“Cryofibrinogen” in a Case of Lung Neoplasm Associated with Thrombophlebitis Migrans

By DONALD R. KORST AND CLYDE H. KRATOVIL

AN ABNORMAL PROTEIN which precipitated when cooled and returned to solution at room temperature was found in the plasma of a man who had migrating thrombophlebitis and pulmonary neoplasia. Thrombophlebitis migrans had been present for 11 months before the diagnosis of lung neoplasm was established. The phenomenon of a cryoprotein has not been previously described in connection with thrombophlebitis migrans and neoplasm. For this reason, it is believed the following case report and experiments with some details of the cryoprotein are of interest.

CASE REPORT

A 41 year old white male was admitted November, 1953, complaining of painful swelling in the right arm. The history was of recurrent thrombosis in veins of all extremities and the neck. There was one episode of chest pain and hemoptysis probably due to pulmonary infarction. Symptoms had been present for 11 months and the inferior vena cava had been ligated at another hospital without change in the course of his disease. Dicumarol therapy was also without benefit. There was no history of cold sensitivity, purpura, or abnormal bleeding. Examination revealed a healthy appearing male with swelling and edema of the right arm and shoulder. Superficial venous thrombosis was evident and the entire extremity was quite painful to touch. Edema was present in the left lower leg and the superficial saphenous veins were thrombosed to palpation bilaterally.

Pertinent laboratory studies revealed: the urine contained 0.1 per cent albumin, the peripheral blood contained 22,300 W.B.C./cu.mm. with 82 per cent neutrophils; platelets slightly increased, and a sedimentation rate (Wintrobe) of 20 mm./hour. The bleeding time (Duke) was 9 minutes, the Lee-White coagulation time at 37 C. was 6 minutes; the prothrombin concentration (Quick) 66 per cent; and total serum proteins 7.1 Gm./100 ml. with albumin 4.7. Bromsulphalein retention was 11 per cent in 45 minutes, and the serum alkaline phosphatase 21 King-Armstrong units/100 ml. A needle biopsy of the liver was normal. Sternal marrow aspiration revealed a normally cellular marrow with some increase in megakaryocytes. X-rays of the bones were normal, but the chest film revealed a soft tissue bulge along the right mediastinum at the level of the aortic arch with a small amount of fluid at the left base.
Marked rouleaux formation was present in smears of the peripheral blood. It was noted that venous blood placed in the routine oxalate or citrate blood bottles would form a soft gelatinous clot. In 24 hours at 37°C there was about 50 per cent decrease in size of the clot, and it remained quite friable. A heparin specimen of blood did not clot. This jelly-like clotting of the blood in the presence of the usual amounts of citrate or oxalate led to the suspicion that an abnormal plasma protein might be present. Cooling the plasma which had been treated with an excess of citrate, oxalate or heparin revealed immediate formation of an abundant white flocculent precipitate. This re-dissolved completely on warming to room temperature. Cold precipitation was not noted in serum samples on repeated examination.

The patient received intramuscular heparin therapy and there was marked lessening of thromboses and disappearance of the cryoprotein and rouleaux formation. Heparin therapy was stopped and in several days there was a recurrence of superficial and deep thromboses. When heparin administration was restarted there was marked improvement and disappearance of most of the thromboses. Examination of pleural fluid revealed numerous malignant cells thought to be from an anaplastic neoplasm of the lung or pleura. The cryoprotein was also present in the pleural fluid.

Chymotrypsin was given in an attempt to decrease the circulating fibrinogen, but this was discontinued because of the appearance of new thromboses. Heparin was reinstituted and again he improved. Roentgen ray therapy to the mediastinum was given, but his condition became worse. Total serum proteins were reduced to 4.3 Gm. with 2.8 Gm. albumin. Evidence of hepatic damage increased as the total bilirubin rose to 7.9 mg./100 ml. and alkaline phosphatase to 33 units/100 ml. The hemoglobin then was 13.8 Gm. and leukocytes 51,000. The presence of the cryoprotein persisted in the blood and pleural fluid. Pleural effusion became marked, ascites developed, and malignant cells were observed in the ascitic fluid. Supraclavicular lymphadenopathy developed. Death ensued two months after the hospital admission and 13 months after the appearance of the first thrombosis. Autopsy was not performed.

**Experimental**

On chilling the patient’s oxalated plasma (fig. 1, tube 4) a fine white flocculent precipitate (fig. 1, tube 5) was noted in the tube. As the mixture was returned to room temperature, the precipitate dissolved (fig. 1, tube 7). Blood was allowed to clot and the separated serum (fig. 1, tube 3) on two determinations failed to reveal any cold precipitate.

The precipitate in cold plasma was separated by centrifuging at 4°C and then washed three times with cold saline (0.85 per cent). The cryoprotein was then soluble at 37°C in saline, but not in water. A dilute solution of bovine thrombin (Parke, Davis–Topical) was prepared so that 0.1 ml. added to 0.2 ml. of normal plasma produced a fibrin clot in 15 seconds. When this thrombin was added to the plasma of our patient at room temperature, a definite fibrin clot formed in 15 seconds as shown in figure 1, tube 1. An equal sample of this plasma was cooled and the precipitate removed by centrifugation. Thrombin was added to the clear supernatant plasma, and a fibrin clot was formed in 15 seconds (fig. 1,
tube 2), but this was decidedly smaller, indicating that a portion of the fibrinogen had been removed. A saline solution of the cold precipitate was clear at room temperature. On adding thrombin to this, a fibrin clot formed in 20 seconds (fig. 1, tube 6). Addition of thrombin to the cold precipitable material led to the formation of a typical fibrin clot. The fibrin clot was compressed to remove as much trapped solution as possible. The remaining supernatant solution revealed no further cold precipitate. In view of this procedure, we did not feel there was a significant amount of cold insoluble globulin accompanying the fibrinogen. All of these observations were repeated on plasma drawn from the patient at three different times. Heparin used as the anticoagulant in vitro did not change the amount of cryoprotein present in vitro.

A portion of the plasma was heated to 56 C. for 10 minutes and, as expected, the fibrinogen precipitated in a denatured form and was then insoluble. The supernate did not clot when thrombin was added. This same supernatant serum revealed no further change when cooled to 4 C. The clotting properties of the cryoprotein in 0.15 M NaCl were destroyed by heating at 56 C. for 10 minutes. The plasma was cooled slowly from 37 C. and observed with each five degree decrease in temperature. No change was noted until 15 C. was reached when the plasma began to appear cloudy. At 5 C. there was a fine precipitate and then a definite fine white flocculation which settled to the bottom of the tube. On gradual warming of the tube, there was a dissolving of the flocculation complete at 25 C. Gradual heating produced an irreversible precipitate at 56 C.

Whenever the patient demonstrated the “cryofibrinogen” and progressive thromboses, rouleaux formation was also noted. This phenomenon disappeared when the patient’s thromboses lessened while receiving heparin. Saline washed normal erythrocytes when smeared and stained showed no abnormality (fig. 2B).
A drop of the saline dissolved “cryofibrinogen” added to 0.5 ml. of washed normal erythrocytes produced definite rouleaux formation (fig. 2A).

**Total Fibrinogen Determinations**

The fibrinogen determinations were performed by diluting plasma in saline, adding calcium and collecting the fibrin on a glass rod. This fibrin was then analyzed for total nitrogen content. In table 1 it can be seen that the total amount of fibrinogen was never high, and when new thromboses were noted there was actually a low total circulating fibrinogen. A definite difference in fibrinogen level existed before and after cooling in six determinations. Chymotrypsin, as shown in table 1, produced little change in the plasma fibrinogen before and after the three day course of twice daily intravenous administration. There was only a slight decrease in plasma fibrinogen in specimens taken immediately before and after the infusion. New thromboses occurred during the three day course of therapy, and the cryoprotein persisted.

Sedimentation diagrams (Spinco ultracentrifuge) of the cold precipitate from both plasma and the pleural fluid are present in figure 3 (A & B). It will be noted that the plasma precipitate contained a trace amount of a fast sedimenting component with the major component having an $s_{20\text{w}} = 7.93$ S., which is consistent with the reported values for fibrinogen. On removing this sample from the analytic cell and adding thrombin, a typical fibrin clot formed. Because of the small amount of precipitate available, it was not possible to obtain the sedimentation constants over a range of protein concentrations in order to extrapolate to an S value at infinite dilution.

The pleural fluid cold precipitate, however, demonstrated a multiplicity of components. A component with an $s_{20\text{w}} = 3.81$ S. was quite consistently present in several examinations of the pleural fluid precipitate. This component is represented by the sharp peak in figure 3B. The cold precipitate from the pleural fluid was tested for fibrinogen “B” according to the method of Lyons. A clot formed with betanaphthol or menadione whereas normal plasma controls were negative to this test. Lyons’ tests for “profibrin” (clot formation with 50 per


Table 1—Laboratory Observations Correlated with the Clinical Course

<table>
<thead>
<tr>
<th>Hospital Day</th>
<th>Presence of Cryoprotein</th>
<th>Therapy Status</th>
<th>Patient Status</th>
<th>Total Fibrinogen mg.%</th>
<th>Fibrinogen in Plasma After Cold Precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>+++</td>
<td>0</td>
<td>Thrombosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>Heparin</td>
<td>Improvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Tr.</td>
<td>Heparin</td>
<td>Improvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>+++</td>
<td>0</td>
<td>New Thromboses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>+++</td>
<td>Chymotrypsin</td>
<td>Progressively Worse</td>
<td>178</td>
<td>143</td>
</tr>
<tr>
<td>31</td>
<td>+++</td>
<td>Chymotrypsin</td>
<td>No Improvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>+++</td>
<td>Chymotrypsin</td>
<td>No Improvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>Heparin</td>
<td>Improvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>++</td>
<td>Heparin</td>
<td>No Change</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Immediately before infusion.
† Immediately after infusion.

Fig. 3—Ultracentrifuge Sedimentation Diagrams of the Cold Precipitates. A. Major component $s_{20,w} = 7.93 S.$ consistent with plasma fibrinogen. B. Multiple components with most consistent fraction at $s_{20,w} = 3.81 S.$


cent NaCl saturation and clot formation with 15 per cent ethanol) failed to clot the cold insoluble material.

In figure 4, the paper electrophoresis diagram of the patient’s serum is presented for comparison with a normal pattern and that of a patient with multiple myeloma. A decrease in the albumin area along with an increased alpha-1
globulin and a decreased alpha-2 globulin and gamma globulin can be seen. Electrophoresis in the conventional Tiselius apparatus was attempted, but the turbidity and precipitate in the plasma at 3 C. prevented a successful experiment.

DISCUSSION

Trousseau first associated thrombophlebitis with neoplasia of internal organs in 1865. Osler and McCrae in 1900 reported a case of multiple thromboses that could be easily squeezed out of the opened vein with little evidence of reaction in the vein wall. Autopsy revealed carcinoma of the stomach with spread to the mediastinum and liver. A recent review of the literature summed up 44 cases of migratory thrombophlebitis in which the latency of the tumors was stressed. The organs mainly involved by tumor were pancreas, 16 cases; lung, 10 cases; and stomach, 6 cases. Edwards points out that the thrombus is a bland type with slight or no surrounding inflammatory reaction and no involvement by neoplasm of the vessel wall. Sproul found in an analysis of 4,258 autopsies, an incidence of 2.5 per cent multiple thromboses in carcinoma of the lung. The involvement in 56 reported cases of carcinoma associated with thrombophlebitis migrans are in order of frequency: the body and tail of the pancreas, the lung, the stomach, and the gall bladder. Abnormalities in the clotting mechanism have not been proven in any of the reported cases. However, one case of thrombosis associated with lung tumor responded initially to heparin therapy with regression of the thrombus formation.

In the literature concerning cold precipitates in plasma and serum, the ma-
majority of reports concern serum cryoglobulins. Wintrobe and Buehl described a case of multiple myeloma in 1933 in which a cold insoluble portion of the plasma was observed. Small amounts of cold precipitating proteins have been noted in cases of kala azar, tuberculosis, endocarditis lenta, malaria, multiple myeloma, arthritis and liver disease. In 1947, Lerner and Watson proposed the term cryoglobulin to represent a group of proteins having the common property of precipitating (or gelifying) as a result of cooling serum. Abrams, Cohen and Meyer described a cryoglobulin obtained from both serum and lymph nodes in a case of lymphosarcoma. One case with a cold precipitate in serum was thought to be related to marked rouleaux formation of the erythrocytes. In a patient with multiple myeloma the serum clotted in the syringe unless warmed, and clotted in the presence of the usual anticoagulants except heparin. Recent reviews stress the view that cryoglobulinemia is usually associated with multiple myeloma.

There has been no report of a cold-precipitating fibrinogen directly related to multiple thrombus formation. Profibrinogen has been described as a flocculent precipitate found after freezing and thawing a solution of fibrinogen. It was speculated that this was a soluble fibrin formed as an intermediary step in the conversion of fibrinogen to fibrin. In a recent discussion of this subject it has been pointed out that some solutions of fibrinogen may show a white sediment on chilling. This flocculent precipitate is a form of fibrinogen, or an intermediate product in the polymerization of fibrinogen to fibrin. Fibrinogen A and B have been described as fractions in the conversion to fibrin. Lyons in 1945 described fibrinogen B as an intermediary step in fibrin formation. This was obtained from cold stored blood, or aged citrated plasma, and was not found in fresh plasma. Turpentine abscesses in rabbits were found to produce large amounts of fibrinogen B in the plasma and the fibrinogen B also produced rouleaux formation in normal blood.

In two previous reports of cold-insoluble fractions of fibrinogen, that obtained by P. R. Morrison and colleagues was found to be nonclottable. Our fraction is quite similar to their fraction I-1, except that our supernatant following clotting with thrombin revealed no further cold precipitation. Also, the Tiselius electrophoresis of the patient's serum revealed no unusual components. We were unable to demonstrate an associated cold-insoluble globulin which precipitated with the clottable fraction. The fraction described by I. R. Morrison clotted on standing in the cold. Of special interest is the recent report by Thomas, Smith and Von Korff in which they observed in vitro a cold precipitate in heparinized rabbit plasma drawn after the injection of a bacterial endotoxin. A similar precipitate was observed in human plasma drawn from normals and from patients with acute rheumatic fever.

It appears that this "abnormal" fibrinogen may indeed be a normal component of plasma, but because of the small quantities present, it is not recognized. The routine use of heparin as an anticoagulant in investigating the plasma of these patients may permit obtaining more of this protein for further investigation.

A cryoprotein has been identified in a case of thrombophlebitis migrans and lung carcinoma. The physical properties are primarily those of fibrinogen, hence
it is designated as "cryofibrinogen" which made up 10-20 per cent of the total circulating fibrinogen. Heparin administration prevented the clinical manifestation of thrombosis in vivo and prevented spontaneous coagulation in vitro. Cold precipitation of a fibrinogen associated with multiple thrombosis has not been previously reported. The other properties were similar to previously described forms of abnormal fibrinogens postulated as transition products in the conversion of fibrinogen to fibrin.

SUMMARIO IN INTERLINGUA

Un proteina anormal que se precipitava sub temperaturas abassate sed retornava al stato de solution sub le temperatura normal del interior esseva incontrate in le plasma de un homine blanc de 41 annos de etate qui habeva thrombophlebitis migrante e neoplasia pulnunar. Thrombophlebitis migrante habeva essite presente durante 11 menses ante que le diagnose de neoplasia pulnunar esseva estabilite.

Le caracteristicas physic del cryoproteina esseva primarimente le caracteristicas de fibrinogeno. Pro iste ration illo es designate como "cryofibrinogeno." In le caso hic reportate illo representava inter 10 e 20 pro cento del circulanste fibrinogeno total.

Administrationes de heparina prevermiva le manifestationes clinic de thrombosis in vivo e coagulation spontanea in vitro. Le altere caracteristicas del cryofibrinogeno eseva simile al caracteristicas previemente reportate pro anormal fibrinogenos postulate como productos de transition in le conversion de fibrinogeno in fibrina.

Le phenomeno de un cryoproteina ha non previemente essite reportate in connection con thrombophlebitis migrante e neoplasia. Pro iste ration le presente caso e le experimentos executate in illo es digne de attention.

REFERENCES


"Cryofibrinogen" in a Case of Lung Neoplasm Associated with Thrombophlebitis Migrans

DONALD R. KORST and CLYDE H. KRATOCHVIL