Quantitative Assay of Fibrinogen and Fibrinolytic Activity

By Herbert A. Perkins and Mary R. Rolfs

Recent years have brought increased appreciation of the importance of the role of fibrinolysis in the regulation of normal hemostasis and in the production of abnormal bleeding. The mechanisms by which active fibrinolysis is induced are far from clear, but it does seem likely that it can be brought about by shock, severe emotional stress, injection of adrenalin, anoxia and liberation of activators from damaged tissue. The vastly complex procedure of open heart surgery provides many opportunities on a theoretical basis for activation of fibrinolysis. Reports in the literature leave no room to question the fact that severe degrees of fibrinolysis have been responsible for massive bleeding in a few cases. The extent to which moderate degrees of fibrinolysis may aggravate the degree of post-operative bleeding in most cases is not clear. A number of groups have addressed themselves to the problem of evaluating the degree of fibrinolysis during and following open heart surgery.

The present study was undertaken in the belief that there was a need for more accurate quantitation of the over-all fibrinolytic state during open heart surgery. In the course of these investigations, a technic was evolved which appears to measure over-all fibrinolytic activity in a sensitive quantitative way providing information which is more readily related to other changes going on in the patient at the same time. The method is based on duplicate fibrinogen determinations. One tube of each pair contains an inhibitor of fibrinolysin; during the period of incubation after fibrin has been formed, the inhibitor prevents destruction of the fibrin. In the other tube, fibrin is lysed to an extent proportional to the fibrinolytic activity. Comparison of the results obtained quantitatively in the two tubes provides an index of fibrinolysis.

In an effort to evaluate this approach, studies were carried out to answer the following questions: (1) Are the level of fibrinogen and the degree of fibrinolytic activity stable in the sample during the period prior to assay? (2) Does the fibrinolysin inhibitor interfere in any way with the results of the assay? (3) How much inhibitor is needed to prevent lysis in the control tube? (4) What period of incubation provides the most convenient and satisfactory discrimination between normals and those with increased fibrinolytic activity?

In the course of this study, information was obtained on the effects of various modifications of technic on the accuracy of the fibrinogen assay. Normal values were established for this and for the fibrinolysin assay. The fibrinolysis technic...
was demonstrated to detect with considerable sensitivity minor degrees of fibrinolytic activity in the plasma of patients at the conclusion of open heart surgery.

**Methods**

**Collection of Samples**

Blood from normal blood bank donors was collected at the end of phlebotomy by disconnecting the bottle or bag and immediately allowing blood to drain from the donor’s vein through the indwelling needle and attached tubing into a siliconed graduated centrifuge tube. Samples from patients undergoing heart surgery* were taken from the venous return line just before the bypass was stopped. Samples were removed through a three-way stopcock directly into a siliconed graduated centrifuge tube. The side arm of the stopcock was first flushed free of stagnant blood by passage of 3 to 5 ml. of blood before collection.

In all instances 9 ml. of blood were added to 1 ml. of an acid citrate anticoagulant. The tubes were immediately covered with parafilm and the contents well mixed. They were placed at once in a bath of melting ice. They were centrifuged within 30 minutes of collection or else discarded. Samples from the open heart surgery cases were centrifuged at 1180 g for 10 minutes at room temperature; those from the blood donors at 11,900 g at 4 C. for 10 minutes. (Facilities for high speed centrifugation in the cold were not available at the hospital. The slower spun plasmas must have had a higher platelet count than the more rapidly centrifuged samples, but the number could not have been sufficiently high for the antifibrinolytic activity of platelets to have an effect on the results. All plasma samples were stored at temperatures below −20 C., and were tested within 2 weeks of collection. They were thawed rapidly with constant shaking in a water bath at 37 C. and used without any further delay.

**Technics**

Fibrinogen assays were performed by a modification of the technic of Ratnoff and Menzie. To a 40 ml. round bottom Pyrex centrifuge tube were added 0.5 Gm. (approximately) of 0.2 mm. glass beads, 10 ml. of 0.85 per cent sodium chloride, 1 ml. of 0.025 M calcium chloride, 0.1 ml. thrombin, and 1 ml. of the plasma to be tested. A duplicate tube contained in addition 0.2 ml. of one per cent epsilon amino caproic acid (EACA) in saline. The tubes were mixed continuously with a swirling motion during clotting to trap the glass beads in the clot, and then incubated for 4 hours in a 37 C. water bath. The rest of the procedure followed the Ratnoff-Menzie technic. Tyrosine concentrations were measured in a Coleman Jr. spectrophotometer.

**Results**

Fibrinogen Assays

1. Reproducibility of results: The technic described under Methods was evolved after considerable experimentation (see Discussion). The extent to which results could be reproduced is indicated in table 1. Duplicate assays were carried out on a series of nine dog plasmas by one of us (M. R. R.). The second set was performed after the plasmas had been stored at a temperature

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*Operations by Dr. Benson Roe, University of California Medical Center, San Francisco, Calif.
†Glass beads, Ct. No. VF 16220, Aloe Scientific, St. Louis, Mo.
‡Thrombin, Topical, 1000 units per ml., Parke-Davis.
§Epsilon amino caproic acid, Lederle. Available as 25 per cent solution for intravenous administration.
Table 1.—Fibrinogen Assay Reproducibility of Results

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Fibrinogens mg./100 ml. Plasma 4/27/62</th>
<th>5/15/62</th>
<th>Per Cent Difference</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>316</td>
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<td>5</td>
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<tr>
<td>2</td>
<td>272</td>
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<td>4</td>
<td>218</td>
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<tr>
<td>5</td>
<td>250</td>
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<td>10</td>
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<td>6</td>
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<td>7</td>
</tr>
<tr>
<td>8</td>
<td>272</td>
<td>260</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>272</td>
<td>263</td>
<td>3</td>
</tr>
<tr>
<td>Average per cent difference</td>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

Assays were performed on 1 ml. samples of dog plasma. The clots were incubated for 10 minutes only; no EACA was added.

of −20 C. for 18 days. The technician was not aware that the same samples were being assayed again. Average results did not differ by more than five per cent.

2. Normal values: Fibrinogen assays were carried out on samples of plasma obtained from 100 normal human blood donors. The clots were incubated for 4 hours in the presence of EACA as described in the Methods section. Figure 1 indicates values obtained. Results for male and female donors are indicated separately.

3. Effect of age of subject: Figure 2 indicates the fibrinogen values of the same 100 donors, each value being plotted against the age of the donor.

Fibrinolytic Activity

1. Stability of fibrinogen and fibrinolytic activity: Table 2 demonstrates that the technic of collection did not permit lysis of fibrinogen prior to the time
that EACA was added in the assay, even when there was demonstrable fibrinolytic activity in the sample. Lytic activity persisted without alteration during prolonged storage under the conditions used (table 3).

2. Effect of EACA on the assay: EACA was shown to have no effect on the fibrinogen assay itself. Results were the same whether blank tubes were prepared as usual or in the presence of 0.2 ml. 25 per cent EACA. Addition of various concentrations of EACA to plasma samples before assay showed no alteration of results in the presence of the highest concentration available (table 4). The same table shows that the 1 per cent concentration used in the routine tests was adequate to neutralize the amount of fibrinolytic activity encountered in open heart surgery samples. This concentration provides a level in the plasma approximating that which should result with doses found effective in vivo in the presence of marked clinical fibrinolysis.13

3. Optimal period of incubation of clot: The length of time for which the formed fibrin should be incubated was investigated in the following manner: Initially, the tubes were incubated overnight for a period of 18 hours. This provided an extremely sensitive technic for estimating fibrinolysis—so sensitive, in fact, that normal blood donors usually demonstrated lysis of as much as 60 per cent of their fibrin. When this incubation period was reduced to 4 hours, however, normal blood donors failed to show lysis; whereas, on the other hand, the majority of open heart surgery samples showed extensive degrees of lysis by this technic, despite the fact that their whole blood clots never did lyse. We have, therefore, standardized with a 4 hour period of incubation.
FIBRINOGEN AND FIBRINOLYTIC ACTIVITY

Table 2.—Stability of Fibrinogen and Fibrinolytic Activity (Samples Obtained from Patients Following Open Heart Surgery)

<table>
<thead>
<tr>
<th>EACA present in anticoagulant on collection</th>
<th>EACA added to plasma before assay</th>
<th>No EACA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment No. 1</td>
<td>212</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>152</td>
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<td></td>
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<td>152</td>
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<td>5</td>
<td>120</td>
</tr>
<tr>
<td>Mean values</td>
<td>165</td>
<td>172</td>
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Table 3.—Repeated Assays on Aliquots of a Single Sample (mg. of Fibrinogen per 100 ml. Plasma)

<table>
<thead>
<tr>
<th>Days of Storage</th>
<th>With EACA</th>
<th>Without EACA</th>
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<tr>
<td>5</td>
<td>133</td>
<td>93</td>
</tr>
<tr>
<td>11</td>
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<td>90</td>
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<td>18</td>
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<tr>
<td>46</td>
<td>127</td>
<td>82</td>
</tr>
</tbody>
</table>

Table 4.—Effect on Fibrinogen Assay of Various Concentrations of EACA*

<table>
<thead>
<tr>
<th>Concentration of EACA (%)</th>
<th>25</th>
<th>12.5</th>
<th>6</th>
<th>3</th>
<th>1.5</th>
<th>0.75</th>
<th>0.37</th>
<th>Saline Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment No. 1</td>
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<td>215</td>
<td>223</td>
<td>212</td>
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<td>207</td>
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<td>128</td>
<td>125</td>
<td>125</td>
<td>130</td>
<td>136</td>
<td>85</td>
</tr>
</tbody>
</table>

*0.2 ml. EACA was added to 1.0 ml. plasma from patients following open heart surgery.

4. Normal values: The results of assays for fibrinolytic activity in a series of 100 presumably normal blood donors are summarized in figure 3. Results are expressed as per cent difference in the level of fibrinogen in the tube incubated without EACA as compared with the level in the paired sample with EACA. The number of samples without EACA which was above the control sample was equal to that which was below, and it thus appears that the difference between the two series were entirely due to the technical error of the method. The results point out that the difference between two consecutive assays on the same plasma under normal conditions by this technic can be expected to be within ± 16 per cent in 95 per cent of cases. In view of the fact that this difference represents the summation of the technical error of two fibrinogen assay procedures, this seems to be an acceptable degree of agreement. For practical purposes one can accept a loss of more than 20 per cent of the original fibrinogen level as significant.

5. Fibrinolysis in donor "reactor" blood: One point which was investigated as this study proceeded was whether the donor who had a "reaction," in the sense that he felt weak or sweaty, or grew pale or faint, showed evidence of
Fig. 3.—Comparison of fibrinogen values in normal plasma with and without EACA (per cent difference).

Fig. 4.—Fibrinolysis in plasma of blood donors who “reacted” to the donation.

having a greater degree of fibrinolytic activity than the donors who responded to donation without subjective or objective discomfort. Figure 4 shows that the plasma of the few “reactors” did not demonstrate increased lytic activity.

6. Fibrinolysis following open heart surgery: Figure 5 provides evidence that this technic is very sensitive in quantitating minor degrees of fibrinolytic activity in situations where crude observations for whole clot lysis shows none. The majority of cases of open heart surgery have shown striking activity by this technic.

Discussion

Fibrinogen Assays

Over-all evaluation of the potentiality for abnormal bleeding following open heart surgery requires an accurate assay for fibrinogen as well as an estimate
of fibrinolysis. Assays for fibrinogen provide the only quantitative chemical approach available to measure the level of any coagulation factor. Nonetheless, the wide variety of technics proposed to measure this substance indicates dissatisfaction with the ease and accuracy of the methods.

The general approaches have been to separate fibrinogen from other plasma proteins by technics which take advantage of its: (1) relative insolubility, (2) relative sensitivity to heat denaturation, and (3) ability to be converted to fibrin by the enzyme thrombin. The separated fibrinogen can then be dried and weighed, or digested and quantitated by its nitrogen or tyrosine content. The formation of precipitates of fibrinogen by salting out or by heat, although a relatively simple procedure, is quantitatively inaccurate and does not provide a direct indication of the biochemical reactivity of the molecule.

The property of fibrinogen which is of biologic interest and the one which we therefore wish to assay is its ability to be clotted by the enzyme thrombin. Fibrinogen can be altered to the extent of making it incoagulable without interfering with its physical characteristics enough to prevent assay by other technics.\textsuperscript{14}

Having formed a precipitate or coagulum of fibrin, it is essential to remove all other plasma proteins without, in the process, losing any of the fibrin. A coagulum of undiluted plasma provides such a dense gel that it is very difficult to wash away occluded proteins. For this reason, it is necessary to dilute the plasma before the fibrin meshes form, then syneresis of the fibrin mesh separates fibrin from the other contaminating proteins. This procedure, simple in theory, has many pitfalls in practice, and none of the technics which have been recorded provide an ideal solution. The simplest approach, detailed for example by Quick,\textsuperscript{15} is to wind the fibrin on a glass rod and then wash it under running water. In many instances it is impossible to gather all of the small fibrin particles onto the glass rod and once this occurs, no amount of manipulation makes it possible to recover the complete amount of fibrin. A simple alternative approach has been suggested by Adelson.\textsuperscript{16} The clot and its
surrounding diluted protein solution is poured through a thin filter of glass wool; the clot is trapped on the filter and the other proteins are washed through. The technic is simple and works well in many cases. The difficulty with it is that there is no way of being certain that all other proteins have been washed out of the glass wool filter. Time and again when serum was tested as a control in this assay, appreciable amounts of protein were retained in the filter which could not have been fibrin.

A variant of this procedure was tried in this laboratory which, it was hoped, would avoid this difficulty. The contained clot and its associated protein solution were passed through a nylon filter made by cutting off a short length of filter tubing, such as is used in transfusion sets, and heat-sealing the bottom. This technic let most occluded proteins through every easily and trapped the fibrin very well—in fact, the nylon filter material had such a strong affinity for fibrin that it was necessary only to shake the filter in the fibrin-containing suspension. However, a very bulky clot trapped in or around the nylon filter released its occluded non-fibrin proteins with great difficulty. Removal of these contaminants by centrifugation or by squeezing the clots against the side of the test tube with a glass rod was unsuccessful. The latter method was particularly unsatisfactory because standardization of squeezing of the clot by different individuals was impossible.

This experience led us to return to the technic of Ratnoff and Menzie, which we had used for the most part in recent years, because this method has the advantage of producing a standardized squeeze of the clot. The diluted plasma is clotted in the presence of fine glass particles; on centrifugation, these particles pull the attached fibrin webs to the bottom of the tube. The Ratnoff technic is annoyingly time-consuming, requiring three washes and centrifugations before all occluded proteins are satisfactorily removed, but we have found no alternative method which gives the same degree of accuracy. The Ratnoff technic has been followed in complete detail with the exception of two modifications. The first is addition of a volume of 0.025 M calcium chloride equal to that of the plasma being assayed. This is added in addition to the thrombin because of evidence that a firmer clot is produced in the presence of calcium. The second modification was an attempt to standardize the glass powder which, according to Ratnoff, is made by crushing pyrex glass tubes in a mortar. Glass beads (0.2 mm. diameter) prepared for use in homogenizing mills worked very satisfactorily in this procedure. The Ratnoff technic calls for the use of approximately half a gram of powdered glass. Investigation demonstrated that with the beads or with the powdered glass the volume of glass used played no role in the final answer obtained in the sense that nothing on the glass reacted with the reagents being used to produce a color such as would be produced by tyrosine. On the other hand, it became apparent that if the amount of beads was not quantitated roughly, we ran the risk of using volumes of beads which did increase the inaccuracy of the technic. Using amounts of the beads approximating 2 Gm., results were far less reproducible, and it was concluded that the volume of beads was great enough to interfere with the ability to wash away non-fibrin proteins. Results have been very satisfactory when the glass beads are weighed very roughly to approximate 0.5 Gm. (table 1).
The Ratnoff method calls for incubation of the clot for 10 minutes before
washing. Addition of a fibrinolysin inhibitor, as in the procedure described
here, permits much longer periods of incubation without variation in the
result obtained.

Using the present technic, plasmas of 100 normal blood donors have been
assayed for fibrinogen. The mean value obtained was 225 mg. per 100 ml. of
plasma with a range from 147 to 319 mg. and a standard deviation of 36 mg.
Only a few of these blood donors were female, but figure 1 shows no reason
to believe that the fibrinogen levels were related to the sex of the donor.
Previous reports have suggested that fibrinogen levels tend to increase with
age. In this series (fig. 2) there does appear to be a very slight tendency for
higher levels to be obtained at older ages, but the results are not statistically
significant.

The normal range obtained here is considerably lower than the usually
quoted 200 to 400 mg., but previous studies were based on smaller series of
presumably normal patients selected from a hospital population. The accuracy
of our standard tyrosine curve was rechecked repeatedly with freshly prepared
solutions of tyrosine as the study progressed, and good agreement was found
between duplicate assays by us and in the laboratory of the hematology clinic
at the University of California Medical Center in San Francisco. The latter
laboratory used the same technic, but standardized their instrument separately.
In both instances, Ratnoff’s factor of 11.7 for conversion of tyrosine equivalents
into fibrinogen levels was used.

Fibrinolytic Activity

Fibrinolytic activity is under intensive study at the present time, and a wide
variety of assay technics has been used to approach this problem. It is generally
accepted that fibrinolytic activity arises from a complex interaction of a num-
ber of factors including activators, precursors and inhibitors. Specific assays of
each of these individually is the only approach which, in the long run, will
provide an understanding of the mechanism by which alterations occur, but
in terms of clinical evaluation of a bleeding situation, the first and most im-
portant question is an overall estimate of the degree of fibrinolytic activity
without necessarily being concerned as to how much of this is due to increase
in activator, decrease in inhibitor, etc. Specific assays for activators, precursors
and inhibitors alone provide no direct picture of the net result.

The discovery that the activators and fibrinolysin could be separated from
their inhibitors by precipitating euglobulin has been widely applied in analysis
of fibrinolytic activity, with inhibitors removed, clots lyse much more quickly.
This lessens the time spent waiting for results and also provides the possibility of finding evidence of activity even in normal people. The disad-
vantge of this technic is that, since inhibitors have been completely removed,
one has no idea of the over-all net effect in the patient. It is conceivable that
increased fibrinolytic activity in the euglobulin precipitate could be more than
balanced by an increase in inhibitor, and vice versa.

Simple observation of clotted whole blood provides valuable evidence of
lysis in clinical situations, but definite lysis of a whole blood clot within a few
hours occurs only with massive activation of fibrinolysis. A more sensitive
index of milder degrees of activation can be obtained by clotting diluted plasma, but this requires prolonged observation of tubes over many hours and is only roughly quantitative. Lysis of recalcified plasma clots can be recorded automatically by the thrombelastograph of Hartert, but the results are difficult to express in quantitative terms, and the number of samples that can be tested in a single day is very limited.

A number of technics have been devised for quantitative evaluation of the amount of clot dissolved away under standardized conditions. The best known of these is the Astrup fibrin plate method; here a clot of fibrin is produced in a Petri dish and a drop of test material is placed upon it. Following incubation, the area of the zone lysed is determined, and in a roughly quantitative sense this area corresponds to the degree of fibrinolytic activity. This type of approach has been made more quantitative by Von Kaula who forms his clots in the narrow neck of a special tube; the tube is then rotated in an incubator, and the slow digestion of the clot down the length of the tube is quantitated by graduations on the side of the tube. Other technics have attempted to provide quantitation of the degree of lysis by labeling the fibrin clot with radioactive materials. Sherry’s technic using $^{131}$ provides satisfactory quantitation, but the use of radioactive materials makes this a bit more complex.

The present technic provides the advantage that quantitative chemical analysis of the degree of fibrinolysis can be carried out. The analysis is possible because the plasma with its own contained fibrinogen is clotted and then on prolonged incubation one finds how much of the fibrin which has been formed disappears. An early approach of this type was described by Adelson who set up fibrinogen determinations in duplicate, carried one to completion at the end of 10 minutes incubation and assumed this was 100 per cent, and allowed the other to incubate for a varying period of hours, tracing in the process the consumption of fibrin from the clot. This approach is somewhat cumbersome because this laborious assay has to be carried out at several different time intervals.

The discovery of a potent inhibitor of fibrinolysis (epsilon amino caproic acid) suggested to a number of observers that this might be employed in a useful manner to investigate fibrinogen levels and the extent of fibrinolysis. Blix added epsilon amino caproic acid (EACA) to tubes in which he was collecting blood samples for fibrinogen assays so that he could be certain lysis had not occurred by the time the assay was carried out. Similarly Nilsson collected blood in two tubes (one containing EACA) and carried out routine fibrinogen determinations assuming that the tube with EACA would have no fibrinolysis and the one without it, if it had a lower value, would thereby indicate the extent of fibrinolysis. Bergström, Blömbach and Kleen performed fibrinogen assays in duplicate, incubating them for 2 hours, using lysine ethyl ester in one to inhibit fibrinolysis. Our technic is based very closely upon this latter approach but differs from it somewhat and has been explored to the point of finding the necessary details of collection, storage and assay of samples which make it practical for general use in analysis of problems in the hospital.
Fibrinogen assays were done on each plasma sample in duplicate, one of the two tubes for each sample containing EACA. The value obtained in the tube with EACA is assumed to represent the initial fibrinogen level in the patient; the difference between that and the value obtained in the tube without EACA indicates the degree of lysis. Initial fibrinogen values in the samples vary, of course, and it is possible to report results either as micrograms of fibrinogen per milliliter of plasma lysed or as the per cent of the initial value which disappeared. Bergström et al. reported their results in micrograms. We have reported our results as per cent lysis for the following reasons: (1) There was no evidence that the result expressed in either way was related to the original fibrinogen level of the sample in our series of normals. This was established by graphing fibrinogen levels against lysis, and showing that there was no correlation. (2) If normal results were considered those in which the number of micrograms of fibrinogen which disappeared was below a certain arbitrary limit, patients whose initial fibrinogen level was below that limit could not be shown to have abnormal degrees of fibrinolysis. It is, of course, the patients with low fibrinogen levels following surgery who are most likely to have activated their fibrinolytic system. When results are expressed as per cent lysis, abnormalities can be detected in these patients.

To minimize the amount of blood taken from patients, we did not wish to add EACA to a portion of the sample at the time of collection. This was done in a number of instances, however, to ensure that the technic of collection and storage of samples did not result in loss of activity. The lability of fibrinolytic activity is well-established, and most technics insist on the performance of tests within 30 minutes of collection of the sample. Our results indicate that our technics of collection and storage of samples did not result in loss of fibrinogen in samples collected without immediate addition of a fibrinolytic inhibitor. The degree of lytic activity remained unchanged with periods of storage well in excess of those customarily used. Addition of the fibrinolytic inhibitor did not alter the values obtained, except by the desired action of preventing lysis.

Results in 100 normal blood donors showed no evidence of fibrinolysis by this technic. The differences between the paired samples (with and without EACA) were within the range of error of the fibrinogen assay. Note was made whether any of the donors had a “reaction” after donation, evidence for this being pallor, sweating, weakness or fainting. The few “reactors” tested in this series showed answers within the normal range. In most instances the reaction took place at the time or immediately after the blood donation had been completed and the samples collected for our assays. It is conceivable, therefore, that a sample taken at the later point might have shown activation of fibrinolytic activity. It has been well demonstrated by others that severe emotion and secretion of adrenalin can do this. The important thing from our point of view is that it does not seem to do this in the donor blood itself and, therefore, it is unlikely that the donor blood can be implicated as the cause of fibrinolysis in recipients of such blood.

The sensitivity of the technic in detection of mild degrees of pathologic
fibrinolytic activity is apparent in the results on samples from patients subjected to open heart surgery. The euglobulin lysis test confirmed activation of fibrinolysis in these plasmas. The striking findings in this group were not associated with whole clot lysis, indicating that relatively mild degrees of activity were being detected. It has not yet been established to what extent, if any, this relatively mild degree of activity plays a role in the occurrence of abnormal bleeding following open heart surgery.

The technic for estimating fibrinolysis provided a quantitative answer with very little work added to the routinely done fibrinogen estimations. Careful attention to conditions of collection, processing and storage of plasma permitted work with frozen plasma without loss of activity. The technic is believed to give as good an estimate of over-all fibrinolytic activity as any currently available. One possible theoretical defect is the need to work with diluted plasma, which minimizes the role of inhibitors in the reaction. This objection does not appear to interfere with the goal of comparing the degree of over-all activation of the fibrinolytic system under various situations.

**SUMMARY**

A variety of technics for assay of fibrinogen were investigated. Greatest accuracy was obtained with a slightly modified Ratnoff-Menzie procedure. One hundred presumably normal blood donors had an average fibrinogen level of 225 mg per 100 ml of plasma, with a range from 147 to 319 mg. The levels were not appreciably influenced by age, sex or "reactions" to donation of blood.

Using this assay, over-all fibrinolytic activity was estimated by duplicate assays employing 4-hour incubation of the diluted clot, lysis being inhibited in one of the tubes by epsilon amino caproic acid. The technic showed significant degrees of fibrinolytic activity in the plasma of most patients after open heart surgery as compared with the normal controls.

**SUMMARIO IN INTERLINGUA**

Un varietate de technicas pro le essayage de fibrinogeno esseva investigate. Le plus alte grado de accuratia esseva obtenite con un levemente modificate procedura de Ratnoff-Menzie. Cento presumitemente normal donatores de sanguine habeva un nivello medie de fibrinogeno de 225 mg per 100 ml de plasma, con un campo de variationes ab 147 ad 319 mg. Le nivellos non esseva influentiate appreciabilemente per etate, sexo, o reactiones al donation de sanguine.

Per medio de iste essayo, le activitate fibrinolytic general esseva estimate per duple essayos, con incubationes del diluite coagulo durante 4 horas e inhibition del lyse in un del tubos per acido epsilon-aminocaproic. Le technica monstrava grados significative de activitate fibrinolytic in le plasma del majoritate del patientes post chirurgia cardiac aperte in comparation con normal subjectos de controlo.

**ADDENDUM**

After this article was accepted for publication, Nilsson reported a further modification of her approach which has much in common with the procedure reported here. It differs in the following ways: Fibrinogen was measured by collection of the clot on a cloth of...
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fine texture. EACA was added to the control blood sample on collection, and to the sample tested for fibrinolysis after incubation of the citrated whole blood for 2 hours at 37 C. Slightly larger amounts of EACA were used, and it was also added to the diluent and washes used in the fibrinogen assay. The technic measures fibrinogenolysis rather than fibrinolysis.

REFERENCES


Quantitative Assay of Fibrinogen and Fibrinolytic Activity

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