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Pathological and biochemical changes in rats infected concurrently with *Nippostrongylus brasiliensis* and *Trypanosoma congolense*

by **J. O. SIMAREN** and **F. M. BAMMEKE**

Laboratory of Parasitic Diseases and Biochemistry, Department of Biological Sciences, University of Ife, Ile-Ife, Nigeria

Résumé

Tous les rats ont reçu la dose-étalon, soit de 1.000 *N. brasiliensis*, soit de 10.000 trypanosomes, soit les deux. Il résulte de l'infection simultanée que les trypanosomes qui apparaissent sont bien moins nombreux que ceux qui apparaissent lors de l'infection unique. On a vu une quantité progressivement croissante de trypanosomes se manifester beaucoup plus dans les infections doubles que dans les infections uniques.

Le taux de glucose est plus haut dans les infections à *Nippostrongylus* que dans les infections des autres groupes. Les résultats montrent que les infections à *T. congolense* et à *N. brasiliensis* changent le métabolisme des hydrates de carbone du rat. Sauf pour les infections à *Nippostrongylus* dans lesquelles on a vu une augmentation du taux de l'albumine, les globulines alpha et béta ne se modifient pas beaucoup pendant la même période, alors que l'albumine diminue.

Summary

All the rats received the standard dose of either 1,000 infective *N. brasiliensis* or 10,000 trypanosomes or both. In the concurrent infection the total number of trypanosomes produced was considerably lower than in the single infection. A gradual rise in trypanosome population was more observed until necropsy in double infections than in single infections.

The amount of glucose in *Nippostrongylus* infections was higher than in other infected groups. The result shows that there is an alteration in the carbohydrate metabolism of the rats by both *T. congolense* and *N. brasiliensis* infections. Albumin decreased and alpha and betaglobulins did not differ much in the same period except in the *Nippostrongylus* infections where albumin increased.

Introduction.

Of recent, the techniques of studying parasitic infections in man and animals in elucidation of host parasite relationship have now shifted to includ immunological, biochemical, cellular and microbiologic alterations of the host to the parasite. Sokolic et al 1963, observed acquired immunity in sheep infected with *Dictyocaulus filaria.* Cox 1964 reported immune response to *Trypanosoma cruzi* and *T. lewisi* infection in rat and mice. Herlich and Merkel (1963) observed a relative increase in gamma-globulin in calves infected with *Trichostrongylus axei.* Sadun et al 1965 reported significant differences in glucose levels in mice infected with spargana of *Spirometra mansonoides* than in the uninfected controls.

Larsh and Donaldson (1944) demonstrated that mice infected concurrently with *Nippostrongylus muris* and *Hymenolepis nana* exhibited a marked immunity to the tapeworm by harbouring one-half of the number of cysticercoids observed in the controls. Simaren (1969, 1970) observed some cellular and striking pathological changes in rats infected with *N. brasiliensis* and *T. brucei* with particular references to their growth and development. He attributed these changes, reduced egg production,, stunting and loss of the adult parasitic nematode to physiological factors associated with the beginning of an acquired immunity of the host to the infection. The purpose of this investigation therefore is to find out further, biochemical and qualitative cellular changes occuring in rats infected concurrently with *Trypanosoma congolense* and *Nippostrongylus brasiliensis.*

Materials and Methods.

Three groups 10-12 weeks old white laboratory bred rats (6 per group) were selected for infection. The strain of *N. brasiliensis* and the trypanosome species used were being maintained in our parasitology laboratory for the past 3 years. Procedures for the maintenance of these test agents are similar to those described by Haley 1966a, Yogogawa 1922, Lincicome and Watkins 1963. Group I was infected with *T. congolense alone.* Group II was concurrently infected with *T. congolense* and *N. brasiliensis.* Group III received *N. brasiliensis* only while group IV was the uninfected control. Each rat received either 1,000 Nippostrongylus larvae or 10,000 Trypanosomes or both. All infected rats were individually kept in clean cages and were fed with commercial food pellets and water. Quantitative standardization of the try-

panosomes count was accomplished by determining the trypanosomes in 1 ml of blood suspension by use of hemocytometer and blood pipettes. The white blood cells and the red blood cells were counted using appropriate blood pipette and mixture of diluting fluid (0.75 ml. of 1 % Saponin in normal NaCl ; 0.5 ml. of 1 % Toluidine Blue in normal NaCl ; 4 ml. of 10 % Formalin, in normal NaCl ; and 4.75 ml. of 1 % sodium citrate) for Trypanosome count. A solution of 16.5 ml. of acetic acid glacial, 6.25 gm. sodium sulphate crystal both in 100 ml. distilled water was used for red blood cell count ; and 2 ml. acetic acid glacial in 98 ml. distilled water with two drops of Methyl violet 6 B for white blood cell count (Stiff, Clough and Branham, 1948). The degree of trypanosome parasitemia was checked daily.

Blood collected by cardiac puncture from infected rats in each group were chemically analysed. Glucose level determination was by Folin and Wu method (1920). The protein free blood filtrate were heated with alkaline copper solution using special tube to prevent reoxidation. The cupprous oxide formed was treated with a phosphomolybdic acid solution to produce blue molybdenum oxide whose concentration was directly proportional to the amount of glucose present. The glucose amount was estimated with a spectrophotometer at 420 mu. A modified method of paper électrophoresis (Chen 1959) using a Shandon type of electrophoresis cell was carried out on three serum samples (20 ml. each) from each group experiment. Separate strips of Whatman paper No. 3 MM (28 × 5 cm) previously moistened with phthalate buffer (pH 5.6) were spotted and a current of 17.5 mM on 150 volts was applied to the strips and ran for 3 hrs. The strips were then stained with 0.1 Bromophenol blue in absolute methanol, containing 6 % $HgCl_2$ washed in running water and dried at room temperature. Quantitative determination of each protein fraction was individually eluted in 5 ml. 50 % alkaline methanol. The colour intensity of the eluate was spectophotometrically estimated at 595 mu. Daily body temperatures of all rat were rectally taken and recorded.

Results and Discussion.

In the four groups of experiments performed, the course of infection and individually traced degree of parasitemia was interpreted by means of trypanosome count, white and red blood cell count, changes in body temperature, glucose and protein levels of the infected rats sera.

From the data collected the prepatent period for *N. brasiliensis* was normal. The daily average number of trypanosomes per mm^3 was significantly higher, 289,600 in single infection and 198,000 in the concurrent infection on the 9th day post infection (Tables I et II).

Daily qualitative rate of trypanosomes developments was slower in concurrent than single infections with the former attaining a peak on the 11th day. Higher body temperatures were recorded for consecutive three days preceeding death in the double infections than in the single infections.

Table I

AVERAGE DAILY HEMOCYTOMETER ESTIMATION OF TRYPANOSOME POPULATION, WBC, AND RBC PER mm^3, 6 RATS INOCULATED *Intraperitoneally with* 10,000 *of T. congolense*

DAYS AFTER INFECTION

	5	6	7	8	9	10*
Temp.	96.2	96.8	97.2	97.6	96.6	
Tryps/Wet Mt	—	+	+	++	++	
Tryps/mm^3	2×10^2	5×10^2	207×10^2	219×10^2	2.896×10^2	
WBC/mm^3	43.5×10^2	46×10^2	66×10^2	70×10^2	73.5×10^2	
RBC/mm^3	85.2×10^4	76.2×10^4	65×10^4	56.3×10^4	47.4×10^4	
Tryps/10^5 RBC	23	66	3,185	38,969	65,316	

— or.
+ Density of Tryanosome.
* All dead.

Table II

AVERAGE HEMOCYTOMETER ESTIMATION OF TRYPANOSOME POPULATION, WBC AND RBC PER mm^3. RATS INFECTED SUBUCTUTAEEOUSLY WITH 1,000 LARVAE *N. Brasiliensis* AND SIMULTANEOUSLY WITH 10,000 *T. congolense*

DAYS AFTER INFECTION

	5	6	7	8	9	10	11
Temp.	98.3	97.5	97.6	97.4	99.0	99.9	101.0
Tryps/W et Mt	—	+	+	++	++	++	++
Tryps/mm^3	2×10^2	4×10^2	44×10^2	433×10^2	1.913×10^2	1.920×10^2	1.980×10^2
WBC/mm^3	57.3×10^2	51.8×10^2	64.3×10^2	66.5×10^2	77.8×10^2	84×10^2	86×10^2
RBC/mm^3	80.6×10^4	72.3×10^4	67.3×10^4	63.7×10^4	53.7×10^4	54.8×10^4	48.10×10^4
Tryps/10^5 RBC	25	55	654	6.782	33,577	35,036	41,250

—/+ Density of Trypanosome.
* All dead.

Table III

AVERAGE NUMBER OF WBC AND RBC MM^3 IN TAIL BLOOD OF 6 RATS INFECTED SUBCUTANEOUSLY WITH 1,000 LARVAE OF *N. brasiliensis*

DAYS POST - INFECTION

	5	6	7	8	9	10	11	12
Temp.	98.6	97.4	97.0	97.3	98.6	97.5	99.9	98.5
WBC/mm^3	35×10^2	36.5×10^2	56.5×10^2	56×10^2	35×10^2	47.8×10^8	48.8×10^2	47×10^2
RBC/mm^3	77.8×10^4	84.4×10^4	91.6×10^4	93×10^4	96.2×10^4	92.6×10^4	91.7×10^4	88.3×10^4

Table IV

AVERAGE NUMBER OF WBC AND RBC PER MM^3 IN TAIL BLOOD OF 6 UNINFECTED CONTROL RATS

DAYS POST - INFECTION

	5	6	7	8	9	10	11	12
Temp.	98.7	97.4	97.7	97.1	97.4	97.7	97.8	97.4
WBC/mm^3	46×10^2	66.3×10^2	81.3×10^2	83×10^2	66.8×10^2	69.3×10^2	73.3×10^2	65×10^2
RBC/mm^3	193×10^4	185×10^4	190×10^4	191.9×10^4	195×10^4	191.9×10^4	188×10^4	190×10^4

In group IV (uninfected control) the daily average RBC/mm³ was stabilized with an average of 856×10^4 for days 5-9 post infection. For the corresponding days post infection, group III rats infected with *N. brasiliensis* alone showed a decrease of $443 \times 10^4 \times 10^4$ RBC/mm³, while group II infected with both *N. brasiliensis* and *T. congolense* gave a lower $337{,}6 \times 10^4$ RBC/mm³. The lowest average indicated by group I infected with *T. congolense* alone was 330.3×10^4 RBC/mm³ (Tables I - IV and Fig. 1).

The destrution of RBC is more pronounced in group I and II than in group III compared with group IV. These confirm the report of Simaren (1970) and emphasise more quantitatively the pathobiological changes occuring by using heavier doses of the infective agents. The decrease in RBC in groupe II as compared with group III further indicates the effect of parasitemia of *T. congolense* coupled with the ingestion of blood and destruction of intestinal tissue by *N. brasiliensis* (Taliaferro and Sarles 1939, Porter 1935b, and Simaren 1964, 1967, 1969, 1970). Furthermore, remarkable differences in glucose level were observed between uninfected control, the single and double infections. The result here is parallel to the work of Sadun et al 1965 and Symon 1960c who reported similar significant changes in glucose level in mice infected with Spargana of *Spirometra masonoides* compared with the controls. However, in our experiments the amount of glucose in sera from trypanosome infections alone showed a much absorbance of glucose by *T. congolense* and *N. brasiliensis* (Table V). Traces of the different protein fractions are shown with beta-globulin

Table V

DATE SHOWING THE AVERAGE AMOUNT OF GLUCOSE IN BLOOD OF INFECTED *and 6 Uninfected rats at the Peak of Infection*

* No. to Experiment groups	Days after infection	No. of Parasites per Rat	No. of Rats necropsied	Glucose mg/100 ml. bld.
1	8	10,000 *T. congolense*	3	8
2	9	10,000 Tryps + 1,000 Nippo	3	1.5
3	10	1,000 *N. brasiliensis*	3	27.5
4	10		3	36

* 1 Infected with *T. congolense* alone.
2 Infected with *T. congolense* and *N. brasiliensis.*
3 Infected with *N. brasiliensis* alone.
4 Uninfected control.
Tryps — *T. congolense;* Nippo — *N. brasiliensis.*

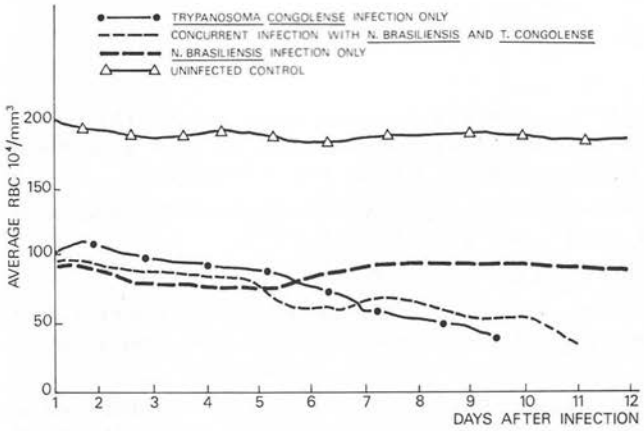

FIG. 1. — Comparative average RBC/mm^3 in rats infected concurrently with *T. congolense* and *N. Brasiliensis* singly with both *T. congolense* and *N. Brasiliensis*, and in the uninfected control

absent in group III infected rats, while post-albumin fraction were absent in groupe I and II infections (Table VI). This depressed protein digestion in rats infected with *N. brasiliensis* was likewise observed by Symons (1960d) Kagan et al (1961). The degree of parasitemia found to be higher in single infections than in the concurrent infections is probably due to the competition for food nutrients between the parasites in the concurrent infections.

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Table VI

DATA SHOWING THE EXTINCTION VALUES OF PROTEIN FRACTIONS FROM SERA OF INFECTED AND UNINFECTED RATS

* No. to Experiment group	No. of rats necropsied	Mean Absorbance			
		Albumin	Post-Albumin	α-globulin	β-globulin
1	3	.66	—	.192	.11
2	3	.56	—	.18	.155
3	3	1.38	.182	.16	—
4	3	.99	.08	.156	.28

* As in Table V.

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