CONTRIBUTION TO THE PATHOGENESIS OF HEMOPHILIA

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IN STUDYING the mechanisms which alter normal hemostasis in hemophilia consideration must be given to the two principal factors, since both appear to be involved, although in different proportions.

The alterations in the hematic factor, represented by a delayed coagulation, have puzzled a great number of competent investigators. Alterations in the vascular factor have been less seriously considered, although in our opinion they play a definite role.

In this paper we shall refer to the various studies on hemophilic coagulation, which is characterized by a lengthening of the coagulation time, apparently caused by a deficit in the thromboplastic factor. To explain this deficit there are three acceptable theories:

(1) *Slow liberation of thromboplastin by an excessive stability of the platelets*

This theory was supported by Sahli (1910),[1] Fonio (1914–36),[2,3] Minot and Lee (1916),[4] Howell and Cekada (1926),[5] Fiessinger and Letard (1940),[7] and was refuted by Feissley (1923),[8] Patek and Stetson (1936),[9] Howell (1939),[10] and Quick (1942).[11] (See also Tocantins.[6])

(2) *An actual deficiency of thromboplastin*

Schmidt in 1893 (quoted by Quick[11]) suggested that the coagulation defect in hemophilic blood is determined by a deficiency in thromboplastin, since zymoplastic substances shorten the coagulation time of hemophilic blood. This theory was subsequently supported by Sahli (1905),[12] Weil (1906),[13] Morawitz and Lessen (1908),[14] Kottmann and Linsky (1910),[15] Schloessmann (1912),[16] and others.

In 1936 new investigations were begun in the United States and Holland (Bendien and Van Creveld[17]) which supported this latter theory. The researches carried out in the Thorndike Memorial Laboratory began with the study of Patek and Stetson,[9] who were supported later by Taylor,[18–22] Pohle, Lozner, and Kark.[23,24] Finally Lewis, Tagnon, Davidson, Minot, and Taylor[25] summarized this work and concluded that in normal whole blood, or platelet-free plasma, there exists a substance (globulin fraction) which shortens the coagulation time of hemophilic blood. The same fraction extracted from hemophilic plasma contains little coagulant power, from which they inferred that hemophilic blood was deficient in some factor or factors present in normal blood. They termed this fraction "antihemophilic globulin."

Howell in 1939[10] called this fraction "thromboplastin" instead of globulin but he agreed that hemophilic blood contains a diminished quantity of thromboplastin.

(3) *Increase of anticoagulant substances which inhibit normal thromboplastin activity*

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The presence of anticoagulant substances has been mentioned by several investigators although their precise nature and their exact mode of action have remained largely obscure. Weil in 1906[18] discovered an increase of antithrombin in hereditary hemophilic blood, which did not exist in sporadic cases. This observation could not be confirmed by Sahli.[1] According to Laclette,[26] "Hydeck of Bratislava in 1923 considered that the most important factor in hemophilia was an excess of antithrombin, the production of which might be under the control of the sexual glands." Feissly in 1924[8] suggested the presence in hemophilic blood of a substance which could prevent the normal transformation of prothrombin into thrombin. On the other hand, in 1931 Evans and Howell[27] discarded the theory of an excess of anticoagulants, either antithrombin or antiprothrombin. Chalier (1935)[28]

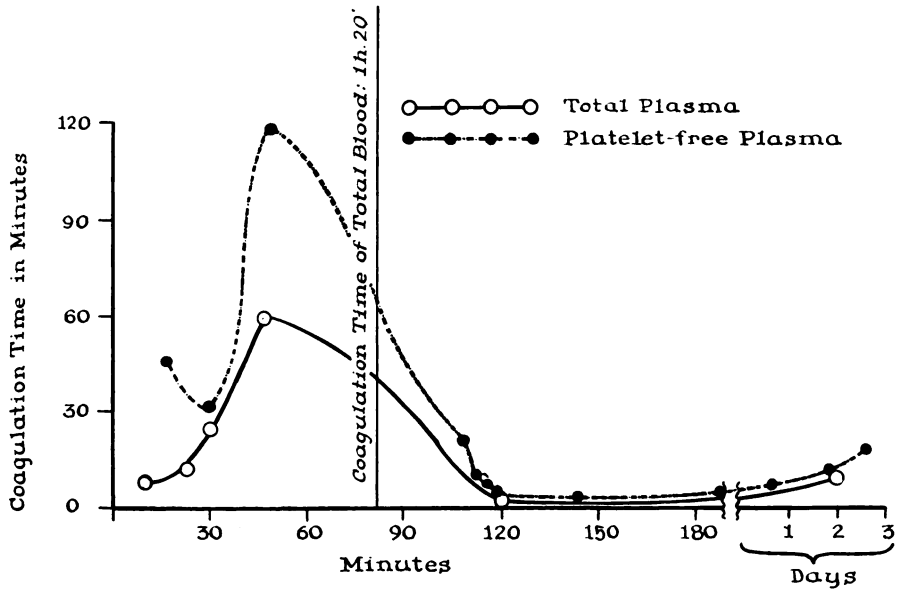

FIG. 1. PERIOD BETWEEN COLLECTION AND RECALCIFICATION OF BLOOD

also disbelieved in the existence of anticoagulant substances. Fonio (1936)[3] in his review concluded that it had not been possible to prove the presence of these anticoagulant substances. Lawrence and Johnson (1942)[29] observed that the blood of a hemophilic patient, added to that of another hemophilic, lengthened the coagulation time of the latter. They maintained that the former's blood contained an anticoagulant substance which was neither heparin nor antithrombin.

In 1942 Tocantins[30] suggested the possibility of the presence of antithromboplastic substances in hemophilia. In 1943[31] he verified the presence of antithromboplastin in both normal and hemophilic blood. He maintained that these antithromboplastic substances acted during the first stages of coagulation, and were later neutralized by the coagulant factors, which are formed in the last stages. It is the presence of these factors that may explain the difficulties found in identifying the

antithromboplastic substances. This antithromboplastic substance may be exhaustible, having a different degree of specificity in the various species; it becomes inactive when heated to 65° C. for 5 minutes; its activity decreases slowly if kept in paraffined or lusteroid tubes. Tocantins found that hemophilic plasma has an anticoagulant activity from five to eight times greater than that of normal plasma. The author termed this substance "anticephalin." In a more recent paper Tocantins[32] gave details of his investigations by which he demonstrated that the increased stability (tendency to remain fluid) of hemophilic blood is due to an uncompensated excess of anticephalin activity.

In 1942 Quick[11] maintained that "the existence of an agent which can neutralize thromboplastin has never been established, nor have any substances been found or prepared which can antagonize by direct action the activity of this clotting factor. This negative evidence does not preclude the possibility that such an agent may ultimately be found." In 1944 Quick[33] seemed to support the theory that the antithromboplastin present in normal blood is abnormally increased in hemophilic blood.

From our own observations, we consider that anticoagulant substances are present in hemophilic blood. These probably act on the coagulant globulin fraction diminishing its activity.

OBSERVATIONS

In 1941, in collaboration with M. R. Castex,[34] we determined the clotting time of recalcified plasma of hemophilic blood, and noted variations in some cases, according to the time at which the test was determined. If the determination was made immediately after collection, the coagulation time of hemophilic recalcified plasma was longer than that of normal plasma; but if the test was made some time after the collection, a period that varied according to the coagulation time of the patient's whole blood, it was definitely shorter. Similar results were obtained by Quick in 1941.[11]

At first we attributed this shortening to a slow disintegration of the hemophilic platelets, which freed the thromboplastin with equal slowness. This hypothesis was discarded after making the same test with platelet-free recalcified plasma, when the same diminution in coagulation time was noted and which could, therefore, not be attributed to the platelets. That indicated the possibility that some anticoagulant substance might be present in hemophilic plasma, which would become inactivated, leaving free the coagulant factors, which would then act as in normal blood.

Subsequently,[35] following the observations of Patek and Stetson,[9] we fixed the optimal doses of total blood, plasma, or serum required to reduce the clotting time of each patient. It was found that the amounts of material required varied with the clotting time of the hemophilic blood, so that for a longer coagulation time, the injection of a larger quantity of normal blood, plasma, or serum was required. One of our patients required very large amounts of blood (more than 600 cc.) in contrast with others who required only 140 cc.

Following observations in vitro (in collaboration with Castex and Simonetti[36])

we verified the influence of the proportion of sodium citrate used in the solutions added to the blood. When instead of using a 3.8 per cent solution (1 to 10 of blood) we used a 7.5 per cent solution of this salt, an anticoagulant effect was noted. In all further experiments we therefore used the 3.8 per cent solution.

With the same collaborators we studied the action of the hemophilic blood added to the blood of other hemophilic patients, and observed a paradoxical fact. We found that occasionally (in vitro) the blood of some of the hemophilic patients with a greatly prolonged clotting time (1 hr. 20 mins.) when added to other hemophilic blood with a much shorter clotting time (40 mins.) possessed a coagulant action nearly as effective as normal blood. Acting on this observation, we transfused plasma from the patient with the longest clotting time to one of the other patients (table 1). The results of this experiment made us doubt whether a deficiency in a coagulant substance (globulin fraction) really existed; for if it did exist, it would be illogical to obtain a coagulant action with hemophilic blood which contained a smaller quantity of coagulant substance.

TABLE 1.—*Transfusion of 100 cc. of Plasma from Hemophiliac 1 to Hemophiliac 9*

Hemophiliac H9	Coagulation time: 30 minutes
Hemophiliac H1	Coagulation time: 80 minutes

Time elapsed after the transfusion of plasma from hemophiliac H1	Coagulation time of hemophiliac H9
Before the transfusion	30 minutes
½ hour later	6 minutes
24 hours later	13 minutes
48 hours later	28 minutes

If we admit that hemophilic blood has a deficit of globulin fraction, we consider that this deficit might be caused not by an original deficiency, but by the action of anticoagulant substances that would render it inactive. With the idea of studying this hypothesis we tried to diminish the coagulant action in normal persons by the help of anticoagulant substances. By using heparin by injection or dicoumarin orally (Pavlovsky and Simonetti[37]) we showed that the globulin fraction had less coagulant activity when coagulation was most delayed. From this experiment we deduced that if the globulin fraction of the hemophilic blood had less coagulant activity, it might be due to the presence of anticoagulant substances that would neutralize its action.

In further studies[38] we determined the coagulant activity of the globulin fraction in different patients and found that it varied, but not in accordance with the length of clotting time. In some of our patients with a greatly prolonged clotting time, the globulin fraction possessed a coagulant activity almost equal to that of normal blood. This indicated the possibility that if an anticoagulant fraction existed, it had a different stability or a different activity in each patient; thus in some the proportion might be greater than in others, hence their longer or shorter clotting time. This substance might become inactive in varying periods of

time after the blood has been extracted, this being the cause of the discordant variations in the coagulant activity of the globulin fraction in the different hemophilic patients.

With this thought in mind, we tried to obtain inactivation of the anticoagulant substance by precipitating the globulin fraction of the hemophilic plasma, in two ways. One sample was obtained by precipitating the globulin immediately after collection of the blood, the other, by leaving the blood 24 hours in a refrigerator before precipitating the protein fraction. In the second test we verified that in some cases the globulin fraction had increased its coagulant power. We thought that this might be due to the inactivation of the anticoagulant fraction.

In accordance with the preceding experiment, we gave our patients autotransfusions of blood, verifying that if we extracted the blood and injected it again immediately afterwards no modification was obtained in the coagulation time; but if the blood was injected after having been kept in a refrigerator for 48 hours, a coagulant effect was obtained that varied according to the patient. We proved that the effect was more obvious if we used whole blood instead of plasma.

From the foregoing evidence, we deduced that it might be possible to solve the problem of the coagulation time in hemophilia by inactivation of anticoagulant substances. It has been established that these anticoagulant substances are neither heparin nor dicoumarin.

We tried also (in vitro) to shorten the clotting time of hemophilic blood by the addition of protamine, without attaining a satisfactory result. It is generally acknowledged that protamine reduces the anticoagulant activity of heparin (Chargaff and Olson,[39] Jaques and Waters,[40] and Jorpes[41]). Despite these negative results, we believe that investigations should be continued to discover a medium that would inactivate these hypothetic anticoagulant substances.

This will be the aim of our further investigations.

SUMMARY

The causes of the delayed coagulation of hemophilic blood seem to become clearer as time advances. On one side we have the investigations of the school of Minot, indicating a deficit in the globulin fraction; and on the other side are the works of those who maintain that there exists an excess of anticoagulant substances. We support the latter theory, although in our opinion the two theories do not contradict each other, since it might be possible that this anticoagulant substance would act on the globulin fraction diminishing its coagulant power. This substance could be identified with the anticephalin fraction of Tocantins.

With the idea that this substance might be less stable than the coagulant fractions (fibrinogen, prothrombin, and thromboplastin) we have tried to render it inactive by keeping the blood in a refrigerator for a certain length of time. Inactivity was obtained in some of our experiments. We know that the stability of this substance varies from one patient to the other, but we have not been able to fix the cause of these variations.

In conclusion, we consider that other means of neutralizing the action of this

anticoagulant substance should be investigated. This inactivity once obtained, we should have advanced far in solving the intricate problem of hemophilia.

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