

## A Case of Autoimmune Hemolytic Anemia with Circulating Cold Agglutinins Presenting for Many Years as "Hypersplenism" and Terminating in Leukemia

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**T**HE CONCEPT of a preleukemic phase of leukemia was proposed by Block et al.<sup>1</sup> who described in 12 patients the occurrence of acute leukemia preceded by a hematologic disorder of variable duration. A few similar observations have been reported by others.<sup>2-4</sup> The usual clinical picture is that of anemia, leukopenia and thrombocytopenia, or some combination thereof. The anemia is sometimes hemolytic though not necessarily associated with reticulocytosis.<sup>2</sup>

We report here an unusual case which presented the features of autoimmune hemolytic anemia and "hypersplenism" for 18 years and finally developed fulminating leukemia.

### CASE REPORT

A painter, born in Poland in 1894, immigrated to Israel in 1926. The patient's mother died young of a heart condition. His father and four brothers died in German concentration camps. There was no history of jaundice or anemia in the family members.

His protracted illness, including 12 hospitalizations, will be described in three periods. The hematologic and other laboratory data obtained in the course of 18 years are summarized in figure 1 and table 1.

#### *First Period*

In 1933 the patient suffered from upper abdominal pain and jaundice. From 1942 to 1947 he was hospitalized three times for similar complaints and cholelithiasis was diagnosed. A firm, enlarged spleen was found on every occasion. The liver was normal on palpation.

Repeated laboratory investigations during these admissions showed in the serum a direct (+) and indirect (+++) Van den Bergh reaction. Bilirubin and increased amounts of urobilinogen were found in the urine. The Weltman coagulation band was 8-10. A false positive Wasserman reaction was obtained on one occasion. Blood counts showed a moderate normochromic anemia, leukopenia and thrombocytopenia. Although the cytopenic changes showed some variations, blood counts only rarely reached normal levels. The osmotic fragility of the red cell was increased on one occasion (0.54 — 0.40) but normal on subsequent examinations.

#### *Second Period*

From 1949 to 1951 he had several episodes of swelling of the lips, and nose, accompanied by a bluish discoloration of the ear lobes.

In 1952 he was admitted to the Department of Dermatology, with severe pains and cyanosis of both ear lobes. The ears were edematous and covered with hemorrhagic blisters. The upper lip was swollen and cyanotic. There were petechial hemorrhages in the oral

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*Submitted May 1, 1962; accepted for publication Mar. 1, 1963.*

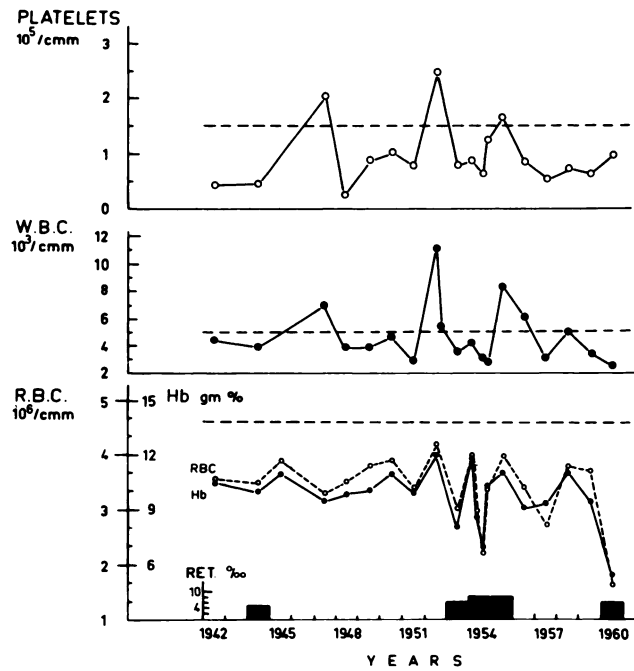


Fig. 1.—Eighteen-year follow-up of hematologic data. The values beneath the stippled lines are regarded as below normal.

mucosa, and the left conjunctiva. Laboratory findings: ESR 127 mm. (Westergren), Hb 12 Gm. per cent, RBC 4,200,000, WBC 5,000–11,000, and thrombocytes 150–250,000. Bleeding time, coagulation time and clot retraction were normal. L.E. “preps” were negative. Serum albumin 3.5 gm. per cent, globulin 3.7 gm. per cent; cephalin flocculation and thymol turbidity were negative. Serum bilirubin 0.7 mg. per cent, prothrombin 32 per cent. A bone marrow smear was normal. A biopsy from the auricular region show a state of chronic “frost-bite,” with signs of acute and subacute inflammation. Skin sensitivity tests revealed a positive reaction to amidopyrin. The final dermatologic diagnosis was “erythema fixum.”

In 1953 the patient was admitted because of painful swelling in the right ankle region, tachycardia and fever. The overlying skin was hot, bluish red and very tender, and movements in the joint were limited. Similar patches were present over the upper lateral aspect of the left foot. Serum uric acid level was 8–9 mg. per cent. Blood counts showed pancytopenia. The osmotic fragility of RBC was normal.

In view of the pancytopenia and hyperuricemia associated with cutaneous and articular manifestations, leukemia was suspected, but two bone marrow examinations were normal. The patient’s symptoms subsided completely on ACTH treatment.

In 1954, following another episode of jaundice, cholecystectomy was performed. At operation the liver was slightly enlarged and the gall bladder was filled with black stones. The common bile duct was widened but contained no stones. The spleen was enlarged to about half the size of the liver and showed an old infarct 3 cm. in diameter. A liver biopsy showed widened portal spaces infiltrated by lymphoid cells and some granulocytes and bile plugs. Some of the parenchymal and Kupffer cells contained a considerable amount of iron pigment. A spleen biopsy showed a fibrotic capsule, hypertrophy and hyperplasia of the cells in the red pulp, and the presence of Gandy-Gamma bodies.

The postoperative period was complicated by severe transitory hemolytic jaundice and oliguria. The hemoglobin dropped to 6.8 gm. per cent, the bilirubin rose to 52 mg. per

Table 1.—Laboratory Findings

Year	Hemoglobin (Gm. %)	RBC (mill. per cu. mm.)	WBC (per cu. mm.)	Platelets (per cu. mm.)	Reticu- locytes (%)	ESR (West- gren)	T. P. (Gm. %)	Alb. (Gm. %)	Glob. (Gm. %)	Abnormal Serologic Findings
1942	10.5	3.5	4500			25				
1944	10	3.5	3800	45000	0.5					
1945	11	3.9	—	—	—	74				
1947	9.5	3.3	6900	208000	—	67				Wassermann +
1948	9.8	3.5	3800	25000						
1949	10	3.8	3800	94000		45	7.3	3.9	3.4	
1950	11	3.9	4300	100000			7.8	4.2	3.6	
1951	9.8	3.4	2800	80000			9.6	3.6	6.3	
1952	12	4.2	11000— 5000	250000— 50000		127	7.2	3.5	3.7	
1953	8	3.0	3500	82000	0.5	130				
1954	11.8	4.0	4250	84000	0.8	90	6.8	3.2	3.4	
1955	11	4.0	8300	162000	0.8	59	6.8	3.1	3.7	
1956	9.1	3.4	6000	82000		135				cold agglutinins—1:64000, cryofibrinogen, Coombs direct +
1957	9.3	2.7	3100	50000						
1958	11	3.8	5100	—		82	7.3	3.2	4.1	
1959	9.4	3.7	3300— 5400	60000						
1960	5.4	1.6	2600	95000	0.6	158	6.5	3.2	3.3	Coombs direct + leuko-agglutinins + + +

cent and the blood urea to 280 mg. per cent. The Coombs test was negative and no cold agglutinins were present. The osmotic fragility of the RBC was normal. The patient was discharged 3 weeks after operation. He was readmitted because of a urinary tract infection which was treated with chloramphenicol. However, this drug had to be discontinued because of a drop in WBC from 6,000 to 2,800.

In 1956 he developed pneumonia. A chest x-ray revealed massive shadowing at the base of the right lung. ESR was 135 mm. There was spontaneous agglutination of his RBC at room temperature. Cryofibrinogen was demonstrated on repeated occasions and cold agglutinins were present to a titer of 1:64,000. The direct Coombs test was positive. The patient was treated with Achromycin and recovered.

In 1957 he had severe epistaxis and a year later several hemoptyses. Cystic bronchiectasis was diagnosed.

The same year the patient had two episodes of loss of consciousness and convulsions which were diagnosed as epilepsy of unknown cause. A lumbar puncture was normal. The E.E.G. showed a diffuse disturbance pattern predominantly on the left side. The hematologic and biochemical findings were similar to those found in previous investigations. During the next 18 months he received Luminal and bromural. In addition, Nicotibin had been prescribed by a chest clinic.

### *Third Period*

In November 1959 he had a short episode of fever and a minor hemoptysis and received a total of 15 Gm. of chloramphenicol. Subsequently his condition deteriorated and he lost weight. A month later the fever recurred and was accompanied by pain and redness in the left auricular region. He again received 4 Gm. of chloramphenicol over a period of 2 days, and was admitted to hospital in January 1960.

Physical examination revealed a severely ill patient with high fever, tachycardia and rapid shallow respirations. In the left mastoid region there was a tender, fluctuating swelling about 1 cm. in diameter. The liver was palpable 1 cm. below the costal margin. The spleen was firm and tender, and reached to about 15 cm. below the left costal arch.

*Laboratory findings:* Hb 5.4 Gm. per cent, RBC 1,600,000, hematocrit 14 per cent MCV 87 cu.  $\mu$ ., MCHC 38 per cent, reticulocytes 0.6 per cent, thrombocytes 90,000, WBC 2,600. The differential count showed 1 promyelocyte, 2 myelocytes, 10 band forms, 40 segmented, 39 lymphocytes, 7 monocytes and 1 blast. Serum iron was 76  $\mu$ g. per cent. Osmotic fragility was normal. Bone marrow examination revealed nests of primitive pathologic cells of moderate size (figs. 2 and 3) with round and sometimes irregular nuclei containing one to three light nucleoli surrounded by a sharp border. Their cytoplasm was very basophilic and contained several round vacuoles. There were no inclusion bodies. The vacuolar material did not stain with PAS and was negative for lipids. The urine contained increased urobilinogen. The total protein level was 6.8 Gm. per cent. Electrophoresis showed albumin 28.5 per cent,  $\alpha_1$  globulin 7 per cent,  $\alpha_2$  globulin 14.2 per cent, beta globulin 16 per cent and gamma globulin 29.6 per cent. Blood urea was 80 mg. per cent, uric acid 12 mg. per cent, bilirubin 1.8 mg. per cent. The Coombs test was again positive. Incubation of the patient's serum with amidopyrin showed a high titer of leukoagglutinins.

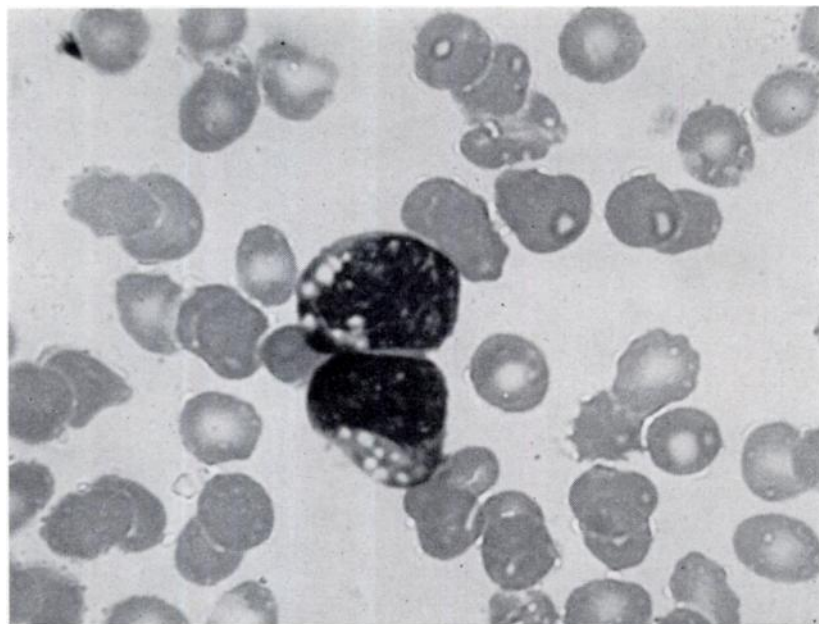
On the 15th day after his admission, the leukocytes rose to 11,000 with 60 per cent blast cells in the peripheral blood. The patient died 1 week later.

### *Autopsy Findings*

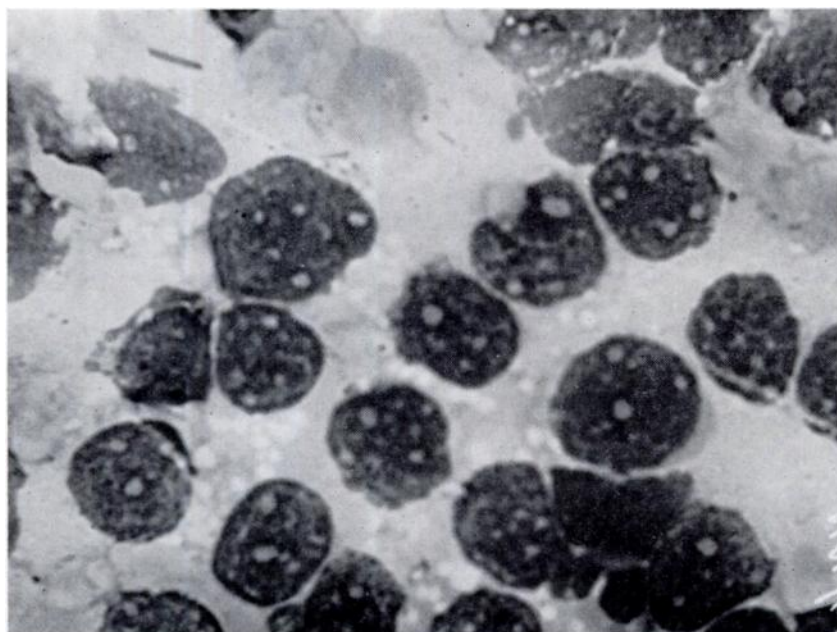
The spleen, which weighed 1,800 Gm., was irregularly scarred and of firm consistency. The greater part of the cut surface showed a homogenous grayish appearance, the normal architecture being completely obliterated. Many wedge-shaped infarcts were seen.

The liver weighed 2650 Gm. and was of normal configuration and consistency. A few slightly enlarged lymph nodes about 15 mm. in diameter were found in the hepatoduodenal ligament. They were of normal consistency and well delimited.

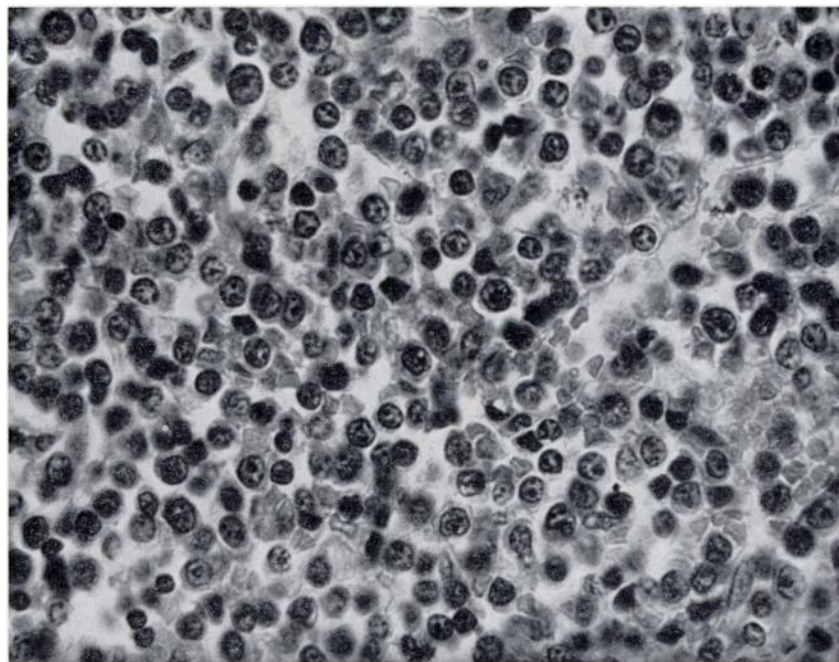
The bone marrow in the femur was grayish pink in color.



**Fig. 2.**—Bone marrow smear taken 3 weeks before patient's death showing vacuolization of primitive cells. x1600.



**Fig. 3.**—Bone marrow smear taken a few days before patient's death. x1600.



**Fig. 4.**—Bone marrow showing homogenous infiltration by almost only one cell type. x680.

*Histologic examinations:* Bone marrow showed a homogenous infiltration by almost only one cell type (fig. 4). No normal hemopoietic foci could be identified and the fatty tissue was almost completely replaced by the infiltrate. The latter consisted of quite uniform cells with scanty cytoplasm and ill-defined borders.

*Spleen:* Both red and white pulp were replaced by cells similar to those in the bone marrow. Most of the endothelial and reticular cells were plump and many of them showed phagocytosis.

*Liver:* The basic architecture was well preserved. The portal tracts were heavily infiltrated by the same cells described above. A striking number of them was seen within the sinusoids (fig. 5). The Kupffer cells were hyperplastic and frequently contained phagocytosed debris.

*Lymph nodes:* These were free from infiltrations and showed a preserved architecture. Marked hyperplasia and phagocytic activity were seen in the reticuloendothelial cells.

*Kidneys, stomach, esophagus, epicardium and underlying tissue, lungs and areolar tissue* showed infiltrations with the same cells as described above.

The calyceal system of both kidneys contained large amounts of urate gravel.

#### DISCUSSION

Several tentative diagnoses were made during the life of this patient. Apart from the gallstones the patient suffered from splenomegaly with the three main signs of so-called hypersplenism: anemia (hemolytic), leukopenia and thrombocytopenia. Hereditary spherocytosis was diagnosed during one of the admissions, but at subsequent repeated examinations the osmotic fragility of the red cells was always normal, while the positive Coombs test and other immunoreactions pointed to an acquired type of hemolytic anemia.



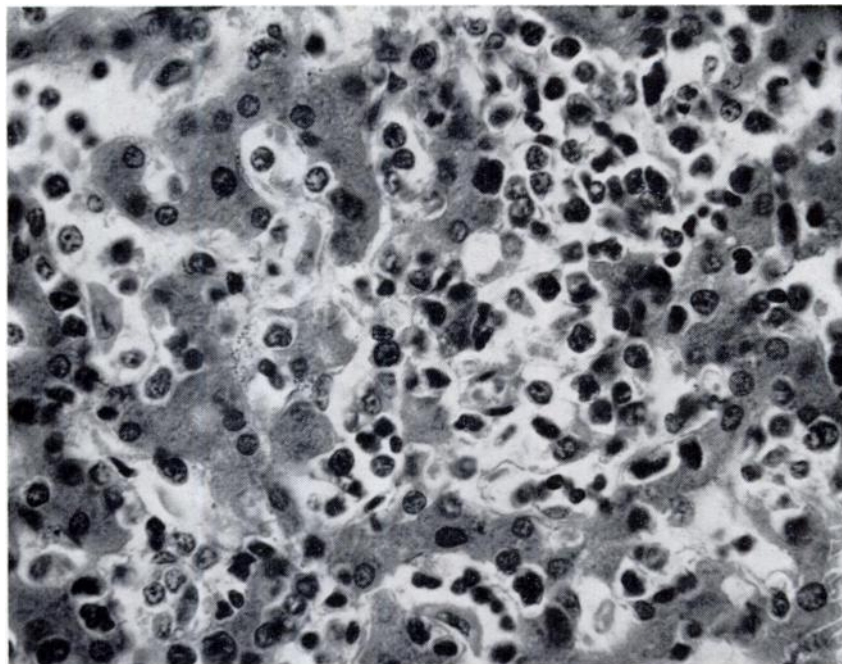


Fig. 5.—Liver showing heavy infiltration of the portal tracts and sinusoids by leukemic cells. x500.

The protracted hyperglobulinemia and elevated sedimentation rate were probably the expression of an abnormal immunologic (auto-immune) response resulting in a high titer of cold agglutinins and leukoagglutinins, a positive Coombs test and a false positive Wassermann. The latter suggested the possibility of systemic lupus, which however, was ruled out by the negative L. E. "preps" and the findings at autopsy.

The pathologic findings were characterized by the involvement of spleen, bone marrow and other tissues by a proliferative disorder and the absence of structural changes in the lymph nodes; although there could be no doubt about the diagnosis now, the cytologic characteristics were not conclusive and no definite classification of the type of leukemia could be established. Although leukemia was considered 7 years before death, repeated bone marrow aspirations performed during many admissions had never disclosed leukemic infiltrations, except in the final stage. Whether the patient had an unrecognized chronic leukemia and died of acute leukemia, or whether the final leukemic state was "grafted" on a preceding non-leukemic hematologic disorder could not be decided with absolute certainty, but in our opinion the course of events fits in best with the latter concept.

The next question to be answered is about the nature of the disease the patient had been suffering from for so many years. The clinical picture resembles most the description of "idiopathic" cold agglutinin disease.<sup>5</sup> This syndrome is characterized by sensitivity to cold manifesting itself in frost bites and peripheral gangrene, acquired hemolytic anemia, hemoglobinuria,

jaundice and the presence of circulating cold agglutinins. Ritzmann and Levin<sup>5</sup> have presented evidence suggesting that this cold agglutinin disease and macroglobulinemia are clinically and biochemically indistinguishable. Because of lack of facilities at that time, no ultracentrifugation or immunoelectrophoretic studies could be performed in our patient, but otherwise his case would fit into this picture.

The subsequent development of leukemia is especially interesting in view of the possible relationships between autoimmunity and leukemia to which Dameshek and Schwartz have drawn attention.<sup>6</sup> Ritzmann and Levin suggest that cold agglutinin disease may be due to "a random mutation in a clone of cells resulting in uncontrolled malignant proliferation of the cold agglutinin specific clone of the beta-2-M immunoglobulins."<sup>5</sup> There is a growing tendency to regard the leukemia cell also as a mutation of one of the elements of the leukopoietic tissue. In the light of these concepts, the present case would support not only Dameshek's and Schwartz's views about the relationship between autoimmune disease and leukemia, but could also give a clue as to how such a relationship could be visualized.

The vacuoles in the blasts of the bone marrow in this case deserve a brief comment. Ackerman<sup>7</sup> described vacuoles in 1.5 per cent of a large group of cases of monocytic granulocytic leukemia. Vacuoles have also been observed in cases of leukemia treated with folic acid antagonists,<sup>8</sup> and in cryoglobulinemia.<sup>9</sup> Recently, chloramphenicol has been shown to induce vacuolar changes in early erythroid cells and sometimes also in the white cells.<sup>8,10,11</sup> As this patient had received chloramphenicol before his last admission, it is not impossible that this drug caused the vacuolization of the leukemic cells, but the phenomenon might have also been an expression of an immune reaction *in vivo*.

#### SUMMARY

A case of splenomegaly of unknown origin with autoimmune hemolytic anemia, leukopenia and thrombocytopenia (clinically classified as "hypersplenism") has been observed for 18 years. The blood showed a high sedimentation rate, hyperglobulinemia, cold agglutinins, cryofibrinogen, a false positive Wassermann reaction, a positive Coombs test and contained leukoagglutinins in high titer. The patient died of acute leukemia for which it is suggested that the preceding hematologic condition may have prepared the soil, possibly by a process of autoimmunization.

#### SUMMARIO IN INTERLINGUA

Un caso de splenomegalia de incognoscite origine con auto-immun anemia hemolytic, leucopenia, e thrombocytopenia (clinicamente classificate como "hypersplenismo") esseva observate durante 18 annos. Le sanguine monstrava un alte rapiditate de sedimentation, hyperglobulinemia, cryoagglutininas, cryofibrinogeno, un reaction de Wassermann falsemente positive, un positive test de Coombs, e un alte titro de leuco-agglutininas. Le patiente moriva de leucemia acute. Il es suggerite que le antecedente condition hematologic ha



possibilmente preparate le campo pro ille leucemia. Il es possibile que le mecanismo esseva un processo de auto-immunisation.

#### ACKNOWLEDGMENTS

The authors are indebted to Dr. L. Nelken for supplying some of the clinical and laboratory data, to Prof. H. Ungar and Dr. W. Dameshek for helpful criticism, and to Dr. G. Izak and Dr. D. Nelken and their respective staff of the Laboratory of Hematology and Microbiology.

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