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Host response and biologic differentiation of *Nippostrongylus brasiliensis*

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Résumé

Des expériences de groupe dans lesquelles des rats ont été infectés par des larves *Nippostrongylus brasiliensis* âgées de 8, 14, 21, 28 et 35 jours par voie sous-cutanée, intraveineuse et intrapéritonéale ont été entreprises.

On a comparé l'évaluation de la réponse de l'hôte au développement du parasite. La réduction du nombre des œufs produits et la perte progressive des vers entre les groupes montre un abaissement de l'infectivité larvaire, résultat de l'augmentation de l'âge des larves.

On a vu, par l'analyse des résultats et des données fournies par l'âge du rat-hôte et les expériences *in vitro* que les larves filariformes ont vraiment besoin d'oxygène et utilisent l'oxygène du sang pour se développer et produire des œufs. Le pourcentage des larves introduites oralement, qui parviennent à maturité est très faible. La réponse et la survie décevante obtenues chez les rats infectés par voie orale et péritonéale s'expliquent par l'effet nocif produit par la présence de vers dégénérés dans la cavité péritonéale, par l'augmentation de l'activité enzymatique et par la sensibilisation de l'hôte à l'infection vermineuse.

Summary

Group experiments in which rats infected with *Nippostrongylus brasiliensis* larvae of 8, 14, 21, 28 and 35 days of age subcutaneously, intravenously, intraperitoneally and orally were carried out.

An evaluation of the host response to the development of the parasite was compared. The reduced number of eggs production

and the gradual loss of worms between the groups illustrate a decreasing larval infectivity with an increasing larval age.

Analysis of results and data collected from development of these various larval age in the rat host and the *in vitro* tests show that the filariform larvae indeed need oxygen and utilize blood oxygen for development and eggs production. The percentage of larvae introduced orally reaching maturity was very small. A possible adverse effect of disintegrated worms in the peritoneal cavity, increase enzymes activity coupled with the sensitivity of the hosts reaction to the infective worms orally injected are associated with the poor response and survival in the rats infected by these two routes.

Introduction.

In the last two decades many investigators have reported that the ability of *N. brasiliensis* to develop to maturity in its normal host was influenced by host age, sex, larvae age, immunity and route of infection (Haley 1962, Chandler 1935, Simaren 1964, Simaren and Fabianek 1967).

The purpose of the present paper is to consider in detail the host response and survival relationship between the varied larval age including total and daily efficiency of egg production by the worms. Development of these stages and the survival rate in *in vitro* tests are also quantitatively compared.

Materials and methods.

White laboratory rats 8 weeks old were used as experimental hosts. The infective larvae of the test agent *N. brasiliensis* were obtained by a culture method similar to that of Yokogawa (1922). All cultures were kept in a fly-proof enclosure in an air-condition room. Room temperature ranged between 21 °C and 27 °C throughout the period of study. Isolated larvae from culture were concentrated, centrifuged and washed 4-times with 0.85 % NaCl. With a dissecting binocular microscope required larvae were counted and each rat infected received 600 larvae. Only female rats were inoculated subcutaneously, intravenously, intraperitioneally and orally with larvae 8, 14, 21, 28, 35 days in age. The number of eggs passed daily for 15 days was determined from every 24 hours fecal collection by a modification of Stoll's (1923) dilution egg-count method. From each group, rats were randomly selected, sacrificed and examined for adult worm population on the 10th day after infection.

For *in vitro* tests, live adult worms were isolated. Fifty larvae were placed in separate 100-ml flask containing 0.85 % NaCl. Five flasks were left open for air and incubated at 37 °C for 1 1/2 hours. The air of five other flasks was replenished by bubbling CO_2 into them for 6 minutes and sealed there after. A third set was

similarly treated with N_2 and incubated at 37 °C for 1 1/2 hours. At the end of 1 1/2 hours worms in all flasks were removed and the live ones counted. Worms which where previously subjected to CO_2 and N_2 atmosphere were now placed in open 100-ml flask containing 0.85 % NaCl and incubated at 37° C for another 11/2 hours. The live worms were sorted and counted.

Results and discussion.

The course of infections produced by the subcutaneous, intravenous, intraperitoneal and oral injections of larvae 8, 14, 21, 28 and 35 days old into rats was followed individually by daily egg and worm counts, sex-ratio and range in size of recovered adult worms were criteria utilized (Tables 1-5).

Table I

Average of Eggs Count in thousands from rats (8 per group) infected subcutaneously with 600 larvae of *N. brasiliensis*

Experiments	I	II	III	IV	V
	Age of larvae in days				
	8-control	14	21	28	35
Days after infection					
5	0	0	0	0	0
6	53	28	19	12	4
7	180	26.2	15	3	5.2
8	163	24	8	14.2	2.4
9	140	14	7	9	3.6
10	78	11	3	3.9	2.8
11	42	10	2	1.6	1.0
12	14.2	6.4	1.1	0.9	0.4
13	6.6	2	0.8	0.3	0.2
14	2.0	0.3	0.2	0	0.2

Data from these findings demonstrated the existence of marked differences in daily and total egg output with highest by the 8 days old larvae (subcutaneous control) and a gradual decrease shown by the 14, 21, 28 and 35 days age larvae in the other three routes (Table 5). The ascent of the daily egg counts in all groups and routes rapidly reached their maxima on the 7th and 8th day respectively followed by an equally abrupt descent to almost zero on the 14th day post infection.

Table II

Average Egg Count in thousands from rats (8 per group) infected intravenously with 600 larvae of *N. brasiliensis*

Experiments	I	II	III	IV	V
	Age of larvae in days				
	8-(control)	14	21	28	35
Days after infection					
5	0	0	0	0	0
6	7	4	5	2.0	1.4
7	98	12	3.2	4.3	1.1
8	172	25	2.4	2.9	1.3
9	124	36	6.1	2.1	0.5
10	113	18	3.0	1.7	1.2
11	22	9	1.9	1.4	0.9
12	2.9	1.7	3.0	0.3	0.3
13	0.14	0.3	1.1	0.8	0.4
14	0.08	0.01	0.6	0.2	0.1

Table III

Average of Egg Count in thousands from rats (8 per group) infected intraperitoneally with 600 larvae of *N. brasliiensis*

Experiments	I	II	III	IV	V
	Age of larvae in days				
	8-(control)	14	21	28	35
Days after infection					
5	0	0	0	0	0
6	1.2	4.8	2.9	0.3	0.4
7	17.6	6.3	4.3	1.6	1.5
8	75	9.4	3.8	3.0	0.1
9	74	5.7	3.6	1.0	2.1
10	32	6.0	5.4	2.4	0.6
11	18	5.2	2.5	1.4	1.3
12	3.5	2.3	0.3	0.5	0.2
13	1.3	0.9	0.3	0.4	0.0
14	0.2	0.6	0.1	0.1	0.0

The subcutaneous infection gave the highest average worm burden ranging between 457 and 46 that is for the rats infected with the youngest (8-days old) to the oldest larvae (35-days age). A decrease number of recovered worms resulted from the intravenous route (highest 365, lowest 60), intraperitoneal (274 highest, 77 lowest) and oral (128 highest, 30 lowest) with a corresponding highest females (277) and lowest females (30). More female than male worms were recovered from

Table IV

Average Egg Count in thousands from rats (8 per group) infected orally with 600 larvae of *N. brasiliensis*

Experiments	I	II	III	IV	V
	Age of larvae in days				
	8-(control)	14	21	28	35
Days after infection					
5	0	0	0	0.	0.
6	10.5	6.2	4.5	1.8	0.2
7	12.5	8.1	2.6	0.3	0.7
8	11.9	5.2	4.1	1.2	0.4
9	8.4	6.3	3.6	1.6	0.6
10	4.8	4.0	1.1	0.8	1.0
11	3.3	2.1	1.4	0.3	0.3
12	3.2	1.8	0.7	0.4	0.1
13	2.6	0.9	0.5	0.1	0.3
14	2.0	0.6	0.1	0.1	0.2

all the different four infection routes with their varied larval age 10 days after infection (Table 5). The pattern of the results are in general accordance with those obtained by Africa (1931) Yokogawa (1922) and Rogers (1939) where a single stain age and route were studied.

The percentage of development of 8-days old larvae injected subcutaneously, intravenously, intraperitoneally and orally were 76.1, 60.8, 45.6, 21.3. With 14 days old larvae it was a high of 36.1 percent and low of 6.6 percent ; for 21 days age 19 percent highest, 12.6 percent lowest ; for 28 days old 14 percent highest, 5.1 percent lowest and for 35 days age it is a high of 12.8 percent and a low of 5.0 percent.

Table V

Summary of Results obtained from Rats infected with *N. brasiliensis*

Experimental Routes	No. of Rats Infected	No. of Larvae Given	Av. Egg passed 5-15 days (in thousands)	No. of Rats Sacrificed	Average Adult Worm Population Recovered Mean-Length mm						
					M	F	Total	% F	% developed	M	F
Subcutaneous Infection :											
Age of larvae in Days :											
8	8	600	676.8	4	160	277	457	63.3	76.1	4.1	5.6
14	8	600	121.9	4	90	120	210	57.1	35		
21	7	600	56.1	4	40	75	115	65.2	19.0	3.8	5.3
28	8	600	44.9	4	33	51	84	60.7	14.0	3.8	4.7
35	7	600	19.8	4	21	25	46	54.3	7.6	3.0	3.9
Intravenous Infection :											
Age of larvae in Days :											
8	7	600	541.1	4	151	214	365	58.6	60.8	4.1	4.9
14	8	600	106	4	110	96	206	46.6	34.3	3.7	4.6
21	8	600	34.3	4	27	63	90	70	15.0	3.8	3.9
28	8	600	15.7	4	22	43	65	66.1	10.8	3.4	3.8
35	8	600	10.8	4	19	41	60	68.3	10.0	3.3	4.0
Intraperitoneal Infection :											
Age of larvae in Days :											
8	8	600	222.8	4	110	164	274	60	45.6	2.9	5.4
14	8	600	41.3	4	85	132	217	60.8	36.1	3.5	4.7
21	8	600	23.2	4	37	74	101	73.2	16.8	3.4	4.4
28	7	600	10.7	4	18	36	54	66.0	9.0	3.3	4.3
35	7	600	6.2	4	26	51	77	63.6	12.8	4.0	3.8
Oral Infection :											
Age of larvae in Days :											
8	8	600	59.2	4	50	78	128	60.9	21.3	4.1	4.2
14	7	600	35.2	4	10	30	40	75	6.6	3.9	4.1
21	8	600	18.6	4	28	47	75	62.6	12.6	3.2	3.7
28	7	600	6.6	4	17	15	32	46.8	5.3	3.6	3.5
35	8	600	3.6	4	11	19	30	63.3	5.0	3.6	3.2

The recovered adult worms showed normal range in lengths. Fewer stunted and shorter recovered worms were found more in oral than in intraperitoneal infections.

Il is not clear why all the intraperitoneally infected rats produced lower egg and worm count. However less than half of the infective larvae developed in four of the five groups in this route. One possible explanation is that the disintegration of the worms which were unable to migrate after death in the peritoneal cavity may

Table VI

Survival Rate of 50 *N. brasiliensis* during 45 minutes in each atmosphere tested

Flask No.	Worms alive after 45 mins.	Percent alive	Worms alive in 45 mins. after return to air	Percent alive
Flask containing Air :				
A	50	100		
B	48	96		
C	49	98		
D	50	100		
E	46	92		
Flask containing Co_2 :				
A	21	42	28	54
B	10	20	26	52
C	18	36	20	40
D	12	24	19	38
E	16	32	30	60
Flask containing N_2 :				
A	38	76	41	82
B	47	94	48	96
C	36	72	37	74
D	44	88	46	92
E	38	76	43	86

have had some influence on the tissue migration and development of the worms in the intestine (Table 5). This deficient migration and response could be attributed to the inaccessibility of the peritoneal cavity vessels coupled with some peculiarity of the cavity preventing normal transition to parasitic existence.

A careful examination of the feces collected from some of the different groups of rats infected orally revealed a few dead larvae which did not migrate from the intestine but were being passed in the feces. This might have been caused by a

marked adverse effect of increase enzymes activity stimulated in the digestive tract on the arriving infective larvae introduced orally into these rats. The oral route seems abnormal prevented somewhat the migration of all the parasites through the lungs. Consequently the hosts sensitiveness to the introduction of the larvae through this route is a sufficient factor evoking a minimum migration and infectivity of the parasite by this route.

Table VII

Survival rate of 50 *N. brasiliensis* worms in atmosphere of Co_2, N_2 and air after 1 1/2 hours in each atmosphere

Flask N°	Worms alive after 1 1/2 hr.	Percent alive	Worms alive in 1 1/2 hr. after return to air	Percent alive
Flask containing air				
A	40	80		
B	32	64		
C	29	58		
D	34	68		
E	42	84		
Flask containing Co_2				
A	14	28	23	46
B	11	22	22	44
C	12	24	20	40
D	8	16	28	56
E	16	32	31	62
Flask containing N_2				
A	20	40	25	50
B	40	80	42	82
C	15	30	17	34
D	38	76	40	80
E	30	60	34	68

The *in vitro* tests revealed that substantial worms placed in environments of CO_2 and N_2 showed no signs of life and motility until they were returned to an environment containing oxygen (Table 6 and 7) seem to indicate that it is possible the worms in their development and egg production utilize blood oxygen considerably. With the poor response shown by the older larvae, future work on the utilization of food reserves would be investigated.

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