
CASE REPORT

HEMATOLOGIC OBSERVATIONS IN A CASE OF KALA-AZAR

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THE hematologic changes generally seen in kala-azar are characterized by a striking reduction of red cells, white cells, and platelets. The mechanism of these profound changes might be diminished production in the bone marrow of all these elements, either due to metaplasia by proliferating reticulo-endothelial cells or due to some toxic inhibition by the infective agent, or an inhibitory effect upon the bone marrow of an enlarged spleen. Another explanation might be sought in an increased destruction of the blood elements by the hyperplastic organs of the reticulo-endothelial system, mainly the spleen. In this case the cell production in the bone marrow might proceed at a normal or even at an increased rate. The methods available for the elucidation of this problem are mainly limited to the red cell series. The type of the anemia, the number of reticulocytes, the composition of the bone marrow, and the quantitative determination of the bile pigments in blood and excreta might furnish valuable data for the understanding of the mechanism of the anemia. The variations of these data under the influence of specific therapy might be of particular importance. With these considerations in mind the studies on the following case were carried out.

CASE REPORT

A young man, 18 years old, was admitted to our department on June 12, 1946. He was born in Palestine and had never left the country. Until one year ago he lived in an agricultural settlement, where he slept in a tent. During the last year he had lived in a town (Haifa). His past history was essentially negative. Four months previous to admission he began to complain of weariness and loss of appetite. One month later slight fever developed. At the same time he noticed that the whites of his eyes became yellowish. He was then examined for the first time and it was discovered that the spleen and liver were moderately enlarged. On April 9 he was admitted to the Municipal Hospital, Tel Aviv. During his two month's stay there he ran a subfebrile temperature and his spleen gradually increased in size to such a degree that it occupied the left half of the abdomen and caused abdominal distress. The liver also increased in size, but not considerably. The blood examination showed: hemoglobin 45% (Sahli), red cells 2.78 M./mm³, leukocytes 2800/mm³. The sedimentation rate was 20 minutes (Linzenmeier's method). The Takata-Ara, cephalin flocculation test and formol-gel reaction were strongly positive; the Weltmann test was IX. Repeated examination of the blood for malaria gave negative results. A careful search of the bone marrow punctate for *Leishmania donovani* bodies (including culture) also gave negative results. In spite of repeated blood transfusions and iron medication the patient's condition grew worse and he lost considerable weight.

On admission to our department, the patient was found to be in a poor nutritional state. His temperature was 38.2° C. He was very pale, and the sclerae were yellowish. There was no edema and there were no hemorrhages on the skin or mucous membranes. The pulse was 100, regular; blood pressure 100/70.

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A systolic murmur was heard at the base of the heart. Outstanding physical findings were confined to the abdomen, where a huge spleen occupied the entire left half of the abdomen, extending to the right of the umbilicus into the pelvis. It was of hard consistency and not tender; its surface was smooth. The liver was enlarged, its lower border being three fingerbreadths below the costal margin. It was also fairly hard, smooth, and not painful. Ascites was not detected.

Laboratory Findings: Urine: Albumin slightly positive, urobilinogen strongly positive, but no bilirubin; sediment negative.

Blood: Hemoglobin 8 gram %; red cells: 2.3 M./mm³; hematocrit 23 %; color index: 1.16; mean corpuscular volume: 100 cu. micra; mean corpuscular hemoglobin 34; mean corpuscular hemoglobin concentration 34%. Fragility test (NaCl solutions) 0.42-0.30%. Leukocytes: 1200/mm³. Differential count: neutrophils 28%, eosinophiles 2%, lymphocytes 54%, monocytes 16%. Reticulocytes: 1.5-5%. Thrombocytes: 110,000/mm³. Coagulation time: 7 minutes. Bleeding time: 3 minutes. Sedimentation rate: 7 minutes (Linzenmeier's method).

Blood chemistry: Urea 17 mg.%, glucose 82 mg.%. Total protein 9.40 g.%. Albumin 5.05 g.%. Globulin 4.35 g.%. Euglobulin strongly increased. Total fibrinogen 0.20 g.%. Formol-gel reaction positive within 4 minutes. Icteric index (Meulengracht) 25 units. Van den Bergh reaction: direct neg-

TABLE 1.—*Differential Bone Marrow Counts Before, During, and After Treatment*

	6/12/46	8/13/46	9/10/46
Myeloblasts.....	4%	1%	1%
Promyelocytes.....	10%	2%	
Myeloc. Neut.....	23%	16%	17%
Myeloc. Eos.....	1%		
Metamyeloc. Neut.....	7%	14%	10%
Stab. Neut.....		20%	28%
Segment. Neut.....		9%	10%
Lymphocytes.....		2%	
Proerythroblasts.....	6%		
Erythroblasts.....	30%	8%	3%
Normoblasts.....	15%	26%	30%
Reticulum & Plasma cells.....	4%	2%	1%

ative, indirect positive. Takata-Ara 4 plus positive, cephalin flocculation test strongly positive, thymol test 25. Total cholesterol 84 mg.%, free cholesterol 56 mg.%, cholesterol ester 28 mg.%. Calcium 10.9 mg.%.

The examination of the bone marrow obtained by sternal puncture showed hyperplasia of the granulocytic and the erythroblastic tissues. The differential count showed myeloblasts 4%, promyelocytes 10%, neutrophile myelocytes 23%, eosinophile myelocytes 1%, neutrophile metamyelocytes 7%, proerythroblasts 6%, erythroblasts 30%, normoblasts 15%, plasma cells and reticulum cells 4%. Occasional erythroblasts in mitotic division were seen. Malaria parasites and Leishmania bodies were not found. On July 1 the bone marrow puncture was repeated and a culture for Leishmania was taken.

On the basis of the clinical picture characterized by hepatosplenomegaly, fever, leukopenia, and anemia, and in view of the negative results of examinations for malaria and Leishmania, the possibility of Hodgkin's disease was considered, and it was decided to irradiate the spleen with x-rays. During this treatment no improvement was noted and the spleen did not decrease in size. The number of red and white cells in the blood decreased. On July 17, i. e., on the seventeenth day after the culture of the bone marrow was taken, growth of Leishmania parasites was obtained. Thus the diagnosis of kala-azar, which was already suspected during his stay in the first hospital, was definitely proved.* Therapy with stilbamidine

* It is instructive to note that in some cases of Mediterranean kala-azar the number of parasites in bone marrow is too small to be detected in smears. But the diagnosis can almost always be established by culture. It is, therefore, important in all suspected cases not to rely on smears alone but to make cultures.

(4,4'-diamidino stilbene) intravenously was started on July 18. At first 0.5 mg. per kilo body weight was given daily; this dose was gradually increased to 1.5 mg./kg. All together 50 injections were administered. The response to the treatment was striking; after the eighth injection the temperature became normal and remained so. At the same time there was a rapid improvement in the patient's general condition. The spleen gradually decreased in size and at the end of the treatment its lower border was felt two fingerbreadths below the costal margin. The subicterus soon disappeared and the liver also diminished in size.

There was a gradual rise in the red and white blood cells which reached normal values at the end of the treatment. The percentage of the reticulocytes also gradually diminished. Subsequent bone marrow examinations revealed diminution of the immature white and red cells, the differential count becoming normal toward the end of the treatment (table 1). Bone marrow culture for *Leishmania* became negative.

The quantitative examinations of fecal and urine urobilinogen before and during the treatment with stilbamidine gave the following results. The daily excretion of urobilinogen in the feces amounted to 441, 539, and 569 mg./24h, on three examinations. These values decreased slowly during the treatment, and essentially normal amounts (226 mg.) were found at the end of treatment. The daily excretion of urobilinogen in the urine was markedly increased before treatment (52.5 and 55 mg./24h) and diminished gradually to almost normal values at the end of the treatment (5.9 mg.).

The only persistent pathologic findings were the changes in the serum proteins, the globulin fraction even slightly increasing after completion of the treatment. The formol-gel test was also still positive at this time.

COMMENT

The analysis of the hematologic data obtained before treatment strongly suggests that we were dealing with a hemolytic type of anemia, which was macrocytic and hyperchromic. The increased rate of red cell production was manifested by reticulocytosis ranging from 1.5 per cent to 5 per cent and by the hyperplastic bone marrow with a predominance of immature cells. Chatterie¹ reported the presence of a red bone marrow with a hyperplasia of the erythropoietic system and an increased number of "megaloblasts" in kala-azar. Also in experimental inoculation of monkeys with *Leishmania* a generalized hyperplasia of the bone marrow has been described (Shortt,² Meleney³). These findings are similar to ours and may be considered as a reaction to increased destruction of the blood cells. The increase of the bilirubin of the indirect type in the blood was another indication of augmented red cell destruction. More conclusive evidence was furnished by the quantitative determination of urobilinogen excretion in feces and urine, which was considerably increased before treatment and varied from 493 mg./24h to 594 mg./24h. These values are particularly high when the low red cell count is considered (Watson,⁴ Miller, Singer, and Dameshek⁵). It is highly probable that the huge spleen was the most important site of red cell destruction. The elimination of the hyperactivity of the spleen by a chemotherapeutic agent affecting the organism responsible for the splenic enlargement, proceeded gradually and steadily in our patient. It was impressive to observe the parallelism between the reduction of the size of the spleen and the subsidence of the signs of its hyperactivity. The similar behavior of the red and white cells in regard to their reduced number in the peripheral blood before treatment, the hyperplasia of both elements in the bone marrow, and the effect of treatment causing a simultaneous rise of both, support the contention that the leukopenia was also due to increased destruction exceeding production. In case of the red cells their increased destruction could be objectively

proved by the determination of the urobilinogen excretion. No such objective evidence could be produced for a possibly increased destruction of the white cells. The estimation of uric acid excretion in the urine, the patient being on a special diet, did not reveal increased values. Although the uric acid content of the blood was within normal limits, this did not exclude the possibility of increased

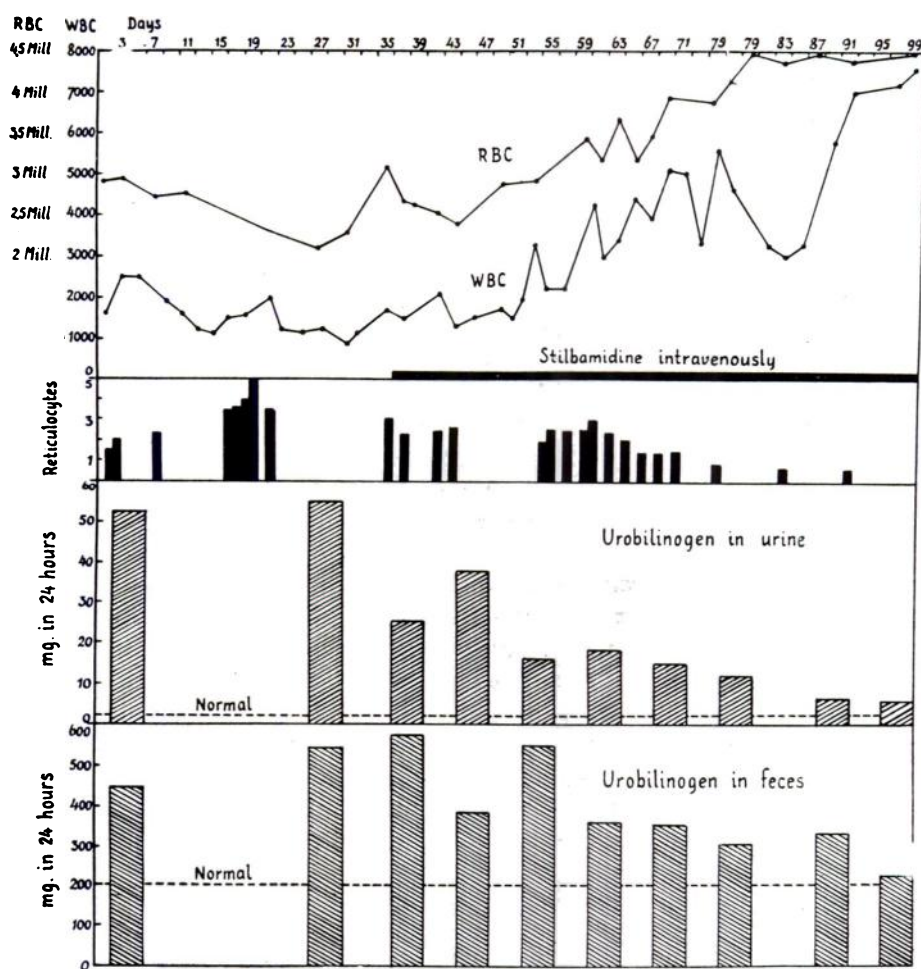


FIG. 1

leukocytolysis. Since the number of phagocytic cells in the spleen and other organs is known to be increased in kala-azar, it is reasonable to assume that phagocytosis was responsible for the increased destruction of all the blood elements. Adler⁶ has demonstrated indiscriminate phagocytosis of blood cells of all varieties by reticulo-endothelial cells in kala-azar. According to Napier⁷ the anemia in kala-azar may be due partly to excessive phagocytosis by the numerous macrophages. The conception of increased destruction of blood cells by a hyperactive phagocytizing spleen conforms with the observations of Doan et al.⁸ and Muether et al.⁹ in splenic

neutropenia. Using the supravital staining technic on fresh splenic parenchyma, Doan actually observed increased granulocyte inclusions along with red cells in the numerous reticulo-endothelial phagocytes. In these cases splenectomy resulted in normalization of the blood. The effects of the enlarged spleen on hemopoiesis may also be explained by a splenic hormone regulating bone marrow activity. The liberation of excessive amounts of this hormone by the enlarged hyperactive spleen may thus lead to interference with the normal production and delivery of blood cells. This explanation, rather than that of increased phagocytosis, is advanced by Dameshek¹⁰ to account for the extreme neutropenia, thrombocytopenia and anemia of many cases with splenomegaly due to various causes, including chronic infection. Dameshek points to the lack of any clear-cut evidence indicating phagocytosis as the cause of the neutropenia and thrombocytopenia. It should also be stated that little direct evidence for the existence of hormonal activity has been produced thus far.

The analysis of the data obtained by the estimation of the urobilinogen excretion shows that the quantity of urobilinogen excreted in the urine was excessively high (52 mg. instead of 2 mg.). This fact points to a marked disturbance in the function of the liver in metabolizing the increased amounts of urobilinogen offered by the portal circulation. The liver in our case was definitely enlarged, and its disturbed function was also manifested by the positive cephalin flocculation test and the decrease of the cholesterol ester in the blood.

SUMMARY

The hematologic findings in a case of kala-azar under the influence of specific treatment are described. The type of the anemia, the hyperplastic bone marrow, the increased urobilinogen output before treatment, and the subsequent changes following treatment strongly suggest increased red cell destruction (most probably by phagocytosis) as the cause of the anemia.

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