Platelet Functional Defects in Thrombocytopenic Purpura

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THERE IS now considerable evidence to suggest that the hemorrhagic manifestations of thrombocytopenic purpura and certain other disorders are probably due largely to deranged platelet function rather than to a reduction in platelet numbers. It is, of course, true that associated vascular changes also play an important role in thrombocytopenic purpura. Wald and McAuley,[1] Stefanini et al.,[2] Cronkite et al.[3] and Hirsch and Dameshek[4] have all demonstrated a dissociation between platelet numbers and the consumption of prothrombin. Wald and McAuley[1] showed that a rapid improvement in prothrombin consumption occurred without a coincidental rise in platelet numbers immediately following splenectomy in a case of idiopathic thrombocytopenic purpura. They ascribed this phenomenon to an improvement in platelet function.

By means of the thromboplastin generation test, Hardisty and Wolff[5] demonstrated a mild thromboplastic platelet defect in five cases of hemorrhagic thrombocythemia. This finding has been confirmed by the author in this disease and in one similar patient with polycythemia vera. In addition, Stacey was able to show that the mean platelet serotonin content was below normal in three of the patients recorded by Hardisty and Wolff.[5] It therefore appears that the platelet functional defects in these diseases are multiple.

In a previous publication,[6] a thromboplastic defect was demonstrated in the platelets of patients with thrombocytopenic purpura and in normal platelets after contact with these patients' sera. It seemed reasonable to assume, therefore, that the platelet defect of these patients was induced by a factor present in their plasma and serum which also induced a similar defect in normal platelets. It was also shown that cortisone therapy produced a rapid return to normal of platelet thromboplastic function in idiopathic thrombocytopenic purpura, a partial improvement in the aplastic type of thrombocytopenic purpura, and that there was a very definite relationship between platelet thromboplastic function and the occurrence of hemorrhagic manifestations. This work has now been confirmed and extended.

In addition to the platelet thromboplastic defect, all but two patients with the severe idiopathic type of thrombocytopenic purpura (megakaryocytic type as distinct from the amegakaryocytic and leukemic types) have shown a serum defect in the thromboplastin generation test. It is the purpose of the present paper to produce evidence which suggests that another platelet functional defect is primarily at fault, which causes this serum defect possibly through the failure of activation of an essential serum thromboplastic component.

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Materials and Methods

1. *Quick's One-Stage 'Prothrombin' Tests*

One-stage 'prothrombin' tests were carried out as described by Quick[7] using 5 per cent human brain extract.

2. *Thrombin Generation Tests*

Thrombin generation tests were performed by a modification of the method described by Fantl.[8] 0.5 ml. citrated whole blood and 0.5 ml. normal saline solution were mixed and 0.5 ml. M/40 calcium chloride solution was added. At minute intervals 0.1 ml. samples were added to a series of tubes containing 0.4 ml. aluminium hydroxide-adsorbed normal plasma to which had been added a fourth part of 2 per cent pyrocatechol in veronal buffer (pH 7.2 to 7.6).

3. *Platelet Function Studies and Thromboplastin Generation Tests*

Appropriate volumes of venous blood were withdrawn using siliconized glassware. Direct platelet counts were made on samples of low-spun plasma. For the thromboplastin generation tests and platelet function studies, platelet suspensions were prepared in saline from volumes of low-spun plasma calculated to yield suspensions of 1,600,000 platelets per cu. mm. as previously described.[6] Serum and aluminum hydroxide-adsorbed plasma (alumina plasma) were prepared and diluted as described by Biggs and Douglas.[9] With this test, a clotting time of 8.5 seconds or less of the substrate normal plasma can usually be obtained at this laboratory when normal reagents are used, and fresh normal reagents were always prepared whenever these clotting times were not obtained. This is important if a comparison of platelet function from day to day is to be made.

4. *The Demonstration that the Serum Thromboplastic Defect Was Corrected by the Presence of Normal Platelets during Clotting*

Using siliconized glassware, the low-spun plasma from both patient's and normal blood was obtained. Platelet counts were performed on each. The plasma samples were then centrifuged at 4,000 r.p.m. for 45 minutes in an M.S.E. major centrifuge to obtain the buttons of platelets and the platelet-poor plasma. Both the normal and patient's platelets were washed and resuspended in volumes of saline solution calculated to give a suspension of 450,000 platelets per cu.mm. Two samples of the patient's platelet-poor plasma were taken and an equal volume of the patient's platelet suspension was added to one sample and an equal volume of normal platelet suspension to the other. Equal volumes of M/40 calcium chloride solution were then added to each and both were thus allowed to clot in the presence of approximately 150,000 platelets per cu.mm. After incubation at 37 C. for one hour, the serum from each tube was separated and examined by the thromboplastin generation test using normal platelet suspension and normal alumina plasma. The sera were diluted 1 in 3 for the tests instead of the usual 1 in 10 dilution because the plasma samples had already been diluted 1 in 3 by the addition of the platelet suspensions and calcium chloride solution.

Results

The type of bone marrow reaction, the primary disease where known and other data concerning the six patients showing a serum thromboplastic defect, are shown in table 1.

The results of the whole-blood platelet counts, one-stage "prothrombin" times, thrombin generation tests and platelet function studies performed shortly after admission to hospital are shown in table 2. In general, the one-stage tests were only very slightly prolonged and were not different from the clotting times of the one-stage tests performed on other purpuric patients who did not show the

TABLE 1.—*Clinical Data*

Patient	Type of bone marrow reaction	Primary cause or disease	Clinical notes and result
Case 1	Amegakaryocytic at first. Totally aplastic later.	Possibly due to chloromycetin given for virus pneumonia.	Sudden severe purpura and gross hemorrhage. Died.
Case 2	Megakaryocytic	Acute idiopathic thrombocytopenic purpura (I.T.P.)	Sudden severe purpura and hemorrhage. Cerebral hemorrhage. Died.
Case 3	Megakaryocytic	Acute I.T.P.	Severe purpura and mild hemorrhage. Responded to Cortisone.
Case 4	Marrow not examined.	Acute thrombocytopenic purpura induced by Quinidine	Sudden severe purpura and mild hemorrhage. Responded to Cortisone.
Case 5	Megakaryocytic	Acute thrombocytopenic purpura associated with collagen disease.	Minimal purpura. Severe hemorrhage; 2 episodes relieved by ACTH. Died of cardiac failure and debility.
Case 6	Megakaryocytic	Acute I.T.P.	Heavy purpura. No frank hemorrhage. Responded to Cortisone.

TABLE 2.—*Relevant Laboratory Findings*

Patient	Platelets (per cu. mm. whole blood)	One stage 'Prothrombin' time (seconds)	Maximum thrombin generation (units)	Platelet thromboplastic function (per cent) (in terms of thromboplastin generated)
Normal	150,000–250,000	11.5	2.8–6.5 in 3–5 minutes	133–150
Case 1	20,000	14	3 in 7 minutes	3
Case 2	40,000	12.5	1.7 in 9 minutes	2.5
Case 3	16,000	16	3.2 in 7 minutes	8
Case 4	30,000	13.5	Not performed	38
Case 5	45,000	14	Not performed	12
Case 6	56,000	12.5	2.6 in 6 minutes	48

serum thromboplastic defect. The serum defect, therefore, did not appear to involve factor VII (accelerator factor VII). The thrombin generation tests, however, did show reduced and delayed thrombin generation but the platelet thromboplastic factor was also defective in these patients which would contribute to the abnormal results.

The defects in the six patients' serum thromboplastic components are shown in table 3. These examinations were performed shortly after admission to hospital before any treatment was commenced. The sera were then stored at −20 C. for varying periods of time until the defects could be further studied at a more opportune moment. The unexpected finding that each serum showed no defect after storage is also illustrated in table 3. The findings in the first five patients were known before case 6 was studied. Samples of the serum of case 6 were therefore stored at −20 C. and one was examined daily to determine the period of storage required for the serum to return to normal. A progressive daily im-

TABLE 3.—*Thromboplastin Generated Using the Patients' Sera both Fresh and after Storage at −20 C.*

Patient	Thromboplastin generated (per cent)		Period of storage
	Using fresh patient's serum (other reagents normal)	Using stored patient's serum (other reagents normal)	
Normal	133–150	133–150	
Case 1	100	133	5 months
Case 2	80	150	4 months
Case 3	70	150	3½ months
Case 4	45	150	7 weeks
Case 5	21	150	9 days
Case 6	48	150	3 days

TABLE 4.—*The Improvement in Thromboplastin Generation with Storage of the Patient's Serum (Case 6)*

Period of storage of serum	Thromboplastin generated (per cent) (using patient's serum, other reagents normal)
Fresh (1 hour after coagulation)	48
24 hours	100
48 hours	100
72 hours	150

provement in serum thromboplastic efficiency was found with complete return to normal after 72 hours, as shown in table 4.

Platelet thromboplastic function and serum thromboplastic efficiency were examined in case 6. The results are recorded in table 5. It can be seen that two defects were present which were cumulative when the patient's platelet factor (numbers reconstituted to normal) and serum components were examined together.

From these findings it seemed likely that an essential serum thromboplastic component was not being immediately activated as normally occurred during clotting, and that slow progressive activation occurred during storage at −20 C. Since at least one platelet factor was shown to be defective it seemed possible that the same or another defective platelet factor might be responsible for the lack of activation of this serum component. A saline suspension of 150,000 normal platelets per cu.mm. was therefore added to the defective serum but on subsequent examination there was no improvement. The normal platelet factor responsible is apparently necessary during coagulation, as shown in table 6. When the platelet-poor plasma of case 6 was clotted in the presence of washed normal platelets, the resulting serum showed no defect while the defect was still present in another sample of this plasma clotted in the presence of a similar number of its own defective platelets.

The effect of ACTH or cortisone therapy on the serum defect has been dramatic on all occasions when it has been tested. The exact time taken for the serum to return to normal has not been determined but no defect has been detected in any patient's serum on the day following commencement of these

TABLE 5.—*Thromboplastin Generation. Illustrating the Defects in Both the Patient's Platelet and Serum Thromboplastic Components (Case 6)*

Reagents	Thromboplastin generated (per cent)
Normal platelets (other reagents normal)	140
Patient's platelets (other reagents normal)	48
Normal serum (other reagents normal)	140
Patient's serum (other reagents normal)	48
Normal platelets and normal serum (Alumina plasma normal)	140
Patient's platelets and patient's serum (Alumina plasma normal)	25

TABLE 6.—*The Effect on Serum Thromboplastin Components of Adding Washed Normal Platelets to the Patient's Plasma before Clotting (Case 6)*

Reagents (Platelet factor and alumina plasma normal)	Thromboplastin generated (per cent)
Normal serum (from clotted whole blood)	150
Patient's serum (from clotted whole blood)	50
Patient's serum (washed patient's platelets present during clotting)	60
Patient's serum (washed normal platelets present during clotting)	150

drugs. The return to normal has preceded a similar return to normal of the platelet thromboplastic function but it is not clear whether improvement in these two defects is dissociated or not.

The serum defect has so far been found in five patients with the megakaryocytic type and one (case 1) with the amegakaryocytic (aplastic) type of thrombocytopenic purpura, although the defect in the latter was minimal (table 3). All were clinically severe hemorrhagic patients (table 1) except case 6 who was heavily purpuric but not hemorrhagic, and all except case 6 showed a severe platelet thromboplastic defect (table 2). The serum defect has not been found in the less severe cases of the megakaryocytic type nor in any case of thrombocytopenic purpura associated with acute leukemia, whether hemorrhagic and associated with a severe platelet thromboplastic defect or not. However, two severe hemorrhagic patients of the megakaryocytic type (acute idiopathic thrombocytopenic purpura) with platelet thromboplastic functions of 5.5 per cent and 11.5 per cent, respectively, did not show a serum thromboplastic defect.

COMMENT

The defective serum thromboplastic component has not been identified. From the results of the one-stage tests it would appear that factor VII is not involved and this type of serum defect must be distinguished from that of another patient studied who suffered from liver disease in addition to thrombocytopenic purpura. In this instance the clotting time in the one-stage test was appreciably prolonged. It now seems that either factor VII is not concerned in intrinsic blood coagulation and is only concerned with the activation of tissue thromboplastin,[10] or that it has two components, only one of which is necessary for the formation of blood thromboplastin.[11] The existence and role of factor X does not seem to

be definitely established as yet. Unfortunately it has not been possible to determine whether these defective sera have any corrective effect on the sera of patients with Christmas disease (P.T.C. deficiency) as no such patients have been found in South Australia. However, it is of interest that one of the serum thromboplastic components appears to require a platelet factor for its immediate activation and proper function in the coagulation mechanism.

The platelet factor concerned in this activation mechanism was also not identified. The serum defect was often present in severe purpuric states of the megakaryocytic type in association with a very low platelet thromboplastic function. However, two such severe cases have not shown a serum defect. It was present in only one of two patients with severe forms of the aplastic type of thrombocytopenic purpura and has not been found in cases of acute leukemia. The response of the two defects to cortisone and ACTH has also been dissociated. These findings would thus suggest that separate platelet factors are responsible for each.

The occurrence of a defect of a serum thromboplastic component has some practical application. The test of platelet thromboplastic function provides a very useful means of following the course and effectiveness of therapy in cases of thrombocytopenic purpura.[12] However, the hemorrhagic state seems to be roughly proportional to the total thromboplastic efficiency rather than to the platelet thromboplastic function alone, so that a patient's platelet factor and serum must be examined together when estimating the hemorrhagic state.

Summary

A defect in a serum thromboplastic component was found in six patients with severe thrombocytopenic purpura in addition to the platelet thromboplastic defect.

The defective sera returned to normal spontaneously upon storage at −20 C. The addition of washed normal platelets during coagulation completely corrected the serum defect in the one instance in which it was possible to carry out the experiment.

It was postulated that a platelet factor was primarily at fault, resulting in the lack of immediate activation of a serum thromboplastic component.

The two thromboplastic defects are cumulative and are possibly dependent upon separate platelet factors.

Summario in Interlingua

Un defecto del componente thromboplastic del sero esseva constatete in sex patientes con sever purpura thrombocytopenic, a parte le defecto thromboplastic del plachettas.

Le defective seros retornava spontaneemente al stato normal post immagasinage a un temperatura de minus 20 C. Le addition de lavate plachettas normal durante le coagulation resultava in le complete correction del defecto seral in le un caso in que il esseva possibile executar iste experimento.

Esseva postulate que un factor plachettal esseva primarimente a blasmar, con le resultato de un insufficiente activation immediate de un componente thromboplastic del sero.

Le duo defectos thromboplastic es mutualmente cumulative e depende possibilemente de distincte factores plachettal.

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