THE BIOLOGICAL SIGNIFICANCE OF THE THROMBOPLASTIC PROTEIN OF BLOOD*

BY ERWIN CHARGAFF AND RANDOLPH WEST

(From the Departments of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia University, and the Medical Service of the Presbyterian Hospital, New York)

(Received for publication, August 5, 1946)

The activators of blood clotting present in mammalian tissue, i.e. the agents responsible for the conversion of prothrombin to thrombin, have been shown to be lipoproteins of a very high particle weight (1-3). These substances readily form sediments in a strong centrifugal field (31,000g), but remain in solution when subjected to weaker centrifugal forces (5000g).

The consideration of the manner in which thromboplastic substances occur in blood is of importance for an understanding not only of the physiology of normal blood coagulation but also of bleeding disturbances, such as hemophilia. The rôle of the blood platelets, usually regarded as the main source of thromboplastic material, in the clotting of normal and of hemophiliac blood has often been considered. (Compare the surveys in (4, 5).) But whether an additional factor, exhibiting the centrifugal characteristics of the thromboplastic protein of tissue cells, is present in blood has remained unknown. A brief report on the effect of high speed centrifugation of plasma on its coagulation time (6) appeared, however, suggestive of the existence of such a factor.

The orienting studies presented here include a comparative investigation of the effect of high speed centrifugation on the clotting time of normal and of hemophiliac plasma. They were prompted by the opportunity of studying an interesting case of an acquired hemophilia-like condition in a female patient.

The literature contains only few reports on an acquired bleeding disturbance in the female exhibiting, in a more or less typical fashion, the characteristics of hemophilia, except for the important feature of hereditary transmittal (8, 9). The case which furnished the opportunity for the study of the blood clotting defect presented here was, in addition, characterized by the presence of a circulating anticoagulant, a phenomenon occasionally observed in the past (10, 11). The other blood specimens used in this study were obtained from a genuine hemophiliac with authentic family history.

* This work has been supported by a grant from the John and Mary R. Markle Foundation.
1 The effects of centrifugation at low speed have been compared by Quick (7).
Origin of Blood Specimens—The blood samples designated N were collected from normal young adults. Those marked H came from an authentic hemophiliac. The blood specimens listed as F were obtained from a female patient with an acquired bleeding disturbance. A brief history of this case is given in the following paragraph. All blood samples were collected before breakfast and examined immediately.

Case History—(Presbyterian Hospital, Unit No. 784026.) A housewife of 33 years was admitted with diffuse, recent subcutaneous hemorrhages and a large hemorrhage beneath the tongue. There were slight limitation of motion of both knees, secondary anemia, moderate leucocytosis. Blood clotting time, 80 to 100 minutes. Considerable prolongation of clotting time of normal blood on admixture of small amounts of the patient's blood. Prothrombin time, 23 seconds. Platelets, 165,000. Serum protein 6.1 gm. per 100 cc. Alkaline phosphatase, 2.4 Bodansky units. Cephalin flocculation, negative. Electrophoretic pattern of plasma normal, including fibrinogen. No unusual capillary fragility. Blood is Rh-positive. The present illness probably dates from 7 months before admission when the patient had her last delivery. Several weeks after a normal delivery, hemorrhages, chiefly in the arms and legs, appeared about every 2 weeks. There was subcutaneous bleeding, intramuscular and within joints. Previous bleeding history: hemorrhages following spontaneous abortion in 1942 and after a tooth extraction in 1943. Three out of five pregnancies resulted in miscarriage; neither of the living children has any bleeding tendency.

Protamine Titration—In view of the presence of a circulating coagulant in blood F it appeared of interest to examine it for the presence of heparin. The determination of the clotting time of blood and plasma, following the addition of varying small amounts of salmine, is based on the observation that this strongly basic protamine abolishes the anticoagulant action of heparin both in the circulation and in vitro (12). The determinations on whole blood were carried out with venous blood, freshly drawn (without any addition) before breakfast. The plasma was obtained 50 minutes later from the same blood sample without centrifugation by allowing the blood cells to settle in the refrigerator. The results, summarized in Table I, furnish no indication of the presence of an anticoagulant of the heparin type. The drop in clotting time, observed on addition of 2.5 γ of protamine, cannot be explained at present.

Effect of Centrifugation on Plasma Clotting Time—The effect of differential centrifugation on the clotting time of normal human plasma has been discussed in a recent publication (13). In the present study a similar approach was employed for a comparison of the behavior of plasma obtained from the patients F and H with that of normal plasma. The blood, collected before breakfast, was mixed immediately with one-ninth its volume of 0.1 M sodium oxalate solution. Plasma samples were removed following centrifugation at 1500 R.P.M. (260g) for 3 minutes (Experiment 1, Table II). The remain-
ing mixture was then centrifuged at 4000 R.P.M. (1900g) for 20 minutes in a refrigerated angle centrifuge. The clear supernatant was siphoned off carefully, and samples were removed for testing (Experiment 2, Table II). 10 cc. portions of the plasma samples were then subjected to a centrifugation at 20,000 R.P.M. (31,000g) for 150 minutes in a refrigerated International centrifuge equipped with a multispeed attachment, which brought about

<table>
<thead>
<tr>
<th>Clotting time</th>
<th>Salmine in experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood.........</td>
<td>25 min. 10 min. 5 min. 2.5 min. 0 min.</td>
</tr>
<tr>
<td>Plasma........</td>
<td>40 min. 40 min. 40 min. 30 min. 35 min.</td>
</tr>
</tbody>
</table>

**TABLE II**

Effect of Centrifugation on Plasma Clotting Time

The experiments were performed at 37° by mixing 0.1 cc. of plasma with 0.2 cc. of a 0.01 M calcium nitrate solution (containing 0.42 per cent of sodium chloride). In experiments in which more than one clotting time is reported, the figures record the span between the first appearance of fibers and the formation of a clot. N = normal; F = female bleeder; H = hemophiliac.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Duration of centrifugation</th>
<th>Centrifugal force</th>
<th>Plasma clotting time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 min.</td>
<td>260</td>
<td>N 2 min., 50 sec.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 9 min., 45 sec. to</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 5 min., 10 sec. to</td>
</tr>
<tr>
<td>2</td>
<td>20 min.</td>
<td>1,900</td>
<td>N 3 min., 20 sec. to</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F 15-27 min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 10 min., 40 sec. to</td>
</tr>
<tr>
<td>3</td>
<td>150 min.</td>
<td>31,000</td>
<td>N 5 min., 50 sec. to</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F No clot within 81 min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 12 min., 35 sec. to</td>
</tr>
</tbody>
</table>

The small pellets sedimented by the centrifugation of 10 cc. of plasma at 31,000g were suspended in 1 cc. of physiological saline (corresponding to a 10-fold concentration with respect to the original plasma). The clotting

2 All centrifugations were carried out in lusteroid tubes.
Effect of these suspensions on the plasma samples that had undergone centrifugation at 31,000g is presented in Table III.

It appeared of interest to determine whether a fraction possessing the centrifugal characteristics of the thromboplastic protein was contained in the pellets sedimented from normal plasma at 31,000g. As has repeatedly been shown in this laboratory (2, 3, 13), the thromboplastic protein of various tissues is sedimentable at 31,000g, but not at 5000g. A sample of normal oxalated plasma was subjected to a fractional centrifugation, as described before. Following centrifugation at 1900g for 20 minutes, the clotting time of the recalcified plasma was 217 to 250 seconds; following centrifugation at 31,000g for 150 minutes, 340 to 690 seconds (see also Table II). The suspension of the high speed sediment (from 10 cc. of plasma) in 1 cc. of borate buffer of pH 8.4 was centrifuged at 8000 R.P.M. (5000g) for 30 minutes. The almost clear supernatant, when tested in the arrangement presented in Table III, produced a firm clot in 160 seconds. The amount of

**Table III**

*Effect of High Speed Sediments on Clotting Time of Plasma Centrifuged at High Speed*

The experiments were carried out at 37° by mixing 0.1 cc. of plasma with 0.1 cc. of the saline suspensions of the high speed sediments (or, in control experiments, of physiological saline) and 0.2 cc. of a 0.01 M calcium nitrate solution (containing 0.42 per cent of sodium chloride). The components of the mixtures are indicated by plus signs. For the explanation of the clotting intervals indicated, compare Table II. Assays A and B were carried out at different times of admission to the hospital of patient F and with different specimens of normal plasma. N = normal; F = female bleeder; H = hemophiliac.
thromboplastic protein found in normal human plasma, when expressed in
terms of the most active preparation isolated from human tissues (3), may
be estimated tentatively as between 0.1 and 1 γ per cc. of plasma.

Anticoagulant Effect of Plasma—Admixture of plasma F to normal plasma
(corresponding to the plasma samples listed as Experiment 2, Table II)
cau sed definite clotting inhibition. With 7 parts of the patient's plasma
and 3 parts of normal plasma or with equal parts of both plasma samples,
an extremely slow fibrin deposition was observed which led to the formation
of a very soft coagulum in about 29 minutes.

**Table IV**

**Effect of Thromboplastic Protein of Beef Lung on Clotting Time of Plasma
Centrifuged at High Speed**

The experiments were carried out at 37° by mixing 0.1 cc. of plasma with 0.1 cc.
of physiological saline containing the indicated amounts of thromboplastic protein
and 0.2 cc. of a 0.01 M calcium nitrate solution (containing 0.42 per cent of NaCl).
For the explanation of N, F, H, A, and B, see Table III.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Thromboplastic protein</th>
<th>Clotting time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>149</td>
</tr>
<tr>
<td>5</td>
<td>0.01</td>
<td>248</td>
</tr>
<tr>
<td>6</td>
<td>0.001</td>
<td>295-540</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>365-690</td>
</tr>
</tbody>
</table>

**Plasma Antithrombin**—The plasma of patient F appeared to contain no
more than the normal amount of antithrombin. (For a discussion of this
factor, see a recent review article (14).) Albumin fractions prepared by
techniques described previously (15) exhibited an antithrombin effect that
was only slightly higher than that shown by comparable preparations from
normal plasma.

**Effect of Thromboplastic Protein**—The action of preparations of the throm-
boplastic protein on human plasma, centrifuged at 31,000g, has recently
been discussed (3, 13). In the experiments here presented a highly purified
preparation of the thromboplastic protein of beef lung (2) was employed.
The results, shown in Table IV, demonstrate that with less than 1 γ of the
thromboplastic protein per 0.1 cc. of plasma the coagulation times of both
pathological plasma samples were abnormally long, though with more this
difference vanished. In what may be considered a region of thromboplastin excess (1 γ or more) the hemophilic plasma H and the hemophilia-like plasma F behaved essentially similar to normal plasma.

**Incubation of Thromboplastic Protein with Plasma**—In view of the apparent deficiency of the pathological plasma specimens in thromboplastic protein, it was of importance to determine whether these plasma samples contained an agent able to destroy the thromboplastic protein. Oxalated plasma (prepared by centrifugation at 31,000g for 150 minutes) was incubated at 37° with a very potent preparation of the thromboplastic protein of beef lung (in concentrations of 20 and 1 γ per cc. of plasma) and the clotting time of the recalcified mixture determined after various time intervals. No important departure from the behavior of normal plasma was observed: the rates at which the clotting times of all plasma specimens responded to the length of incubation were essentially similar (Table V).

It is, in fact, doubtful whether the gradual prolongation of the clotting time may be attributed to the thromboplastic protein which, at least in the absence of plasma proteins, is known to be extremely stable (2). It is pos-

### Table V

**Effect of Incubation of Thromboplastic Protein with Plasma**

Mixtures of 1 cc. of plasma (centrifuged at high speed) with 1 cc. of saline containing the indicated amounts of thromboplastic protein were kept at 37°. The clotting times were determined at stated intervals on 0.2 cc. portions of the mixtures, following recalcification as in Table II. The first appearance of fibers is recorded as the clotting time. N = normal; F = female bleeder; H = hemophilic.

<table>
<thead>
<tr>
<th>Thromboplastic protein per 1 cc. plasma</th>
<th>N</th>
<th>F</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation time</td>
<td>Clotting time</td>
<td>Incubation time</td>
</tr>
<tr>
<td>γ 20</td>
<td>0 min.</td>
<td>85 sec.</td>
<td>0 min.</td>
</tr>
<tr>
<td>10</td>
<td>105 min.</td>
<td>105 sec.</td>
<td>17 min.</td>
</tr>
<tr>
<td>25</td>
<td>102 min.</td>
<td>102 sec.</td>
<td>31 min.</td>
</tr>
<tr>
<td>40</td>
<td>115 min.</td>
<td>115 sec.</td>
<td>48 min.</td>
</tr>
<tr>
<td>60</td>
<td>118 min.</td>
<td>118 sec.</td>
<td>67 min.</td>
</tr>
<tr>
<td>110</td>
<td>132 min.</td>
<td>132 sec.</td>
<td>118 min.</td>
</tr>
<tr>
<td>150</td>
<td>180 min.</td>
<td>180 sec.</td>
<td>101 min.</td>
</tr>
<tr>
<td>240</td>
<td>268 min.</td>
<td>268 sec.</td>
<td>258 min.</td>
</tr>
<tr>
<td>1 20</td>
<td>0 min.</td>
<td>150 sec.</td>
<td>0 min.</td>
</tr>
<tr>
<td>20</td>
<td>170 min.</td>
<td>170 sec.</td>
<td>35 min.</td>
</tr>
<tr>
<td>77</td>
<td>255 min.</td>
<td>255 sec.</td>
<td>92 min.</td>
</tr>
<tr>
<td>120</td>
<td>285 min.</td>
<td>285 sec.</td>
<td>135 min.</td>
</tr>
</tbody>
</table>
sible that partial inactivation of prothrombin is responsible for the observed effect.

**DISCUSSION**

Tables II to IV may be considered to summarize the most important results of this study. When oxalated plasma, following centrifugation at 4000 R.P.M. (1900g), is subjected to prolonged centrifugation at 20,000 R.P.M. (31,000g), its coagulation time is extended very noticeably. This effect is even more conspicuous in hemophiliac plasmas in which occasionally complete absence of clotting is observed (Table II). The clotting factor of which the plasma is deprived by high speed centrifugation is found in a minute pellet whose sedimentation is brought about by this operation. When the effects of these sediments on normal and pathological plasma specimens are compared, it can be seen that the plasma from patients exhibiting a marked bleeding tendency contains considerably less of the clotting activator than does normal plasma (Table III). Thromboplastic protein preparations, when added in sufficient, though still very small, amounts, induce the pathological plasma specimens to clot with normal speed (Table IV).

The studies of the coagulation defect in the case exhibiting an acquired bleeding tendency resembling hemophilia (patient F) indicate that it is attributable to two causes, (1) a pronounced lack of thromboplastic factor in the plasma of the patient, (2) the presence in this plasma of a clotting inhibitor different from heparin. It might be argued that these two causes are in reality facets of the same phenomenon; namely, the occurrence in the patient of an agent that destroys the activity of the thromboplastic protein. However, the experiments on the effect of incubation of the thromboplastic protein with plasma (Table V) furnish no support to this assumption, at least with respect to the presence in the blood of such a factor, nor is there evidence of the existence of such an agent in the authentic hemophiliac plasma H.

It appears likely that a thromboplastic protein not unlike that isolated from tissue cells occurs in extravasated normal blood and contributes to its clotting properties. The particulate fraction sedimented at 31,000g probably includes, in addition to the thromboplastic agent, a variety of minute breakdown products of the blood corpuscles. Whether the thromboplastic protein exists in circulating blood remains, unfortunately, an inherently unanswerable question. In any event, the results summarized in Table III point to a remarkable parallelism between the clotting behavior of the whole plasma and the activity of the high speed sediment derived from it. If the activation experiments presented in Table IV are plotted as previ-
ously described (13), it can, for instance, be computed from the results of Experiments 2 and 6 in Table III that the high speed sediment from 1 cc. of normal plasma exhibited an activity corresponding to 0.4 $\gamma$ of the thromboplastic protein from beef lung, whereas the sediment from the pathological plasma F contained only 0.03 $\gamma$.

The reasons for this abnormality are not clear. Recent work in this laboratory (3, 13) has demonstrated that practically the entire thromboplastic activity of tissue cells is confined to one particulate fraction, the thromboplastic protein, which is probably derived from the cytoplasm. The specific cell types responsible for the thromboplastic effect are not yet known. In the absence of additional information, the explanation of bleeding disturbances that are due to the low concentration of the thromboplastic protein in blood (hypothromboplastinemia) will have to be limited to the statement that these conditions could be due either to the failure of certain tissue cells to produce, or to release, sufficient quantities of the thromboplastic factor or to the occurrence in the organism of an agent that destroys this factor. Concerning the anticoagulant whose presence has been shown to be probable in plasma F, all that can be said at present is that it appears not to be heparin.

The assistance of Mrs. Helen Fabricant Saidel is gratefully acknowledged.

**SUMMARY**

The effects of high speed centrifugation on the clotting behavior of normal and hemophiliac plasma are compared. The coagulation defect in the pathological specimens is shown to be attributable to a marked deficiency of the blood in a clotting factor similar to the thromboplastic protein of tissue cells. The blood of a female patient exhibiting a non-hereditary bleeding disturbance was, in addition, characterized by the presence of a coagulation inhibitor different from heparin. In connection with the finding that the coagulation defect can be overcome by the addition of the purified thromboplastic protein of beef lung, the evidence of the presence in normal blood of a thromboplastic protein fraction, sedimentable by high speed centrifugation, similar to that isolated from tissue cells is discussed.

**BIBLIOGRAPHY**

THE BIOLOGICAL SIGNIFICANCE OF THE THROMBOPLASTIC PROTEIN OF BLOOD
Erwin Chargaff and Randolph West


Access the most updated version of this article at [http://www.jbc.org/content/166/1/189.citation](http://www.jbc.org/content/166/1/189.citation)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/166/1/189.citation.full.html#ref-list-1](http://www.jbc.org/content/166/1/189.citation.full.html#ref-list-1)