

Worm burden, pathophysiology and indirect nutritional effect of *Nippostrongylus brasiliensis* on the host

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Résumé

Trente-deux rats infestés avec 500, 1 000 et 4 000 larves de *Nippostrongylus brasiliensis* réagirent de différentes manières. Les autopsies effectuées 48 heures après l'infection montrèrent une rétention plus faible de vers dans les poumons dans les infections moyennes que dans les fortes infections. Des diminutions modérées du contenu total des protéines ont été observées dans les sérums des rats des expériences B et D, cependant que dans l'expérience C, le chiffre obtenu était même nettement inférieur à celui des témoins. Les réductions varièrent de 23 à 37 pour cent. Le diamètre moyen des muscularis externa dans les expériences D et E était presque le double de celui observé dans les expériences C et B, par rapport aux témoins. La population moyenne de vers adultes récoltés à la fin de chaque expérience était proportionnelle à la dose de larves donnée à chaque infection.

Les infections avec de moyennes et fortes doses de vers ont réduit de 40 à 68 pour cent le contenu en glucose du sérum par rapport aux témoins. La réduction dans le gain de poids fut plus prononcée chez les rats très infectés que dans les groupes modé-

rément infectés. Ces différences qualitatives et quantitatives obtenues dans cette étude de faibles, moyennes et fortes infections, démontrent que les vers produisirent des gradients de compétition ainsi que de vraies compétitions pour le régime alimentaire de l'hôte. Les diminutions quantitativement observées par tests biochimiques, furent pathologiquement importants, et en conséquence, furent des résultats indirects de la diminution de digestion et absorption des aliments disponibles dans l'intestin de l'hôte.

Les observations histo-pathologiques et physiologiques, et les faibles gains de poids des rats infectés, furent directement causés par les interactions hôte-parasite associées au mécanisme nutritionnel agissant pendant l'infection.

Summary

Thirty-two rats infected with 500, 1 000 and 4 000 *Nippostrongylus brasiliensis* larvae generated different responses. Autopsies performed 48 hours post infections showed a retention of fewer worms in the lungs of moderate infections than the heavy infections.

Moderate decreases in total protein were observed in sera of rats in experiments B and D, except that the average found in experiment C was markedly lower than the control's. The reduced levels varied from 23-37 per cent. Average diameter of muscularis externae in experiments D and E were almost twice or twofold the measurements found in experiments C and B compared with the control's. The mean adult worm population recovered from each experiment appeared proportional to the larval dose given per infection.

Infections with moderate and heavy burdens produced lower glucose figures in the serum compared with the control's. The range of reduction was 40-68 per cent. Reduction in weight gains were more pronounced in the rats heavily infected than in the groups which were moderately inoculated. These qualitative and quantitative differences obtained in this study of light, moderate and crowded infections demonstrate that gradient and true competitions for the host's diet by the worms existed. The decreased results collected from the biochemical tests were pathologically important and were consequently indirect results of reduced digestion and absorption rate of available food materials in the host's intestine.

The histo-pathological and physiological findings, and the poor weight gains shown by the infected rats were directly caused by the host-parasite interactions associated with the operating nutritional mechanism during the infection.

Introduction

Several reports and reviews on *Nippostrongylus brasiliensis* have appeared in the literature in recent years. These reports have been on the increase, because this organism is an excellent example in that its study provides a better understanding of many phases of biological systems. *Nippostrongylus brasiliensis* infection in rats produces a serious and fatal disease, the intestinal pathology of which is complex and accompanied by marked physiological derangements. Observation has shown that the infection is very similar to some pathological conditions of unknown etiology in man; such as ulcerative colitis (Symons and Fairbairn, 1962, Ogilvie and Jones, 1971). While parasitologists continue their research efforts to improve the known metabolic knowledge of the organism, major literature highlights on the parasite include its biology, development, effect of drugs, digestion and absorption (Haley, 1962, Simaren, 1964, 1970; Ashley, 1962; Symons and Fairbairn, 1962, Symons, 1966).

Africa (1931), Lindquist (1950) and Haley (1958) observed innate resistance on the parasite in hamsters and acquired resistance in rats. Both forms of resistance affect female worms more than the males. Studies on adaptation of the parasite were accomplished in mice and hamsters (Solomon and Haley, 1966) except in rabbits where only few worms reached adulthood (Thorson, 1953 *a*). Cellular changes in the skin, alterations in fat and electrolytes in the jejunum tissue have also been recently reported (Mulligan et al., 1965, Neilson, 1969 *a*, Symons, 1966, Taliaferro and Sarles, 1939, Symons and Fairbairn, 1961, Symons and Jones, 1970). Since the methods, objectives and results of these reports varied and the investigations were restricted directly to the diseased intestine or other tissue of the hosts used, the present paper describes experiments on the qualitative and quantitative changes in *Nippostrongylus* infections based on worm burden, patho-physiology and indirect nutritional effect of its parasitism on the rat host.

Materials and methods

White female albino rats (7 weeks old) used in this investigation were obtained from our laboratory breeding stock. These rats were maintained separately in wire cages over sawdust-covered pans and were fed commercial food pellets and water ad libitum. The strain of *N. brasiliensis* used through-out this study has been maintained in white laboratory rats for the past four years in our parasitology laboratory. The life cycle of this test agent was reviewed by Haley (1962). The culture method of the organism, isolation and preparation methods of infective larvae from culture for injection have already been described (Simaren, 1963, Simaren and Ogunkoya, 1970).

Desired standardized doses of infective larvae (7-days old) collected from the cultures were injected subcutaneously into each rat in all experiments except the control. Five groups of experiments (A, B, C, D and E) with 8 rats per group were conducted. Group A served as uninfected control. Group B rats were each infected

with 500 *Nippostrongylus brasiliensis* larvae. The C group rats were also each injected with 1 000 larvae, while group D and E rats were respectively challenged with 2 000 and 4 000 larvae subcutaneously.

The number of eggs passed daily for every 24 hour fecal collection for fifteen days was determined by a modification of Stoll's dilution egg count method as previously reported (Simaren 1963, 1964). The number of adult worm population found in the lungs and small intestine of the rats was quantitatively determined by direct counts. Autopsies were performed after 48 hours and on the 10th day after infection on rats randomly selected from each group. These rats were necropsied by etherization in order to avoid hemorrhages in the lungs. The infected lungs were qualitatively evaluated and gross histopathological preparations made from them and the intestine. The small intestines were cut into small pieces, slit and spread open in petri-dishes. The worms were carefully teased and counted under correct light adjustment of a dissecting binocular microscope. Microscopic examinations were performed by pressing small sections of the lungs between two glasses for possible revelation of worms trapped and retained in the lungs. Measurement of diameters of muscularis externae were recorded. The lengths of twenty adult worms (male and female) from each group isolated were measured by a method previously reported (Simaren, 1963). Weights of all infected rats were taken before the infection. All measurements were also repeated on the 5th, 10th and 15th day post infections. From this data the percentage of weight gain was calculated.

Determination of blood glucose level on the 10th day after infection was made by a macro-procedure method (Folin and Wu, 1920, 1929). This method permits the interaction of protein-free filtrate with alkaline copper solution. The cuprous oxide formed is treated with phosphomolybdic acid given a blue molybdenum oxide whose concentration is directly proportional to the amount of glucose present in the solution. Readings were made spectrophotometrically at 420 mu. For protein measurement, the method of Henry, Sobel and Berkman (1957) was adopted. The serum proteins reacted with alkaline copper reagent to give a bluish-purple complex (Biuret reaction). Spectrophotometric readings were made to determine the amount of protein present at 545 mu.

Results and discussion

Symons and Fairbairn (1962) and Simaren (1964) have respectively described the jejunum pathology and the development of *N. brasiliensis* infections. The following detail account is limited to the qualitative and quantitative results of our investigation presented in this communication.

In *Nippostrongylosis* the main characteristic features include the enlargement of normal jejunum due to hyperplasia of mucosa and hypertrophy of the muscularis externa, although the extent of histopathology depends upon the severity of the infection. No such severe traces were observed in experiment B rats, except instances

of migratory passages seen in the lungs. The average egg production between 5th and 15th day after infection by group B rats was 721,000. Group C rats produced a mean of 1,114,000 eggs while rats in group D and group E yielded 858,000 and 996,000 eggs in the same period. These results indicate that in infections of rats with *N. brasiliensis*, moderate worm burdens have greater capacity for egg production than heavier worm loads (Table I, expts. B to D).

The adult worm population found in the intestines in experiments B and C were well developed and appeared normal. The magnitude of worm burden in each experiment is expressed as mean per cent of the number of larvae given. The mean worm burden in group B was 70 per cent. Group C rats produced 60 per cent, while decreases of 42 per cent and 31 per cent were found in group D and E rats. There were noticeably fewer, underdeveloped and stunted worms found in group D than in group E (Table 1).

TABLE I

Comparative Summary of Qualitative and Quantitative Data Collected from Rats Infected with Nippostrongylus brasiliensis

Group Expts.	No. of rats infected	No. of larvae given	Av. Eggs produced (thousands) per 2 wks	No. of rats autopsied	Mean per cent Adult worm burden
A control .	8	—	—	4	—
B	8	500	721	4	70
C	8	1 000	1,114	4	60
D	8	2 000	858	4	42
E	8	4 000	996	4	31

The mean lengths of male and female worms measured in groups B through E were within the normal range reported by Yokogawa (1920) and Simaren (1964). These findings confirm previous reports on the relationships between development and routes of infection of *N. brasiliensis*. The present result further shows that moderate to high density of infective *Nippostrongylus* larvae does not inhibit the growth of the parasite. Microscopic examination of the lobes of the lungs of rats in group D and E exhibited severe to moderate dark brown hemorrhagic spots in the alveoli (Figs. 5 and 6). These hemorrhages were due to intense inflammatory reaction which were more pronounced in group E. Slightly fewer worms were found trapped in the lungs of heavily infected rats than in the moderately infected groups (Table 2).

Histological and morphological changes of the small intestine of infected rats

in group D and E showed the nematode parasites were adhering to the mucosa. These worms were found embedded in reddish to mucoid nodular pockets or aggregates of varied clusters in the small intestines, especially in group E rats (Figs. 1-4). The intestinal villi and crypts were also found inflamed coupled with hypertrophy and hyperplasia. Another aspect of the pathology noticed in the study was the abnormal size of brown watery fluids which filled the thick and flabby jejunum. The picture was more prominently displayed in the intestine of group E rats than in group D but were less depicted in group B. The muscularis externae of rats in groups E, D, C and B compared with those of A group (control) were found to be in increasing degrees of thickness as the challenging dose increased in the experiments (Table 2, Figs. 2-4). Symons (1960 *d*) observed thickened muscularis externa in rats infected with *N. brasiliensis*. No measurement data were reported. These pathological changes were probably caused by the absorptive feeding action of the parasite — on the gut tissues or functional defect of the intestine initiated by the infections. It is interesting to report that two of the rats infected in the group E experiment became lethargic and diarrhetic a few days post infection, and were ignoring their food.

Average weight gains by the heavily infected and moderately infected animals were much lower than the controls (Table 2). The observed changes in weight measurement of the infected rats were a definite response to the various degrees of inoculations. The reduced growth rate was aggravated in part by the worm loads coupled with the rats' poor appetite.

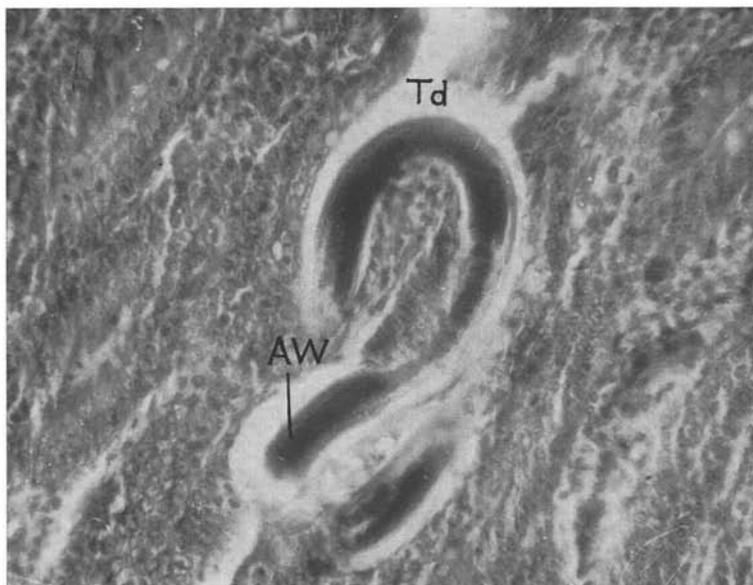


FIG. 1. — Section through the intestine showing adult worm deeply embedded in the submucosa. Movement, and feeding of the worm accompanied by tissue destruction of the worm is visibly depicted in the light area. Hematoxylin stain $\times 5.5$

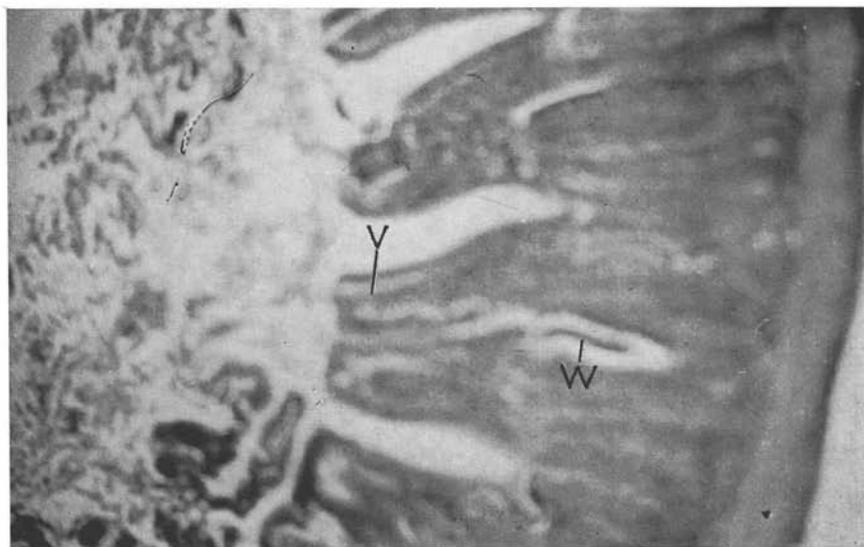
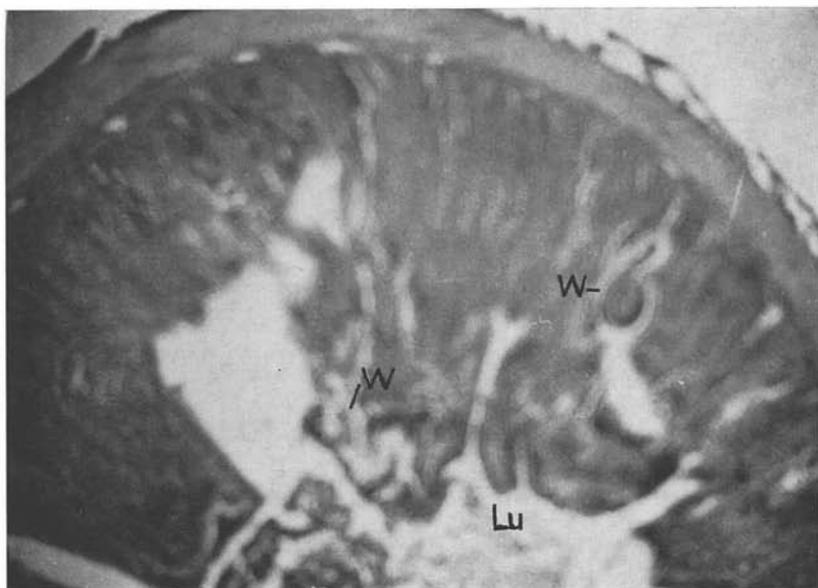


FIG. 2 and 3. — Cross sections of the intestine showing *Nippostrongylus* worms among several villi. Some of the worms are underdeveloped. Hematoxylin stain

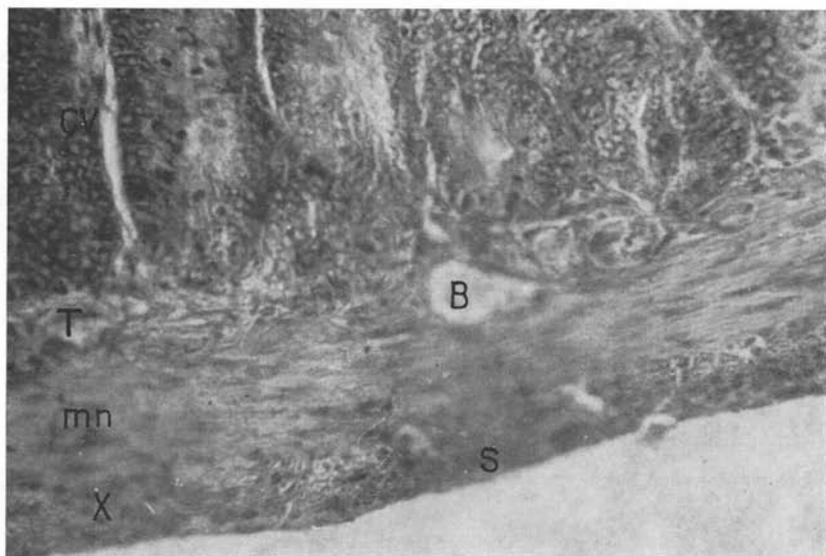


FIG. 4. — Intestinal cross section showing dilation of a blood vessel. Columnal villi, Connective tissue, Circular muscle, Longitudinal muscle and Serosal layer. Hematoxylin stain

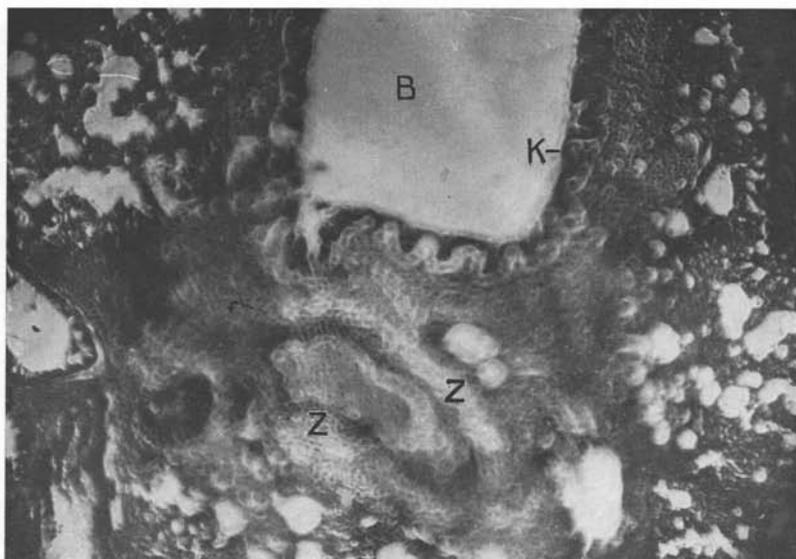


FIG. 5. — A cross section of an infected lung showing a blood vessel with its thickened wall, exceptionally dilated and filled blood. Inflamed lungs characteristic of heavy infection of experiment E is represented. Hematoxylin and Eosin stain

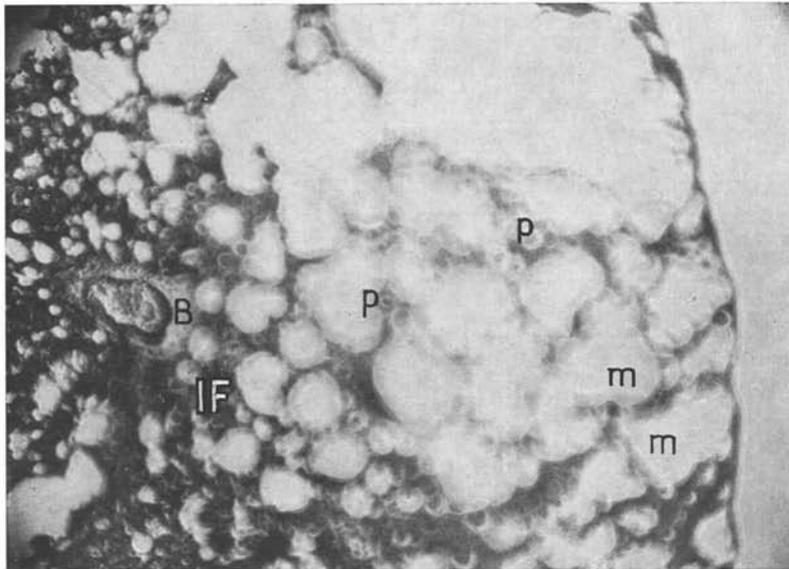


FIG. 6. — Another cross section of the infected lung with *N. brasiliensis*, showing small blood vessel, migratory routes, and lesions in the tissue. These and pigments in the cells are prominently displayed. Hematoxylin stain

Analytical results of the amount of glucose and protein present in the blood on the 10th day after infection in the control rats gave average readings of 48.1 mg per cent for protein and 35.4 per cent for glucose. Biochemical determination of blood serum from rats infected with 500 larvae (Group B) produced 34.1 per cent protein and 19.1 per cent glucose. At the same time, protein and glucose tests were separately made from serum collected from rats in groups C, D and E. The spectro-photometric readings for C yielded 29 per cent protein and 12.6 per cent glucose, while the mean levels of glucose and protein obtained from the serum of group D animals were 13.4 per cent and 34.0 per cent respectively. One unusual observation found here was the existing low levels of glucose noticed in serum of B and C experiments. In experiment E, a similar situation occurred where glucose and protein test readings showed only 11 per cent for the former and 37.5 per cent for the latter (Table II). Symons (1960 *d*) reported that jejunal protein digestion and absorption were reduced in rats infected with *N. brasiliensis*. Symons and Jones (1970) also claimed that no depression of absorption of glucose and histidine was observed from the entire intestine. However, Herlich (1962) successfully produced marked clinical signs and reductions in serum phosphorus, glucose and protein in his studies of mixed experimental infections of *O. ostertagi*, *T. axei*, *T. colubriformis* and *C. punctata* in calves. Layrisse et al. (1961) produced evidence of iron deficiency, reduced level of Vitamin B 12 in serum, impairment of folic acid absorption, reduced nitrogen substances in human patients with

TABLE II

Comparative Summary of Qualitative and Quantitative Data Collected from Rats Infected with *Nippostrongylus brasiliensis*

Group Expts	Mean length recovered worms (mm)	Mean per cent worm in lungs	Mean diameter muscularis externae (microns)	Mean per cent weight gained by rat	Mean serum glucose value mg/100 mb	Mean Serum protein value mg/100 mb
A control	—	—	65	25.4	35.40	48.20
B	M 3.8 F 4.5	4.8	78	22.6	19.10	34.11
C	M 3.4 F 4.6	9.3	130	17.5	12.60	29.05
D	M 3.6 F 4.4	16.2	150	10.3	13.10	34.05
E	M 3.1 F 4.5	21.7	176	7.5	11.07	37.50

severe hookworm infections. Several reports including that of von Bonsdorff (1956) on the pathogenesis of *Diphyllobothrium latum* in man have demonstrated evidence of anemia and deficiency of vitamin B 12 in the host partly caused by the worm's competition with the host for this dietary extrinsic factor. Concomitantly, our results of this investigation strongly suggest occurrences of similar competitive phenomena. The histopathological differences and the serum biochemical changes reported here indicate direct and indirect parallels to these authors' findings. These effects on the host may be attributed to the bioecology of the parasites, accompanied by their yet unknown bursting activities within.

The qualitative results further indicate that, while clinical and other signs are often recognised in chronic gastro-intestinal diseases, severe and detrimental serum changes frequently occur in such acute infections. The data presented definitely demonstrate that in moderate to heavy infections, reproductive capacity and infectivity can be suppressed. Tissue pathological changes and serum biochemical alterations often occur as infective dose increases. Attempts to further define the histopathology of this host-parasite relationship have been demonstrated.

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Key to the Abbreviation of Figures

AW	Adult worm.
W	Underdeveloped worms.
B	Lumen of Blood vessel.
Cu	Columnal villi.
IF	Lung inflammatory lesion.
K	Thickened wall of blood vessel.
Lu	Intestinal lumen.
M	Migratory route in the lung.
Mn	Circular muscle.
P	Pigments in lung tissue cells.
S	Serosa.
T	Connective Tissue.
Td	Destruction of tissue in the intestine.
V	Villi if intestine.
X	Longitudinal muscle.
Z	Inflamed lung alveoli.
