

The Production of Schistocytes by Fibrin Strands (A Scanning Electron Microscope Study)

By BRIAN S. BULL AND IRVIN N. KUHN

A FORM OF HEMOLYTIC ANEMIA characterized by thrombocytopenia and the presence of fragmented red cells in the peripheral blood has been called microangiopathic hemolytic anemia by Brain, Dacie and Hourihane.¹ These workers showed that in this disease the amount of hemoglobinemia and the degree of erythrocyte fragmentation correlated closely with the severity of microangiopathy. This microangiopathy, most prominent in the kidneys, was characterized by fibrinoid necrosis of the arterioles, necrotizing arteritis, and the presence of intraluminal hyalin thrombi.

When intravascular coagulation is precipitated in animals under appropriate experimental conditions, fragmented erythrocytes and a microangiopathy are produced.²⁻⁴ The tissue from these experiments provides strong evidence that erythrocyte fragments or schistocytes result when rapidly moving red cells encounter fine fibrin strands. Acute defibrination in animals initially causes the formation of large numbers of microclots composed of very fine fibrin strands. Subsequently a microangiopathy results, with similar histology to that found in postmortem kidney sections of patients who, during life, exhibited the classical symptoms of microangiopathic hemolytic anemia.

Despite this considerable body of evidence incriminating fine fibrin strands as the major etiologic factor in schistocyte production, these strands are rarely observed postmortem in humans. Bull and Brain in a study of experimental models of microangiopathic hemolytic anemia suggested that the fine fibrin strands, being the most delicate of the clot components, would be the first to undergo lysis at death.⁵ The patient herein described suffered from severe microangiopathic hemolytic anemia during life and at death his vasculature contained large numbers of microclots composed of fine fibrin strands.

This case is presented in some detail because it documents the essential identity of the pathogenesis of microangiopathic hemolytic anemia in man with the hemolytic anemia that accompanies induced intravascular coagulation in animals.

CASE REPORT

The patient, a Caucasian male born in 1908, was admitted to the University Hospital

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on January 11, 1968, with the complaint of moderate low back pain for two weeks which had become severe 24 hr. prior to hospitalization.

A physical examination showed no petechiae, ecchymoses, or abnormally enlarged lymph nodes. The heart and lungs were normal. There was no hepatosplenomegaly, abdominal tenderness, or distention. The vibration sense was decreased in both lower extremities but pain sensation was intact.

In the laboratory the hemoglobin was 14.5 Gm./100 ml., PVC 43 per cent, and white blood count 3600/mm³. There was a normal white blood count differential, and adequate platelets were observed on the peripheral blood film. Sedimentation rate was 60 mm. at one hour (Wintrobe). X-rays of the back revealed considerable demineralization of bone and a possible narrowing of the intervertebral spaces in the area of the back pain.

The patient was treated symptomatically for his back pain with bed rest and intermittent traction, but his pain became progressively more severe. Fifteen days after hospitalization severe pallor, rapid in onset, was noted. Laboratory studies now revealed a hemoglobin of 7 Gm./100 ml. with a PCV of 23 per cent. Nucleated red cells were present in large numbers on a peripheral blood film. There was microcytosis, poikilocytosis, and polychromasia. Three days later the polychromasia was even more evident and a reticulocyte count was 28 per cent, uncorrected. The MCV was 71 μ^3 , MCH 23 $\mu\mu\text{g.}$, and MCHC 33 per cent. The TIBC was 340 $\mu\text{g.}$ per cent and serum iron 150 $\mu\text{g.}$ per cent. The direct and indirect Coombs tests were negative and a G-6-PD screening test was normal. The plasma iron disappearance showed a T 1/2 of 32 min. X-ray examination of the chest, and the GI tract including a barium enema and upper GI studies, revealed no significant lesions except for an equivocal stiffening of the distal portion of the stomach during the fluoroscopic examination.

Because of the precipitous drop in hemoglobin and stiffening of the distal stomach, an exploratory laparotomy was performed on February 15, 1968. An infiltrating adenocarcinoma of the stomach was found with metastases in the regional lymph nodes. Prior to surgery a serum bilirubin had been 2.2 mg. per cent total with 1.2 mg. per cent indirect. Three days after surgery the total bilirubin was 16 mg. per cent, of which 15 mg. per cent was direct. Thereafter the jaundice deepened rapidly, the patient became febrile and developed shaking chills. Ecchymoses were noted in the dependent portions of the body and platelet counts during the last five days ranged between 26,000 and 28,000/mm³. He expired on February 24, 1968. At postmortem examination, scirrhous carcinoma of the stomach was found. There was widespread metastasis to diaphragm, vertebral bone marrow, regional lymph nodes, small intestines, and retroperitoneum. During the last 24 days of life the patient received a total of 23 units of blood administered on 11 occasions. These transfusions, given at approximately 48 hr. intervals, maintained the hemoglobin level at 7-9 Gm./100 ml.

MATERIALS AND METHODS

Tissue specimens removed at the time of postmortem examination 23 hr. after death were fixed in 10 per cent formalin and processed in standard fashion for study by light microscopy. For the *in vitro* studies, clots were prepared in a mechanical model of the circulatory system. This circuit, identical to that described previously,⁴ consisted of silicone rubber tubing around which blood could be pumped. A metal disk perforated with several thousand 0.2-mm. holes was placed in the circuit to simulate a microvasculature. Clotting was initiated by infusing minute amounts of thrombin into the rapidly moving blood, and the fibrin microclots which resulted were fixed in a buffered isotonic sodium chloride solution containing 0.25 per cent glutaraldehyde. A pressure differential across the clot of approximately 100 mm. of mercury was maintained through the fixation period; thus fixation took place while the clot was exposed to the fluid flow generated by a normal blood pressure.

Both sets of tissues were subjected to the usual histologic processing routines of dehydration, infiltration, and wax embedding. At the completion of the wax embedding step those sections to be examined by light microscopy were cut at 5 μ , stained with phosphotungstic acid hematoxylin or with hematoxylin and eosin, and mounted on glass slides. Duplicate sections were cut at 8 μ , mounted on cover slips, dewaxed, returned through

Fig. 1.—Red cells from peripheral blood obtained 21 days prior to death. The film is characterized by thrombocytopenia and schistocytosis. ($\times 950$) light microscope.

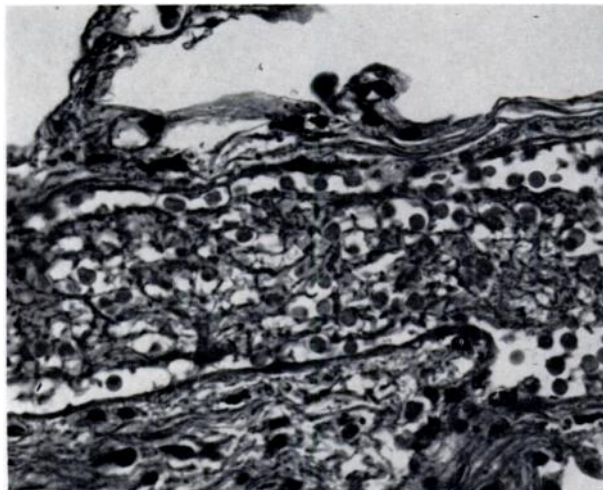
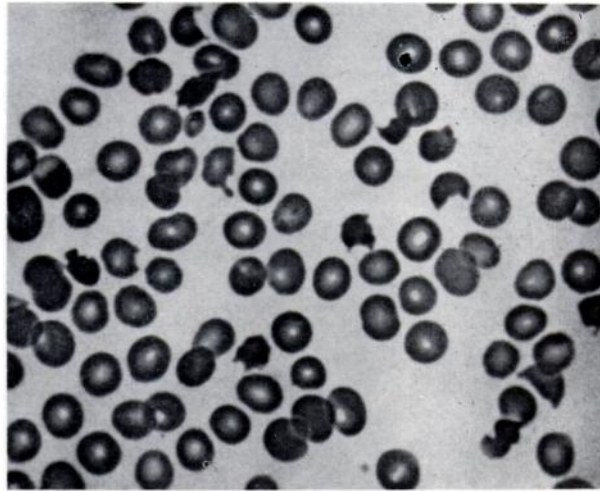


Fig. 2.—Fibrin microclot in the lung. Note relative diameters of fine fibrin strands and red cells ($\times 570$) light microscope.

graded alcohol solutions to water, and permitted to air dry in a refrigerator at 4°C . The cover slips were then fractured and the pieces mounted on aluminum pedestals, shadowed with a gold platinum alloy at an atmosphere of 1×10^{-4} Torr, and then examined in a Cambridge scanning electron microscope.

RESULTS

The peripheral blood during the last three weeks of life was characterized by the absence of platelets and the presence of schistocytes. A typical blood film is shown in Fig. 1. At autopsy, fibrin microclots were present throughout the vasculature and were particularly prominent in lung sections (Figs. 2, 3). These microclots showed all the identifying characteristics of antemortem clots. They consisted of fibrin bands $0.25\text{--}2 \mu$ in diameter, twisted and matted into a tangled skein. Tumor cells could be identified in many of the smaller clots and were present in most of the larger clots when sufficient sections were examined. The fibrin bands running vertically to the plane of section appeared as bright

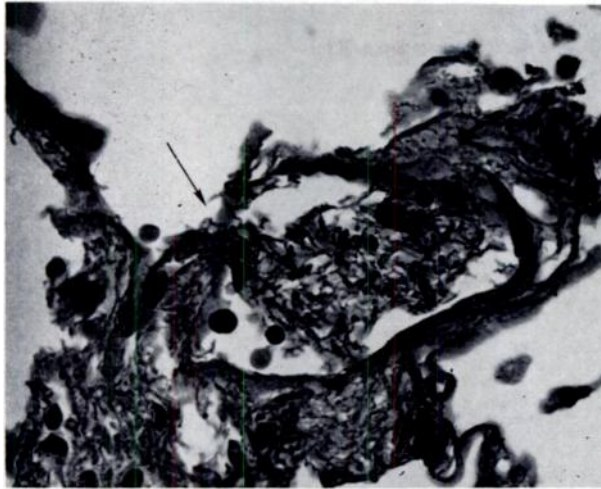
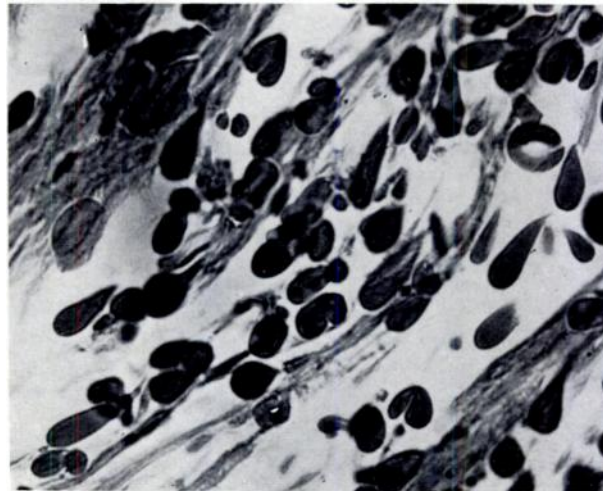


Fig. 3.—Fibrin microclot arrested at junction of pulmonary arteriole and capillary. Clots arrested at sites such as these usually do not occlude either vessel, blood flow continues, and schistocytes are produced. ($\times 570$) light microscope.

Fig. 4.—Section of clot from in vitro model fixed under pressure while schistocytes were being produced. Normal red cell is present, upper right. Remaining cells, in contact with fine fibrin strands, are deformed and fragmented. ($\times 270$) light microscope.



refractile dots at intervals along the strands. The spaces between adjacent strands were irregular in shape, often with sharply angular corners. Red cells were rarely present, and when present they were few in number. Platelets could not be identified in the sections. White cells were frequently present.

The fibrin architecture of the clots prepared in the mechanical circulatory-system model was very similar to the clots recovered from the human material. The strands varied in thickness from about 0.2μ up to $2-3\mu$. There were, in addition, large heavy fibrin pillars formed of accumulations of smaller fibrin strands. Larger numbers of red cells were present. The majority of these cells were in physical contact with the fibrin strands and showed marked distortion (Fig. 4).

Scanning Electron Microscope Studies

Duplicate tissue sections examined under the scanning electron microscope showed large numbers of fibrin microclots present throughout the vasculature.

Fig. 5.—Fibrin microclot in pulmonary vasculature from patient W.H. Note matted and tangled fibrin bands. Upper surface of this 8- μ section has been distorted by microtome blade. Some red cells have shrunk, the rest have sphered, as a result of fixation in unbuffered 10 per cent formalin. ($\times 2000$) scanning electron microscope.

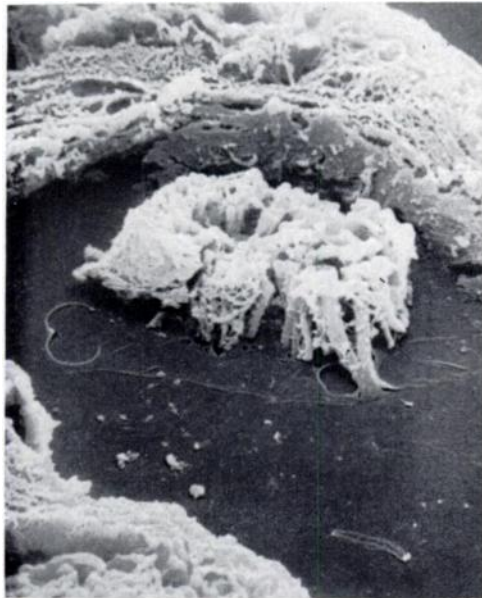
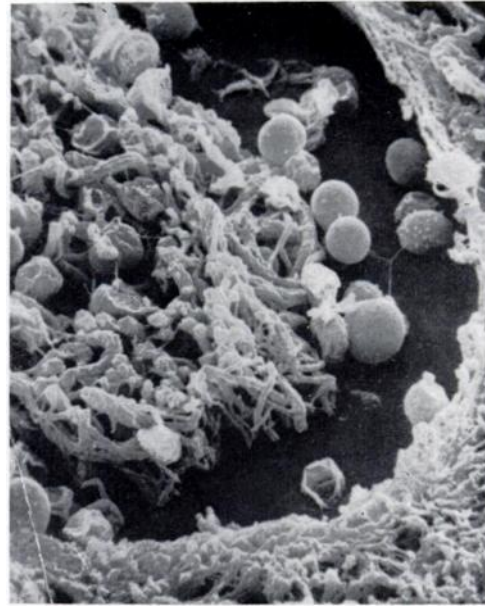


Fig. 6.—Fibrin microclot which is no longer adhesive enough to trap red cells and is becoming more amorphous. In the center the original fine fibrin strands are still visible. ($\times 2200$) scanning electron microscope.

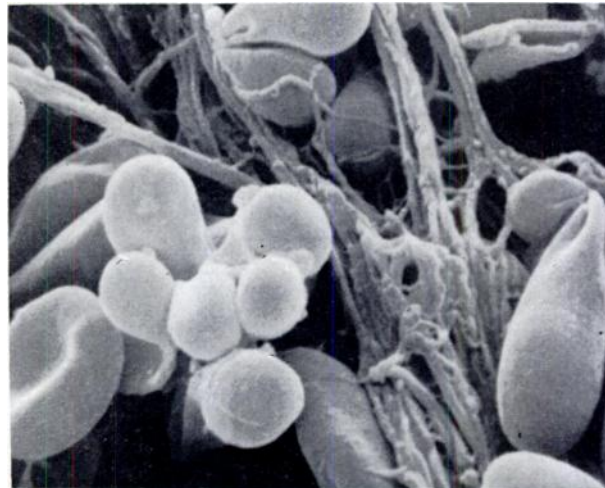
These fibrin microclots consisted of rounded fibrin strands of varying thickness which were matted and tangled. The red cells present in the sections were spherical and shrunken. The cytoarchitecture of these clots had been disturbed for some distance on either side of the plane of section by the microtome blade, but in an 8- μ -thick slice the fibrillar structure of the fibrin bands was clearly evident throughout the central 5- μ thickness (Figs. 5, 6).

The scanning electron microscope findings on the clots prepared in vitro



Fig. 7.—Section of clot from in vitro model (see Fig. 4). Normal red cell at left center. Effect of fibrin strands on red cells clearly evident. ($\times 2000$) scanning electron microscope.

Fig. 8.—Enlargement of section of Fig. 7 which contains normal red cell. Extreme delicacy of finer fibrin strands is well shown. ($\times 5100$) scanning electron microscope.



are depicted in Figs. 7–9. The fine structure of the fibrin clot is clearly evident as is the marked distortion produced in the large numbers of attached red cells.

DISCUSSION

This patient, during the last three weeks of life, suffered from a severe anemia which, in addition to being hemolytic, was characterized by thrombocytopenia and schistocytosis. The most likely cause of this microangiopathic hemolytic anemia was intravascular coagulation, precipitated by tumor emboli from the disseminated adenocarcinoma. The process increased in severity until death supervened.

Fig. 9.—Hanged red cell! Dense fibrin band in background formed from accumulations of finer strands, some of which are still evident. It is only these denser, more amorphous structures that typically persist postmortem. ($\times 5200$) in vitro model, scanning electron microscope.



Microangiopathic hemolytic anemia is seen in several other human diseases such as placental abruption, the hemolytic uremic syndrome, renal cortical necrosis, polyarteritis, and uremia. In most of these disease states the inciting cause for the intravascular coagulation is brief in duration and may precede the patient's demise by several days or weeks. Thus, the usual microangiopathy seen at postmortem is characteristic of the burned out phase of the disease when schistocytes are no longer being produced. In widely disseminated adenocarcinoma, the underlying cause of the intravascular coagulation and hence also of the schistocytosis would be expected to persist and even increase up to the time of death. It is thus of considerable interest that the microangiopathy seen in this patient so closely mimics the findings reported from experimental animals where tissues may be obtained and fixed during the height of the hemolytic process. The initial response to intravascular activation of the coagulation mechanism is the formation of showers of stringy, fibrin microclots. These, the effective agents in schistocytosis, are continually being incorporated into more dense and more amorphous portions of the clot (Fig. 6). It is these heavier and thicker deposits that characterize the usual postmortem histology. Only rarely are the initial stringy microclots seen in such large numbers as in this case, although this fibrillar fibrin is invariably present in animal experimental models.

Observation of the actual fragmentation process in fixed tissue places even more stringent requirements upon fixation. The fine fibrin microclots must still be forming at the time of death and postmortem fibrinolysis must be minimal or absent. Fixation must take place while the red cells are traversing formed and forming fibrin strands at normal flow rates. This requires maintenance of systolic blood pressures throughout fixation, a state of affairs that is impossible to achieve except in an in vitro model. Fixation under any other circumstance will fail to preserve the distortion that occurs in a red cell prior to schistocyte production. In the present case although the fibrin microclots are well preserved the red cells have been quite poorly fixed. They are shrunken due to fixation in 10 per cent formalin, which is inadequate at

preserving tissues for electron microscopy. In addition, because they could not be fixed under the pressure of normal blood flow, they fail to show the prefragmentation distortion seen in the tissues from the in vitro model.

SUMMARY

The relationship between intravascular coagulation and schistocyte formation has been adequately documented in animal experiments and in in vitro models. A case report is presented of a patient who suffered from microangiopathic hemolytic anemia during life as a result of disseminated gastric adenocarcinoma. The fibrin microclots found in his vasculature on postmortem examination indicate that in intravascular coagulation, both in the human and in the experimental animal, fibrin initially polymerizes in the form of fine fibrin strands. These strands, the main etiologic factor in producing schistocytes, are constantly being lysed or rendered amorphous, and rarely persist in postmortem tissues except under unusually favorable conditions. Postmortem histologic study of one case where such favorable conditions did exist indicates the essential identity of the pathologic process in man and in experimental animals.

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