Acute Promyelocytic Leukemia

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ACUTE leukemia associated with a low fibrinogen concentration and a serious bleeding tendency was first described some years ago in three independent case reports.[1-3] Since then about 70 such cases have been observed with distinctive clinical and hematologic features.[4-7] Since our first case,[2] we have seen three additional patients with this disease who form the basis of the present report.

Clinically this is a hemorrhagic disorder and large ecchymoses are particularly prominent. A notable feature is the absence of hepatosplenomegaly or lymphadenopathy. The disease runs a rapidly fatal course and cerebral hemorrhage is the most frequent terminal event. Only five patients have survived longer than four months.

The bone marrow has a preponderance of abnormal myelocytes and promyelocytes, and, because of this, the disorder has been called "acute promyelocytic leukemia".

The outstanding features of the clotting mechanism are a low fibrinogen concentration with rapid lysis of a normally formed clot, prolonged prothrombin time and thrombin time, and a reduction in factor V and the prothrombin consumption time.

The association of the low fibrinogen with rapid clot lysis has led most observers to conclude that the hypofibrinogenemia was due to pathologic fibrinolysis. However, attempts to demonstrate the presence of fibrinolytic activity by more specific tests have been unsuccessful.[4, 5, 7] In one case,[7] there was evidence of intravascular coagulation with temporary improvement following heparin therapy; this intravascular coagulation was confirmed at autopsy. In another case[5] heparin failed to raise the fibrinogen and factor V levels. Our own studies indicate that an activator of plasminogen is present, which produces the hypofibrinogenemia and the associated clotting changes.

MATERIALS AND METHODS

Routine hematologic procedures were performed as described by Cartwright.[8] One-stage prothrombin times were determined by the method of Quick[9] using Simplastin. Factor V, plasma prothrombin, prothrombin consumption time and factor VIII were determined by methods previously described.[10] In addition, the thromboplastin generation test,[11] fibrinogen[12] and euglobulin lysis time[13] were determined. After the clotting time was established, the specimens were observed in a water bath at 37.5° C. for evidence of clot lysis. Thrombin times were performed at room temperature using 10 units of human thrombin (Fibrindex) mixed with 0.2 ml. of fresh oxalated plasma. Dried human fibrinogen prepared by Connaught Medical Research Laboratories, University of Toronto, was used in the *in vivo* and *in vitro* experiments. The epsilon amino caproic acid (Amicar) was supplied by Lederle Laboratories.

CLINICAL FINDINGS

The four patients were all adults ranging in age from 21 to 55 years. There were three men and one woman. All presented with bleeding. Ecchymoses were present in all and three had bleeding gums. The woman also had considerable metrorrhagia. Petechial hemorrhages were not a significant feature until later in the illness when the platelets became more markedly reduced. With the marked thrombocytopenia, the hemorrhagic features became more severe, usually ending in the death of the patient. One man (Case 4) presented with a huge hematoma and ecchymoses following an injection of penicillin into his buttocks administered because of an infected finger. None of these patients had adenopathy or palpable livers and spleens.

All had a normochromic anemia. A leukopenia was found in three and a normal white count in one initially, but a preterminal leukocytosis developed in the three patients who survived longer than one week. There were 0 to 18% blast cells and 1 to 85% promyelocytes and myelocytes in the peripheral blood. On initial examination the platelets ranged from 120,000 to 200,000 per c.mm. but marked thrombocytopenia developed in all as the disease progressed (Table I). The sedimentation rate was 20 mm. or less in one hour in all patients. The leukocyte alkaline phosphatase of the neutrophils was studied in Case 4 on a number of occasions and ranged from 1 to 9, a significantly low value.

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TABLE I.—HEMATOLOGIC DATA IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

Case	*Age and sex*	*Course (weeks)*	*Cause of death*	*Hb. (g.%)*	*WBC*	*Blasts (%)*	*Promyelocytes*	*Platelet count*	*Leukocyte alkaline phosphatase score**
Normal	—	—	—	12 to 16 g.%	5 to 10,000	0	0	200,000 to 400,000	30 to 100
1	49 M	1	Cerebral hemorrhage	10.6	3000	3	24	120,000 to 36,000	—
2	46 F	2	Cerebral hemorrhage	7.0	8100 to 113,000	1	85	160,000 to 80,000	—
3	21 M	4	Retroperitoneal hemorrhage	9.5	1100 to 22,000	18	22	128 000 to 22,000	—
4	55 M	14	Cerebral hemorrhage	8.0	2200 to 50,000	0	1 to 93	200,000 to 8000	9 and 1

*This score is determined by a histochemical technique where the mature neutrophils are counted and graded 0 to 4 plus, according to the degree of staining. The sum of the grades per 100 neutrophils constitutes the score.

The bone marrow in all revealed almost complete infiltration with abnormal myelocytes and promyelocytes (Fig. 1). These cells had immature nuclei which were often lobed or of

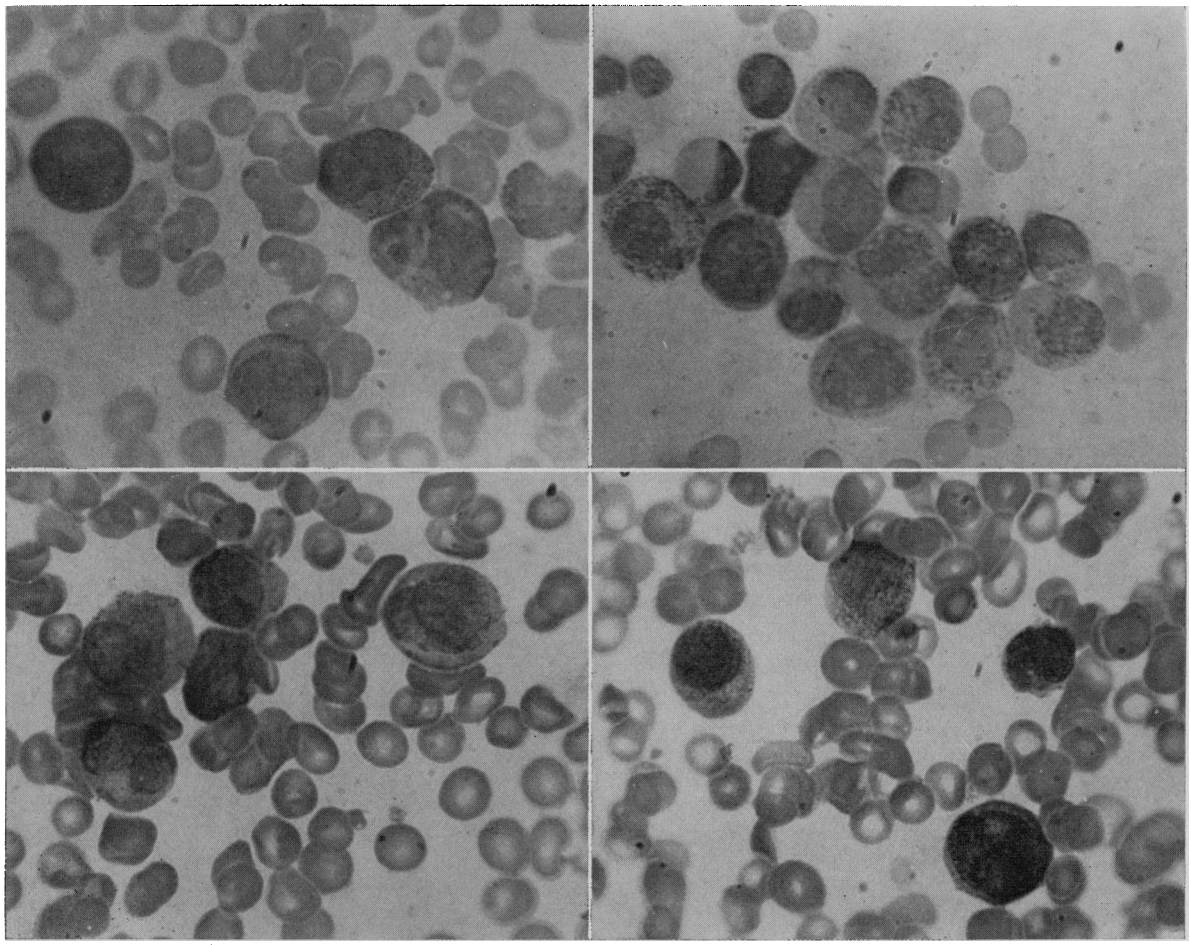

Fig. 1.—Bone marrow specimens of four patients with acute promyelocytic leukemia. The immature, frequently irregular nuclei and the abundant coarse granulation are the characteristic features.

TABLE II.—COAGULATION STUDIES IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

Case	*Coag'n time (min.)*	*Lysis time (hr.)*	*Prothrombin time (sec.)*	*Prothrombin consumption time (sec.)*	*Thrombin time (sec.)*	*Fibrinogen (mg.%)*	*Factor V (%)*	*TGT (%)*	*Euglobulin lysis time*
Normal	<20 min.	No lysis	13 sec.	>20 sec.	<6 sec.	200 - 400	75 - 125	>80	> 4 hr.
1	12	8	18.0	14.8	—	78	—	—	—
2	10	16	22.4	16.0	90	142	42	40	—
3	15	18	19.4	13.4	—	130	—	—	—
4	12	2	19.4	15.2	38	52	52	0	51 min.

irregular shape and contained one or two nucleoli. There was abundant cytoplasm containing large azurophilic granules as well as some with normal neutrophilic granulation. Occasional Auer rods were also present. These granules were peroxidase-positive in the one case where the test was applied. The appearance of these cells suggests a dissociation of maturation, the cytoplasm being more mature than the nuclei.

All patients died in coma with clinical evidence of a cerebral hemorrhage. An autopsy was performed on the first three patients. In addition to widespread hemorrhages and microscopic leukemic infiltrations there was massive subdural and subarachnoid hemorrhage in all. In Case 3 there was considerable retroperitoneal bleeding as well. None showed any enlargement of the liver, spleen or lymph nodes although there was leukemic infiltration in these organs. There was no postmortem evidence of intravascular coagulation in these cases.

COAGULATION STUDIES

The coagulation time was normal in all four patients and a normal-appearing clot formed which soon dissolved spontaneously. Complete clot lysis took place in from one to 18 hours (Table II). During the course of the illness, the time and degree of dissolution often varied in the same patient (Fig. 2). Occasionally a small clot persisted, but as a rule the clot lysis was complete in less than 24 hours. The prothrombin time was prolonged in all cases and factor V was reduced. When normal plasma was mixed with patient's plasma in a ratio of one to nine, the prothrombin time was only partially corrected, from 23 to 16 seconds (control: 13 sec.). The prothrombin consumption time was reduced and the thrombin time was prolonged. Factor VIII was reduced in Case 4. It returned to normal during remission but fell again during relapse. The thromboplastin generation test was impaired in Cases 2 and 4. In Case 2 there was 40% generation on one occasion and 25% on another. Similar results were obtained when the patient's

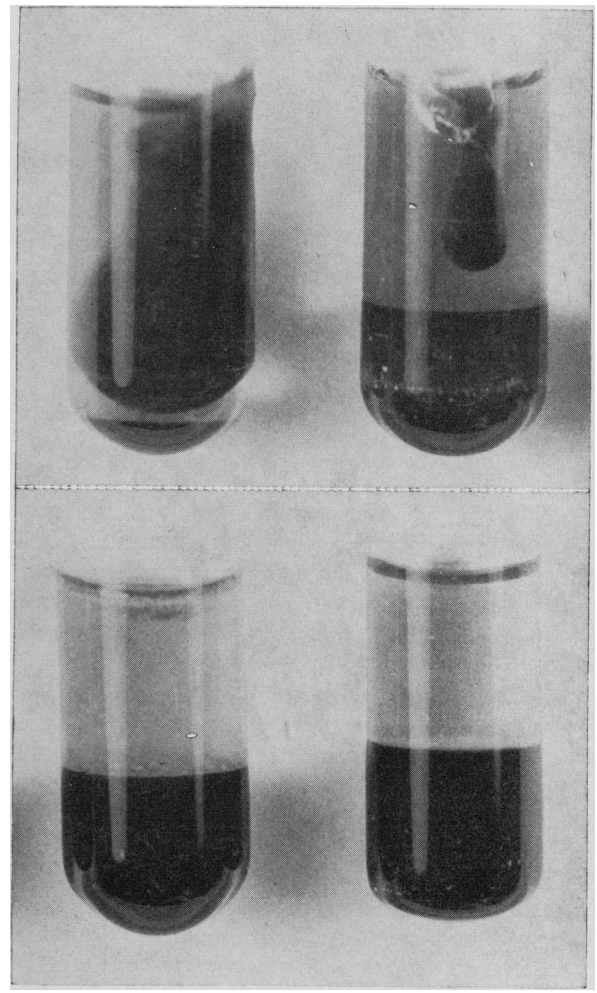

Fig. 2.—Variable degree of clot dissolution from day to day (tube on top left is control). Blood from Case 4 with a moderate-sized clot is in second tube (top right). Only a tiny clot persists at the surface in the specimen taken on the following day and complete lysis is seen in the blood taken on the third day (bottom-left and right).

adsorbed plasma was substituted in the control, suggesting a factor VIII deficiency. When the control adsorbed plasma was added to the patient's plasma in a ratio of one to nine, the generation of thromboplastin was normal. The fibrinogen concentration was reduced in all patients, ranging from 17 to 140 mg. The thrombin

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TABLE III.—CLOTTING CHANGES IN CASE 4

Date	Coagulation time (min.)	Clot lysis time	Euglobulin lysis time	Fibrinogen (mg. %)	Prothrombin time	Factor V (%)	Specific prothrombin (%)	Prothrombin consumption time	Factor VIII (%)
Normal	<20 min.	No Lysis	> 4 hrs.	200 - 400	13 secs.	75 to 125%	75 to 125%	> 20 secs.	50 to 150%
Nov. 5/64	12	1 hour	51 min.	52	19.4 secs.	83	60	—	14
Nov. 10/64	—	2 "	85 "	—	23.6 "	52	60	—	0
Nov. 11/64	—	—	85 "	52	21.5 "	52	—	—	6
No.v 27/64	13	5 "	85 "	17	18.0 "	66	64	15.2 sec.	0
Nov. 30/64	13	Incomplete in 24 hr.	—	21	—	—	—	—	—
Dec. 9/64	11	"	92 "	42	18.8 "	100	68	15.4 "	—
Dec. 16/64	12	"	—	42	17.2 "	85	80	15.2 "	9
Dec. 23/64	10	Normal clot retraction	4 hr.	124	14.8 "	90	97	16 "	—
Jan. 6/65	10	"	—	105	15.2 "	90	95	16.2 "	78
Jan. 13/65	13	—	2¼ hr.	84	16.8 "	88	95	15.0 "	—
Jan. 20/65	16	—	—	44	21.1 "	65	90	14.6 "	50
Jan. 27/65	21	Incomplete in 24 hr.	2 hr.	40	23.6 "	55	90	14.8 "	—
Feb. 3/65	21	4 hr.	—	21	26.0 "	54	88	14.4 "	28

time was 90 seconds and 38 seconds in the two patients on whom the test was performed. Control values were six to eight seconds. The serial clotting studies of Case 4—the patient who had the longest survival and went into temporary remission—are shown in Table III.

The following additional studies were performed in this patient. The euglobulin lysis time was persistently reduced. It returned to normal only during remission but was reduced again during relapse (Table III). This indicates the presence of increased plasminogen activator or plasmin activity.[14]

Serial dilutions of lyophilized human fibrinogen were prepared in 0.1-ml. amounts to provide total fibrinogen concentrations varying from 40 to 750 mg. %. After 1 ml. of fresh blood from the patient was added to each tube and mixed, there was complete lysis of the clots within five hours (Table IV). This again indicates the presence of potent fibrinolytic activity in the patient's blood. Since plasminogen is usually present in the fibrinogen fraction, the lytic activity was probably mediated through an activator of plasminogen. With higher concentrations of fibrinogen the blood was often found to be incoagulable.

When 65 mg. of ε-aminocaproic acid was added to a similar series of fibrinogen concentrations as above, and mixed with the patient's blood, lysis was inhibited in all tubes (Table IV). The drug also inhibited lysis of the patient's blood without added fibrinogen. Since it is a known inhibitor of plasminogen activator, this seems to confirm that this patient's blood contained an activator.

After an intravenous infusion of 7.2 g. of human fibrinogen over a period of two hours, the patient's fibrinogen level rose from 21 mg. % to

TABLE IV.—EFFECT OF ADDED FIBRINOGEN AND EPSILON AMINOCAPROIC ACID ON CLOT LYSIS IN CASE 4

Tube No.	0.1 ml. human fibrinogen in concentration equivalent to (mg.%)	Lysis time after 1 ml. fresh blood added (hr.)		EACA 65 mg. in 0.25 ml. saline + 0.1 ml. fibrinogen + 1 ml. pt. blood
		Patient	Control	
1	750	5	No lysis after 48 hr.	No lysis after 48 hr.
2	610	5	"	"
3	430	5	"	"
4	330	5	"	"
5	250	5	"	"
6	150	5	"	"
7	75	2	"	"
8	40	2	"	"
9	0	2	"	"
10	0	2	"	"

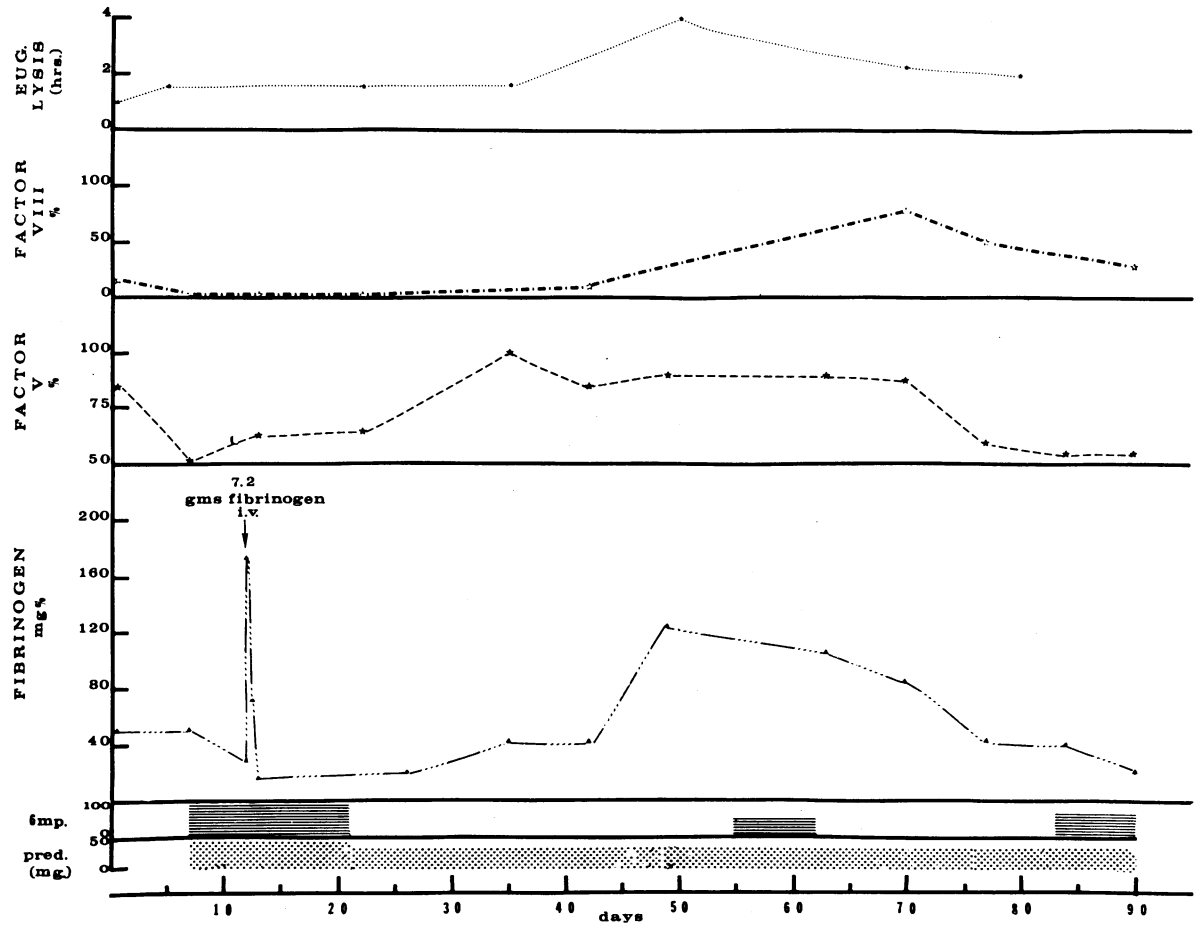

Fig. 3—Euglobulin lysis time, Factor VIII, Factor V and fibrinogen levels in Case 4. The rapid rise in fibrinogen level after infusion, as well as the rapid fall in 24 hours, is clearly demonstrated. The improvement in all parameters is evident during the temporary remission.

175 mg. % (immediately after the injection) but fell to 47 mg. % in 24 hrs. (Fig. 3). This short survival of fibrinogen indicates rapid *in vivo* destruction and may be considered as evidence of increased plasmin activity. One cannot, however, exclude the possibility that there was increased fibrinogen utilization, as in intravascular thrombosis.

Mixtures of the patient's blood and control blood were prepared in a series of test tubes after the blood was withdrawn simultaneously from both individuals by two technicians. Only the patient's blood was placed into the first tube, 80% patient's blood and 20% control blood into the second, 60% and 40% into the third, 40% and 60% into the fourth, 20% and 80% into the fifth and 100% control blood into the sixth tube. There was a total of 2 ml. in each tube. After these specimens were mixed by inversion and allowed to clot, they were observed for evidence of clot dissolution in the water bath at 37° C. There was clot lysis only in the patient's blood and in the mixture containing 80% patient's blood and 20% control blood. The absence of lysis in the other mixtures that contained lesser amounts of the patient's blood may be due to inactivation of the activator, and possibly of plasmin as well, by the respective inhibitors in the control blood.[14] Pisciotta and Schulz,[3] however, were able to demonstrate lysis in all such mixtures.

Equal amounts of patient and control plasma were mixed (Table V). The fibrinogen content

TABLE V.—FIBRINOGEN CHANGES AFTER INCUBATION OF PLASMA

	Before incubation	*20 hours after incubation*
Control plasma fibrinogen (mg.%)	346	347
Patient plasma fibrinogen (mg.%)	21	10
50% patient + 50% control plasma fibrinogen (mg.%)	168	121

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of the mixture and of the patient's and control plasma alone was determined before and after incubation for 20 hours.[5] The reduction in fibrinogen in both the patient's plasma and in the mixture suggests plasmin activity.

DISCUSSION

There appears to be little question now that acute promyelocytic leukemia is a distinct form of leukemia. Although the clinical features—a profound bleeding tendency and absence of hepatosplenomegaly—are characteristic, these may be seen in other forms of acute leukemia. The pathognomonic findings are the abnormal promyelocytes and myelocytes in the bone marrow and rapid clot lysis of the normally formed clot. The vast majority of cases have, in addition, a variable hypofibrinogenemia, a factor V deficiency, a prolonged prothrombin time and probably a factor VIII deficiency.

A universal finding in acute promyelocytic leukemia has been the rapid dissolution of a normally formed clot. The degree of dissolution is variable. Although complete dissolution has been reported by most observers, Rosenthal, in a large series, found a small clot remaining in every case.[4] Rosenthal concluded that the clot dissolution reported by most workers is "apparent lysis" rather than real. He believes that with the low fibrinogen concentration only small amounts of fibrin can appear and hence only a small clot which can easily be missed. Thus, contrary to general belief,[14] he concludes that the appearance of clot dissolution does not necessarily indicate the presence of abnormal fibrinolysis. Didisheim *et al.*[5] also deny that clot lysis occurs. They believe that the observed clot dissolution when the fibrinogen levels were low is due to excessive fragility of the clot, which may disintegrate almost completely after repeated tilting of the tubes in checking for lysis. These workers take a fibrinogen concentration of 160 mg. % as the lower limits of normal, and emphasize that hypofibrinogenopenia was observed in 86% of cases of acute promyelocytic leukemia. In Pisciotta and Shulz's case,[3] the fibrinogen level was 214 mg. %, and in Nitzberg and Dameshek's case[6] complete clot dissolution persisted even though the plasma fibrinogen had risen to normal levels. Thus, when clot lysis is observed in the presence of normal fibrinogen levels one cannot attribute clot dissolution to low fibrinogen alone or to an apparent lysis. Similarly, with the addition of fibrinogen in high concentrations to the patient's blood *in vitro*, clot lysis still occurs, indicating again that clot dissolution is not related to the concentration of fibrinogen alone. The inhibition of clot lysis with ε-aminocaproic acid when the fibrinogen was low again excludes the low fibrinogen as the sole cause of the clot dissolution. It is generally accepted that whole-blood-clot lysis, partial or complete, indicates active fibrinolysis due either to an activator of plasminogen, or to plasmin or both.[14]

Most workers report markedly reduced platelets at the initial examination. In our experience, the platelets have been only slightly reduced at the onset. However, marked thrombocytopenia did develop in all patients as the illness progressed, the hemorrhagic features became more severe, and terminated in death from cerebral hemorrhage. Rosenthal[4] and Baker *et al.*[7] were unable to increase the fibrinogen level in their patients by the administration of fibrinogen. However, both Didisheim *et al.*[5] and Nitzberg and Dameshek[6] found that the fibrinogen level could be increased by this means. The fibrinogen increase was short-lived,[5, 6] and this was our experience as well. One cannot determine from this whether the shortened fibrinogen survival is due to an increased rate of destruction or to an increased rate of utilization, as in the defibrination syndrome.

Many of our results are at variance with those of others. Rosenthal[4] found an increased concentration of factor VIII, whereas we found a persistently reduced factor VIII level. The latter is compatible with the almost universally observed reduction in the prothrombin consumption time. In addition, in two of our patients the impairment in thromboplastin generation was due to a plasma factor defect. Thus, it is not likely that the impaired prothrombin consumption time is due to the low platelets alone. The euglobulin lysis time was impaired in our patient but was normal in the two reported by Didisheim *et al.*[5] and in the one reported by Baker *et al.*[7] The thrombin time was prolonged in two of our patients, as well as in three others reported,[3, 5] but was normal in another.[7] Plasmin in low concentrations has antithrombin activity. In higher concentrations which produce free fibrinolytic activity, fibrinogen breakdown products appear which also prolong the thrombin time.[15] Thus it appears that the prolonged thrombin time may be taken as evidence of plasmin activity.

There are now many clinical states associated with evidence of diffuse intravascular clotting. In some of these there is associated fibrinolytic activity. In others the fibrinolytic features predominate without evidence of intravascular thrombosis. Attempts have been made to distin-

guish between primary thrombosis and primary thrombolysis,[14] but the clinical distinction is usually very difficult in a given case. Both mechanisms result in low fibrinogen, factor V and factor VIII levels. Even in the absence of overt fibrinolytic activity, a prolonged thrombin time is believed to be evidence of fibrinolytic breakdown products.

It is now generally held by most investigators that diffuse intravascular clotting is the predominant feature[16] and that thrombolysis follows as a reactive process. On the other hand, activated factor XII has been found to initiate not only the clotting mechanism but the lytic mechanism as well.[17] Thus both processes may be initiated simultaneously by the same mechanism, but, under given circumstances, preferential thrombogenic or thrombolytic activity may predominate. This may explain the variable results that have been described in patients with acute promyelocytic leukemia. Our own experience with this disease is that the fibrinolytic mechanism appears to be dominant and that there is no convincing evidence of diffuse intravascular coagulation.

One can only speculate whether the abnormal myelocyte of this type of leukemia may secrete a plasminogen activator which is responsible for the clotting changes. High activator activity in bone marrows has been described,[18] and marrow eosinophils have been implicated in profibrinolysin production.[19] Similar studies of the bone marrows of patients with acute promyelocytic leukemia may help to solve this problem.

Response to therapy in acute promyelocytic leukemia is very poor. Only one of our patients had a short-lived remission with prednisone and 6-mercaptopurine. In one patient, 8 to 17 g. of ϵ-aminocaproic acid daily for four days did not control the bleeding and did not produce any changes in the prothrombin time, thrombin time or whole blood clot lysis. In one report[20] larger doses of ϵ-aminocaproic acid appeared to control the bleeding after seven days' therapy. Heparin was believed to alter the clotting changes in one patient[7] but not in another.[5] The principal treatment should be directed to the leukemia, because the clotting disturbances are secondary and probably continuously stimulated. Only the induction of a remission resulted in the control of bleeding and improvement in the coagulation changes towards normal.

Summary Acute promyelocytic leukemia is a disease of adults that presents as a severe bleeding disorder and is usually rapidly fatal, death being due to cerebral hemorrhage. The bone marrow is infiltrated with abnormal myelocytes and metamyelocytes and examination of it is diagnostic. The bleeding may precede the appearance of significant thrombocytopenia but becomes more severe as the platelets decrease. The clotting changes include the rapid dissolution of a normally formed clot, a low fibrinogen concentration, a prolonged prothrombin time, a reduction in factors V and VIII, and a reduced euglobulin lysis time. Further investigation suggested that there is a plasmin activator that is responsible for the bleeding tendency. There was no evidence of intravascular thrombosis in the three patients on whom an autopsy was performed.

Résumé La leucémie promyéloïde aiguë est une maladie hémorragique grave de l'adulte. En général, elle est rapidement fatale, par suite d'hémorragie cérébrale. La moëlle osseuse est infiltrée de myélocytes et de métamyélocytes anormaux. Leur recherche constitue une méthode diagnostique. L'hémorragie peut précéder l'apparition de la thrombopénie, mais s'aggrave à mesure que le nombre de plaquettes diminue. Parmi les modifications de la coagulation figurent la dissolution rapide d'un caillot normalement formé, une faible concentration de fibrinogène, un temps de prothrombine prolongé, une réduction des facteurs V et VIII et une diminution du temps de lyse des euglobulines. Des études ultérieures permettent de croire à la présence d'un activateur de la plasmine, qu'on croit pouvoir accuser de la tendance à l'hémorragie. Il n'y avait aucun signe de thrombose intravasculaire chez les trois malades qui ont été autopsiés.

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