THE CLINICAL AND HAEMATOLOGICAL RESPONSE TO TREATMENT IN CHILDREN WITH KALA AZAR.

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by

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D E C L A R A T I O N

This Dissertation is my original work and has not been presented for a Degree in any other University.

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This Dissertation has been submitted for examination with my approval as University Supervisor.

Signed N.O.Bwibo----------

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S U M M A R Y

This is a prospective study of eighteen children with kala azar (Visceral Leishmaniasis) seen at the Kenyatta National Hospital, Nairobi. The clinical and haematological data before and for each week during treatment is presented and discussed.

Sixteen patients were given the standard dose of pentostam. The treatment was continued daily without interruption until the patients were parasitologically negative on splenic aspirates. Only one out of these sixteen patients failed to respond after twelve weeks of pentostam. Two patients known to be resistant to pentostam were treated with a combination of pentostam and allopurinol.

From this study, it is apparent that patients respond differently to treatment with pentostam. The duration of treatment ranged from four to eleven weeks. Each case of kala azar should therefore be viewed on its own merits as far as duration of treatment is concerned,. Complete cure can only be determined with time, it is recommended that patients should be closely followed up after discharge from hospital.

I N T R O D U C T I O N

Kala azar in children, particularly the East African kala azar, is very difficult to manage. The disease is unusually refractory to treatment. It has been observed that inadequate or unsuccessful treatment with antimony of any patient with kala azar, may subsequentl render that particular case of infection less amenable to antimony (1). If interrupted or subcurative antimony treatment is continued a stage is reached when the infection becomes completely refractory to the maximum tolerated dose of the drug (1). Therefore, when a therapeutic course of treatment with antimony is begun, it should be continued to its completion without interruption.

The majority of kala azar patients improve clinically on commencement of treatment with antimony (2). Thus, often patients are discharged from hospital without being confirmed parasitologically negative of infection. A number of such patients come back within a few weeks to about three months with the same infection. Such patients when discharged and not sufficiently followed up, are likely to be wrongly diagnosed as relapses or re-infections. To my knowledge, there has been no study in Kenya to show the response to continous treatment with Sodium antimony gluconate (pentostam) in children with kala azar so as to delineate those

patients who are likely to be refractory to a therapeutic dose of the drug. This lack of a follow-up study in patients treated either adequately or without cure prompted this prospective study to elucidate the clinical and haematological response in Kenyan children on continuous treatment with pentostam.

MATERIALS AND METHODS

Patient Collection

Only those patients with a confirmed diagnosis of kala azar were included in the study. All the patients selected were admitted in one particular ward of the Kenyatta National Hospital throughout the length of treatment. The study was began in August 1979 and follow-up of patients is still going on.

Diagnosis

For each patient a comprehensive evaluation was made which included the following:-

1. Patient identification by name, age, sex, tribe, hospital number(s) and a full residential address.
2. History which included the duration of illness, the main complaints and details of specific treatment received previously.
3. A thorough physical examination including the weight, general condition of the patient, the nutritional status as determined by the clinical impression and the weight,

and the spleen size which was staged using the Hackett's classification (3) as shown below:-

Class of Spleen	Description
0	Normal spleen not palpable even on deep inspiration
1	Spleen palpable on deep inspiration
2	Spleen palpable below the costal margin, but not projected beyond a horizontal line half way between the costal margin and the umbilicus, measured along a line dropped vertically from the left nipple.
3	Spleen with the lowest palpable point projected more than half way to the umbilicus but not below a line drawn horizontally through it.
4	Spleen with lowest palpable point below the umbilical level but not projected beyond a horizontal line situated half way between the umbilicus and the symphysis pubis.
5	Spleen with lowest point palpable beyond the lower limit of class 4.

4. Haematological Data:-

a) Haemoglobin in grammes per decilitre (Hb gm/dl).

b)= Packed Cell Volume in volumes percent (PCV%)

c) Red Cell Count in millions per millilitre($RBCx10^{12}L$)

d) Mean Corpuscular Volume in femtolitres (MCVinfl).

e) Mean Corpuscular Haemoglobin in picogrammes (MCH in pg).

f) Mean Corpuscular Haemoglobin Concentration in grammes per decilitre (MCH gm/dl).
The MCH and MCHC were calculated from the Hb, PCV, RBC and MCV counts which had been previously evaluated using the Coulter Counter Model ZB1.

g) Reticulocyte Count as a percentage of total RBC (Retic %).

h) Platelet count per cubic millilitre as described by Brecher et al (4).

i) Erythrocyte Sedimentation Rate in millimeters per hour (ESR/hr).

j) Peripheral thick film for malaria parasites.

5. Total Serum proteins in grammes per decilitre using the Biuret Method (5).

6. Cysts and ova in stool using the method described by Kato and Miura (6).

7. Mantoux test.

8. Postero-anterior x-ray view of the chest.

9. Urinalysis for proteinuria, blood and sugar.

10. The specific complement fixation test for kala azar. This test was also performed on other patients with malaria, pulmonary tuberculosis, pneumococcal meningitis and

schistosomiasis for comparison.

11. The Leishmanin skin test (Montenegro skin test), first described by Montenegro (7).

12. Confirmation of diagnosis by the demonstration of the Leishman-Donovan (LD) bodies, (the amastigote forms of the parasites), on splenic aspirate as described below.

The splenic aspirates were performed using a 21 gauge size needle and a 10 cc. plastic disposable syringe. Tiny amounts, about one drop (less than 0.1cc) of material for each aspirate were obtained. From each aspirate two thick slides were made and stained with the Giemsa stain. Some of the aspirate was injected into a tube containing the Schneider's insect liquid medium for culture as described by Hockmayer et al (8) and Hendricks et al (9,10). Following the splenic aspirates, all the patients were kept strictly in bed and observed for twenty four hours following the procedure. The blood pressure, pulse and temperature, were recorded during the observations.

Two experts working independently examined the slides for the LD bodies using a light microscope with a X100 objective. The whole of the culture medium was examined by a third expert for the mobile forms of the parasites, the promastigotes. The first examination of the culture was made after twenty four hours of incubation and then daily for seven days before being discarded . A slide was examined for at least half an hour by each of the two experts, before being declared negative . Similarly, one hour was spent examining the culture medium before being termed negative.

Treatment

Each of the patients 1-16 received pentostam; by deep intramuscular injections given once daily. The dose was culculated at 0.1ml per kilogram body weight (10mg/kg) ranging from 2ml to 4 ml a dose, as recommended by Hunter (11). Patients 17-18 were given allopurinol in combination with pentostam. Intercurrent diseases were treated by appropriate methods.

Follow-up

Weekly assessments of the patients' progress were recorded. These were the physical examination including the spleen sizes, the haematological data, the total serum proteins and the splenic aspirates. Treatment was stopped and patients discharged after two consecutive negative aspirates on both smear and culture. The patients were given the first follow-up appointment at the Parasitology Clinic six weeks after treatment was stopped, and at three monthly intervals thereafter. At each follow-up visit, a physical examination was carried out, blood was taken for the haematological investigations and stool was examined for cysts and ova.

R E S U L T S

At the time of analysis of the results, eighteen patients had been followed up long enough to provide the data presented.

TABLE I PATIENTS, AGE, SEX AND DISTRICT OF ORIGIN

PATIENTS	AGE IN YRS	SEX	DISTRICT
1	9	M	Machakos
2	11	M	"
3	10	M	"
4	8	M	"
5	6	M	Kitui
6	8	F	Machakos
7	12	F	"
8	10	F	"
9	7	M	"
10	10	M	Kitui
11	10	M	"
12	16	M	"
13	$1\frac{1}{2}$	F	Kitui
14	15	M	Machakos
15	8	M	Kitui
16	10	M	Machakos
17	$3\frac{1}{2}$	F	"
18	7	M	Kitui.

There were twelve boys and six girls. The age ranged from one and a half years to sixteen years. The two youngest children were both girls. Patients Nos. 6, 7 and 8 were sisters. Twelve of the patients came from the Machakos District and six from Kitui District.

All patients were Kambas with the exception of patient No. 9 who was a Kikuyu born and brought up in Machakos district.

Four patients, Nos. 3,13,17 and 18 had had treatment with pentostam. Patient No. 3 had been treated with an adequate dose but for only 30 days three months previously. On discharge, no investigations had been done to indicate the response to treatment.

Patient No. 13 had been treated for six weeks two months previously with only 1ml of pentostam daily. On discharge this patient had shown some clinical improvement but she was still anaemic with an Hb of 7.5gm/dl. The spleen was reported to have regressed a little but the aspirate before discharge was negative.

Patient Nos. 17 and 18 were referred from Machakos District Hospital as "resistant" cases. They had been treated for at least three interrupted courses each at different health institutions before being admitted at the district hospital where a fourth course was then started. Each course lasted thirty consecutive days but the interval between the courses was not consistent and varied from one month to about six months. Clearly, these two patients had failed to respond to pentostam and although their data is presented along with the other data, they are not included in the final analysis as they received both allopurinol and pentostam as a second line of treatment.

TABLE II DURATION ON ILLNESS

Duration	No. of Patients
Few days to 3 months	6
> 3 months but <6 months	5
> 6 months but <1 year	4
More than 1 year	1
Not known, but a long time	2
Total	18

The duration of illness was only an approximation from the history given by the attendant and/or the patient. In the majority of patients, twelve out of eighteen, the illness was of a long duration, of more than three months.

TABLE III CHIEF COMPLAINTS ON ADMISSION

Complaint	No. of Patients
Fever	14
Abd.Dist. or Swelling	12
General ill health	9
Abd. pain or discomfort	6
Cough	6
Epistaxis	6

Fever and abdominal distension or swelling were the commonest complaints. Half of the patients complained of general ill health.

TABLE IV MAIN FINDINGS ON ADMISSION

Physical Findings	No of Patients
Pallor	18
Splenomegaly	18
Hepatomegaly	18
Ill looking	8
Fever	7
Poor nutrition	6
Skin and Hair changes	
a) with poor nutrition	4
b) with good nutrition	3

All the patients had pallor, splenomegaly and hepatomegaly. Eight of the patients looked ill but the other patients were in good general health. Seven patients were actually febrile at the time of admission although fever was the commonest complaint.

The skin changes were those of general hypopigmentation or patches of hypopigmentation on the face and particularly around the eyes. The hair changes too were those of hypopigmentation with loss of lusture.

TABLE V CONCOMITANT ILLNESSES IN PATIENTS WITH KALA AZAR

Type of illness	No. of Patients
Scabies	9
Throat congestion	6
Otitis media	5
Intestinal parasites	4
Dental caries	2

Nine of eighteen patients had scabies. Throat congestion and otitis media were found in eleven patients.

Intestinal parasites were found in four patients:- Patients Nos. 6, 7 and 8, the sisters, had both hookworm and Schistosoma mansoni each, while the fourth patient with intestinal parasite was patient an No.5 who had Giardia lamblia cysts. Two patients had dental caries.

Treatment of Specific Illnesses

Scabies was treated with benzyl benzoate applied for three consecutive days. This course was repeated where the infection did not clear in the first three days. Patients with infected scabies were treated with septrin in addition to the benzyl benzoate.

Hookworm infection was treated with Bephenium hydroxynaphthoate (Alcopar) at a dose of 2.5 grammes daily for three consecutive days, and Schistosoma mansoni infection with oxamniquine at 50 grammes per Kilogramme body weight given as a single dose for two consecutive days.

Patients Nos. 6 and 15 received ferrous sulphate 200 milligrammes three times daily and folic acid 5 milligrammes once daily towards the end of kala azar treatment as they had features of iron deficiency. Patient No. 6 was one of the three sisters with hookworm and Schistosoma mansoni infections but patient No.15 had only kala azar infection.

Suppurative otitis media was found only in patient No. 16,and for this she was given septrin and boric acid ear drops. None of the other patients with otitis media and throat congestion received antimicrobials.

TABLE VI: WEEKLY HAEMOGLOBIN VARATION IN gm/dl-

Patients	Time in weeks																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	1st Rev.	13	14	15	16	17	18	19	20
1	4.9	4.6	5.3	8.1	9.4	10.8	10.2	10.6						11.8								
2	9.9	9.3	10.4	10.0	10.1									10.7								
3	4.7	4.6	4.6	6.0	6.9	8.1	7.8	8.1	7.8	6.8	5.7	4.8	4.8		5.1	7.5	9.2	10.4	9.9	10.8	11.4	
4	7.0	8.7	9.0	9.5	9.7	10.3								11.2								
5	7.8	9.2	9.7	9.4	10.1	9.8	12.5	11.7	11.6	11.8	10.8	12.9	13.0									
6	3.8	3.8	5.0	6.3	5.6	5.9	6.9	6.9	6.5	7.7	8.8	8.7	8.4		6.9							
7	7.7	10.5	12.2	11.5	10.0	11.1								11.9								
8	4.7	3.6	4.4	4.8	5.3	5.9	6.8	8.3	9.1	10.8	11.8	11.8		13.1								
9	9.7	9.7	11.2	11.1	11.3	11.4	12.7	12.7						13.5								
10	5.8	6.9	8.8	11.0	11.1	12.2	12.5							12.5								
11	5.7	5.7	5.8	7.1	7.3	7.7	8.8	9.0	10.1					12.2								
12	6.6	6.5	7.8	8.1	8.9	9.3								11.1								
13	5.8	6.9	9.6	10.1	9.9	10.6								11.4								
14	9.2	8.0	8.4	10.0	9.2	10.2								13.1								
15	6.9	6.1	8.4	9.2	9.9									12.0								
16	7.0	8.4	9.4	9.4	10.2	11.3	11.1															
17	4.7	5.8	6.9	8.7	8.2	9.3	9.8	8.7	10.4	8.8	8.9	8.6	8.9		7.3	7.8	9.3	9.1	9.7	7.9	8.3	8.5
18	5.4	7.0	7.9	7.4	8.3	8.3	9.5	9.4	9.7	8.5	5.8	4.6	4.8		5.2	8.0	8.6	7.2	8.1	7.9	7.1	6.3

FIG.I. : HAEMOGLOBINS DURING TREATMENT

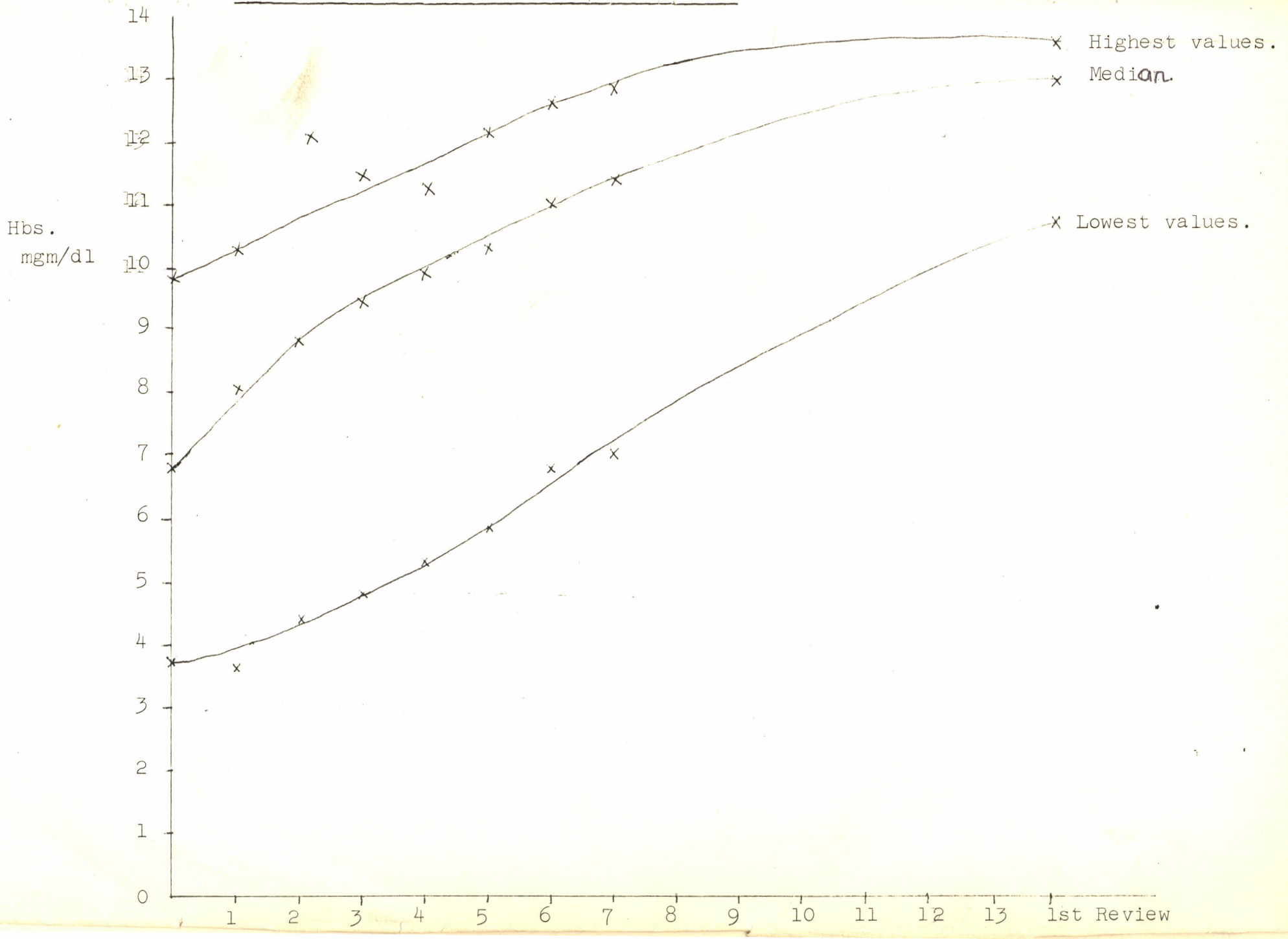

Table VI shows haemoglobin (Hb) response by weeks of treatment. Hbs on Week 0 were the samples taken before treatment was started. There was a general rise in Hbs during treatment. The Hb range on admission was 3.8 to 9.2 gm/dl, on discharge it was 9.3 to 12.7 gm/dl and on the first review visit it was 10.7 to 13.5 gm/dl. The Hb of patient No. 3 did not show any significant change during treatment in the first twelve weeks on pentostam. He did not respond to pentostam hence he was given allopurinol 100 milligrammes three times daily for four weeks on which he improved. During treatment with allopurinol the Hb rose significantly.

Figure I shows the haemoglobin response in a graph plotted using the highest, lowest and median values for each week. These values rose with time and tended to merge after treatment.

TABLE VII : RED BLOOD CELL RESPONSE DURING TREATMENT

Patients	Time in weeks 0	1	2	3	4	5	6	7	8	9	10	11	12	1st Rev	13	14	15	16	17	18	19	20	21	22	23
1	1.66	1.64	1.69	2.90	3.12	3.54	3.68							4.11											
2.	3.97	3.58	4.00	3.92	3.97									4.53											
3.	1.81	1.73	1.81	2.39	2.86	4.47	3.42	3.66	3.42	2.78	2.53	2.19	2.09												
4.	3.28	4.16	4.02	4.19	4.30									4.66											
5.	3.05	3.38	3.43	3.40	3.88	3.77	4.60	4.59	4.31	4.50	4.08	4.67													
6.	1.73	1.78	2.46	3.45	3.33	3.68	4.31	4.19	4.13	4.60	5.10	4.81													
7.	3.15	4.20	4.72	4.43	3.76																				
8.	2.70	1.60	1.97	2.13	2.71	2.91	3.43	4.04	4.37	4.98	5.39	5.51													
9.	4.38	4.47	5.14	4.84	5.22	5.11	5.59							6.29											
10.	2.72	3.07	3.67	4.30	4.50	4.87																			
11.	2.62	2.57	2.58	3.35	3.50	3.67	3.87																		
12.	2.81	2.86	3.23	3.45	3.58																				
13	2.66	3.10	4.00	4.19	4.28	4.80																			
14	3.88	3.32	3.42	3.88	3.52	3.79																			
15.	2.67	2.96	3.91	4.28	4.79																				
16.	3.22	3.55	3.61	3.48	3.69	4.33	4.56																		
17.	2.07	2.38	2.68	3.30	2.99	2.62	4.00	3.57	4.23	3.59	3.87	3.51	3.53		2.97	3.18	3.76	3.76	3.88	3.18	3.32	3.57	---	3.69	
18.	2.40	2.92	3.31	3.14	3.16	3.69	4.30	4.27	4.03	3.72	2.63	2.14	2.08		2.39	3.18	3.66	2.66	2.85	2.72	2.60	2.34	2.03	2.04	

On admission the RBC counts were very low but they rose in all patients showing response. Patient No. 3 showed no significant change in the first twelve weeks.

These results are plotted on figure II using the highest, lowest median values of RBCs of each week.

FIG.II. RBC COUNTS DURING TREATMENT

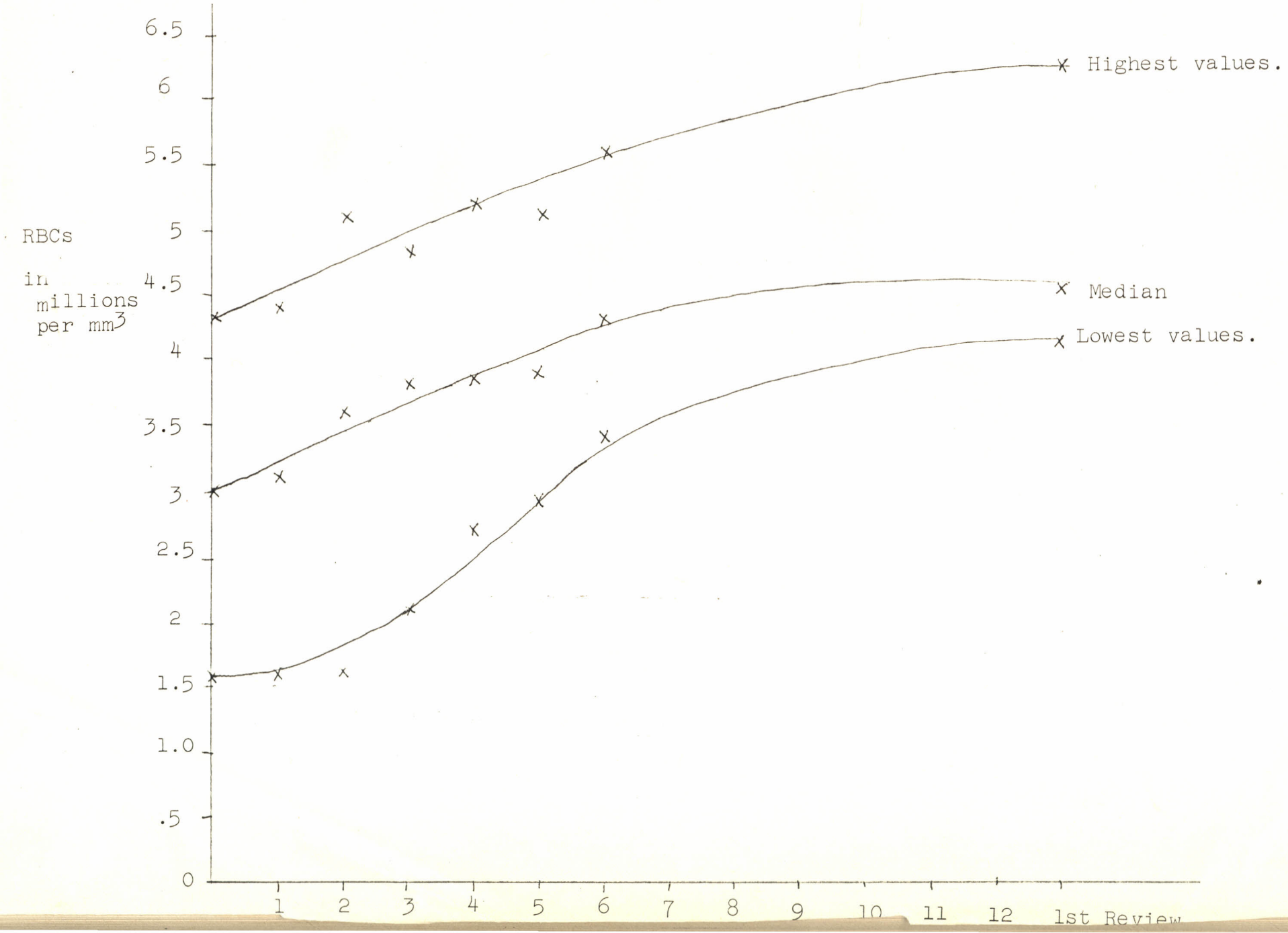

TABLE VIII; WBC RESPONSE DURING TREATMENT

Patients	Time in weeks 0	I	2	3	4	5	6	7	8	9	10	11	12	1st REV	13	14	15	16	17	18	19	20	21	22	23
1.	2520	1260	2081	1993	2654	3875	3354							3661											
2.	3150	2890	3980	3630	3640									6577											
3.	3100	3605	2587	4904	2745	4146	5083	5167	4146	3329	3119	3324	3438												
4.	3074	4332	3996	5594	6568									10325											
5.	2362	3575	5949	5160	2647	6049	6239	5349	7751	9059	4218	5976													
6.	5040	2952	6131	5426	1610	6006	7825	7180	7377	9179	9292	5614													
7	2400	4330	4267	4559	3792									5974											
8.	1886	2372	1887	1527	2412	2219	2154	1832	3450	3377	4554	4376		7993											
9.	2539	5827	6739	4920	5149	6305	6596							7987											
10.	2610	5271	3483	4088	8204	7256																			
11.	1731	2038	2360	2523	3317	3038	3260							5662											
12.	1566	1692	2403	1504	2344									4253											
13.	3477	3930	5174	5813	7619	7504								9575											
14.	2530	1755	3604	3228	2175	3524								4116											
15.	3277	3069	2155	3792	3721									7192											
16.	1990	4445	2107	2193	4906	3266	1824																		
17.	5080	4474	5302	3684	3475	4678	4366	3835	9516	3755	4646	4168	3424		2215	3958	8710	5340	3568	3267	4399	4558	---	5189	4476
18.	1810	7039	1805	1515	4080	6440	5378	4105	2083	3512	1494	1285	1608		2534	5363	6604	6280	4998	2896	1963	3299	2324	2052	3075

Table VIII shows total WBC counts of each week. On admission the values were low ranging from 1,566 to 5080. These results showed an inconsistent rise during treatment as the figures were fluctuating.

Table IX shows the WBC differential counts in percentages of the total WBC count, both on admission and discharge. There was a relative lymphocytosis with granulocytopenia. On admission there was almost complete absence of eosinophils with a range of 0-4% while on discharge the range was 1 to 23%.

In patients No. 17 and 18 who were still on treatment at the time of analysis of the results, the differential counts showed no significant change. Eosinophilia was not observed in patient No. 3 during treatment with pentostam but was observed when this patient was successfully treated with allopurinol.

3.	57	14	24	3	0	0	2	---						89	1	9	0	0	0	1	0				

TABLE IX WBC DIFFERENTIALS

Patients	ON ADMISSION								ON DISCHARGE							
	LYMPH	PMN SEG	BAND	MONO	EOSIN	BASO	META	MYELD PROM-YELO	TOTAL LYMPH	PMN SEG	BAND	MONO	EOSIN	BASO	META	MYELO PROM-YELO
1.	79	12	1	0	4	4	0	--	51	17	22	4	6	0	0	0
2.									55	27	12	0	6	0	0	0
3.	58	23	17	1	1	0	0	--	61	20	14	0	5	0	0	0
4.	86	1	10	2	1	0	1	---	50	22	14	1	13	0	0	-
5.	93	0	3	4	0	0	0	---	61	19	17	1	2	0	0	-
6.	72	5	21	0	2	0	0	--	44	20	23	3	10	0	0	-
7.	79	5	15	0	1	0	0		67	14	10	0	7	2	0	0
8.	78	5	16	0	0	1	0	---	63	26	6	0	5	0	0	-
9.	88	1	8	2	0	1	0	---	56	28	12	0	4	0	0	0
10.	87	1	9	1	1	0	1	---	54	12	15	4	15	0	0	0
11.	98	0	0	2	0	0	0	---	55	37	7	1	0	0	0	-
12.	80	5	14	1	0	0	0	---	39	7	81	0	23	0	0	-
13.	85	5	9	0	0	0	1	0	66	21	12	0	1	0	0	-
14.	43	10	43	3	1	0	0	---	53	22	20	1	4	0	0	-
15.	85	1	13	1	0	0	0	---	81	6	9	0	4	0	0	0
16.	85	6	5	1	3	1	0	----	47	37	14	1	1	0	0	-
17.	65	9	18	6	1	0	0	---	78	2	19	0	1	0	0	0
18.	57	14	24	3	0	0	2	---	89	1	9	0	0	0	1	0

TABLE X: RECTICULOCYTE COUNT IN PERCENTAGE

Patients	Weeks of treatment																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	1st REV	13	14	15	16	18	19	20	21	22	23	24
1.	7.0	---	13.2	6.8	5.4	5.4	3.4	2.0						0.9											
2.	3.2	5.4	1.8	2.6	2.0	---	1.4																		
3.	12.8	9.4	8.8	17.8	6.8	7.2	11.2	8.6	9.6	6.4	10.1	23.2	18.6	12.6	12.4	5.8	4.2	2.6	6.4						
4.	1.4	3.6	1.2	3.2	2.8	1.0								2.2											
5.	4.6	7.0	5.4	2.0	1.4	4.6	1.0	2.0	1.8	1.6	1.6	2.6	2.6		4.4										
6.	11.8	6.4	11.8	7.4	3.6	7.0	3.2	3.2	5.8	6.1	7.6	3.0	4.0												
7.	3.2	6.2	1.6	5.8	3.7	2.9								2.4											
8.	3.8	16.0	11.0	5.2	14.4	13.9	14.0	8.8	5.0	3.2	6.4	5.0	1.6												
9.	3.6	1.0	5.4	3.4	2.8	7.0	4.6	4.0						0.8											
10.	3.2	13.4	11.2	2.6	2.7	1.1	1.0																		
11.	5.4	5.4	5.9	8.1	9.8	4.0	3.0	3.4	4.2																
12.	2.6	5.8	4.4	9.6	5.0	4.8																			
13.	0.2	---	5.2	---	4.8	12.7	14.6	14.2	4.4	2.6	1.4	1.8													
14.	2.4	3.2	6.0	8.0	5.2	4.4																			
15.	8.6	15.4	7.2	1.8	2.0																				
16.	---	6.8	7.4	---	4.0	4.8	3.2	1.0																	
17.	---	12.0	7.0	6.0	6.6	4.2	3.6	4.2	3.4	7.2	5.2	12.0	15.8	7.4	6.8	8.4	8.4	6.8	9.6	7.8					
18.		9.4	6.6	1.6	6.4	6.8	6.0	4.0	1.8	1.2	1.2	3.9	8.8	9.8	9.6	6.0	6.2	9.2	14.2	11.4					

. The recticulocyte counts were high on admission ranging from 1.4 to 12.0% and remained above normal during treatment. On discharge the range was 1.0 - 6.4%.

TABLE XI: PLATELET COUNTS

Patients	Time in weeks																						
	0	I	2	3	4	5	6	7	8	9	10	11	12	1st REV	13	14	15	16	17	18	19	20	21	22
1.	196	110	112	106	---	248	184							120										
2.	206	190	142	100	170									---										
3.	78	60	---	103	128	142	148	142	116	146	138	252	198											
4.	76	166	---	163	150									354										
5.	100	---	97	118	134	192	192	159	174	198	128	200												
6.	72	---	145	134	168	172	174	136	158	246	276	152												
7.	144	114	242	198	268									100										
8.	66	126	104	128	166	154	201	144	132	166	142	226												
9.	146	178	370	209	198	334	268																	
10.	112	388	102	178	310	220																		
11.	82	94	60	90	104	90	90																	
12.	104	100	122	128	356									249										
13.	188	358	278	234	320	146																		
14.	168	198	170	330	388	220								322										
15.	162	265	266	342	352																			
16.	82	114	156	---	193	165	208																	
17.	96	76	130	---	81	98	104	126	138	124	122	128	130		132	176	174	118	164	48	36	82	---	80
18.	122	86	52	---	175	136	260	206	232	134	96	130	74		162	160	128	148	198	88	102	88	70	78

In keeping with the pancytopenia, the platelet levels were low on admission. Seven patients had platelet counts of less than 100,000 $mm,^3$ of these, four had given a history of epistaxis.

No correlation could be made betwen platelet levels and spleen sizes.

TABLE XII ESR in mm/hr.

Time in weeks

Patients	0	1	2	3	4	5	6	7	8	9	10	11	12	1st REV	13	14	15	16	17	18	19
1	-	79	75	65	61	54	58	55						51							
2																					
3	70	--	78	70	62	60	--	55	60	66	76	75	75	--	74	61	55	52	52	36	15
4	69	--	--	60	59	52								53							
5	--	59	60	61	54	56	39	45	45	40	--	23	29								
6	73	79	72	64	39	28	28	15	22	67	20	26	20	12	28						
7	66	28	30	23	56	61															
8	77	81	78	75	71	66	63	43	35	26	22	30		12							
9	42	50	49	30	36	27	23	58						22							
10	75	69	61	58	57	51	46														
11	71	70	67	67	69	61	41	31	49					37							
12	70	67	66	66	65	60								47							
13	70	67	56	52	37	40								26							
14	58	61	62	67	62	59								43							
15	67	68	59	53	35																
16	59	60	55	39	25	21	17														

(Time in weeks)

	13	14	15	16	17	18	19	20	21	22	23
17	66	65	56	59	60	61	62	60	--	56	60
18	72	60	26	62	63	63	68	72	77	78	78

The ESR's were invariably high on all patients on admission ranging from 42 to 75 mm per hour. These dropped slowly but were still above normal on discharge ranging from 17 to 60 per hour. On the first review visit the range was 12 to 53 per hour.

TABLE XIII: MCH

Patients	Time in weeks 0	1	2	3	4	5	6	7	8	9	10	11	12	1st REV	13	14	15	16
1	29.77	28.05	31.36	29.60	30.13	30.86	27.72							28.71				
2	29.	25.95	26.00	25.51	25.44	24.18	23.62											
3	25.97	26.59	25.41	25.10	24.13	17.90	22.9	22.13	22.81	21.94	22.53	21.95	22.97	23.72	23.15	24.21	22.81	23.24
4.	21.34	20.91	22.39	22.67	22.56	22.74								24.03				
5	25.85	27.22	28.28	27.65	26.37	26.20	27.17	25.49	26.91	26.22	26.47	27.62	27.48					
6	21.02	21.35	20.33	18.26	16.82	16.03	16.05	16.47	15.74	16.74	17.25	18.68	17.80					
7	24.44	25.00	25.85	25.96	26.60	26.88												
8	17.41	22.50	22.34	21.13	19.56	20.27	19.83	20.54	20.82	21.69	21.89	21.42	22.54					
9	22.15	21.70	21.96	22.93	21.65	22.31	22.72	22.28										
10	21.32	22.48	23.98	25.58	24.67	25.05	25.99											
11	21.76	28.18	22.48	21.19	20.86	20.98	22.74	22.50	22.05									
12	23.49	22.73	24.15	23.48	24.86	24.28												
13	21.04	19.63	18.38	22.27	21.96	21.80	22.26	24.00	24.11	23.13	22.08	21.76						
14.	23.71	24.10	24.63	25.77	26.14	26.91												
15	25.04	20.54	21.48	21,50														
16	21.74	23.66	26.11	27.01	27.64	26.10	24.34											
17	22.71	24.37	25.75	26.36	27.42	25.69	24.50	24.37	24.59	24.51	23.00	24.50	25.21	24.58	24.53	24.73	24.20	25.00 24
18	22.50	23.97	23.87	23.57	22.87	22.49	22.79	22.61	24.07	22.85	22.05	21.50	21.63	21.76	25.16	25.60	27.07	28.42 29

TABLE XIV MCHC

Patients	Time in weeks																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	1st REV	13	14	15	16	17	18
1	36.78	37.10	40 77	36.94	35.21	38 30	35.54	35.22						37.11						
2	36.7	33.21	35.49	35.34	36.20	36.36	38.63													
3	33.57	36.80	33.09	34.29	34.85	33.61	35.45	33.89	34.51	34.08	36.77	32.00	34.29	33.33	32.75	35.11	37.14	36.53		
4	32 41	35.37	34.88	32.81	34.04	35.03								36.25						
5	37.07	35.38	37.60	36.58	34.83	36.16	37.43	35.24	37.30	38.44	38.57	40.69	39.16							
6	35.24	30.40	32.26	27.63	30.77	31.22	30.53	33.17	32.50	32.92	31.21	36.40	31.58							
7	35.16	36.21	36.31	36.51	38.31	38.95														
8	41.59	33.03	34.38	34.62	32.92	35.76	29.06	35.93	36.55	36.86	36.99	38.56	37.99							
9	32.44	35.40	37.33	37.00	36.33	37.01	39.32	35.38												
10	33.33	34.33	35.06	33.23	35.13	38.01	37.09													
11	35.63	36.08	30.85	35.68	32.02	35.48	38.77	33.96	37.00											
12	37.71	36.72	39.80	38.39	38.53	36.76														
13	31.33	30.24	29.58	34.23	33.68	35.15	35.94	36.23	37.97	36.67	36.30	37.25								
14	38.33	39.02	39.07	38.31	38.49	37.50														
15	40.59	32.80	34.71	36.22																
16	34.83	35.44	35.21	35.34=38.06		35.19	34.58													
17	33.10	33.72	35.75	33.46	37.10	34.07	34.39	35.80	37.41	36.21	34.23	35.10	36.33	36.87	35.45	35.77	31.93	36.60	36.57	
18	35.29	30.84	37.09	34.74	34.16	33.20	33.68	34.69	36.60	35.86	36.02	37.70	35.16	34.67	33.90	36.13	37.50	37.85	37.09	

TABLE XV

MCV

	Time in weeks																			
Patients	0	1	2	3	4	5	6	7	8	9	10	11	12	1st REV	13	14	15	16	17	18	19
1	83	77	78	81	85	80	77							75							
2	77	77	72	71	71									62							
3	78	73	77	73	69	68	64	65	62	66	67										
4	66	59	64	69	66									67							
5.	72	77	76	75	75	72	73	73	72	69	72	68									
6	61	70	63	65	54	51	53	50	49	51	54	50									
7	69	69	72	71	69									65							
8	62	68	64	62	60	57	69	59	59	61	58										
9	68	61	60	63	61	61	59							59							
10	63	66	69	71	71	67															
11	62	62	74	62	67	59	59														
12	63	63	61	66	64																
13	63	62	66	63	63	73															
14	64	62	62	67	68	71															
15	63	62	60	59	58																
16	63	67	69	76	73	74	70														
17	70	73	72	78	74	75	71	68	66	68	67	70	68		67	69	69	67	68	68	69
18	65	75	64	68	67	57	66	64	63	63	62	58	65		63	70	71	72	75	79	77

Tables XIII, XIV and XV are best looked at together. Patients Nos. 6 and 15 had features of iron deficiency and were treated with ferrous sulphate and folic acid.

TABLE XVII : SPLEEN SIZES BY HACKETT'S CLASSIFICATION

Patients	On Adm.	On Disch.	1st Review
1	5	3	3
2	3	3	2
3	5	Refractory to pentostam	
4	5	2	2
5	4	2	No review
6	3	2	0
7	4	3	2
8	5	3	2
9	5	2	2
10	3	2	2
11	4	2	2
12	5	2	2
13	3	2	2
14	3	3	1
15	3	2	2
16	4	2	No review
17	5	3	No review
18	5	on Re.	

The spleen sizes ranged between Hackett stage 3 to 5 on admission, 2 to 3 on discharge and 0 to 3 on the first review.

Patient No. 3 did not show any splenic regression while on treatment with pentostam, but he did while on allopurinol.

There was no correlation between the spleen size and duration of illness.

TABLE XVIII: TIME IN WEEKS TAKEN FOR ASPIRATES TO BECOME NEGATIVE AND LENGTH OF TREATMENT WITH PENTOSTAM

Patient	Time for Negative aspirate	Length of treatment
1	3	4
2	5	6
3	-	Resistant
4	3	4
5	10	II
6	10	11
7	3	4
8	10	11
9	5	6
10	4	5
11	5	6
12	3	4
13	4	5
14	4	5
15	3	4
16	5	6

Treatment with pentostam was continued while awaiting the culture results, hence all patients were treated for one week longer after aspirates became negative.

Five patients were treated for four weeks, three for five weeks, four for six weeks and three for eleven weeks.

Patient No. 3 did not show significant clinical or haematological response after twelve weeks of treatment with pentostam. He was persistently positive on splenic aspirate during this time. The length of treatment did not correlate with the duration of illness given in the history.

All patients had a positive complement fixation test, normal chest xrays and negative Mantoux Tests. The Leishmanin skin tests performed on sixteen patients were negative.

D I S C U S S I O N

The results of this study help to throw light on some aspects of East African Kala azar as seen in Kenya where the disease has become endemic in some areas as well as appearing sporadically (13).

The disease in most cases runs a chronic course with a long incubation period of about two to six months but there are considerable variations (1). The onset is insidious but may be acute and dramatic. From the results of this study, the duration of illness lasted several months in the majority of patients, but due to the characteristic absence of prostration, patients were seen well advanced in the disease. Despite the lenghth of illness, about half of the patients were in good general condition. There was no correlation between the duration of illness, and the response to treatment. This could be due to the fact that one could not tell from the history how long the patient had had the infection or also because the host may respond differently to the parasite.

Fever in kala azar is nonspecific and irregular (1,2,3,11). Fourteen of the patients gave a history of fever at some time during the illness but only seven were actually febrile at the time of admission. Irregular spikes of fever were recorded in the majority of patients in the first few days to about two weeks after admission, but all patients previously febrile

and showing response to treatment, became afebrile before they were negative on splenic aspirates. Continuing fever after about two weeks of treatment indicates poor or no response, or an additional cause.

In well established kala azar cases, pallor and hepatosplenomegaly are invariable and patients often seek medical attention because of symptoms related to the anaemia and the abdominal distension. Rarely do patients complain of pain, but they may have abdominal discomfort from the greatly enlarged spleen and to a lesser extent the liver. Pallor, splenomegaly and hepatomegaly were found in all the patients in the study. The general impression was that the spleen was relatively more enlarged than the liver. With successful treatment, the spleens regressed but were still palpable on discharge, the time when parasites were no longer recoverable from the aspirates. As shown on Table XVII, the spleens continued to regress even after discharge while on no treatment suggesting that a great part was being played by the host towards achieving complete cure. There was no correlation between the duration of illness and the size of the spleen.

In contrast to the Indian kala azar (3), the skin changes as seen in Kenya are those of either generalized hypopigmentation or hypopigmented patches on the face particularly around the eyes. The hair which may be lustureless and brittle, is also lighter in colour even in patients of good nutritional status. Normal colour of skin and hair was restored during treatment. These skin and hair changes were observed in four patients who were in poor nutritional status and in three others who looked apparently healthy. From these observations, poor nutrition may contribute to the skin and hair changes, but some patients will have these changes despite their apparently good nutrition. No prognostic significance could be attributed to these features.

Epistaxis was found to be unrelated to the platelet levels or the degree of anaemia. In most cases it was mild but attracted the attention of both parents and patients. It was observed to occur in patients with normal levels of platelets as well as in those with thrombocytopenia.

In the group under study, scabies was the commonest concomitant illness, probably because the infection is common in the area the patients came from. Otitis media and throat inflammation were also common.

These are common problems in the age-group studied but Manson-Bahr (2) has reported the demonstration of the amastigote forms of the parasites on nasal and throat swabs as well as from normal skin. One must also remember that the chronic ill health and pancytopenia may render kala azar patients more susceptible to other infections. Although a number of patients had presented with cough, none had any clinical or radiological features of pneumonia. Pneumonia has been said to be relatively common in patients with kala azar either due to secondary infections or due to generalized leishmanial infection (2). Manson-Bahr reports that about ten percent of patients have pulmonary tuberculosis as an associated infection (2,3). In the group studied, though small, there was no patient with symptoms and signs suggestive of pulmonary tuberculosis.

Any concomitant illness may complicate the clinical presentation of kala azar and the response to treatment. In particular, other parasitic infections such as hookworm infection and schistosomiasis may contribute to the degree of anaemia and hence its response to treatment. In this study patient No.6, had features of iron deficiency anaemia with both hookworm infection and schistosomiasis. She was given ferrous sulphate and folic acid.

The absence of malaria in this group which came from a malaria endemic area was attributed to the fact that antimalarial drugs are commonly and arbitrarily given to patients with fever and splenomegaly in many health units in the country. They are also easily obtainable from the grocery shops without a prescription.

The most characteristic haematological finding in kala azar is the anaemia which is almost always found in established cases (14-18). In many cases the anaemia, which becomes progressively more severe with the duration of illness, is responsible for much of the morbidity in the disease. The anaemia is associated with pancytopenia and peripheral reticulocytosis. The mechanism of its causation is multifactorial and probably complex. The following have been shown to take part:- reduced survival of the circulating erythrocyte due to haemolysis in the spleen and also as an autoimmune phenomenon (14,17,18); the pooling of the erythrocytes in the spleen (20,21); reduced erythrocyte maturation in the marrow; and intramedullary destruction of erythrocyte precursors. Hypervolaemia with consequent red cell dilution may play a part. This could be due to hypersplenism per se or to increased plasma osmolarity due to high plasma protein levels found in the disease. Woodruff (14) observed the abscence of anaemia in a patient with a heavy infection of kala azar but who had been previously

splenectomised, suggesting that the spleen has a major part to play in the causation of anaemia. Blood loss may also occur particularly in the gastrointestinal tract secondary to the thrombocytopenia. Lastly, anaemia may be aggravated by the presence of other infections such as hookworm.

From the results of this study, patients who showed response to treatment had steady and consistent rising levels of the haemoglobins and the red cell counts as shown on Tables VI and VII and Figures 1 and II. The bone marrow in kala azar is hyperplastic(22) despite the pancytopenia. Although bone marrow aspirations were not used for the diagnosis and follow-up of the patients in this study, the persistent reticulocytosis was an indication of an active marrow.

Leucopenia, particularly the granulocytopenia was most striking. As with the anaemia, leucopenia may be due to a number of factors some of which are the reduced life span, pooling and destruction of the leucocytes in the spleen and a decreased marrow reserve(13). The differential count showed relative lymphocytosis and an almost complete abscence of eosinophils. The monocytes and basophils did not show significant variations from normal. The eosinopenia was noted to occur uniformly in all patients regardless of the presence of other parasites such as scabies and intestinal parasites which would normally provoke

an eosinophilia. As the patients responded to treatment, the total white count rose to levels near normal, but the differential count showed an eosinophilia. Eosinophils were also noted to be absent on splenic aspirate smears before treatment only to appear as the patients responded to treatment. This abscence of eosinophils, followed by eosinophilia as patients responded to treatment, was so regularly and uniformly observed, that it could be used as a criterion for both diagnosis and response to treatment. In kala azar the cellular immune system is depressed (1,2 & 3). The eosinophil is dependent on the T cells and it is known that in patients who have an antigen which strongly stimulates eosinophilia, depression of the cellular immune system of such patients, produces an eosinopenia even in the continued presence of the antigen(25). This is, most likely, the explanation for the observation in kala azar. If this is true of kala azar, one would postulate that one of the possible mechanisms of the action of pentostam is by stimulating the celullar immune system.

Platelets are reduced in advanced cases of the disease but not invariably so. The thrombocytopenia may be responsible for the epistaxis in some patients, however, as mentioned above, epistaxis was noted to occur in patients with normal platelet levels as well as

in those with thrombocytopenia. Thrombocytopenia is accounted for by splenic pooling with increased peripheral consumption (26). The platelet count rose during treatment to levels sometimes above normal. The ESR was raised in all patients and fell slowly during treatment but was still above normal levels in all patients even on discharge.

The total serum proteins were raised throughout the length of treatment and even at the time of discharge. Soon when facilities are available, serum protein electrophoresis will be done on all the samples to provide more information. However, the high level of serum proteins has been shown to be due to a rise in the gamma globulin fraction while there is a fall in the albumin fraction (1,2,3) The levels of serum proteins in these patients were of no prognostic value. The compliment fixation test was performed on fifteen of the patients using an antigen prepared from parasites cultured from an adult Kenyan patient in the hospital. This test was positive in all the fifteen patients, but negative in all the other patients, tested for comparison.

The Leishmania skin test (Montenegro skin test) is a good indicator of development of delayed hypersensitivity to the leishmania antigen and therefore a measure of cellular immunity. It becomes positive in kala azar

only after cure (21,27,28). It was negative in all the sixteen patients on whom it was done during active infection. This test is helpful as a criterion for cure and also in the field studies as a measure of immunity to kala azar. The Mantoux test was negative in all the eighteen patients who included thirteen who had had BCG vaccinations. This test, like the Leishmanin skin test is a good indicator of the cellular immunity. One patient, patient No. 12 reverted to a positive Mantoux test by six weeks after treatment. His Leishmanin skin test then, was still negative.

As with all parasitic infections the diagnosis of kala azar fundamentally rests on the recovery and recognition of the parasites. It has been shown that splenic aspirates give the highest yields as compared to the bone marrow and liver biopsies (11). Splenic aspiration is a simple and safe procedure which gives valuable information by demonstrating the parasites in an enlarged spleen. Widal was probably the first person to use splenic material as a means of diagnosis (29,30). Splenic aspirates have been recommended as a means of diagnosis in other diseases with splenomegaly such as anaemias, malaria, polycythemias (29,30). It should be

noted that in this study, splenic aspirations rather than biopsies were performed using small sized needles. Splenic aspirates in Kenya have been employed almost exclusively for confirmation or exclusion of the diagnosis of kala azar(31,32,33). The main precaution is that the doctor should be well conversant with the procedure which should take less than a second to perform. There was no morbidity in these patients that could be attributed to the splenic aspirates. The majority of patients could not indicate the site of puncture when asked. Using this procedure, the diagnosis of kala azar can be confirmed within minutes, by examining the smears. This makes the procedure a valuable tool in areas where laboratory support is minimal.

The Schneider's insect media was preferred to the NNN media as it gives quicker and more accurate results (8,9,10). Care should be taken not to inject too much blood in the media as this inhibits growth of the parasites on culture (12). It is the author's impression that a good examination of the slides gives just as good, or even better, results than the culture. This means that one can rely on the examination of the slides for diagnosis cutting down on the expense for the media.

A high proportion of cases of Kenyan kala azar can be treated successfully with sodium antimony gluconate (pentostam), a pentavalent antimonial. This drug is well tolerated with little or no significant side effects. It is quickly excreted in the urine so the chance of accumulation is only a remote one. In the group of patients studied no significant side effects were noted. Only one patient, No. 3, did not respond to pentostam. It is interesting to note that this patient had had treatment with the same drug three months previously. From these results it can be seen that patients differ widely in their response to treatment. In adults, patients may take as short a time as only two weeks to become parasitologically negative (34). In the study group this ranged from three weeks to ten weeks. The duration of treatment was however four weeks to eleven weeks because the treatment was continued while awaiting culture results for the confirmation of negative slides. From this study, the duration the patients took to become parasitologically negative and hence the length of treatment had no apparent correlation with the other features in the disease. A comparative study of adults and children would help to show whether age could have any correlation.

It has been impossible to forecast the possible line of response a patient is likely to follow from either the clinical or the haematological features. There was no special feature in patient No. 3, except that he had been treated previously, to indicate the possible failure to respond. He probably had not been successfully treated in the first instance. One of the three sisters No. 7 took three weeks to become negative while the other two took ten weeks. One would have expected that in these three patients, the response to the infection and to the treatment would be similar as the patients had the closest genetic make-up and were presumably infected with the same strain of the parasite. Looking, at all the clinical and haematological parameters, one cannot tell how soon a patient is likely to become parasitologically negative even after an initial response. Some patients may look very sick but show a better and quicker response than those not so sick. There are no obvious features from this study which would help one to predict the outcome of a patient with kala azar. Each case of kala azar must therefore be judged on its own merits. It could be argued that patients need not clear their parasites before discharge. However in this study patients were discharged only when parasitologically negative

due to difficulties on the part of the patient, to travel long distances for follow up at the hospital. Similarly some patients were unable to return to the Parasitology Clinic for the reading of the skin tests.

C O N C L U S I O N S

1. There is a male preponderance in kala azar even in children of the age group studied.
2. Spleen sizes alone cannot be used as criteria for cure but should be used in conjunction with splenic aspirates and close follow-up of patients after treatment.
3. Eosinophilia develops in all patients who show response to treatment and is therefore a reliable indicator for response.
4. Patients take different times to become parasitologically negative and hence need different lengths of treatment even when their haematological parameters and physical examinations suggest cure. Therefore a rigidly standardised regime of treatment is not advisable.

RECOMMENDATIONS

1. More work needs to be done as regards the immune system in kala azar, particularly on eosinophils, to help understand the pathological mechanisms of the disease. This will also help in determining the possible mechanism of action of pentostam which is still unknown.
2. The complement fixation test is a specific and reliable test and should be used in doubtful cases of diagnosis and in situations where a splenic aspirate cannot be done such as in an early case of infection without splenomegaly.
3. As far as possible a second splenic aspirate should be done to determine response, after at least four weeks of treatment. While awaiting the results of the aspirate, treatment should be continued without interruption. A second line of treatment should be followed if there are no features showing response after ten to twelve weeks of treatment.
4. All patients should be followed up after treatment until complete cure occurs as indicated by a positive Leishmanin skin test. This can only be determined with time.

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