CIRCULATING anticoagulants may be defined as "abnormal endogenous components of blood which inhibit the coagulation of normal blood." An anticoagulant directed against antihemophilic factor has been recognized for several decades. This inhibitor and others directed against the various stages of coagulation have been the subject of recent extensive reviews.

Investigation of the mode of action of anticoagulants has lagged behind clinical description because of the lack of simple, reproducible, in vitro assay systems for the factors being measured. Recently Biggs and her associates have applied the thromboplastin generation test to the study of the anticoagulant directed against antihemophilic factor and have described some of the kinetics of the anticoagulant-antihemophilic factor reaction.

In the present study, an assay system for antihemophilic factor has been devised by modifying Margolis's kaolin clotting time method. This system has been applied to the study of the kinetics of the reaction between antihemophilic factor and its specific circulating anticoagulant. This study suggests that the anticoagulant directed against antihemophilic factor inactivates this substance in an enzymatic fashion.

MATERIALS AND METHODS

Citrated plasmas were obtained by venipuncture from normal persons and patients with various coagulation disorders by methods previously described. The plasma was used immediately or stored in silicone-coated Lusteroid containers at −20 C. until used.

Normal plasma, or Cohn fraction I thereof, was used as a source of antihemophilic factor.

Hemophilic plasma was obtained from patients with classic hemophilia, that is, hemophilia due to a deficiency of antihemophilic factor. Prior to use, these plasmas were shown not to have anticoagulant activity by previously published methods. This plasma was used as the source of all coagulation factors other than antihemophilic factor.

Plasmas containing anticoagulants directed against antihemophilic factor were obtained from a patient with classic hemophilia (case H. B., ref. 1), a woman with an anticoagulant...
which appeared within two months postpartum (case L. J., ref. 1), and a 70 year old woman whose case has not been reported before:

E. S., a 70 year old white female was seen in July, 1961, with the complaint of spontaneous ecchymoses dating from May, 1959. Her family history was non-contributory. She had never been pregnant nor received a blood transfusion or penicillin. The ecchymoses usually appeared in the vicinity of joints and were preceded by pain in the area involved. There had never been any joint swelling or limitation of motion.

On physical examination many large ecchymotic areas were seen on the legs and several small ones on the left breast.

Laboratory studies revealed a clotting time in Pyrex tubes of 155 minutes (normal 17–31 minutes), poor prothrombin "consumption" and absent antihemophilic factor. The prothrombin time, bleeding time, and platelet count were normal. Her blood prolonged the clotting time of normal blood.

Each anticoagulant-containing plasma was made deficient in all known coagulation factors by methods previously described. Ten volumes of the citrated plasma was incubated with one volume of aluminum hydroxide gel* diluted to a concentration of 0.55 per cent aluminum oxide in uncoated Lusteroid tubes at 37 C. for 3 minutes. It was then centrifuged at 3000 rpm for five minutes. The supernatant, deficient in vitamin K-dependent factors, was heated in a 56 C. water bath for 30 minutes and centrifuged at 3000 rpm for five minutes to remove the precipitated fibrinogen.

Hageman factor and PTA were then removed by the following method. The adsorbed, heated plasma was diluted to twice its volume by the addition of 0.15M sodium acetate, pH 5.2, and sufficient IM acetic acid to bring the pH of the mixture to 5.2. The acidified plasma was then stirred with 75 mg. carboxymethylcellulose per ml. of original plasma volume at room temperature for 10 minutes and centrifuged at 3000 rpm for 5 minutes. The supernatant was dialyzed against 0.15M barbital-saline buffer, pH 7.5, for 12 hours.

This treated plasma, or Cohn fractions II or III* of this treated plasma, was used as a source of anticoagulant; it was deficient in all the recognized clotting factors.

A 0.1 per cent suspension of Gliddex-"P"* in normal saline was used in place of platelets or cephalin. Gliddex-"P" is a crude phospholipid fraction of soybeans free of thromboplastic activity. The Gliddex suspension was divided into 5 or 10 ml. portions and stored at -20 C. until used. On the day of use, kaolin† was carefully mixed with Gliddex suspension at a concentration of 5 mg. per ml.

Test tubes used for all clotting studies were Pyrex 10 x 75 mm. with an internal diameter of 8 mm.

Barbital-saline buffer, pH 7.4-7.5, contained 7.3 Gm. NaCl., 2.76 Gm. barbital, 2.06 Gm. sodium barbital and distilled water to make the volume one liter.

Acetate buffer, 0.15M at pH 5.2, contained 2.48 ml. concentrated acetic acid, 11.47 Gm. anhydrous sodium acetate, and distilled water to make the volume one liter.

Methods

The assay for antihemophilic factor in the presence of circulating anticoagulant was performed by a modification of the method of Margolis and Rapaport et al. A volume of 0.1 ml. hemophilic plasma was mixed with 0.1 ml. of kaolin-Gliddex suspension in a Pyrex tube and preincubated in a water bath at 37 C. for 8 minutes. At the end of this interval, 0.1 ml. of the plasma to be tested, suitably diluted in barbital-saline buffer, and 0.1 ml. of 0.025M

*Cutler aluminum hydroxide gel kindly supplied by Cutter Laboratories, Berkeley, Calif.
†Gliddex-"P" was provided through the kindnss of the Chemurgy Division, The Glidden Co., Chicago, III.
calcium chloride were added. Incubation at 37 C. was continued for another 30 seconds. At this time the mixture was tilted continually until a clot appeared. The interval between the addition of calcium and the appearance of a clot was recorded as the clotting time.

The conditions selected for this assay provided, in our hands, a sharper end-point and possibly more reproducible values than in Margolis's original method. Duplicate assays were consistently within one or two seconds and the clotting time was recorded as an average of the two values. The concentration of kaolin used, 5 mg. per ml. of Gliddex suspension, was optimal for this assay under the conditions used. The temperature selected for the assay, 37 C., gave more reproducible results than lower temperatures; incubation at higher temperatures resulted in spontaneous disappearance of antihemophilic factor during the assay. The end-point of the assay was sharpened by the use of Gliddex-“P” rather than crude rabbit brain “cephalin.” The use of Gliddex-“P”, however, necessitated lengthening the incubation time from 4 to 8 minutes; identical results were obtained when the incubation period was varied from 6 to 16 minutes. The pH at which the assay was performed (7.4–7.5) seemed to improve reproducibility. At higher or lower pH values, the stability of the antihemophilic factor during incubation or storage was decreased. Under the conditions of this assay, the logarithm of the concentration of antihemophilic factor was an inverse linear function of the logarithm of the clotting time (fig. 1).

In the method outlined, the mixture to be tested for antihemophilic factor was not added until the end of the preliminary incubation period. In this manner it was possible to avoid any effect of the circulating anticoagulant during this 8-minute period. In the absence of anticoagulant activity, the material to be tested could be added at the beginning of the incubation. Under such conditions the clotting time was appreciably shortened but the curves were similar (fig. 1).

Anticoagulant activity was tested by incubating suitable amounts of anticoagulant plasma, or its fractions, with normal plasma in Pyrex tubes. The time of the incubation was arranged so that, at its completion, the resulting mixture could be diluted in barbital-saline buffer and tested immediately for antihemophilic factor to obviate further action of the anticoagulant. To do this, anticoagulant-antihemophilic factor mixture and the hemophilic plasma-kaolin-Gliddex mixture were incubated separately but simultaneously. When the incubations were complete, 0.2 ml. of the anticoagulant-antihemophilic factor mixture was diluted with 0.8 ml. barbital-saline buffer. One-tenth ml. was transferred immediately to the hemophilic plasma-kaolin-Gliddex mixture, calcium was added, and the clotting time measured. Each experiment for determination of anticoagulant activity was quantified by serially diluting the normal plasma used and assaying each dilution for antihemophilic factor. In this manner the amount destroyed by the anticoagulant could be expressed in per cent of the antihemophilic factor present in whole plasma.

The antihemophilic factor present in normal plasma, measured by this assay system, spontaneously deteriorated significantly at 37 C. when incubated longer than 10 minutes. For this reason the assays of anticoagulant activity were carried out for 10 minutes or less or corrected for spontaneous disap-
Fig. 1.—Assay curves of normal plasma for antihemophilic factor activity plotted on a doubly logarithmic scale. Curve A—Effect of incubation of hemophilic plasma-kaolin-Gliddex mixture prior to the addition of normal plasma (source of antihemophilic factor) and calcium. B—Effect of incubation of normal plasma with hemophilic plasma-kaolin-Gliddex mixture together prior to addition of calcium.

appearance of antihemophilic factor during the time period utilized.

The optimal pH range for the action of the anticoagulant was tested by adjusting the normal plasma and the anticoagulant plasma to the desired pH range by addition of sodium acetate buffer (pH 5.2) or 0.2M sodium hydroxide, and reading the final pH of the mixture on a Cambridge pH meter. This mixture was incubated for 10 minutes at 37°C and then 0.2 ml of the anticoagulant-antihemophilic mixture was diluted in 0.8 ml of barbital-saline-buffer, so that final pH measured 7.3-7.5, and tested for antihemophilic factor. The results were compared to normal plasma incubated without anticoagulant under the same conditions and in the same dilutions for the same time interval.

Assays of other factors associated with coagulation disorders were done by means of the published methods.1,12,14,15

Diisopropyl fluorophosphate*-treated anticoagulant plasma was prepared by incubation of the plasma with diisopropyl fluorophosphate in the manner previously described.10 The final concentrations of diisopropyl fluorophosphate were 10⁻⁴, 10⁻⁵, and 10⁻⁶M. A diisopropyl fluorophosphate control and a plasma control without diisopropyl fluorophosphate were run with each experiment and results of the treated groups compared with these controls.

Mercuric chloride, iodoacetate† and p-hydroxymercuribenzoate‡-treated anti-

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*Diisopropyl fluorophosphate: K and K Labs, Long Island City, N. Y.
†Iodoacetate—Iodacetic acid: Eastman Kodak Co., Rochester, N. Y.
‡P-hydroxymercuribenzoate-sodium crystalline: Sigma Chemical Co., St. Louis, Mo.
STUDIES ON THE ANTICOAGULANT-AHF REACTION

Fig. 2.—Effect of time on the anticoagulant-antihemophilic factor reaction. Concentration of anticoagulant equal to a 1:80 dilution of plasma. Note: These are net values corrected for the spontaneous inactivation of antihemophilic factor during the time interval used (see text).

Coagulant plasmas were prepared by dilution of the anticoagulant plasma to 1:20 with barbital-saline buffer containing these compounds; the final concentration of these compounds was $10^{-4}$ M. The diluted plasmas were incubated for 10 minutes at 37 C. before they were tested for anticoagulant activity. Normal plasma was incubated with barbital-saline buffer containing these compounds at the same concentration as a control for their effect on the antihemophilic assay itself.

Agar-gel double diffusion was performed by using Ion Agar* plates with a center well and six peripheral wells. Citrated whole normal plasma was placed in the center well. Various barbital-saline dilutions of the anticoagulant plasma were placed in the peripheral wells and the intervening agar observed for lines of antigen-antibody interaction at 5 C. for 10 days. Serum from a rabbit immunized against whole human plasma was used as a control in one of the peripheral wells.

RESULTS

The incubation of anticoagulant plasma with normal plasma resulted in a loss of antihemophilic factor from the normal plasma in a predictable manner.

Table 1.—Effect of pH on Action of Anticoagulant

<table>
<thead>
<tr>
<th>pH</th>
<th>Per cent antihemophilic factor lost during 10 minute incubation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8</td>
<td>60</td>
</tr>
<tr>
<td>7.0</td>
<td>63</td>
</tr>
<tr>
<td>7.5</td>
<td>81</td>
</tr>
<tr>
<td>8.0</td>
<td>64</td>
</tr>
</tbody>
</table>

*These are net values corrected for the spontaneous disappearance of antihemophilic factor activity under the experimental conditions.

Table 2.—Effect of Temperature on Anticoagulant-Antihemophilic Factor Reaction

<table>
<thead>
<tr>
<th>Temperature in degrees centigrade</th>
<th>Total per cent antihemophilic factor lost during incubation with anticoagulant</th>
<th>Total per cent antihemophilic factor lost during incubation without anticoagulant</th>
<th>Net per cent antihemophilic factor inactivated by anticoagulant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>37</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>45</td>
<td>64</td>
<td>8</td>
<td>56</td>
</tr>
</tbody>
</table>

Thus the reaction was time dependent (fig. 2), the disappearance of antihemophilic factor most rapid during the first 10 minutes and decreasing in velocity thereafter. The reaction was also demonstrated to be pH dependent (table 1) and optimal inactivation occurred at pH 7.5.

The reaction between anticoagulant and antihemophilic factor was temperature dependent. No inactivation of antihemophilic factor occurred at 0°C during a 10-minute period. Progressively more antihemophilic factor was inactivated during a 10-minute incubation as the temperature was increased to 45°C (table 2).

As the concentration of anticoagulant was increased, a proportional increase in the destruction of antihemophilic factor occurred until the anticoagulant was present in such large amounts that the rapid destruction of its substrate, antihemophilic factor, limited the velocity of the reaction (fig. 3).

As the concentration of normal plasma (the source of antihemophilic factor) was increased, the amount of antihemophilic factor inactivated by the anticoagulant at first increased linearly. At higher concentrations of substrate, further increases in the velocity of the reaction were proportionally less, as if the velocity was approaching a maximal value (fig. 4).

The anticoagulant-antihemophilic factor reaction was allowed to proceed at 45°C and a quantitative relationship was established between the two components. Doubling the concentration of anticoagulant doubled the amount of antihemophilic factor lost during a given time interval. Thus, the amount of antihemophilic factor inactivated by a 10 per cent solution of anticoagulant in 2.5 minutes closely approximated the amount inactivated by a 5 per cent solution in 5 minutes or a 2.5 per cent solution in 10 minutes (fig. 5).

Incubation of anticoagulant with antihemophilic factor did not result in a loss of activity of the anticoagulant. To demonstrate this the incubation was begun with a concentration of anticoagulant of 10 per cent. This was incubated
Fig. 4.—Effect of varying concentration of anticoagulant on its inhibition of antihemophilic factor. In this experiment whole normal plasma was arbitrarily assigned a value of 100 units of antihemophilic factor and a 1:160 dilution of anticoagulant plasma was arbitrarily designated one unit of anticoagulant.

Fig. 4.—Effect of substrate concentration on the anticoagulant-antihemophilic factor reaction when anticoagulant concentration was constant. Whole normal plasma was arbitrarily assigned a value of 100 units of antihemophilic factor.

For 10 minutes, tested for residual antihemophilic factor and new substrate was added, reducing the concentration of anticoagulant to 5 per cent. This mixture was incubated for 10 minutes more, retested and the procedure repeated, the concentration of anticoagulant now being 2.5 per cent that of the original plasma. Parallel tests of similar concentrations of anticoagulant plasma revealed that there had been no loss of anticoagulant activity during incubation (table 3).
Fig. 5.—Effect of varying concentration of anticoagulant on inactivation of anti-hemophilic factor when incubation is carried out at 45 C. Curve A—10 per cent solution of anticoagulant; B—5 per cent solution of anticoagulant; C—2.5 per cent solution of anticoagulant. Whole normal plasma was arbitrarily assigned a value of 100 units of antihemophilic factor.

No lines indicative of antigen-antibody interaction were demonstrated between the anticoagulant and antihemophilic factor by means of double agar-gel diffusion (fig. 6).

Anticoagulant activity against antihemophilic factor was detected in the plasmas of the three different types of patients tested, and the three anticoagulants were identical in all respects. An identical amount of anticoagulant was found in the plasma and serum of E. S. when tested by this method.

Anticoagulant activity was preserved in plasma which had been adsorbed with aluminum oxide, heated at 56 C. for 30 minutes, and adsorbed with carboxymethyl-cellulose. It was stable in plasma, or fractions of plasma prepared by Cohn's method, for prolonged periods at −20 C. Cohn fractions II and III of L. J.'s plasma, tested by this method, retained their anticoagulant activity after 5½ years storage. The anticoagulant proved to be relatively heat stable; it lost 80 per cent of its activity after heating at 60 C. for 10 minutes; 90 per cent after heating at 70 C. for 10 minutes; and 100 per cent after heating at 80 C. for 10 minutes. The anticoagulant activity was not lost after incubation with diisopropyl fluorophosphate, mercuric chloride, iodoacetate, or p-hydroxymercuribenzoate in the concentrations used.

The anticoagulant was specific for antihemophilic factor, having no activity against Christmas factor, plasma thromboplastin antecedent, or Hageman factor.

Fractions of plasmas from L. J. and E. S., prepared by the method of Cohn,
Fig. 6.—Double agar-gel diffusion of anticoagulant vs. antihemophilic factor. The center well contained whole normal plasma (source of antihemophilic factor). The peripheral wells contained: 1) 1:4 anticoagulant plasma; 2) 1:8 anticoagulant plasma; 3) 1:16 anticoagulant plasma; 4) 1:32 anticoagulant plasma; 5) 1:64 anticoagulant plasma; 6) rabbit anti-whole normal human plasma.

reconstituted in barbital-saline, and tested by the method described revealed anticoagulant activity only in fractions II and III.

DISCUSSION

Circulating anticoagulants directed against antihemophilic factor have been observed in three types of patients: 1) patients with classic hemophilia, 2) women who develop a hemorrhagic disorder resembling hemophilia within a year postpartum, and 3) individuals in whom hemophilia-like disease appears spontaneously without previous transfusions, pregnancies or a family history of bleeding disorders. The anticoagulants seen in these patients cannot be distinguished by their physiochemical characteristics and actions.

One view has been that the anticoagulant is an antibody which has been formed in response to the antigen, antihemophilic factor, which is foreign to the hemophiliac and transfused into him therapeutically. This view is supported by the fact that the anticoagulants usually appear in the more severe hemophiliacs who have received blood or its products. In addition, some authors have been able to demonstrate precipitin reactions in the majority of their patients with this anticoagulant.
The antibody hypothesis has been questioned by many investigators because it does not adequately explain the development of anticoagulant activity in the mild hemophiliac who has never been transfused, the newborn child, and cases in which it appears spontaneously. Furthermore, precipitin tests have not been observed universally and the validity of these reactions has been questioned since precipitated fibrin may be mistaken for a precipitate. The inability to detect precipitin reactions or complement fixation during the anticoagulant-antihemophilic factor reaction has been reported previously from this laboratory. In the current study, the use of another immunologic method, double agar-gel diffusion, has also given negative results.

The hypothesis that the anticoagulant could be enzymatic in nature has been proposed. This hypothesis is consistent with the clinical data reported concerning this anticoagulant. Its appearance after transfusions directly parallels the many instances in nature where an enzyme present initially in small amounts or in an inactive form becomes active or increases in concentration when its substrate increases in amount. A spontaneous increase in the enzyme could explain its appearance in the cases described without a previous history of bleeding diathesis or transfusion therapy. The tendency for the anticoagulant to disappear gradually when transfusions are not given to the hemophiliac is also consistent with the enzyme hypothesis. It is well documented that an enzyme which appears in response to a new substrate will decrease in concentration or disappear when this substrate is removed from the organism’s environment.

The previously described properties of the anticoagulant are consistent with the enzymatic hypothesis. Munro demonstrated that anticoagulant activity was not lost after prolonged incubation with antihemophilic factor, and Lawrence and Craddock reported that the reaction between anticoagulant and antihemophilic factor was time dependent. Biggs and Bidwell, confirming these observations, postulated that the anticoagulant was the inhibitor of an enzyme.

The data obtained during this study are consistent with the hypothesis that the anticoagulant is enzymatic in nature. Thus the inactivation of antihemophilic factor by anticoagulant has been shown to increase with increasing time intervals, to be dependent on the temperature and pH in which the reaction is allowed to proceed, and to approach a maximum rate when the substrate concentration is increased. These characteristics are more indicative of an enzymatic reaction than an immunochemical one. The proportional relationship established between the anticoagulant and antihemophilic factor during their interaction and the fact that the anticoagulant retains full activity after completing the inactivation of antihemophilic factor are strong evidences in favor of this hypothesis.

The inconsistencies in the antibody hypothesis of anticoagulant action, the dependence of the anticoagulant on the environmental conditions in which it acts, its substrate dependence, and the preservation of its full activity after interaction with antihemophilic factor strongly support the hypothesis that it is enzymatic in nature. It is also conceivable that the spontaneous deterioration...
of antihemophilic factor in normal plasma stored for prolonged periods is on the basis of an enzymatic inactivation. Studies are underway at the present time to define this phenomenon more completely.

Modification of the kaolin clotting time has produced a reliable, simple assay system for antihemophilic factor. This system has been utilized for an investigation of the nature and mode of action of the circulating anticoagulant directed against antihemophilic factor.

The anticoagulant has been shown to be present in equal amounts in plasma and serum, to be associated with fractions II and III of plasma prepared by the method of Cohn, to be relatively heat stable and to be stable for prolonged periods at -20 C.

Investigation of the kinetics of the anticoagulant-antihemophilic reaction has demonstrated its temperature, time, pH, and substrate concentration dependency. The anticoagulant is not inactivated during the reaction with antihemophilic factor, and there is a proportional relationship between the anticoagulant and the antihemophilic factor during their interaction.

No antigen-antibody manifestations could be detected during the anticoagulant-antihemophilic factor reaction. These characteristics support the hypothesis that the inactivation of antihemophilic factor by specific circulating anticoagulants is enzymatic.

**Table 3.—Effect of Incubation with Antihemophilic Factor on Activity of Anticoagulant**

<table>
<thead>
<tr>
<th>Mixture*</th>
<th>Per cent antihemophilic factor inactivated</th>
<th>Expected per cent antihemophilic factor inactivated by this concentration of anticoagulant</th>
<th>Per cent of expected value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original (10 per cent concentration of anticoagulant)</td>
<td>75</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>Original (1) plus new substrate (5 per cent concentration of anticoagulant)</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Original (2) plus new substrate (2.5 per cent concentration of anticoagulant)</td>
<td>25</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

*Original mixture was carried throughout the experiment as a source of anticoagulant. All incubations were carried out for ten minutes at 37 C.

**SUMMARY**

Modification of the kaolin clotting time has produced a reliable, simple assay system for antihemophilic factor. This system has been utilized for an investigation of the nature and mode of action of the circulating anticoagulant directed against antihemophilic factor.

The anticoagulant has been shown to be present in equal amounts in plasma and serum, to be associated with fractions II and III of plasma prepared by the method of Cohn, to be relatively heat stable and to be stable for prolonged periods at -20 C.

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**SUMMARIO IN INTERLINGUA**

Un modification del determination del tempore coagulatori a kaolin ha producite un simple e fidel sistema de essayage pro le factor antihemophilic.
Iste systema esseva utilitate pro le investigation del natura e le modo de action del circulante anticoagulante dirigite contra factor antihemophilic.

Esseva demonstrate que le anticoagulante es presente in quantitates equal in plasma e in sero, que illo es associate con le fractiones II e III de plasma preparate per le methodo de Cohn, que illo es relativemente thermostabile, e que illo es stable durante periodos prolongate a −20 C.

Investigation del cinetica del reaction inter anticoagulante e factor antihemophilic ha demonstrate le dependentia de ille reaction de variationes de temperatura, del tempore, del pH, e de del concentration del substrato. Le anticoagulante non es disactivate durante le reaction con factor antihemophilic, e un relation proportional es mantenite inter le anticoagulante e factor antihemophilic durante lor interaction.

Nulle manifestationes de antigeno e anticorpore esseva detecte durante le reaction inter anticoagulante e factor antihemophilic. Iste caracteristicas supporta le hypothese que le disactivation de factor antihemophilic per specific anticoagulantes circulante es de character enzymatic.

ACKNOWLEDGMENTS

We are indebted to Dr. Norman L. Wright and Dr. Melvin F. Strockbine for referring patients L. J. and E. S. to us for study. Dr. E. W. Davie kindly prepared the disisopropyl fluorophosphate-treated plasmas used in this study.

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Oscar D. Ratnoff, M.D., Professor of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio; Career Investigator of the American Heart Association.
Studies on the Nature of the Circulating Anticoagulant Directed against Antihaemophilic Factor: With Notes on an Assay for Antihaemophilic Factor

ROBERT T. BRECKENRIDGE and OSCAR D. RATNOFF