RELATION OF CONTACTING SURFACE AND ANTICEPHALIN ACTIVITY TO THE MAINTENANCE OF THE FLUIDITY AND COAGULABILITY OF BLOOD

By Leandro M. Tocantins, M.D.

Tentative explanations of the mechanism of coagulation of the blood must necessarily be concerned as well with the equally important problem of the maintenance of its fluidity in the circulation. Fluidity and coagulability are properties of the blood that are in a sense antagonistic to each other. To understand what promotes coagulation, one must also be familiar with the factors that discourage this change and thereby keep the blood in a fluid state. Both physical states are essential in their proper places. Excessive bleeding may result when the blood is so stable that it remains liquid longer than it should after it leaves the vessels; if, on the other hand, the blood clots within the vessels, where it should not, an interruption of the circulation may follow. It is by preserving a balance between fluidity-inducing (anticoagulant) and coagulation-promoting (coagulant) factors that the blood maintains itself as a circulating medium.

Two theories of blood coagulation, with substantial experimental support—those of Bordet1 and of Howell2—have attempted to account for the state of fluidity of circulating blood as well as for the development of coagulation in shed blood. Bordet divided the reactions involved into three phases:

1. Proserozyme (inactive prothrombin) on contact with a suitable surface or with lipid cytozyme = serozyme (active prothrombin).
2. Serozyme (active prothrombin) + cytozyme (lipid from platelets and tissue cells) in the presence of calcium = thrombin.
3. Thrombin + fibrinogen = fibrin.

Howell also recognized the existence of three successive reactions:

1. Prothrombin / antiprothrombin (heparin) + platelet or tissue factor (cephalin) = free prothrombin.
2. Free prothrombin + calcium = thrombin.
3. Thrombin + fibrinogen = fibrin.

With the exception of Pickering3 and Fuchs,4 most workers, especially in publications within the last ten years, do not mention the first phase and therefore leave unexplained the question of the maintenance of the fluidity of circulating blood.

In common with Bordet, Howell recognized that the prothrombin in circulating blood differs from that in shed blood. According to Howell, the circulating prothrombin is bound with antiprothrombin (heparin) and is freed from the latter by the platelet or tissue factor that neutralizes the antiprothrombin. With this point Bordet could not bring himself to agree, and much of the evidence that he brought against it received further support from Pickering,3 Mellanby,5 and others. Bor-
det also questioned the hypothesis that prothrombin can be activated by calcium alone, and insisted that the lipid (cytozyme) activator is necessary.

According to Bordet's theory, the initial step toward clotting is a change of the inactive prothrombin (proserozyme) into an active one (serozyme) when the blood is brought into contact with a suitable surface or with the lipid from platelet and tissue cells (cytozyme). Just how contact brings about this change, which requires a variable period of time, was not clear to Bordet. He recognized, however, that the inactive form of prothrombin (proserozyme) may simply represent prothrombin protected by an inhibiting substance that can be inactivated or displaced by a suitable contacting surface, thereby setting the prothrombin free.

The existence of forces that influence the conversion rate of prothrombin was therefore clearly recognized by Bordet and Howell. Within recent years, principally perhaps because of the great interest in the quantitative determination of prothrombin, the role of the contacting surface and the existence of prothrombins of differing reactivity have been to some extent overlooked. The process of coagulation has generally been divided into the two phases originally proposed by Mcrawitz:

1. Thrombokinase (or thromboplastin) + prothrombin + calcium = thrombin.
2. Thrombin + fibrinogen = fibrin.

The impression is conveyed (unwittingly perhaps) that prothrombin can be instantly changed into thrombin in vivo or in vitro by the action of thromboplastin in the amount ordinarily present in the blood. The fact that a great excess of thromboplastin is necessary for the rapid conversion of prothrombin to thrombin in the quantitative determination of prothrombin, should not lead one to forget that such working conditions are artificial and designed to remove natural obstacles (presence of inhibitors, variations of prothrombin conversion rate) that have in the past defeated efforts toward the development of quantitative methods. Attention has been drawn to the errors that may be caused by this oversight. The tendency to attribute to the amount of prothrombin in the blood the dominating role in coagulation overlooks the fact that, under natural conditions, the conversion rate of prothrombin (independent of its amount) really governs the speed of the reaction. For example, what seem to be quantitative differences in the prothrombin of the blood of different species have been shown to represent variations in the respective prothrombin conversion rates. It becomes of some importance therefore to determine the factors involved in the conversion of prothrombin to thrombin.

The remarks that follow will deal principally with the natural forces involved in the changes preceding the activation of prothrombin in man. There is general agreement that the lipid from platelets and tissue extracts promotes the coagulation of blood by accelerating the transformation of prothrombin into thrombin. If the amount of prothrombin is held fixed, the speed of its conversion into thrombin is substantially determined by the quantity of lipid factor available. Whether the lipid influences the conversion of prothrombin by combining directly with it, as Bordet, Mellanby, and others have maintained, or by removing an inhibitor, is a point on which general agreement has not been reached. The evidence brought out by modern methods seems to indicate that thrombin results from
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a chemical combination between the lipid factor and prothrombin. The question that remains unanswered is: What factor keeps the prothrombin relatively invulnerable to the amounts of the thromboplastic lipid that may be released in the circulating blood, and how is that factor removed from the blood as it is shed?

The existence in the blood of a natural antiprothrombin, or of a substance that acts on prothrombin itself and presumably opposes its transformation into thrombin, is not generally conceded. Natural antithromboplastins likewise have until recently not been seriously considered. These doubts have been reflected in the attitude of some workers, who have been unwilling to accept the assumption of an inhibitor as an explanation of the fluidity of circulating blood. This viewpoint is all the more understandable when one considers that the inhibitor, if present at all, would make itself felt only in the early stages of coagulation, before adverse surrounding conditions or release of the lipid, platelet, or tissue thromboplastic factor, rendered it partially or wholly ineffective. It would therefore have a fleeting existence in vitro. The difficulty of distinguishing between antiprothrombin and antithromboplastic activity contributes further to the uncertainty. Since, for activating prothrombin, thromboplastin is required, the slowing of this activation might be attributed with equal plausibility to a deficiency in thromboplastin, to antiprothrombin, or to antithromboplastic activity. In view of what follows, it is not unlikely that much that has been described as antiprothrombin may actually be antithromboplastic activity.

Observations of the past four years seem to indicate that a natural antithromboplastin exists in normal human plasma. This substance (or group of substances), the activity of which may be detected in plasma separated from normal blood collected with special precautions, reduces the clot-accelerating action of dilute extracts of brain tissue when the plasma is incubated with the extracts before recafcification. The inhibiting activity is exhaustible, has a certain degree of species specificity, is made ineffective by heating (to 65°C. for five minutes) or dilution, diminishes slowly on standing in paraffin- or collodion-coated tubes, and more rapidly in glass vessels when in contact with platelets or other blood and tissue cells. Hemophilic plasma has an inhibiting activity from five to eight times greater than that of normal plasma in relation to certain dilute thromboplastin solutions. In shed hemophilic blood, more free thromboplastin and a longer time are necessary for the inactivation of antithromboplastin than in normal blood.

The thromboplastin of aqueous brain extracts is a lipoprotein; its clot-accelerating properties reside chiefly in the lipid moiety, the active constituent of which is a cephalin. It seems that it is the cephalin portion that is vulnerable to the antithromboplastic activity of the plasma. The well known increased resistance of hemophilic plasma to activation by cephalin appears to be due to its excessive antithromboplastic activity. This activity, which may then be properly referred

* The term cephalin as used in this and preceding papers designates the alcohol-insoluble lipid fraction extracted with ethyl ether from acetone-dried human brain. Cephalin suspensions so obtained are obviously not pure. Most indications point to a cephalin as the active fraction of the thromboplastic lipoprotein, and it may be so considered until convincing evidence to the contrary is available. The term anticephalin is intended to designate that activity of plasma which is directed against the thromboplastic action of cephalin.
to as due to *anticephalin*, bears some resemblance to the anticoagulating effect of the protamines, which are known to oppose the thromboplastic action of cephalin by combining with it and forming indissociable inert compounds.

Plasmas that have been exposed to contact with materials rich in silica (kaolin, asbestos wool fibers, infusorial earth, glass), or that have been dialyzed against water, lose their anticephalin activity and in this state are easily activated by cephalin, in the form either of tissue or platelet extracts or of cephalin suspensions. A study of the rate of conversion of prothrombin of these plasmas (hemophilic, normal, and adsorbed) has disclosed that the prothrombin of the adsorbed plasma is more rapidly converted into thrombin than that of the normal and hemophilic plasmas. Dilutions of the plasma to 5 per cent or less may be required to equalize the reaction to cephalin as between normal and hemophilic plasmas.

On the other hand, in order to equalize the coagulability of adsorbed plasma, normal plasma requires 320 times and hemophilic plasma about 1000 times as much cephalin as adsorbed plasma (table 1).

**Table 1.** — *Clotting Time* (in Seconds), in Glass Tubes, of Normal, Hemophilic, and Adsorbed Plasmas to Which Increasing Dilutions (0.85 per cent Sodium Chloride) of Cephalin Have Been Added

<table>
<thead>
<tr>
<th>Type of Plasma</th>
<th>Dilution of Cephalin Suspension</th>
<th>0.85 C. NaCl</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>X 5</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>Hemophilic</td>
<td></td>
<td>169</td>
</tr>
<tr>
<td>Adsorbed†</td>
<td></td>
<td>27</td>
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</table>

* 0.3 cc. of plasma, 0.1 cc. of cephalin suspension, 0.1 cc. of 0.074 mol calcium chloride.
† Hemophilic plasma in contact with asbestos wool fibers for two hours at 20 C. (10 mg. of asbestos per 1 ml. of plasma).
Prothrombin content (two stage method) of normal and hemophilic plasma, 100 per cent of normal; of adsorbed plasma, 78 per cent of normal.

The fact that contact with certain surfaces accelerates blood coagulability seems therefore to be linked with the removal (by adsorption or inactivation) of anticephalin from the plasma, with a consequent increase in its prothrombin conversion rate. Plasmas with an excess of anticephalin (e.g., hemophilic) appear to be better protected not only from activation by cephalin but also from the effect of contact with glass. The slower rate of coagulation in paraffin- and collodion-coated tubes seems to be due to the fact that they preserve anticephalin activity longer, thereby holding the conversion of prothrombin to a slow rate. Differences between the coagulability of blood in glass and in collodion tubes generally parallel the magnitude of its anticephalin activity. The high direct correlation between these two variables led to the proposal that the extent of the difference between the rate of coagulation of blood in glass and in collodion tubes might be used as an approximate estimate of blood anticephalin activity. The greater magnitude of the electrical charge on a glass surface in contact with water, when compared with that on a paraffin surface, led Gortner and Briggs to suggest that a positively charged substance in blood standing in a glass tube, may be adsorbed by the negatively charged...
glass surface, thereby modifying the coagulability of the blood. That such forces may be involved in changes in the plasma after contact with glass, has been indicated by Lozner and Taylor. Whether anticephalin is simply adsorbed on the contacting surface or also inactivated is not possible to state at present; efforts at elution from the adsorbents have, so far, yielded inconclusive results.

Potent suspensions of cephalin may be injected intravenously without visible change in the animal, except for a transient blood hypercoagulability. Circulating blood seems to adapt itself to the injection of thromboplastic substances intravenously, provided the mechanism of adjustment is not overwhelmed by too rapid injection of concentrated solutions. From the foregoing it seems justifiable, therefore, that anticephalin should be included among the various factors contributing to the maintenance of the fluidity of blood.

What relation does this activity have to heparin, the anticoagulant extracted from tissues? It is generally agreed that, with the aid of plasma cofactors, heparin acts as an antithrombin and in addition is capable of retarding or preventing the transformation of prothrombin into thrombin, an effect that could with equal plausibility be considered antithromboplastin or antiprothrombic. That the anticephalin activity of the plasma, in itself, is not due to heparin alone and cannot be replaced by it, is indicated by the fact that once anticephalin is removed from plasma by contact with adsorbents, or by dialysis (with restoration of salt), heparin can no longer block the conversion of the prothrombin of such plasma. Moreover, as Chargaff points out, heparin cannot neutralize cephalin, as both substances are strongly acidic. Since, in plasma brought in contact with adsorbents, anticephalin activity may be removed while prothrombin is preserved—though addition of heparin before or after contact will not retard prothrombin conversion in such adsorbed plasmas—the designation of heparin as an antiprothrombin seems open to question. Furthermore, hemophilic blood with a known increase in anticephalin activity does not contain, according to Howell, an excess of heparin. Experiments under way seem to indicate that heparin blocks the conversion of prothrombin to thrombin by intensifying or enhancing the natural anticephalin activity of the plasma. These considerations make it seem desirable, for the time being, not to regard the natural anticephalin activity of the plasma as a manifestation of the presence of heparin.

With the foregoing in mind, let us see how anticephalin activity and its so far known characteristics may be fitted into an explanation of the mechanism for maintenance of the fluidity and coagulability of blood. Circulating blood seems to have within and about itself all that is required to delay or promote coagulation. The stability of the blood depends on the extent of dominance of one of the following opposing groups of factors over the other:

<table>
<thead>
<tr>
<th>Anticoagulants (Fluidity-promoting agents)</th>
<th>Coagulants (Coagulation-promoting agents)</th>
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<tbody>
<tr>
<td>1. Intact vascular endothelium (or a surface like collodion)</td>
<td>1. Damaged vascular endothelium (or a surface like clay or glass)</td>
</tr>
<tr>
<td>2. Anticephalin (antithromboplastin)</td>
<td>2. Cephalin (platelets, leukocytes, tissue juices)</td>
</tr>
<tr>
<td>3. Antithrombin</td>
<td>3. Prothrombin Ca ++</td>
</tr>
<tr>
<td>4. Fibrinolysin</td>
<td>4. Fibrinogen</td>
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It should follow from the foregoing that the coagulability of a given sample of blood may be enhanced by an increase in the effective amount of the coagulation-promoting factors or by a decrease in the fluidity-promoting elements. Conversely, blood may become unusually stable if there is an uncompensated increase in the anticoagulants or a decrease in the coagulants.

THE CONTACTING SURFACE

The internal area of a blood vessel is lined with endothelium, which offers to the blood a neutral contacting surface—neutral in the sense that it does not readily precipitate the changes that precede the inception of clotting. In this respect the surface endothelium of the vessel resembles a collodion membrane. As long as the stability of the blood is maintained by an adequate content of clot-inhibiting substances, surfaces like collodion or the endothelial lining of blood vessels will aid in maintaining this stability. When a vessel is severed, the entrance of tissue juices into the circulation at the point of contact in the ruptured area, and the destruction of the continuity of the endothelium, will interfere with the fluidity of the blood (a) by supplying thromboplastin, which inactivates the available anticephalin and thus accelerates the conversion of prothrombin, and (b) by offering a surface favorable to the removal of anticephalin and the liberation of more cephalin from disintegrating platelets. Blood itself may, on the other hand, even without previous vascular injury, lose its anticephalin activity and consequent stability, for one reason or another (stasis, hemorrhage). A neutral undamaged endothelial surface will then afford it little protection and intravascular clotting may conceivably take place, even though the vessel surface remains intact.

DEGREES OF STABILITY OF THE BLOOD

The stability of blood is therefore influenced by the surface with which it comes into contact and the extent of the blood anticephalin activity, among other factors. Table 2 presents examples of blood with varying degrees of stability. Hemophilic blood resists contact with glass and activation by cephalin, and displays more anticephalin activity than normal blood. On the other hand, normal blood has greater stability than blood obtained after a severe hemorrhage. The latter is more readily activated by cephalin, has less anticephalin activity, and is not greatly influenced by differences in contacting surfaces.

It seems probable that blood of low stability with respect to coagulation may lose its fluidity readily in the circulation, even when in contact with surfaces like the endothelium of blood vessels, which probably contribute to the stability of blood by preserving anticephalin activity. On the other hand, blood like that of the hemophilic will maintain its stability, even though the vessel wall has been traumatized and the character of the endothelial surface rendered favorable to coagulation. The excess of anticephalin in this blood will protect it against these surface conditions, just as it protects shed blood of this kind against glass surfaces.

These remarks have been intended principally to cover the factors involved in the first phase of coagulation in their relation to the maintenance of the stability of blood. It is obvious that increased stability can also result, for example, from a marked increase in antithrombin (rarely encountered in man) or from a diminution...
in the prothrombin of blood while its anticephalin activity is maintained; an
absence of fibrinogen would produce a similar effect. It must be kept in mind, how-
however, that a simple diminution in prothrombin may not in itself increase the
stability of the plasma if there is an equal or greater decrease in anticephalin
activity. Increased fluidity or coagulability must, in the end, result from an un-
compensated increase or decrease in the anticoagulant or coagulant factors.

There have been observations27, 28 of patients in whose plasma a decrease in the
amount of prothrombin has apparently been compensated by an increase in its
convertibility. In such patients the rate of coagulation of blood may remain un-
altered and may even sometimes become accelerated. After severe hemorrhage,
although the amount of prothrombin may not change or may even be reduced,

there is often a diminution in anticephalin activity,28 with a consequent accelera-
tion of the prothrombin conversion rate and of the blood clotting time.

Slight or moderate increases in stability of the blood, on the other hand, occur
spontaneously in man, but are generally undetected, principally because the rate of
coagulation of whole blood is usually measured in tubes of glass, a surface least
suited to study of such changes. The blood of 2 patients with histories of bleeding
that suggested hemophilia, yielded clotting times in glass tubes not significantly
greater than normal (table 3). The cephalin and whole blood clotting times in
plastic tubes revealed the true nature of their disorder. Similar discordant results
as regards the coagulability of hemophilic blood, with or without addition of
cephalin, are often found after a transfusion of normal blood. There may be a sig-
nificant shortening of the clotting time in glass tubes, while little or no change will
be noted in paraffin or plastic tubes. Attention has been drawn by Davidson and
McDonald to the advantages of plastic tubes for detection of slight changes in blood coagulability induced by dicumarol.

Plasma separated (high speed centrifuging in cold) from normal or hemophilic blood within five minutes after collection with special precautions and without use of anticoagulants, displays unusual stability when kept in paraffin- or collodion-coated tubes but clots promptly when placed in contact with glass. If

<table>
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<tr>
<th>TABLE 3.—Comparison of Coagulation Time of Venous Blood and Plasma of 2 Mildly Hemophilic Subjects with That in Normal Men</th>
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<tr>
<td>Blood</td>
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<tr>
<td>Clotting time (sec.)</td>
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<td>± S.D.</td>
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<td>615*</td>
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<tr>
<td>Cephalin clotting time (sec.)</td>
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<td>± S.D.</td>
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<tr>
<td>124*</td>
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<tr>
<td>Plasma</td>
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<td>Cephalin clotting time (sec.)</td>
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<td>± S.D.</td>
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<td>91†</td>
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<td>2o' incubation</td>
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<tr>
<td>± S.D.</td>
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<tr>
<td>114†</td>
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* Means of 26 determinations in 19 normal men.
† Means of 33 determinations in 26 normal men.
Prothrombin content of blood of hemophilic subjects, 100 per cent of normal.

<table>
<thead>
<tr>
<th>TABLE 4.—Clotting Time, in Glass and Collodion tubes, of Mixtures of Hemophilic and Normal Plasma in Various Proportions (0.3 cc. of Plasma, 0.1 cc. of Calcium Chloride)</th>
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<tbody>
<tr>
<td>Plasmas</td>
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<tr>
<td>Glass tube</td>
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<tr>
<td>Collodion tube</td>
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the effect of addition of hemophilic blood or plasma to normal blood or plasma is tested in glass tubes, there is a striking reduction of the clotting time toward normal. In collodion tubes, however, the results differ. A clot-delaying effect may be detected after the addition of one part of hemophilic to nine parts of normal plasma (table 4). This effect may be decreased or even erased by dilution, heating, or exposure of the hemophilic plasma to glass or certain adsorbents. From the data (table 4) one might with just as much propriety maintain that the coagulability
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of normal plasma is decreased by the hemophilic plasma as that the clotting time of the latter is accelerated by the normal plasma. These difficulties of interpretation are intrinsic to tests of this type. The apparent clot-accelerating effect of normal plasma, however, becomes even less convincing when the mixtures of the normal and hemophilic blood or plasma are made at once after collection, in tubes of plastic, paraffin, or similar composition.

Some of the earlier workers on blood coagulation were able to retain, either through choice or necessity, almost completely natural conditions in the planning and execution of their experiments. It was principally on the basis of observations on the behavior of blood in intra- and extracorporeal segments of vessels and in glass tubes that Lister\(^1\) was able to state:

The blood as it exists within the vessels, has no spontaneous tendency to coagulate, and therefore . . . the notion of any action on the part of the blood-vessels to prevent coagulation is entirely out of the question. The peculiarity of the living vessels consists not in any such action upon the blood, but in the circumstance, remarkable indeed as it is, that their living membrane, when in a state of health is entirely negative in its relation to coagulation, and fails to cause that molecular disturbance or, if we may so speak, catalytic action which is produced upon the blood by all ordinary matter. . . .

The real cause of the coagulation of the blood when shed from the body, is the influence exerted upon it by ordinary matter, the contact of which for a very brief period effects a change in the blood, inducing a mutual reaction between its solid and fluid constituents, in which the corpuscles impart to the liquor sanguinis a disposition to coagulate.

SUMMARY

A review of the various factors in the blood that have to do with the promotion and the retardation of coagulation is presented.

Circulating blood seems to have within and about itself all the factors required to delay or to promote coagulation. The stability of blood (i.e., its tendency to remain fluid) depends on the extent of the dominance of the anticoagulant (fluidity-inducing) group of factors over the coagulant (coagulation-promoting) group.

Among the anticoagulant factors are the natural anticephalin activity of the plasma and the intact vascular endothelium; the latter is simulated by such contacting surfaces as collodion and paraffin films.

Alterations in the stability of blood result from uncompensated increases or decreases in one or more of the anticoagulant or coagulant factors.

The increased stability of hemophilic blood, due to an uncompensated excess of anticephalin activity, enables it to resist activation by cephalin or by contact with injured walls of blood vessels or with surfaces like glass. Blood obtained from normal individuals after severe hemorrhage has a decreased stability owing to an uncompensated diminution in anticephalin activity; such blood is readily clotted by cephalin and may not remain stable even when in contact with undamaged vascular endothelium or surfaces like collodion or paraffin.

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