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# Expulsion of *Nippostrongylus brasiliensis* from rats protected with serum

## I. THE EFFICACY OF SERA FROM SINGLY AND MULTIPLY INFECTED DONORS RELATED TO TIME OF ADMINISTRATION AND VOLUME OF SERUM INJECTED

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**Summary.** Several of the parameters related to the efficacy of passive protection against *Nippostrongylus brasiliensis* were studied in female hybrid (PVG/c × DA)$F_1$ and outbred Wistar rats. The time after infection at which immune serum was given did, to some extent, alter the degree of protection conferred. There was substantial protection 8 days after challenge in rats given hyperimmune serum (HIS) either as daily injections 4–7 days post-infection or as a single dose on day 4. Eight separate experiments in which HIS was injected 4 days after challenge with 1000 $l_3$ resulted in expulsion of $96 \pm 2\%$ of the worm burdens by day 8. In a further six experiments, following infection with 2000 $l_3$ and using the same protocol, $85 \pm 3\%$ of the worm burden was expelled by day 8. A lag of 2 days between serum transfer and commencement of worm expulsion was consistently observed and, within the space of a further 2–4 days, more than 95% of the parasites were expelled. Transplanted 'normal' and 'damaged' adult worms were also susceptible to the passive transfer of HIS. Sera recovered from rats immunized with two or three challenges (hyperimmune sera) were more protective than sera from rats given one challenge. Serum from rats challenged for the first time 6 days previously conferred significant protection against a 1000 $l_3$ infection and sera recovered 8 and 10–12 days post-infection with 4000 $l_3$ protected recipients with increasing effectiveness. Thoracic duct lymph collected on the tenth day of infection with 4000 $l_3$ was also protective. The response to both primary infection and hyperimmune serum was dose-dependent. The consistently good protection achieved in the present study when compared with the variable success of previous experiments (reviewed by Ogilvie & Jones, 1971) may be a function of the strain and sex of the rats used, together with modifications of the immunization protocols. The relevance of these findings to mucosal defences against *N. brasiliensis* is discussed.

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## INTRODUCTION

Experiments in inbred laboratory animals have demonstrated that immune lymphocytes confer protection against a variety of intestinal nematodes (Ogilvie & Love, 1974; Wakelin, 1978). The protective capacities of the transferred cells, however, differ

according to the strain of animal used and are affected by the class, source, and dose of cells given and by the stage of infection at which donor cells are collected (Wakelin, 1978; Ogilvie, Love, Jarra & Brown, 1977; Crum, Despommier & McGregor, 1977; Nawa & Miller, 1978). Immune lymphocytes have the capacity to transfer cell-mediated immunity, to regulate antibody synthesis, and to influence the localization of accessory cells such as basophils, eosinophils and mast cells (Ogilvie & Love, 1974; Wakelin, 1978; Nawa & Miller, 1979). They also influence mucosal epithelial architecture and differentiation in parasitized animals (Ferguson & Jarrett, 1975; Miller & Nawa, 1979a).

Any interpretation of the nature of the protection conferred by immune lymphocytes is compromised by the varied functions of the cells and by their ability, once transferred, to proliferate, differentiate and localize in different tissues. Passive transfer of immune serum, an alternatve method of augmenting the intestinal immune response has, in recent years, received less attention as a means by which intestinal protective mechanisms can be studied. One reason for this may be the variable success achieved by passive protection experiments (reviewed by Ogilvie & Jones, 1971; Wakelin, 1978). However, recent data have shown that, in common with cell transfer studies, the protective capacity of immune serum is influenced by the strain and sex of both the donors and recipients of serum (Dobson & Owen, 1978).

The kinetics of primary infection and the parameters for adoptive immunization against the rat intestinal nematode *Nippostrongylus brasiliensis* have been defined in several rat strains (Jarrett, Jarrett & Urquhart, 1968; Ogilvie *et al.*, 1977; Nawa & Miller, 1978, 1979). Similarly there have been a number of studies of passive protection against this parasite, in which a variable degree of success has been achieved (reviewed by Ogilive & Jones, 1971). The purpose of the present study was, therefore, to re-evaluate the protocols for the passive transfer of immune serum and, where possible, to compare the results with cell transfer studies (Nawa & Miller, 1978) in the same strain of rat.

A consistently high degree of protection against adult *N. brasiliensis* was achieved with hyperimmune serum, and serum and lymph from donors harbouring primary infections were also protective. The methodology described in this study may thus provide a useful alternative approach to the understanding of mucosal immunity against this parasite.

## MATERIALS AND METHODS

*Animals*

Female (PVG/c × DA)$F_1$ and outbred Wistar rats 11–13 weeks old were used as serum recipients. Serum donors were 10–15 week old females of the same two strains. Female Wistar rats 10–15 weeks old were used to maintain the parasites as described previously (Nawa & Miller, 1978).

*Parasitological techniques*

The methods of culturing Nippostrongylus larvae, of infection with third stage larvae ($l_3$), and of recovering and counting adult worms were those described previously (Nawa & Miller, 1978). The techniques for the intraduodenal implantation of adult worms have also been recorded (Nawa & Miller, 1978).

*Preparation of immune serum*

Primary infection serum was obtained 6, 8, 10–12 and 13–17 days post infection (p.i.) of female (PVG/c × DA)$F_1$ and Wistar rats with 4000 $l_3$. Hyperimmune serum was recovered from the same two rat strains given primary infections of 1000–4000 $l_3$ and one or two subsequent infections of 4000 $l_3$ in the space of 4–6 weeks. After ether anaesthesia, rats were bled out by section of the carotid artery. Hyperimmune serum donors were killed 5–8 days after the final challenge. Serum samples were stored at $-20°$ until sufficient were collected to be pooled into a single volume of at least 150 ml and in which there were equal proportions of (PVG/c × DA)$F_1$ and Wistar serum. The pooled samples were sterilized by Millipore filtration and were stored at $-20°$ in 20 ml vials. Five pools of hyperimmune serum varying between 150 ml (pool A) and 1600 ml (pool D) were collected. The passive cutaneous anaphylaxis (PCA) titres of each pool were assessed at intervals and little or no loss of activity was detected during the period of storage (4–8 months).

*Preparation of adult worm antigen*

Adult worm antigen was prepared by the method of Ogilvie (1964) and adjusted to 3000 worm equivalents/ml with sterile saline. One millitre samples were stored at $-70°$ until use.

*Homologous passive cutaneous anaphylaxis (PCA)*

The level of specific IgE antibodies against *N. brasiliensis* were assayed by PCA and the sensitized rats

were challenged intravenously with 0·5 ml worm antigen and 0·5 ml 1% Evans' blue (Ogilvie, 1964).

*Passive immunization*

Serum recipients were lightly anaesthetized with ether and were injected intraperitoneally with immune serum using 1, 5, or 10 ml syringes and 20 gauge needles.

## RESULTS

### Time of administration of serum

The effect of passive transfer of hyperimmune serum (HIS) on day 0 of a 2000 $l_3$ challenge was compared with serum transfer on day 4, after the worms had established in the gut. An additional group of rats was injected daily (days 4–7 p.i.) with 1 ml HIS/100 g body weight. All rats were killed 8 days after challenge. Control groups included untreated rats and a group given 4 ml/100 g body weight of normal serum. The results in Table 1 show that administration of serum on day 0 was more protective than when given on day 4 ($P<0·01$) or on days 4–7 p.i. ($P<0·01$). However, there was no difference between the latter two treatments, and normal serum had no effect on the parasite burden (Table 1).

In subsequent experiments, it became apparent that daily injections of hyperimmune serum were less protective than single doses given 4 days after challenge (compare Tables 2 and 3). The passive transfer of HIS at a dose of 4 ml/100 g body weight conferred protection against 1000 $l_3$ infections (Table 3) which was comparable to that achieved by adoptive immunization with TDL (Nawa & Miller, 1978). However, the same doses of hyperimmune serum given to rats harbouring 2000 $l_3$ infections resulted in a less complete expulsion of the parasites (Table 3).

### Dose response experiments

The results of several dose–response experiments using three different pools of hyperimmune serum are recorded in Table 4. In all of the experiments hyperimmune serum was injected daily on days 4–7 p.i. and the doses ranged from 0·125 ml/100 g body weight per day (cumulative dose 0·5 ml/100 g body weight) to 2 ml/100 g body weight per day (cumulative dose 8 ml/100 g body weight). As little as 0·5 ml of pool C/100 g body weight conferred significant protection ($P<0·05$) against a 2000 $l_3$ infection and increasing protection was conferred with increasing doses of serum (Table 4). The results of the third experiment using pool C have been plotted on a logarithmic scale and demonstrate the inverse relationship between the volume of serum injected and the number of parasites remaining in the small intestine (Fig. 1).

### Kinetics of worm expulsion

Female (PVG/c × DA)$F_1$ rats were infected with 2000 $l_3$ and were allocated to two groups. Group 1 comprised untreated controls, five to eight rats being killed daily for worm counts between days 4 and 14 after infection. The rats in the second group were injected intraperitoneally with 4 ml/100 g body weight pool C hyperimmune serum 4 days after challenge. Groups of six to eight rats were killed on each of days 5–10 p.i.

**Table 1.** The timing of administration of hyperimmune serum (HIS); its effect on passive protection in (PVG/c × DA)$F_1$ rats

| Protocol | | Worm burden ± S.E. | Worm expulsion (%) | $P$* |
|---|---|---|---|---|
| Untreated control | (5) | 1136 ± 51 | — | — |
| HIS 4 ml/100 g BW day 0 | (5) | 107 ± 25 | 90 | <0·001 |
| HIS 4 ml/100 g BW day 4 | (6) | 315 ± 54 | 72 | <0·001 |
| HIS 1 ml/100 g BW days 4–7 | (6) | 376 ± 57 | 69 | <0·001 |
| Normal serum 4ml/100 g BW day 4 | (5) | 1114 ± 100 | 2 | NS |

* Student's $t$ test, treated groups versus untreated control infection.

NS = not significant, $P>0·05$.

All groups were killed 8 days after challenge with 2000 $l_3$. The number of rats/group is recorded in parentheses.

**Table 2.** Passive protection by daily injections of hyperimmune serum (HIS) into (PVG/c × DA)$F_1$ rats infected with 1000 $l_3$ or 2000 $l_3$

| Experiment | HIS pool | PCA titre (HIS) | Worm burden ± SE | | Expulsion (%) | *P** |
|---|---|---|---|---|---|---|
| | | | Control | HIS | | |
| 17 | A | 640 | 633 ± 67 | 149 ± 86 | 76 | < 0·01 |
| 25† | A | 640 | 417 ± 27 | 75 ± 5 | 82 | < 0·001 |
| 28† | B | 320 | 833 ± 54 | 252 ± 64 | 70 | < 0·001 |
| 32† | B | 320 | 421 ± 58 | 180 ± 20 | 57 | < 0·01 |
| 38 | B | 320 | 526 ± 36 | 156 ± 44 | 70 | < 0·001 |
| | (1000 $l_3$) | Mean ± SE | 566 ± 77 | 162 ± 28 | 71 ± 4 | |
| 36 | B | 320 | 1357 ± 119 | 373 ± 32 | 80 | < 0·001 |
| 41 | C | 1280 | 1111 ± 13 | 200 ± 42 | 82 | < 0·001 |
| | (2000 $l_3$) | Mean ± SE | 1234 ± 174 | 236 ± 52 | 81 ± 1 | |

* Student's *t* test, serum recipients versus controls.
HIS was injected i.p. daily (days 4–7 or days 3–6†–post infection) in doses of 1 ml/100 g body weight.
Rats were killed 24 h after the last injection.

**Table 3.** Protection conferred by a single injection of hyperimmune serum (HIS) in (PVG/c × DA)$F_1$ and Wistar rats infected with 1000 or 2000 $l_3$

| Experiment | HIS pool | PCA titre (HIS) | Worm burden ± SE | | Expulsion (%) | *P** |
|---|---|---|---|---|---|---|
| | | | Control | HIS | | |
| 49 | D | 640 | 523 ± 25 | 38 ± 21 | 93 | < 0·001 |
| 57 | D | 640 | 517 ± 40 | 22 ± 8 | 96 | < 0·001 |
| 63† | D | 640 | 490 ± 24 | 3 ± 3 | 99 | < 0·001 |
| 72 | D | 640 | 464 ± 36 | 16 ± 5 | 97 | < 0·001 |
| 74† | D | 640 | 600 ± 40 | 7 ± 2 | 99 | < 0·001 |
| 77† | E | 64 | 527 ± 21 | 0 | 100 | < 0·001 |
| 78 | E | 64 | 719 ± 13 | 127 ± 18 | 82 | < 0·001 |
| 79† | E | 64 | 535 ± 45 | 2 ± 1 | 100 | < 0·001 |
| | (1000 $l_3$) | Mean ± SE | 547 ± 28 | 27 ± 15 | 96 ± 2 | |
| 44 | C | 1280 | 1423 ± 80 | 249 ± 38 | 82 | < 0·001 |
| 47 | C | 1280 | 1256 ± 50 | 177 ± 21 | 86 | < 0·001 |
| 50 | D | 640 | 1066 ± 23 | 293 ± 128 | 73 | < 0·001 |
| 55 | C | 1280 | 864 ± 35 | 143 ± 26 | 83 | < 0·001 |
| 55 | D | 640 | 864 ± 35 | 116 ± 28 | 87 | < 0·001 |
| 63† | D | 640 | 1059 ± 84 | 21 ± 8 | 98 | < 0·001 |
| | (2000 $l_3$) | Mean ± SE | 1089 ± 90 | 166 ± 40 | 85 ± 3 | |

* Student's *t* test, serum recipients versus controls
† Denotes Wistar strain.
HIS was injected i.p. 4 days after infection in doses of 4 ml/100 g body weight.
Rats were killed on day 8 of infection.

**Table 4.** The effects of different doses of hyperimmune serum on 1000 $l_3$ and 2000 $l_3$ infections in (PVG/c × DA)$F_1$ rats

| | Pool A 1000 $l_3$ infection | | Pool B 2000 $l_3$ infection | | Pool C 2000 $l_3$ infection | |
|---|---|---|---|---|---|---|
| Cumulative volume of HIS (ml/100 g BW) | Worm burden ± SE | Expulsion (%) | Worm burden ± SE | Expulsion (%) | Worm burden ± SE | Expulsion (%) |
| Untreated control | 633 ± 67 | — | 1357 ± 119 | — | 1111 ± 13 | — |
| 0·5 | ND | ND | ND | ND | 860 ± 86 | 23 |
| 1 | 324 ± 21 | 49 | ND | ND | 652 ± 55 | 41 |
| 2 | 320 ± 48 | 51 | 412 ± 57 | 70 | 410 ± 89 | 63 |
| 4 | 149 ± 86 | 76 | 273 ± 32 | 80 | 200 ± 42 | 82 |
| 8 | ND | ND | 161 ± 28 | 88 | 96 ± 26 | 91 |

Serum was injected daily (days 4–7 p.i.) in volumes ranging from 0·125 to 2 ml/100 g BW.
Rats were killed 24 h after the last injection.
ND = not done.

The kinetics of worm expulsion are shown in Fig. 2 together with the PCA titres of the passively immunized rats. None of the control rats had demonstrable PCA titres in their sera at any stage after infection.

For 48 h following injection of hyperimmune serum (days 5 and 6 p.i.) there was no reduction in the worm burden (Fig. 2). Seven days after challenge 53% of the parasites had been expelled and on days 8, 9 and 10 p.i. respectively, the worm burdens were 23, 12 and 4% of the control values (Fig. 2). The PCA titres declined rapidly to reach zero on day 7 and no further activity was detected in any of the passively protected rats. The worm burden in the controls remained unchanged until day 10 post infection and spontaneous cure took place between days 11 and 14 (Fig. 2).

A kinetic study of passive protection was also performed in Wistar rats and the results are tabulated

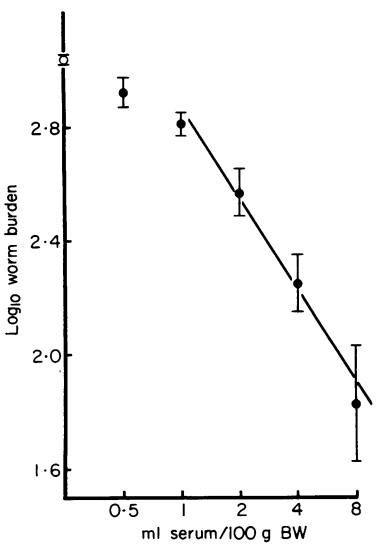


**Figure 1.** The worm burdens in (PVG/c × DA)$F_1$ rats challenged with 2000 Nippostrongylus $l_3$ are plotted logarithmically against the cumulative volume of pool C hyperimmune serum injected daily 4–7 days after challenge. Groups of 6 serum recipients (•) and untreated controls (○) were killed on day 8. Each point represent $Log_{10}$ geometric mean ± SE (Slope − 1·09 ± 0·09).

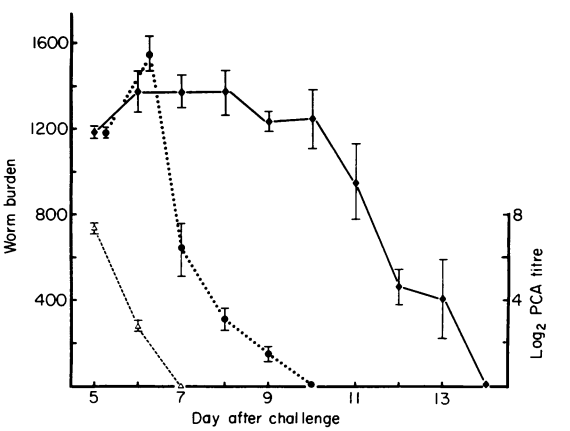


**Figure 2.** Kinetics of worm expulsion in untreated (PVG/c × DA)$F_1$ rats challenged with 200 $l_3$ (diamonds) and in infected recipients of 4 ml/100 g body weight (pool D) hyperimmune serum (PCA titre 1/1280) (circles) given on day 4 post-infection. Also shown are the PCA titres in the serum recipients (triangles). Each point represents the mean ± SE for groups of five to eight rats.

**Table 5.** Kinetics of worm expulsion in Wistar rats passively protected with hyperimmune serum (HIS)

| Day after Challenge (2000 $l_3$) | Worm burden ± SE | | Worm expulsion (%) | $P$* |
|---|---|---|---|---|
| | Control | HIS | | |
| 6 | (4) 1465 ± 126 | (4) 1166 ± 30 | 20 | NS |
| 7 | (5) 995 ± 92 | (5) 178 ± 78 | 82 | < 0·001 |
| 8 | (4) 1059 ± 84 | (5) 21 ± 8 | 98 | < 0·001 |

* Student's $t$ test.
NS = not significant $P > 0{\cdot}05$
Rats were injected i.p. with 4 ml/100 g BW HIS (pool D) 4 days post-infection. Values in parentheses represent number of rats/group.

**Table 6.** Time-lag between injection of hyperimmune serum and expulsion of the parasites in (PVG/c × DA)$F_1$ rats

| Experiment | Day after infection | Worm burden ± SE | | Worm expulsion (%) | $P$* |
|---|---|---|---|---|---|
| | | Control | HIS | | |
| 1 | 6 | (5) 1165 ± 50 | (5) 971 ± 99 | 17 | NS |
| | 8 | (5) 1256 ± 50 | (6) 177 ± 21 | 86 | < 0·001 |
| 2 | 6 | (6) 1371 ± 59 | (6) 1549 ± 81 | — | — |
| | 8 | (6) 1136 ± 51 | (6) 315 ± 54 | 72 | < 0·001 |
| 3 | 6 | (5) 1246 ± 100 | (5) 1388 ± 141 | — | — |
| | 8 | (5) 1240 ± 121 | (6) 367 ± 56 | 70 | < 0·001 |

* Student's $t$ test
NS = not significant, $P > 0{\cdot}05$.
Serum (4 ml/100 g BW) was injected i.p. on day 4 (experiments 1 and 2) and recipients were killed on days 6 and 8 post-infection. In experiment 3 serum was injected daily (1 ml/100 g BW) on days 4–7. Numbers of rats/group are shown in parentheses.

**Table 7.** The effect of injecting hyperimmune serum (HIS) 4 or 6 days after challenging Wistar rats with 1000 $l_3$

| Protocol | Worm burden ± SE | | Worm expulsion (%) | $P$* |
|---|---|---|---|---|
| | Control | HIS | | |
| HIS injected day 4 | (11) 600 ± 40 | (6) 7 ± 2 | 99 | < 0·001 |
| HIS injected day 6 | (11) 600 ± 40 | (5) 461 ± 62 | 23 | NS |

* Student's $t$-test.
NS = not significant, $P > 0{\cdot}05$.
Recipient rats were given 4 ml/100 g BW of serum Pool D. Number of rats per group is shown in parentheses. All rats were killed 8 days post-infection.

(Table 5). Again, hyperimmune serum (pool D) had no significant effect before day 6 but this was followed by rapid and virtually complete expulsion of the parasites between days 7 and 8 of infection (Table 5).

The lag phase between injection of immune serum and expulsion of the parasites has been noted on several previous occasions (Neilson, 1969; Ogilvie & Jones, 1971). The consistency of this finding is illustrated in Table 6 wherein no effect on the parasites is demonstrable for 48 h after injection of immune serum, despite the fact that at 96 h less than 30% of the worm burden remained (Table 6).

There may be several reasons for this latent phase of inactivity, for example, mucosal permeability may be insufficient to permit the pathotopic transfer of antibodies (Murray, 1972). Alternatively immune serum may be specific for a certain stage of parasite development (Bell, McGregor & Despommier, 1979) or the parasites must be exposed to antibody for a certain period of time before they are damaged sufficiently to be expelled (Ogilvie & Jones, 1971). Consequently, the effects of transferring serum on day 4 p.i. were compared with those following transfer on day 6. The results of this experiment show that a 48 h delay in the transfer of serum substantially reduces the protection conferred (Table 7). This would indicate that the effects of serum are unlikely to be stage-specific.

**Passive protection against 'normal' and 'damaged' worms**

Adult intestinal parasites harvested 6–9 days after infection ('normal' worms) will, when transferred to naive recipients, survive for a further 7–9 days (Ogilvie & Jones, 1971). Parasites harvested after day 10 ('damaged' worms) are rapidly expelled from naive recipients (Ogilvie & Jones, 1971). Expulsion of 'damaged' worms is thought to be a non-specific process, mediated by sensitized lymphocytes and bone marrow cells (Ogilvie & Love, 1974), consequently it was of interest to compare the effects of serum transfer on recipients of 'normal' and 'damaged' worms. In agreement with previous experiments (Neilson, 1969; Ogilvie & Jones, 1971), 'normal' worms were more rapidly expelled in passively protected $(PVG/c \times DA)F_1$ rats when compared with controls given normal serum (Table 8). However, 'damaged' worms were also susceptible to hyperimmune serum (Table 8) which again points to a lack of stage specificity in the ability of immune serum to protect against adult worms.

**Protection conferred by primary infection serum and lymph**

The preceding experiments established that each of five large pools of hyperimmune serum were protective against the intestinal phase of *N. brasiliensis* infections. Immune serum recovered after a single *N. brasiliensis* infection will also confer significant protection (Mulligan, Urquhart, Jennings & Nielson, 1965; Ogilvie & Jones, 1971) but there is no evidence to suggest that serum recovered from donors harbouring a pri-

**Table 8.** Passive protection against implanted 'normal' and damaged' adult worms

| Recipients of 'Normal worms' (495 ± 8)* | Worm burdent ± SE Control | | Worm burdent ± SE HIS | | % Worm expulsion | P† |
|---|---|---|---|---|---|---|
| Day 3 | (6) | 418 ± 35 | (6) | 316 ± 22 | 24 | < 0·05 |
| Day 4 | (6) | 327 ± 43 | (6) | 107 ± 28 | 67 | < 0·01 |
| Recipients of 'Damaged' worms (506 ± 7)* | | | | | | |
| Day 3 | (5) | 183 ± 13 | (5) | 103 ± 9 | 44 | < 0·01 |
| Day | (6) | 87 ± 26 | (7) | 22 ± 5 | 75 | < 0·05 |

* Number of worms transplanted.

† Student's *t* test.

Control rats were given 1 ml/100 g BW normal serum on days 0–3 of infection. The same doses of pool B hyperimmune serum were also given to infected rats. The number of rats/group is recorded in parentheses. Recipients of serum and worms were killed 3 and 4 days post-infection.

**Table 9.** Protective capacities of primary infection serum and thoracic duct lymph in (PVG/c × DA)$F_1$ rats infected with 1000 $l_3$

| Experiment | Protocol | Worm Burden ± SE control | | Worm Burden ± SE Recipients | | % Worm expulsion | *P** |
|---|---|---|---|---|---|---|---|
| 32 | Day 10 lymph | (6) | 421 ± 58 | (6) | 220 ± 32 | 48 | < 0·02 |
| | Pool A HIS | (6) | 421 ± 58 | (6) | 180 ± 20 | 57 | < 0·01 |
| 57 | Day 10-12 serum | (8) | 517 ± 40 | (6) | 243 ± 30 | 53 | < 0·001 |
| | Pool D HIS | (8) | 517 ± 40 | (6) | 22 ± 8 | 96 | < 0·001 |
| 63 | Day 13-17 serum | (5) | 445 ± 47 | (5) | 154 ± 63 | 65 | < 0·01 |

* Student's *t* test.

For Experiment 32, lymph and serum were injected daily on days 3–6 post-infection in doses of 1 ml/100 g BW. All groups were killed on day 7. In Experiments 57 and 63 sera were injected on day 4 (4 ml/100 g BW) and all groups were killed 8 days after infection. Values in parentheses are the numbers of rats per group.

mary infection is able to influence the parasite burdens.

The adult worms in Wistar and in hybrid (PVG/c × DA)$F_1$ rats are expelled 12–14 days after challenge with 3000–4000 $l_3$ (Nawa & Miller, 1978, 1979). Consequently, the protection conferred by primary infection serum and by ten times concentrated, defatted, thoracic duct lymph (kindly provided by Dr Y. Nawa) recovered from donors harbouring a 4000 $l_3$ infection was compared with that provided by similar volumes of hyperimmune serum. Primary infection serum and lymph harvested from infected donors conferred significant protection (Table 9). Similarly, serum harvested from donors at about the time of or just after spontaneous cure (days 13–17 p.i.) also conferred substantial protection (Table 9). However, neither primary infection serum nor lymph were as protective as hyperimmune serum (Table 9).

A dose–response experiment was also performed, in which the same pool of day 10–12 primary infection serum was injected (doses ranging from 2 to 16 ml/100 g body weight) into the hybrid rats 4 days after infection with 1000 $l_3$. Those rats receiving the highest dose (16 ml/100 g body weight) were given 8 ml/100 g body weight on each of days 4 and 5 p.i. All groups, each comprising 5–8 rats, were killed on day 8 and the worm burdens were counted. Figure 3 demonstrates that,

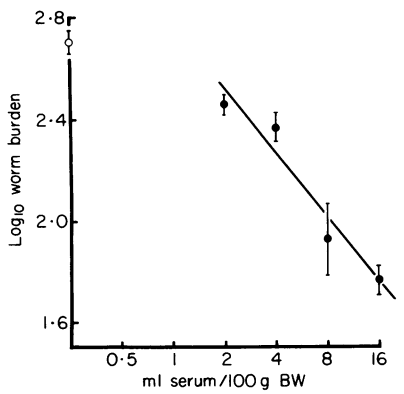


**Figure 3.** Dose–response curve showing the relationship between worm burden and volume of day 10–12 serum injected (●). (PVG/c × DA)$F_1$ rats were challenged with 1000 $l_3$, were injected with day 10–12 serum on day 4 (and 5) and were killed 8 days after challenge. The untreated infected control group (○) comprised eight rats and there were six rats per group in recipients of 2, 4 and 8 ml serum/100 g body weight. The remaining group (16 ml/100 g BW) contained five rats. Each point depicts $\log_{10}$ geometric mean ± SE (slope −0·846 ± 0·155).

**Table 10.** Protective capacities of primary infection serum in Wistar rats

| Protocol | Worm Burden ± SE Worm | | % Worm expulsion | *P** |
|---|---|---|---|---|
| Untreated control | (8) | 631 ± 23 | — | — |
| Day 6 serum | (6) | 448 ± 67 | 29 | < 0·05 |
| Day 8 serum | (5) | 379 ± 42 | 40 | < 0·001 |
| Day 10–12 serum | (4) | 6 ± 3 | 99 | < 0·001 |

* Student's *t* test.

Serum was recovered 6, 8, and 10–12 days after primary infection with 4000 $l_3$. Recipient rats were injected (8 ml/100 g BW) 4 days after infection with 1000 $l_3$ and were killed on day 8. Number of rats per group is shown by values in parentheses.

like hyperimmune serum, day 10–12 serum confers protection in a dose-dependent manner.

Since day 10 lymph and day 10–12 sera were harvested from donors which had yet to expel the bulk of their parasite burdens, the possibility that sera might be protective when recovered from donors at earlier stages of infection was also tested. Wistar rats infected with 1000 $l_3$ 4 days previously were injected with sera (8 ml/100 g body weight) recovered 6, 8 and 10–12 days after infection with 4000 $l_3$.

Significant protection ($P < 0{\cdot}05$, Table 10) occurred with day 6 primary infection serum and sera harvested 8 and 10–12 days p.i. conferred increasing degrees of protection (Table 10). These results establish that protective factors are present in the sera of rats infected with 4000 $l_3$ several days before spontaneous cure commences.

## DISCUSSION

The range of experiments described in this paper establish protocols by which a consistently high degree of passive protection against adult *N. brasiliensis* worms can be achieved. The transfer of immune serum confers protection in a dose-dependent manner. Furthermore, protective factors are present in serum and thoracic duct lymph of rats harbouring primary infections. The efficacies of the primary infection serum pools increased with time after infection but they were not as potent in their protective capacities as hyperimmune serum.

Doubts regarding the significance of passive protection experiments against *N. brasiliensis* infection have been raised on two main issues: (1) immune sera were usually sporadically protective, and there was no anamnestic response following hyperinfection (Ogilvie & Jones, 1968) and (2) protection was conferred by post-infection sera and not by sera harvested during infection, thereby implying that factors in serum played, at best, a secondary role in the mechanisms of spontaneous cure. The present results demonstrate that, given the right conditions, all except pools of normal serum are protective, and that hyperimmune serum is more effective than primary infection serum.

The fact that primary infection serum is protective lends further support to the evidence provided by Ogilvie and co-workers (Ogilvie & Jones, 1968; Ogilvie & Hockley, 1968; Jones & Ogilvie, 1971) that antibody-mediated damage is an early event during primary infection (Henney, Maclean & Mulligan, 1971). However, it does not answer the question as to whether worm damage is mediated directly by antibody or indirectly via antibody-mediated host responses (Wakelin, 1978).

The experiments by Jones, Edwards & Ogilvie (1970) have shown that passive protection is conferred predominantly by an IgG fraction which would suggest, *a priori*, that immunoglobulin is the principal protective factor in serum. It would, however, be premature to exclude the existence of other non-immunoglobulin factors.

The half-life of IgG in infected rats is reduced when compared with that in normal controls (Jones & Ogilvie, 1971) and there is also a rapid catabolism of transferred worm-specific IgE (Fig. 2). It is, therefore, interesting that the earliest demonstrable effect on the parasites is 72 h after transfer of serum and that, once initiated, the expulsion mechanism proceeds to completion despite the rapid catabolism of the transferred immunoglobulins. The present data suggest that this delay is not related to any stage-specificity (Bell *et al.*, 1979) of the transferred serum which was active against both 'normal' and 'damaged' worms. There are, in addition, a number of cellular changes in the intestinal mucosa which are temporally related to worm expulsion (Taliaferro & Sarles, 1939 and reviewed by Ogilvie & Jones, 1971). Several of these changes are accelerated in both adoptively, and passively immunized infected rats, although their development occurs over a period of several days (Nawa & Miller, 1979; Miller & Nawa, 1979b; Miller, 1979). An additional element in the delayed effects of serum transfer may thus be related to the delay in the development of these mucosal changes.

The kinetics of *N. brasiliensis* expulsion are governed not only by the strain (Jarrett *et al.*, 1968; Ogilvie *et al.*, 1977; Nawa & Miller, 1978; 1979) but also the sex of the infected rats (Murray, Jarrett & Jennings, 1971). Recently Dobson & Owen (1978) described a strain and sex susceptibility of mice to the passive transfer of resistance against *N. dubius*. The relative success of the present study when compared with previous data (reviewed by Ogilvie & Jones, 1971) may, therefore, reflect good fortune in the choice of rat strains and the fact that young adult female donors and recipients were used for all experiments. Modifications in the immunization protocols and, in particular, the time at which recipient rats were killed may also have contributed to the present results.

The relative importance of cell-mediated versus humoral immunity during primary spontaneous cure

of *N. brasiliensis* remains unresolved. For example, approximately 1·5 plasma volumes of hyperimmune serum or 3–4 volumes of day 10–12 serum injected 4 days after challenge confer the same degree of resistance against a 1000 $l_3$ infection as $1 \times 10^8$ day 10 TDL transferred on day 0 of infection (Nawa & Miller, 1978). Moreover, the transfer of either cells or serum leads to a final common pathway for several of the cell changes in the infected mucosa (Nawa & Miller, 1979; Miller & Nawa, 1979a and b; Miller, 1979). Consequently, it would be interesting to determine whether the much more rapid protection achieved by transferring both cells and serum (Ogilvie & Love, 1974) is reflected by a more rapid development of these mucosal changes.

Whilst the present results do not resolve the mechanisms of primary spontaneous cure, they do open up a useful alternative approach to studies of mucosal immunity against *N. brasiliensis*. The ability to purify and test the various active components in immune serum (Jones *et al.*, 1970) offers an obvious advantage over cell fractionation procedures (Ogilvie *et al.*, 1977. The adoption of this alternative approach may eventually permit a more precise dissection of the complex mechanisms controlling mucosal immune responses to helminth infection.

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