A New Diagnostic and Therapeutic Approach  
Fibrinolysis and Hemorrhage

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The following study gives evidence that the enzyme present in the plasma of a patient with acute fibrinolysis was able to digest a protein substrate different from fibrinogen or fibrin. On the other hand, this enzyme was found to be markedly inhibited in vitro by the soy bean trypsin inhibitor (S.B.I.). On the basis of this observation, an attempt was made to treat the patient by means of the intravenous administration of S.B.I., and the results of this treatment are reported here. This study represents the first observation of the treatment of fibrinolysis of shock in humans by means of S.B.I.

Material and Methods

Venous blood samples were obtained by syringe and needle. In addition, blood flowing from the thoracic wound was collected in test tubes.

Citrated plasma was obtained from citrated blood (one volume of 3% sodium citrate solution for 9 volumes of blood) by centrifuging for 30 minutes at 3000 r.p.m. The samples of citrated plasma were quick frozen and stored at −20 C. until they were studied. Certain samples were quick frozen immediately after separation of the plasma, while others remained at room temperature for 3 hours before being quick frozen; this delay was due to a certain degree of confusion in the operating room during the acute emergency.

Soy bean inhibitor. A crystalline preparation following the method of Kunitz1 was used.

Observation of fibrinolysis. The presence or absence of fibrinolysis in samples of fresh whole blood (or citrated plasma) was detected by the method previously described.2

Measurement of fibrinogen in plasma. This was carried out on plasma by a modification of the method of Cullen and Van Slyke as follows. To ½ ml. of plasma diluted with 20 ml. of physiologic saline, 1 ml. of 3 p.c. CaCl2 solution was added. The mixture was incubated in the same fashion for a short and long time in order to find out whether digestion of fibrin took place during the period of incubation. Another control sample was prepared by the addition of 200 µg. of S.B.I., in the form of a solution containing 2 mg. per ml., to the mixture. At the end of the period of incubation, the clotted protein in each sample was collected on a glass rod and a total nitrogen determination carried out. The purpose of the control sample containing the S.B.I. was to evaluate by comparison with the two other samples the inhibitory activity of the S.B.I. on the plasma enzyme.

Measurement of proteolytic activity of plasma. A substrate of fibrinogen or fibrin cannot be used to measure the proteolytic activity of plasma because it is often contaminated with plasminogen, which may become activated into plasmin during the measurements, thereby altering the results.

If a casein substrate is used, tyrosine measurements as an end-product of the proteolysis are not practical in the presence of plasma or serum because of their high content of tyrosine-like substances as measured by the Folin reagent or by the U.V. absorption, thus giving high and variable blank values.
Finally the following method of measurement based on the use of casein tagged with \(^{131}I\) was employed: 9 Gm. of Hammarsten casein (Merck) were dissolved at 50 °C–60 °C. in 120 ml. of M\(^{-15}\) phosphate buffer at pH 7.4. The solution was boiled for a few seconds, cooled and filtered. Iodination was carried out by the addition, drop by drop, with constant stirring, of the following solution: 1 ml. of 0.01 M KIO\(_3\); 1 ml. of 0.01 M KI; 10 mc. of \(^{131}I\) in the form of NaI; 1.5 ml. of 0.05 M HCl. The iodinated casein solution was dialyzed at 4 °C. against running tap water for 15 to 20 hours. After dialysis, the solution was added with approximately 1 ml. of molar KI, then heated at 80 °C. for one hour. The final solution was dialyzed for several hours against distilled water and rendered isotonic by the addition of solid NaCl. Buffering was done by the addition of phosphate buffer or no buffer was used. The pH was adjusted to 7.4 by the addition of 0.5 M NaOH. The final protein concentration was between 7.5 Gm.% and 8.28 Gm. % There was no release of free iodine in this solution when kept at 4 °C. for 10 days. The detailed method of preparation of this casein substrate will be published elsewhere.

Determination of proteolytic activity. One ml. of plasma to be tested was incubated with 1 ml. of the casein substrate for 6 hours at 29 °C. The reaction was stopped by the addition of 2 ml. of ammonium sulfate solution at \(\frac{5}{12}\) saturation. The final ammonium sulfate saturation was \(\frac{5}{12}\). Filtration was done through a 803 Green filter. Two ml. of the filtrate were measured for radioactivity in a scintillation counter. The blank was made of the same reagents incubated separately and mixed at the end of the period of incubation just before the addition of the ammonium sulfate solution. In the experiments reported here, results were expressed in mg. of digested casein. By digested casein is meant the increase in radioactivity in the fraction soluble in \(\frac{5}{12}\)-saturated ammonium sulfate solution at 22 °C. Milligrams of digested casein were calculated by dividing the increase in radioactivity of the filtrate by the specific activity of the substrate. Two different batches of marked casein were used in this study. The first batch was used for the first sample of blood (obtained at 1:15 P.M.), the other for all subsequent samples. Calculations and control measurements showed that an increase in radioactivity exceeding \(\frac{12}{12}\) of the blank, that is 0.100 mg. for a first batch of casein and 0.134 mg. for a second, was significant as indicating true digestion of the substrate.

Complete standardization of proteolysis in terms of a reference enzyme was not attempted. However, as a comparison, it was found that 1 ml. of a human plasmin preparation, containing 0.05 mg. of plasmin (Fraction III\(_t\) of Cohn) incubated with 1 ml. of this substrate for 15 hours at 22 °C., digested 0.248 mg. of casein.

Prothrombin time determinations. These were carried out by the method of Quick and the results expressed in seconds. Each determination was repeated with the addition of Ac globulin in the form of a hundred times diluted fresh beef serum.

**Case Report**

A 28-year-old female four months pregnant, was admitted on January 29, 1957, to the Lying-in Department of the St. Pierre Hospital in Brussels. Her chief complaint was vaginal hemorrhage for the last two months.

Family history was not contributory. Past history revealed a normal pregnancy in 1952 followed by another pregnancy in 1953, which was terminated by an abortion with curettage. The patient had a serofibrinous pleuresy in 1953, following which a diagnosis of bilateral pulmonary tuberculosis was made. This was treated by the administration of streptomycin, isoniazid and sanatorial care. The tuberculosis was of a severe, rapidly progressing type.

On admission the positive findings were: the presence of extensive, ulcer-
tive, bilateral pulmonary tuberculosis, continuous vaginal hemorrhage and a marked anemia with 2.9 million red corpuscles per cubic mm. of blood and 7.6 Gm. of hemoglobin per 100 ml. of blood. The decision was made to interrupt pregnancy because of the severity of the tuberculosis and the vaginal bleeding, and the patient was prepared for cesarean section. Anesthesia was by intravenous pentothal with curare, completed by nitrous oxide and cyclopropane.

The following is a chronological account of subsequent events. At 10:30 A.M. the abdominal cavity was opened and blood transfusion began. Eight liters of blood were transfused between the hours of 10:30 A.M. and 4 P.M. At 11 A.M. the heart stopped beating. Thoracotomy and cardiac massage were resorted to at 11:10 A.M. At 11:20 A.M. the heart was observed to have resumed normal function.

The patient received an intracardiac injection of 0.5 mg. of epinephrin in 5 ml. of saline at 11:15 A.M.; at the same time 10 ml. of a 10 per cent calcium gluconate solution were given intravenously. At 11:20 A.M. an intravenous injection of 0.25 mg. of epinephrin and 10 ml. of calcium gluconate solution was given, followed immediately by 0.8 mg. of cedilanid by the intravenous route.

Following this treatment, pulse and skin color returned to normal. From 11:30 A.M. to 12 noon, the heart was beating normally, but blood began to ooze from the thoracotomy wound which was sutured. At 12:30 P.M. profuse bleeding was observed from the thoracic as well as the abdominal wound. The decision was then made to interrupt the operation in view of the severe bleeding and impending shock. At 12:40 P.M. the abdominal incision was oozing blood profusely. At 12:45 P.M. 1½ liters of blood had been collected from the thoracic drain. Between 12:45 P.M. and 2:14 P.M. several blood samples were obtained from the thoracic drain as well as by venous puncture. All these blood specimens remained unclotted at 37 C. for several hours. The blood pressure was unobtainable from 11:00 A.M. until 1:45 P.M. when pressure was 70 systolic. The diastolic pressure could not be obtained. At 1:50 P.M., a diagnosis of bleeding associated with a fibrinopenemia, possibly associated with fibrinolysis, was made and the patient received an injection of 300 mg. of soy bean trypsin inhibitor in 150 ml. of \( \frac{M}{6} \) Na lactate, followed five minutes later by 5 Gm. of a human fibrinogen preparation in 250 ml. of Alzheimer solution. At 2:14 P.M., bleeding was distinctly observed to decrease: a sample of venous blood obtained at this time clotted in four minutes but redissolved completely in 30 minutes at 37 C. A sample of venous blood was obtained at 3 P.M. and clotted in 3 minutes at 37 C. At 4:04 P.M., the venous blood clotted in three minutes and redissolved almost completely in 70 minutes at 37 C. By this time the systolic blood pressure reached the value of 100 mm. and remained at this level thereafter. At 5 P.M. the patient received a second intravenous injection of 400 mg. of soy bean trypsin inhibitor in 150 ml. of \( \frac{M}{6} \) Na lactate, followed at 5:50 P.M. by 5 Gm. of the same fibrinogen preparation. From there on, all venous blood samples which were tested clotted spontaneously and no evidence of fibrinolysis was observed during a period of observation of six hours at 37 C.
At 7:40 P.M., a dead fetus was spontaneously delivered. It weighed 425 Gm. and was not macerated; the placenta weighing 585 Gm. followed shortly thereafter.

The subsequent course of the patient was without hemorrhagic episodes. However, the patient never regained consciousness and died two days later.

Postmortem examination revealed the following significant findings: multiple hemorrhages in the bronchi, mediastinum, the pleural spaces and the meningae; bilateral caseous pulmonary tuberculosis; marked lesions of the basal ganglia and the brain cortex. The lesions consisted of picnotic nuclei with retraction and acidophilia of the cytoplasm. In addition, an unexpected finding was the presence of an astrocytoma in the cerebellum.

RESULTS

1. Observations of fibrinolysis on whole blood

The blood samples collected from the thoracic wound never clotted, and the addition of thrombin showed that they were devoid of fibrinogen. As far as venous samples are concerned, the diagnosis of fibrinolysis was based on the observation of the clot of whole blood. Therefore, it could not be made on samples obtained during the period when no clot formed in the blood specimens because of the absence of fibrinogen. The blood samples obtained at 2:14 P.M. clotted normally and the clot redissolved completely in 30 minutes at 37 C. The blood sample obtained at 3 P.M. clotted normally and redissolved completely in 150 minutes at 37 C. The clot of whole blood obtained at 4:04 P.M. redissolved almost entirely in 30 minutes at 37 C., but a very small residue of solid material remained unchanged during an additional incubation of six hours at 37 C. Therefore, this sample cannot be said to have shown unmistakable fibrinolysis as defined by the method of observation applied here.

All blood samples obtained after 4:04 P.M. clotted normally but did not show fibrinolysis during a period of observation of six hours at 37 C.

2. Measurements of the level of fibrinogen in blood plasma

The results are shown in table 1.

As far as venous blood is concerned, the following observations of fibrinolysis were made. The plasma obtained from blood withdrawn at 4:04 P.M. showed marked fibrinolytic activity in vitro. This was demonstrated by the difference in the amounts of fibrin recovered from the plasma after one and 2 H.15 incubation, respectively, at room temperature. The lower level of fibrin recovered after the longer incubation period reflects the digestion of the fibrin by the fibrinolytic principle. This was confirmed by measuring the amount of fibrin recovered from the same plasma incubated with an excess of S.B.I., which was found to be distinctly greater than the amounts recovered without addition of S.B.I. These results indicate that when a fibrinogen determination is carried out on plasma exhibiting fibrinolytic activity, it is necessary to add...
Table 1.—Fibrinogen Level of Plasma with Variable Incubation Time and With and Without Addition of S.B.I.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Incubation Time in hours</th>
<th>Room t °C</th>
<th>Fibrinogen (mg./100 ml. of plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Without S.B.I.</td>
</tr>
<tr>
<td>2-14-57</td>
<td>*1:15 P.M.</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4:04 P.M.</td>
<td>1</td>
<td></td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>2.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5:17 P.M.</td>
<td>1</td>
<td></td>
<td>272</td>
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<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>5:37 P.M.</td>
<td>1.30</td>
<td></td>
<td>237</td>
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<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6:00 P.M.</td>
<td>1.30</td>
<td></td>
<td>337</td>
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<tr>
<td></td>
<td>3</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>7:30 P.M.</td>
<td>1.30</td>
<td></td>
<td>345</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8:45 P.M.</td>
<td>1.30</td>
<td></td>
<td>371</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9:45 P.M.</td>
<td>1.30</td>
<td></td>
<td>371</td>
</tr>
<tr>
<td></td>
<td>3</td>
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<td></td>
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<tr>
<td>2-15-57</td>
<td>9:00 A.M.</td>
<td>3</td>
<td></td>
<td>382</td>
</tr>
<tr>
<td>2-16-57</td>
<td>12:15 A.M.</td>
<td>3</td>
<td></td>
<td>180</td>
</tr>
</tbody>
</table>

*This sample of thoracic blood plasma did not clot after addition of calcium and 10 U. of thrombin.
†Five minutes after death.

Adequate amounts of S.B.I. in order to prevent fibrinolytic destruction of the fibrinogen during the period of incubation.

No fibrinolytic activity was detected in the plasma from blood samples obtained after 4:04 P.M., as shown by the fibrinogen determinations with and without incubation or addition of S.B.I. (table 1).

Effect of intravenous injection of S.B.I. The effect of the injections of S.B.I. is difficult to correlate with the fluctuations of the plasma fibrinogen level, because this drug was administered concurrently with the fibrinogen. As far as the effect on fibrinolysis is concerned, the blood sample obtained after the first injection still showed fibrinolysis, but immediately after the second injection, fibrinolysis disappeared completely and remained absent until the end of the course.

3. Casein digestion as a measure of the proteolytic activity of blood

The results are shown in table 2.

Proteolytic activity as measured by casein digestion was present in the sample of plasma from thoracic blood and in the venous sample obtained at 4:04 P.M. Subsequent samples of plasma showed no proteolysis by this method. These results correlate well with the results of the fibrinogen determinations described previously.
Table 2.—Proteolytic Activity of Plasma on Casein

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample</th>
<th>Digested casein (mg.) without S.B.I.</th>
<th>with S.B.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-14-57</td>
<td>1:15 P.M.</td>
<td>0.440</td>
<td>0.348</td>
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<td></td>
<td>4:04 P.M.</td>
<td>0.670</td>
<td>0.220</td>
</tr>
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<td></td>
<td>5:17 P.M.</td>
<td>0.122</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>5:37 P.M.</td>
<td>0.137</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>6:00 P.M.</td>
<td>0.000</td>
<td>0.060</td>
</tr>
<tr>
<td></td>
<td>7:30 P.M.</td>
<td>0.096</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>8:45 P.M.</td>
<td>0.132</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>9:45 P.M.</td>
<td>0.000</td>
<td>0.040</td>
</tr>
<tr>
<td>2-15-57</td>
<td>9:00 A.M.</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>2-16-57</td>
<td>12:15 A.M.</td>
<td>0.100</td>
<td>0.113</td>
</tr>
</tbody>
</table>

*Thoracic blood plasma.
†Five minutes after death.

Table 2 also shows that the addition of S.B.I. in vitro to the mixture of plasma and casein produced definite inhibition of proteolysis in venous blood, while the inhibition was doubtful in the case of the blood obtained through the thoracic drain.

4. Prothrombin time determinations

The results of these determinations on plasma samples are shown in table 3. There was a marked prolongation of the prothrombin time in samples 4 to 7, which was not corrected by the addition of the Ac globulin preparation.

Discussion

The data presented here are admittedly incomplete because blood samples were not obtained before the period of shock and during the early phase of this period. The type of clinical emergency represented by this patient did not lend itself to careful planning of the collection of blood samples. Nevertheless, sufficient samples were obtained to provide a basis for a diagnosis of fibrinolysis by several methods and for the evaluation of the results of treatment in this case.

The data show that this patient had fibrinolysis of the blood during the hemorrhagic episode which occurred during the operation.

An attempt was made in this work to introduce a quantitative evaluation of its intensity. The first method used was based on the measurement of the level of fibrinogen in the plasma after different lengths of incubation with and without the addition of S.B.I. It is simple but cannot be used if fibrinogen is absent. The second one, based on the use of marked casein, appears to be the most reliable and capable of giving a true quantitative estimation of the intensity of fibrinolysis. The use of casein as a substrate eliminates the possibility of contamination of the substrate by plasminogen. The measurement of radioactive end products of the reaction of plasma enzymes on casein offers the advantage that the plasma used as a source of enzyme does not contribute any end product to the reaction, and therefore does not contribute to the blank and readings of the method.
The presence of fibrinolysis in the case reported here was confirmed by these two methods. Fibrinolysis was present when the patient was bleeding. It would seem that the state of anoxemia precipitated by the combination of bleeding and cardiac arrest was responsible for the appearance of fibrinolysis.2

Fibrinolysis associated with severe bleeding is an acute emergency with an immediate threat to the life of the patient; several cases have been reported which terminated in death of the patient.4 Blood transfusions are able to replace the blood loss, but as long as fibrinolysis persists, hemostasis may remain deficient and bleeding continue. The administration of large amounts of human fibrinogen in the form of Fraction I of Cohn has been advocated and seems to be successful in a number of cases of bleeding due to a-fibrinogenemia.5,6 However, it should be noted that this fibrinogen preparation is often contaminated with plasminogen, the precursor of plasmin.7 Therefore the possibility exists that this plasminogen may become activated into plasmin in the circulating blood and add to the fibrinolytic activity. Another objection to the use of this preparation is the possibility of transmitting viral hepatitis; such an occurrence has been reported in the literature.8

A more logical approach to the treatment of the hemorrhagic manifestations of fibrinolysis is to stop the activity of the enzyme responsible for the digestion of fibrinogen. Tagnon and Soulier in 1946 demonstrated that the trypsin inhibitor from soy bean is a strong inhibitor of the enzyme plasmin.2 They demonstrated in 1948 that S.B.I., injected intravenously into dogs, increased several times the antifibrinolytic activity of the plasma.10 In 1952, Tagnon and his associates11 reported the first use of crystalline purified S.B.I. in the treatment of fibrinolysis associated with metastatic cancer of the prostate. The toxicity and pharmacology of S.B.I. have been investigated,12 and it was shown that the therapeutic dose in humans was much smaller than the toxic dose, and that the drug could be injected safely into humans. The data reported here indicate that S.B.I. effectively inhibited in vitro the fibrinolytic enzyme present in the venous blood of our patient. The small extent of the inhibition in vitro of proteolysis of thoracic blood by S.B.I. is not explained except, perhaps, by the fact that admixture of other factors to this blood may have rendered S.B.I. less effective.

As far as the therapeutic use of S.B.I. in humans is concerned, this is the first published report of the injection of S.B.I. for the treatment of fibrin-
Fibrinolysis and hemorrhage

Fibrinolysis of shock in humans. The first injection was given concurrently with 5 Gm. of fibrinogen. For the sake of evaluation it is unfortunate that S.B.I. was not given alone, but the physician in attendance felt that the critical state of the patient required the use of all available therapeutic means. The results of this first injection on fibrinolysis are therefore impossible to evaluate. The second injection was given without fibrinogen. Following this administration, the proteolytic activity of plasma, as measured on casein, was found to be nil, while the sample obtained prior to the injection had marked activity. This may represent the effect of the injected S.B.I. However, in view of the fact that fibrinolysis may disappear spontaneously, this observation alone does not demonstrate unequivocally that S.B.I. stopped fibrinolysis. Nevertheless, if one considers that S.B.I. inhibited in vitro the proteolytic activity of the plasma of our patient, it seems logical to consider as probable that the same effect was produced in vivo. This observation indicates that S.B.I. in the single instance in which it was used was followed by the expected therapeutic effect on fibrinolysis of shock. The use of this preparation is therefore justified in other cases of the same disorder in order to confirm its therapeutic effectiveness. No manifestations of toxicity were observed following the injections, and this is in accord with our studies on toxicity carried out on animals.12

Summary

1. The case is presented of a patient who underwent a cesarean section during which cardiac arrest occurred. Cardiac massage was successful in re-establishing normal heart action.
2. A severe hemorrhagic diathesis associated with fibrinolysis appeared during the period of shock associated with cardiac arrest.
3. The plasma of the patient obtained during the period of fibrinolysis was able to digest its own fibrin as well as a substrate of casein marked with radioactive iodine.
4. A new method of measurement of fibrinolysis based on the use of tagged casein is presented: the main advantage of this method is that the substrate is not contaminated with plasminogen or plasmin. In addition the products of the reaction are measured by their radioactivity. Therefore, the blank and readings in this method depend solely upon the substrate and are not contributed to by the other reagents used.
5. The proteolytic activity of the plasma from a patient with fibrinolysis was shown to be inhibited by the trypsin inhibitor from soy bean.
6. The intravenous injection of trypsin inhibitor in this patient was followed by the disappearance of fibrinolysis in her blood.

Summario in Interlingua

1. Es presentate le caso de un paciente in qui arresto cardiac occurreva in le curso de un section cesareee. Massage cardiac succedeva a restablir le action normal del corde.
2. Un sever diathese hemorrhagic associate con fibrinolyse appareva durante le periodo de choc que eseva associate con le arresto cardiac.
3. Le plasma del paciente, prendite durante le periodo de fibrinolyse, se
monstrava capace a digerere su proprie fibrina e etiam un substrato de caseina marcate con iodo radio-active.

4. Es presentate un nove metodo pro le meusurazione de fibrinolyse. Illo es basate super le uso de caseina radio-marcate. Le principal avantage del metodo es que le substrato non es contaminate con plasminogeno o plasmina. In plus, le productos del reaction es mesurate per lor radioactivitate. Assi, le "blanco" in iste metodo depende exclusivemente del substrato e non recipe uille contribution ab le altere reagentes usate.

5. Le activitate proteolytic del plasma ab le patiente con fibrinolyse esseva inhibite per le inhibitor de trypsina ab soja.

6. In iste patiente le injection intravenose del inhibitor esseva sequite per le disparition de fibrinolyse in su sanguine.

REFERENCES


12. ——: To be published.
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