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RECENT ADVANCES IN BLOOD COAGULATION WITH REGARD TO ANTICOAGULANT THERAPY, FIBRINOGEN, HEMOPHILA AND PLATELET DISORDERS*

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Studies in blood coagulation which have been pursued for many decades in clinical entities such as hemophilia have suddenly become important in laboratory medicine because of the use of the oral anticoagulants. The problem of intravenous clotting is not well understood and a great deal of attention is being given to it. Since it is not known what elements in blood render it hypercoagulable, one therapeutic approach is to render the blood less coagulable by the administration of oral anticoagulants. Prothrombin time is prolonged when any coumarin or phenindione drug is given.

The prothrombin time (Table I) is a general test involving the addition of tissue

TABLE I

PROTHROMBIN TIME	
PLASMA, OXALATED	0.1 ML.
$CACL_2$	0.1 ML.
TISSUE THROMBOPLASTIN	0.1 ML.
CLOT APPEARS IN 14 SECONDS	

thromboplastin and calcium to plasma and observing the time that the plasma takes to form a clot. If excess or normal amounts of prothrombin and accelerators are present a short prothrombin time of approximately 14 seconds results. Our concepts of coagulation are shown simply in Table II. The prothrombin time measures the components involved in the activation of prothrombin in the presence of tissue thromboplastin. The right side of the Table represents the activation of prothrombin without tissue thromboplastin but with platelets and the various co-factors of platelets which are normally found in plasma. Tissue thromboplastin is not normally present in the circulating blood and is thought to be released only from the surrounding tissue by a break in the surface of the blood vessel caused by trauma. The oral anticoagulants reduce prothrombin and autoprothrombin I, both of which affect the prothrombin time by prolonging it, and autoprothrombin II. Autoprothrombin II is involved in one of the hemophilias and its concentration does not affect the prothrombin time.

It is possible that the oral anticoagulants reduce or alter some other factor which has not yet been identified and that this unidentified component moves parallel to the two factors, prothrombin and autoprothrombin I, which do affect the prothrombin

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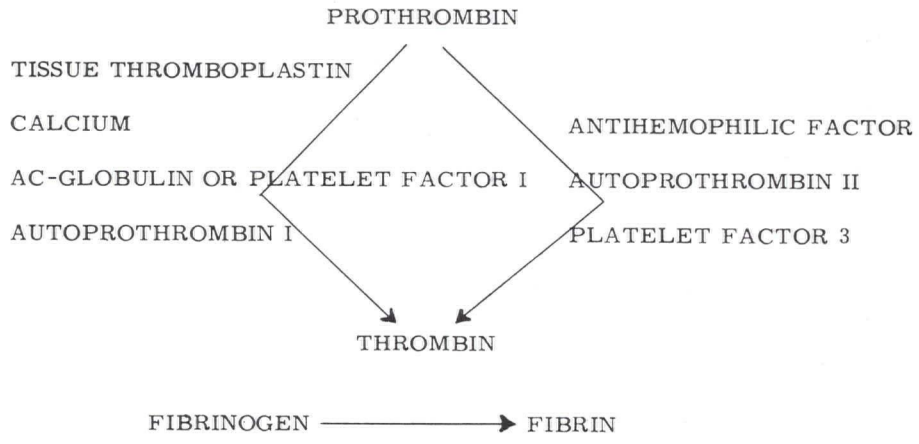
time. This unidentified component may be the more important factor in the prevention of thrombosis. It is known that the prothrombin time gives an accurate gauge of the dosage of the oral anticoagulant, and for this reason it is an important test in blood coagulation.

Table II shows that a small quantity of thrombin, just a fraction of a unit, will greatly shorten the prothrombin time by converting the fibrinogen to fibrin and eliminating the prothrombin to thrombin step. If enough anticoagulant has been given

TABLE II



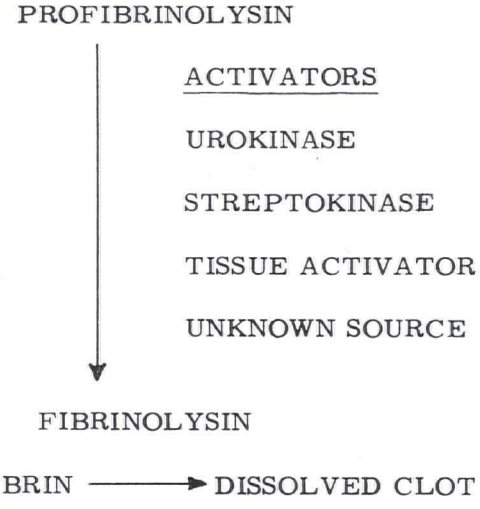
CONCEPT OF COAGULATION

to prolong a 14 second prothrombin time to 30 seconds, a little thrombin can shorten this to 17. A physician may note a 17 second prothrombin time, expect 30 and increase the dose of anticoagulant. The next day instead of 30 seconds as expected he finds 45. The small amount of thrombin should be kept out of the blood in the first place. The blood is mixed in the tube with sodium oxalate immediately after it is drawn. The tissue thromboplastin in the form of tissue juices initiates the coagulation processes and that small amount of prothrombin is converted to thrombin and the cycle begins. Contrary to popular belief the prothrombin time of a specimen of plasma is constant throughout a day. Prothrombin and the accelerators involved in the activation of prothrombin are fairly stable for eight hours or so at room temperature. What this means is that a prothrombin time is rarely falsely prolonged, but only falsely shortened.

Except in the laboratory measurement of dosage of anticoagulant, the field of blood coagulation has contributed little to the management and understanding of thrombosis. The most important aspect of blood coagulation in the clinical sphere in the five next years will be, no doubt, in the area of clot dissolving or fibrinolysim. While the method of preventing the formation of the clot is not yet available, it does appear that more is known about how to dissolve the clot once it has formed. Within the next five years an intravenous fibrinolysim will probably be developed for thrombophlebitis, intravascular thrombosis and even coronary thrombosis. The problem is that

normal blood contains a profibrinolysin in an inactive form and how it is activated to active fibrinolysim has never been discovered. (Table III) It may be an activator which is called urokinase because it is found in urine. Whether or not this is the actual in vivo method is not yet known for certain. Streptokinase is another activator of fibronolyism but is not the natural one.

TABLE III



POSSIBLE ACTIVATORS OF PROFIBRINOLYSIN

In addition to finding the best and, if possible, the natural activator of profibrinolysin, another problem exists. Some investigators believe that once the clot has formed from the whole blood and sufficient profibrinolysin is incorporated into the clot, lysis will take place if activator alone is added so only one of the experimental intravenous preparations contains activator. Another intravenous preparation contains excess activator, as well as some profibinolysin, and, of course, the various activators vary. One of the most exciting races in the pharmaceutical industry is in this field. While intravenous clot prevention is probably a long way off, lysis of the intravenous clot is near at hand.

The two aspects of blood coagulation so far discussed, namely, anticoagulant dosage control and administration of fibrinolysin are recent. Hemophilia, which has been known for centuries, is the time honored problem in blood coagulation. Prior to World War II there was some debate about whether hemophiliacs bled due to a defect in their platelets or to a plasma defect. Brinkhous[1] showed that the hemophilic platelets were normal when removed from normal blood but that the plasma recalcification time was long. The prothrombin time is normal. This is easy to understand with reference to Table II. The antihemophilic factor acts with platelets to activate prothrombin in a somewhat slower fashion than tissue thromboplastin but in a rather analagous way. Therefore formation of activity between platelets and the antihemo-

philic factor is called blood thromboplastin and can be measured by the thromboplastin generation test. The formation of blood thromboplastin requires neither extravascular materials nor a break in the lining of the vessel. For these reasons, tissue thromboplastin added to the plasma will mask this formation of blood thromboplastin, and normal and hemophilic plasma will give a normal prothrombin time. (Table IV)

TABLE IV

LABORATORY TESTS IN HEMOPHILIA	
PROTHROMBIN TIME	NORMAL
PLASMA CLOTTING TIME (RECALCIFICATION TIME)	PROLONGED
CLOTTING TIME	PROLONGED
PROTHROMBIN CONSUMPTION TIME	ABNORMALLY SHORT

Classical hemophilia is concerned with a deficiency in the antihemophilic activity but the kind of defect is not agreed upon by investigators in this field. A controversy exists between those who believe that it represents the actual lack of a factor and those who believe that the antihemophilic factor is present but its activity is masked by association with an inhibitor. For practical purposes it does not matter as treatment would be the same regardless of the mechanism of the defect. The antihemophilic factor is unstable in whole blood or plasma. Perhaps it becomes conjugated with its inhibitor unstable in whole blood or plasma. Perhaps it becomes conjugated with its inhibitor as it does when blood clots for there is no antihemophilic activity in serum. Treatment of a hemophiliac is one of the rare instances in which fresh blood should be given. Many investigators recommend giving a bleeding hemophiliac blood which is less than 12 hours old.

The above remarks pertain to classical hemophilia. In 1952 independently in California and in England another kind of hemophilia was described. The term hemophilia has been enlarged to include any defect in the plasma factors which act with platelets. There are now considered to be at least two factors and some investigators believe there are more. Aggeler and associates[2] called their deficient factor plasma thromboplastin component and the disease PTC. Biggs and associates[3] named it Christmas disease from the name of their patient. (Table V)

Whether the PTC factor or Christmas factor acts with the antihemophilic factor together with platelets or whether one acts with platelets to form a product which then acts with the other is not known. But the two components, the antihemophilic factor and PTC, do act with platelets in some rather complicated fashion. Hemophilia is a rare disaese. Most clinical bleeding problems are concerned with platelet disturbances. However, there are those who feel that platelets have no relation to coagulation.

The thrombocytopenias, primary or secondary, comprise the largest group of bleeding problems. From an investigative point of view the coagulation of blood without platelets is a fascinating concept. It is considered that a platelet count of 50,000 or less will cause bleeding and yet the whole blood clotting time or Lee White

TABLE V
TYPES OF HEMOPHILIA

NAME OF DISEASE	FACTOR INVOLVED
CLASSICAL HEMOPHILIA	ANTIHEMOPHILIC FACTOR
PTC DEFICIENCY OR CHRISTMAS DISEASE	PTC OR CHRISTMAS FACTOR
PTA DEFICIENCY	PTA?

of such blood is normal. Blood will clot in the thrombocytopenic state but it clots differently when an abundance of platelets is available. Blood clots differently when platelets are reduced as evidenced by the fact that the prothrombin consumption time is greatly shortened in thrombocytopenic serum.

The prothrombin consumption time is based on the Quick prothrombin time, the difference being that the prothrombin consumption time is carried out on serum. In normal serum the prothrombin is reduced from about 250 units per millilitre to about 20 units and some accelerators of prothrombin activation are formed as blood clots. If prothrombin time is carried out on normal serum, that is the addition of thromboplastin and fibrinogen, instead of a value of 14 seconds one of 30 or more is found. So the normal prothrombin consumption time is prolonged and if the coagulation is incomplete and residual prothrombin remains in the serum the prothrombin consumption time is shortened. The prothrombin time is easily carried out if the serum is carefully defined such as whole blood that has been permitted to clot for one hour at a standard temperature. The end products of coagulation differ considerably depending on the time that serum is examined. The thrombin, which forms when blood clots, disappears due to the action of antithrombin. As a result, serum 30 minutes old contains thrombin which is almost completely absent in serum 60 minutes old.

If platelets are reduced to the thrombocytopenic state the prothrombin consumption time on such blood is shortened abnormally. However, there is little or no residual prothrombin in the serum. The short prothrombin consumption is due to the formation in abnormal amounts of an accelerator when blood clots. It is probable that this accelerator is a derivative of prothrombin because chemically it resembles prothrombin. From an investigative point of view the mechanism of action of blood coagulation in the thrombocytopenic state is an intensely interesting one. However, at the moment the therapeutic problem must be approached from the other end, that is, replacement of platelets.

Platelet transfusions and platelet substitutes are used in various parts of the country. Whole fresh human platelets are the logical source but economically this presents problems. However, most platelets in bank blood are lost because the platelets have disintegrated before the blood is transfused. It is feasible to collect the platelets from each pint of blood as the blood comes into the blood bank and to lyophilize the platelets and store them till needed. The most active factor in platelets, platelet factor 3 or the thromboplastic factor of platelets, is destroyed with lyophilization which is a disadvantage of this method. Huge quantities of platelets are required to control

bleeding. An attempt to use a purified platelet factor 3 preparation from bovine sources in secondary thrombocytopenia has been made at the Henry Ford Hospital with some success.[4] There have been no antigenic problems, perhaps because the reticulo-endothelial system is damaged in these patients. This bovine platelet preparation is sterilized using beta-propriolactone and ultraviolet radiation.[5] However, as yet the best platelet substitute has not been worked out.

Another aspect of platelet work is concerned with a group of fifteen patients who bleed because of defective platelets.[6] The condition is designated as thrombocytopathy. This group of fifteen patients had mild bleeding manifestation, and most laboratory findings were normal except the bleeding time which was over 20 minutes and the prothrombin consumption time which was 14 seconds. This suggested a platelet dysfunction and the platelets were collected from 100 ml. of blood and assayed in the thromboplastin generation test. It was found that a suspension of equal numbers of normal platelets generated more thromboplastic activity than those of the patients. There are many platelet factors but we feel that the one most responsible for activation of prothrombin and generation of blood thromboplastin is probably platelet factor 3. This platelet activity is also specific for platelets. There is no comparable activity in plasma. Using a specific platelet factor 3 assay involving the activation of purified prothrombin in the presence of the antihemophilic activity the platelets from these patients activated less than one hundred units of prothrombin to thrombin while platelets activated 800 units per millitre.

Rebuck and associates[7] studied these platelets morphologically using the phase and electron microscopes. It is considered that platelets pass through four stages in the process of disintegration. The round or circulating type which is opaque to electrons is believed to become the dendritic form. The dedritic form changes into the intermediate form and then to the less dense spread type. Electron micrographs of normal blood show most of the platelets in the dendritic stage. The platelets of these patients, however, are mostly in the spread stage. A platelet count of the blood of seven of the patients was carried out under the electron miscroscope and revealed about 26 per cent of their platelets to be in the dendritic stage, compared to 80 per cent of the platelets in normal blood. It is possible that this represented a kind of platelet that did not rupture easily and so could account for the pile-up at this later stage. This would mean that the platelets of these patients had adequate amounts of platelet factor 3 but could not release it. This was tested by disintegrating the platelets in a sonic oscillator and measuring the platelet factor 3 activity. The activity of these patients' platelets has increased from the ability to activate 200 units per millilitre of purified prothrombin to thrombin to 600 units per millilitre, when they are thoroughly disintegrated. We feel that we have elucidated, in part, the fault in these platelets but what the satisfactory therapeutic measure will be is another problem.

Another aspect of platelet dysfunction has been found in uremia. Some uremics bleed. The laboratory tests in coagulation appear to be normal except that the prothrombin consumption test in some cases is abnormally short. Like the thrombocytopenic state and thrombocytopathy this short prothrombin consumption was not due to residual prothrombin but probably due to the formation of an accelerator. Platelets from the uremic patients are obtained by differential centrifugation from

100 ml. whole blood, washed in saline three times, and then resuspended in saline at a known concentration per cu.mm. These fresh whole platelets were used to determine clot retraction by the method suggested by Hartman and Conley.[8] The uremic plasma and platelets were compared with that of normal and found to have normal retraction.

Platelet factor 3 assays were carried out and found to be markedly decreased. Previous studies on thrombocytopathy A showed that platelets deficient in factor 3 would give normal activity after being fragmented by sonic treatment. This procedure was carried out on the uremic platelets, but no increase in activity was obtained. This suggests that the defect in uremia is different from that in thrombocytopathy A. It has been postulated that in thrombocytopathy A the platelet factor 3 activity is present in normal amounts; however, the platelets do not disintegrate normally to release it. But in the uremic condition the platelets appear to be actually deficient in the activity. Preliminary studies done by electron microscopy have shown the uremic platelets to be normal morphologically.[9] A considerable study on morphology of thrombocytopathy A platelets have shown them to be abnormal morphologically. Possibly this difference in morphology is closely related to the difference in the activity obtained in thrombocytopathy A after sonic treatment and the lack of activity obtained from uremic patients' platelets.

Thromboplastin generation tests were done using platelets harvested from uremic platelets and these were found to generate far less thromboplastin than platelets from normal individuals. Since many metabolites in the blood are altered in uremia, the possibility arises that they could be responsible for the destruction of platelet activity. To test this possibility, normal platelets were suspended in uremic plasma and incubated at 8° C for 18 hours. Then the platelets were centrifuged and washed and their activity tested. The platelet factor 3 activity of this platelet extract was greatly decreased. The thromboplastin generation test was also done and it was found the platelets which had been incubated in uremic plasma did not generate as much thromboplastin as those incubated in normal plasma. It is too early in this investigation to be able to draw any definite conclusions, but the cause of the loss of platelet factor 3 activity will probably be closely associated with the metabolites of uremia.

BIBLIOGRAPHY

1. Brinkhous, K.M.: Clotting defect in hemophilia: deficiency in a plasma factor required for platelet utilization. Proc. Soc. Exper. Biol. & Med. 66:117, 1947.

2. Aggeler, P. M., and others: Plasma thromboplastin component (PTC) deficiency: a new disease resembling hemophilia, Proc. Soc. Exper. Biol. & Med. 79:692, 1952.

3. Biggs, R., and others: Christmas disease: a condition previously mistaken for hemophilia, Brit. M. J. 2:1378, 1952.

4. Johnson, S. A., Monto, R. W. and Riddle, J. M.: A new approach to the thrombocytopathies (Thrombocytopathy A). Presented at the Symposium on Blood. Wayne State University, 1958.

5. LoGrippo, G. A.: Sterilization of platelet factor 3 with beta-propiolactone and ultraviolet irradiation, Fed. Proc. 17:524, 1958.

6. Johnson, S. A., Monto, R. W. and Caldwell, M. J.: A new approach to the thrombocytopathies. (Thrombocytopathy A). Thrombosis et Diathesis Haemorrhagica. In press.

7. Rebuck, J. W., Johnson, S. A., Riddle, J. M., and Monto, R. W.: Ultrastructural platelet disturbances as a cause of abnormal bleeding. Presented before Am. Soc. Hematologists, 1958.

8. Hartmann, R. C., and Conley, C. L.: Clot retraction as a measure of platelet function; effects of certain experimental conditions on platelets in vitro. Bull. Johns Hopkins Hosp. 93:355, 1953.

9. Cahalane, S. F., Johnson, S. A., Monto, R. W. and Caldwell, M. J.: Acquired thrombocytopathy: observations on the coagulation defect in uremia. Am. J. Clin. Path. In press.