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Nippostrongylus brasiliensis in the rat: Failure to relate intestinal histamine and mast cell levels with worm expulsion

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In adult rats, the intestinal nematode, *Nippostrongylus brasiliensis*, is expelled by a mechanism which involves at least two separate and sequential steps. In the first, worms are damaged by antibodies and then, in a further step which may be pharmacological, the worms are expelled (Jones & Ogilvie, 1971). It appears that in rats infected before 9 weeks of age, the first step develops normally (Ogilvie & Hockley, 1968), but the second step does not develop properly (Jarrett, 1971). The present work deals with the problem whether intestinal mast cells and/or intestinal histamine are involved in the second, expulsive step of worm elimination.

MATERIAL AND METHODS

Rats. Colony-bred rats of the Osborne–Mendel strain were used throughout this work.

Parasites. Methods for maintenance and recovery of *N. brasiliensis* and for counting eggs in rat faeces were as described earlier (Keller, 1970*b*).

Examination of the small intestine. The small intestine from the pyloric sphincter to the end of the small intestine was removed immediately after death. In order to compare equivalent regions, the intestine was cut into pieces using a device as described by Wells (1962), and the pieces were rinsed with phosphate buffered saline, and weighed.

Histamine assay. In these experiments, the entire small intestine of neonatal, young or adult rats (approximately 120 days old) was cut into 16 pieces of equal length. The pieces were then incubated for 12 h in 10% trichloroacetic acid, and the supernatant neutralized and assayed for histamine on the isolated, atropinized guinea pig ileum.

Histology. After death, the entire small intestine was immediately removed, opened, rinsed cautiously with phosphate buffered saline and cut into pieces of 2 cm, using the device as above. Tissue samples situated about 4, 12, 20, 24, 32, 42, 50 and 76 cm below the pyloric sphincter were fixed for 60 min in Carnoy's fluid, dehydrated, cleared in methylbenzoate containing 1 % celloidine, and embedded in paraffin wax. Paraffin sections (5 μm) were stained with Astrablue (Gurr) and counterstained with Safranin O (Gurr) as described by Enerbäck (1966). The number of mast cells (and globule leucocytes) lying in the lamina propria and sub-

mucosa between two crypts and in the villus immediately above was counted (Jarrett, Jarrett, Miller & Urquhart, 1968*b*). Twenty 'villus-crypt units' were counted in each of the above regions.

RESULTS

Age dependence of the histamine and mast cell levels in the small intestine of the rat

In this experiment, the histamine content per g tissue along the whole small intestine was assessed in neonatal rats, and in rats aged 20, 37, 50 and 60 days, and the values compared with adult rats aged approximately 120 days. Each group consisted of 15–20 rats, and equivalent pieces of young and adult rats were compared. Since within the same animal, the histamine concentration along the entire length of the small intestine was found to be fairly constant, only the mean values per group were shown in Table 1. These results show that, in neonatal rats, the intestinal histamine content per g tissue is very low. From birth to the adult

Table 1. *Histamine content (μg base) per g tissue in the small intestine of rats of different age*

Experimental group	Neonatal rats (1)	Rats aged 37 days (2)	Rats aged 50 days (3)	Rats aged 60 days (4)	Adult rats 120 days (5)
Histamine content (±) standard deviation	1·00 (±0·67)	3·72 (±0·95)	10·10 (±2·83)	13·13 (±5·69)	47·00 (±14·88)

Statistical evaluation (variance analysis). The differences between all groups were statistically highly significant ($P < 0{\cdot}001$), except for (3) against (4) ($P < 0{\cdot}1$).

Table 2. *Mast cell counts per 20 'villus-crypt units' in the small intestine of normal rats of different age (mean of 12 rats per group)*

Samples* ...	1	2	3	4	5	6	7	8
Age of rats								
Neonatal	5	4	3	4	3	3	2	3
40 days	321	234	255	207	184	235	160	166
60 days	588	520	482	460	430	395	382	388
120 days	742	784	740	744	760	740	682	712

* For histological examination, the various samples were withdrawn at equivalent distances from the pyloric sphincter.

age, there is a gradual rise in the histamine concentration which concerns all parts of the small intestine equally and which is especially marked between the age of 60 and 120 (adult rats) days. Statistical evaluation of the data by variance analysis revealed that the differences of the means between two neighbouring groups were highly significant ($P < 0{\cdot}001$) except for the values obtained for 50- and 60-day-old rats; here, only a moderate difference ($P < 0{\cdot}1$) was established.

Tissue mast cells were counted in equivalent regions of the small intestine of rats of different age, and the results are given in Table 2. In the small intestine of neo-

natal rats, mature mast cells were found only occasionally; with increasing age, a gradual increase in the mast cell number of both the lamina propria and the submucosa along the entire length of the small intestine was observed. Thus, during growth from birth to the adult age, intestinal mast cell counts show a similar rise as intestinal tissue histamine.

Changes in the histamine concentration and its distribution along the small intestine of the adult rat during a primary infection with Nippostrongylus brasiliensis

In this experiment, adult rats (180–220 g) were infected with 4000 larvae of *N. brasiliensis*. On days 6, 8, 9, 12, 13, 14 and 16 of the infection, 10 rats each were sacrificed, the location of worms and the histamine content of the pieces assessed, and the latter compared with that of controls of the same age and weight.

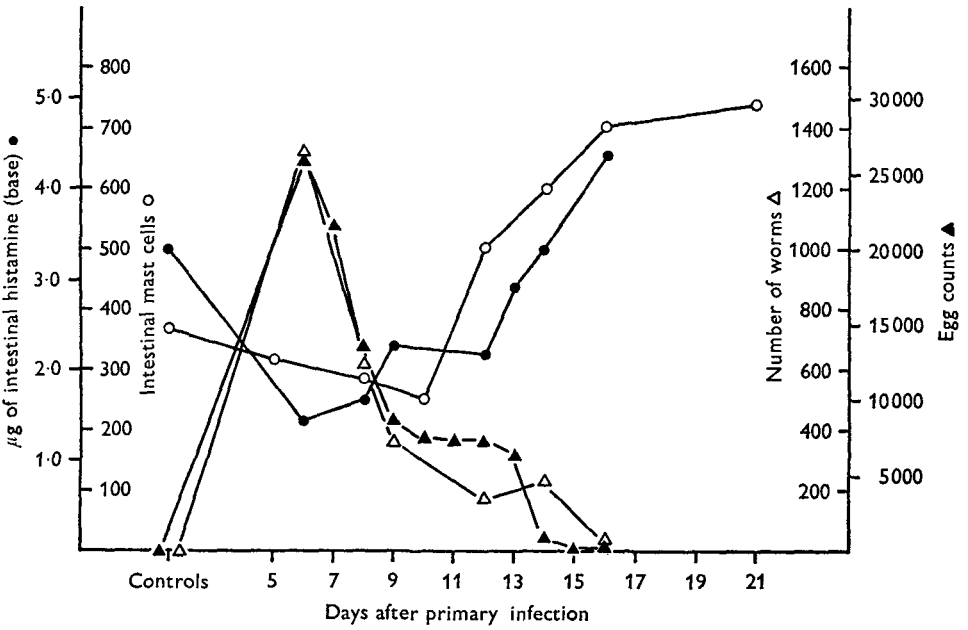

Fig. 1. The course of the numbers of intestinal parasites, of egg counts, of intestinal histamine concentration (μg base) and the number of tissue mast cells in the course of a primary infection with *N. brasiliensis*.

The results listed in Table 3 show that from day 6 till day 14 of the worm infection, histamine concentration is clearly lower than in controls. On day 6–8, these changes were especially marked and occurred throughout the entire length of the small intestine. On days 9–14 after a primary infection, the lowering in intestine histamine was clearly restricted to the regions where most of the worms were located; by this time, the regions of the intestine not directly affected by worms were already showing increased histamine levels. On day 16 after primary infection, all parts of the small intestine showed clearly higher values than controls (Table 3; Fig. 1).

Table 3. *Histamine content (μg base) of intestinal pieces taken at various distances from the pyloric sphincter to the end of the small intestine of controls and of animals sacrificed* 6 *to* 16 *days after an initial infection with* 4000 *larvae of* Nippostrongylus brasiliensis

Distances from the pyloric sphincter (cm)	Controls		Worm-infected rats: Day 6		Day 8		Day 9		Day 12		Day 13		Day 14		Day 16	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	3·69	0·89	3·01	1·18	3·07	0·82	4·12	1·17	4·59	0·98	3·13	2·52	5·95	0·04	5·99	2·07
4	3·17	0·71	2·54	1·18	2·42	0·38	3·54	0·39	3·63	1·70	3·66	0·42	5·12	0·88	5·51	0·32
8	3·04	0·60	2·04	0·95	2·22 |	0·28	2·81 |	1·03	4·03 |	1·59	4·00	0·89	4·11	0·30	6·10	1·45
12	3·31	1·04	1·84 |	0·94	1·89 |	0·65	3·01 |	1·21	2·13 |	0·28	3·98	1·00	5·07	1·24	5·25	2·43
16	3·39	1·17	1·26 |	0·52	1·26 |	0·70	2·02 |	1·32	1·20 |	0·32	3·29	2·02	3·32	0·56	4·23	2·86
20	3·51	1·07	0·91 |	0·48	0·98 |	0·55	1·63 |	1·35	1·52 |	0·67	2·43	3·01	1·42	1·41	4·09	2·20
24	3·45	1·15	0·65 |	0·53	1·08 |	0·56	1·12 |	0·61	0·89 |	0·76	0·95	0·49	1·35	0·92	4·41	1·41
28	3·95	1·14	0·76 |	0·59	1·06 |	0·43	1·48 |	0·46	1·33 |	0·87	1·51	0·48	1·98	1·00	4·67	0·66
32	3·80	1·42	1·24 |	0·73	1·72 |	0·62	2·04 |	1·23	1·74 |	1·34	2·40	1·24	2·46	1·73	4·62	0·39
38	3·21	0·96	1·49 |	0·93	1·93 |	0·56	2·26 |	0·65	2·58 |	2·20	3·58	0·69	3·67	1·64	4·33	0·45
42	3·26	0·98	1·83 |	0·85	2·06 |	0·40	2·64 |	1·03	2·31 |	1·82	2·64	1·13	4·61	0·41	4·03	0·19
46	2·94	0·76	1·86 |	0·92	2·10 |	0·15	2·86 |	0·25	2·89 |	1·96	3·31	0·77	4·50	0·60	3·67	0·36
50	3·01	0·60	2·14 |	1·12	2·22 |	0·31	3·34 |	0·70	3·37	1·86	4·66	1·99	4·38	0·79	3·05	1·24
54	2·95	0·69	2·30	1·04	2·56	0·20	3·58	0·77	3·58	1·88	4·40	0·78	4·77	0·30	3·82	1·52
58	3·03	1·07	2·10	1·06	2·81	0·26	3·83	0·87	3·22	1·66	4·33	0·63	4·47	1·35	3·91	1·34
62	3·53	0·94	2·11	1·05	2·93	0·44	4·18	0·70	3·54	1·37	4·60	0·47	6·10	0·34	3·97	1·64
66	4·15	1·81	2·39	0·96	2·93	0·71	4·35	1·17	4·33	0·86	6·04	1·35	5·80	0·81	5·03	1·81
70	3·88	1·46	2·32	1·00	2·79	0·34	4·38	0·96	4·36	1·14	6·22	0·87	5·37	1·21	5·37	2·53
74	3·02	1·59	2·18	0·98	2·66	0·45	4·43	0·23	4·38	0·98	5·54	0·85	4·88	2·01	5·93	2·87
78	2·75	1·45	1·78	0·17	2·96	0·52	4·14	0·57	4·01	0·21	5·42	1·73	4·88	1·13	5·80	3·07

$\bar{x}$ = mean values; s = standard deviation; | = region where worms were located.

Changes in mast cell counts in the small intestine during Nippostrongylus brasiliensis *infection*

Mast cell counts of corresponding regions of the jejunum were done in rats initially infected with 4000 larvae of *N. brasiliensis* and sacrificed on days 5, 8, 10, 12, 14 and 16 respectively, and in controls. Each experimental group consisted of 6 rats. In controls the number of mast cells present in both the lamina propria and the submucosa was fairly constant (Table 4). On days 8 and 10 of a primary infection, mast cell counts were found to be markedly decreased in the region where most of the worms were located, i.e. at about 12–50 cm below the pyloric sphincter (Table 4). From day 12 on, mast cell counts showed a clear tendency to rise, and on days 16–21, the counts reached approximately double the control values. Moreover, changes in mast cell counts were always most marked in the region where the main worm burden was located.

Table 4. *Mast cell counts per* 10 *'villus-crypt units' in controls and worm-infected adult rats in various parts of the small intestine*

Samples* ...	1	2	3	4	5	6	7	8
Controls (12 animals) ...	371	392	370	372	380	370	341	356
Worm-infected rats (6 rats per day)								
Day 5	232	298	310	310	300	338	472	310
8	320	330	242	250	240	240	326	316
10	331	250	251	68	245	380	323	203
12	578	330	542	570	462	560	523	560
14	643	663	563	650	582	570	493	600
16	837	670	760	653	690	743	633	650
21	730	810	718	782	707	880	600	553

* For histological examination, the various samples were withdrawn at approximately the following distances below the pyloric sphincter: 1, 4 cm; 2, 12 cm; 3, 20 cm; 4, 24 cm; 5, 32 cm; 6, 42 cm; 7, 50 cm and 8, 76 cm.

Time relationship between worm expulsion and the changes in intestinal histamine and mast cells

To assess the time relationship between worm expulsion and the changes observed in intestinal histamine and mast cell counts, the course of egg and worm counts during a primary infection was followed in rats of the same age and weight as before and infected with the same pool of larvae as in experiments reported previously. Results depicted in Fig. 1 demonstrate that both faecal egg counts as well as the number of worms located in the small intestine show a rapid decrease on days 7–9 which is rather common in the strain of rats used in these experiments. Numbers of eggs and worms have been found to be decreased as early as day 9, and very low counts were reached on days 12–14 of an initial infection. In Fig. 1 the time course obtained for tissue histamine and mast cells within the region of the main worm burden, i.e. at approximately 12–50 cm below the pyloric sphincter, was compared with that of worm and egg counts. Comparison of the results demon-

strate that, in the jejunum, mast cells and histamine show rather low values during the main expulsion phase and begin to rise at a time when most of the worms have already been expelled.

Variation of the histamine and mast cell concentration in peritoneal cells during a primary infection with Nippostrongylus brasiliensis

Results of histamine assays and mast cell counts from peritoneal cells harvested at various periods after an initial infection with 4000 larvae of *N. brasiliensis* were related and compared with those of controls. The data summarized in Table 5 demonstrate that, in the peritoneal cavity, mast cell counts and total histamine undergo drastic changes during the worm infection. Namely, both of these parameters fall to very low values between days 9 and 12 after primary infection and then gradually increase to levels which were often higher than that of controls. A similar course was recognized when the histamine content per mast cell was calculated.

Table 5. *Mast cells and histamine in the peritoneal cavity during a primary infection with* Nippostrongylus brasiliensis

Day of infection	Histamine (μg base)		Mast cells ($\times 10^3$)	
	4000 larvae	Controls	4000 larvae	Controls
2	—	—	185	203
4	—	—	198	261
6	3·27	3·27	165	175
7	—	—	115	188
9	1·46	2·82	22	195
11	—	—	6	182
12	0·75	3·46	25	175
14	2·41	3·22	55	210
16	3·02	3·73	90	—
19	2·45	2·12	120	168
21	—	—	210	190
25	—	—	230	186

—, Not done.

DISCUSSION

Present experiments on age dependence of the histamine and mast cell levels in the small intestine show that only very low amounts of histamine per gramme tissue are present in the gut of the neonatal rat; similarly, mature mast cells are found only very rarely. With increasing age, a gradual increase in the histamine concentration and the number of mature mast cells of all parts of the small intestine occurs. The increase in intestinal histamine was rather slow between birth and the age of 9 weeks, but was clearly more marked from then on till the adult age. It has been reported by several groups of workers that in neonatally infected rats, the worm population is not expelled at the end of the second week as it is in adult rats (Jarrett *et al.* 1966, 1968*a*, *b*; Kassai & Aitken, 1967; Ogilvie & Jones, 1967; Ogilvie & Hockley, 1968). According to Jarrett (1971), the expulsion response of

the animals to the parasite did not mature until 9 weeks of age, although damage to worms occurs almost as rapidly as in adult rats (Ogilvie & Hockley, 1968). When more recent knowledge on the mechanism of worm expulsion is considered (Jones & Ogilvie, 1971), the above findings might be interpreted by suggesting that the first step of worm expulsion, the immunological damage to the parasite, is fully developed even in the very young rat. Obviously, the second step involving expulsion of damaged worms from the intestine is not fully mature until approximately 9 weeks of age. Some reports suggest that the release of amines from tissue mast cells might be involved in some way in the process of worm expulsion (Urquhart, Mulligan, Eadie & Jennings, 1965; Barth, Jarrett & Urquhart, 1966; Keller, 1970*a*; Murray, Miller, Sanford & Jarrett, 1971). The present data, which show that intestinal histamine and mast cell levels are very low in neonatal rats and then gradually increase till the adult stage make it conceivable that histamine or other mast cell derived agents might be the component decisive for the deficiency in worm expulsion of very young rats. It must be kept in mind however, that other agents possibly engaged in this process might show a similar course of development.

Present experiments demonstrate that at the height of the intestinal phase of a primary infection in adult rats and during the main period of worm expulsion (days 6–11), both histamine and mast cell levels in the small intestine are depressed compared with the levels in uninfected controls (Fig. 1). After day 12–13, histamine and mast cells begin to rise and on day 16 reach levels which were obviously higher than in controls. Moreover, the changes occurring in levels of histamine and mast cells of the intestinal wall during the nematode infection were also reflected in free peritoneal cells (Table 5). These findings thus largely agree with those reported by Wells (1962) but conflict with Jarrett *et al.* (1968*b*) and Murray *et al.* (1971) who observed a sudden and marked increase in the number of intestinal mast cells at the beginning of the exponential phase of worm expulsion. Possibly, this discrepancy might be explained by a difference in reaction between the strains of rats used. Thus, the present data on the intestinal levels of histamine and tissue mast cells in the course of a primary infection with *N. brasiliensis* do not provide evidence for a pharmacological expulsion mechanism mediated by histamine or other agents originating from tissue mast cells. Earlier findings showing that cortisone (Ogilvie, 1965; Urquhart *et al.* 1965) reserpine (Sharp & Jarrett, 1968) and the chemical histamine liberator, compound 48/80 (Keller, 1970*a*) inhibit worm expulsion, must not necessarily be interpreted to be due to an effect on intestinal mast cells; for example, it could as well be the consequence of an anti-inflammatory effect. And earlier observations have demonstrated that treatment with compound 48/80 does not affect mast cells and histamine of the small intestine of the rat (Riley, 1959; Keller 1970*a*).

The changes in intestinal histamine and tissue mast cells occurring in the rat infected with *N. brasiliensis* could be explained in different ways. First, they might be the result of the acute inflammatory response locally elicited by the worms; acute inflammation is known to be accompanied by a marked decrease in local tissue mast cells and histamine which is followed by a reactive phase. The origin of reappearing mast cells is not known, and both immigration or local new

formation of cells might be responsible for their rapid rise at the end of the expulsion phase. Alternatively, a specific immune reaction leading to histamine release must be considered. Mast cell counts and intestinal histamine decrease long before reaginic antibodies appeared in regional lymphoid tissues or in circulation which suggests that an immune mechanism is unlikely to induce the fall in these parameters. Thirdly, a release of mast cell degranulating agents by the worm could be the cause for the changes observed. Indeed, extracts from normal (day 6) worms do contain such activities. On the other hand, no such activity could ever be detected in metabolic products of normal worms (Keller, unpublished). Thus, further studies will be necessary to a better understanding of the mechanisms involved in the postulated second step of worm expulsion.

SUMMARY

This paper describes experiments to determine whether intestinal tissue mast cells and/or intestinal histamine are involved in the second, expulsive step of worm elimination. In neonatal rats, intestinal tissue contains only very little histamine and mature mast cells are encountered only sporadically. From birth to the adult age, there was a gradual rise in both intestinal mast cells and histamine.

During *Nippostrongylus brasiliensis* infection in the adult rat, the concentration of histamine in the small intestine was clearly lower than in uninfected controls. Especially low histamine values were found to occur on days 6–12 of a primary infection in the region where the main worm burden was located. Similarly, the number of tissue mast cells present in the epithelium of the jejunum was decreased in the same region and during the same period of time. From the observation that the bulk of the parasites are expelled at a time when histamine and mast cell levels are low, it was concluded that mast cells and their constituents were not an essential factor in the second step of worm elimination.

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