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Experimental *Trypanosoma brucei* Infection in Lions

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Introduction

Trypanosoma brucei was isolated by SACHS et al. (1967) from two lions in the Serengeti National Park in Tanzania by subinoculation of lion blood into white rats. Slides made with these lion blood samples did not show trypanosomes by microscopic examination.

Unidentified trypanosomes were found in the blood of a lion by WECK (1914); SACHS et al. (1967) found 22 out of 32 blood smears of lions containing unidentified trypanosome species. BAKER (1960) and BAKER et al. (1967) were able to demonstrate *Trypanosoma congolense* in the blood of two shot lions.

We had the opportunity to infect two six months old lion cubs with *Trypanosoma brucei* and to follow up the course of this infection. The results of our observations and records of a lot of parameters will be summarized in this paper.

Material and Methods

Two six months old lion cubs, one male (A) and one female (B), born and bred at the Zoo of Antwerp, were used in this experiment. The animals were in a relatively poor condition since weaning, as they were suffering from bone growth disorders.

Trypanosoma brucei E.A.T.R.O. 1125 mavubwe strain was used to infect the animals. The inoculum consisted of an intramuscular injection of heparinised rat-blood containing about 100,000 parasites. The 15th laboratory animal passage of the strain was used since it was isolated from a bushbuck in 1966 in Uganda.

Splenectomised young Wistar rats (100 g) were used to control the presence of parasites in the peripheral blood of the lions during the course of infection.

Parasitaemia in lions and rats was controlled by microscopic examination of wet slides and Giemsa-stained thick drop preparations. Blood samples withdrawn from the tail of rats were used in these experiments.

Blood samples from the jugular vein of the lions were withdrawn when fully anaesthetised by a mixture of Methoxymol® and Fentanyl®. The heparinised lion blood was furthermore inoculated intraperitoneally into two rats at a dose of 1 ml.

Our experiment started on 10. 12. 1970 when the first recording of parameters could be made. The infection was done on 23. 12. 1970, which was day 0. Further recordings could be realised on day 12, 22, 29, 42 and 50 after infection; at that moment both the animals were sacrificed as they were in a very poor state.

The following parameters could be followed up: parasitaemia in the peripheral blood, erythrocyte number, total and differential leucocytic counts, hemoglobin content, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total protein, albumin and globulin by paper-electrophoresis, glucose, alcaline phosphatase, SGOT, SGPT, total bilirubin, thymol and zincsulfate tests.

Results

The animals did not show any visible sign of illness due to trypanosomiasis during the first weeks of observation; they continued to eat and were aggressive; no eye lesions were observed as it can be seen in dogs and domestic cats during the acute stage of the disease. At the day of sacrifice as before both the animals had to be caught by nets before injection with the narcotic mixture. The initial body weight of the animals had dropped for lion A from 45 kg at the start of the experiment to 36 kg at the end and for lion B from 34 kg to 25 kg; their general condition became very poor and weak.

Table 1 and 2 show the evolution of some blood cell parameters of lion A and B. Table 3 and 4 give data on the evolution of some blood serum and plasma components during the course of infection.

Table 1. Red and white blood cell changes in lion A

Days before and after infection	–14	0	+12	+22	+29	+42	+50
R.Bl.C./mm³	5,575,000	5,575,000	5,900,000	5,400,000	5,000,000	5,150,000	3,450,000
Hematocrit %	37	37.5	38	37	32.5	37	23
Hg level g %	12.4	12.4	12.95	12.4	10.8	10.95	7.1
Mean Hg conc. g %	33.5	33.1	34.1	33.5	33.7	29.6	30.8
Mean cell volume μ3	66	68	65	68	64	72	67
W.Bl.C./mm³	10,500	14,700	10,200	7,650	7,050	37,200	10,100
Total neutro %	87.5	79	73.5	85.5	75	72	66
Mature neutro %	80.5	77	70	84	66	41	52.5
Band neutro %	7	2	3.5	1.5	9	31	13.5
Lymphocyte %	12.5	17	19	14	21	24	31
Monocyte %	0	0.5	3.5	0	3	4	2.5
Eosinophil %	0	3.5	4	0.5	1	0	0.5

Table 2. Red and white blood cell changes in lion B

Days before and after infection	–14	0	+12	+22	+29	+42	+50
R.Bl.C./mm³	6,530,000	5,825,000	4,750,000	5,150,000	4,375,000	4,600,000	4,810,000
Hematocrit	42	39.5	32.5	33	29	31	32
Hg level g %	14	12.6	10.8	10.8	10.2	10.5	10.35
MCHC g %	33.4	31.9	34.2	33	35.2	33.8	32.3
MCV μ3	64	67	69	64	66	67	67
W.Bl.C./mm³	11,300	11,350	10,400	6,800	9,250	15,300	12,250
Total neutro %	89	76.5	84.5	82.5	79.5	82	79
Mature neutro %	82.5	75	80.5	78	79	72	59
Band neutro %	6.5	1.5	4	4.5	0.5	10	20
Lymphocyte %	11	20.5	13.5	12	17.5	13.5	14
Monocyte %	0	1	1	4.5	1.5	2.5	2
Eosinophil %	0	2	1	1	1.5	2	5

Table 3. Blood serum and plasma changes in lion A

Days before and after infection	−14	0	+12	+22	+29	+42	+50
Glucose mg %	114	133	82	73	106	86	82
Total protein g %	6.78	7.35	7.39	7.56	7.19	7.6	6.6
Albumin %	54.5	53.9	42	37.3	33.8	30.8	36.9
Globulin α_1 %	3.2	3.5	2.6	2	3.6	3.9	2.1
Globulin α_2 %	8	4.7	6.3	3.1	3.6	4.3	2.6
Globulin β %	13.9	13.6	17.4	18.9	14.4	20.6	7.8
Globulin γ %	20.4	24.3	31.5	38.7	44.6	40.4	50.6
Alc. phosph. U	17.8	21	6.6	10	7.3	2.7	1.4
SGOT U	14	23	18	13	29	40	28
SGPT U	10	14	5	6	13	7	14
Total bilirubin mg %	0.2	0.2	0.25	0.2	0.25	0.6	0.5
Thymol (MacLagan)	0.4	0.25	1.5	5.75	5.1	3.4	2.8
$ZnSO_4$ (Kunkel)	0.15	0.5	2.5	10.5	9.65	9.4	12.75

Table 4. Blood serum and plasma changes in lion B

Days before and after infection	−14	0	+12	+22	+29	+42	+50
Glucose mg %	102	95	78	74	72	78	74
Total g %	6.81	6.40	6.86	7.35	7.54	6.40	7.24
Albumin %	55	52	41.9	28.5	33.9	34.5	37.1
Globulin α_1 %	3.5	4.4	3.4	2.2	3.4	2.5	4.5
Globulin α_2 %	6.9	6.9	5.1	5.6	6	5	6.3
Globulin β %	13.8	7.6	18.6	17.9	16.9	14	8.6
Globulin γ %	20.8	29.1	31	45.8	39.8	44	43.5
Alc. phosph. U	19.8	20.6	2.6	2.6	2.3	4	4.6
SGOT U	31	7	10	13	13	5	13
SGPT U	25	5	5	6	5	5	5
Total bilirubin mg %	0.3	0.25	0.15	0.2	0.3	0.35	0.1
Thymol (MacLagan)	0.3	0.1	1.75	5.35	5.85	3.55	3.25
$ZnSO_4$ (Kunkel)	0.15	0.4	2.8	9.80	11.75	10.65	9.15

Table 5. Trypanosoma brucei found in the peripheral blood of lion A and B

Days after infection	+12	+22	+29	+34	+42	+50
Lion A						
Wet slide	−	+	−	−	−	−
Thick drop	−	+	−	−	−	−
Rat inoculation	+	+	+	+	+	+
Lion B						
Wet slide	+	−	−	−	−	−
Thick drop	+	−	−	−	−	−
Rat inoculation	+	+	+	+	+	+

Table 5 summarizes the results of parasitaemia obtained by microscopic examination of peripheral blood of lion A and B and by subinoculation of the lion blood into white rats.

Discussion

The results summarized in table 5 confirm those obtained by SACHS et al. (1967) in the field, who could not find *Trypanosoma brucei* parasites in blood smears but who were able to demonstrate them by rat inoculation; even in experimental *Trypanosoma brucei* infection we had generally to inoculate rats to demonstrate the presence of parasites in two lions.

These experimental animals developed a parasitaemia which could be observed by microscopic examination only during the first weeks after infection; even then the parasites were very rare.

Both the animals developed a progressive anaemia with a slow decrease in the hemoglobin content, the red blood cell count and the hematocrit value; the mean corpuscular hemoglobine concentration (MCHC), however, did not seem to be changed significantly, thus the anaemia seems to be normochromic.

The most striking point of the white blood cell formula is the increase, after one month of infection, in the young band neutrophils, whereas the total neutrophil count did not change seriously in lion B nor in lion A.

In general a slight progressive increase in the mononuclear white blood cells accompanied by a slight decrease in the polynuclear cells seems to dominate the general white blood cell count during the course of the infection.

The data of lion A recorded for day 42 and 50 have to be interpreted with certain reserves as the animal developed at that time a purulent skin infection which had to be cured by local and parenteral antibiotic treatment.

The biochemical blood serum and plasma parameters show a heavy decrease in blood glucose, alcaline phosphatase and albumin.

The drop of the blood glucose content in the early stages of the disease is a well known phenomenon in experimental *Trypanosoma brucei* infection in different animal species; it could be observed in these lions too.

As the animals were young and suffering from severe bone formation disorders, we could normally expect increased values of alcaline phosphatase; on the contrary an early and heavy drop of this enzyme level accompanies the onset of infection.

The total protein content does not change but the albumin/globulin ratio is reversed; the heavy and progressive increase in the gamma fraction of the globulins seems to be responsible for it. However, at day of sacrifice, the albumin content rises again; it could be due to the extremely poor condition of the animals accompanied by starvation and thirst.

The MacLagan thymol test and the Kunkel zincsulphate test confirm the evolution observed for the globulin fraction.

The data obtained for the transaminases SGOT and SGPT give irregular lines and do not give any particular information.

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