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Actions and properties of platelet factor I

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THE ACTIONS AND PROPERTIES OF PLATELET FACTOR I

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The existence of a blood coagulation factor that we will call platelet factor I is rather controversial at this time, although the preponderance of the evidence points away from its existence. Most of the arguments center around whether platelet factor I is the same as factor V, or a now obsolete substance called serum accelerator globulin which results from the action of thrombin on labile factor, or whether platelet factor I is a separate, distinct entity. (See figure I)

Figure I

Stage I:
AHG, PTC, PTA, PFIII, Stuart factor, and calcium act together and form thromboplastin

Stage II:
calcium, thromboplastin, Stuart factor, factor V, factor VII, and PLATELET FACTOR I act on prothrombin to give thrombin. (Labile factor, V, accelerates the conversion of prothrombin to thrombin. Formerly it was thought that thrombin activated factor V to factor VI, serum ac-globulin, which really accelerated the conversion process.)

Stage III:
calcium, thrombin, PFII, act on fibrinogen to give fibrin

AHG.....antihemophilic globulin
PTC.....plasma thromboplastin component
PTA.....plasma thromboplastin antecedent
PFII.....platelet factor II
PFIII.....platelet factor III

In this paper I plan to discuss this controversy,

as well as the controversy about platelet factor I being absorbed or adsorbed from plasma or manufactured in the platelets. I plan to present pertinent findings in these regards from a review of the literature and supplement those findings with some research data from our laboratory.

Bizzozzero was probably the first man to realize the unique importance of platelets in physiologic hemostasis. "Bordet and Gengou in 1904 first described platelet accelerator (platelet factor I) and obtained an impure lipoid substance that they called cytozime."#25 In 1912 Bordet and Delange #3 confirmed these earlier studies and noted that platelet accelerator shortened the prothrombin time and the recalcified clotting time of human blood. In 1947 Mann et al #15 did some studies on prothrombin conversion, and they found a substance they called platelet factor I which was precipitated by centrifugation at 3000 rpm for 30 minutes. It would shorten the prothrombin time by potentiating the action of thromboplastin on stored plasma. They found that their extract from platelets was more potent

than plasma or serum in shortening the prothrombin time. They also thought that this factor was present in circulating blood.

Platelet factor I has many physical, chemical, and physiological properties closely resembling those of labile factor and serum Ac-globulin. The latter factor is generally considered to be the same as labile factor or activated labile factor which results from the action of thrombin on labile factor. The latter concept is thought to be false by many experts. Serum Ac-globulin, also known as factor VI, is now considered an obsolete term by the International Committee for Nomenclature of Blood Clotting Factors.

Stefanini and Dameshek in their excellent text, The Hemorrhagic Disorders #26 say that platelet factor I may be the same as serum Ac-globulin even though in earlier work #25 Stefanini noted that platelet accelerator (PFI) had chemical charachteristics clearly distinguishable from those of serum accelerator, (serum Ac-globulin).

Creveld and Paulssen #4 in 1951 confirmed the findings of McClaughry and Seegers #16 that platelet factor I exists, and that it closely resembles serum

Ac-globulin in action. Luscher #14 thinks that platelet factor I is actually labile factor absorbed from plasma. Johnson and Seegers #12 state that platelet factor I is an entity, but its actions and properties are closely similar to serum Ac-globulin. McClaughry and Seegers #16, referred to above, noted that platelet factor I acted identically with serum Ac-globulin in their clotting experiments, but they differed in some of their chemical properties. Ware and Seegers #29 and Lewis and Ware #13 say that serum AcOglobulin is actually factor V.

The action of platelet factor I, whatever the substance really is, is to accelerate the conversion of prothrombin to thrombin according to this equation, about which few people will argue:

> Calcium, thromboplastin, PLATELET FACTOR I, factors V and VII, Stuart factor act on prothrombin to form thrombin. An obsolete theory was that thrombin in turn, activated labile factor, factor V, to serum Ac-globulin which was the active form.

Platelet factor I has the following properties, according to several authors:

1. activity is lost at 53 degrees Centigrade (C); 87% in 30 minutes
2. precipitatable with half-saturated ammonium sulfate

3. sedimented by centrifugation at 32,00 rpm for 45 minutes

4. high molecular weight protein

5. can not be dialyzed

6. is not adsorbed on barium sulfate

7. has activity in a pH range of 4 to 10.5

8. activity is destroyed by incubation with trypsin

9. water soluble

10. stable at 4 degrees C

In comparison, labile factor has the following properties:

1. activity is lost at 37 degrees C

2. precipitatable with half-saturated ammonium sulfate

3. not sedimented at 32,000 rpm for 45 minutes

4. has the charachteristics of a globulin

5. can not be dialyzed

6. is not adsorbed on barium sulfate

7. active in a pH range of 4 to 10.5

8. activity is destroyed by incubation with trypsin

9. water soluble

10. gradually loses its activity at 4 degrees C

Labile factor and platelet factor I have the

same charachteristics and properties except that labile factor is more potent in converting and accelerating the conversion of prothrombin to thrombin.

From an examination of the properties of these two substances, it seems apparent to this writer that the two are one and the same substances. The differences are due to technical errors, even though the same properties were found by several authors working independently. The differences in heat lability is somewhat controversial because some authors merely state that labile factor's activity is lost at a certain temperature. They fail to investigate how much lower the temperature may be to get the same results. In this way, false weight is given to the identity of labile factor or platelet factor I.

I was unable to find any reports of where labile factor may be sedimented except that Ware et al #28 noted that it did not sediment at 32,000 rpm. Hjort et al #9 says that platelet factor I does precipitate at 32,000 rpm because it is factor V adsorbed to platelet fragments.

Some of the similarities, for example inactivation by trypsin, pH range of activity, and precipitation,

with 50% ammonium sulfate; are very striking and very difficult to disregard. Nevertheless, from the evidence presented in the literature, it is my feeling, after reviewing these properties, that labile factor and platelet factor I are one and the same substance. I also feel that serum Ac-globulin is obsolete and does not exist.

J. Roskam in 1922 originated the platelet sponge concept. #1 Today, a controversy exists about whether platelet factor I is manufactured in platelets if it exists at all, or is a material adsorbed or absorbed from plasma, even though platelet factor I may or may not be identical with labile factor. Luscher #14 feels that platelet factor I is actually labile factor so tightly adherent to the platelets that it cannot be washed off by routine methods. Mann et al #15 feel that platelet factor I is in plasma as well as in platelets. Turpini #27 noted that the Ac-globulin in platelets is like the plasma Ac-globulin but is reduced in quantity. McGlaughry and Seegers #16 feel that platelet factor I is an integral part of the platelets. Adelson et al #1 believe that one of the main functions of the platelet is to adsorb and concentrate coagulation

coagulation factors and carry them in the blood stream. Hjort et al #9 confirmed earlier work demonstrating that platelet factor I was adsorbed from plasma and was actually factor V. Several authors #'s 23, 11, 17 give supporting evidence that patients who have a congenital deficiency of factor V also have a deficiency of platelet factor I, thus suggesting that they are one and the same. Of course, if platelet factor I does not really exist, it cannot be manufactured anywhere, and the substance we call platelet factor I is some other substance--labile factor?

Below I present my findings on some of the properties and identity of platelet factor I.

The purpose of these experiments is to confirm or disprove various authors' claims about the properties and identity of platelet factor I.

In these experiments we used plasma and platelets from apparently normal hospitalized patients and medical students. We prepared stored plasma by collecting it in 0.1 normal EDTA and incubating plasma for 24 hours at 37 degrees C and then storing it in a freezer until it was needed. We used siliconized tubes and syringes and venous blood via a clean venipuncture.

"Platelet factor I" was extracted by the following method: Twenty ml. of whole blood was collected in siliconized syringes and tubes in 0.1 normal EDTA from an apparently normal person. The blood was then centrifuged at 10,000 rpm for 10 minutes, and the plasma was drawn off and recentrifuged at 15,000 rpm for 20 minutes to precipitate the platelets. The platelet sediment was washed in normal saline to remove adsorbed and residual plasma. The platelet sediment was then suspended in 3 ml. of distilled water to lyse them. The platelets were agitated and broken with a stick, and finally, the suspension was frozen overnight and re-agitated the following morning. The suspension was re-centrifuged at 15,000 rpm for 20 minutes, and the supernatant removed. Microscopic exam of the precipitate revealed about 90% of the platelets were broken, allowing their contents to diffuse in the solution. To the supernatant was added 100 mg. of barium sulfate per ml. of fluid. This was mixed for 10 minutes at 37 degrees C and centrifuged at 32,000 rpm for 35 minutes at 7 degrees C in the ultracentrifuge. The white precipitate at the bottom was dissolved in 2 ml. of distilled water. This suspension was then used in our tests as platelet factor

I (PFI). Several preparations were made, as we needed them; thus explaining the different control values.

Tissue thromboplastin and 0.1 normal calcium chloride were used in our tests. All of our tests were run with the reagents at 37 degrees C to closely simulate in vivo conditions.

Table A

No.	stored plasma	$BaSO_4$ adsorbed Plasma from Na-oxalated blood platelet poor	$BaSO_4$ adsorbed Na Oxalate Platelet rich	$BaSO_4$ adsorbed EDTA blood platelet poor	$BaSO_4$ adsorbed EDTA blood platelet rich	thrombo-plastin	0.1N CaCl	Time
1	0.1 cc					0.1 cc	0.1cc	68 sec
2		0.1 cc				0.1 cc	0.1cc	above 2 min
3			0.1 cc			0.1 cc	0.1cc	above 2 min
4				0.1 cc		0.1 cc	0.1cc	above 2 min
5.					0.1cc	0.1 cc	0.1cc	above 2 min
6						0.1 cc	0.1cc	above 2 min
7		0.3cc				0.1 cc	0.1cc	28 sec
8			0.3cc			0.1 cc	0.1cc	24 sec
9					0.3 cc	0.1 cc	0.1cc	35 Sec
10				0.3cc		0.1 cc	0.1cc	35 sec

This experiment indicates that oxalated plasma lyses platelets and shortens the prothrombin time, but EDTA allows the platelets to remain intact and thus,

the increased numbers of platelets do not decrease the prothrombin time. With this evidence and the evidence of L. V. Dahlquist #5 who did platelet counts on EDTA and oxalated plasma and demonstrated more lysed platelets with oxalate, we used EDTA as our anticoagulant rather than sodium oxalate.

Table B

Number	Stored plasma	Platelet Factor I	thrombo-Plastin	0.1 NCaCl	time
1	0.1 cc		0.1 cc	0.1 cc	60 sec
2		0.1 cc	0.1 cc	0.1 cc	above 2 min
3	0.1 cc	0.1 cc	0.1 cc	0.1 cc	50 sec
4	0.1 cc	0.15 cc	0.1 cc	0.1 cc	45 sec
5	0.1 cc	0.18 cc	0.1 cc	0.1 cc	43 sec
6	0.1 cc	0.2 cc	0.1 cc	0.1 cc	40 sec
7	0.1 cc	0.3 cc	0.1 cc	0.1 cc	46 sec

Examination of the above results show that platelet factor I shortens the prothrombin time of stored plasma. The more platelet factor I used, the shorter the prothrombin time until one has the factor of dilution which we apparently have when using 0.3 ml. of platelet factor I suspension. The control on platelet factor I illustrates that it is the combination of the

two materials, platelet factor I and stored plasma, that are decreasing the prothrombin time and not just platelet factor I alone.

In the experiment below we tried to show that heating platelet factor I to 53 degrees C for 20 minutes destroys much of its activity.

Table C

Number	stored Plasma	Platelet Factor I extract (Fresh)	Platelet Factor I extract – heated to 53°C for 20 min	thrombo-plastin	0.1 N $CaCl_2$	Time
1	0.1 cc	0.2 cc		0.1 cc	0.1 cc	30 sec
2	0.1 cc		0.2 cc	0.1 cc	0.1 cc	40 sec

In the above experiment we have shown that heating platelet factor I to 53 degrees C for 20 minutes does destroy a good share of its activity.

In table D is shown the result of an experiment in which we attempted to compare the potency of platelet factor I extract solution with fresh EDTA platelet poor plasma. From the results it can be seen that one gets the maximum shortening when even a small amount of platelet poor plasma is added to stored plasma and that the addition of platelet factor I does not shorten the prothrombin time any further.

Table D

Number	stored plasma	saline	Platelet factor I	fresh platelet poor plasma	thrombo-plastin	0.1 N CaCl2	time
1	0.1 cc				0.1 cc	0.1 cc	60 sec
2			0.1 cc		0.1 cc	0.1 cc	above 2 min
3				0.1 cc	0.1 cc	0.1 cc	above 2 min
4	0.1 cc				0.1 cc	0.1 cc	35 sec
5	0.1 cc		0.2 cc		0.1 cc	0.1 cc	30 sec
6	0.1 cc	0.1 cc		0.1 cc	0.1 cc	0.1 cc	25 sec
7	0.1 cc		0.1 cc	0.1 cc	0.1 cc	0.1 cc	25 sec

In table E we tried to show the effects platelet factor I has on the prothrombin time of stored plasma in relation to the effect of plasma from a patient who has been on Coumadin, 5 mg. per day.

Table E
(continued on the next page)

Number	stored plasma	saline	Platelet factor I	Coumadin-ized plasma	thrombo-plastin	0.1 N CaCl2	time
1			0.1 cc		0.1 cc	0.1 cc	above 2 min
2				0.1 cc	0.1 cc	0.1 cc	47 sec
3	0.1 cc				0.1 cc	0.1 cc	60 sec
4	0.1 cc	0.2 cc			0.1 cc	0.1 cc	75 sec
5		0.2 cc		0.1 cc	0.1 cc	0.1 cc	38 sec

No.	Stored Plasma	saline	platelet Factor I	Coumadinized Plasma	thrombo-plastin	0.1N CaCl2	time
6	0.1cc		0.2cc		0.1cc	0.1cc	40 sec
7			0.1cc	0.1cc	0.1cc	0.1cc	35 sec
8	0.1cc			0.2cc	0.1cc	0.1cc	35 sec

The action of Coumadin is to inhibit the formation of prothrombin in the liver. Platelet factor I should shorten the prothrombin time somewhat by accelerating its conversion to thrombin, even though prothrombin is decreased in concentration, but the main point under observation is the comparison of the effects of platelet factor I and coumadinized plasma on the prothrombin time of stored plasma, since with these two substances we are replacing labile factor activity.

By examining the results, we see that platelet factor I shortens the prothrombin time of stored plasma, (done to prove its potency) and also decreases the prothrombin time of coumadinized plasma, up to a certain point (df. #'s 6 & 3 and 7 & 2), but the coumadinized plasma is more potent than platelet factor I (cf. 6 & 8). Some of the results are rather puzzling and are undoubtedly due to laboratory error, for example (cf. 5 & 2). These two

tests seem to show that water shortens the prothrombin time but not as much as does platelet factor I (cf. 8 & 2). Platelet factor I solution must be so dilute that it increases the prothrombin time of coumadinized plasma, after a certain amount has been added.

In the experiment below we attempted to show the comparative potencies of platelet factor I and dicumarolized plasma on stored plasma, or in other words, compare the potency of labile factor since labile factor is all that is lacking in stored plasma; the same experiment as above except that here we used dicumarolized plasma. Normal plasma could have been used in place of dicumarolized plasma, but the addition of more prothrombin might give false results due to a mass reaction. All we need to add is labile factor activity. The dicumarolized plasma was taken from a patient on the drug with a prothrombin time stable at 25 seconds with a control of 13 seconds.

Examination of these results show that dicumarolized plasma lowers the prothrombin time of stored plasma more than platelet factor I, but the significance

of this is a little hazy since dicumarolized plasma clots in 38 seconds, and platelet factor I does not clot at all. The number of seconds or the percentage shortened cannot be calculated in this case. I think it is safe to assume from these results that plasma labile factor is more potent than platelet factor I extract.

Table F

Number	stored plasma	Platelet Factor I	Dicumerolized Plasma	Thrombo-plastin	0.1 N $Ca\,Cl_2$	Time
1	0.1cc			0.1cc	0.1cc	85 sec
2		0.1cc		0.1cc	0.1cc	above 2 min.
3			0.1cc	0.1cc	0.1cc	38 sec
4	0.1cc	0.1cc		0.1cc	0.1cc	65 sec
5	0.1cc		0.1cc	0.1cc	0.1cc	22 sec

In the last three experiments, we have taken different sources of labile factor activity and compared it to platelet factor I. I believe the results can be explained by variations in concentration of labile factor activity in the various preparations.

Table G*

Number	Stored plasma	Platelet factor I, fresh	Platelet factor I incubated at 37°C for 24 hrs.	thrombo-plastin	0.1 N $CaCl_2$	Time
1	0.1 cc			0.1 cc	0.1 cc	52 sec
2		0.1 cc		0.1 cc	0.1 cc	above 2 min
3	0.1 cc	0.1 cc		0.1 cc	0.1 cc	38 sec
4	0.1 cc	0.15 cc		0.1 cc	0.1 cc	40 sec
5	0.1 cc	0.2 cc		0.1 cc	0.1 cc	40 sec
6	0.1 cc			0.1 cc	0.1 cc	60 sec
7			0.1 cc	0.1 cc	0.1 cc	above 2 min
8	0.1 cc		0.1 cc	0.1 cc	0.1 cc	60 sec
9	0.1 cc		0.15 cc	0.1 cc	0.1 cc	60 sec
10.	0.1 cc		0.2 cc	0.1 cc	0.1 cc	60 sec

*Note: Stored plasma in #6 was 24 hours older than #1.

In the above experiment we attempted to show whether labile factor and platelet factor I were identical or two separate entities by extracting platelet factor I, assuring its activity, and then incubating it at 37 degrees C for 24 hours. If they are one and the same, the activity should be gone, but if they are different, the accelerating action should remain. This property is the one

most people rely on when they claim two separate entities. Examination of the results (cf. #'s 3 & 8) shows that the activity disappeared and thus, they are one and the same substance. Numbers 4, 5, 9, and 10 were done to check for amount of activity.

The bulk of the data seems to indicate that platelet factor I is actually labile factor adsorbed on platelets. The last experiment shown above left no doubt in my mind as to the identity of platelet factor I and all of the other experiments lend support to this hypothesis.

In summary, then, the literature is divided in thought about the identity of platelet factor I, although a preponderance of the more recent literature illustrates that labile factor and platelet factor I are identical. The people who favor this viewpoint have more impressive studies to support them than the other investigators. The illustration of people with factor V deficiency also having platelet factor I deficiency, and that platelets from these patients adsorb platelet factor I activity if incubated in normal plasma is very impressive support. The many

physical and physiological charachteristics of the two substances being very similar or identical lend to the "identical substance" viewpoint. The differences in charachteristics, such as difference in sedimentability, can be explained. In this case what actually sediments is platelet fragments which have a small amount of labile factor adsorbed to them. The differences of heat lability is very controversial; nearly every investigator lists different figures. It would appear that carefully performed experiments would show that even this property is identical in the two extracts.

The case of platelet factor I may be closed. It is really only labile factor found clinging to platelet fragments.

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