

Hemolysis In Vitro and the Anemia of Leukemia

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A HEMOLYTIC system has been described in the blood of some patients with acute leukemia and other disseminated neoplasms.¹ Hemolysis occurs in vitro when heparinized or defibrinated blood from such patients is incubated for more than 6 hours at 37 C. The intensity of the reaction can be modified by altering pH and Ca^{++} concentration. It was suggested that the hemolytic system depends on alteration of the plasma which permits a harmful reaction between Ca^{++} and the red cells.

Most patients with leukemia and other disseminated neoplasms are anemic, and it has been shown in many of these cases that the red cell life span is less than normal, indicating the hemolytic disease contributes to the severity of the anemia.² The present study was undertaken to learn if the Ca^{++} related hemolytic system shortens the red cell life span in patients with leukemia. Included are some observations on the incidence of the hemolytic phenomenon and its behavior during the course of acute leukemia.

MATERIALS AND METHODS

The studies were performed on patients on the Hematology Service of Walter Reed General Hospital. The hemolytic test was performed as described¹ by incubating at 37° under mineral oil 2 ml. aliquots of sterile blood drawn from a fasting patient. After 24 hours, the sedimented red cells were carefully resuspended, the test tube was centrifuged, and the concentration of hemoglobin in the supernatant plasma was measured.³ In most of the trials, 7 tubes were incubated to determine the effect of several additives. The volume of additive in each tube was 0.1 ml. To whole blood was added 2 per cent potassium oxalate; 4 per cent sodium citrate; 10 units of heparin; a second tube with heparin was separated immediately to establish the original plasma hemoglobin concentrations. One tube of whole blood with no additive was permitted to clot. To defibrinated blood was added 0.9 per cent sodium chloride; 5 per cent glucose and 5 per cent 3-methyl glucose. The additives were placed in 11 x 75 mm. test tubes under oil. The blood was added and mixed immediately by gentle rotation of the tube. The tubes were not disturbed during the 24 hours incubation.

A typical normal result is shown in table 1. Plasma hemoglobin in the heparin (H) tube using normal blood was never greater than 20 mg. per 100 ml. after incubation. In normal defibrinated blood (Sal) the value was never in excess of 60 mg. On this basis the values reflected in figure 1 were divided into "normal and abnormal."

Life span of patients' red cells was related to the rate of decay of radioactivity of the patients' blood after autotransfusion of Cr^{51} -labelled red cells.⁴ Normal values for Cr^{51} $t/2$ are in excess of 25 days. Blood volume was determined by isotope dilution when the labelled cells were injected. Normal RBC mass is between 25 and 33 ml. per Kg. of body weight.

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**Table 1.—Four Cases of Acute Granulocytic Leukemia in Adults
Results of 24-Hour Incubation Test Done at Time of Admission**

	P	O	C	H	S	G	3MG	Sal
Case 1	1	30	10	110	1	65	720	355
Case 2	1	25	5	50	10	25	165	145
Case 3	2	60	10	32	30	25	110	35
Case 4	1	5	5	5	2	30	85	55
Normal	2	20	3	10	6	30	40	25

Results are given as mg. Hb per 100 ml.

P = unincubated plasma Hb.; O = oxalate; C = citrate; H = heparin; S = serum of incubated clot. Last 3 values are from defibrinated blood with various additives. G = glucose; 3MG = 3-methyl glucose; Sal = 0.9 per cent NaCl.

RESULTS

In table 1 are shown the results on four cases of acute granulocytic leukemia in young adults, one of them strongly positive, one moderately positive and two considered to be negative. The results of a test of normal blood are presented for comparison.

These tests demonstrate the variability of the hemolytic system in clinically similar cases of acute leukemia. In addition, the low values associated with citrate and oxalate show the sensitivity of the hemolytic system to Ca^{++} .¹ The system is also sensitive to pH; where there is an excess of glucose to metabolize, the pH goes down and there is little hemolysis. Defibrination, on the other hand, raises the pH and hemolysis is increased.¹

Table 2 demonstrates the results of the test performed repeatedly on the blood of a young woman with acute lymphocytic leukemia. During her illness she had two remissions, the first on 6-mercaptopurine, the second on amethopterin. With each remission the autohemolysis test became normal; with each relapse the test again became abnormal. In this patient, as in some others with acute leukemia, reversion of the test to normal was often the first parameter to indicate the beginning of a remission.

Table 2.—Results of the Autohemolysis Test in a Case of Acute Lymphocytic Leukemia*

	P	O	C	H	S	G	3MG	Sal
1 January	5	30	10	360	20	50	470	540
16 January	1	8	2	25	1.4	30	25	2.4
23 January	1	13	3	2	3	17	9	3
27 February	1	22	1	4	—	2	15	10
2 April	10	50	20	130	20	55	140	135
11 April	1	6	25	5	7	5	10	40
23 April	1	27	7	3	5	60	35	35
9 May	2	4	6	8	10	30	30	20
20 May	—	10	6	80	7	70	115	65
15 June	1	10	1	210	21	290	360	355
6 July	1	10	10	6	2	15	20	30

See table 1 for key to column headings.

*In January the patient, a young woman, developed a remission on 6-mercaptopurine. She relapsed in April, developed a second remission on amethopterin, then relapsed and died in July. The test reverted prior to death without remission.

The autohemolysis test has been performed at the time of initial admission to hospital on 55 cases of acute leukemia. To demonstrate typical results in all 55 cases, the heparinized blood and the defibrinated blood are representative and the results are presented in figure 1. In the heparin tube, 20 of 54 trials (37 per cent) were abnormal with hemoglobin in the plasma in excess of 50 mg. per 100 ml. In the defibrinated blood, 32 of 44 trials (74 per cent) were abnormal with hemoglobin concentrations in excess of 100 mg. Defibrinated blood is a more sensitive indicator than heparinized blood of the presence of this hemolytic system. There was no obvious correlation between results of the test and the type of acute leukemia, nor did the initial test have any prognostic value as to remission.

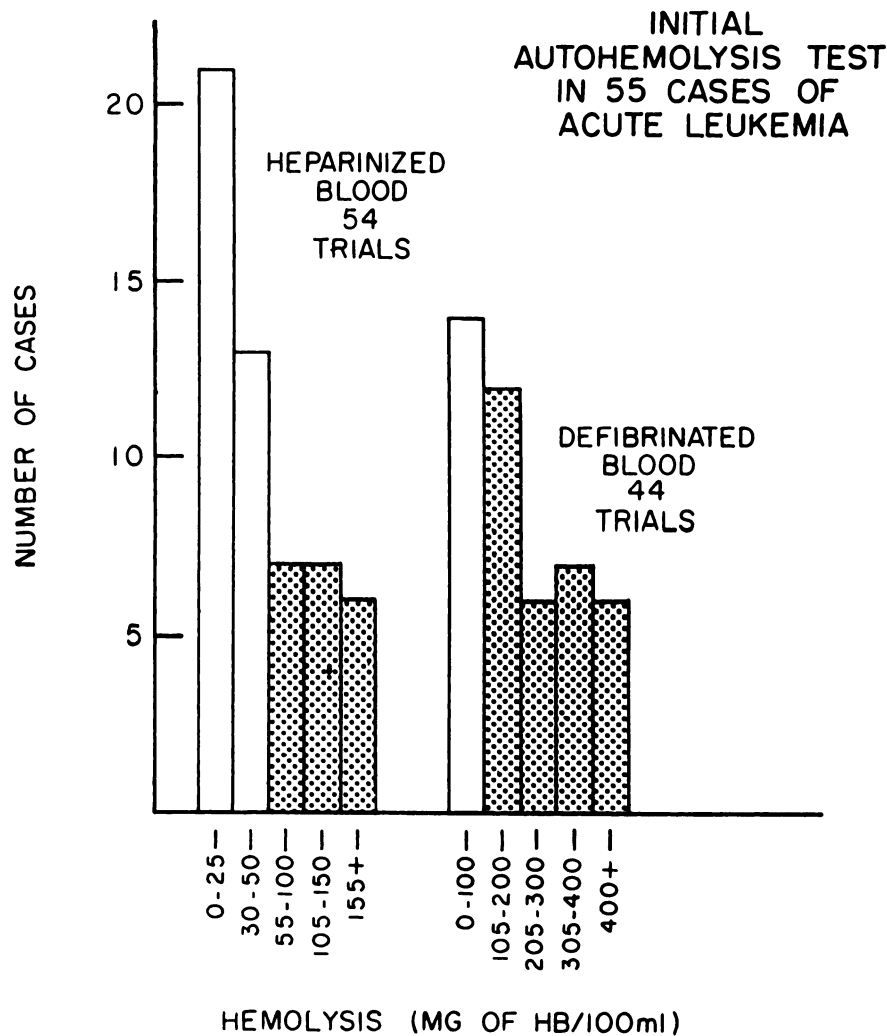


Fig. 1.—The initial autohemolysis test in 55 consecutive cases of acute leukemia. The shaded areas are considered to be abnormal results.

Table 3.—*In Vivo Versus In Vitro Hemolysis in Acute Leukemia—The Results of Red-Cell Life Span Tests in Seven Cases of Acute Leukemia*

Case number	Diagnosis	Hemolysis in vitro defibrinated blood (Hb mg./100 ml.)	RBC mass (ml./Kg.)	Hemolysis in vivo Cr ⁵¹ RBC t/2 (days)
1	AGL	80	12	9
2	AGL	155	10	15
3	ALL	230	20	30
4	ALL	355-30	15	20
5	AGL	355	19	19
6	AGL	360-122	12	18
7	AGL	760-430	25	34

Table 3 shows the results of the incubation test performed at the time of measuring red cell life span. In some of the patients the test was positive, in others negative. There was no correlation between results of the incubation test and the life span of the red cells. Neither was there any correlation between the results of the test and the severity of anemia. Of special significance were those cases, numbers 3 and 7, in which there was a strong hemolytic reaction in vitro and the red cell life span in vivo was not shortened. These cases indicate that the hemolytic system is an in vitro phenomenon, and it probably does not contribute to the patients' anemia. Still in question are the nature and significance of the plasma abnormality which permits the hemolytic reaction in vitro.

SUMMARY

Autohemolysis occurs in vitro in the blood of some patients with leukemia and other disseminated neoplastic diseases. It is known that patients with such diseases are frequently anemic and hemolytic disease frequently contributes to the anemia. The present study has demonstrated that the in vitro auto-hemolytic phenomenon is not necessarily associated with a short red cell life span. This suggests that the abnormality does not damage the red cells while they are in the circulation.

SUMMARIO IN INTERLINGUA

Autohemolyse occurre in vitro in le sanguine de certe patientes con leucemia e altere disseminate morbos neoplastic. Es cognoscite que patientes con tal morbos es frequentemente anemic e que morbo hemolytic contribue frequentemente al severitate de lor anemia. Le presente studio ha demonstrate que le phenomeno de autohemolyse in vitro non es necessarimente associate con un curte longevitate del erythrocytos. Isto suggere que le anomalitate non affice le erythrocytos durante que illos se trova in le circulation.

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