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THE ANEMIA OF KALA-AZAR

José Murilo MARTINS [1], Joaquim Eduardo de ALENCAR [2]
and Valdenor Benevides MAGALHÃES [3]

SUMMARY

One hundred and ninety cases of visceral leishmaniasis (Kala-azar) were observed at the Preventive Medicine Institute of the University of Ceará in a period of 6 years.

The mean values for red blood cell count was 2,578,600 per mm^3, hemoglobin 7.45 g%, hematocrit 22.1% and reticulocyte count 4.5%. Nucleated red blood cells were seen in 13 patients. Microcytic anemia was present in 57.7% of the cases. Bone marrow studies showed hyperplasia of the erythroid series.

No relationship was found between the degree of anemia and the duration of the disease. The anemia was more severe under the age of 10, in females and in patients with great splenomegaly.

A marked degree of anemia was seen in patients with fatal outcome. The mean red blood cell count in 10 patients was 1,942,000 per mm^3 and hemoglobin 6.1 g%.

The anemia was more severe in the group of patients with Kala-azar and hookworm infestation, meanwhile it was less severe when there was Kala-azar and schistosomiasis association.

A review of the treatment of anemia in Kala-azar was performed. Emphasis was given to Amphotericin B.

The pathogenesis of anemia was discussed. It was felt that several factors contribute to the development of anemia.

INTRODUCTION

Anemia is quite frequent in Kala-azar. It is observed in the majority of the patients and may be absent in the very early stages of the disease [16]. The degree of anemia may be severe, but usually is moderate and subsides with specific therapy. A large number of papers have been published on the subject [1, 5, 6, 12, 16, 17, 20, 21, 25, 26, 27, 29, 33, 35, 38, 40, 41] and up till now investigators did not come to terms concerning its pathogenesis.

In the present study we show our experience with the problem at the University of Ceará, Fortaleza, Ceará, Brazil.

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(1) Associate Professor and "Livre Docente" of Medicine, Universidade do Ceará

(2) Director, Preventive Medicine Institute. Professor of Preventive Medicine and "Docente Livre" of Parasitology. Faculdade de Medicina da Universidade do Ceará, Ceará, Brasil.

(3) Assistante of Infectious and Tropical Diseases. Faculdade de Medicina da Universidade do Ceará, Ceará, Brasil

1 — PRELIMINARY DATA

1.1 — One hundred and ninety cases of Kala-azar were observed at the Kala-azar Service of the Preventive Medicine Institute of the University of Ceará and "Departamento Nacional de Endemias Rurais" in the period of August 1956 to April 1963. The diagnosis was established by the demonstration of *L. donovani* in the smears obtained by sternal puncture. Occasionally spleen puncture was required. The patients in which the parasite was not demonstrated were not included in the study. Ten additional cases seen in the months of May, June and July 1963 were included in this paper for reticulocyte counts, bone marrow studies, serum iron and osmotic fragility determinations.

1.2 — One hundred and fourteen patients were males and 76, females. The age varied from 10 months to 63 years; 95 patients (50%) had less than 5 years and 148 (78%), less than 10 years. All cases came from the hinterland of the State of Ceará, Brazil, where the disease is found to be endemic.

1.3 — Serum iron was determined after the method of BARKAN & WALKER [3], modified by SCHALES and osmotic fragility of red cells by the method of DACIE [7].

2 — HEMATOLOGICAL DATA

The figures of red blood cell count, hemoglobin and hematocrit determination obtained by other authors (brazilian and non-brazilian) can been analysed in the Tables I and II.

2.1 — *Red blood cell count* — The results of the red blood cell count performed in 163 cases of Kala-azar before the onset of therapy are presented in the Table III. The mean value was 2,578,600 per mm³, ranging from 950 000 to 4.210,000. By comparing our findings with the Figures of the Tables I and II, the degree of anemia was more severe in the present series.

The red blood cell count varied from 2 to 3 millions per mm³ in 95 patients (58%). One hundred and twenty four cases (76%) had the count below 3,000,000 and only 5 (3%) had above 4,000,000 per mm³ (Figure 1).

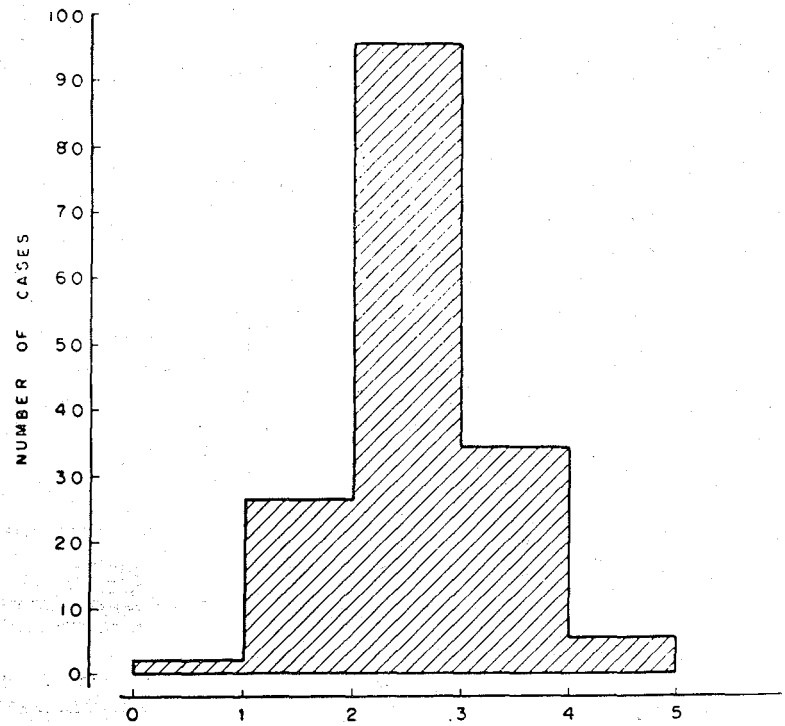

Fig. 1 — Degree of anemia in 163 cases of Kala-azar. Red blood cell count in millions per mm³.

2.2 — *Hemoglobin determination* — The mean value for hemoglobin determination done in 163 untreated cases of Kala-azar was 7.45%, ranging from 3.0 g% to 14.0 g%.

Twenty two cases (13.5%) had the hemoglobin below or equal 5 g%. Hookworm infection was present in 9 out of 13 patients in which feces where examined.

2.3 — *Hematocrit* — Red blood cell packed volume was determined in 45 patients. The range varied from 10 to 33%, with the mean value of 22.1%.

3 — BLOOD INDICES

TRINCÃO [40] in 1948 studied for the first time the blood indices of Wintrobe in 3 cases of Kala-azar (Table I). Since then several brazilian investigators studied the problem and their figures are presented in the Table II.

We determined the mean corpuscular hemoglobin in 163 cases and the mean corpuscular volume and the mean corpuscular hemoglobin concentration in 45 untreated patients with Kala-azar. The mean values are shown in the Table III.

Eight patients (17.7%) had the mean corpuscular volume within the limits of normal, 11 (24.6%) above and 26 (57.7%) below normal (Figure 2). Microcytic anemia was prevalent in our series.

4 — PERIPHERAL BLOOD SMEARS

4.1 — In the various reports on Kala-azar there is a great variation in the number of nucleated red blood cells in the peripheral smears. NAPIER & SHARMA [25] and KEEFER et al. [12] did not find normoblast in the periphery, while KUROYA et al. [13] referred the presence of normoblast in 26 out of 151 patients and CARTWRIGHT et al. [5],

TABLE I

Hematological data in Kala-azar (non Brazilian authors)

Author, Nation, Year	Number of cases	RBC (X 10^3 per mm^3)	Hb (g%)	Hematocrit (%)	MCH (yy)	MCV (μ^3)	MCHC (%)
NAPIER & SHARMA (India, 1933)	47	2,880	7.08	—	—	—	—
KUROYA et al. (China, 1939)	151	2,930	—	—	—	—	—
MOST & LAVIETES (U. S. A., 1947)	28	3,200	—	—	—	—	—
TRINCÃO (Portugal, 1948)	3	2,670	7.3	—	27.5	90.3	29.9
CARTWRIGHT et al. (China, 1948)	18	3,220	9.5	—	—	—	—
Mean value	—	2,969	7.73	—	—	90.3	—

TABLE II

Hematological data in Kala-azar (Brazilian authors)

Author Year	Number of cases	RBC (X 10^3 per mm^3)	Hb (g%)	Hemato-crit (%)	MCH (yy)	MCV (μ^3)	MCHC (%)
RAMOS (1955)	18	2,766	7.58	23.71	27.53	85.6	31.83
PRATA (1957)	22	2,868	7,35	24.10	25.10	83.7	30.00
SILVA (1957)	77	3,070	7.40	24.20	23.90	79.0	29.50
NEVES (1958)	13	2,858	8.57	25.40	29.70	89.5	34.20
MOURÃO (1960)	17	2,610	7.53	23.50	28.80	88.5	31.40
Mean value	—	2,930	7.53	24.16	25.60	82.5	30.49

TABLE III

Hematological data in 163 untreated cases of Kala-azar
(MARTINS, ALENCAR & MAGALHÃES, 1963)

RBC (X 10^3 per mm^3)	Hb (g%)	Hematocrit (%)	MCH (yy)	MCV (μ^3)	MCHC (%)
2,578,6	7.45	22.10 *	29.40	82.02 *	33.05 *

* Mean value of 45 cases

in 5 out of 27. In our series, nucleated red blood cell was found in the periphery in 13 patients.

4.2 — Slight poikilocytosis is described in Kala-azar. One of us (J.E.A.), in a previous paper, found poikilocytosis 39 times and anisocytosis 24 times in 123 patients [1]. Polychromasia and stippling are rarely seen [5].

5 — RETICULOCYTE

A mild degree of reticulocytosis is present in untreated Kala-azar (see Table IV). The reticulocyte count varies from 1 to 10% and exceptionally may attain high levels, as in the BURCHENAL et al. [4] case.

The mean value for the reticulocyte count done in 6 patients was 4.5% with the range

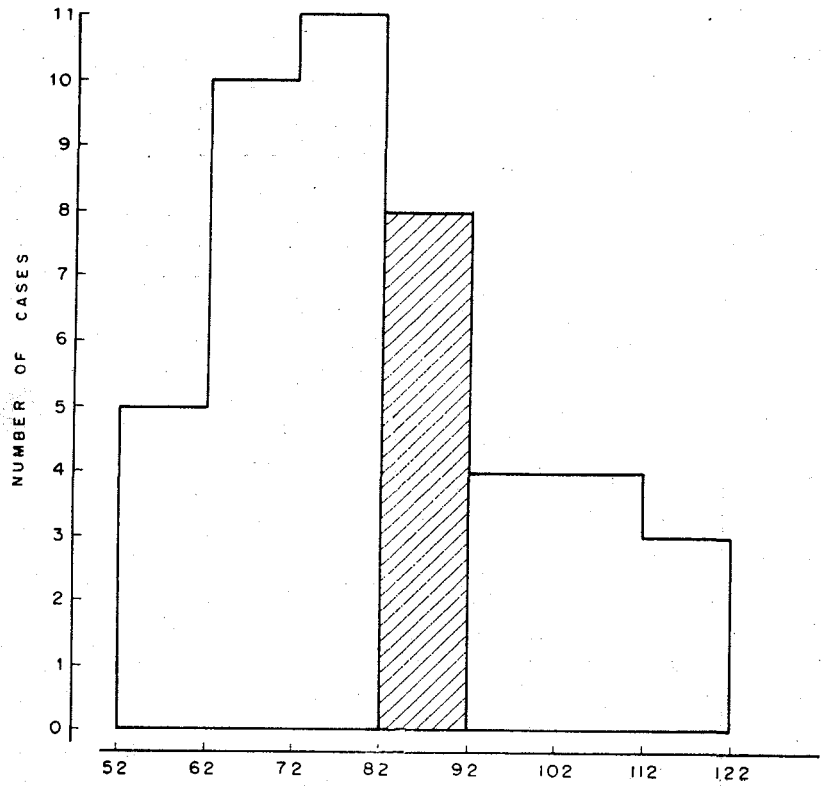

Fig. 2 — Mean corpuscular volume in μ^3 (45 cases of Kala-azar)

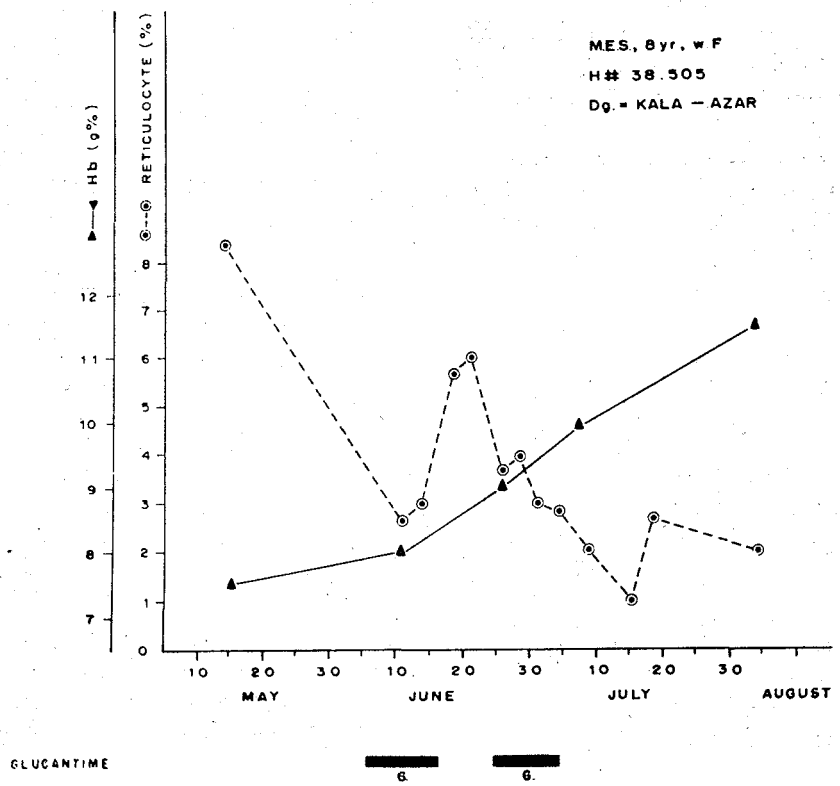

Fig. 3 — Reticulocytes response in Kala-azar

TABLE IV

Reticulocyte count in untreated Kala-azar

Author Year	Number of cases	(%)	Range
NAPIER & SHARMA (1933)	47	2.07	—
RAMOS (1955)	18	3.27	1.1 — 8.4
PRATA (1957)	15	4.20	1.5 — 13.4
SILVA (1957)	21	2.80	1.0 — 6.7
NEVES (1958)	4	4.20	2.4 — 7.3
SWARUP et al. (1958)	20	5.53	0.8 — 15.5
MARTINS et al. (1963)	6	4.50	1.7 — 7.5

of 1.7% to 7.5%. The counts became normal after therapy. Figure 3 shows a typical reticulocyte response to Kala-azar specific treatment.

MOST & LAVIETES [21] observed a significant rise in the reticulocyte count appearing shortly after the onset of therapy with the peak occurring in 2 weeks. We did not find this type of response.

6 — RATE OF DEVELOPMENT OF ANEMIA

Anemia is one of first appearing symptoms of Kala-azar. MOST & LAVIETES [21] analysed the blood counts done in 34 american soldiers, infected during the Second World War, from the first symptoms up to the establishment of the diagnosis few months later. They noted that the anemia was present in 6 cases that had the cell counts done within 10 days after the symptoms appeared. A considerable degree of anemia developed in the early stage of the illness, but did not progress to extreme values. CARTWRIGHT et al. [5] also observed that a remarkable degree of anemia was present shortly after the onset of symptoms with a gradual progression, if not treated.

It is difficult to correlate the degree of anemia and the duration of disease, because the majority of patients do not know when symptoms began. In the present series no relationship was found between the duration of symptoms and the anemia, which was practically unchanged for over one year. We must emphasize that all cases, but one arrived at the Service within one or more than one month after the onset of symptoms, when a significant degree of anemia is expected (Table V).

TABLE V

Relationship between the degree of anemia and the duration of symptoms in 161 cases of Kala-azar

Duration of symptoms (in months)	Number of cases	RBC (X 10^3 per mm^3)	Hb (g%)
0 — 4	56	2,579	7.34
4 — 8	60	2,624	7.70
8 — 12	31	2,511	7.22
> 12	11	2,580	7.50

7 — RELATIONSHIP BETWEEN THE DEGREE OF ANEMIA AND THE AGE, SEX OF THE PATIENT AND THE SIZE OF SPLEEN

7.1 — LAVERAN [14] and NAPIER & SHARMA [25] observed that anemia in Kala-azar was more severe in children than in adults. ALENCAR & CARNEIRO [1] noted that in childhood hypochromic anemia was seen more frequently. This was explained by depletion of the iron stores due to alimentary deficiency.

The mean value for the red blood cell count and hemoglobin determination done in 124 untreated patients with Kala-azar under the age of ten was 2,506,000 per mm^3

and 7.22 g% and in 39 patients over 10, was 2,806,000 per mm³ and 8.20 g% (Table VI). Statistical analysis showed there is a relationship between the degree of anemia and the age, but this relationship can not be measured after the present data.

TABLE VI

Relationship between the degree of anemia, age, sex of patient in 163 cases of Kala-azar

		Number of cases	RBC (X 10^3 per mm³)	Hb (g%)
Age (years)	0 — 10	122	2,506	7.2
	> 10	38	2,809	8.2
Sex	Male	96	2,602,4	7.56
	Female	64	2,542,9	7.28

7.2 — NAPIER & SHARMA [25] noted that the anemia was more severe in women. The mean values for the red blood cell count and hemoglobin determination performed in 64 women was 2,543,000 per mm³ and 7.28 g% compared with 2,602,000 per mm³ and 7.56 g% of 99 men. Statistical analysis also showed a relationship between anemia and sex that could not be measured with the present findings.

In normal condition the red blood cell count and the hemoglobin determination are lower in females than in males during the fertile period of life. This physiological factor probably did not affect our hematogical mean values because only 6 women were over the age of 12; all them had either amenorrhea or normal menstruations.

7.3 — According to CARTWRIGHT et al. [5] a moderate degree of anemia is present when the spleen becomes palpable and progress with the spleen enlargement. A relationship between the size of spleen and degree of anemia was present in our study (see Fig. 4).

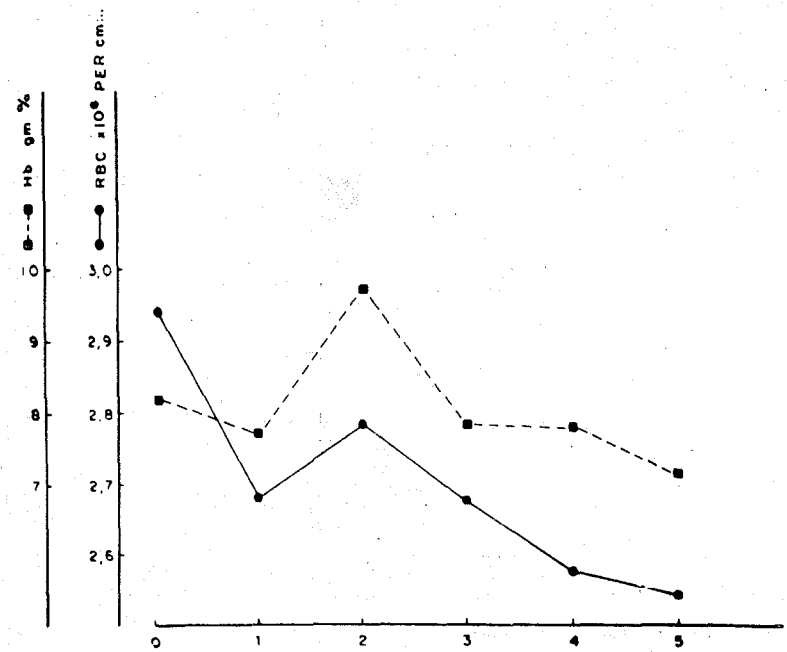

Fig. 4 — Size of spleen (Hackett). Relation between the size of spleen and the red blood cell count and hemoglobin.

8 — PATIENTS WITH FATAL OUTCOME

8.1 — Eleven cases died of the following causes: cachexia, bacillary disentery, heart failure and undetermined. The red blood cell count and hemoglobin determination were performed in 10 untreated patients with the mean value of 1,942,000 per mm³ and 6.1 g%. Eight patients died before Glucantime R was administered and only one was resistant to chemotherapy.

The degree of anemia was more severe in patients with fatal outcome than in the whole group. Blood loss could not be blamed as the main cause of the anemia because only 2 patients had intestinal bleeding due to bacillary disentery. Purpura was present in 3 cases but not severe enough to explain the degree of anemia and there was no history of blood loss in the remainder patients.

We feel that the severity of anemia in some patients with fatal outcome can be explained by a more severe protozoal infection.

9 — ASSOCIATION OF KALA-AZAR WITH OTHER PARASITARY DISEASES

9.1 — *Kala-azar and ankylostomiasis* — Ankylostomiasis produces an iron deficiency anemia due to chronic blood loss through the intestinal tract. The problem has been reviewed by LAYRISSE et al. [15] with radiochromium technic. They showed that a variable amount of blood can be lost through the bowels according to the degree of infestation. Kala-azar is frequently associated with ankylostomiasis and one would expect the aggravation of the anemia with this association.

The mean value for the red blood cell count performed in 46 patients with Kala-azar and ankylostomiasis was 2,534,000 per mm³ with standard deviation of 679.2 and in 28 with negative feces examinations was 2,658,500 per mm³ with standard deviation of 707.1. Analysis revealed that the hookworm increases the anemia of Kala-azar, but the increment was statistically insignificant with CRAME index of association of $p = 0.0184$ (Table VII).

9.2 — *Kala-azar and schistosomiasis* — The association of Kala-azar with schistosomiasis is seen frequently at the areas where the diseases are found endemically. RAMOS [30] refers 6 out of 18 cases, PRATA [27] 9 out of 22, NEVES [26] 5 out of 13 and, in our experience, only 3 cases of Kala-azar had schistosomiasis.

The Brazilian authors figures of the association of the 2 parasitosis are presented in the Table VIII. Only RAMOS [30] found the mean values for the red blood cell count and hemoglobin determination lower than the mean value of the whole group. PRATA [27] states that there is a parasitary competition making anemia milder in the association of Kala-azar with schistosomiasis.

TABLE VII

Hematological data in Kala-azar with and without ankylostomiasis

	Number of cases	RBC (X 10^3 per mm³)	Hb (g%)	Hematocrit (%)	MCH (yy)	MCV (μ^3)	MCHC (%)
Kala-azar with Ankylostomiasis	46	2,534	7.0	19.7 *	28.2	77.9 *	34.0 *
Kala-azar without Ankylostomiasis	28	2,658,5	7.6	23.5 **	29.3	91.6 **	31.4 **

* Mean value of 25 cases
** Mean value of 9 cases

TABLE VIII

Hematological data in Kala-azar associated with schistosomiasis

Author Year	Number of cases	RBC (X 10^3 per mm^3)	Hb (g%)	Hematocrit (%)	MCH (yy)	MCV (μ^3)	MCHC (%)
RAMOS (1955)	6	2,510	6.56	20.83	26.61	83.5	31.91
PRATA (1957)	9	3,111	7.64	25.20	23.70	80.6	29.50
NEVES (1958)	5	3,200	10.18	28.00	30.30	85.9	33.90
MARTINS et al. (1963)	3	2,583	7.5	24.00 *	29.10	88.8 *	27.00 *
Mean value	—	2,904	7.89	24.56	26.59	83.0	31.11

* 1 case

10 — BONE MARROW STUDIES

Several studies have been done on bone marrow changes in Kala-azar [5, 6, 22, 26, 27, 30, 35, 40]. Most of the authors agree that there is a reticulo-endothelial and erythroid hyperplasia. Other common findings are: maturation arrest of the granulocytic series at metamyelocyte and stab levels, absence or decrease of the eosinophils, asynchronism in the maturation of the nucleus and cytoplasm and abnormality in the nucleus of the orthochromatics. Normoplasia or hyperplasia is the rule, but hypoplasia or aplasia may be observed in severe cases.

The hyperplasia of the erythrocytic series is a common finding in all studies. Some authors feel that there is a block in the maturation at the polychromatic normoblast level. MOURÃO [22] and SILVA [35] did not find this block in their studies where orthochromatic was prevalent in number. Erythroid hyperplasia was present in 7 out of 9 patients that we made differential bone marrow count. Orthochromatic was also prevalent in number and nucleus changes were seen frequently.

11 — THE TREATMENT OF THE ANEMIA OF KALA-AZAR

11.1 — The treatment of the anemia of Kala-azar is directed to the destruction of the leishmania bodies. Improvement of the anemia after hospitalization due to adequate diet, rich in blood maturation factors, has been referred by NAPIER & SHARMA [25]. Iron, vitamin B12, folic acid, proteins, blood transfusions, etc., may be used with advantage, but they are of no value without specific therapy.

11.2 — *Antimony therapy* — We have used with success the N-methyl glucamine antimoniate (Glucantime R) with daily doses of 75 to 100 mg per kg of body weight, during 10 days, by intramuscular or intravenous route. The medication can be repeated after an interval of 10 days. In general, 1 to 4 series are necessary for cure of the patient. Cure is based in the clinical improvement, normalization of the blood count and disappearence of *L. donovani* from the bone marrow. In the Figure 5 we can see the red blood cell count and the hemoglobin determination response in one case of Kala-azar treated with Glucantime R.

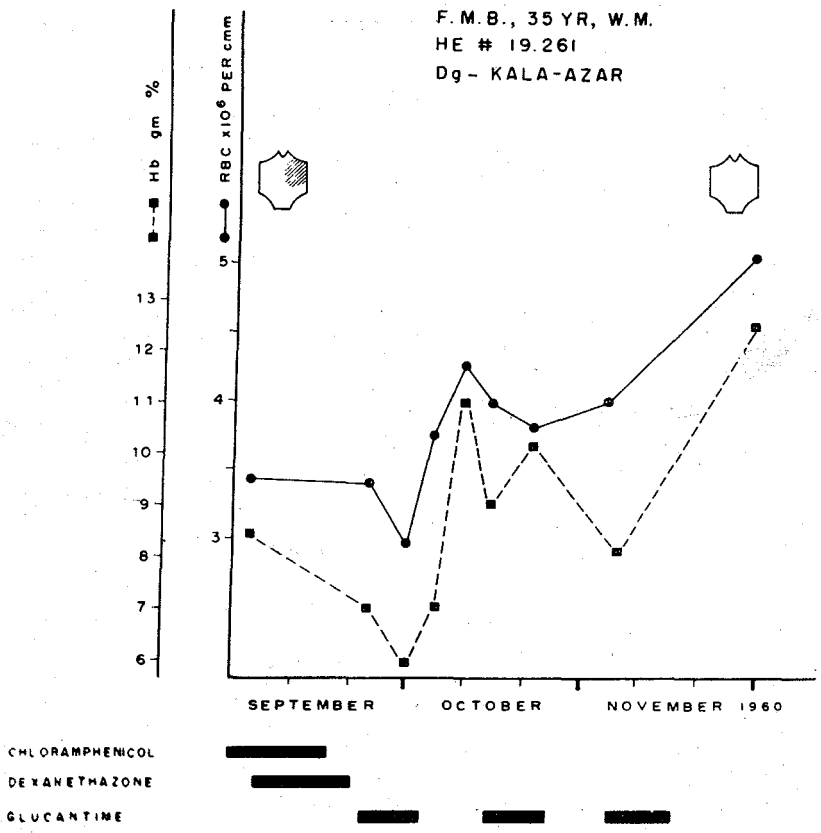

Fig. 5 — Red blood cell and hemoglobin response to specific therapy (Glucantime [R]) in Kala-azar. No blood maturation factors were given to the patient.

11.3 — *Pentamidine* — Pentamidine (Lomidine [R]), an aromatic diamidine, was employed in cases with antimony resistance. The recommended daily dose is 3-4 mg per kg of body weight, given by intramuscular route, every other day performing a total of 15 injections.

11.4 — *Amphotericin B* — Amphotericin B (Fungizone [R]), an antibiotic largely employed in the deep disseminated mycosis has been used experimentally by brazilian authors [2, 8, 28, 34], in cases of leishmaniasis. It was first tried in resistant cases of american cutaneous leishmaniasis [8, 34], with great success. PRATA [28], in the State of Bahia, Brazil and ALENCAR et al. [2] among us, used the drug in Kala-aar, with good results. Amphotericin B is given daily or every other day, in the dose of 1 mg per kg of body weight, dissolved in dextrose at 5%, in slow intravenous drip. Antipyretics must be used in order a to avoid hyperthermia and steroids may be added to the intravenous fluid in order to prevent phlebitis. The total doses in the cases of PRATA [28] and ALENCAR et al. [2] varied from 226.5 to 1,255 mg given in a variable period of 2 to 3 months. In all cases there was cure of the disease.

Disadvantages of Amphotericin B are the prolonged intravenous drop and the untowards effects of fever, nausea, vomiting, phlebitis and renal failure with raise in the blood urea nitrogen. Due to great Glucantime [R] efficacy and Amphotericin B side reactions, this antibiotic must be used only when antimony salts and aromatic diamidine failed. Figure 6 shows the hemoglobin and the red cell count performed in a 5 year-old-boy with Kala-azar resistant to an-

timony and pentamidine therapy. After 358,5 mg of Amphotericin B the patient improved, the clinical symptoms subsided, the spleen diminished in size, the hemoglobin raised from 5 to 11 g% and the leishmania bodies disappeared from the bone marrow.

11.5 — Improvement of anemia is noted after the beginning of specific therapy, and may be present even before bone marrow and pancytopenia that did not answer to anti-leishmania drug.

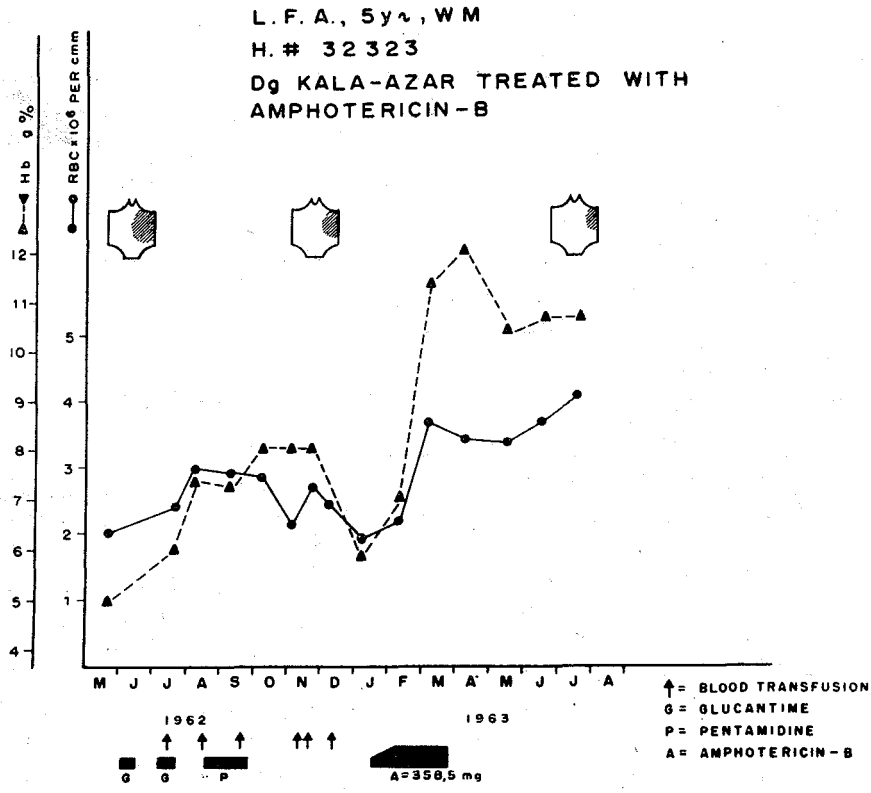

Fig. 6 — Red blood cell and hemoglobin response to Amphotericin B in a resistant case to Glucantime R and Lomidine R

11.4 — *Splenectomy* — Splenectomy was performed for the first time in a case of Kala-azar in 1910 by SOUZA [37] in Portugal, before the era of antimony therapy. Even after specific drugs appeared, the portuguese surgeon continued to perform splenectomies in search of the cure of anemia and not for the "disappearance of the parasite from bone marrow, liver and peripheral blood". SOUZA [36] reviewed the records of the Pediatric Service of the School of Medicine of Lisboa and reported that, among 38 splenectomies in Kala-azar, 27 were cured.

At the present time, splenectomy is limited to the cases with persistant splenomegaly becomes negative. Red blood cell count and hemoglobin determination were done in 65 patients between 1 to 2 months after the beginning of treatment, in 32 between 2 to 4 months and in 11 cases between 4 to 6 months. There was a raise of 34% from the mean value for the red blood cell count in the period 1 to 2 months after starting therapy and of 33% in the hemoglobin determination. The raise in the 2nd to 4th month-period and in the 4th to 6th month-periods were of 49 and 55% for the red blood cell count and of 48 and 73% for hemoglobin determination. (Fig. 7 and Table IX).

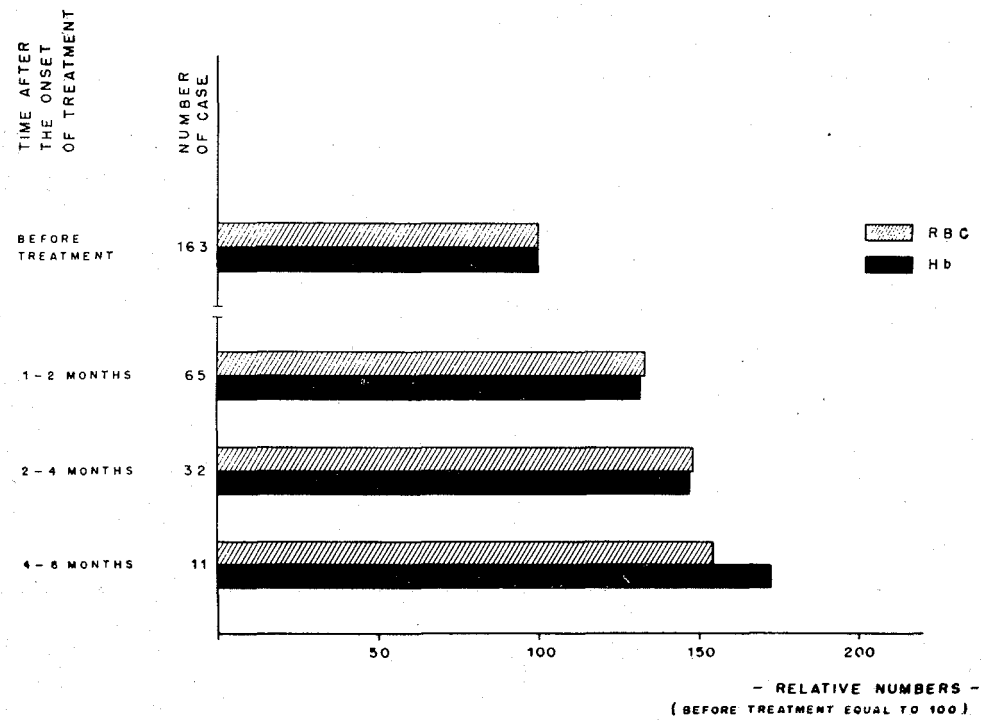

Fig. 7 — Hematological changes following specific therapy

TABLE IX

Hematological data following specific therapy

Months after the onset of therapy	Number of cases		RBC (X 10^3 per mm^3		Hb (g%)	
	Absolute numbers	Relative numbers	Absolute numbers	Relative numbers	Absolute numbers	Relative numbers
0	163	100	2,578,6	100	7.45	100
1 — 2	65	41	3,460,0	134	10.00	133
2 — 4	32	20	3,853,0	149	11.10	148
4 — 6	11	7	4,015,0	155	13.00	173

Six patients had the blood count performed after 6 months of the beginning of specific drugs. They were excluded from the study because only serious cases with severe anemia had such a prolonged hospitalization.

In spite of the remarkable improvement of the anemia with therapy, a complete normalization of the blood count was not achieved in most of the patients. A slight degree of anemia was always present at the time of the hospital discharge. GIRAUD et al. [10] found an inconstant and usually mild degree of anemia in a great number of cases clinically cured for a variable period of 8 months to 12 years. They felt that Kala-azar may leave profound and lasting disturbances in the bone marrow and in the whole reticulo-endothelial system.

12 — PATHOGENESIS OF THE ANEMIA OF KALA-AZAR

Several theories were proposed to explain the pathogenesis of the anemia of Kala-azar. The anemic state may be due just to one cause, but it is most likely to be the result of several conjoined factors.

12.1 — *Blood volume* — MURANO & SAGGESE [23] were the first investigators to call the attention for the blood volume changes in Kala-azar. They noted an increase in the blood volume at the expenses of the plasma volume. The anemia of Kala-azar was supposed to be more apparent than real, as it happens in schistosomiasis [19]. This must be confirmed because only few studies have been done in this field.

12.2 — *Hypochlorhydria* — VOUDOURIS [42] found hypochlorhydria in the majority of cases of Kala-azar, which subsides with specific drugs. It has been claimed that hypochlorhydria may be one factor in the development anemia, due to the lack of iron absorption from the intestinal tract.

12.3 — *Iron* — GALLO [9], in 1905, made iron determinations in the whole blood and tried to explain the anemia of Kala-azar in the iron deficiency basis. The problem was reviewed in 1948 by TRINCÃO [39, 40] who found normal or increased values for serum iron in 16 patients with visceral leishmaniasis. Based on his findings the portuguese hematologist excluded iron deficiency as the cause of anemia of Kala-azar.

SWARUP et al. [38] did iron determinations in a group of patients with Kala-azar in India. They found that in the average case the serum iron is low, the iron binding capacity is depressed and the oral absorption is decreased. This is the pattern observed in chronic infection.

Serum iron was estimated in 4 untreated cases: slightly increased values were found (Table X). Weekly determinations of the serum iron done in those patients showed a progressive decrease to normal levels. Figure 8 shows a typical fall of the serum iron.

TABLE X

Serum iron in 4 patients with Kala-azar

Patient, age and sex	Serum iron (μg%)	
	Before treatment	After treatment
J.F.L., 13 years, Male	172	149
M.E.S., 8 years, Female	169	150
L.F.A., 19 years, Male	150	152
R.J.E.S., 6 years, Male	157	147

Lack or iron would be unlike to be present in those cases. In iron deficiency anemia one would expect low serum values increasing slowly to normal, and the opposite pattern was found.

It is difficult to give an explanation for the fall in serum iron. MARTINS et al. [18] showed an increase in serum glutamic oxalacetic transaminase in 80% of cases of Kala-azar with higher values before treatment and tendency to become normal with cure. They felt that the increase in the enzyme was due to slight degree of liver cells necrosis that subsides with treatment. As serum iron can increase in the same fashion as transaminase in liver cell necrosis, the same explanation can be given to the return to the normal of the serum levels [31, 32].

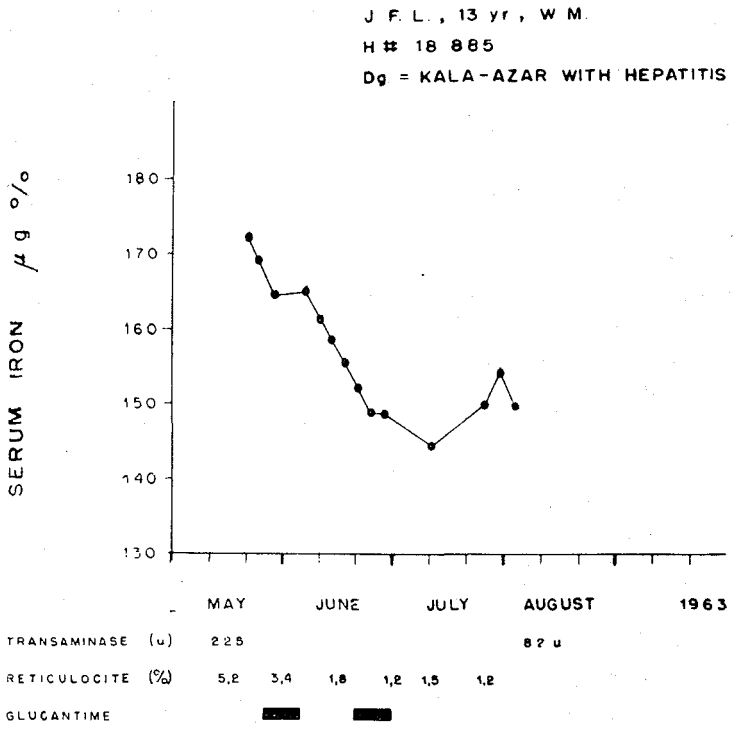

Fig. 8 — Serum iron in Kala-azar

Serum iron is increased in hemolytic anemia. SWARUP et al.[38] based on the reticulocytosis and normoblastic hyperplasia of the bone marrow believed that a hemolytic component must be present in cases of Kala-azar; this disappears with treatment. The return to normal of serum iron levels may as well be explained on the disappearance of the disease. In our cases reticulocytosis and erythroid hyperplasia were present before the onset of specific therapy and disappeared with cure.

12.4 — *Hyperhemolysis* — Based on the enlargement of the spleen, increase of indirect bilirubin, urobilinuria and reticulocytosis, NAPIER & SHARMA[25] and NAPIER[24] stated that hyperhemolysis is an important causative factor of the anemia of Kala-azar. RECHMILEWITZ et al.[29] agree with them and explain the hemolysis under the hypersplenism of DOAN and WRIGHT. More recently SWARUP et al.[38] using modern technics demonstrated the existence of an hemolytic component in 13 out of 21 cases. They felt that in some patients the hemolysis developped only on the basis of an auto-immune mechanism and in others it was probably hypersplenic in origin.

CARTWRIGHT et al.[5] felt that the increase in bilirubin and urobilinuria may be explained under spleen enlargement and liver cell damage. Hemolysis would explain only the hematologic changes present in the erythrocytic series but never in the granulocytic and thrombocytic series.

We made red cell osmotic fragility study in 14 patients, 7 before and 7 after treatment (Fig. 9). In all but one it was within the normal limits. One patient resistant to antimony and pentamidine therapy presented an increased osmotic fragility shortly after Amphotericin B treatment that slowly returned to normal. This increased osmotic fragility was correspondent to a reticulocytosis of 17% (Fig. 10).

Hyperhemolysis probably is not the chief mechanism responsible for the Kala-azar anemia but an hemolytic component is actually present in a great number of cases.

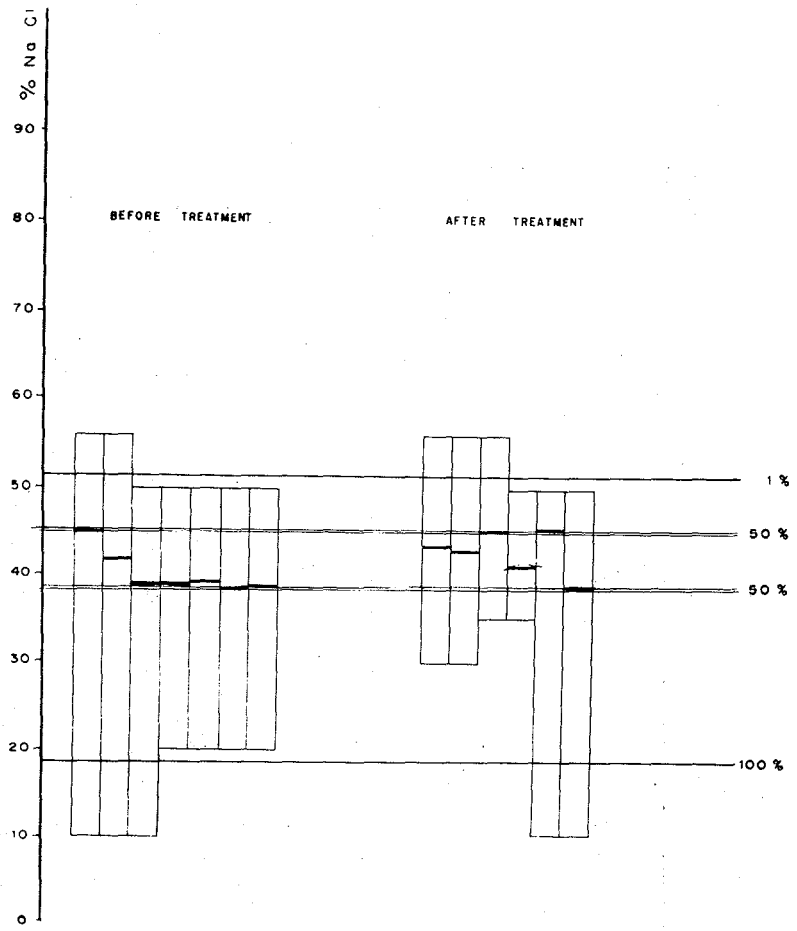

Fig. 9 — Osmotic fragility in 13 patients with Kala-azar (before and after treatment).The result of the patient L.F.A. is presented in the figure 10. Each bar is one patient; 1% of hemolysis is the top line of the bar, 100% the lower line and 50% the thick line. The horizontal lines represent the mean value for 1% and 100% of hemolysis determined in 9 normals. The two double horizontal lines represent the upper and the lower normal limits for 50% of hemolysis.

12.5 — *Hypersplenism* — LEE & CHUNG [16], CARTWRIGHT et al. [5] and TRINCÃO [40] agree that the pancytopenia of Kala-azar can be explained under DAMESHEK hypersplenic mechanism. Peripheral blood cytopenia with bone marrow hyperplasia, progressive enlargement of the spleen followed by worsening of the anemia, maturation arrest of the erythrocytic and granulocytic series and improvement of the blood count after splenectomy strengths this idea. Aplastic anemia and agranulocytosis observed in severe cases would be due to progressive myelodepressive action of the spleen.

Brazilian authors [11, 22, 35] do not accept hypersplenism as the cause of pancytopenia. SILVA [35] did not find the maturation block in the erythrocytic series and MOURÃO [22] stated that there is not an arrest in the granulocytic series. Even though we found a relationship between the size of spleen and the anemia, we think that both, anemia and spleen enlargement, are the expression of the progression of the disease. The improvement of the pancytopenia after splenectomy can be explained under the "release crisis" of the bone marrow observed after spleen removal in cases of aplastic anemia [22]. Fur-

thermore the concept of hypersplenism is changing in the current days. "Pure cases" of hypersplenism such as acquired hemolytic anemia and idiopatic thrombocytopenia purpura frequently recur after splenectomy [11].

12.6 — *Hyperplasia of the reticulo-endothelial system* — A great degree of reticulo-endothelial hyperplasia is present in human and experimental cases of visceral leishmaniasis. This hyperplasia, by obstruction, would give a reduction or complete absence of hematopoiesis, developing a myelophtisic anemia — in the same way of bone marrow carcinomatosis. This mechanism has not been accepted by all authors. MOURÃO [22] believes that hyperplasia, as a single factor, would never produce a mechanic obstruction of the function because hyperplasia without cellular dysfunction would result in benefit to hematopoiesis.

MEIRA et al. [20] in 1948 reported a case of complete aplasia of the bone marrow. Studying the problem they felt that the hyperplasia of the reticulo-endothelial system would change the reticulum cells from their normal blood formating function into phagocytosis and plasmocytic formation. The parasited reticulum cell fighting against leishmania would loss their normal cytoevolutive and hematopoietic function, leading to pancytopenia.

12.7 — *Conclusion* — In the pathogenesis of the anemia of Kala-azar several factors may be present. It has been felt that the chronic diffuse and focal infection of the bone marrow and the removal of the reticulum cell from its normal blood formating function are the principal mechanisms. An hemolytic component may be present in most

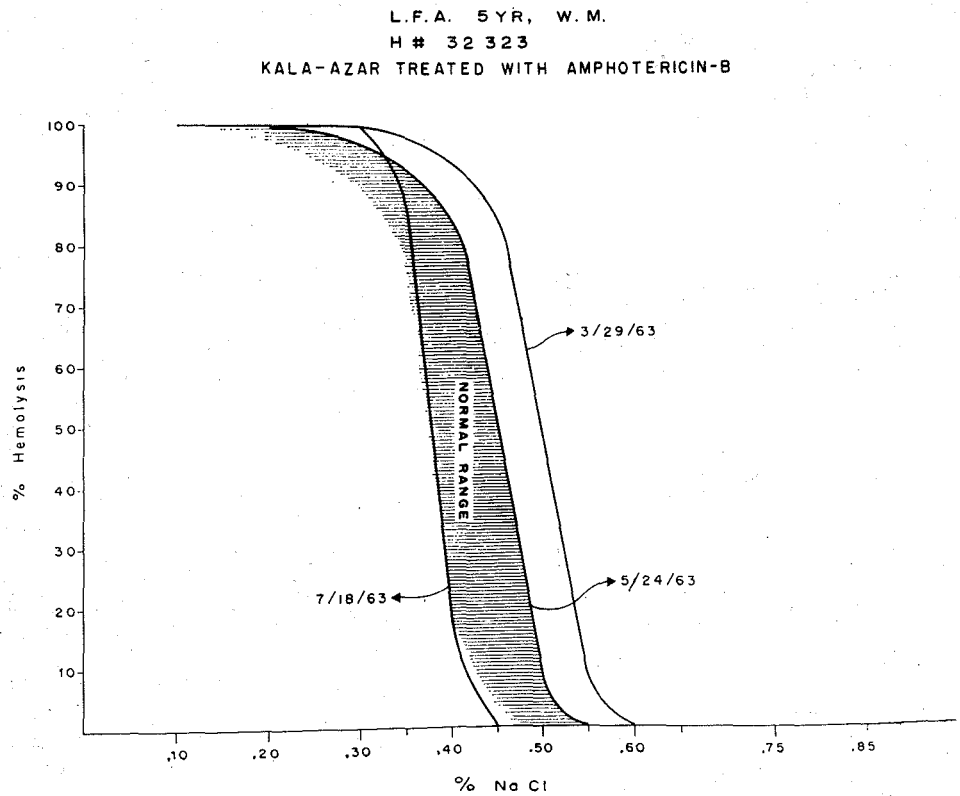

Fig. 10 — Osmotic fragility in Kala-azar. Note that the patient had an increased osmotic fragility shortly after finishing the treatment with Amphotericin B in March 29, 1963, with slow return to normal.

of the cases. Further studies on blood volume and radioisotopes technics must be done in order to explain some unsolved questions.

RESUMO

Anemia no calazar

Os autores observaram 190 casos de calazar no Instituto de Medicina Preventiva da Universidade do Ceará, em período de 6 anos.

Os valôres hematológicos médios encontrados antes do tratamento foram os seguintes: hemácias 2.578.600 por mm³, hemoglobina 7,45 g% (163 casos), hematócrito 22,1% (45 casos) e reticulócitos 4,5% (6 pacientes). Havia hemácias nucleadas em 13 enfêrmos. A presença de anemia microcítica alcançou 57,7% dos casos. Estudo da medula óssea revelou hiperplasia da linhagem vermelha.

Não se observou relação entre o grau de anemia e a duração da doença. A anemia era mais intensa nos indivíduos com menos de 10 anos, nas mulheres e nos pacientes com grande esplenomegalia.

A anemia era mais acentuada nos pacientes que tiveram êxito letal. Os valôres médios encontrados em 10 pacientes foram: 1.942.000 por mm³ para as hemácias e 6,1 g% para a hemoglobina.

A anemia era mais grave ainda no grupo de pacientes com calazar e ancilostomose, enquanto que era menor na associação calazar e esquistossomose.

Os AA. fizeram revisão do tratamento do calazar, dando ênfase à Anfotericina B. Discutiram a patogenia da anemia do calazar e acreditam que vários fatôres contribuem para o seu desenvolvimento.

REFERENCES

1. ALENCAR, J. E. & CARNEIRO, P. A. M. — Índices eritrocitários no diagnóstico do calazar. *Paper presented before the XII Brazilian Congress of Hygiene.* Fortaleza, 1956.

2. ALENCAR, J. E.; MAGALHÃES, V. B. & SAMPAIO, V. D. — Tratamento de 3 casos de calazar pela Anfotericina B. *Rev. brasil. med.* 19:669-678, 1962.

3. BARKAN, G. & WALKER, B. S. — *Apud* SIMMONS, J. S. & GENTZKOW, C. S. — *Medical and Public Health Laboratory Methods.* Philadelphia, Lea & Febiger, 1956, p. 417.

4. BURCHENAL, J. H.; BOWERS, R. F. & HAEDICKE, T. A. — Visceral leishmaniasis complicated by severe anemia; improvement following splenectomy. *Am. J. Trop. Med.* 27:699-709, 1947.

5. CARTWRIGHT, G. E.; CHUNG, H. L. & CHANG, A. — Studies on the pancytopenia of Kala-Azar. *Blood* 3:249-275, 1948.

6. COUTINHO, A. & LIRA, A. — Leishmaniose visceral em Pernambuco: considerações clínicas e hematológicas sôbre um nôvo caso. *Rev. brasil. med.* 11:89-97, 1954.

7. DACIE, J. V. — *The haemolytic anaemias; congenital and acquired.* London, J. A. Churchil, 1954. *Apud* MIALE, J. — *Laboratory Medicine. Hematology.* St. Louis, Mosby, 1958.

8. FURTADO, T. A. — Resultados clínicos obtidos pela Anfotericina, administrada por via oral e intravenosa, no tratamento da Leishmaniose americana. *In Antibiotics Annual,* 1960. Medical Encyclopedia, New York, 1959-1960, p. 631-637.

9. GALLO, C. — Ricerche sul contenuto in ferro del sangre nelle anemie spleniche e nella leishmaniosi infantile. *Pediatria* 33: 251-256, 1925.

10. GIRAUD, P.; ORSINI, A. & Mme. ANSALDI — Estudio sobre el futuro hematológico de los enfermos convalecientes de leishmaniosis visceral (Kala-azar). *Sangre* 6:354-360, 1961.

11. JAMRA, M. — Patogenia das alterações hematológicas da leishmaniose visceral (Calazar). *Rev. Assoc. med. brasil.* 7:170-177, 1961.

12. KEEFER, C. S.; KHAW, O. K. & YANG, C. S. — The anemia of Kala-Azar with special reference to treatment. *Nat. med. J. China* 15:731-742, 1929.

13. KUROYA, M.; YUONG, S.; TANG, L. C. & HONG, L. Y. — Kala-azar in Pi-Hsian District, Kiangsu Province, China; blood picture and sedimentation rate of red blood cells in Kala-azar. *J. Shanghai Sc. Inst.* 4(Sect. 4):165-227, 1939.

14. LAVERAN, A. — *Leishmaniosis.* Paris, Masson, 1917. *Apud* NEVES, 26.

15. LAYRISSE, M.; BLUMENFELD, N.; DUGARTE, I. & ROCHE, M. — Vitamin B_{12} and folic acid metabolism in Hookworm-infected patients. *Blood* 14:1269-1279, 1959.

16. LEE, C. V. & CHUNG, H. L. — A clinical study of early manifestations of chinese Kala-azar. *Chinese M. J.* **49**:1281-1300, 1935.

17. LINS, F. M. — Leishmaniose visceral infantil em Pernambuco; aspectos clínicos, hematológicos e electroforéticos. *An. Fac. Med. Univ. Recife* **18**:215-239, 1938.

18. MARTINS, J. M.; FONTENELE, Z. & ALENCAR, J. E. — Estudo da transaminase glutâmico-oxalacética no calazar. *Hospital* (Rio de Janeiro) **61**:123-133, 1962.

19. MEIRA, J. A. — *A esquitosomiase hépato-esplênica.* Thesis, São Paulo, 1951.

20. MEIRA, J. A.; JAMRA, M. & LIMA, M. L. M. T. — Leishmaniose visceral americana. Considerações clínicas, hematológicas e anátomo-patológicas a propósito de um caso. *Arq. Fac. Hig. Saúde Púb.* **2**:253-300, 1948.

21. MOST, H. & LAVIETES, P. H. — Kala-azar in american military personnel. *Medicine* **26**:221-284, 1947.

22. MOURÃO, O. G. — *A medula óssea no calazar.* Thesis, Belo Horizonte, 1960.

23. MURANO, G. & SAGGESE, M. — L'emovolumetria nella leishmaniosi interna. *Bol. Soc. ital. Biol. Sper.* **23**:389-393, 1947.

24. NAPIER, L. E. — *The Principle and Practice of Tropical Medicine.* New York, Mac Millan, 1946.

25. NAPIER, L. E. & SHARMA, L. R. — The anemia of Kala-azar. *Indian M. Gaz.* **68**: 545-554, 1933.

26. NEVES, J. — *Calazar em Minas Gerais.* Thesis. Belo Horizonte, 1958.

27. PRATA, A. — *Estudo clínico e laboratorial do calazar.* Thesis, Salvador, Fundação Gonçalo Moniz, 1957.

28. PRATA, A. — Treatment of Kala-azar with Amphotericin B. (Em publicação).

29. RACHMILEWITZ, M.; BRAUN, K. & VRIES, A. de — Hematologic observations in a case of Kala-azar. *Blood* **2**:381-385, 1947.

30. RAMOS, H. — *Contribuição para o estudo do mielograma na leishmaniose visceral.* Thesis. Rio de Janeiro, 1955.

31. REISSMANN, K. R.; CHRISTIANSON, J. F.; BOLEY, J. O. & KITTLE, C. F. — The relation of serum iron changes, hepatic function, and histologic findings in experimental hepatic injuries of various etiologies. *Surgery* **37**:738-753, 1955.

32. REISSMANN, K. R.; BOLEY, J.; CHRISTIANSON, J. F. & DELP, M. H. — The serum iron in experimental hepatocellular necrosis. *J. Lab. & Clin. Med.* **43**:572-582, 1954.

33. ROGERS, L. — The diagnostic and prognostic value of leukopenia of cachexial fever and Kala-azar. *Brit. Med. J.* **1**:750, 1905. *Apud* NEVES, 26.

34. SAMPAIO, S. A. P.; GODOY, J. T.; PAIVA, L.; DILLON, N. L. & LACAZ, C. S. — The treatment of American (Mucocutaneous) leishmaniasis with Amphotericin B. *Arch. Dermatol.* **82**:627-635, 1960.

35. SILVA, J. R. — *Leishmaniose visceral (Calazar).* Thesis. Rio de Janeiro, Serviço Nacional de Educação Sanitária, 1957.

36. SOUZA, C. S. de — *Apud* TRINCÃO, 40.

37. SOUZA, J. S. de — Kala-azar infantile à Lisbonne. *Med. contemp., Lisboa,* **28**:91, 1910.

38. SWARUP, S.; CHATTERJEA, J. B. & SEN GUPTA, B. C. — Haemolysis in Kala-azar. *Proc. VII Congr. Int. Soc. Hematol.* **2**:1593-1598, 1958.

39. TRINCÃO, C. — A sideremia no Kala-azar. *An. Inst. Med. trop., Lisboa* **5**:131-134, 1948.

40. TRINCÃO, C. — *Sindroma anemico de Kala-azar.* Lisboa, Soc. Ind. Tipográfica, 1948.

41. VERONESI, R.; JAMRA, M.; SILVA, O. R. S.; CRUZ, O. & FIORILLO, A. — Leishmaniose visceral (Kala-azar). Estudo do quadro clínico, hematológico e electroforético. *Rev. Hosp. Clin. Fac. Med. Univ. São Paulo* **9**:13-50, 1954.

42. VOUDOURIS, E. — Le chemisme gastrique dans le Kala-azar infantile et ses modifications sous l'influence du traitement. *Rev. franc. Pediat.* **12**:96-101, 1936.

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