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Mast Cells and Macromolecular Leak in Intestinal Immunological Reactions

THE INFLUENCE OF SEX OF RATS INFECTED WITH *NIPPOSTRONGYLUS BRASILIENSIS*

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Summary. The onset of the exponential expulsion of *Nippostrongylus brasiliensis* worms in the rat is associated with a sharp burst of intestinal mast cell activity and increased permeability of the bowel wall. It was found that the onset of maximum velocity of worm expulsion occurred earlier in female rats than in male rats and proceeded at a faster rate. There was a corresponding difference in the timing of the mast cell rise and macromolecular leak between the sexes. This suggested that there is a relationship between these events. Cortisone, a drug known to stop worm expulsion and to suppress the mast cell response, also prevented the macromolecular leak. Electron microscopy showed that during the period of increased permeability a pathway for protein tracers had opened up between the epithelial cells and it is suggested that this is the route for enhanced antibody transfer across mucous membranes.

We suggest that a stimulus or stimuli from the parasite cause synchronous development of new populations of mast cells, IgE-producing plasma cells and plasma cells synthesizing antibodies of other classes possessing an anti-worm effect. It is also suggested that these mast cells discharge their pharmacological mediators by an allergen–reaginic antibody mediated system and that these mediators create a pathway through the intestinal mucosa for the translocation of antibody.

INTRODUCTION

There has been considerable interest recently in the mechanism of translocation of antibody across mucous membranes. The discovery of the identity of IgE with homocytotropic, reaginic or mast cell-sensitizing antibody has raised questions as to its immunological function. This paper describes experiments which would support the hypothesis that certain parasites produce a range of substances which result in synchronous production of new populations of mast cells, IgE-producing plasma cells and plasma cells synthesizing antibodies of other classes possessing an anti-parasite effect. These phenomena act in combination in a system which causes the release of large amounts of antibody at the site of the parasite.

In some immunological reactions, it would appear that there is probably a sudden large release of antibody into the lumen of the intestine. Parasitic infections offer an

excellent model for studying the mechanism of antibody release. The model employed in this study was *Nippostrongylus brasiliensis* infection in the rat. This has the advantage that large numbers of animals can be used, and the progress of the infection and the immunological reaction measured directly and accurately. In addition, the parasite remains localized in the gut lumen and does not multiply within the body, while the site of the reaction can be identified easily because of the size of worms.

N. brasiliensis larvae were injected subcutaneously into Hooded Lister rats. The larvae travel via the lungs to the small intestine where they establish a stable population within 3 days. Around day 12, a sudden phase of expulsion begins and the worms are expelled in a logarithmic fashion over the next few days (Jarrett, Jarrett and Urquhart, 1968a). This rejection is caused by an immune reaction and the slope of the line showing the kinetics of the worm population, i.e. the expulsion rate coefficient, is a measure of the immune status of the animal. Second and third infections show an increased speed of expulsion (Jarrett *et al.*, 1968a).

Evidence has accumulated over a number of years that a local hypersensitivity mechanism might play a part in worm expulsion (see Discussion) and Barth, Jarrett and Urquhart (1966) provided evidence that antibody transfer might be related to gut hypersensitivity. It was found that infected rats given hyperimmune serum and a heterologous anaphylactic shock expelled worms sooner than rats given hyperimmune serum alone and that anaphylactic shock by itself did not cause expulsion.

The present study was designed to measure the kinetics of worm expulsion and the subepithelial intestinal mast cell population more precisely than previously. Using isotopically labelled macromolecules, an assessment was made of the changes in the permeability of the parasitized intestinal mucosa. In addition, a group of rats treated with cortisone, a drug known to suppress worm expulsion (Ogilvie, 1965; Urquhart, Mulligan, Eadie and Jennings, 1965; Jarrett, Jarrett, Miller and Urquhart, 1968b) was studied in the same way. Structural changes associated with an alteration in bowel permeability to macromolecules were examined with the electron microscope using protein-tracing techniques.

MATERIALS AND METHODS

I. Worm burden kinetics

Eighty male and eighty female Hooded Lister rats weighing approximately 150 g and aged 8–10 weeks were infected subcutaneously with 3000 *N. brasiliensis* larvae. The parasite culture was prepared after the modified method of Bakarat (1951) adapted by Jennings, Mulligan and Urquhart (1963). Groups of eight to ten male and female rats were killed on days 7, 10, 11, 12, 13, 14, 15 and 16 after infection. The technique of worm recovery from the small intestine was the same as that described by Mulligan, Urquhart, Jennings and Neilson (1965). Total worm counts were done in all cases in order to eliminate the sampling error caused by the clumping of worms. Daily faecal egg counts were done after the method of Gordon and Whitlock (1939).

II. Subepithelial intestinal mast cell population kinetics

Fifty male and fifty female Hooded Lister rats weighing approximately 150 g and aged 8–10 weeks were each infected subcutaneously with 3000 *N. brasiliensis* larvae. Groups of four to five rats were killed on days 7, 8, 9, 10, 11, 12, 13, 14, 15 and 19 after infection.

Tissue for histological examination was taken in all cases from the jejunum at a point 20 cm from the pylorus. We had previously determined that this was the site of regular maximum concentration of worms at the time of initiation of expulsion. The excised piece of intestine was laid on dry filter paper. On opening along the line of mesenteric attachment, the tissue automatically flattened itself on the paper. The paper and attached tissue was then immersed in Carnoy's fluid. The samples were trimmed longitudinally. This method gave the best sections (Miller and Jarrett, 1971) because of the transverse orientation of the leaf-like and tongue-like villi in the rat intestine. Twenty-four to 48 hours later, the tissues were dehydrated and cleared in an alcohol–amyl acetate–chloroform series and embedded in paraffin wax. Sections were stained with the Astra blue–safranin technique at pH 0·3 (Enerback, 1966; Murray, Miller and Jarrett, 1968). This technique is a highly sensitive one for detecting mast cells and the number of mast cells and globule leucocytes was counted in twenty villus/crypt (VC) units and expressed as the number/VC unit (Jarrett *et al.*, 1968b; Miller and Jarrett, 1971). We have shown that globule leucocytes are mast cells which are discharging or have discharged some or all of their granule content (Murray *et al.*, 1968), a fact that must be taken into consideration in counting mast cells.

III. Permeability of the intestinal mucosa

Measurement of intestinal permeability. In each experiment, ^{131}I-labelled polyvinylpyrrolidone ([^{131}I]PVP), (average M.W. 40,000) in succinate buffer solution was prepared in a 0·5 per cent solution of Evan's Blue in normal saline. One ml of the solution containing 10 μCi of ^{131}Iodine was injected intravenously into each rat and the animal killed 4 hours later, after removing a blood sample. The 4-hour interval between injection and death was chosen after a preliminary experiment had shown that this period allowed maximum leakage of plasma into the small intestine. The small intestine was removed and the intestinal contents were gently squeezed out using phosphate-buffered saline. The radioactivity of the intestinal contents, the intestinal wall and the plasma in the blood sample were measured in a scintillation counter. By measuring the radioactivity of the plasma in the blood sample, the amount of plasma in the small intestine was estimated indirectly from the level of radioactivity in the small intestine. This was expressed as microlitres of plasma in the small intestine.

Experiment 1. Sixty male Hooded Lister rats weighing approximately 150 g and aged 8–10 weeks were infected subcutaneously with 3000 *N. brasiliensis* larvae. After administration of [^{131}I]PVP the rats were killed in groups of seven to ten on days 11, 12, 13, 14, 15 and 16 of the infection. A group of ten uninfected rats were treated in the same way and used as controls on day 11.

Experiment 2. One hundred and eighty Hooded Lister rats (sixty males and 120 females) weighing approximately 150 g and aged 8–10 weeks were infected subcutaneously with 3000 *N. brasiliensis* larvae. From day 9 of the infection, sixty females were each given subcutaneous injections of 0·1 mg cortisone (Betsolan, Glaxo Ltd) and 2·5 mg terramycin each day until the end of the experiment.

After administration of [^{131}I]PVP, groups of eight to eighteen male and female rats and cortisone-treated rats were killed on days 10, 11, 12, 13, 14 and 15 after infection. In addition, ten uninfected male and ten uninfected female rats were treated as above at the start of the experiment (day 10) to establish control levels.

Experiment 3 (Electron microscopy). Tissues were removed from forty female Hooded Lister

rats, weighing approximately 150 g and aged 8–10 weeks, infected subcutaneously with 3000 *N. brasiliensis* larvae. On day 14 of the infection, each rat was given an intravenous injection of 50 mg horseradish peroxidase (HPO, M.W. 40,000 Type II Sigma*), prepared in isotonic saline to which sucrose was added to give a 2·5 per cent solution. Graham and Karnovsky (1966) have described a cytochemical technique for the ultrastructural localization of this enzyme. The rats were killed in groups of five, at 1, 2, 4, 6, 8, 10, 20 and 30 minutes after injection. Forty parasite-free rats were treated in the same way. The tissue samples were taken from the jejunum 20 cm from the pylorus and fixed in paraformaldehyde–glutaraldehyde; subsequent treatment was as described by Karnovsky (1965, 1967) and Graham and Karnovsky (1966). The tissues were embedded in an epoxy resin (Epikote 812). Thin sections were cut on an LKB Mark III ultratome,† and mounted on copper mesh grids. The sections were stained with saturated uranyl acetate in methanol followed by lead citrate (Reynolds, 1963). They were examined with an AEI electron microscope 6B.‡

TABLE 1

WORM BURDEN IN MALE AND FEMALE RATS INFECTED WITH 3000 *N. brasiliensis*

Days after infection	Geometric mean		$\log_{10}$ GM		$\log_{10}$ SE	
	Male	Female	Male	Female	Male	Female
7	1629	1489	3·2121	3·1730	0·0170	0·0301
10	1640	1372	3·2147	3·1374	0·0189	0·0371
11	1343	1178	3·1274	3·0711	0·0246	0·0418
12	1189	1053	3·0750	3·0224	0·0406	0·0681
13	1264	431	3·1018	2·6343	0·0414	0·1508
14	703	191	2·8466	2·2800	0·1056	0·1069
15	435	39	2·6385	1·5950	0·1752	0·2540
16	178	29	2·2515	1·4676	0·0986	0·2053

TABLE 2

EGGS/g IN FAECES IN MALE AND FEMALE RATS INFECTED WITH 3000 *N. brasiliensis*

	Days after infection											
	5	6	7	8	9	10	11	12	13	14	15	16
Male	0	40,400	94,700	85,800	78,700	34,200	30,500	16,300	650	0	0	0
Female	0	41,500	85,900	84,800	61,500	23,200	16,100	2,100	400	0	0	0

RESULTS

I. WORM POPULATION KINETICS

The course of a primary infection in male and female rats can be followed from the worm counts and egg counts given in Tables 1 and 2 and Fig. 1. It can be seen that approximately 50 per cent of the injected dose became established in the small intestine as a

* Sigma Chemical Co. Ltd. 12 Lettice Street, London, S.W.6.
† LKB Produktes, AB, Stockholm, Sweden.
‡ GEC-AEI Electronics, Scientific Apparatus Division, Harlow, Essex, England.

stable population until day 10 of the infection after which there was a loss of the worm burden. Three major points are obvious.

1. A drop in faecal egg counts occurred earlier in the female rats.

2. Worm expulsion can be divided into two phases (Fig. 1), the first being associated with a slower rate of expulsion than the second. The first phase lasted from days 10 to 12 in the females and from days 10 to 13 in the males.

3. The second phase of expulsion, which started earlier in the females, was exponential and proceeded at a faster rate. The expulsion coefficient (k) is the slope of the line showing the change in population numbers with time. The females have a k value of 0·46 and the males of 0·28, i.e. female rats were expelling worms at more than 1·5 times the speed of male rats.

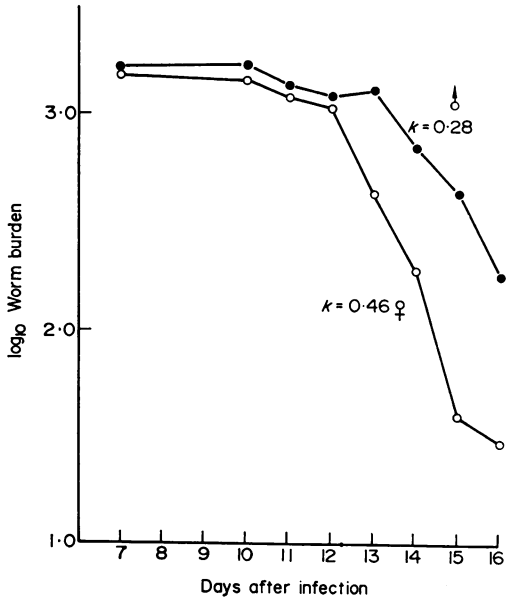

FIG. 1. Kinetics of worm burden in male and female rats infected with 3000 *N. brasiliensis*.

II. SUBEPITHELIAL INTESTINAL MAST CELL POPULATION KINETICS

The presence of adult worms in the small intestine resulted in complete disappearance of subepithelial intestinal mast cells at the site of maximum worm concentration (Table 3 and Fig. 2). In the female rats, mast cells could be recognized again after day 11 and in male rats they started to appear after day 12. Subsequently, the number of mast cells increased to seven or eight times greater than normal (in non-parasitized rats ten to twelve mast cells are found per villus/crypt unit). During this phase mast cells were found in mitosis and the majority of them appeared to be losing some or all of their granule contents. It was at this time that large numbers of mast cells migrated into the epithelial sheet and became transformed to globule leucocytes.

From these results two major points can be made. First, in both male and female rats the onset of the phase of maximum worm expulsion was associated with a marked increase in mast cell proliferation and discharge. Secondly the onset of mast cell activity in the female

rats occurred at least 1 day earlier than in the male rats, a similar difference to that found in the onset of the worm expulsion phase.

The rats in this experiment were accidentally deprived of water on day 2 of the infection. We believe that this delayed the onset of mast cell activity in both male and female rats.

TABLE 3

MAST CELLS/VC UNIT IN MALE AND FEMALE RATS INFECTED WITH 3000 *N. Brasiliensis*

Days after infection	Arithmetic mean ± SE	Geometric mean	$\log_{10}$ GM	$\log_{10}$ SE
7 Male	0·49 ± 0·49	—	—	—
Female	0·03 ± 0·02	—	—	—
8 Male	0·08 ± 0·05	—	—	—
Female	0·07 ± 0·05	—	—	—
9 Male	0·17 ± 0·17	—	—	—
Female	1·48 ± 0·95	—	—	—
10 Male	0	—	—	—
Female	0·22 ± 0·18	—	—	—
11 Male	0·97 ± 0·19	—	—	—
Female	0·58 ± 0·06	—	—	—
12 Male	1 ± 0·62	—	—	—
Female	2 ± 0·88	—	—	—
13 Male	5 ± 4	2	0·2332	0·2683
Female	12 ± 3	11	1·0265	0·1283
14 Male	6 ± 1	5	0·7182	0·0920
Female	26 ± 7	23	1·3701	0·1197
15 Male	35 ± 12	22	1·3341	0·2473
Female	37 ± 7	34	1·5368	0·0930
19 Male	68 ± 5	67	1·8271	0·0300
Female	55 ± 1	55	1·7412	0

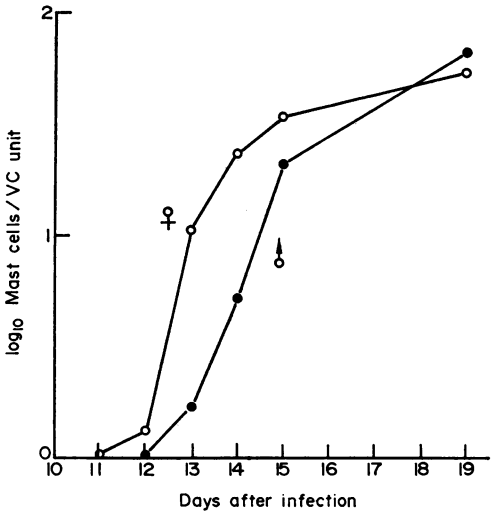

FIG. 2. Changes in the intestinal mast cell population in male and female rats infected with 3000 *N. brasiliensis*.

This conclusion was supported by findings in other experiments in which mast cells appeared a day or so earlier in both male and female rats (Murray, Miller, Sanford and Jarrett, 1971).

III. PERMEABILITY OF INTESTINAL MUCOSA

The foregoing results show that the onset of worm expulsion is related to the rise in the number of mast cells and previous work has shown that these cells are discharging their vasoactive compounds at this time (Miller, Murray and Jarrett, 1968; Murray *et al.*, 1968, 1971; Miller, 1970; Jarrett, Miller and Murray, 1969). Since it appeared that the release of vasoactive amines might be responsible for an increased permeability of the bowel wall this was studied with PVP labelled with ^{131}I and by electron microscopy using the tracer enzyme horseradish peroxidase.

TABLE 4

MICRO LITRES OF PLASMA IN SMALL INTESTINE OF MALE RATS INFECTED WITH 3000 *N. brasiliensis*

Days after infection	Arithmetic mean ± SE
11	1000 ± 190
12	1300 ± 190
13	2500 ± 620
14	5100 ± 980
15	2400 ± 310
16	1700 ± 320
Uninfected controls	600 ± 145

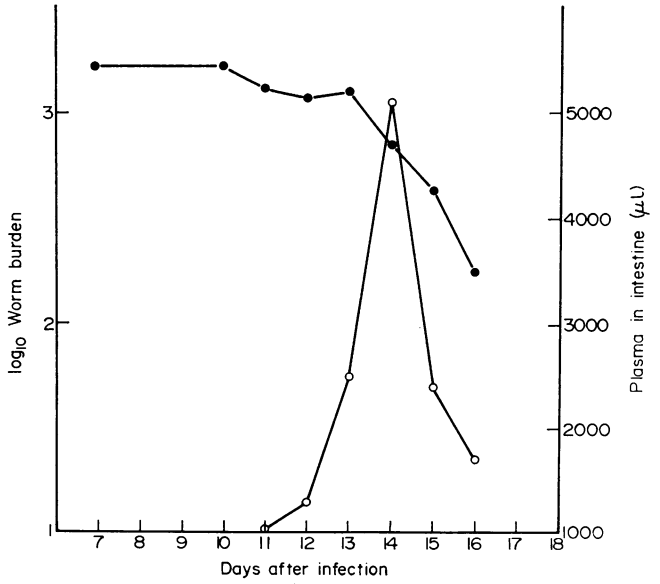

FIG. 3. Leak of plasma [^{131}I]PVP into the small intestine of male rats infected with 3000 *N. brasiliensis*. ○, Calculated plasma in intestinal contents; ●, kinetics of worm burden in male rats (from Fig. 1).

Experiment 1

This experiment was performed at the same time as the worm burden kinetics study and was done on male rats. On days 11–16 of a primary infection the permeability of the bowel wall to macromolecules was increased (Table 4). On day 14, however, there was a very

sharp increase in permeability. This coincided very closely with the onset of the maximum velocity of worm expulsion in male rats (Fig. 3).

Experiment 2

This experiment was designed to compare the permeability of the bowel to macromolecules in parasitized male and female rats. In addition, a group of parasitized rats was treated with cortisone, a drug known to inhibit the expulsion phase (Ogilvie, 1965; Urquhart *et al.*, 1965; Jarrett *et al.*, 1968b).

TABLE 5

MICRO LITRES OF PLASMA IN SMALL INTESTINE OF MALE AND FEMALE RATS INFECTED WITH 3000 *N. brasiliensis*

Days after infection		Arithmetic mean ± SE
10	Male	1500 ± 180
	Female	2200 ± 340
11	Male	1900 ± 340
	Female	2300 ± 380
12	Male	1900 ± 290
	Female	2700 ± 190
13	Male	3000 ± 430
	Female	2700 ± 220
14	Male	2800 ± 480
	Female	4000 ± 630
15	Male	4300 ± 1050
	Female	2500 ± 400
Uninfected controls	Male	1100 ± 165
	Female	500 ± 70

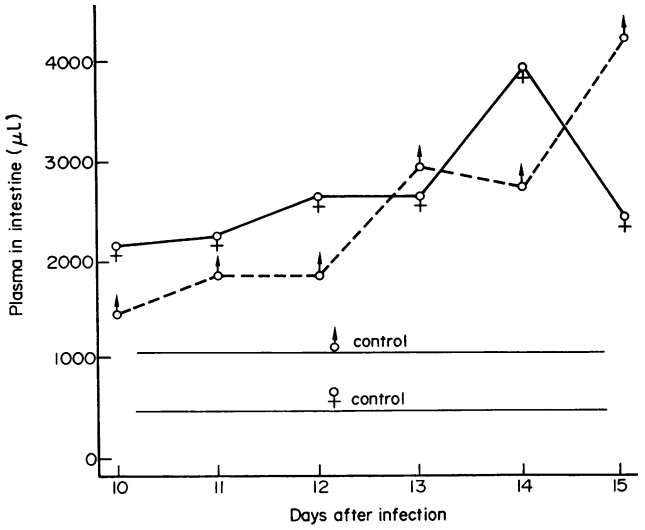

FIG. 4. Leak of plasma [^{131}I]PVP into the small intestine of male and female rats infected with 3000 *N. brasiliensis*. ○, Calculated plasma in intestinal contents.

In both male and female rats the permeability of the intestine was increased between days 10 and 15 of infection (Table 5 and Fig. 4). The greatest increase occurred on day 14 in the females and on day 15 in the males. In the males this was a day later than in the

previous experiment. The rats used in this experiment were from the same group as the mast cell kinetics study. As stated earlier, these rats suffered water depletion on day 2 of infection and there was evidence that this delayed the mast cell response and therefore possibly delayed the change in bowel wall permeability.

In the cortisone-treated rats, there was very little change in bowel wall permeability, the levels of plasma found in the intestine being only slightly above control levels (Table 6).

TABLE 6

MICRO LITRES OF PLASMA IN SMALL INTESTINE OF FEMALE RATS INFECTED WITH 3000 *N. brasiliensis* AND TREATED WITH CORTISONE FROM DAY 9

Days after infection	Arithmetic mean ± SE
10	1000 ± 100
11	1300 ± 290
12	1500 ± 200
13	1100 ± 135
14	1100 ± 200
15	700 ± 105

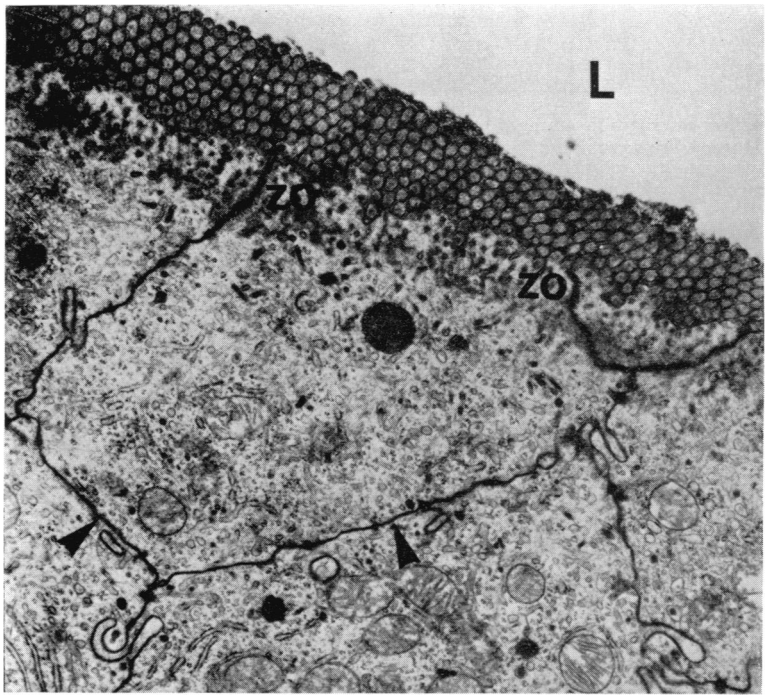

FIG. 5. Electron micrograph of intestinal mucosa of a parasite-free rat. Horseradish peroxidase (arrows) lies between the epithelial cells up to the level of, but not beyond the zonula occludens (ZO). L, lumen. × 11,000.

Experiment 3 (Electron microscopy)

Epithelial cells lining the rat intestine are joined by a tripartite junctional complex. In every complex, the juxtaluminal lateral plasmalemmata form a complete seal between adjacent epithelial cells, the zonula occludens. The use of horseradish peroxidase in non-parasitized rats showed that this tracer did not pass beyond the zonula occludens (Fig. 5)

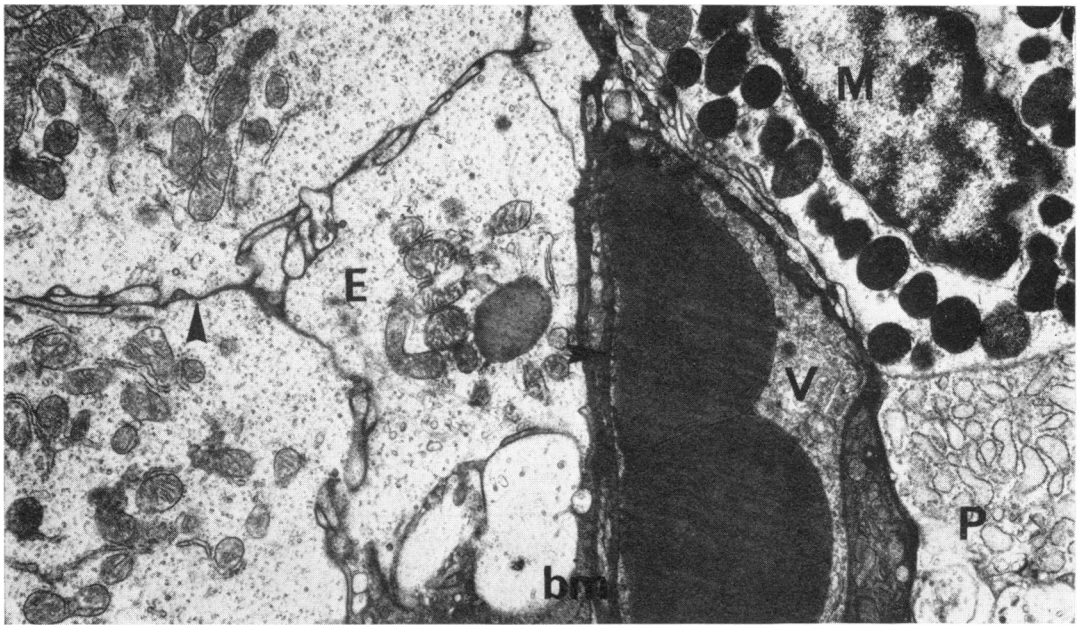

FIG. 6. Electron micrograph of the intestinal mucosa of a parasitized rat on the fourteenth day of infection. Horseradish peroxidase (arrows) is leaking from a post capillary venule (V) via fenestrae into the lamina propria and between epithelial cells (E). Note the close apposition of the mast cell (M), the plasma cell (P) and the post capillary venule. BM, basement membrane. × 10,800.

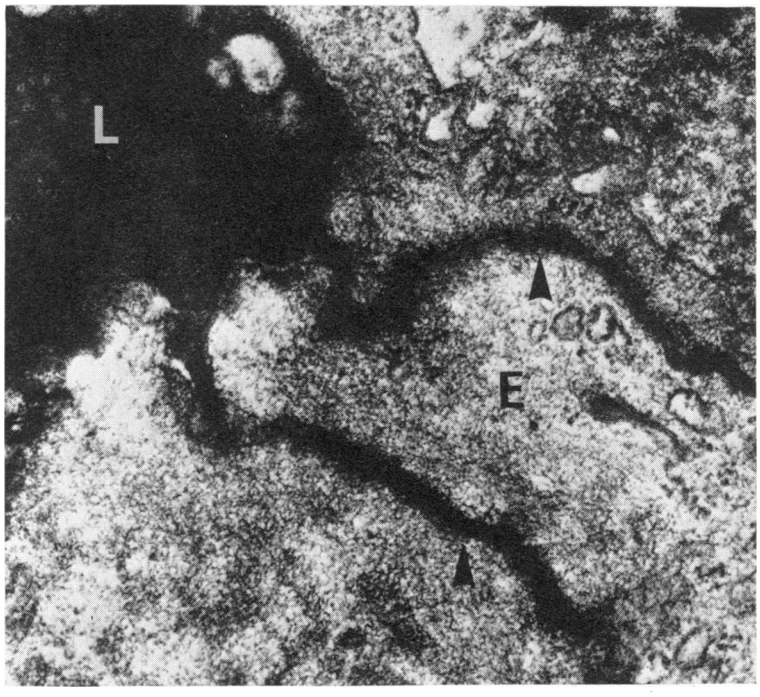

FIG. 7. Electron micrograph of the intestinal mucosa of a parasitized rat on the fourteenth day of infection. Horseradish peroxidase (arrows) lies between separated epithelial cells (E) and in the lumen (L) of the gut. × 46,000.

although it readily 'leaked out' of the circulation, mainly via fenestrae in post-capillary venules into the lamina propria and between epithelial cells.

On day 14 of the infection there was severe mucosal damage affecting the epithelium and the lamina propria. Some epithelial cells were sloughed off into the lumen. Others had a degenerate appearance and gaps approximately the width of a single cell were often observed within the epithelial sheets. Usually the cells in the adjacent lamina propria which included mast cells, plasma cells, eosinophils and macrophages, showed extensive damage. Many were fragmented, vacuolated, and had their plasmalemmata disrupted. Venular and capillary endothelia were likewise damaged.

In these circumstances, horseradish peroxidase passed rapidly from the circulation into the lamina propria via fenestrae, open junctional complexes, and damaged endothelial cells (Fig. 6). It passed between epithelial cells into the lumen of the intestine through disrupted cell junctions (Fig. 7). This was most marked in the areas of severe mucosal damage described above. The enzyme was also found occasionally within severely damaged and degenerate cells. In more normal areas where the junctional complexes were intact, it occurred within the cytoplasm in membrane-delimited vesicles and vacuoles which appeared to bud off from the lateral plasmalemmata. These vesicles and vacuoles were commonly found in epithelial cells lining the non-parasitized mucosa.

DISCUSSION

In *Nippostrongylus* infections in the rat it has been suggested that a two-stage process might be involved in the immunological expulsion of worms from the intestine; these stages are: (1) worm antigens stimulate the production of anti-worm antibodies; and (2) the worm secretes an allergen which induces the formation of reagins. The latter attach to mast cells in the lamina propria and on contact with allergen cause the discharge of vasoactive amines which increase mucosal permeability. This causes a release of anti-worm antibody at the site of the parasite (Jarrett *et al.*, 1969). We propose that the process is a three-stage one in which the third component is a factor (or factors) causing a population expansion of mast cells located in the lamina propria.

A striking temporal relationship exists between the onset of worm expulsion and the rise in the numbers of intestinal mast cells (Jarrett *et al.*, 1968b; Miller and Jarrett, 1971). The present results show that the phase of maximum velocity of worm expulsion occurs sooner and proceeds at a faster rate in females than in males. The corresponding differences in mast cell population kinetics and increased intestinal permeability in females and males suggests that the three circumstances are related.

Worm burden kinetics

Worm expulsion occurred in two phases. The first occurred around day 10 in both sexes. It lasted until day 12 in females and day 13 in males. This first slow phase of worm loss with a corresponding drop in egg production possibly indicates the presence of small amounts of antiworm antibody in both sexes. The onset of the phase of maximum worm expulsion occurred a day earlier in females, and there was a corresponding difference in mast cell response and intestinal permeability. Why the mast cell reaction occurs earlier in females is not known but preliminary results of experiments done on castrated male and spayed female rats infected with *N. brasiliensis* showed that male castrates expel their worm burden earlier than entire males and at the same time and speed as entire females, which suggests that sex hormones may play a role (Waddell, Jarrett and Murray, unpublished).

Subepithelial intestinal mast cell population kinetics

During a *Nippostrongylus* infection major changes occur in the mast cell population. In the normal parasite-free young adult rat there are about ten to twelve mast cells per villus crypt unit. Shortly after the young adult worms reach the intestine there is marked degranulation and disappearance of mast cells. We have shown that at this time the worms secrete a degranulator (Jarrett *et al.*, 1969; Miller, 1970). A possibily similar substance has been described in *Ascaris* by Uvnas and Wold (1967). In *Nippostrongylus* this may be part of the worm's feeding mechanism and possibly allows access of worm antigen to host tissue. Injection of carbon black at this stage shows that there is a vascular leak and that this is largely localized in the venules and other vessels of the crypt region (Jarrett *et al.*, 1968b). Histologically, there is marked oedema and enlargement of the villus while injection of Evans' blue shows that the areas of albumin leak coincide exactly with the worm distribution in the gut.

Until about day 10 of the infection there is an almost complete disappearance of demonstrable mast cells from the lamina propria. Towards the end of this phase large numbers of mononuclear stem cells can be seen with the electron microscope; these undergo mitosis and begin to differentiate into mast cells (Miller and Jarrett, 1971). These authors describe a complete range of cells from stem cells through to fully granulated cells and found that the rise in numbers is exponential. The cause of this rise is not known. It is not dependent on the continuing presence of worms. In an experiment in which groups of infected rats were treated with an anthelmintic to expel the worms, it was found that if the worms were removed by day 7, a subsequent mast cell rise still took place (Murray *et al.*, 1971). Two points from other experiments may be of importance here. Ginsburg and Lagunoff (1967) have shown that an antigenic stimulus can cause the appearance of cells in mesenteric lymph nodes and thoracic duct lymph which can differentiate into mast cells *in vitro*, and Morse and Bray (1969) have shown that *Bordetella pertussis*, a potent adjuvant for reagin-mediated reactions, possesses a lymphocyte-stimulating factor. Thus, the possibility might be envisaged that helminths could produce a factor which stimulated mastoblast production. These cells might, like the large lymphocytes of thoracic duct lymph, migrate to the gut lamina propria and differentiate into mast cells.

While the rise of mast cells is taking place, a corresponding increase in the amount of 5-hydroxytryptamine in the intestinal wall at the site of infection can be demonstrated by spectrofluorometry whether the worms are removed or not (Murray *et al.*, 1971). During this period, if worms are present, a marked release of amines from mast cells can be demonstrated by histochemistry and mast cell discharge is also seen on electron microscopy (Miller *et al.*, 1968; Murray *et al.*, 1968). It is at this stage there is marked transformation of mast cells into globule leukocytes (Jarrett *et al.*, 1968b; Murray *et al.*, 1968).

Possible mode of mast cell discharge

It is known that mast cell discharge in the intestinal lamina propria only takes place if worms are present (Murray *et al.*, 1970b); if worms are removed by drug treatment, discharge does not occur. Urquhart *et al.* (1965) found the bowel wall of rats infected with *N. brasiliensis* to be sensitive to the administration of *Nippostrongylus* antigen and proposed that a local anaphylactic reaction might play a part in worm expulsion. It has been shown that *Nippostrongylus* worms produce an allergen (Jones and Ogilvie, 1967; Wilson, 1967) and Ogilvie (1964) found reagin-like antibodies in the serum of rats infected with *N. brasiliensis*. Wilson and Bloch (1968) showed that reagin-sensitized peritoneal mast cells are

present from the 10th day of a primary infection. The appearance of these antibodies at this time makes it tempting to suggest that at least some of the extensive mast cell discharge reaction is caused by an allergen–reagin mediated system, although at present, the action of degranulator (see earlier in Discussion) cannot be excluded.

Consequences of vasoactive amine release

The rise in bowel wall permeability as judged by [^{131}I]PVP experiments corresponded to the rise in mast cells and reached a peak which coincided with the peak of mast cell amine discharge. It also coincided with the time of maximum velocity of worm expulsion. In these studies the differences between male and females again occurred. At the peak of increased permeability there was marked mucosal damage, both epithelial cells and cells in the lamina propria being involved. The maximum accumulation of horseradish peroxidase also occurred at these sites. This tracer enzyme was also found between epithelial cells of both normal and parasitized rats, but only in the latter did it appear to escape into the intestinal lumen. This route may represent the pathway for leakage of circulating or locally-produced antibody into the lumen. The mechanisms involved in mucosal changes are not fully understood. It would appear that the damage and increased permeability are related to the release of vasoactive amines and possibly proteolytic enzymes from mast cell granules as suggested by Jarrett *et al.* (1969). The plasma cell damage which occurs in the lamina propria at this time might result in the release of locally-produced antibody (Miller, 1970).

Inhibition of worm expulsion

Ogilvie (1965) and Urquhart *et al.* (1965) found that cortisone could suppress worm expulsion and Luffau and Urquhart (unpublished results) were able to arrest the process even after it had started by administration of this drug. Cortisone has several modes of action and Urquhart *et al.* (1966) concluded that the effect of cortisone occurred so rapidly that its role in interfering with worm expulsion could not involve a suppression of antibody production. Jarrett *et al.* (1968b) found that administration of cortisone suppressed the mast cell response in *Nippostrongylus* infections in the rat. Correspondingly, the present results show that increased permeability did not occur in cortisone-treated rats.

The effect of specific inhibitors of vasoactive amines lends strong support to the importance of the mast cell discharge reaction in worm expulsion. Urquhart *et al.* (1965), using an antihistaminic, and Sharp and Jarrett (1968), using reserpine (a depletor of 5HT), reduced worm loss. In an experiment in which both antihistamines and anti-5HT drugs were given twice daily commencing just prior to worm expulsion, it was found that the initial gradual loss of worms occurred but that the onset of the rapid phase of worm expulsion was prevented (Murray, Smith, Waddell and Jarrett, 1971). Such results indicate a major role for histamine and 5HT in this reaction.

Conclusion

We propose that a stimulus or stimuli from the worm cause synchronous development of a new population of mast cells, IgE-producing plasma cells and plasma cells synthesizing antibodies of other classes possessing an anti-worm effect. It is suggested that these mast cells discharge their pharmacological mediators by an allergen–reaginic antibody mediated system. The mediators released create a pathway through the intestinal mucosa which allows circulating or locally-produced antiworm antibodies access to the parasites in the

gut. Miller (1970) has also suggested that this reaction causes plasma cell disruption thereby releasing antibodies.

The cytological changes seen in *Nippostrongylus* infections occur in many parasitic diseases in a variety of species; for example, we have found marked mast cell activity and globule leucocyte formation associated with the exponential expulsion of *Ostertagia ostertagi* infections in cattle (Murray, Jennings and Armour, 1970) and *Ostertagia circumcincta* in sheep (Armour, Jarrett and Jennings, 1966). It is therefore possible that this mechanism proposed for antibody release and translocation exists in other diseases.

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