerin (serum Ac-globulin) rests solely upon the finding that the kinetics of prothrombin activation by the two agents are the same.[6, 12] The key experiment that distinguishes proaccelerin from accelerin, the addition of thrombin, has been missing. Our experiments clearly show that thrombin activates the platelet accelerator. The ten fold increase in activity of fresh platelet suspensions equals that found by Lewis and Ware[29] for the thrombin activation of plasma proaccelerin. Platelet accelerator, therefore, behaves like *proaccelerin.* The equal thromboplastin times of platelet-poor and platelet-rich plasma furnishes additional, though indirect, evidence that platelets do not possess preformed accelerin activity.

The lack of significant accelerator activity in parahemophilic platelet suspensions strongly suggests that plasma proaccelerin and platelet accelerator are identical. The trace of accelerator activity which these platelets possess (about $\frac{1}{50}$ that of normal platelets) indicates to us that the parahemophilic plasma contains a trace of proaccelerin. Our previous belief that this plasma is completely free of proaccelerin[30] which was based upon our inability to activate it with thrombin, must be altered. Lewis and Ferguson[31] have recently described another patient with parahemophilia and little platelet accelerator activity.

The finding that trypsin destroys most of the accelerator activity of normal platelet suspensions without changing the appearance of the platelets under the light microscope also supports the belief that the accelerator activity does not reside within the platelet but upon its surface.

The strongest support for the adsorption theory comes from the demonstration that both parahemophilic platelets and trypsinized normal platelets readily adsorb proaccelerin from normal plasma. Most of this adsorbed proaccelerin sticks to the platelets despite repeated washings. This "acquired platelet accelerator activity" behaves like that of fresh normal platelet suspensions.

Ware and his collaborators[6] found that 70–80 per cent of the accelerator activ-

ity of their platelet extracts could be sedimented by centrifugation at 32,000 *g* for 45 minutes. This led them to believe Ac-globulin and platelet accelerator were entirely different proteins for the former does not sediment at this centrifugal force. We have confirmed their experimental results, but interpret them differently. Our platelet extract was not a true solution but a fine suspension of tiny platelet fragments. We believe that the platelet accelerator sediments because it is bound to these particles. No conclusions can be drawn about the sedimentation properties of the accelerator itself.

The finding that platelet accelerator is not a specific platelet protein but adsorbed plasma proaccelerin should not detract from its as yet unevaluated biologic significance. A thrombus begins with the accumulation of platelets at a site of vessel wall injury. The local concentration of proaccelerin that results certainly favors the deposition of fibrin that follows.

Summary

1. Human platelets possess an accelerator activity equal to about six per cent of the proaccelerin activity of normal citrated plasma. This accelerator activity is only slightly reduced by 10 washings.

2. Thrombin increases platelet accelerator activity as much as ten fold. This means that platelet accelerator activity behaves like proaccelerin (plasma Ac-globulin) rather than accelerin (serum Ac-globulin).

3. Platelets from a patient with parahemophilia (congenital proaccelerin deficiency) possess only a trace of accelerator activity. They acquire a normal amount of accelerator activity after contact with normal platelet-poor plasma.

4. Trypsin destroys 90 per cent or more of the platelet accelerator activity of normal platelets without altering their appearance. Trypsinized platelets regain normal accelerator activity upon incubation with normal platelet-poor plasma.

5. These findings strongly suggest that the platelet accelerator (platelet factor 1) is adsorbed plasma proaccelerin.

Summario in Interlingua

1. Plachettas human possede un activitate accelerator equal a circa 6 pro cento del activitate de proaccelerina in normal plasma citrate. Iste activitate accelerator exhibi solmente un leve reduction post 10 lavages.

2. Thrombina augmenta le activitate accelerator del plachettas usque decuplemente. Isto significa que le activitate accelerator del plachettas se conduce como proaccelerina (globulina Ac del plasma) plus tosto que como accelerina (globulina Ac del sero).

3. Plachettas ab un patiente de parahemophilia (carentia congenite de proaccelerina) monstra solmente un tracia de activitate accelerator. Illos acquire un grado normal de activitate accelerator post contacto con plasma normal a basse contento de plachettas.

4. Trypsina destrue 90 pro cento o plus del activitate accelerator de plachettas normal sin alterar lor apparentia. Plachettas trypsinate reattinge grados normal

de activitate accelerator post incubation con plasma normal a basse contento de plachettas.

5. Iste constatationes indica fortemente que le accelerator plachettal (factor plachettal 1) es proaccelerina plasmatic adsorbite.

REFERENCES

[1] Morawitz, P.: Die Chemie der Blutgerinnung. Ergebn. d. Physiol. *4:* 307, 1905.

[2] Quick, A. J.: Studies on the enigma of the hemostatic dysfunction of hemophilia. Am. J. Med. Sc. *214:* 272, 1947.

[3] McLean, J.: The thromboplastic action of cephalin. Am. J. Physiol. *41:* 250, 1916.

[4] Feissly, R.: Recherches sur la nature et l'origine de la thrombokinase plasmatique. Helvet. Med. Acta *12:* 215, 1945.

[5] Mann, F. D., Hurn, M., and Magath, T. B.: Observations on the conversion of prothrombin to thrombin. Proc. Soc. Exper. Biol. & Med. *66:* 33, 1947.

[6] Ware, A. G., Fahey, J. L., and Seegers, W. H.: Platelet extracts, fibrin formation and interaction of purified prothrombin and thromboplastin. Am. J. Physiol. *154:* 140, 1948.

[7] van Creveld, S., and Paulssen, M. M. P.: Significance of clotting factors in blood-platelets, in normal and pathological conditions. Lancet *2:* 242, 1951.

[8] Stefanini, M.: Basic mechanism of hemostasis. Bull. New York Acad. Med. *30:* 239, 1954.

[9] Maupin, B.: Les plaquettes sanguines de l'homme. Paris, Masson and Lie, 1954, p. 137.

[10] Baserga, A. and de Nicola, P.: Le malattie emorragiche. Milan, Societa Editrice Libraria, 1950, p. 78.

[11] Marbet, R. and Winterstein, A.: Neuere Auffassungen ueber den Mechanismus der Blutgerinnung. Experientia *10:* 273, 1954.

[12] McClaughry, R. I. and Seegers, W. H.: Prothrombin, thromboplastin, Ac-globulin and platelet accelerator: quantitative interrelationships. Blood *5:* 303, 1950.

[13] Mann, F. D., Hurn, M., and Barker, N. W.: Platelets and the coagulation defect caused by Dicumarol. Am. J. Clin. Path. *21:* 814, 1951.

[14] Biggs, R., and Macfarlane, R. G.: Human blood coagulation and its disorders. Oxford, Blackwell, 1953, p. 74.

[15] Owren, P. A.: The coagulation of blood. Oslo, J. Chr. Gundersen, 1947, p. 19.

[16] —: New clotting factors, in Blood Clotting and Allied Problems, Trans. Fifth Conference, Josiah Macy, Jr., Foundation, 1952, p. 92.

[17] Aas, K.: Prokonvertin og konvertin. Thesis, Oslo, Akad. Trykningssentral, 1952.

[18] Owren, P. A., and Aas, K.: The control of Dicumarol therapy and the quantitative determination of prothrombin and proconvertin. Scand. J. Clin. & Lab. Invest. *3:* 201, 1951.

[19] Hjort, P., Rapaport, S. I., and Owren, P. A.: A simple, specific one-stage prothrombin assay using Russell's viper venom in "cephalin" suspension. J. Lab. & Clin. Med., in press.

[20] Owren, P. A.: A quantitative one-stage method for the assay of prothrombin. Scand. J. Clin. & Lab. Invest. *1:* 81, 1949.

[21] Ware, A. G., and Seegers, W. H.: Serum Ac-globulin: formation from plasma Ac-globulin; role in blood coagulation; partial purification; properties; and quantitative determination. Am. J. Physiol. *152:* 567, 1948.

[22] Stefanini, M.: Autocatalytic formation of thrombin in blood coagulation. Acta med. Scandinav. *140:* 290, 1951.

[23] DeRobertis, E., Paseyro, P. and Reissig, M.: Electron microscopic studies of the action of thrombin on blood platelets. Blood *8:* 587, 1953.

[24] Quick, A. J.: The physiology and pathology of hemostasis. Philadelphia, Lea and Febiger, 1951, p. 152.

[25] DESFORGES, J. F., AND BIGELOW, F. S.: An action of thrombin on platelets in accelerating clotting. Blood *9:* 153, 1954.

[26] SEEGERS, W. H., STEFANINI, M., AND TOCANTINS, L. M. IN ALBRITTON, E. C.: Standard values in blood. Philadelphia, W. B. Saunders, Co., 1952, pp. 13–15.

[27] SCHULTZE, H. E., AND SCHWICK, G.: Ueber den Mechanismus der Thrombinbildung im isolierten System. Ztschr. Physiol. Chem. *289:* 26, 1951.

[28] QUICK, A. J.: The chain reaction in the coagulation of blood. Its theoretical and clinical significance. Le Sang *25:* 726, 1954.

[29] LEWIS, M. L., AND WARE, A. G.: The mechanism of action of human accelerator globulin and its relation to other clotting factors. Blood *9:* 520, 1954.

[30] RAPAPORT, S. I., AAS, K., AND OWREN, P. A.: The lipid inhibitor of brain: mechanism of its anticoagulant action and its comparison with the soybean inositol phosphatide inhibitor. J. Lab. & Clin. Med. *44:* 364, 1954.

[31] LEWIS, J. H., AND FERGUSON, J. H.: Hypoproaccelerinemia. Blood *10:* 351, 1955.