

IMMUNITY TO TRICHINELLA SPIRALIS

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by

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I dedicate this thesis to my

Mother and Father

## ABSTRACT

Immunity to *Trichinella spiralis*

by

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The major part of this work was concerned with the mechanism of protective immunity which acts against the parenteral phase of a *Trichinella spiralis* infection in mice.

Passively transferred immune serum always gave strong protection, to recipients, against an intravenous challenge of newborn larvae, regardless of whether the donor animals were sensitized by single, multiple, full or parenteral infections of *T. spiralis*. Small doses of immune serum gave significant protection, against challenge, to mice. Sensitized spleen cells and mesenteric lymph node cells, taken from infected donors, varied in their ability to protect recipient mice against challenge. Immune serum was only effective when transferred before a parenteral challenge, suggesting that it affected the newborn larvae and not the muscle larvae. The protective factors in immune serum appeared seventeen days after a single parenteral infection was given; they became progressively more protective after this time and there was evidence of their presence in serum taken five months after infection. The serum factors were protective in the absence of effector T-cells but whether they acted alone, against newborn larvae, or required the cooperation of non specific cells was not fully established.

Genetical studies on the ability of CBA and NIH mice to expel intestinal *T. spiralis* were made. CBA mice responded poorly to infection whereas NIH mice responded well. Cross breeding between the strains and back crosses with the first generation progeny of these showed that the responder characteristic of NIH mice was dominant and its inheritance was polygenic. The ability to expel adult worms was not linked to the genes coding for the colour or sex of the mice.

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## KEY TO ABBREVIATIONS USED IN TEXT

AchE	-	acetylcholinesterase
ALS	-	anti-lymphocyte serum
ATS	-	anti-thymocyte serum
ECF	-	eosinophil chemotactic factor
GALT	-	gut associated lymphoid tissue
HL	-	mice with high antibody production
ICH	-	Institute of Child Health
Ig	-	immunoglobulin
IMS	-	immune mouse serum
i.p.	-	intraperitoneal
Ir genes	-	immune response genes
i.v.	-	intravenous
L <sub>3</sub>	-	third stage filariform larva
L <sub>4</sub>	-	fourth stage filariform larva
LL	-	mice with low antibody production
MLN	-	mesenteric lymph nodes
MLNC	-	mesenteric lymph node cells
NBL	-	newborn larvae
PBS	-	phosphate buffered saline
PEC	-	peritoneal exudate cells
PFC	-	plaque forming cells
PVP	-	polyvinyl pyrrolidone
sIgA	-	secretory IgA
Sp	-	spleen
SpC	-	spleen cells



SRBC - sheep red blood cells  
SRS-A - slow reacting substance of anaphylaxis  
TDLC - thoracic duct lymph cells

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CHAPTER 1

INTRODUCTION

Trichinellosis, as a disease of man, domestic and wild animals, has been, and still is to varying degrees, medically and economically important. The causative nematode parasite Trichinella spiralis (Owen, 1835) Railliet, 1896, is easily maintained in laboratory animals and therefore makes a useful model for parasitological and immunological studies.

Trichinellosis is commonly termed a disease, but the disease state is connected with the intake of high dosages of Trichinella larvae and although this is often seen in man, it is a rare phenomenon compared to the normal condition which is frequently overlooked. In animals the disease state is rarely seen, the observed cases being of domestic, zoo or experimental animals.

For a long time the T. spiralis life cycle was thought to be completely dependent upon predation, but it is now generally accepted that scavenging must also be involved, as the infective larvae are exceptionally tolerant to putrefaction, desiccation, adverse temperatures and salinity. These properties coupled with its cosmopolitan distribution in wild life, suggest that the parasite is of great antiquity.

In this thesis T. spiralis is viewed only as a laboratory model, thus this introduction will simply serve to describe the life cycle of the parasite, it will not encroach upon the vast expanse of epidemiological knowledge of the disease which has been extensively reviewed by Gould (1945, 1970).

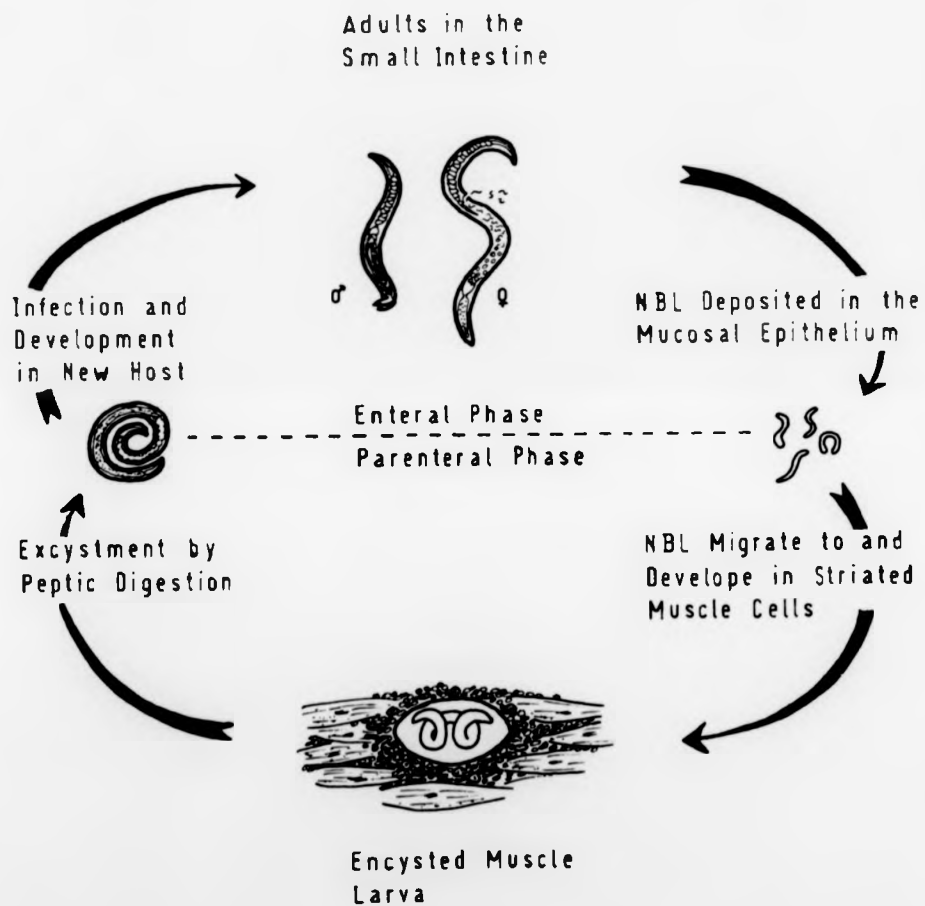
### Life Cycle

T. spiralis is unusual amongst parasitic nematodes in that all stages of its life cycle occur within a single host. The enteral phase of the infection includes 4 larval stages and the adult (Ali Khan, 1966; Kozek, 1970, 1971a). The parenteral phase comprises the migrating, newborn larvae (NBL) and the intracellular muscle larvae [see Figs. 1-3 and Plates 1-3].

The experimental work described in later chapters is concerned with the interrelationship between the host's immune response and the parasite and an understanding of the mechanisms of immunity which act against T. spiralis will be facilitated by a full description of the parasite's development and the niches it occupies within the host.

### Intestinal Phase

The swallowed muscle larvae are liberated from the infected muscle and cyst wall, by peptic digestion, and pass from the stomach to the small intestine, where they burrow into the mucosal epithelium. The worms lie embedded at the base of the villi and in the glandular crypts, completely surrounded by epithelial cells. They remain in this site until expulsion occurs (Gardiner, 1976). The infective larvae undergo four intestinal moults (Vilella, 1958; Ali Khan, 1966; Kozek, 1970, 1971a) before they become adult at 24-30 hours after infection (Vilella, 1958). The larval stages in the intestine are collectively termed pre-adults.

Fig.1 The Life Cycle of T.Spiralis

The ratio of male to female worms varies during the course of infection, but during the initial stages the ratio is approximately 1 male to 1-2 females.

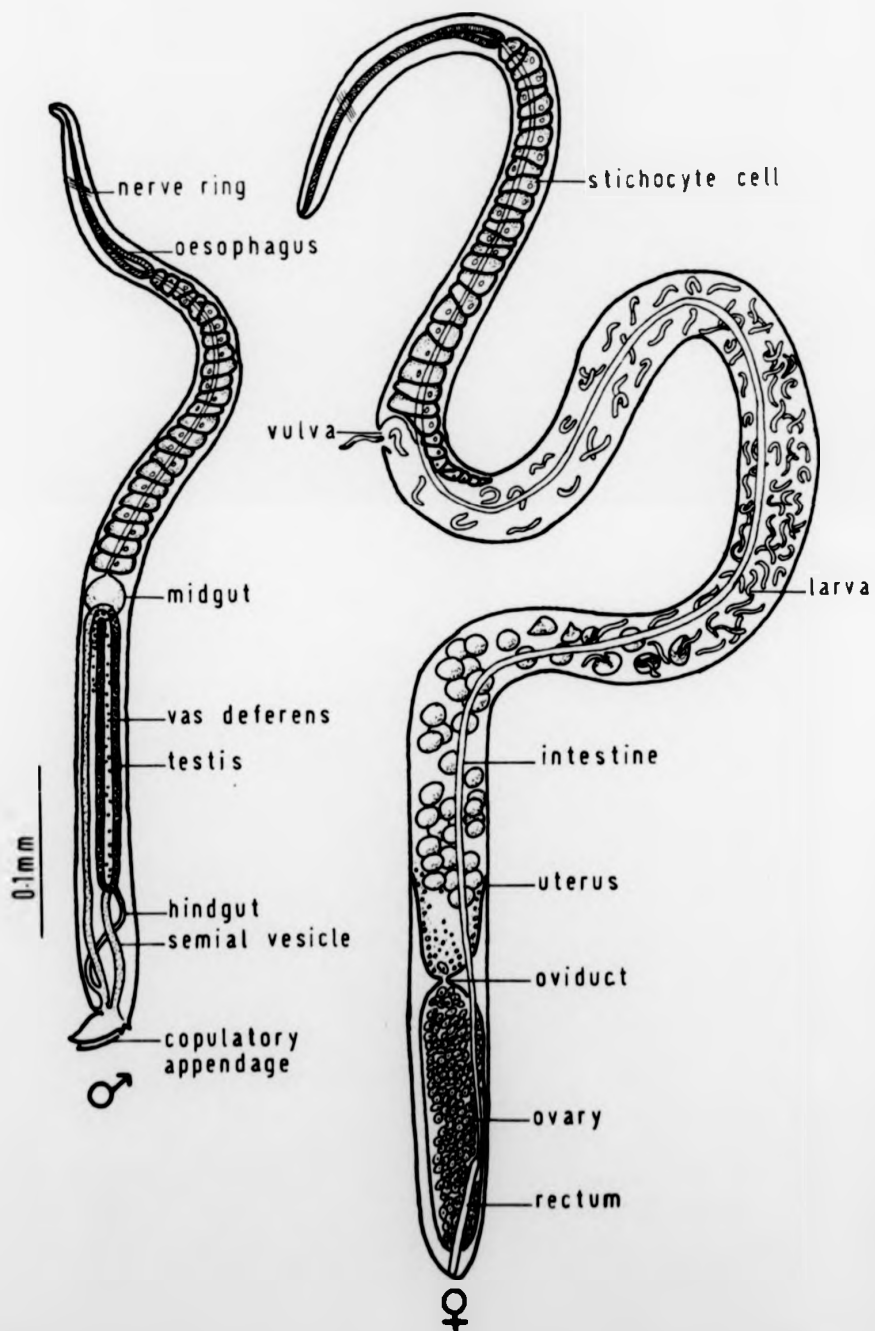
The insemination of female worms first occurs 30-32 hours after infection (Wu and Kingscote, 1957). Gardiner (1976) has shown that, not only will a male inseminate several females, but that insemination occurs throughout the course of infection, and is necessary for the continual output of NBL by the females.

The adults live in the zone of intestinal absorption, and molecules of nutritive materials penetrate through the worms cuticle into the body cavity (Timonov, 1974). The worms feed and grow until the females, at least, reach their maximum size at around 8-10 days after infection, whereafter the effects of the immune response cause a decrease in size (Semrad and Coors, 1951; Campbell, 1965; Kim, 1957; Denham, 1969).

The length of time the adults can abide in the intestine depends upon the development of the host's acquired immunity. In naive mice and rats the worms usually remain for between 7 and 18 days (Gursch, 1949; Larsh, Gilchrist and Greenberg, 1952; Larsh, Race and Jeffries, 1956; Denham, 1968; Wakelin and Lloyd, 1976a).

#### Newborn Larvae

T. spiralis is ovoviviparous; as the embryos pass anteriorly along the uterus they develop progressively inside their egg shells, becoming juveniles which hatch in utero before being born. The first NBL emerge from the vulva about 4-5 days after infection (Gould, Gomberg, Vilella and Hertz, 1957; Harley and Gallicchio, 1971a).

Fig. 2 ADULT T. SPIRALIS WORMS



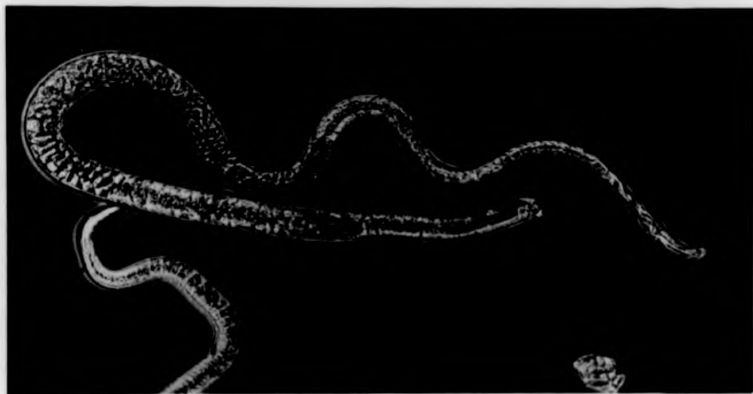


Plate 1. Female T. spiralis worm

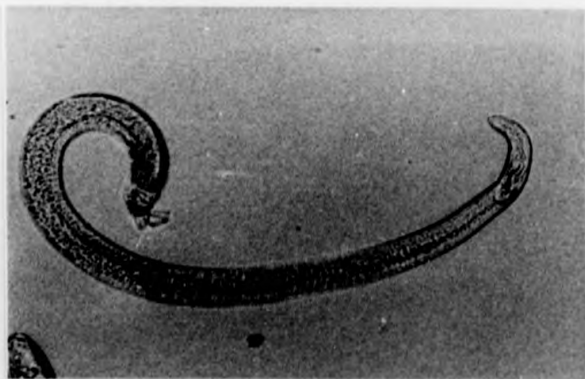


Plate 2. Male T. spiralis worm

The average rate of production of NBL from a female worm is one every half hour (cited by Gould, 1945) but this rate will fluctuate during the course of infection; maximum production being 6 days after infection (James, 1971) and declining thereafter. The number of larvae produced per female has been quoted as ranging from 91-625 (Thomas, 1965) to 1300-1500 (Roth, 1939).

In utero the NBL has a well formed oesophagus, a glycogen store and stichocyte precursor cells which, even at this early stage, contain distinct globules of secretory product (Bruce, 1974a).

At birth the larvae are 5-6  $\mu\text{m}$  in width and 95  $\mu\text{m}$  in length (Harley and Gallicchio, 1971b). They are cylindrical, have blunt ends, an anterior stoma through which a stomatostylet is actively protruded and retracted and they possess a complete alimentary canal (Harley and Gallicchio, 1971b).

During migration further development occurs, there is an increase in length, the larvae being on average 106.60  $\mu\text{m}$  long when they reach the skeletal muscle (Harley and Gallicchio, 1971c). The oesophagus develops further and Bruce (1974a) suggests that hypodermal gland cells have developed by the time the NBL start to invade the muscle.

Thus the NBL is a motile migratory stage, which possesses boring and secretory organs for migration to and penetration of voluntary muscle.

Gardiner (1976) has shown that NBL are deposited, by the female, in the epithelial layer of the mucosa. The larvae then migrate through the connective tissue until they reach a draining lymphatic or blood vessel. There are 3 possible routes of migration to the striated muscle:-

- (1) The larvae penetrate the lymph capillaries and are carried to

the arterial circulation via the thoracic duct.

(2) The larvae enter draining blood capillaries and are carried via the venous system to the heart and thence disseminated.

(3) There is continuous active burrowing through the body tissues.

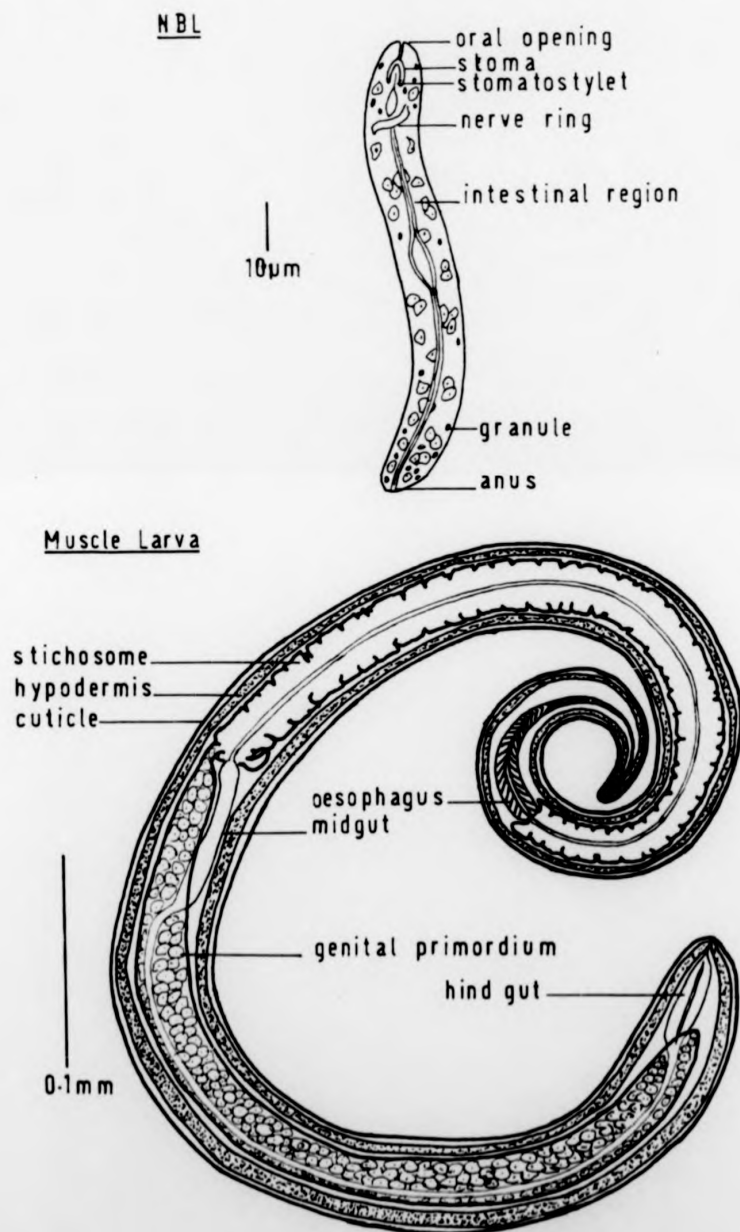
Harley and Gallicchio (1971a) have shown, by catheterizing the thoracic duct, that the majority of the NBL migrate through the lymph system to the arterial circulation.

Harley and Gallicchio (1971a) recovered the first migratory larvae 4 days after infection and found larvae in the diaphragm 5 days after infection, implying that the migration to and penetration of muscle cells takes less than 24 hours. These workers found 70% of the migratory NBL in the thoracic duct lymph, but very few in the arterial blood; because the NBL enter the blood from the thoracic duct lymph, it is likely that they leave the blood, for their preferred site, almost immediately.

#### Muscle Larvae

It is not known what factors cause the NBL to leave the blood stream and penetrate the striated muscles, but Ogielski (1949) suggested that the density of capillary networks around these muscles were attractive to the parasite. This being so, the high oxygen consumption associated with steadily working muscles such as the diaphragm, intercostals and extraocular muscles, would account for the high concentrations of worms found in these sites.

NBL frequently enter other tissue, such as heart muscle and brain, but normal development into infective muscle larvae will only occur in striated muscle and degenerating NBL may be pathogenic

Fig.3 NBL and Excysted Muscle Larva

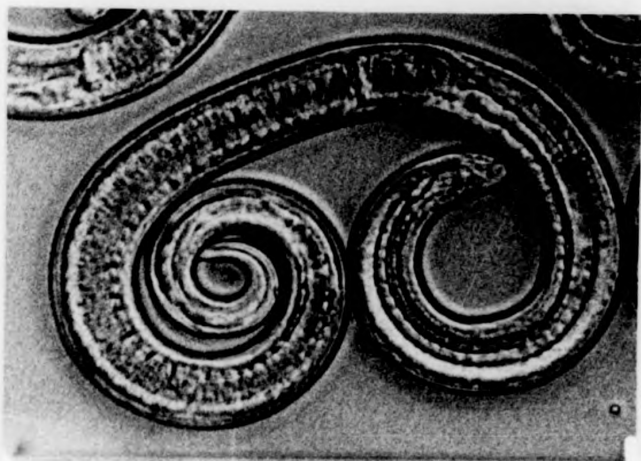


Plate 3. Excysted muscle larva.

in ectopic sites.

Once the larva is within a muscle cell, it induces a number of host cell changes, culminating in a new host unit termed the Nurse cell (Purkerson and Despommier, 1974; Despommier, 1975). Despommier (1975) observed no changes in the architecture of the muscle fibre for the first two days of intracellular infection. 3 days after the invasion of a muscle cell a space containing sarcoplasmic elements developed between the plasma membrane and myofilaments. By day 4 the myofilaments were in a state of partial disarray and the nuclei had enlarged, increased in number and migrated towards the central portion of cell cytoplasm. Sarcomeres were highly disorganised on day 5 and the sarcoplasmic reticulum had increased. On day 8 the 'T system' of transport tubules became distorted and enlarged. Sarcoplasmic reticulum had completely replaced the myofilaments by day 10 and, associated with a thickening of the glycocalyx, there was a hyperinvolution of the plasma membrane. A host derived double membrane, which completely surrounded the larva, developed and adjacent to this were concentrations of rough endoplasmic reticulum and mitochondria, but, by day 20 these had been displaced, towards the outer edge of the nurse cell, by sarcoplasmic reticulum. A well developed matrix of collagen surrounding the parasitised cell was evident by day 32. This can be considered as forming a 'protective' capsule.

The development of the nurse cell is paralleled by the growth of the larva, which occurs in 3 distinct phases (Despommier, Aron and Turgeon, 1975).

(1) An initial growth phase on day 1, when the larva is utilizing its stored glycogen.

- (2) A lag phase, of 3 days, when the nurse cell is not ready to administer to the needs of the larva.
- (3) An exponential growth phase of the worm from day 4-19. This parallels the growth of the nurse cell which is gathering nutrients for itself and the worm. The host's metabolites must be taken in across the nurse cell plasma membrane, traverse the nurse cell matrix, possibly through the t tubules, finally crossing the membrane at the nurse cell-parasite interface and entering the worm. Timonov (1974) suggested that nutrient uptake is across the cuticular surface of the muscle larva.

Most infections of muscle cells result in pathology and ultimate death of the cell. However, it can be seen that the larva of T. spiralis, in some unknown manner, triggers the cell to transform into a nurse cell which is capable of obtaining nutrients and excreting waste products for the parasite. Furthermore, this nurse cell-parasite unit remains alive for the life span of the host (Despommier, 1975).

The association of hypodermal gland cells and functioning stichocytes, with the periods of tissue invasion, feeding and major growth of T. spiralis, implies that both organs play a major role in the physiological relationship between the parasite and the host. The hypodermal gland cells, which are present in the pre-adult, adult worms and young muscle larvae, are totally absent in the mature muscle larvae (Bruce, 1974a). This absence coupled with the non-exportation of secretory globules from the stichocytes (Bruce, 1974b) suggests that the mature, infective muscle larva is a resting stage.

Thus encystment of the muscle larvae completes the life cycle

and the worms then lie dormant, in their nurse cells, to await ingestion by a new host.



CHAPTER 2

A COMPARISON OF THE ACQUIRED IMMUNITY ACTING  
AGAINST NIPPOSTRONGYLUS BRASILIENSIS AND TRICHINELLA SPIRALIS

I. THE INDUCTION AND THE EFFECTS OF IMMUNITY

By the use of experimental models, much progress has now been made in the understanding of acquired immunity to nematodes. This review will compare the existing knowledge of immunity against worms, gained from studies of Trichinella spiralis and Nippostrongylus brasiliensis in rats and mice.

1. Life cycles and infection

N. brasiliensis has a typical nematode life cycle; in the laboratory rats are infected percutaneously or subcutaneously with third stage (L<sub>3</sub>) filariform larvae which migrate to the lungs where they moult to become L<sub>4</sub>s; the second parasitic moult occurs after migration to the small intestine. From 120 hours post infection female worms produce increasing numbers of eggs. The majority of adult worms are expelled from the intestine 12 to 15 days after infection (Sarles and Taliaferro, 1936; Mulligan, Urquhart, Jennings and Neilson, 1965; Ogilvie, 1965a, b).

The life cycle of T. spiralis is unique, as larval migration through the body occurs after the intestinal adult stage and not before. Usually the adult nematode represents the long term phase of infection, and the

migratory larval stage is of comparatively short duration. However, in T. spiralis infections, the newborn larvae (NBL) released from the females in the intestine migrate to the skeletal muscles and develop into encysted muscle larvae which may remain alive for the duration of the host's life (Despommier, 1975). Experimental hosts are infected per os with excysted muscle larvae and the resulting adults will remain in the intestine for 12 to 18 days (Gursch, 1949; Larsh, Gilchrist and Greenberg, 1952; Larsh, Race and Jeffries, 1956; Denham, 1968; Wakelin and Lloyd, 1976a).

T. spiralis adults penetrate and lie embedded in the intestinal epithelium (Gardiner, 1976) whereas N. brasiliensis adults brace themselves against and between the villi, by their cuticular ridges, but tissue damage still occurs (Lee, 1969). The different life cycles and habitats, of these two nematodes, must be taken into consideration when the immunity they elicit is compared.

## 2. Challenge Infections

The sensitisation of rats and mice to a primary infection of N. brasiliensis induces an accelerated expulsion of the worms given in a challenge infection (Chandler, 1932, 1935; Haley, 1962; Ogilvie, 1969; Ogilvie and Jones, 1971, 1973; Love, 1975a). Similarly challenge infections of T. spiralis are eliminated more rapidly than primary infections in mice, rats and guinea-pigs (McCoy, 1931, 1940; Roth, 1939; Culberston, 1942; Larsh, Gilchrist and Greenberg, 1952; Denham, 1968; Love, Ogilvie and McClaren, 1976; Wakelin and Lloyd, 1976a).

3. The effect of immunity on the worms

T. spiralis and N. brasiliensis become stunted and have a reduced fecundity towards the end of both primary and challenge infection periods in the gut (Chandler, 1936; Rappaport and Wells, 1951; Semrad and Coors, 1951; Ogilvie and Hockley, 1968; Despommier and Wostmann, 1969; Denham and Martinez, 1970; Jacqueline, Vernes and Biguet, 1978; Kennedy, 1978; Kennedy, Wakelin and Wilson, 1979). Cytological damage to N. brasiliensis and T. spiralis caused by the immune response also occurs before expulsion (Ogilvie and Hockley, 1968; Lee, 1969; Love et al., 1976).

The damage to N. brasiliensis, caused by the onset of immunity in the host, is reversible if the worms are transplanted into clean hosts less than 10 days after a primary infection (these worms are termed 'normal'); but the damage to worms is permanent after this time period (these worms are termed 'damaged') (Ogilvie and Jones, 1971; Ogilvie and Love, 1974). The damage to T. spiralis is not permanent, as the adults can re-establish and renew larval production in clean hosts even when they have been expelled from the small intestine of the immune host (Kennedy et al., 1979).

N. brasiliensis worms of a challenge infection have the potential to adapt, to the immune status of the host, during their larval stages. The resulting adults (termed 'adapted worms') are stunted and damaged but they establish successfully upon transfer into clean hosts, their cytological damage is repaired rapidly and egg production commences. Adapted worms are less immunogenic than normal or damaged worms and

remain for longer time periods in the host (Ogilvie and Jones, 1971). There is no evidence to suggest that other nematodes adapt to the immune status of the host.

4. The parasitic stages responsible for eliciting protective immunity in the host

The contrived immunological control of nematode infections requires knowledge of the parasitic stages responsible for inducing acquired immunity in the host.

A. The parasitic stages

Irradiation and anthelmintic abbreviation of infections were used to study the immunogenicity of the migratory larvae of N. brasiliensis (Prochazca and Mulligan, 1965; Ogilvie and Jones, 1971) and the pre-adult intestinal stages of T. spiralis (Levin and Evans, 1942; Kim, 1957; Larsh, Race and Goulson, 1959; Campbell, Hartman and Cuckler, 1963; Campbell, 1965; Denham, 1966a). These experiments reported varying degrees of larval immunogenicity, but Ogilvie and Jones (1971) suggested that the larval stages of N. brasiliensis were of minor immunogenicity when compared to the adults and Denham (1966a) proposed that the pre-adult T. spiralis worms were only strongly immunogenic when present in abnormally large numbers or when remaining for long periods of time, as occurs with worms produced by irradiation. The transplantation of mature worms into the small intestine of clean hosts showed that the adults of N. brasiliensis (Ogilvie, 1964, 1965b) and T. spiralis infections (Denham, 1966b; Kennedy et al., 1979) were

the major source of protective intestinal immunity.

Sterile, female N. brasiliensis worms were as immunogenic as normal females (Jennings, Mulligan and Urquhart, 1963) but more than 100 male worms were needed to stimulate an immune response comparable to that produced by 10 females (Ogilvie, 1965b). In contrast, no difference was found in the degree of protective immunity induced by male or female T. spiralis worms (Anderson and Leonard, 1940).

The parenteral phase of a T. spiralis infection also induces strong protective immunity. Freeze-thaw killed newborn larvae (NBL) produced no noticeable immunity; but when viable NBL were injected (i.v.) into rats and allowed to develop into muscle larvae, they gave 95% protection against the parenteral stage, resulting from an oral challenge (Despommier, 1971). Similarly when diffusion chambers, containing muscle larvae, were implanted into the peritoneal cavities of mice, the level of immunity obtained, as assessed by the number of muscle larvae produced, was comparable to that induced by a primary complete infection (Despommier and Wotsman, 1968). However, the same immunising procedure gave no protection against a parenteral challenge (Despommier, 1971) and it was suggested that because NBL do not possess a stichosome, they fail to produce the functional antigens which induce immunity and thus, along with muscle larvae, escape the effects of acquired resistance. The conclusions from this work are contradicted by the strong protective immunity, developed to parenteral immunisation (by i.v. injected NBL), which acts against a parenteral challenge (of i.v. injected NBL) (James and Denham, 1975; Ruitenbergh and Steerenbergh, 1976; James, Moloney and Denham, 1977).

B. The stage specificity of the immune response to *T. spiralis*

The first suggestion of the immunogenicity of the parenteral phase of *T. spiralis* came from Oliver-Gonzalez (1941). His 'dual-antibody' hypothesis suggested that two main humoral responses occurred during infection with *T. spiralis*. An anti-adult worm antibody response was detected first, and this was followed by a peak of antibodies, specific to muscle larvae, which could be demonstrated in vitro. Support for this theory was given by Oliver-Gonzalez and Levine (1962) and Hendricks (1952) who found that the antibody titres produced to infections of sterilized adults were lower than those to full infections. However, Chute (1956) demonstrated that injections of killed muscle larvae were effective in producing immunity against adult worms, and suggested that the dual antibody hypothesis represented a quantitative rather than qualitative antigenic difference between the two stages.

James and Denham (1975) revitalized the 'dual antibody' hypothesis and showed that the immune response to *T. spiralis* was stage specific. They demonstrated that resistance to the intestinal phase was mainly restricted to that phase and had little or no effect upon a parenteral challenge. James et al. (1977) confirmed the immunogenicity of the parenteral phase and showed that the immunity stimulated by this phase did not affect the intestinal worms.

The protective immunity stimulated by *N. brasiliensis* was not stage specific, as larvae developing in rats, previously sensitised with adult worms alone, were affected during the final larval moult as well as during the adult stage (Ogilvie, 1965b).

C. Functional Antigens

Virtually all the components of nematodes have been reported to be antigenic to some extent, but as the contact of tissues with the host is restricted, when the parasites are alive, it is unlikely that these antigens act as immunogens. It is more probable that the antigens which evoke a strong immunity are the worm's secretory products.

The concept, of the protective antigens being released from the parasite, first came from the work of Sarles (1938), who demonstrated precipitates at the orifices of N. brasiliensis larvae in immune serum in vitro. Edwards, Burt and Ogilvie (1971) and Sanderson and Ogilvie (1971) suggested that the enzymes produced by adult N. brasiliensis were the important protective antigens. Four enzymes, aminopeptidase, non-specific esterase, acid phosphatase and acetylcholinesterase (AChE) were detected in the adults (Lee, 1970).

AChE was secreted from the anterior glands of adult and L<sub>4</sub> worms, but not from infective larvae (Lee, 1970) and only this enzyme was considered to be important as an immunogen as its production, by the worms, was closely linked to the immune response, being increased in the presence of antibodies and decreased in their absence (Sanderson, 1969; Lee, 1970; Sanderson and Ogilvie, 1971; Edwards et al., 1971; Jones and Ogilvie, 1972). AChE reduces peristalsis of the gut and aids the worm's maintenance in situ (Symons, 1966; Ogilvie and Jones, 1971). As worms became damaged, the increase in AChE levels was coupled with an alteration in the proportions of its isoenzymes (Edwards et al., 1971). AChE has three isoenzymes, A, B and C and antibodies associated with IgG<sub>1</sub> were detected to all three (Jones,

Edwards and Ogilvie, 1970). The antibody response had its strongest activity against the isoenzyme A, but antibodies were induced more readily by the B and C isoenzymes; no antibodies were found against the worms' non specific esterases, aminopeptidases or acid phosphatases (Ogilvie and Jones, 1971).

The functional antigens of T. spiralis are secreted from the multicellular stichosome (Despommier and Muller, 1970b), an organ which occupies the anterior third of the muscle larvae and adult worms and consists of large stichocyte cells, filled with granules which are secreted through the oesophagus (Despommier and Muller, 1970a). The granules are of 3 types; alpha, beta<sub>1</sub> and beta<sub>2</sub>; the smaller beta<sub>1</sub> granules are the important antigens; they were purified and were shown to be strongly immunogenic and to elicit a protective immune response (Despommier and Muller, 1969; Despommier and Muller, 1970a). Microgram quantities were capable of protecting mice and rats against all stages of a subsequent challenge. However, lower stimulating doses of the antigen served only to reduce female fecundity (Despommier, Campbell and Blair, 1977). This work illustrates the degree of control, on the immune response, available when purified antigenic materials are used.

##### 5. The thymus dependency of the immune response

The majority of antigens are thymus dependent, in that they require the presence of thymus derived cells to induce an immune response. The absence of a functional thymus and thymus derived cells allowed a greatly prolonged life span of adult T. spiralis worms in nude mice (Ruitenbergh, 1974; Ruitenbergh, Elgeroma, Kruizinga and Leenstra, 1977a). Anti-thymocyte serum (ATS) prevented rats and mice



from developing protective immunity to T. spiralis (Kozar, Karmanska, Kotz and Seniuta, 1971; Larsh, Weatherly, Goulson and Chaffee, 1972; Mackinicka, 1972; Walls, Carter, Leuchars and Davies, 1973). The increase in the number of plasma cells in the intestinal mucosa of infected animals was thymus dependent (Ruitenber, Leenstra and Elgersma, 1977b) as was the presence of intra-epithelial lymphocytes (Parrott and de Sousa, 1974; Guy-Grand, Griscelli and Vassalli, 1974), the degranulation of intestinal mast cells coupled with IgE production (Kelly, 1972; Ruitenber, Teppema, Kruizinga, Elgersma and Steerenberg, 1975; Ruitenber and Elgersma, 1976; Ruitenber et al., 1977b) and the blood and tissue eosinophil response (Basten and Beeson, 1970; Walls et al., 1973).

However, some degree of antibody formation, to infection with T. spiralis, has been found in thymectomised and irradiated CBA mice. It was suggested that these immunoglobulins acted as blocking antibodies and that their production was stimulated by thymus independent antigens (Ljungström and Ruitenber, 1976). No antibody response to T. spiralis was noted in nude mice (Ruitenber et al., 1977a). This disparity could be due to the incomplete destruction of the thymus and thymus derived cells of the CBA mice or their possible genetically determined ability to respond to T-independent antigens.

The development of protective immunity to N. brasiliensis is also totally thymus dependent (Ogilvie and Jones, 1967; 1971; 1973; Kelly, 1972; Ferguson and Jarrett, 1975).

II. THE COMPONENTS INVOLVED IN THE IMMUNE EXPULSION OF T. SPIRALIS  
AND N. BRASILIENSIS FROM THE INTESTINE OF THE HOST

1. Factors involved in the immunological response

T. spiralis and N. brasiliensis infections of the gut induce antibody formation, a cell mediated response and an inflammatory reaction. The infiltration of plasma cells, mononuclear cells, eosinophils and mast cells/basophils into the intestine have all been described (Taliafferro and Sarles, 1939; Larsh and Race, 1954; Ogilvie and Jones, 1973).

Two main theories have been proposed to describe the mechanism of immunological expulsion of T. spiralis and N. brasiliensis from the gut. Briefly, Larsh and his co-workers (reviewed by Larsh and Race, 1975; Larsh and Weatherby, 1975) proposed that expulsion of these worms from the host was due directly to the inflammatory changes brought about by antigen sensitised lymphocytes and involved no humoral response. Ogilvie and Love (1974) suggested that immune expulsion was a complex mechanism requiring the sequential action of antibodies and sensitised lymphocytes. Antibodies caused cytological damage to the intestinal and genital cells of the worms (Ogilvie and Hockley, 1968; Lee, 1969; Love, Ogilvie and McClaren, 1976), while sensitised lymphocytes, with the possible involvement of inflammatory cells, effected their expulsion from the host (Ogilvie and Love, 1974).

Present day knowledge suggests that the immune response against these worms may not be as stereotyped as suggested by these theories (discussed later).

## 2. The humoral response

### A. Systemic antibodies

The majority of work on the humoral response to T. spiralis and N. brasiliensis has concentrated on the systemic immunoglobulins, yet it is difficult to correlate protection with circulating antibody levels. Following a primary infection, systemic antibodies, specific to the worms, are generally detected after N. brasiliensis adults have been expelled (Block, Cygan and Waltin, 1973; Poulain, Laffau and Perry, 1976; Sinski and Holmes, 1977) and during the latter stages of expulsion of T. spiralis (Crandall and Crandall, 1972; Ottesen, Smith and Kirkpatrick, 1975). Nevertheless, the serum levels of IgG<sub>1</sub>, IgG<sub>2</sub>, IgM, IgE and IgA were all elevated after infection with either worm (Crandall, Cerbra and Crandall, 1967; Kagan, Maddison and Norman, 1968; Jones et al., 1970; Crandall and Crandall, 1972; Perrudet-Badoux, Binaghi and Bouesac-Aron, 1976; Jarrett and Bazin, 1977; Sinski and Holmes, 1977).

Remarkably high levels of circulating IgE are produced in response to T. spiralis and N. brasiliensis infection (Catty, 1969; Keller, 1969; Ogilvie and Jones, 1971; Jarrett and Bazin, 1977) but again the elimination of these worms can occur in its absence (Jones et al., 1970; Rivera-Ortiz and Nussenzweig, 1976).

### B. Intestinal antibodies

Secretory IgA (sIgA) is the major immunoglobulin component of intestinal secretions (Lamm, 1976). Dimeric, 11s, IgA is produced

locally by plasma cells in the lamina propria of the gut, it passes across the basement membrane into the interepithelial cell space, where it picks up "secretory piece", a membrane receptor on epithelial cells, which allows its secretion into the lumen of the gut (Brandtzaeg and Baklien, 1977). As little dimeric IgA is found in blood, this glandular transport constitutes a very effective specific clearing mechanism (Heremans, 1974). IgM can also be secreted in the same way, but secretory IgM is not as stable as sIgA and it has a shorter functional survival time in gastrointestinal secretions (Haneberg, 1974a; b). Monomeric 7s IgA is mainly found in the blood and has little bearing on intestinal infections; however it can be found in the gut and its presence there occurs not by secretion but by leakage. Inflammatory responses in the gut will allow leakage of small amounts of monomeric IgA and IgG, into the lumen i.e. pathologic potentiation (Lamm, 1976).

It might therefore be assumed that sIgA is in the correct place and concentration to act against nematode worms, with a possible minor role being played by IgM and IgG and yet specific antiworm IgA has rarely been identified (Cypess, Ebersole, Molinari, 1977; Sinski and Holmes, 1977). However, it is only recently that mucosal antibodies have been considered important in the protective immunity against nematodes and as it is difficult to analyse intestinal immunoglobulins, most work has concentrated on serum antibodies.

Crandall and Kozek (1972) showed, by radial immunodiffusion, that the IgG<sub>1</sub>, in the intestinal contents, remained at a low level during infection with *T. spiralis*, but that the levels of IgA increased. These observations suggest an active production and secretion of sIgA against the parasite, and the leakage of systemic IgG<sub>1</sub>. Similarly,

Sinski and Holmes (1977) demonstrated a tenfold increase in specific antiworm sIgA during the first 12 days of a N. brasiliensis infection; serum IgG increased gradually and, unlike sIgA, had no temporal relationship with the expulsion of worms.

60-80% of the immunoglobulin containing cells in the intestine of rats, infected with T. spiralis, were found to be of the IgA type (Schäfers, 1975). Moreover, B lymphocytes, which were characteristic of IgA producing cells, were collected from immune rats and transferred into naive syngeneic recipients and found to accelerate the expulsion of T. spiralis from the gut (Despommier, McGregor, Crum and Carter, 1977).

Thus, this recently acquired knowledge suggests that sIgA plays a role in the effector mechanism of worm expulsion.

#### C. Passively transferred antibodies

If the major part of humoral immunity is mediated by locally produced antibody then it is not surprising that there is conflicting evidence about the protective properties of passively transferred serum and that the effects of this antiserum are usually weak.

Several workers have obtained varying degrees of protection with serum transfers against T. spiralis in rats and mice (Culbertson and Kaplan, 1938; Oliver-Gonzales, 1941; Culbertson, 1942; Hendricks, 1953; Love, Ogilvie and McLaren, 1976) but other antiserum transfers have been unsuccessful (McCoy and Bond, 1941; Larsh et al., 1964; Larsh, 1967; Denham, 1969; Larsh, Goulson, Weatherly and Chaffee, 1970; Crum, Despommier and McGregor, 1977).

Similarly, immune serum varied in its ability to passively protect rats against N. brasiliensis (Chandler, 1937; Neilson, 1965; Ogilvie and Jones, 1968; Jones et al., 1970). Large amounts of serum were required to transfer marginal protection (Sarles and Taliaferro, 1936; Chandler, 1938; Ogilvie and Jones, 1968; Jarrett and Urquhart, 1971). This marginal passive protection may occur by the secretion of IgM and the leakage of small amounts of monomeric IgA and protective IgG<sub>1</sub> antibodies into the lumen of the gut.

Neonatal mice could be passively protected against T. spiralis by antibodies received in the mother's milk (Duckett et al., 1972, Perry, 1974). Similarly, young rats suckling immune mothers were resistant to a challenge of N. brasiliensis larvae (Jones and Ogilvie, 1967). The milk, from sensitised lactating mothers, has a high concentration of protective antibodies (mainly sIgA) and these might affect the worms before they can establish in the gut, which would account for antibody mediated expulsion without cellular cooperation. IgG from milk is rapidly absorbed through the wall of the gut (Lamm, 1976) so this antibody might affect the migrating larvae of N. brasiliensis.

D. Antibody, an essential component of the expulsion process?

Although the involvement of the humoral response in the expulsion of worms has been well documented, doubts about its essentiality to the process have arisen with the demonstration that a normal expulsion pattern of N. brasiliensis occurred in mice which were treated with anti- $\mu$  serum and were incapable of mounting a significant systemic antibody response (Jacobson, Reed and Manning, 1977). Mice, under

anti- $\mu$  suppression lack IgA producing cells in their intestine (Lawton, Asofsky, Hylton and Cooper, 1972).

Therefore it can only be concluded that immunoglobulins are not essential factors in the process which results in damage to worms. It is probable that the well organized and finely balanced immune response, against worms, responds to the lack of immunoglobulin and increases the production of other factors which may normally act with sIgA in mediating the damage observed in worms. Such factors could be prostaglandins, histamine or serotonin, these have been related to the expulsion of N. brasiliensis (but especially Trichostrongylus colubriformis) (Dineen, Kelly, Goodrich and Smith, 1974; Rothwell, Love and Evans, 1978) and it was suggested that they act by making the environment unfavourable to the worms.

Adult N. brasiliensis which were cultured in normal serum became damaged (Love et al., 1975) in a similar manner to that produced by the immune response in vivo (Ogilvie and Love, 1974). These in vitro damaged worms, when transplanted, showed the same expulsion pattern as in vivo damaged worms (Love et al., 1975). This also suggests that an unfavourable environment will damage worms, and may support the theory that antibody acts, with anaphylactic factors, in the first stage of the expulsion process.

3. Adoptive transfers and the effector stage of the expulsion of worms

After intestinal stimulation the lymphocyte traffic is from the gut associated lymphoid tissue (G.A.L.T.) through the thoracic duct lymph to the lamina propria (Rose, Parrott and Bruce, 1976). These sensitised lymphocytes injected intravenously will home to the mucosal

layer of the small intestine in recipients. This pattern of homing has been shown to be enhanced during infection with intestinal nematodes (Dineen, Wagland and Ronai, 1968; Rothwell and Dineen, 1973; Rose, Parrott and Bruce, 1976; Despommier et al., 1977; Love and Ogilvie, 1977).

It would therefore appear, that the most likely places to find lymphocytes sensitised to intestinal worms would be in the G.A.L.T. and thoracic duct lymph.

Many attempts at adoptively protecting rats and mice against N. brasiliensis and T. spiralis have been made. In all cases syngeneic donor and recipient hosts were used. The majority of adoptive transfer experiments with N. brasiliensis utilized the technique of transplanting damaged worms (taken from rats at least 10 days after a primary infection) into the small intestine of recipients which had received cells taken from immune donors i.e. sensitised cells. Thus the necessity of the first step, in immune expulsion, was by-passed in the recipients.

The operation of factors which mediate cytological damage to worms plus an effector lymphocyte function seem to be necessary for worm expulsion to occur. Recipients of sensitised mesenteric lymph node cells (MLNC) expelled damaged N. brasiliensis worms more rapidly than normal worms (Dineen, Ogilvie and Kelly, 1973a). A normal pattern of damage to N. brasiliensis occurred in neonatal and lactating rats, but there was no expulsion of worms because of a failing in the effector lymphocyte function of these hosts (Ogilvie and Hockley, 1968; Jones and Ogilvie, 1971; Kelly and Ogilvie, 1973). Heavily irradiated rats could not be passively protected by antiserum and they could not expel damaged worms (Jones and Ogilvie, 1971) unless they



were given MNC (Keller and Keist, 1972). The cells responsible for transferring protection against N. brasiliensis and T. spiralis appeared in the thoracic duct lymph and mesenteric lymph nodes, of donor rats and mice (respectively), before damage in the primary worm burden of these hosts was evident (Wakelin and Wilson, 1977a; Ogilvie, Love, Jarra and Brown, 1977). This suggests that the effector cells cannot cause expulsion until damage to the worms has occurred.

T. spiralis worms, given in a challenge infection, are damaged and expelled very rapidly from rats (Love et al., 1976) and some strains of mouse (Wakelin and Lloyd, 1976a). However, several days elapse before sensitised cells, transferred to competent recipients, can effect normal T. spiralis worms (Wakelin and Wilson, 1977a). These authors suggested that the delay in expulsion was not due to the time taken to damage T. spiralis worms but rather that the transferred cells had to cooperate with factors of the hosts immune response. It is possible that antibodies are produced by the transferred B cells and that the transferred effector T cells promote the infiltration, to the gut, of the host's non-specific cells and the ultimate release of amines.

Non specific cells are probably involved in causing damage to worms, but they may also be necessary for the expulsion phase of immunity. Several experiments have suggested that sensitised cells need the cooperation of a myeloid derived component to effect the elimination of damaged N. brasiliensis and normal T. spiralis worms.

The transfer of sensitised MNC, to sublethally irradiated rats, resulted in the expulsion of damaged N. brasiliensis (Dineen, Kelly, and Love, 1973b; Kelly, Dineen and Love, 1973 ) but immune regeneration in these hosts had occurred before expulsion, thus giving them the

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potential to cooperate in the response (Kelly et al., 1973 ).

Similarly sublethally irradiated rats, given sensitised thoracic duct lymph cells (TDLC), could expel normal T. spiralis, but expulsion occurred 2 days later than in normal rats given sensitised TDLC (Despommier, McGregor, Crum and Carter, 1977) suggesting that some degree of immune regeneration of the host was necessary to achieve damage to worms and/or expulsion.

The expulsion of normal T. spiralis worms from sublethally irradiated mice required both sensitised MLNC and differentiated bone marrow cells (Wakelin and Wilson, 1977b). This disparity between rats and mice, as hosts of T. spiralis, may be due to differences in the susceptibility of their immune components to irradiation.

Lethally irradiated rats, given sensitised MLNC, could not expel damaged N. brasiliensis worms (Dineen and Kelly, 1973a; Kelly et al., 1973 ) unless they were also given bone marrow cells (Dineen and Kelly, 1973a).

However, Ogilvie et al. (1977) demonstrated that the expulsion of damaged worms from lethally irradiated rats could occur within 5 days, after the transfer of sensitised MLNC or TDLC alone. The removal of cells bearing immunoglobulin did not alter the protective capacity of the TDLC. Thus inferring that this rapid expulsion, of damaged worms, was brought about by the action of T lymphocytes without the involvement of bone marrow derived cells. Ogilvie et al. (1977) also found that MLNC, but not TDLC, varied greatly in their ability to cause worm expulsion from irradiated rats and suggested that this was the cause of the discrepancy between their work and that of Kelly et al. (1973 ) and Dineen and Kelly (1973a). It is also possible that lethal irradiation caused more damage to the intestinal epithelium of the rats used

by Ogilvie et al. (1977) thus making their worms more susceptible to adoptive immunity.

Despommier et al. (1977) separated the TDLC of immune rats into T and B cell populations; they found that transferred B cells caused expulsion of normal T. spiralis, from sublethally irradiated recipients, more rapidly than T cells. The B cells were characteristic of IgA plasma cells and it was suggested that resistance to T. spiralis was mediated locally by IgA forming cells.

The idea of B cell mediated expulsion of T. spiralis in rats (Despommier et al., 1977) and T cell mediated expulsion of N. brasiliensis in rats (Ogilvie et al., 1977) are not as contradictory as they seem. Consider, only damaged N. brasiliensis worms were employed and thus expulsion did not require the action of B cells or non-specific cells in mediating damage. The transfer of B cells alone to sublethally irradiated recipients, before infection with T. spiralis, resulted in worm expulsion because as worm damage was occurring, the T cell population was probably regenerating and was thus available to cause worm elimination when required. The transferred T cells took longer to induce expulsion possibly because their effector action was delayed until B cell and non-specific cell regeneration had occurred.

In contrast to the T. spiralis/rat system, Wakelin and Wilson (1978) suggested that sensitised T cells were more effective than B cells in causing the expulsion of T. spiralis from sublethally irradiated mice. It is possible that non-specific cells are more efficient at mediating damage to worms in mice than they are in rats, and this may be supported by the expulsion of N. brasiliensis from mice which were depleted of antibodies (Jacobson et al., 1977).

It has been suggested that both sIgA and factors released from

non-specific cells are capable of mediating damage to worms. However the IgA response in gut associated lymphoid tissue has little or no immunological memory (Lamm, 1976). Therefore the first phase in the rapid expulsion of worms from some challenge infections may be brought about by non-specific cells without the involvement of antibody.

It would seem that the mechanism of immunological expulsion of worms may well encompass both the theory put forward by Larsh and Race (1975), and that proposed by Ogilvie and Love (1974) (pp. 36 ). Furthermore, the involvement and cooperation of the components of immunity will be dependent upon the experimental conditions employed.

#### 4. Inflammation - the third component?

Sensitised effector T lymphocytes release a variety of lymphokines which can activate additional cell populations to react to antigen in a non-specific manner. These lymphokines include macrophage inhibition and activation factors; blastogenic factors which cause lymphocyte transformation and chemotactins which attract macrophages, neutrophils, eosinophils and other lymphocytes. It is the attraction of these cell populations which causes the inflammatory response seen in infected tissues. Antibody activity can also result in inflammation, as the activation of complement produces anaphylatoxins and opsonins.

Inflammatory changes in the intestine of mice infected with T. spiralis began, on about the 4th day of infection, with an infiltration of T lymphoblasts into the lamina propria (Rose, Parrott and Bruce, 1976). This was superceded by an influx of lymphocytes, large mononuclear cells, plasma cells, eosinophils, neutrophils and mast

cells (Larsh and Race, 1954; Larsh, Race and Yarinsky, 1962; Race, Larsh, Martin and Pate, 1972). Similar inflammatory changes have been reported in rats infected with N. brasiliensis (Taliaferro, and Sarles, 1939).

The association of inflammatory cells with infection and the necessity for the involvement of non-lymphoid components in restoring the expulsion capacity of certain heavily irradiated hosts, infected with N. brasiliensis and T. spiralis (Dineen and Kelly, 1973a; Wakelin and Wilson, 1977b) suggests the involvement of myeloid cells in the immune expulsion of these worms. Two such cell populations, the mast cells and eosinophils have been extensively studied with regard to nematode infections.

#### A. Mast cells

The plasma membranes of mast cells contain non-specific receptors for the Fc portion of IgE and IgG<sub>1</sub>. The antigens attach to the specific IgE, carried on the mast cells, and this ultimately results in the release of amines from the cells. The main amines are histamine, serotonin and slow reacting substance of anaphylaxis (SRS-A).

Urquhart et al. (1965) and Barth et al. (1966) proposed a 'Leak-Lesion' hypothesis, in which they suggested that the increased mucosal permeability caused by the vasoactive amines released from mast cells, altered the environment to the detriment of N. brasiliensis and also allowed antibodies to reach the worms by leakage i.e. pathotopic potentiation. It has been suggested that amines play a major role in the expulsion of T. colubriformis from guinea pigs (Rothwell, Dineen and Love, 1971; Rothwell, Love and Evans, 1978) and Ogilvie and Jones

(1971) suggested that amines could act directly on N. brasiliensis adults. However, there is evidence which suggests that mast cells are not requisite for the expulsion of N. brasiliensis. In neonatal and lactating rats damage to worms occurred independently of mast cells (Ogilvie and Hockley, 1968; Keller, 1971; Kelly and Ogilvie, 1973). Expulsion, itself, could also occur without the aid of mast cells as irradiated rats, reconstituted with sensitised MLNC, lost their worms before mast cell regeneration occurred (Keller and Keist, 1972).

The fact that damage to worms can occur in the absence of either mast cells or antibodies could lend support to the suggestion that both are involved in the first stage of worm expulsion (see pp. 41 ).

#### B. Eosinophils

Increased levels of circulating and tissue eosinophils are characteristic of nematode infections. However knowledge of the role they play in the immune response is limited. Eosinophils are closely associated with mast cells, being invariably found at the site of mast cell degranulation and histamine may exert a degree of chemotaxis on them (Archer, 1963; 1965; Fernex, 1968). After contact with antigen, lymphocytes generate a factor which is chemotactic for eosinophils (E.C.F.) (Cohen and Ward, 1971). Antibody-antigen complexes are also chemotactic for eosinophils which phagocytose these particles on contact (Archer and Bosworth, 1961; Sabesin, 1963; Litt, 1964). Immune adherence to a target can occur as eosinophils have receptors for the complement components C5a and C567 (Hobarth and McConnel, 1975); they also possess receptors for the Fc portion of IgG and in the presence

of specific antiserum, in vitro, they attach to nematodes and release enzymes (McLaren, Mackenzie and Ramalho-Pinto, 1977).

Eosinophils can release peroxidases, phospholipases and prostaglandins (Larsh et al., 1974b; Hubscher, 1975; McLaren et al., 1977) and like amines, these enzymes have been thought to damage worms by directly affecting them or by altering the intestinal environment. When synthetic prostaglandin E was injected into the lumen of the intestine, it caused the expulsion of N. brasiliensis from rats (Dineen, Kelly, Goodrich and Smith, 1974) and potent inhibitors of prostaglandins prevented the expulsion of N. brasiliensis (Kelly, Dineen, Goodrich and Smith, 1974). Peroxidase was released from myeloid derived cells located in the lamina propria, its activity was increased during T. spiralis infection and in vitro it was found to kill adults but not muscle larvae (Castro, Roy and Schanbacher, 1975).

However, Grove, Mahmoud and Warren (1977) have suggested that the depletion of eosinophils has no effect upon the ability of the host to expel adult T. spiralis.

Macrophages are an essential component in the induction of the immune response against worms, but Keller and Keist (1972) indicated that activated macrophages were not required at the effector stage of worm expulsion.

Rats depleted of complement showed a normal expulsion pattern of N. brasiliensis (Jones and Ogilvie, 1971) but in normal animals complement may be involved in the induction of inflammation and immune adherence.

#### C. Non-specific cross immunity

Inflammatory changes, although triggered specifically, will act



non-specifically on worms. To illustrate this, Bruce and Wakelin (1977) used concurrent infections of T. spiralis and Trichuris muris, which inhabit different niches in the host's intestine, and stimulate a similar type of immune response. No cross immunity was demonstrated between these parasites and interspecific competition did not occur. It was found that the inflammatory response acting against T. spiralis in the small intestine resulted in an early expulsion of T. muris from the large intestine. When the timing of the infections was changed so that the inflammatory response was initiated by T. muris, it had no effect on the T. spiralis. This non-reciprocal effect suggests that T. muris were affected by the amine and enzyme products of inflammation being carried down the intestine by peristalsis.

These results support the theory of Larsh and Weatherly (1975) because non-specific inflammation, alone, caused the early expulsion of T. muris. However, T. muris does not mature till the fourth week of infection (Wakelin, 1967), so it is possible that expulsion occurred because the worms were not fully established in the host. T. spiralis establishes very quickly and so could withstand the initial effects of inflammation.

It is still not clear if inflammatory cells are crucial components of the effector stage of worm expulsion. A normal pattern of worm expulsion occurs in the absence of the individual non-specific cells involved in inflammation, but this could be due to compensatory effects by the other components in the immune response.

III. THE IMMUNE RESPONSE WHICH ACTS AGAINST THE LARVAL STAGES OF  
T. SPIRALIS AND N. BRASILIENSIS

A wealth of information concerning the type and mechanism of immunity directed against the adults of N. brasiliensis and T. spiralis infections now exists; but surprisingly little is known about the immunity which acts against the larvae of these worms.

The larvae of N. brasiliensis are of minor immunogenicity when compared to the adults (Ogilvie, 1964 ; 1965a; Wilson, 1967) and no stage specificity of the immune response is evident (Ogilvie, 1965a). There does, however, appear to be a difference between the type of immunity which acts against developing larvae and that which acts against adult worms (Love, 1975b).

The combined transfer of sensitised cells and immune serum, to naive rats, before a larval infection of N. brasiliensis, caused a decrease in the intestinal worm burden, which could occur as early as 48 hours after challenge (Ogilvie and Love, 1974). However, the effects of this adoptive and passive immunity did not become evident until the larvae had migrated to the intestine (Ogilvie and Love, 1974). Whereas the immunity which developed after a full infection, affected the migrating larvae as well as the L<sub>4</sub> and adults of a challenge infection (Ogilvie, 1965b; Lee, 1976). The developing L<sub>4</sub> larvae were more susceptible to the combined action of cells and anti-serum than were adult worms, suggesting a quantitative difference between the requirements for expulsion of larvae and adult worms (Love, 1975b).

The humoral response is intact and functional in neonatal rats (Ogilvie and Hockley, 1968), and they damage worms in the normal

manner, but there is a deficiency in their effector cell mechanism which prevents the expulsion of N. brasiliensis (Keller and Keist, 1972; Dineen and Kelly, 1973b; Ogilvie and Love, 1974). This deficiency was not repaired in neonatal rats which were given sensitised MLNC and BM from mature donors (Love and Ogilvie, 1975).

When neonatal rats acquired antibodies passively, before a larval infection, a reduction in the worm burden resulted (Jones and Ogilvie, 1967; Greenberg, 1971), but when antibodies were given after the worms reach maturity there was no expulsion (Ogilvie and Hockley, 1968; Jarrett and Urquhart, 1971). Similarly sensitised MLNC from adult donors given to neonates, prior to larval infection caused worm expulsion (Dineen and Kelly, 1973b; 1974). Thus although antibodies cannot cause the expulsion of adult worms without the cooperation of T cells, it is possible that they can effect migrating larvae with the help of non-specific cells only. It is postulated that unseparated cell transfers to neonates are successful because antibody is produced.

It has been shown that a strong stage specific immunity can be induced by the parenteral phase of T. spiralis (James and Denham, 1975; Ruitenbergh and Steerenbergh, 1976; James et al., 1977), but apart from Despommier et al. (1977) demonstrating that TDLC sensitised to pre-adults only, will not confer protection against a challenge with NBL, no passive or adoptive transfers have been made to unravel the components which are involved or to assess at which stage they effect protection.

When NBL are released from adults, they migrate to skeletal muscles, via the lymph and blood, and would seem vulnerable to attack in this environment, especially as they do not possess a thick and resistant cuticle, like the other stages (Villèle, 1970).

McLaren et al. (1977) have shown that antibody mediated attachment of eosinophils in vitro occurs to all stages of T. spiralis and N. brasiliensis worms. The in vitro studies of Kazura and Grove (1978) showed that only the NBL of T. spiralis were susceptible to attack and destruction by eosinophils. This process involved specific opsonic antibodies and could occur in the absence of complement. Depletion of eosinophils, from the peritoneal cell populations, prevented the killing of NBL as did the preabsorption of immune sera with NBL.

Mackenzie, Preston and Ogilvie (1978) showed that the surface of the infective larvae and adults of T. spiralis and N. brasiliensis activated complement, but that the surface of NBL did not. Rats, infected with these worms, produced antibodies which were specific to the surface of each stage of the life cycle. These antibodies mediated the attachment of eosinophil enriched peritoneal exudate cells to the worms in vitro and attachment was enhanced by complement. Over 90% of the NBL were killed after incubation in fresh immune serum and eosinophil enriched peritoneal exudate cells taken from normal or infected rats.

In vivo studies have also suggested that eosinophils contribute to the immunity against the parenteral phase of T. spiralis. Mice which were depleted of eosinophils by monospecific anti eosinophil serum, showed a similar pattern of adult worm expulsion when compared to controls but had twice the burden of muscle larvae (Grove, Mahmoud and Warren, 1977). It was suggested that treatment with anti eosinophil serum had no effect on adult worms but facilitated the migration of NBL or their intramuscular development. However, no studies on the fecundity of adults were made and their expulsion was not monitored

adequately.

Perrudet-Badoux, Binaghi and Boussac-Aron (1978) studied a parenteral infection of T. spiralis in mice genetically selected for high and low antibody production. After a single full infection the mice with low antibody production (LL) had twice the burden of muscle larvae when compared to the high antibody producers (HL). Following a second, full infection, the LL mice were fully resistant to the parenteral phase whereas the HL mice were not. The authors suggested that the HL mice produced antibody early enough to affect the parenteral phase of a primary infection, whereas in a secondary infection both HL and LL mice had antibodies present and here the immunity was dependent upon the catabolic rate, of their macrophages or eosinophils, which was higher in LL mice. They concluded that immunity against the parenteral phase of T. spiralis was mediated by antibody and non-specific cells. It is difficult to accept this conclusion as the authors did not monitor the effects of immunity directed against the adults; there were no controls for the challenge infection and the experiment was not repeated. Moreover the strong immunity normally elicited against parenteral challenge (Despommier, 1971; James and Denham, 1975; Ruitenbergh and Steerenberg, 1976; James et al., 1977) was not evident in their HL mice, even though intestinal immunity should have enhanced the protection.

It has been assumed that the parenteral phase of T. spiralis is responsible for the elevated levels of circulating eosinophils developed in infected animals (Gould, 1970). However, Despommier, Weisbroth and Fass (1974) showed that parenteral infections alone did not produce increased levels of eosinophils; whereas exposure to terminated adult worm infections resulted in circulating eosinophil levels, which were comparable to those induced by a full infection.

Soon after NBL penetrate muscle cells, changes are induced which result in the formation of a nurse cell surrounded by a protective collagen capsule (Purkerson and Despommier, 1974; Despommier, 1975). While this nurse cell and capsule are being formed, inflammatory changes take place in the muscle (Gould, 1970) yet this response does not seem to adversely affect the developing muscle larvae (Ruitenbergh, 1974).

Gould (1970) showed that inflammation involved the invasion of interstitial connective tissues by polymorphonuclear cells, lymphocytes, tissue histiocytes and eosinophils; but mast cells seldom occurred. The inflammatory response in deprived mice, treated with thymectomy, irradiation and reconstitution, was slower to develop, involved fewer cells and was inadequately sustained (Walls et al., 1973). It has been generally thought that inflammation aids in the process of worm encapsulation and Michaloka and Karmanska (1976) showed that the inhibition of mast cell degranulation resulted in a decrease in the establishment of muscle larvae. However, Walls et al. (1973) noted no alteration of the encapsulation process in T-cell deprived mice.

A reduction in the inflammatory response to muscle larvae was also demonstrated by Grove and Warren (1976) who treated their mice with niridazole, a suppressant of cellular, but not humoral immunological responses; they suggested that the inflammatory response was due to cellular, rather than humoral, immunity.

Production of macrophage inhibition factor was not detected during the intestinal phase of T. spiralis, but marked macrophage inhibition occurred during the muscular phase of infection (Banki and Alföldy, 1975). Warren, Karp, Pelly and Mahmoud (1976) found that soluble muscle larvae antigens enhanced the migration of eosinophils taken from mice infected with T. spiralis.

It is not clear if muscle larvae can be affected by protective immunity. The in vitro work of Kazura and Grove (1978) and Mackenzie et al. (1978) suggests that it may well be the NBL which are arrested by the action of antibodies and eosinophils but in vitro results cannot be extrapolated to encompass the response which exists in vivo.

#### IV. CONCLUSIONS

The immunity induced by the intestinal phases of N. brasiliensis and T. spiralis are remarkably similar, and the actual mechanism of response to these parasites, in rats, may well prove to be the same.

Normally, cytological damage to these worms is necessary before their expulsion. Damage probably results from the combined action of sIgA and factors released from non-specific cells; but the absence of one of these components may result in the other effecting damage alone. Whether these non-specific cells are needed to cooperate with T cells in effecting expulsion is not clear. For although the expulsion of N. brasiliensis can occur in the absence of accessory cell populations and the expulsion pattern of both T. spiralis and N. brasiliensis are unaltered by depletion of the individual factors involved in inflammation: mast cells; eosinophils; macrophages and complement, it is probable that, in a naturally developing immune response, all such factors are involved. The depletion of one or more of these components would probably result in a compensating effect by the others; thus portraying a seemingly normal response. Most of the experimental designs described have attempted to identify the crucial elements of expulsion rather than to emphasise the probable cooperation between all the constituents of intestinal immunity.

The crucial effector component in the response against developing N. brasiliensis larvae might well be antibody. It is not known if both the muscle larvae and NBL of T. spiralis are susceptible to an immune response, however in vitro studies have indicated that NBL may be open to attack by antibodies and eosinophils.



## CHAPTER 3

MATERIALS AND METHODSThe Parasite

The strain of T. spiralis used in these experiments was the 'London strain' (Nelson and Mukundi, 1963). Professor R. T. Leiper isolated this, at the London School of Hygiene and Tropical Medicine, from an infected cat he obtained in Penrith, Cumberland in 1939. Since then it has been maintained by passage through laboratory rats and, from 1968, through laboratory mice.

Laboratory Hosts

Female T.O. mice (Tizer Original, Tuck and Co.) of any age were used to maintain a stock infection of T. spiralis. For experimental purposes female T.O.s which were 8 to 10 weeks old were used.

Mice of the 'Snell' strain were used in experiments concerned with adoptive transfers. Initially these mice were obtained from the Institute of Child Health (I.C.H.), thereafter a colony was maintained at the London School of Hygiene and Tropical Medicine, with additions to it coming from the colony at the I.C.H. Snell mice carry a gene for a general deficiency of the anterior pituitary gland, but this gene is only expressed in the double recessive mouse. The heterozygous and homozygous dominants being normal in all respects. These mice were originally developed at the University

Institute of Human Genetics, Copenhagen. They were later sent to the Institute of Animal Genetics, Edinburgh, where they were out-crossed to several other strains, including inbred A and CBA, to select for a large body size. The University of Sussex then received a colony and after three years a batch of these was given to the I.C.H., and these have remained as a closed colony for the last 8 years.

Inbred CBA female and male mice (Tuck and Co.) and inbred NIH female mice (Anglia Laboratories) were used in the genetical studies on the resistance of hosts to T. spiralis.

Female Wistar rats (Tuck and Co.) of varying ages were used for the production of adult T. spiralis worms.

#### Recovery of Infective Muscle Larvae

Infected stock mice usually harboured between 50,000 and 100,000 muscle larvae each. These mice were used for the production of excysted muscle larvae, after a minimum infection time of 6 weeks. When required one or more mice were killed, skinned, eviscerated and the surplus fat removed. The excess bone was removed by cutting off the tails, feet and incisors. The carcasses were then passed twice through a kitchen mincer, having first roughly chopped up the tongues as these, when whole, slipped through the mincer without shredding and were, thereafter, resistant to digestion.

The mince was digested immediately; the digestion mixture being freshly prepared, as follows:-

Pepsin - 1:2,500 BDH	15 g
Hydrochloric acid - 31% w/v BDH	10 ml
Warm tap water	1,000 ml

The mince was placed in a bucket with 2-3 litres of digestion fluid and stirred mechanically, at 37°C, for 24 hours. This was not a total digest, but the excysted muscle larvae were more suitable for infection purposes, than those obtained from total digestions.

After the period of digestion the resulting mixture was poured through a 50 mesh Endecote sieve (pore size - 250 µm) in series with a 200 mesh Endecote sieve (pore size - 75 µm). The larvae, retained by the 200 mesh sieve, were washed into a urine glass with a fine jet of tap water, allowed to settle and washed once by precipitation from tap water.

#### Infection with Muscle Larvae

The larval suspension was made up to a fixed volume, with tap water, in a beaker and stirred with a teflon coated magnet, on a magnetic stirrer. When large numbers of larvae were present, further dilutions were made. Aliquots of the suspension were placed in a MacMasters type (Hawksley) counting chamber and the mean of 5 sequential counts was used to calculate the total number of muscle larvae present. The concentration of the original suspension was then adjusted, so that the required number of larvae could be administered to each mouse.

Mice were infected orally with a blunted 14 inch gauge 18

hypodermic needle attached to a 1 ml disposable syringe. The volume of the stirred suspension normally given was between 0.2 and 0.4 ml. A 4 inch gauge 15 needle was used for rats, and volumes between 0.5 and 1.0 ml were given.

#### Determination of the Muscle Larva Burden of Individual Mice

The procedure was as before except that mincing and digesting of the carcass was carried out on individual mice. Each shredded carcass was placed in a litre jar of digesting fluid, which contained 13 ml of HCL per litre as opposed to 10 ml per litre. The contents of these jars were then stirred mechanically, or agitated by bubbling, at 37°C for six hours or less.

The resulting digests were sieved, as before, and the larvae quantitatively washed into urine glasses. The larvae could then be counted at any convenient time. A total count, in a ruled petri dish, was made if only a few hundred larvae were present. Otherwise the larvae were suspended in 150 ml of water, 15 ml aliquots were taken from this and the larvae present were counted in a ruled petri dish. The mean of 4 aliquots was used to determine the total number of muscle larvae present in each digested mouse.

#### Determination of the Number of Adult Worms in the Intestine

Mice were killed by cervical fracture and the small intestine was removed immediately and slit along its length using fine blunt-ended scissors. The slit gut was then shaken in a jar of warm tap

water to remove the excess digesta. This procedure made subsequent examination much easier and worm loss was less than 1% (Denham, 1968). The intestine was transferred to a jar containing 20 to 30 ml of 0.85% saline and incubated at 37°C for 1½ to 2 hours. The gut was then removed, the contents of the jar were tipped into a ruled petri dish and the adult worms counted under a dissecting microscope. The lining of the gut was scraped with the edge of a microscope slide, the mucosa squashed between two glass plates and the number of undetached worms counted. Less than 2% of the worms did not detach.

#### The Production of Newborn Larvae

Rats were infected per os with approximately 10,000 muscle larvae each, and killed 6 days later, as the peak of larval production, by adult females, occurs 6 to 7 days after infection (Denham and Martinez, 1970; James, 1971). The small intestines were removed, slit and washed 3 times in warm tap water, to remove all the digesta, and then incubated at 37°C in normal saline for 1-1½ hours, after this period the guts were carefully removed from the jars and discarded. The contents of the jars were tipped into petri dishes and studied under a dissecting microscope, any conglomerates of gut mucosa were sucked up by pipette and discarded. The remaining worms were poured, via urine glasses, into 10 ml conical test tubes and allowed to settle. They were then cleaned by 3 washes, lasting a total of 30 minutes, in sterile washing fluid. All further preparations for culturing the worms and mixing the wash and culture media

were performed in sterile conditions.

The media were prepared as follows:-

Washing fluid

Tissue culture medium 199 (10 x concentrated BDH)	100 ml
Sodium bicarbonate 4.4% w/v (59009 BDH)	50 ml
Merthiolate (Thiomersal 1 in 1,000 Lilly)	5 ml
Sterile distilled water	845 ml

This medium was stored in 100 ml screw top bottles at 4°C.

Culture medium

Tissue culture medium 199	100 ml
Sodium bicarbonate 4.4% w/v	25 ml
Sterile inactivated calf serum No.1 (Wellcome C508)	100 ml
Sterile distilled water	775 ml

20 ml of culture medium was poured into each of two 20 ml screw top bottles. To the first was added 1,000 mg of Penbritin (Ampicillin sodium, Beecham Research Laboratories) and to the second was added 500,000 units of Mycostatin (Nystatin B.P., E.R. Squibb and Sons). The remaining culture medium was stored in 200 ml screw top bottles and kept at 4°C with the Penbritin and Mycostatin until required.

Adult worms were cultured in 250 ml screw top bottles. To each bottle was added 200 ml of culture medium, 2 ml of Penbritin solution (to give a final concentration of 0.5 mg/ml) and 1 ml of the

Mycostatin suspension (to give a final concentration of 135 units/ml). Up to 20,000 female worms were placed in each bottle, which was then firmly sealed and laid horizontally in a 37°C incubator. The worms were cultured, with occasional agitation, for 12-20 hours.

During the latter stages of this work the cultures became contaminated with bacteria. The bacteria probably originated in the intestines of the rats and were carried into the culture medium by the adult worms, despite their numerous washings. Although the medium appeared normal the mice given i.v. injections of NBL, taken from it, died within 24 hours.

2 infected mice were autopsied, when showing symptoms of contamination, and their spleens and livers were homogenised. Samples of liver, spleen and blood from the infected mice, and samples from a naive mouse were then plated individually onto Blood agar and MacConkey's agar. The plates were incubated overnight, at 37°C. The plates from naive mice remained clear but all the ones from the infected mice grew gram negative rods, which were seen in direct smears and identified by colour and the positive oxidase test as Pseudomonas spp.

A diagnostic sensitivity test was then carried out. Agar was inoculated with the contaminated culture medium and Gentamicin and Carbemcillin discs were applied at varying concentrations. The Pseudomonas bacteria were found to be sensitive to these drugs and thereafter the culture medium was made up containing 0.1 mg/ml of Gentamicin.

After culturing, the contents of the bottles were passed, through a 200 mesh Endecote sieve, into a beaker. The adult worms were retained on the sieve. The culture medium containing the NBL

was poured into 40 ml conical centrifuge tubes and centrifuged for 5 minutes at 1,000 r.p.m. The supernatant from each tube was discarded and the sedimented larvae were washed in warm Phosphate Buffered Saline (P.B.S.). When mice were to be injected only once, the NBL were washed twice with P.B.S. However when multiple injections of NBL were given, to avoid anaphylaxis, it was essential that no protein, from the culture, remained with the larvae, and in these cases the NBL were washed four or more times in P.B.S.

After washing the sedimented larvae were pooled and resuspended in a fixed volume of P.B.S. The suspension was agitated with a Pasteur pipette, a 25  $\mu$ l aliquot was withdrawn and the number of viable NBL in it counted, on a 2 mm x 2 mm ruled slide, under a dissecting microscope. The mean of 4 aliquots was used to determine the total number of NBL present.

#### Infection with NBL

The concentration of NBL in P.B.S. was adjusted, so that the required number of NBL per mouse could be administered, in a suitable volume (usually 0.2 ml). The suspension was agitated with a pipette, the larvae withdrawn in a 1 ml syringe, and injected into the lateral tail veins of the mice, using a 'sabre' 25 g 5/16" needle. During injection the mice were contained within a perspex cylinder, held in a clamp. The cylinder had a longitudinal slit, to allow for the protrusion of the tails.



#### Preparation of Serum

Snell and T.O. mice were used for the production of immune and normal serum. After sensitisation to T. spiralis the mice were anaesthetised with Nembutal and bled, by cardiac puncture, using a 2 ml disposable syringe and 1½ inch x 21 g needles. The blood was allowed to clot at 37°C for 30 minutes and then at 4°C for 5, or more, hours. The clots were removed and to sediment the loose blood cells the serum was centrifuged at 3,000 r.p.m. for 10 minutes. The serum was then pooled and stored in 1 or 5 ml aliquots at -70°C.

Recipient mice received the serum by intraperitoneal (i.p.) injection.

#### Preparation of Cell Suspensions

Snell mice were used as donors of immune and normal spleen cells (SpC). To obtain SpC the donor mice were killed, the spleens removed aseptically and pushed, gently, through a sieve, into a small container of medium 199 on ice, thus trapping the fibrous capsule and releasing the SpC into the medium.

The cells were concentrated by centrifugation at 1,000 r.p.m. for 2 minutes, at 4°C and washed 3 times with cold medium 199. The cells were suspended in a fixed volume of 199. After dilution, the lymphocytes were counted in a 'Neubauer' haemocytometer, the viability of the cells being determined by the trypan-blue exclusion test. The concentration of the SpC was adjusted, so that the required number of viable lymphocytes could be administered to each recipient.

In some experiments mesenteric lymph node cells (MLNC) were also used, these were prepared in the same manner as SpC.

$4 \times 10^7$ , normal or sensitised, SpC were injected into the lateral tail vein of each recipient mouse. The cells were given in a volume of 0.4 ml. Normal MLNC could also be injected intravenously (i.v.), but the i.v. administration of sensitised MLNC resulted in the instantaneous death of recipients - for undetermined reasons. Dineen and Kelly (1973b), working on Nippostrongylus brasiliensis infections in rats, showed that 3 to 4 times as many lymphoid cells must be transferred i.p. to achieve worm expulsion of a similar magnitude to that which results from the same cells given i.v. Thus, in the experiments reported here, recipient mice were given  $8 \times 10^7$  MLNC i.p., in a volume of 0.4 ml.

## CHAPTER 4

THE GENETICAL CONTROL OF RESISTANCE TO T. SPIRALIS  
IN TWO STRAINS OF MICE

During the search for a suitable inbred strain of mouse to use in the adoptive transfer experiments, reported in Chapter 6, it was noted that NIH mice were extremely competent at expelling enteral T. spiralis worms whereas CBA mice responded poorly to the infection. Thus, NIH and CBA mice appear to fall into the categories of responder and non-responder mice respectively and as little is known about genetically controlled mechanisms of helminth elimination in the host NIH and CBA mice could perhaps provide suitable tools for such investigations.

REVIEW

The degree and type of immune responsiveness is controlled by a variety of genes. The location and influence of certain of these genes is understood and at least two groups can be recognised (Hobarth and McConnell, 1975).

- 1) Where the capacity for response is associated with the inheritance of V-region germ line genes which code for the antibody.
- 2) Where the specific responses to some antigens have been shown to be under the control of autosomal dominant genes which are inherited in a strict Mendelian fashion and are linked to the major histocompatibility loci; these are known as immune response or Ir genes (McDevitt and Benacerraf, 1969).

There are also Ir genes which control the overall level of antibody responses, as shown by Biozzi mice, in which the development of low and high responder strains is not antigen specific (Biozzi, Stiffel, Mouton, Bouthillier and Decreusfond, 1972).

Several host-parasite systems will be described in which a particular host strain or individuals within a strain are non-responsive to a parasitic helminth. The expulsion of worms is a complex process involving a number of interacting components (Ogilvie and Love, 1974), any or all of which could be under genetic control. Thus, the recognition of worm antigens, antibody production and cell mediated activity could all be controlled by the Ir genes an animal possesses; and non-responsive mice may have few or none of these Ir genes. Nevertheless, susceptibility to infection does not necessarily imply a total lack of response to worm antigens. Non-responsive mice infected with Trichuris muris produce circulating anti-worm antibody (Wakelin, 1967). Therefore, these mice might only have the Ir genes concerned with antigen recognition and antibody production, but not the genes involved in the cell mediated phase of reaction which is necessary for worm expulsion to occur.

Genetically determined resistance to helminths has often been suggested (Ackert, 1942) and the differences in development of parasites in different strains of host as well as in individuals within a strain has long been known. Ackert, Pratt and Freeman (1936), using Ascaridia lineata in chickens, were the earliest workers to selectively breed for host resistance. A number of other authors have considered the genetic control and inheritance of resistance in a variety of helminth systems such as: Ostertagia circumcincta in sheep (Gregory, 1937; Stewart,

Miller and Douglas, 1937); Nematospiroides dubius in mice (Liu, 1966); Schistosoma mansoni in mice (Moor, Yolles and Melener, 1949; Stirwalt, Shepperson and Lincicome, 1965); Trichostrongylus colubriformis in sheep (Whitlock, 1955; 1958; Whitlock and Madsen, 1958); Echinococcus multilocularis in mice (Lubinsky, 1964; Ali Khan, 1974); C. fasciolaris in mice (Dow and Jarret, 1960); T. muris in mice (Wakelin, 1969); T. spiralis in mice (Duckett, 1971) and Brugia pahangi in rats (Sucharit and Macdonald, 1973).

Wassom, De Witt and Grundman (1974), working on variation in the acquired resistance to Hymenolepis citelli in the deer mouse, found that resistance was controlled by a single autosomal dominant gene. This may suggest that the responsible Ir gene is linked to the major histocompatibility loci. These workers attempted an analysis in immunological terms and transferred resistance to non-responsive hosts with sensitised lymphocytes from responsive animals although not with immune serum. The ability of competent hosts to respond was depressed by treatment with anti-lymphocyte serum (ALS) (Wassom et al., 1974).

Few other workers have attempted to relate variations in infection to specific immunological events. Wakelin (1975a) showed that the variability of response to T. muris in the outbred CFLP mouse strain and the uniformity of response in inbred NIH mice was a consequence of the genetic control of the immune response. Irradiation of the mice reduced variation in the response to T. muris and the factors responsible for worm expulsion which appeared to work on the fifth day of infection were inactivated by cortisone acetate. Cross breeding experiments showed that responsiveness to the parasite was inherited as a dominant characteristic in simple Mendelian fashion, suggesting that relatively

few genes are involved in determining the period required for the expulsion of worms.

Subsequent work by Wakelin (1975b) demonstrated that populations of Schofield mice have a genetically controlled bimodal variation in their ability to achieve immune expulsion of T. muris and either produced a strong immune response against the parasite or none at all. The number of genes involved appeared to be small as separation of the two phenotypes was achieved after a few generations of selection. Again, cross breeding showed that earlier expulsion was inherited as a dominant characteristic.

Similar experiments were carried out by Rothwell, Le Jambre, Adams and Love (1978) on T. colubriformis infections in outbred guinea-pigs. After two generations of selective breeding two lines of guinea-pigs were developed which were significantly different in their response to the parasite. They suggested that the variation in susceptibility to infection with T. colubriformis was dependent upon genetically determined differences in the rate of immune expulsion of the worms, as immunosuppression, by thymectomy and ALS treatment abolished resistance in the non-susceptible guinea-pigs. The pattern of separation of the phenotypes implied that resistance was controlled by dominant alleles. This host-parasite system resembled T. muris infections in some mouse strains (Wakelin, 1975a) but differed from T. muris infections in Schofield mice and H. citelli infections in deer mice where the hosts fell into the categories of strong responders or non-responders (Wakelin, 1975b; Wassom et al., 1974).

It can be seen that little definitive work has been carried out on the genetics of the variation of the immune response to parasitic helminth infections. This chapter describes preliminary experiments which

attempt to define genetically the variability of response to T. spiralis in CBA and NIH mice.

#### MATERIALS AND METHODS

Male and female CBA mice were obtained from Tuck and Co. and NIH females from Anglia Laboratories. At the time of experimentation it was not possible to acquire male NIH mice, so all first generation crosses were made between NIH females and CBA males. All breeding was carried out in groups of one male to two females per cage. The resulting offspring were weaned, separated by type and sex and caged in groups of fifteen to twenty. Mice were infected when aged from 8 to 14 weeks, since they could then be considered as immunologically mature.

#### Experiment 4.1.

This experiment was designed to compare the development of resistance in NIH and CBA mice, to primary and secondary infections of T. spiralis.

50 NIH mice were divided into two groups of 20 and 30 mice. The first group received 300 muscle larvae of T. spiralis per os. 40 days later both groups were given 200 muscle larvae. The mice were killed in groups of 5 at various intervals after infection, to determine the period required for adult worm expulsion to occur. The number of worms recovered from the small intestines are shown in Table 1 and Fig. 4.

35 female CBA mice were sensitised with a primary infection of 300 muscle larvae. They were challenged with 100 muscle larvae 40 days

later, together with 35 control mice. The mice were killed at intervals after infection and worm counts were made. 8 of the challenged mice died from undetermined causes. The results are shown in Table 2 and Fig. 5.

The NIH mice given initial or challenge infections had an inoculum recovery of 55%; the recovery from CBA mice was around 60%. The challenge infection, in NIH mice, showed a sharp decline 3 days after infection and worm expulsion was practically complete by Day 6, with only one worm being found in the 5 mice which were autopsied. The primary infection in NIH mice remained stable until 6 days after infection, thereafter expulsion commenced and the majority of worms were expelled by Day 12. Therefore, the primary expulsion commenced as the secondary expulsion neared completion.

In CBA mice, however, there was little difference between the rates of the worm expulsion in primary and secondary infections. These rates were slow compared to the rapid elimination of worms observed in both the initial and challenge infections in NIH mice. In fact, CBA mice took longer to expel challenge worms than NIH mice took to expel worms from a primary infection.

With regard to challenge infections, Day 7 post infection appeared to be a suitable time for future autopsies involving the two strains of mice or their crosses, as by this time the NIH mice had expelled all of their challenge worms, whereas the expulsion of worms from the secondarily infected CBA mice had not, as yet, commenced.

#### Experiment 4.2.

The mice used to determine the parameters of expulsion in primarily



TABLE 1. Experiment 4.1.

Mean number of adult worms recovered during initial and challenge infections of NIH mice with T. spiralis. There were 5 mice per group.

Days post-challenge	<u>Challenge Infection</u>	<u>Initial Infection</u>
	Mean (SE) number of worms	Mean (SE) number of worms
3	111 (+ 13.9)	-
4	62 (+ 29.8)	109 (+ 5.1)
6	0.2 (+ 0.2)	109 (+ 25.9)
9	0	68 (+ 14.4)
11		10 (+ 5.8)
12		3 (+ 2.4)

TABLE 2. Experiment 4.1.

Mean number of adult worms recovered during initial and challenge infections of CBA mice with T. spiralis.

Days post-challenge	<u>Challenge Infection</u>		<u>Initial Infection</u>	
	Number of mice	Mean (SE) number of worms	Number of mice	Mean (SE) number of worms
3	3	68 (+ 4.3)	5	60 (+ 3.0)
6	4	63 (+ 2.5)	5	60 (+ 3.4)
9	4	55 (+ 2.6)	5	52 (+ 7.0)
11	4	57 (+ 6.3)	5	54 (+ 9.5)
13	5	38 (+ 10.3)	5	51 (+ 8.9)
15	5	4 (+ 3.1)	5	45 (+ 5.1)
17	2	0.5 (+ 0.5)	5	9 (+ 6.4)

The Mean No of Adult Worms Recovered from NIH and CBA Mice Given Primary and Secondary Infections of T. Spiralis

Fig.4 NIH Mice

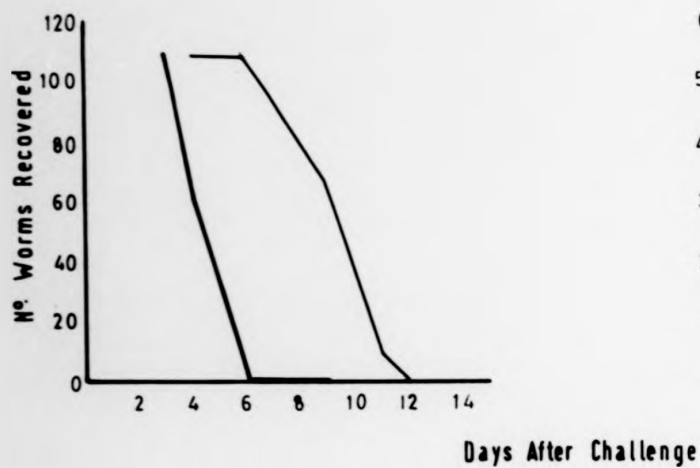
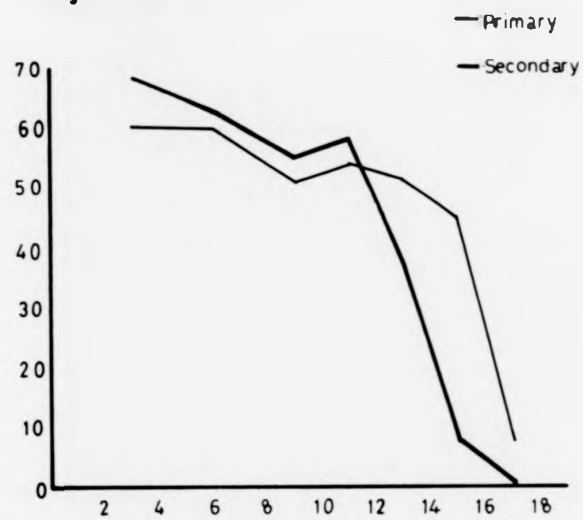


Fig.5 CBA Mice



and secondarily infected hosts were also employed in the calculation of spleen (Sp) and mesenteric lymph node (MLN) weight to body weight ratios during the course of infection. These calculations were made in order to determine when the blastogenesis of lymphocytes occurred in the two strains.

The mice were weighed before they were killed. After the removal and incubation of their small intestines the Sp and MLN were removed, cleaned and weighed. The Sp and MLN to body weight ratios of each mouse were then calculated and the results are shown in Table 3a and b and Fig. 6.

Both the Sp and MLN to body weight ratios of the NIH mice increased markedly in value during primary infection, indicating activation of cell populations in these organs. The Sp and MLN to body weight ratios of NIH mice, given secondary infections, had initially raised values when compared to the ratios from mice given primary infections. This would be expected if mice are responding to a challenge infection.

In contrast, values of the Sp and MLN to body weight ratios of CBA mice experiencing both primary and secondary infections remained stable throughout the period of observation, and the ratios from CBA mice given a challenge infection were lower than from those given primary infections.

#### Experiment 4.3.

In this experiment the times required for worm expulsion in the progeny of crosses between responder and non-responder mice were tested, in an attempt to determine the way in which the responder/non responder character was inherited. An  $F_1$  generation was produced by crossing NIH females with CBA males, but the reciprocal cross could not be made as

TABLE 3a. Experiment 4.2.

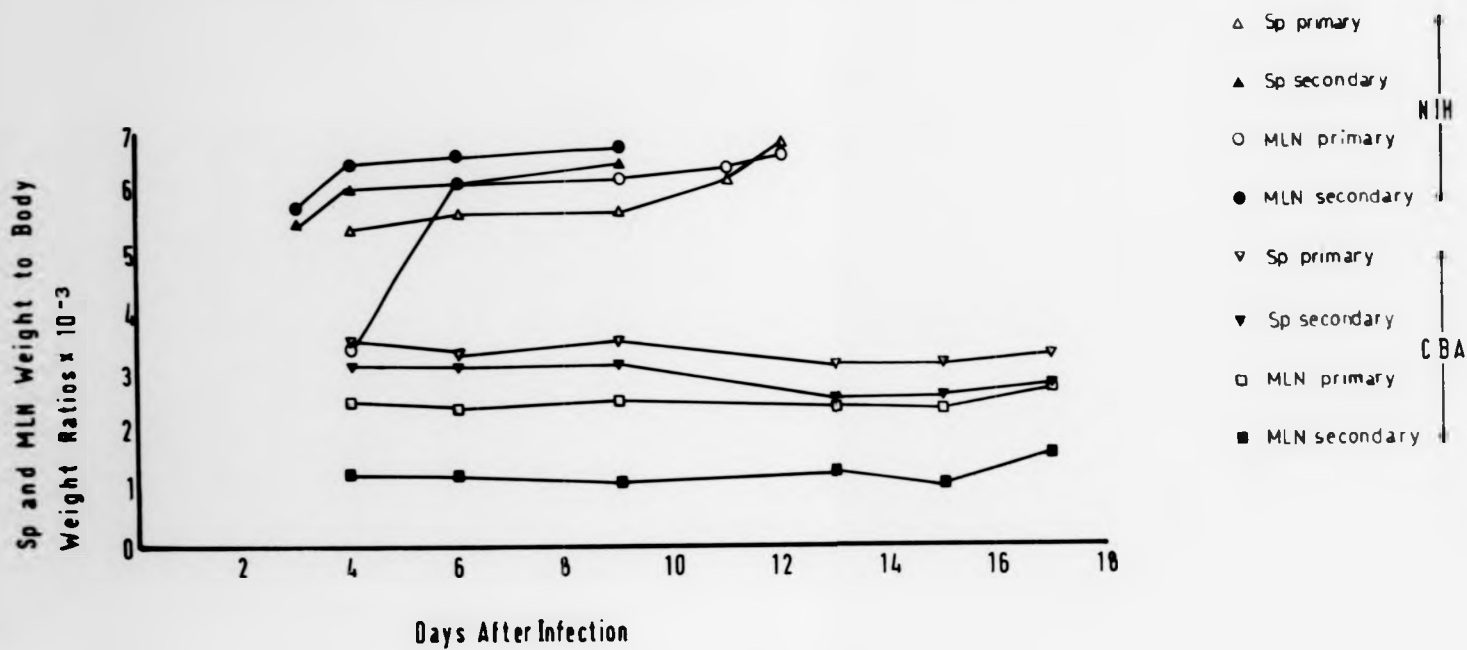
The mean spleen (Sp) weight to body weight and mesenteric lymph nodes (MLN) weight to body weight ratios of NIH and CBA mice during initial and challenge infections with T. spiralis.

Days post- infection	<u>Challenge Infections</u>		<u>Initial Infections</u>	
	Mean (SE) ratios of:		Mean (SE) ratios of:	
	Sp to body weight	MLN to body weight	Sp to body weight	MLN to body weight
3	0.0054	0.0057	-	-
4	0.0060	0.0064	0.0053	0.0034
6	0.0061	0.0066	0.0056	0.0061
9	0.0064	0.0067	0.0056	0.0062
11			0.0062	0.0063
12			0.0068	0.0066

TABLE 3b. Experiment 4.2.

Days post- infection	<u>Challenge Infections</u>		<u>Initial Infections</u>	
	Mean (SE) ratios of:		Mean (SE) ratios of:	
	Sp to body weight	MLN to body weight	Sp to body weight	MLN to body weight
4	0.00311	0.00122	0.00352	0.00236
6	0.00305	0.00123	0.00330	0.00228
9	0.00310	0.00112	0.00355	0.00243
13	0.00252	0.00121	0.00311	0.00234
15	0.00259	0.00111	0.00309	0.00219
17	0.00279	0.00153	0.00318	0.00268

Fig.6 The Mean Spleen(Sp) and Mesenteric Lymph Nodes (MLN) Weight to Body Weight Ratios of NIH and CBA Mice During Primary and Secondary Infections of T.Spiralis



NIH males were unavailable.

5 NIH females, 5 CBA females, 10 F<sub>1</sub> females and 10 F<sub>1</sub> males were infected with 200 muscle larvae each. 4 weeks later all the mice were challenged with 200 muscle larvae, autopsied 7 days later and adult worm counts made. The results are shown in Table 4.

None of the NIH mice harboured T. spiralis adults but the CBA mice had a mean worm count of 26 ( $\pm$  10.6). This figure was lower than would be expected from the results of experiment 4.1. There was no significant difference between the mean worm counts of the male and female F<sub>1</sub> progeny (P = 0.4). 75% of these mice contained no adult worms and only one mouse harboured as many as 16. From these results it is concluded that the great majority of the F<sub>1</sub> progeny responded to infection in the manner of the NIH parenteral strain.

#### Experiment 4.4.

This was essentially a repeat of experiment 4.3. The mice were stimulated with 200 infective larvae and challenged with 200 infective larvae 4 weeks later. Autopsies were carried out 8 days after infection, in an attempt to obtain clearer results from the F<sub>1</sub> progeny. The results are shown in Table 5.

Only 1 worm was found in all 5 NIH mice, although a mean of 34 ( $\pm$  6) worms were recovered from the CBA mice. Again, there was no significant difference between the worm recoveries in the males and females of the F<sub>1</sub> generation. Although worm expulsion in the F<sub>1</sub> groups was still not complete, there was no overlap between the worm recoveries of the CBA and F<sub>1</sub> mice.

TABLE 4, Experiment 4.3.

The numbers of adult worms recovered 7 days after a challenge infection of 200 muscle larvae.

Type of mouse	Number of mice/group	Individual adult worm counts	Mean (SE)	Probabilities
NIH ♀	5	0 0 0 0 0	0	
CBA ♀	5	59 41 21 10 0	26 (11)	
F <sub>1</sub> (NIH ♀ x CBA ♂) ♀	10	16 3 0 0 0 0 0 0 0 0	1.9 (1.6)	↑ 0.005 ↓
F <sub>1</sub> (NIH ♀ x CBA ♂) ♂	10	3 1 1 0 0 0 0 0 0 0	0.5 (0.3)	↑ 0.4 ↓



TABLE 5. Experiment 4.4.

The numbers of adult worms recovered 8 days after a challenge infection of 200 muscle larvae.

Type of mouse	Number of mice/group	Individual adult worm recoveries	Mean (SE)	Probabilities
NIH ♀	5	1 0 0 0 0	0.2 (0.2)	
CBA ♀	5	56 34 32 29 18	338 (6.2)	
F <sub>1</sub> (NIH ♀ x CBA ♂) ♀	7	14 7 3 1 0 0 0	3.6 (2.0)	
F <sub>1</sub> (NIH ♀ x CBA ♂) ♂	6	12 2 0 0 0 0	2.3 (2.0)	

Even though the results, in experiments 4.3 and 4.4, are not resolute, it is evident that the majority of the  $F_1$  progeny showed the early expulsion time associated with the responder NIH mice. This indicated that the responsiveness character showed a strong tendency towards dominance.

#### Experiment 4.5.

In this experiment the  $F_1$  generation from the NIH female to CBA male cross were backcrossed to the parental strains, in an attempt to determine the genetic mechanism associated with expulsion. The NIH to CBA cross was repeated so that the  $F_1$  mice could be tested simultaneously with the backcross progeny. The backcrosses made were as follows: CBA females to  $F_1$  males;  $F_1$  females to CBA males and NIH females to  $F_1$  males. After weaning, the mice of each sex and from each backcross were caged separately. The progeny of the backcross to NIH mice were also segregated by colour.

187 of these mice were infected with 200 muscle larvae and challenged with 200 muscle larvae 4 weeks later. 7 days after challenge, all of the mice were autopsied, within 5 hours of each other, and adult worm counts were made. The results are shown in Table 6.

There was no significant difference between the mean worm burdens of the male and female CBA mice ( $P = 0.3$ ), these being 57 ( $\pm 10.3$ ) and 50 ( $\pm 9.2$ ) respectively. The mean worm recovery from the NIH mice was 2.2 ( $\pm 1.7$ ). However the mean worm burdens of the male and female  $F_1$  generation mice were higher than those observed in experiments 4.3 and 4.4 and were 9.7 ( $\pm 6.1$ ) and 12.4 ( $\pm 3.9$ ) respectively. The progeny of the  $F_1$  backcrosses to the CBA parental strain had significantly

TABLE 6, Experiment 4.5.

The numbers of adult worms recovered 7 days after a challenge infection of 200 muscle larvae.

Type of mouse	Sex	No. of mice per group	Individual counts of adult worms	$\bar{x}$ (SE)	Probabilities
CBA	♀	5	84 70 59 49 23	57.0 (10.3)	↑ 0.3 ↓
CBA	♂	5	73 64 52 41 20	50.0 (9.2)	
NIH	♀	5	9 2 0 0 0	2.2 (1.7)	↑ 0.05 ↓
F <sub>1</sub> (NIH♀ x CBA♂)	♀	12	35 32 28 22 11 10 10 1 0 0 0 0	12.4 (3.9)	↑ 0.35 ↓
F <sub>1</sub> (NIH♀ x CBA♂)	♂	8	41 34 3 0 0 0 0 0	9.7 (6.1)	↑ 0.2 ↓
CBA♀ x F <sub>1</sub> (NIH♀ x CBA♂)♂	♀	13	121 75 58 55 54 48 35 28 18 14 11 3 2	40.1 (9.3)	↑ 0.05 ↓
	♂	11	49 39 28 25 23 19 17 10 7 5 3	20.4 (4.4)	↓
F <sub>1</sub> (NIH♀ x CBA♂)♀ x CBA♂	♀	41	114 109 106 105 102 95 91 62 56 56 53 50 49 47 46 41 38 37 35 35 32 30 29 27 25 24 22 21 18 18 18 16 16 15 15 8 8 8 7 7 3	41.3 (5.0)	↑ 0.35 ↓
	♂	55	121 103 102 78 75 73 70 69 67 67 67 59 58 57 56 55 54 53 51 48 45 41 40 38 37 35 28 28 26 23 21 20 20 19 19 19 18 18 17 15 15 14 11 11 9 7 3 3 2 2 2 2 0 0 0 0	36.2 (4.0)	↑ 0.2 ↓
NIH♀ x F <sub>1</sub> (NIH♀ x CBA♂)♂	White ♀	5	6 5 4 0 0	3.0 (1.3)	↑ 0.2 ↓
	Brown ♀	10	18 12 7 6 5 3 2 1 0 0	5.4 (1.8)	↓
	White ♂	9	16 6 5 3 1 0 0 0 0	3.4 (1.7)	↑ 0.3 ↓
	Brown ♂	8	12 4 2 0 0 0 0 0	2.2 (1.5)	↓

higher mean worm burdens than the progeny of the  $F_1$  backcrosses to the NIH parental strain.

#### DISCUSSION

The NIH mice used in these experiments responded rapidly to both primary and secondary infections of T. spiralis; the parameters of expulsion being similar to those described by Wakelin and Lloyd (1976a) for the same system. In contrast, CBA mice took much longer to expel a primary infection and showed very little immunity to challenge. The parameters of expulsion in CBA mice resembled those described for inbred Swiss Albino mice by Larsh (1963). Thus, in terms of their ability to respond to T. spiralis infections, the NIH mice could be classified as a responder strain and the CBA mice as a non-responder strain.

The immunosuppression of responder hosts reduces their ability to expel H. citelli (Wassom et al., 1974), T. muris (Wakelin, 1975a) and T. colubriformis (Rothwell et al., 1978) infections which led the authors to suggest that the variation in expulsion times between responder and non-responder hosts is a consequence of the genetic control of the immune response. In this report no such conclusions can be drawn from the breeding experiments alone, although the results of experiment 4.2 suggest that the expulsion of T. spiralis is related to the immunological competence of the host. The MLN to body weight ratios of the primarily infected NIH mice increased sharply 4 to 6 days after infection, and this might be correlated to immune activity before the commencement of worm expulsion. The rise in values of both the MLN

and Sp to body weight ratios between days 9 and 12 were probably due to the maximum expulsion activity against adult worms with the additional stimulus of migrating NBL. This pattern of increase in both Sp and MLN to body weight ratios resemble the initial increase in the T. spiralis antigen reactive spleen cells (SpC) and mesenteric lymph node cells (MLNC), of primarily infected rats, measured by lymphocyte transformation (Ottesen, Smith and Kirkpatrick, 1975), and suggests a correlation between Sp/MLN to body weight ratios and the immune status of the host. Ottesen et al. (1975) found that the antigen reactivity of MLNC rose sharply after 12 days of infection, peaked between days 15 and 20 and thereafter fell; whereas the SpC, although less reactive to T. spiralis antigen, fluctuated about their peak reactivity for 60 days. Thus, the work of Ottesen et al. (1975) suggests that the MLNC reacted to a large stimulation from both adult worms and the migrating NBL, whereas SpC were mainly concerned with a smaller, but more consistent stimulation from the muscle larvae.

The Sp and MLN to body weight ratios of the secondarily infected NIH mice were similar in pattern, but the MLN to body weight ratios showed a much greater increase over the initial MLN to body weight ratios obtained from primarily infected mice than did the Sp to body weight ratios. These raised values would be expected if indicative of immunological memory and the results suggest that the MLN contained more memory cells to adult worm antigens than the Sp.

In contrast the Sp and MLN to body weight ratios of both primarily and secondarily infected CBA mice had low values and remained surprisingly static throughout the period of observation. The low values of the primary curves would account for the slow response to initial infection with T. spiralis, found in CBA mice. Higher values would be

expected for the Sp and MLN to body weight ratios of the challenged mice and the observed decrease in their values suggests that the challenged mice were in a depressed state. This could be a result of the primary infection and would account for their deficiency in expressing immunological memory against the challenge worms.

Thus the relative sizes of the Sp and MLN, if correlated to lymphoid activity, suggests that the NIH mice responded rapidly to infection and that their lymphoid organs contained memory cells to a challenge infection. Whereas the organs from the CBA mice showed little activity against primary or challenge infections, indicative of the poor response and lack of immunological memory expressed against T. spiralis by CBA mice.

If the CBA and NIH strains of mice are considered to be highly inbred, then it can be assumed that they are homozygous for the genes affecting the response to infection. If this is so, then the variability in response observed between  $F_1$  individuals in experiments 4.3, 4.4 and 4.5 was due to environmental factors. The results of experiment 4.5 did not show as much dominance of the responder character in the  $F_1$  generation (NIH ♀ x CBA ♂) when compared to experiments 4.3 and 4.4. This makes it difficult to attribute the variability solely to environmental factors and further makes the dominance of the responder characteristic less certain. However the mean worm burdens harboured by the progeny of the NIH ♀ to  $F_1$  ♂ backcross in experiment 4.5 did not differ significantly from the mean worm burden of the NIH mice, which would be expected if the responder characteristic was dominant.

The responder characteristic was not linked to the white/brown gene as none of the progeny from the NIH ♀ x  $F_1$  ♂ backcross (experiment

4.5), which were segregated by colour, differed in their mean worm burdens.

If the responder characteristic is sex linked then the CBA ♀ x F<sub>1</sub> ♂ backcross would give the following inheritance of the different X chromosomes:- (see Fig. 7).

Thus the X Y males of the backcross progeny would be non-responders like the parental CBA strain and the X X females would be like the F<sub>1</sub> females, because the NIH (N) responder characteristic tends towards dominance. However it can be seen, from the results of experiment 4.5, that the male progeny from the CBA ♀ to F<sub>1</sub> ♂ backcross had lower worm counts than their female counterparts. Thus the responder characteristic was controlled by autosomal genes and this would be an expected result as sex linkage is rare.

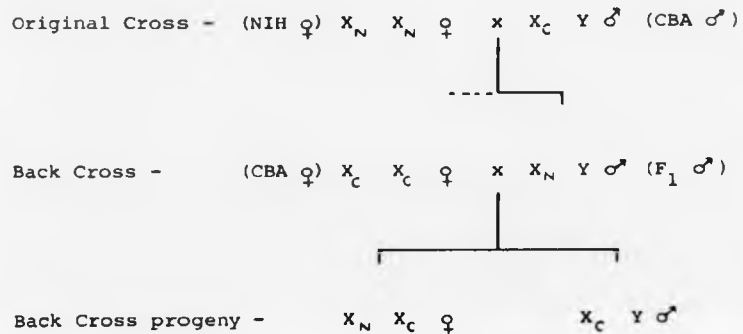
The genetic mechanism of inheritance must involve more than one gene and may be polygenic because in experiments 4.3 and 4.4 and even 4.5 a typical F<sub>1</sub> result was a zero worm score, but such zero scores were rare in the backcrosses to CBA mice. Whereas, with a single gene mechanism, heterozygotes, just like the F<sub>1</sub> progeny would occur in 50% of the backcross progeny:- (see Fig. 8).

An unlinked two gene model would make genotypes like the F<sub>1</sub> rarer in the backcross:- (see Fig. 9).

It can be seen from the unlinked two gene model that only one quarter of the backcross progeny were exactly like the F<sub>1</sub> genotype and would therefore have a good chance of a zero worm score. Table 6 shows that of the 120 backcross progeny tested (CBA ♀ to F<sub>1</sub> ♂ and F<sub>1</sub> ♀ to CBA ♂) only 4 showed zero worm counts. This suggests that more than two genes were involved in the inheritance of the responsiveness character. An unlinked polygenic model would show a similar

Figure 7.

A SEX LINKAGE mechanism for the inheritance of the responder characteristic



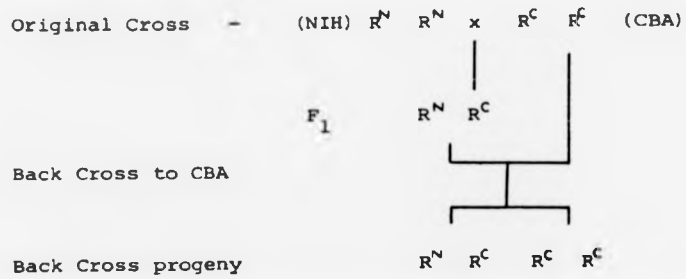
where N = NIH responder characteristic

c = CBA non-responder characteristic



Figure 8.

A single gene mechanism for the inheritance of the responder characteristic

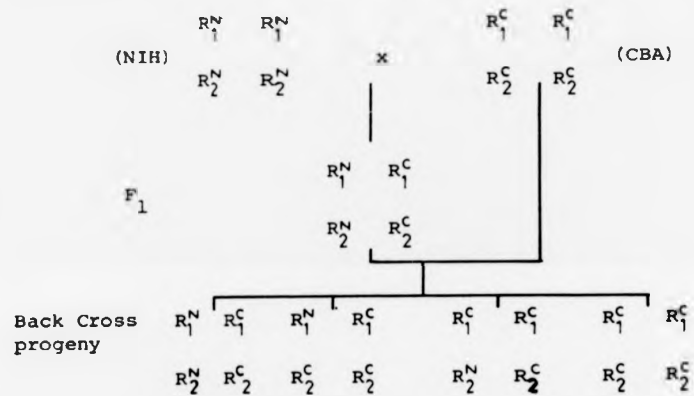


where R is postulated as a single responder locus with alleles  $R^N$  and  $R^C$ .

Figure 9.

An unlinked two gene model for the inheritance of the responder characteristic

Original Cross



where  $R_1$  and  $R_2$  are two postulated loci affecting the response.

C and N symbolise alleles of each locus from the two strains.

tendency to the unlinked two gene model, but the frequency of the  $F_1$  genotype in the backcross progeny would be rarer still.

The early expulsion of T. muris from CFLP, NIH and Schofield mice (Wakelin, 1975a and b) and that of T. colubriformis from guinea-pigs (Rothwell et al., 1978) have each been shown to be inherited as a dominant characteristic and controlled by few genes. The results of the cross breeding experiments, reported in this chapter, show that the early expulsion of T. spiralis tends to be inherited as a dominant characteristic. The expulsion characteristics of the progeny, from the backcross to the CBA parental strain, suggest that more than 2 genes are involved in the control of expulsion. If the mechanism of control of T. spiralis infections in mice is similar to those operating in the T. muris/mouse and T. colubriformis/guinea-pig systems, then it is probable that this polygenic mechanism also involves few genes.

In conclusion, it is suggested that the immunological development of resistance to T. spiralis, in mice, is controlled by autosomal dominant alleles.

## CHAPTER 5

The Parameters of Acquired Immunity to *T. spiralis*  
in Snell and T.O. mice and their suitability as  
Donors of Adoptive and Passive Immunity

The type of immune response which acts against parasites is determined by the genetical make up of the host and the parameters of immunity to *T. spiralis* have been shown to vary considerably (reviewed in Chapter 4). Wakelin and Lloyd (1976a) emphasised the importance of establishing the parameters of immunity to *T. spiralis*, for each strain of host used, before experimental studies were designed.

The following experiments attempt to establish some of the parameters of infection with *T. spiralis*, in two strains of murine host; these being the outbred T.O. strain and the 'semi-inbred' Snell strain. The suitability of Snell mice, for use in adoptive transfers, was also tested.

Experiment 5.1.

This experiment was designed to study the development of resistance, to adult worms in primary and secondary infections, in T.O. mice.

Forty T.O. mice were divided into 2 groups, the first group received 200 muscle larvae of *T. spiralis*, per os. Four weeks later both groups were given 200 muscle larvae. The mice were killed at

intervals, after challenge and adult worm counts were made. The results are shown in Table 7 and Fig. 10.

There was a 75% inoculum recovery in both the primary and secondary infections. Worm expulsion in the primary infection commenced between days 12 and 14, after challenge, by which time the worm recovery had fallen to 99 ( $\pm$  20). Challenge worms were expelled 4 days earlier, as the worm number had fallen to 62 ( $\pm$  11) between days 8 and 10, after challenge.

#### Experiment 5.2.

This experiment tested the response of Snell mice to initial and challenge infections. Thirty-three Snell males were infected with 200 muscle larvae, 4 weeks later these mice and 70 naive Snell mice were challenged with 200 muscle larvae. Both groups were autopsied at intervals, after challenge, and adult worm counts were made. The results are shown in Table 8 and Fig. 11 .

The inoculum recovery from the primary infection fluctuated around a mean of 60%, until day 15, post infection, when worm expulsion had commenced, and the recovery had dropped to 62 ( $\pm$  14) worms. After this initially rapid expulsion rate there seemed to be a plateau until day 24, after which the expulsion proceeded slowly to its completion. The challenged mice showed an inoculum recovery of 72% on day 4, after challenge, and by day 7 this had dropped to a mean of 53 ( $\pm$  10) worms. Day 13, showed a drop to a mean of 30 ( $\pm$  11) worms, and here again a static effect was evident, until after day 15.

Thus in primary and secondary infections, once expulsion had started, there is a rapid ejection of worms which then decelerates

TABLE 7 , Exp. 5.1.

The mean number of adult worms recovered during initial and challenge infections of T. spiralis in T.O. mice.

Days post-challenge	Challenge Infection		Initial Infection	
	Number of mice	Mean (SE) number of worms	Number of mice	Mean (SE) number of worms
4	5	150 (+ 14.1)	5	146 (+ 18.5)
6	5	137 (+ 15.15)	5	123 (+ 14.4)
8	5	111 (+ 22.5)	5	151 (+ 12.6)
10	5	62 (+ 10.6)	5	144 (+ 12.1)
12	5	52 (+ 3.9)	5	139 (+ 16.0)
14	5	29 (+ 7.0)	5	99 (+ 19.9)
16	5	23 (+ 9.6)	5	67 (+ 13.1)
18	5	5 (+ 2.8)	5	40 (+ 7.4)

TABLE 8 , Exp. 5.2.

The mean number of adult worms recovered during initial and challenge infections of T. spiralis in Snell mice.

Days post-challenge	Challenge Infection		Initial Infection	
	Number of mice	Mean (SE) number of worms	Number of mice	Mean (SE) number of worms
4	5	145 (+ 26.4)	5	113 (+ 9.4)
7	5	53 (+ 9.6)	5	117 (+ 11.3)
9	5	56 (+ 5.6)	5	135 (+ 19.4)
11	5	30 (+ 11.0)	5	102 (+ 10.2)
13	5	28 (+ 8.3)	5	131 (+ 30.0)
15	4	29 (+ 12.7)	5	62 (+ 14.0)
18	4	11 (+ 3.5)	5	37 (+ 3.6)
21			5	36 (+ 10.5)
24			5	40 (+ 8.0)
28			5	10.6 (+ 5.8)
32			5	1.4 (+ 0.9)
36			5	0.6 (+ 0.5)
40			5	0.2 (+ 0.1)
41			5	0

The Mean No. of Adult Worms Recovered from T.O. and Snell Mice Given Primary and Secondary Infections of T.Spiralis

Fig.10 T.O. Mice

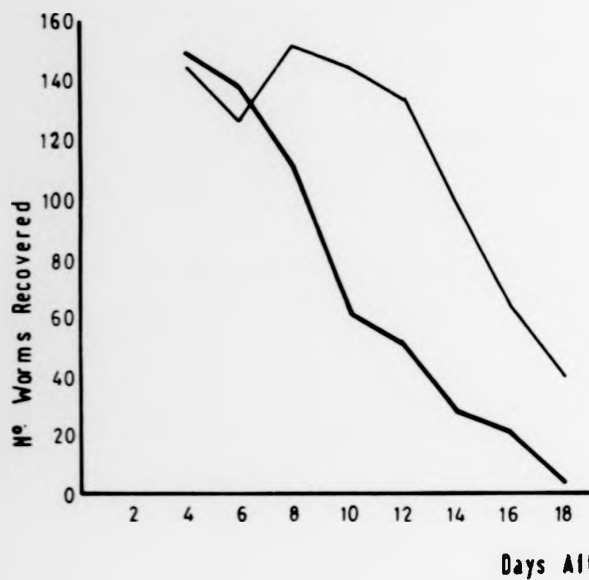
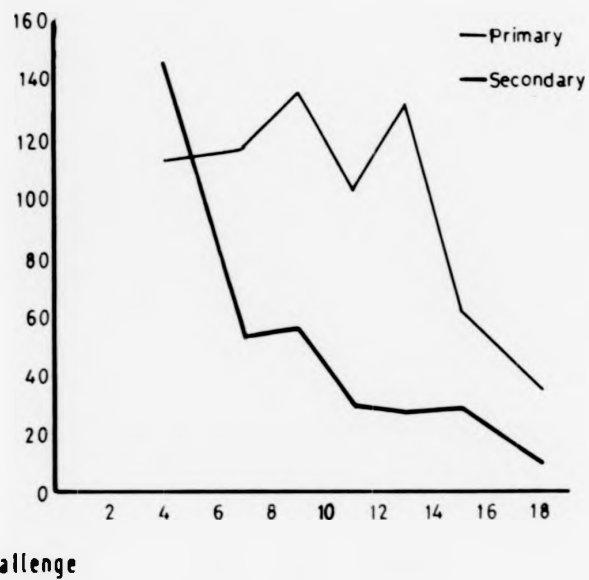


Fig.11 Snell Mice





towards the end of the infection period.

The primary infection pattern is comparable in both T.O. and Snell mice, the former start expulsion between days 12 and 14, after infection while the latter start expulsion around 13-15 days after infection. This suggests that the development of the immune response, to a primary infection, is similar in both strains of mouse. However the Snell mice start expulsion of challenge worms before the T.O. mice, implying that they have a better immunological memory.

#### Experiment 5.3.

The Snell mice were intended for use as the donors of sensitised cells, so this experiment attempted to define the patterns of cell blastogenesis associated with primary and secondary infections. The criteria of spleen (Sp.) to body weight and mesenteric lymph nodes (MLN) to body weight ratios were used as a means of timing cellular activity in the two organs.

The Snell mice used in determining the expulsion times of adult T. spiralis were weighed before being killed. After removal and incubation of the small intestines, the Sp. and MLN of each mouse were taken and weighed, so that Sp. to body weight and MLN to body weight ratios could be calculated. The results are shown in Table 9 and Fig. 12 .

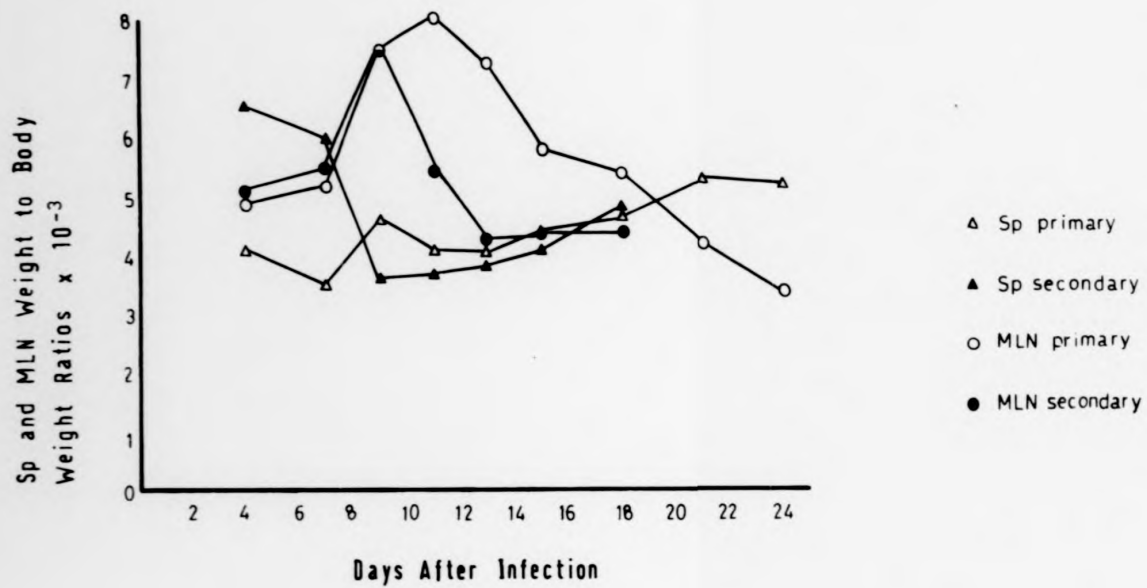
In the primary infection both the Sp. and MLN to body weight ratios rose after 7 days of infection. The Sp. to body weight ratios rose slowly throughout the monitored course of infection, but the MLN to body weight ratios rose sharply to peak 11 days after infection, and thereafter they dropped in value as worm expulsion

TABLE 9 , Exp. 5.3.

The mean Sp. to body weight and MLN to body weight ratios of Snell mice during initial and challenge infections with T. spiralis.

Days post- challenge	Challenge Infection		Initial Infection	
	Mean (SE) ratios of:		Mean (SE) ratios of:	
	Sp. to body weight (SE x 10 <sup>-4</sup> )	MLN to body weight (SE x 10 <sup>-4</sup> )	Sp. to body weight (SE x 10 <sup>-4</sup> )	MLN to body weight (SE x 10 <sup>-4</sup> )
4	0.0065 (2.4)	0.0051 ( 6.4)	0.0041 (6.9)	0.0049 (2.8)
7	0.0060 (7.0)	0.0055 ( 4.2)	0.0036 (2.7)	0.0052 (3.0)
9	0.0037 (2.9)	0.0075 ( 6.8)	0.0047 (4.7)	0.0075 (5.0)
11	0.0037 (6.4)	0.0054 ( 6.3)	0.0041 (4.7)	0.0080 (5.9)
13	0.0039 (2.3)	0.0042 ( 4.3)	0.0041 (3.8)	0.0072 (4.6)
15	0.0041 (2.5)	0.0044 ( 3.4)	0.0044 (2.8)	0.0058 (4.1)
18	0.0049 (5.6)	0.0043 (10.3)	0.0047 (3.5)	0.0054 (4.3)
21			0.0053 (5.2)	0.0042 (2.0)
24			0.0052 (5.9)	0.0034 (2.5)

Fig.12 The Mean Spleen (Sp) and Mesenteric Lymph Nodes (MLN) Weight to Body Weight Ratios of Snell Mice During Primary and Secondary Infections of T.Spiralis



commenced. This suggests that spleen cells are being stimulated by the parenteral phase as well as the intestinal phase of infection; but that mesenteric lymph node cells receive their major stimulation from the adult worms, with possible involvement of the migrating NBL. The Sp. to body weight ratios in the secondary infection had initially raised values, which fell as worm expulsion started. However, there was a further, small increase between days 9 and 18, which might imply that a few NBL were attempting to migrate to and penetrate muscle cells. The MLN to body weight ratios of the challenge infection fell in value after 9 days of infection, again they were probably stimulated by adult worms only.

#### Experiment 5.4.

The experiments in Chapter 6 attempt to describe an artificially produced secondary immune response against the parenteral phase of infection. If, in natural secondary infections, the reduced fecundity of female worms does not allow the production of NBL to occur, then the study of the secondary immune response against NBL is unrealistic.

This experiment was designed to determine the reduction in the fecundity of worms experiencing a secondary immune response by Snell mice.

Six Snell mice were given 300 muscle larvae; 3 weeks later these mice, along with 6 naive mice, were challenged with 300 muscle larvae. The mice were killed 6 days after challenge. A set of worms from each mouse was taken, washed and cultured individually for between 20-22 hours. After this time the NBL produced, from each

set of worms, were counted and the number of NBL produced per female per hour was calculated. The results are shown in Table 10.

Worms from the primary infection produced a mean of 2.0 ( $\pm$  0.39) NBL/female/hour; the worms from the challenge infection bore a mean of 1.65 ( $\pm$  0.38) NBL/female/hour. This only gave a 17.5% reduction in the fecundity of female worms experiencing a secondary immune response.

#### Experiment 5.5.

This experiment was a repeat of experiment 5.4. Ten Snell mice were divided into 2 groups; the first received 300 muscle larvae each, 3 weeks later both groups were challenged with 300 muscle larvae. 6 days after challenge the mice were killed and their worms cultured for 15-18 hours. The number of NBL produced/female/hour was recorded as before and the results are given in Table 10.

Worms from the primary infection produced a mean of 1.47 ( $\pm$  0.09) NBL/female/hour, whereas the challenge worms produced a mean of 0.84 ( $\pm$  0.19) NBL/female/hour. In this experiment there was a 43% reduction in the fecundity of females from a secondary infection.

#### Experiment 5.6.

This experiment tested the effectiveness of the immune response, in T.O. mice, against the parenteral phase of T. spiralis. Thirteen T.O. mice were injected, i.v., with 3 doses of NBL at weekly intervals. Two weeks later 7 immunised mice and 7 control mice were

TABLE 10

The fecundity of female *T. spiralis* worms, *in vitro*, taken 6 days after primary and challenge infections in Snell mice.

Exp. 5.4.

<u>Primary Infection</u>		<u>Secondary Infection</u>	
NBL/female/hour		NBL/female/hour	
	3.30		2.57
	0.57		2.71
	1.86		0.62
	2.35		0.64
	2.56		2.06
	<u>1.34</u>		<u>1.29</u>
Mean (SE) number of NBL produced	2.00 (+ 0.39)	Mean (SE) number of NBL produced	1.65 (+ 0.38)

Exp. 5.5.

<u>Primary Infection</u>		<u>Secondary Infection</u>	
NBL/female/hour		NBL/female/hour	
	2.47		1.31
	1.64		1.16
	1.59		0.77
	1.32		0.73
	<u>1.31</u>		<u>0.21</u>
Mean (SE) number of NBL produced	1.47 (+ 0.09)	Mean (SE) number of NBL produced	0.84 (+ 0.19)

challenged by i.v. injection of 6,000 NBL. The remaining 6 immunised mice acted as unchallenged controls. All the mice were killed and digested 28 days later; their muscle larvae were counted and the results are shown in Table 11 .

The mice immunised and challenged with NBL had a mean of 769 larvae more than the immunised-unchallenged mice, which is 18% of the number of larvae in the challenge controls. Thus these mice were 82% resistant to parenteral challenge.

#### Experiment 5.7.

This experiment tested the response of Snell mice to a challenge infection of NBL. 10 male Snell mice were given 6,000 NBL i.v. Two weeks later 5 of the immunised mice and 5 control mice were challenged by i.v. injection of 2,000 NBL. All the mice were killed and digested after 28 days; the muscle larvae were counted and the results are shown in Table 12.

The mice which were immunised and challenged in NBL had a mean of 225 muscle larvae more than the immunised unchallenged mice. This is 21% of the number of larvae in the challenge controls; thus these mice were 79% resistant to parenteral challenge.

Thus, after parenteral sensitisation, both T.O. and Snell mice are strongly protected against a parenteral challenge.

TABLE 11 , Exp. 5.6.

Immunity against parenteral challenge in T.O. mice.

Number of mice	Mouse group	Mean no. of larvae per mouse (SE)	No. of larvae attributed to challenge
6	Immunised not challenged	3,220 (+ 224)	
7	Immunised-challenged	3,990 (+ 532)	770
7	Challenged	4,360 (+ 453)	4,360

TABLE 12 , Exp. 5.7.

Immunity against parenteral challenge in Snell mice.

Number of mice	Mouse group	Mean no. of larvae per mouse (SE)	No. of larvae attributed to challenge
5	Immunised not challenged	3,954 (+ 526)	
5	Immunised-challenged	4,180 (+ 737)	225
5	Challenged	1,080 (+ 209)	1,080



The suitability of Snell mice for use in adoptive transfers

The Snell mice were not 'truly' inbred, so before using them in adoptive transfers it was necessary to test the histocompatibility of cells between individuals of the strain.

Experiment 5.8.

Skin grafting is a convenient method for testing the degree of histocompatibility between cells and this experiment utilised tail to chest grafts.

Three male Snell mice were killed and their tails were carefully stripped of skin. The skin was flattened, cut into 1 cm<sup>2</sup> pieces and kept moist with Medium 199. 13 male Snell mice were anaesthetised with 0.01 ml of Nembutal/10 g body weight. The right lateral chest area of each mouse was shaved, and a 1 cm<sup>2</sup> area of skin was cut to the underlying capillary bed and removed. Tail skin was placed on this area, coated with 'new skin' and covered with paraffin wax gauze. The chest was then bound with plaster of paris and left for 13 days. After removal of the dressings the grafts were studied for signs of rejection. All the grafts took well, and the first signs of sloughing were seen, in 8 of the mice, 41 days after grafting. Total rejection of all grafts did not occur until 69 days after grafting.

Experiment 5.9.

This experiment tested histocompatibility by tail to tail grafting. 12 female Snell mice were anaesthetised with Nembutal. 2 small pieces of skin, from each tail, were removed to the depth of the capillary layer and each transferred to the tail of another mouse. The grafts were gently pressed into their new sites and the tails were protected for 6 days with a plastic tube held in place by a clip. This method allowed the histocompatibility type of each mouse to be tested against 2 other mice. The first signs of rejection occurred in 3 of the mice, 38 days after grafting. Total rejection of all grafts was evident by day 48.

The two grafting methods used proved that the Snell mice did not differ at the major histocompatibility loci, as rejection would have started within 7 days. It is probable that they differed at one or more of the minor histocompatibility loci. These mice were considered suitable for use in adoptive transfers as graft rejection was a slow process.

Experiment 5.10.

This experiment tested the effect of transferred normal cells on a parenteral infection. 5 mice were given  $4 \times 10^7$  spleen cells (SpC) i.v. and 5 were given  $4 \times 10^7$  mesenteric lymph node cells (MLNC) i.v.; 5 mice were left as controls. All the mice were intravenously injected with 10,000 NBL 1 day after the cell transfers; 28 days later the mice were digested and their muscle larvae

counted. The results are shown in Table 13.

There was no significant difference between the groups, so the transfer of normal cells had no effect upon a parenteral infection.

#### Experiment 5.11.

It was necessary to determine whether or not transferred cells would function in their new sites. This was done by using direct and indirect plaque techniques (Dresser and Wortis, 1965; Cunningham and Szenberg, 1968).

This experiment was designed to assay the number of IgM producing transferred cells in the spleen, by the Direct Plaque Technique. On day 0, 6 donor mice were sensitised with  $1.8 \times 10^8$  sheep red blood cells (SRBC) i.p. On day 8, 8 naive mice were sublethally irradiated with 360 rads of whole body irradiation (30 rads/min. for 12 min.). On day 9 the donor mice were killed and 4 of the irradiated mice along with 4 control mice were given  $4 \times 10^7$  SpC i.v., 4 mice were not treated with either irradiation or cells. On the same day all the mice were challenged with  $1.8 \times 10^8$  SRBC i.p. On day 13 the mice in the 4 groups were assayed for the number of plaque forming cells (PFC) their spleens contained. The results are shown in Table 14 and Figure 13.

In the irradiated groups, the mice given sensitised cells showed a mean 2,700% increase in the PFC/spleen over their controls (or a 730% increase in the PFC/ $10^6$  SpC). So 4 days after their transfer to new mice these cells were showing a marked response to SRBC. The response to SRBC in the controls was low, because after irradiation

TABLE 13, Exp. 5.10.

The mean number of muscle larvae recovered from mice given normal cells and challenged with 10,000 NBL, i.v.

No. of mice	Mouse group	Mean worm recovery (SE)
5	SpC + NBL	5,026 (+ 507)
5	MLNC + NBL	5,272 (+ 678)
5	NBL only	4,843 (+ 325)

TABLE 14 , Exp. 5.11.

The mean number of plaque forming cells in irradiated and non-irradiated recipients of sensitised SpC.

Number of spleens	Mouse group	Mean number of PFC/SP (SE)	Mean number of PFC/10 <sup>6</sup> SpC (SE)
4	+ SpC + SRBC	8,100 (+ 1348)	296 (+ 61)
4	+ SRBC	800 (+ 337)	40 (+ 18)
4	SpC + SRBC	13,750 (+ 2602)	169 (+ 45)
4	SRBC	12,000 (+ 4880)	268 (+ 133)

few of the original SpC, capable of responding, would survive. In the non-irradiated groups, the mice given sensitised cells had a mean of 1,750 PFC/spleen more than their controls. A larger response than this in the sensitised group would not be expected as the assay measures the number of IgM producing cells and these have a poor immunological memory (Roitt, 1974). So the small increase in PFC is simply due to the additive effect of the introduction of donor PFC to the recipients own PFC. This experiment shows that the IgM producing cells of Snell mice will function normally when transferred to recipients.

#### Experiment 5.12.

This experiment was designed to assay the numbers of IgM producing cells and the IgG producing cells, in mice given lymphocytes sensitised to SRBC. 5 mice were sensitised with  $1 \times 10^9$  SRBC i.p., on day 0. On day 7 the donors were killed and their SpC prepared for transfer. 5 clean mice were given  $4 \times 10^7$  SpC i.v. plus  $1 \times 10^9$  SRBC i.p. 5 more mice were given SRBC only. On day 11 all the mice were killed and their spleens assayed by direct and indirect plaques. The results are shown in Table 15 and Fig. 14 .

The mice given sensitised cells showed a mean 20% decrease in the number of IgM producing cells per spleen compared to the controls (or an 18% decrease in PFC/ $10^6$  SpC). This decrease is not unusual, as IgM lymphocytes have little or no immunological memory and in some cases a secondary stimulation will cause a suppression of IgM production (Roitt, 1974) and this presumably has occurred here. The mean total number of PFC/spleen in the mice given sensitised cells

TABLE 15, Exp. 5.12.

The mean numbers of IgM and IgG producing cells per spleen in recipients given sensitised cells.

Number of spleens	Mouse group	Mean number of PFC/SP (SE)	Mean number of PFC/10 <sup>6</sup> SpC (SE)
5	Direct cells + SRBC	2,120 (+ 372)	12 (+ 2.8)
4	Direct SRBC only	2,650 (+ 299)	15 (+ 3.4)
5	Indirect cells + SRBC	10,900 (+ 3300)	63 (+ 18)
4	Indirect SRBC only	-269 (+ 998)	-2.1 (+ 6.2)

IgG cells = Total no. - (Direct no. x KI)    KI = 0.97.

Mean no. of IgG PFC/SP = 8,784

in recipients given sensitised cells.

Mean no. of IgG PFC/10<sup>6</sup> SpC = 51

N° of Plaque Forming Cells(PFC) per Spleen(Sp) in Mice Given Sensitised Spleen Cells(SpC) and SRBC.

Fig 13

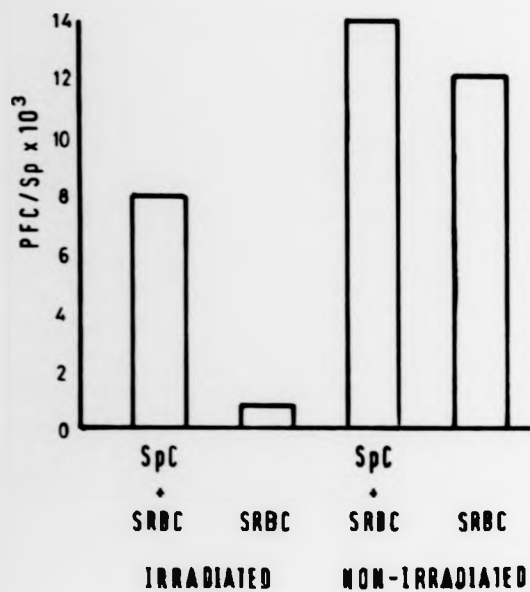
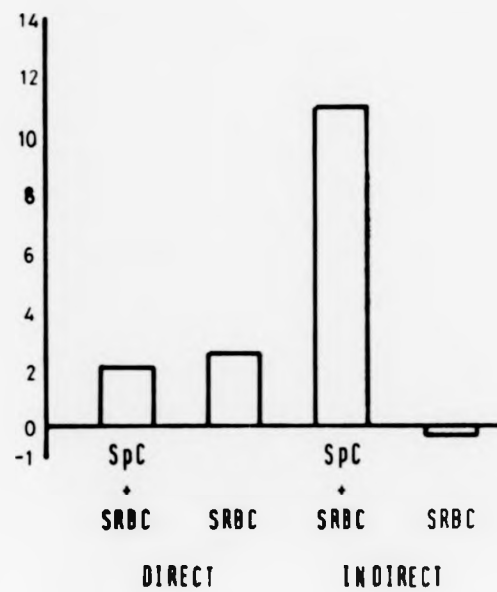


Fig 14





and tested by the indirect plaque technique was 10,900 ( $\pm$  3,300).

The number of IgG producing cells is given by:

$$\text{IgG} = \text{Total no.} - (\text{Direct no.} \times \text{KI}) \text{ where KI} = 0.97.$$

Therefore the mean number of IgG producing cells per spleen was 8,784 (or 51 cells/ $10^6$  SpC), which is a 4,000% increase over the control values. These results show that the sensitised, transferred IgG cells functioned normally in their new site as they had immunological memory and responded to challenge by early activation and an increase in the number of effector cells. The negative control values are reasonable, as these represent the numbers of IgG producing cells responding to a primary stimulation. Secondly stimulated cells will respond within 4 days, which was the time left between challenge and assay in this experiment, but this was not long enough to allow any IgG production from the primarily stimulated cells, and they would require at least 7 days to commence immunoglobulin production (Roitt, 1974).

#### DISCUSSION

The dynamics of primary and challenge infections, which are widely quoted in the literature, suggest that (a) primary infections are expelled towards the end of the second week of infection; (b) after one stimulating infection immunity remains comparatively feeble and (c) repeat infections are necessary to obtain a high level of immunity (Larsh, 1963). The expulsion times found in the primary infections in T.O. and Snell mice are in agreement with the first point. However the T.O. mice, used in these experiments, showed an

accelerated expulsion of challenge worms after one stimulating infection. Similarly Snell mice showed a rapid response to a second infection of T. spiralis and the expulsion of challenge worms commenced 6 days earlier than their primary counterparts. It would therefore seem that Snell and T.O. mice express immunological memory after a single infection of T. spiralis. If T.O. and Snell mice are compared to the CBA and NIH strains, described in Chapter 4, then they would fall into the category of average responders.

The average degree of immunity to parenteral challenge, in T.O. mice, was quoted as being 85% (James *et al.*, 1977). In accordance with this, experiment 5.6 showed that T.O. mice were 82% resistant to parenteral challenge, after 3 parenteral immunisations. Similarly, experiment 5.7 demonstrated that a single parenteral immunisation of Snell mice resulted in 79% protection against challenge. These figures suggest that there is little difference between single and multiple parenteral stimulations. The similarity in response of both T.O. and Snell mice to intestinal and parenteral worms shows the suitability of these 2 strains for conjunctive use in sensitised cell and serum production.

Studies on the fecundity of worms from a challenge infection in Snell mice show that NBL are produced in sufficient numbers to suggest that mice might need to develop an immune response to inhibit their migration to the muscle. This is supported by the spleen to body weight ratios, in Exp. 5.3, which show a gradual rise in values during the time of the production and migration of NBL in a challenge infection. Thus the few NBL which penetrate the gut are stimulating cell activity in the spleen. However the ratios of mesenteric lymph

nodes to body weight, for both primary and challenge infections, would suggest that these nodes are not involved in the latter stages of parenteral infection.

If the patterns of spleen and mesenteric lymph node to body weight ratios are indicative of the format of adoptive transfers then they suggest that the transfer of MLNC against intestinal worms would be most effective 7-11 days after a single immunisation of the donor mice. Similarly Wakelin and Lloyd (1976b) found that one stimulation resulted in the most effective transfer of MLNC, against adult T. spiralis, in NIH mice; however they found no difference in the efficacy of MLNC taken 4 or 8 days after immunisation.

The spleen to body weight ratios reported here suggest that SpC could be transferred most effectively 3 weeks after a primary stimulation or within a week of the last multiple stimulation. The pattern of ratios suggests that spleen cells are more involved with the parenteral rather than the enteral phase of infection. This would be expected, as in general the spleen deals with systemic antigens and the mesenteric lymph nodes with antigens entering from the gut.

The experiments on skin grafts showed that the Snell mice did not differ at the major histocompatibility locus, but that they differed at one or more the minor loci. The transfer of normal cells to recipient mice had no effect upon their worm burdens. It only takes 3 weeks for a T. spiralis infection to reach maturity and the skin grafts showed that the recognition of antigenic difference between the cells did not occur for at least 5 weeks. It is, therefore, reasonable to suppose that the eventual effects of the graft versus host responses will have no bearing on the infection and also

that transferred sensitised cells will have completed their mission before the incompatibility of the cell populations is realised.

The plaque assays only showed the immunoglobulin producing lymphocytes functioned normally in recipient mice; but if the B cells function there is no reason to suppose that the transferred T cells will not.

The experiments described in this chapter suggest that Snell mice and T.O. mice have a similar immunological response to T. spiralis and that Snell mice are suitable for use as the donors and recipients of sensitised cells.

## CHAPTER 6

Immunity against the parenteral phase of  
T. spiralis infections

A stage specific immune response operates against T. spiralis infections (James and Denham, 1975; James et al., 1977). The effector stage of the immune response against intestinal T. spiralis occurs in two sequential stages. The first stage involves the damage of worms by antibodies and non-specific cells (Love et al., 1976; Wakelin and Lloyd, 1976b; Wakelin and Wilson, 1977a) and the second the expulsion of worms by effector T cells and non-specific cells (Wakelin and Wilson, 1977b; Wakelin and Wilson, 1978). Little is known about the mechanisms of immunity which control the parenteral phase of a T. spiralis infection. It is not known whether the migrating NBL or the developing muscle larvae are susceptible to immunity but in vitro work suggests that the antibody mediated attachment of eosinophils to NBL may contribute to parenteral immunity (Mackenzie et al., 1978).

This chapter describes preliminary investigations into the immunological mechanisms which control the parenteral phase of T. spiralis.

Experiment 6.1.

This experiment was designed to study the effects of transferring serum and cells from mice, which had been immunized by the injection of NBL intravenously (i.v.) to naive mice. As donors 18 Snell and 10 T.O. mice were given 3 i.v. injections of 2,000 NBL each at weekly intervals. One week after the last sensitization the mice were killed and serum was

collected from all of them. Spleens were removed from the Snell mice and spleen cell suspensions (SpC) were prepared.

28 Snell mice were allotted to 4 groups; each mouse of the first group was given  $4 \times 10^7$  SpC i.v.; each mouse of the second group was given 1 ml serum i.p.; each mouse of the third group received both cells and serum and the fourth group of mice was left untreated. The following day each mouse was challenged by the i.v. injection of 2,000 NBL each. 28 days post challenge the number of muscle larvae in each mouse was enumerated. The results are summarized in Table 16, and Figure 15.

#### Experiment 6.2.

This experiment was essentially a repeat of 6.1. As donors 14 Snell mice were each given 3,000, 5,000 and 3,000 NBL at weekly intervals and 8 T.O. mice were each given 3,000, 2,000 and 2,000 NBL at weekly intervals. Serum from all the mice and SpC from the Snell mice were collected 7 days after the last immunization. 6 recipient Snell mice were each given  $4 \times 10^7$  SpC i.v.; 5 were each given 1 ml serum i.p.; 6 were each given both 1 ml serum i.p. and  $4 \times 10^7$  SpC i.v. and 6 were left untreated. One day after treatment all the mice were challenged with 1,500 NBL i.v. The results of the muscle larval counts made 28 days later are summarized in Table 16 and Figure 16.

In experiments 6.1. and 6.2. both SpC and serum conferred resistance to parenteral challenge but the two together were not substantially more effective than each alone. A combination of the two experiments shows that SpC conferred 66% immunity, serum 69% and both together 70%.

TABLE 16 Experiments 6.1. and 6.2.

The mean number of muscle larvae recovered from mice treated with parenterally sensitized cells and/or serum and challenged with 2,000 (6.1.) and 1,500 (6.2.) NBL.

Exp.	Treatment	No. mice /group	Mean (SE) no. of muscle larvae recovered	% protection	Significance of difference (p)
6.1.	4 x 10 <sup>7</sup> SpC i.v.	6	222 (40)	63%	
	1 ml serum i.p.	6	247 (18)	59%	
	4 x 10 <sup>7</sup> SpC i.v. + 1 ml serum i.p.	5	262 (30)	57%	
	Untreated control	5	602 (55)	-	
6.2.	4 x 10 <sup>7</sup> SpC i.v.	6	330 (47)	69%	
	1 ml serum i.p.	5	221 (66)	79%	
	4 x 10 <sup>7</sup> SpC i.v. + 1 ml serum i.p.	6	166 (32)	84%	
	Untreated control	6	1057 (50)	-	

Experiment 6.3.

This experiment was designed to study the effects of transferring cells and serum, taken from donors sensitized by full infections, on a parenteral challenge in naive, recipient mice.

22 Snell mice and 18 T.O. mice were immunized four times with 300 infective larvae each at 14 day intervals. Serum from all the donor mice and SpC and mesenteric lymph node cells (MLNC) from the Snell mice were collected 14 days after the last immunization. 41 recipients were assigned to 8 groups; the first group was given  $4 \times 10^7$  SpC i.v.; the second group was given double quantities of MLNC i.p., i.e.  $8 \times 10^7$ , and to control for this route of administration another group of mice was given  $8 \times 10^7$  SpC i.p.; the mice of the fifth group were each given 1.5 ml serum i.p. in two doses; the sixth group of mice was treated with  $4 \times 10^7$  SpC i.v. and 1.5 ml serum i.p.; the seventh group of mice was given  $8 \times 10^7$  MLNC i.p. and 1.5 ml serum i.p. and the final group was left untreated. One day after treatment, all the mice were challenged with 4,000 NBL each and autopsied 28 days later to determine the numbers of muscle larvae which had encysted. The results and protocol of this experiment are summarized in Table 17 and Figure 17.

The 3 groups of mice, treated with SpC or MLNC alone, showed a mean of 60% immunity against challenge. There was no significant difference between the larval burdens of these groups. No protection was obtained when SpC and MLNC were transferred together. The mice given serum alone were 99.7% resistant to challenge, those given serum and SpC were 99.5% resistant and those given serum and MLNC were 99.9% resistant. The degree of protection transferred by serum is remarkable, even taking into account the heavy immunization schedule to which the donors had been subjected.



TABLE 17 Experiment 6.3.

The worm burdens of mice treated with cells and/or serum, from donors sensitized by 4 infections of muscle larvae, and challenged with 4,000 NBL.

Treatment received	No. of mice /group	Mean (SE) no. of worms recovered	% protection	Significance of difference (p)
$4 \times 10^7$ SpC i.v.	6	496 (96)	43%	
$8 \times 10^7$ SpC i.p.	4	310 (54)	64%	
$8 \times 10^7$ MLNC i.p.	4	242 (180)	72%	
$8 \times 10^7$ (SpC + MLNC) i.p.	5	707 (87)	18%	
1.5 ml serum	6	2.3 (1.2)	99.7%	
$4 \times 10^7$ SpC i.v. + 1.5 ml serum	6	4.3 (2)	99.5%	
$8 \times 10^7$ MLNC i.p. + 1.5 ml serum	4	1.25 (0.8)	99.9%	
Untreated control	6	862 (95)	-	

Experiment 6.4.

This experiment tested the ability of cells and serum, derived from donors which had received only one full stimulating infection of muscle larvae, to transfer protective immunity.

12 Snell and 30 T.O. mice were infected with 300 muscle larvae given per os. Cells and serum were collected from the infected, 5 naive Snell mice and 3 naive T.O. mice 24 days later. 50 recipient mice were assigned to 10 groups which, if treated, were given cells and/or serum. One day after the transfers all the mice were challenged with 10,000 NBL i.v. The protocol and results of this experiment are summarized in Table 18 and Figure 18.

There was no significant difference between the number of larvae in the mice given MLNC and SpC i.p. and the mice given SpC i.v. The mean resistance to challenge of these 3 groups was 36% when compared to the untreated control group. The 3 groups which received immune serum showed no significant differences between their larval burdens, thus the combination of cells and serum was not additive. The mean degree of protection in the 3 groups which received serum was 72%, approximately twice the mean level of protection found in the groups treated with cells alone.

The fact that serum from a single full infection was as potent as that derived from the multiple parenteral immunizations, in experiments 6.1. and 6.2., can be accounted for by the potentially large parenteral stimulation resulting from single intestinal infections (Thomas, 1965; Roth, 1939).

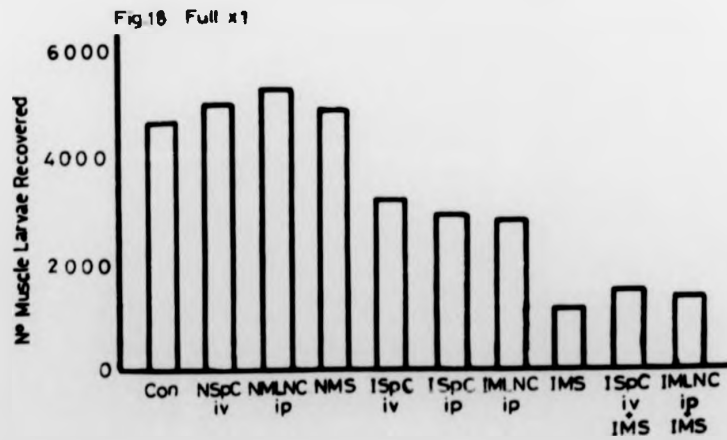
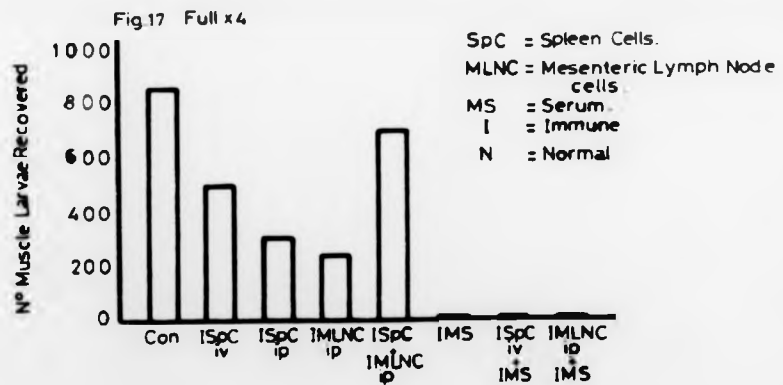
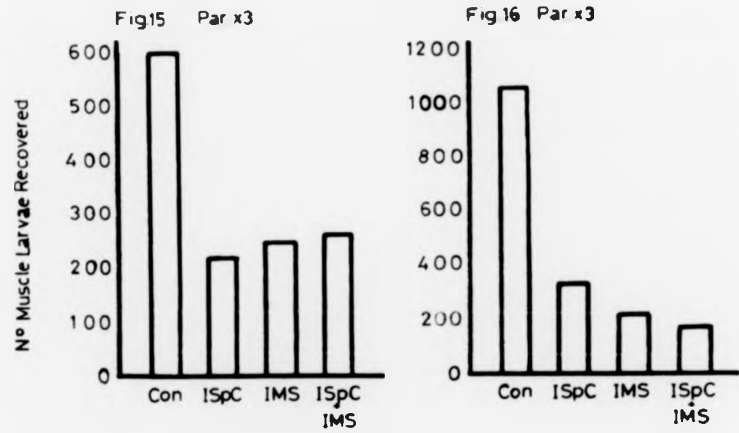
Normal cells and serum had no effect upon the establishment of larvae in the muscles.

TABLE 18 Experiment 6.4.

The worm burdens of mice treated with cells and/or serum, from donors sensitized by 1 infection of muscle larvae, and challenged with 10,000 muscle larvae. There were 5 mice per group.

Treatment received	Mean (SE) no. of worms recovered	% protection	Significance of difference (p)
4 x 10 <sup>7</sup> SpC i.v.	3,238 (519)	31%	0.3
8 x 10 <sup>7</sup> SpC i.p.	2,897 (417)	38%	↓
8 x 10 <sup>7</sup> MLNC i.p.	2,823 (1887)	40%	0.49
1.5 ml serum i.p.	1,148 (553)	76%	0.35
4 x 10 <sup>7</sup> SpC i.v. + 1.5 ml serum i.p.	1,490 (530)	68%	0.4
8 x 10 <sup>7</sup> MLNC i.p. + 1.5 ml serum i.p.	1,352 (277)	71%	↓
4 x 10 <sup>7</sup> normal SpC i.v.	5,026 (513)		
8 x 10 <sup>7</sup> normal MLNC i.p.	5,272 (686)		0.0005
1.5 ml normal serum i.p.	4,892 (401)		0.0005
Untreated control	4,683 (361)		0.2

N° of Muscle Larvae Recovered from Mice Given Cells and Serum from Donors Given Parenteral or Full Infections



Experiment 6.5.

The previous experiments have shown that SpC, sensitized by both full and parenteral infections, conferred varying degrees of protection against parenteral challenge. Experiments 6.3. and 6.4. showed that MLNC, sensitized by full infections, could also transfer immunity against parenteral challenge. In this experiment an attempt was made to ascertain whether MLNC from mice exposed to parenteral infections only, could transfer protection to recipients challenged with NBL.

12 donor Snell mice and 35 donor T.O. mice were each infected twice with 10,000 and 5,000 NBL i.v. with a 4 weeks interval between. 7 days after the second infection serum was collected from all the mice and SpC and MLNC were taken from the Snell mice.

35 Snell mice were divided into 7 groups; 6 of these were treated with cells and/or serum and one day after the transfers, all the mice were challenged with 10,000 NBL each. The mice were autopsied 28 days post challenge; the results and protocol of this experiment are summarized in Table 19.

In this experiment immune serum gave 54% protection against challenge. When  $4 \times 10^7$  SpC (i.v.) were given with serum, there was a marginally significant enhancement of immunity (71%), while this number of SpC (i.v.), given alone, failed to transfer protection. It should be noted, in this context, that  $8 \times 10^7$  SpC (i.p.) given without serum conferred marginally significant protection (38%). Similarly,  $8 \times 10^7$  MLNC (i.p.), given with serum, conferred a level of protection (68%) which was just significantly better than that transferred by serum alone (54%). However, this number of MLNC (i.p.) alone did not transfer immunity.

TABLE 19 Experiment 6.5.

The protective capacity of cells and/or serum, taken from parenterally infected donors, against a challenge of 10,000 NBL in recipient mice.

Treatment	No. of mice per group	Mean (SE) no. of muscle larvae recovered	% protection	Probabilities
$4 \times 10^7$ SpC i.v.	5	3296 (397)	9%	0.025
$8 \times 10^7$ SpC i.p.	4	2260 (129)	38%	0.025
$8 \times 10^7$ MLNC i.p.	5	3386 (307)	7%	0.025
1.3 ml serum i.p.	5	1664 (236)	54%	0.05
$4 \times 10^7$ SpC i.v. + 1.3 ml serum i.p.	5	1066 (204)	71%	0.05
$8 \times 10^7$ MLNC i.p. + 1.3 ml serum i.p.	5	1146 (204)	68%	0.4
Untreated control	5	3630 (392)		0.0005

Experiment 6.6.

It is clear that immune serum has strong activity against an intravenous challenge of NBL. This experiment was designed to determine the amount of serum required to transfer immunity.

150 T.O. mice were immunized with 200 infective larvae each; 50 days later they were given 4,000 NBL each and 14 days later 6,000 NBL each. The mice were bled for serum 21 days after the last infection. 11.75 ml of this serum was used in the dose response determination, the rest was aliquoted and stored at  $-70^{\circ}\text{C}$ , until it was required for subsequent experiments. It was termed Immune mouse serum - experiment 6.6 (IMS - Exp 6.6).

35 T.O. mice were randomly assigned to 7 groups, one group was left untreated. Each of the other groups was given an injection of serum i.p. 9 hours later all the groups were challenged with 5,000 NBL. 28 days later all the mice were killed and the number of muscle larvae in each mouse counted. The protocol and results are summarized in Table 20 and Figure 19.

It can be seen that 0.05 ml serum conferred no significant protection against challenge. However, 0.1 ml of serum gave a passive protection of 37%. Better protection was obtained with 0.4 and 0.6 ml amounts of serum and these values were only just significantly different to the protection derived from treatment with 1 ml amounts of serum. It would thus appear that increasing amounts of serum, from 0.1 ml onwards, transferred progressively better protection against challenge, with NBL, in recipient mice.

TABLE 20 Experiment 6.6.

The dose response of T.O. mice treated with immune serum and challenged with 5,000 NBL. There were 5 mice per group.

Amount of serum administered i.p.	Mean (SE) no. of muscle larvae recovered	% protection	Probabilities
0.05 ml	2636 (253)	6%	
0.1 ml	1754 (104)	37%	
0.2 ml	1772 (202)	37%	
0.4 ml	1194 (250)	57%	0.0005
0.6 ml	1186 (130)	58%	0.0125
1.0 ml	806 (173)	71%	0.35
Untreated control	2796 (375)		0.0025
			0.0125



Experiment 6.7.

This experiment was designed to determine at what time after infection the factors responsible for immunity appeared in the serum.

76 T.O. mice were immunized with 10,000 NBL on day 0, groups of at least 8 mice were exsanguinated at 4 day intervals until day 36 post infection. All the sera obtained were stored at  $-70^{\circ}\text{C}$  until needed.

50 T.O. mice were assigned to 10 groups of 5. Each of 9 groups were given 0.6 ml amounts of one batch of serum, the last group was left untreated. One day after the serum transfers all the mice were challenged with 1,500 NBL. 28 days later the mice were autopsied and their muscle larvae counted. The protocol and results are summarized in Table 21 and Figure 20.

The serum taken from donor mice did not transfer protection until the parenteral infection in the donors was 20 days old. After this time the serum became progressively more effective against challenge until 36 days after infection.

Experiment 6.8.

In this experiment an attempt was made to determine how long protective factors remained in the serum. 28 T.O. mice were immunized with 2 infections of 10,000 NBL, at weekly intervals and a final infection of 5,000 NBL 2 weeks later. These mice were then assigned to 4 groups; which were exsanguinated 1, 5, 9 and 12 months after infection respectively. All the sera were stored at  $-70^{\circ}\text{C}$  until required.

21 T.O. mice were allotted to 5 groups. Each mouse of 4 groups was given serum from either the 1, 5, 9 or 12 month old infection. 5

TABLE 21 Experiment 6.7.

The protective capacity of batches of serum which had progressively longer exposure times to a single parenteral immunization. 0.6 ml of serum was given i.p. to each recipient mouse, there were 5 mice per group and the challenge was 1,500 NBL.

Serum taken days after infection	Mean (SE) no. of muscle larvae recovered	% protection	Probabilities
4 days	210 (32)	16%	
8 days	221 (27)	12%	0.15
12 days	250 (31)	0%	0.15 0.1
16 days	255 (33)	-2%	0.025 0.025
20 days	170 (20)	32%	0.25 0.2
24 days	149 (20)	41%	0.35 0.25
28 days	159 (15)	37%	0.025 0.45
32 days	112 (12)	55%	0.2 0.0125
36 days	92 (21)	63%	0.0005
Untreated control	251 (32)	-	0.25

Fig.19 Dose Response of Mice Treated with Serum

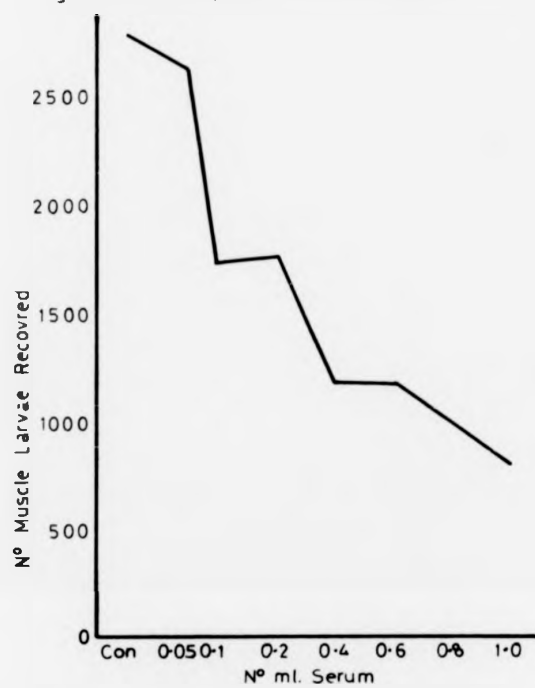
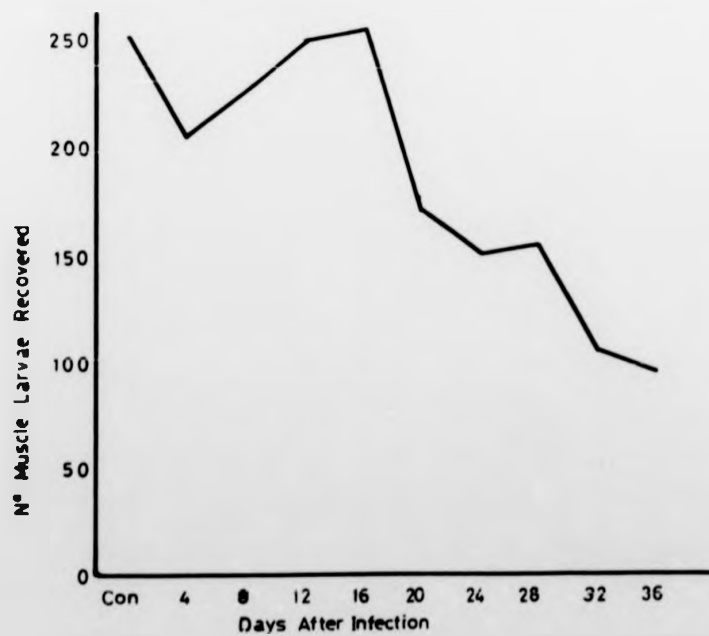


Fig.20 Protection Afforded by Serum After Increasing Exposure to Parenteral Infection



mice were left untreated. One day later all the mice were challenged with 5,000 NBL. The mice were autopsied and their muscle larvae counted 28 days later. The results are shown in Table 22.

Immune factors were still present in the serum taken 5 months after the final immunization but the protective capacity provided by this serum was only half of that afforded by the serum taken 1 month post immunization. Serum taken 9 and 12 months after the last stimulation, transferred no protection to recipient mice.

Experiment 6.9. (i), (ii) and (iii).

Experiment 6.7. showed that protective factors were present in serum taken 20 days after a primary parenteral infection. The next series of experiment attempted to ascertain the time taken for protective cells to appear in the spleen, after a single parenteral immunization.

35 T.O. mice were immunized twice with 10,000 NBL given at 2 weekly intervals, they were exsanguinated 2 weeks after the last immunization. The serum prepared was stored at  $-70^{\circ}\text{C}$  until required to treat the experimental controls in experiments 9 (ii) and 9 (iii).

21 Snell mice and 31 T.O. mice acted as donors for the serially timed production of sensitized cells and serum. These mice were immunized once with 10,000 NBL.

Experiment 6.9. (i)

In the first of these experiments 8 Snell and 10 T.O. mice were killed 6 days after their single immunization and SpC and serum were prepared. 5 Snell mice were each given  $4 \times 10^7$  of these SpC i.v., 5

TABLE 22 Experiment 6.8.

The mean number of muscle larvae recovered from mice treated with serum taken at different times after infection of the donors.

Age of serum post immunization	No. of mice per group	Mean (SE) no. of larvae recovered	% protection	Probabilities
1 month	4	1170 (156)	58%	0.0125
5 months	4	1955 (231)	30%	0.1
9 months	4	2540 (367)	9%	0.05 0.3 0.0125 0.005
12 months	4	2790 (243)	0%	0.45 0.35
Untreated control	5	2796 (375)		

were each given 1 ml of the serum i.p. and 4 were each given both cells and serum. These 3 groups of mice, along with 5 untreated mice, were challenged 1 day later with 10,000 NBL. All the mice were autopsied 28 days later and the muscle larvae counted. The results are summarized in Table 23a.

The muscle larval burdens of the experimental groups were not significantly different to those of the control mice. So, as would be expected from the results of Experiment 6.7., serum taken at this early stage after immunization had no protective capacity against a challenge of NBL. Although lymphocytes would be present in the granulomas forming around the infected muscle cells of the donor mice (Gould, 1970), the SpC taken 6 days after infection could not transfer immunity.

#### Experiment 6.9. (ii)

7 of the Snell and 10 of the T.O. donors were killed 8 days after the immunization and cells and serum were prepared. 25 Snell mice were arranged in 5 groups; the first group was given SpC and the second serum. The third group was treated with the stored, control, immune serum taken from the T.O. donors which had been infected twice; the fourth group was given both SpC and the control immune serum; the last group remained untreated. All the mice were challenged with 15,000 NBL 1 day after treatment and were autopsied 28 days later. The protocol and results are shown in Table 23a.

Again it can be seen that serum and SpC taken 8 days after a single parenteral sensitization, conferred no protection against challenge on recipient mice. The control immune serum, obtained from donors which were stimulated twice, transferred 81% protection against challenge; SpC given with this serum did not increase its protective capacity.

TABLE 23a Experiments 6.9 (i) and (ii)

The mean number of muscle larvae recovered from mice challenged with NBL and treated with serum and/or cells which were taken 6 and 8 days after the single immunization of donor mice with 10,000 NBL. There were 5 mice per group.

Cells and serum taken: (i) 6 days post immunization. Challenge of 10,000 NBL given				(ii) 8 days post immunization. Challenge of 15,000 NBL given		
Treatment	Mean (SE) no. of larvae recovered	% protection	Probabilities	Mean (SE) no. of larvae recovered	% protection	Probabilities
$4 \times 10^7$ SpC i.v.	5747 (499)	1%		6268 (547)	18%	0.05
1 ml serum i.p.	6016 (448)	-4%		7887 (584)	-3%	
$4 \times 10^7$ SpC i.v. + 1 ml serum i.p.	5180* (508)	11%	0.4			0.1
1 ml control immune serum i.p.	-		0.25 0.47	1442 (182)	81%	0.4
$4 \times 10^7$ SpC i.v. + 1 ml control immune serum i.p.	-			1542 (331)	80%	0.0005
Untreated control	5792 (598)			7634 (743)		

\* This group contained 4 mice.

Experiment 6.9. (iii)

In the final experiment of this series, the remaining Snell and T.O. donors were killed 17 days after their immunization and SpC and serum were prepared. 25 clean Snell mice were assigned to 5 groups; the first group was treated with SpC, the second with serum and the third group was given both cells and serum. Mice of the fourth group were given the control immune serum and the last group of mice acted as untreated controls. All the mice were challenged with 7,000 NBL 1 day after the transfers occurred and were autopsied 28 days later. The results of the larval counts are shown in Table 23b.

The control immune serum (from donors which had been immunized twice) conferred 77% protection upon the recipients, a similar measure to that obtained in experiment 6.9. (ii). The experimental serum (1.0 ml amounts), taken 17 days after the single parenteral immunization, conferred marginally significant protection against challenge (28%), which might be expected from the results of experiment 6.7., where serum (0.6 ml amounts), taken 20 days after a single parenteral sensitization, gave 32% protection. SpC, given alone, did not protect the recipients, nor did they enhance the passive immunity (28%), when given with the experimental serum (26%).

Experiment 6.10.

The next series of experiments attempted to determine whether serum acted against a parenteral infection before or after the penetration of muscle cells by the NBL.

14 Snell mice were immunized with 6,000 NBL each and exsanguinated



TABLE 23b Experiment 6.9 (iii)

The mean number of muscle larvae recovered from mice challenged with 7,000 NBL and treated with serum and/or cells which were taken 17 days after the single immunization of donor mice with 10,000 NBL. There were 5 mice per group.

Treatment	Mean (SE) no. of larvae recovered	% protection	Probabilities
$4 \times 10^7$ SpC i.v.	4249 (353)	7%	
1 ml serum i.p.	3306 (254)	28%	0.45
$4 \times 10^7$ SpC i.v. + 1 ml serum i.p.	3366 (176)	26%	0.3
1 ml control immune serum i.p.	1038 (253)	77%	0.0005, 0.025, 0.025
Untreated control	4571 (498)		

4 weeks later along with 10 naive Snell mice. Immune and normal sera were prepared. 22 recipient Snell mice were allotted to 4 groups; 5 mice were each treated with 1.5 ml of the immune serum and 5 were each given 1.5 ml of the normal serum. These mice along with 12 untreated mice were challenged 24 hours later with 2,000 NBL each. 6 of the untreated and challenged mice were left as challenge controls and the other 6 were each given 1.5 ml of immune serum 4 days after challenge. The results of the muscle larval counts, made 28 days later, are summarized in Table 24 and Figure 21.

The mice given immune serum before challenge were only 51% resistant, but this should be related to the light immunization of the donors. The mice given serum 4 days after challenge yielded more larvae than the controls and were obviously not protected. Normal serum had no effect on the establishment of muscle larvae.

#### Experiment 6.11.

This experiment was essentially a repeat of 6.10. 12 T.O. mice were immunized with 10,000 NBL each, 4 weeks later each mouse was given a second infection of 5,000 NBL. One week later their serum was collected. 15 recipient T.O. mice were allotted to 3 groups; serum was given to one group 1 day before challenge with 10,000 NBL and to another group 4 days after challenge, the last group was untreated and acted as the challenge control. The results of the larval counts made 28 days later are shown in Table 24 and Figure 22.

The mice given serum before challenge were 54% resistant, whereas those given serum 4 days after challenge were as susceptible as the controls.

TABLE 24 Experiments 6.10 and 6.11.

The mean number of muscle larvae recovered from mice treated with serum before or after challenge.

	Treatment given i.p.	Time of treatment	No. of mice per group	Mean (SE) no. of muscle larvae	% protection	Probabilities
<u>Exp. 6.10.</u> 2,000 NBL on Day 0	1.5 ml serum	Day -1	5	299 (118)	51%	
	1.5 ml serum	Day +4	6	880 (318)	-45%	
	1.5 ml normal serum	Day -1	5	633 (66)	-4%	
	Untreated control		6	606 (115)	-	
<u>Exp. 6.11.</u> 10,000 NBL on Day 0	1 ml serum	Day -1	5	1664 (236)	54%	
	1 ml serum	Day +4	5	3542 (376)	4%	
	Untreated control		5	3630 (392)		

Experiment 6.12.

This experiment was designed to determine how soon, after challenge, the administration of immune serum ceased to be effective.

The immune serum, used in this experiment, was IMS - Exp 6.6. 5 T.O. mice were given 0.6 ml of this serum and 2 hours later were challenged, along with 30 other mice, with 3,000 NBL. 5 of these 30 mice were left as challenge controls, the rest were randomly assigned to 5 groups and were given 0.6 ml IMS - Exp 6.6 serum each, in groups of 5, at 2, 6, 24, 48 and 72 hours after challenge. All the mice were killed and digested 28 days later. The summary of protocol and results is shown in Table 25 and Figure 23.

The mice given serum 2 hours before challenge were 50% resistant, but all the groups given serum after challenge (2 hours - 72 hours) were not significantly different from the control group.

Experiment 6.13. (i) and (ii)

In vitro studies (Experiment 6.19.) had indicated that the peritoneal exudate cells (PEC), from naive rats and mice, adhered in comparable densities to NBL, in the presence of homologous immune serum, but significantly fewer rat PEC adhered in the presence of mouse serum. If passively transferred serum requires the co-operation of the recipients non-specific cells, then these in vitro studies would indicate that heterologous (rat) serum would be less effective than homologous serum, in conferring passive immunity to mice. This was investigated in experiments 6.13. (i) and 6.13. (ii).

TABLE 25 Experiment 6.12.

The mean number of muscle larvae recovered from mice treated with serum before or after challenge with 3,000 NBL. There were 5 mice per group.

Time at which 0.6 ml serum was given (i.p.) relative to challenge with NBL	Mean (SE) no. of muscle larvae recovered	% protection	Probabilities
-2 hours	366 (42)	50%	
+2 hours	608 (122)	16%	
+6 hours	690 (35)	5%	
+24 hours	848 (54)	-16%	
+48 hours	833 (67)	-14%	
+72 hours	643 (49)	12%	
Untreated control	728 (84)		

The Mean N° of Muscle Larvae Recovered from Mice Given Serum Before or After Challenge

Fig.21.

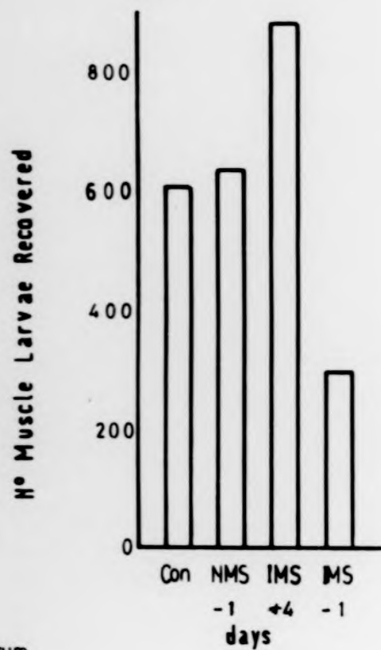
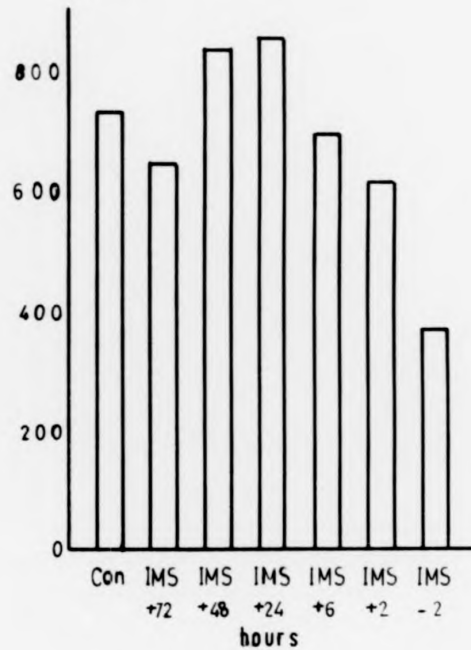


Fig.22.



Fig.23.



NMS=normal mouse serum  
IMS=immune mouse serum

Experiment 6.13.(i)

10 T.O. mice were immunized twice, with 8,000 NBL and 9,000 NBL at weekly intervals and were exsanguinated 2 weeks later. The serum was stored at  $-70^{\circ}\text{C}$ . 4 rats were immunized with 60,000 NBL each, followed one week later by 65,000 NBL each. These rats were bled 2 weeks after the last immunization and the serum was stored at  $-70^{\circ}\text{C}$ . A further 5 donor rats were sensitized with 4,000 infective larvae each, followed by a second infection of 8,000 infective larvae 3 weeks later. These rats were bled 3 weeks after the final immunization and the serum was stored at  $-70^{\circ}\text{C}$ .

16 recipient T.O. mice were assigned to 4 groups, the mice of the first group were each given 0.8 ml of the immune mouse serum; each mouse of the second group received 0.8 ml of the serum from rats sensitized by parenteral infections; each mouse of the third group received 0.8 ml of the rat serum taken from fully infected donors and the last group of mice was untreated. One day after the transfer of serum all the mice were challenged with 10,000 NBL. The results of the muscle larval counts, made 28 days later, are shown in Table 26 and Figure 24.

The mouse serum and the rat serum from parenterally infected donors conferred 62% and 58% protection respectively, on the recipient mice. However, the serum from fully infected donor rats showed no protective capacity.

Experiment 6.13. (ii)

The protection afforded to mice, by the two types of stored rat serum (parenteral and full immunization), used in the previous experiment, was tested again. The control immune mouse serum employed was IMS - Exp 6.6.

18 T.O. mice were assigned to 4 groups; the mice of the first group were each given 1 ml of the immune mouse serum; the mice of the other experimental groups received 1 ml each of the serum taken from parenterally infected rats or 1 ml each of the serum taken from fully infected rats; the last group of mice received no serum. One day after the serum transfers all the mice were challenged with 5,000 NBL. The results of this experiment are shown in Table 26 and Figure 25.

The immune mouse serum conferred 71% protection upon recipient mice. The larval burdens of the 2 groups given rat serum, taken from either parenterally infected or fully infected donors, were not significantly different from those of the untreated group.

#### Experiment 6.14.

In neonatal and lactating hosts the humoral response, against intestinal N. brasiliensis, functions, as a normal pattern of worm damage occurs, but there is a deficiency in the effector T cell response which prevents worm expulsion (Connan, 1972; Love and Ogilvie, 1974). T-cells may be blocked in lactating animals, but the T-cell population is deficient in young animals (Doenhoff, Leuchars, Kerbel, Wallis and Davies (1979)).

This experiment used young and lactating recipient mice, in an attempt to elucidate the action of passive immunity without the possible interference of effector T cells. Neonatal mice could not be used as it was impossible to challenge them intravenously with NBL.

30 T.O. and 12 Snell mice were infected with 400 infective larvae each followed 6 weeks afterwards by 300 infective larvae each; a third infection of 2,000 NBL was given to each mouse 3 weeks later. These donor mice were killed 10 days after the final infection and SpC and

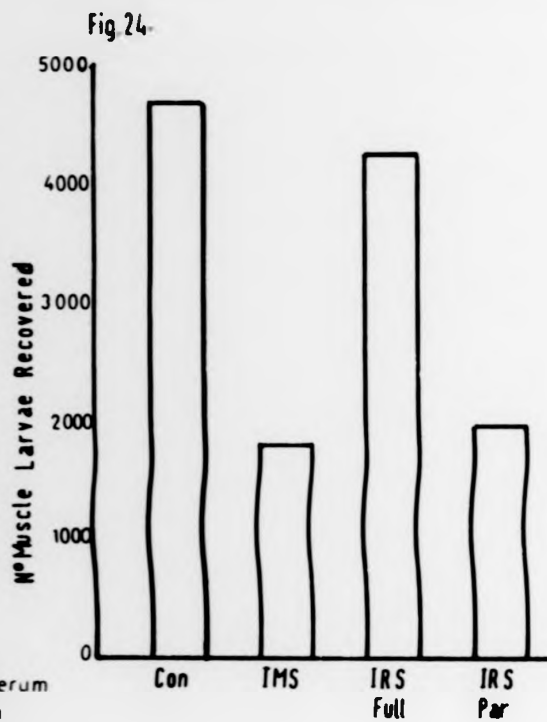


TABLE 26 Experiments 6.13 (i) and 6.13 (ii)

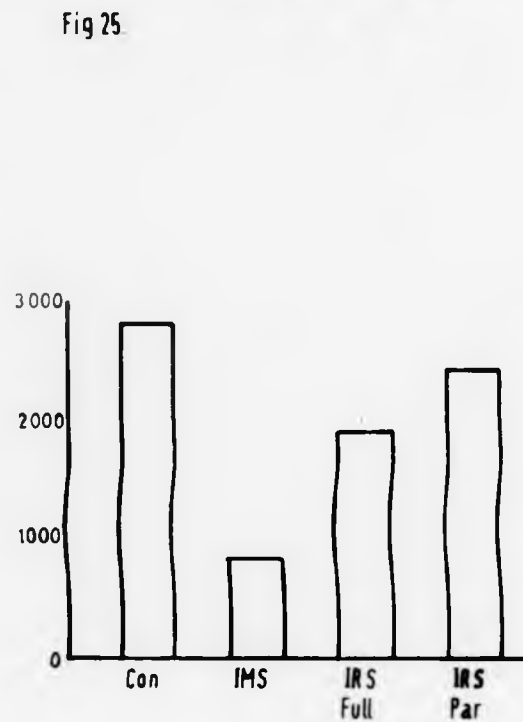
The mean number of muscle larvae recovered from mice treated with immune mouse or rat serum before challenge.

	Treatment	No. of mice per group	Mean (SE) no. of muscle larvae recovered	% protection	Probabilities
<u>Exp. 6.13 (i)</u> challenge of 10,000 NBL	0.8 ml immune mouse serum (i.p.)	4	1785 (649)	62%	0.4
	0.8 ml rat serum (i.p.) immunized parenterally	4	1947 (455)	58%	0.0025      0.025
	0.8 ml rat serum (i.p.) immunized by full infections	4	4237 (322)	9%	0.025 0.35
	Untreated control	4	4640 (900)	-	
<u>Exp. 6.13 (ii)</u> challenge of 5,000 NBL	1 ml immune mouse serum (i.p.)	5	806 (173)	71%	
	1 ml rat serum (i.p.) immunized parenterally	4	2413 (321)	14%	0.0005 0.1
	1 ml rat serum (i.p.) immunized by full infections	4	1883 (384)	33%	0.2 0.1-0.05
	Untreated control	5	2796 (375)	-	

The Mean N<sup>o</sup> of Muscle Larvae Recovered from Mice Given Immune Mouse or Rat Serum



IMS = immune mouse serum  
IRS = immune rat serum  
Full = full infection  
Par = parenteral infection



serum were prepared.

3 types of recipient Snell mice were used. These consisted of 15 normal 8 week old Snell females, 15, 2½-3½ week old Snell females and 12, 10 week old lactating Snell mice, which were within a few days of parturition. Mice of each type were allotted to 3 groups, each mouse of one group and type received either SpC or serum or were left untreated. The mice, in the three cell groups, were each given  $6 \times 10^7$  SpC one day before challenge. The cells were administered i.p. to all groups, as the tail veins of the young mice were still very small and damage to them would have made the i.v. injection of NBL more difficult. Each lactating and normal mouse in the serum group was given 0.7 ml of serum 5 hours before challenge. At the same time, 5 young mice were each treated with 0.5 ml serum i.p. All the treated and untreated mice were challenged by i.v. injection of 8,000 NBL; one infection of an untreated young mouse failed as the NBL could not be injected. The mice were autopsied 28 days later and the individual muscle larval burdens of each were enumerated. The protocol and results of this experiment are summarized in Table 27 and Figure 26.

The immune serum conferred 87% protection on normal recipient mice but functioned less effectively in young and lactating mice (60% and 35% protection respectively). SpC transferred 31% protection to normal recipient mice but failed to protect the young and lactating recipients.

#### Experiment 6.15.

This experiment was designed to determine whether or not macrophages played a role, with serum, in the effector stage of the immune response against a parenteral challenge.

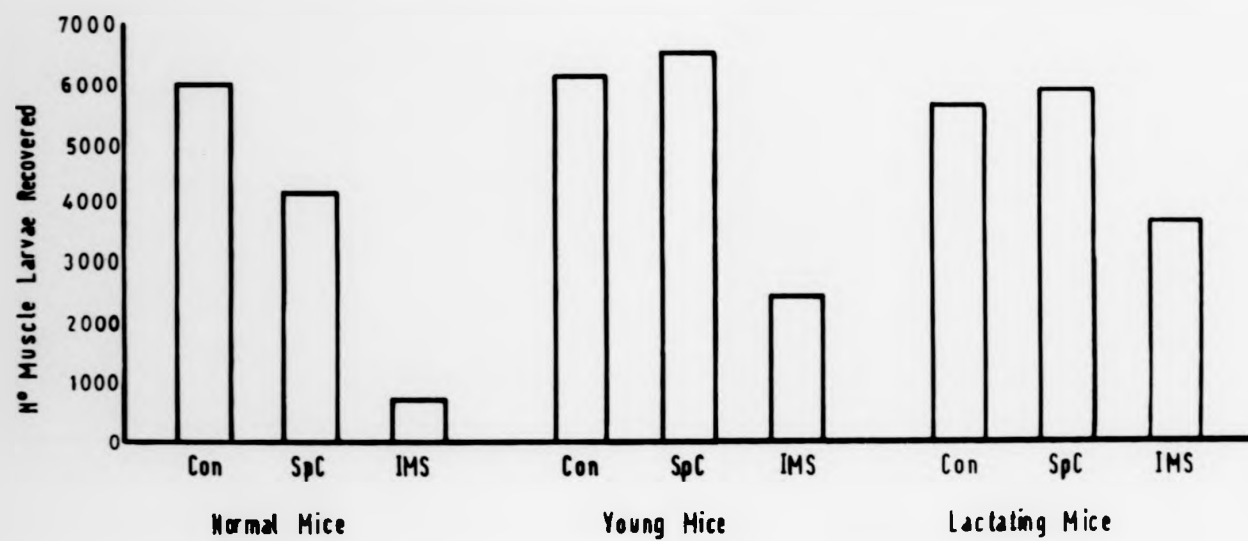
Polyvinyl-pyrrolidone (PVP-360 - average Mol. Wt. 360,000 -

TABLE 27 Experiment 6.14.

The mean number of muscle larvae recovered from normal, young and lactating mice after treatment with immune cells or serum and challenge with 8,000 NBL.

Type of mouse	Treatment	No. of mice per group	Mean (SE) no. of muscle larvae recovered	% protection	Probabilities
NORMAL	$6 \times 10^7$ SpC i.p.	5	4,112 (325)	31%	
	0.7 ml serum i.p.	5	786 (314)	87%	
	Untreated control	5	5,956 (432)	-	
YOUNG	$6 \times 10^7$ SpC i.p.	5	6,476 (700)	-6%	
	0.5 ml serum i.p.	5	2,446 (271)	60%	
	Untreated control	4	6,112 (784)	-	
LACTATING	$6 \times 10^7$ SpC i.p.	4	5,797 (535)	-5%	
	0.7 ml serum i.p.	4	3,589 (429)	35%	
	Untreated control	4	5,525 (440)	-	

Fig. 26 The Mean No. of Muscle Larvae Recovered from Normal, Young and Lactating Mice Given Spleen Cells (SpC) or Serum (IMS)



from Sigma Chemical Co.) is immunologically inert, has a high molecular weight and like Silicone or carbon particles will be phagocytosed to saturation point by macrophages, causing a temporary loss of their phagocytic ability and 10 mg of PVP given i.p. to mice will block the activity of their macrophages (Morgan and Soothill, 1975). 0.2 g of PVP was suspended in 2 ml of distilled water and 0.1 ml of this solution was administered to mice. The serum IMS - Exp 6.6. was used in this experiment.

25 T.O. mice were assigned to 5 groups and each mouse from the first group was treated with immune serum, before the administration of PVP. The second group of mice was given PVP before being treated with serum; the third group of mice was given serum alone and the fourth group PVP alone. The untreated group of mice, along with all the treated mice, were challenged with 5,000 NBL each, 2 hours after the last treatment was administered. The protocol and results of this experiment are summarized in Table 28.

It is not certain that the amount of PVP given was sufficient to block macrophage activity of the mice as a preliminary dose response curve was not made. However, it can be seen from the results that a dosage of 10 mg of PVP per mouse affected neither the protection afforded by immune serum nor the establishment of muscle larvae in naive mice.

#### Experiment 6.16.

This experiment studied the effects that exposure of NBL to immune serum in vitro had on their subsequent infectivity in vivo.

Two types of antiserum were used, one batch was obtained from 10 T.O. mice immunized with 3 infections of 300 infective larvae every 3

TABLE 28 Experiment 6.15.

The mean number of muscle larvae recovered from mice after treatment with PVP and/or serum and challenge with 5,000 NBL at 0 hours. There were 5 mice per group.

Treatment given i.p.	Time of treatment	Mean (SE) no. of muscle larvae recovered	% protection	Probabilities
1 ml serum + 10 mg PVP	-3½ hours -2 hours	1114 (230)	60%	↑ 0.2 ↓
10 mg PVP + 1 ml serum	-4 hours	883 (154)	68%	↑ 0.4 ↓
1 ml serum	-2 hours	806 (173)	71%	↑ 0.025 ↓
10 mg PVP	-2 hours	1930 (497)		↑ 0.1 ↓
Untreated control		2796 (375)		↑ 0.0005 ↓

weeks and bled 3 weeks after the last infection. The other batch was obtained from 10 T.O. mice given 3 injections of NBL every week (3,000, 4,000 and 2,000 NBL respectively) and bled 2 weeks after the last infection.

20,000 NBL were prepared and divided into 4 lots. One lot was incubated in phosphate buffered saline (PBS), one lot in normal mouse serum (NMS), one lot in the serum from mice immunized with infective larvae and the other in serum from mice given NBL. The NBL were incubated at 37°C for 1 hour, washed twice in PBS and injected in batches of 10,000 into normal T.O. mice. After this treatment and before injection approximately 90% of the NBL were motile and no difference could be detected between the activity of larvae in any of the groups. The larval counts made 28 days after infection are shown in Table 29 and Figure 27.

It can be seen that there was little difference between the infectivity of NBL incubated in PBS or NMS but the infectivity of those incubated in serum from sensitized mice was considerably reduced. However, it must be noted that the mean number of muscle larvae recovered after incubation of NBL in serum taken from parenteral infections (2,950) was not significantly different to the means of the two control groups (4,279 and 5,250).

#### Experiment 6.17.

In this experiment an attempt was made to absorb out the anti-NBL effect demonstrated above.

The serum used was obtained from 14 T.O. mice given 2 infections of 300 muscle larvae each at one months interval and 5,000 NBL each 2 weeks later. The mice were bled 2 weeks after the last infection.



2.5 ml of this immune serum was incubated with 200,000 NBL at 37°C for 2 hours. 4 batches of 75,000 NBL were incubated in either PBS, NMS, immune serum or absorbed immune serum for 2 hours at 37°C, washed twice in PBS and injected into mice at 15,000 NBL per mouse. The mice were killed 28 days post infection and the results of the counts of muscle larvae are shown in Table 29 and Figure 28.

As in experiment 6.16., the immune serum reduced the infectivity of NBL but the infectivity of NBL which had been incubated in 'absorbed' immune serum was almost identical with that of the NBL incubated in normal serum.

#### Experiment 6.18.

This experiment attempted to analyse the effects of absorbed immune serum on an in vivo challenge of NBL.

3.5 ml of the immune serum, IMS - Exp 6.6. was incubated for 1 hour, at 37°C, with 100,000 NBL. 5 T.O. mice were given 0.6 ml each of this absorbed serum and 5 mice were given 0.6 ml each of the unabsorbed immune serum. 2 hours later these mice, along with 5 untreated ones, were challenged with 3,000 NBL each. The results of the muscle larvae counts made 28 days after challenge are shown in Table 30 and Figure 29.

The mice given immune serum had significantly fewer larvae than the challenge control mice. However, a significant proportion of the passive protection, afforded by this immune serum, was absorbed out by pre-incubation with NBL in vitro.

TABLE 29 Experiments 6.16 and 6.17.

The mean number of muscle larvae recovered from mice given NBL which had been incubated in vitro in PBS, NMS, immune mouse serum or absorbed immune mouse serum. There were 5 mice per group.

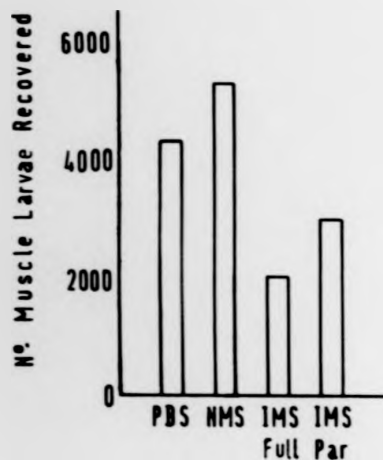
Experiment	Medium used for incubation of NBL	Mean (SE) no. of muscle larvae recovered	% reduction in larval burden compared to mean of the two controls	Probabilities
6.16 challenge of 10,000 NBL	PBS	4279 (519)	-	
	NMS	5250 (997)	-	
	Serum from full infections	2031 (246)	57%	
	Serum from parenteral infections	2950 (816)	38%	
6.17. challenge of 15,000 NBL	PBS	5996 (604)	-	
	NMS	6294 (819)	-	
	Immune serum	2025 (314)	67%	
	Absorbed immune serum	6221 (310)	-1%	

TABLE 30 Experiment 6.18.

The mean number of muscle larvae recovered from mice treated with immune serum or absorbed immune serum and challenged with 3,000 NBL. There were 5 mice per group.

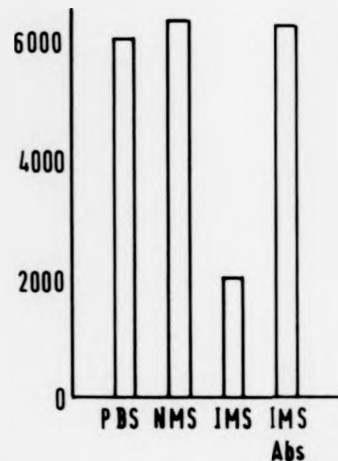
Treatment	Mean (SE) no. of muscle larvae recovered	% protection	Probabilities
0.6 ml immune serum	366 (42)	50%	
0.6 ml absorbed immune serum	496 (118)	32%	
Untreated control	728 (84)	-	

Fig.27 The Infectivity of NBL  
After Incubation in  
Immune Serum



PBS = phosphate buffered saline  
NMS = normal mouse serum  
IMS = immune mouse serum

Fig.28 The Infectivity of NBL  
After Incubation in Immune  
and Absorbed Immune Serum



Full = full infection  
Par = parenteral infection  
Abs = absorbed

Fig.29 The Protection Afforded  
by Immune and Absorbed  
Serum



Experiment 6.19.

In this and the next experiment preliminary investigations were made into the effects of immune serum and normal peritoneal exudate cells (PEC) on NBL in vitro.

PEC were taken from Snell mice and from one Wistar rat. The mice were given between 0.5 and 2 ml of medium 199 i.p., the rat was injected with 5 ml of medium 199 i.p. The abdominal area of each animal was massaged and the peritoneal exudate removed a few minutes later. The cells were washed twice in medium 199 and counted.

300 NBL in 25  $\mu$ l of culture medium were added to each of 54 round bottomed wells in microtitre plates. The wells were divided into 9 groups of 6. 3 types of stored serum were used: IMS - Exp 6.6., which had been tested in experiments 6.6, 12, 13 (ii), 15 and 18 and shown to give, on average, 71% protection in 1 ml doses and 52% in 0.6 ml doses; the rat serum, taken after 2 infections of muscle larvae, tested in experiments 6.13 (i) and 13 (ii) and shown to lack efficacy in mice; and normal mouse serum (NMS). 25  $\mu$ l amounts of each serum were pipetted into their groups of wells and in the absence of serum 25  $\mu$ l amounts of medium 199 were added. 4 types of PEC from unimmunized animals were used:- normal mouse PEC; young mouse PEC; lactating mouse PEC and rat PEC. 150,000 PEC in 25  $\mu$ l volumes were added to each well, thus giving a ratio of 500 cells to 1 larva. The absence of cells was compensated for by the addition of 25  $\mu$ l of medium 199. The microtitre plate was sealed with an adhesive polythene cover and incubated at 37°C. 12 hours later the contents of one well from each group were aspirated, studied, at x 10, by light microscopy and the numbers of dead larvae, those covered by 10 cells or more and those uncovered, were determined.

The remaining wells were aspirated at intervals up to 24 hours after the start of incubation, and the same observations made. The protocol and results of these observations are shown in Table 31 and plates 4-12.

A heavy clumping of cells occurred in all the wells which contained homologous serum and PEC. It was therefore impossible to judge, accurately, how many NBL had more than 10 cells attached to them. Thus, it was assumed that there were 300 NBL, in each of these wells and the number of uncovered larvae present was used to estimate the percentage number of NBL which had cells attached to them. None of the groups showed a significant worm kill during the 24 hours of incubation. Where clumping occurred viable worms could be seen wriggling amongst the cells and no straight bodies were observed in these masses. Little or no cellular adhesion occurred on the NBL incubated with NMS and PEC or PEC alone. PEC from young and lactating mice appeared to adhere as readily to NBL, in the presence of immune serum, as those cells from normal mice. Rat PEC showed a high percentage adherence in the presence of immune rat serum. However, over the time period studied, on average, only 26% of the NBL were covered by mouse PE cells in the presence of rat immune serum. This low percentage attachment can be correlated to the in vivo results of experiments 6.13 (i) and 13 (ii), where, immune rat serum lacked efficacy, in mice, in 3 out of 4 passive transfers. It is suggested that rat antibodies are less efficient than mouse antibodies at opsonising NBL for mouse cells.

#### Experiment 6.20.

This experiment followed the same procedure as above, except that rat PEC were not used and the cell to larva ratio was decreased to

TABLE 31 Experiment 6.19.

The percentage of NBL covered by PEC after incubation with PEC and/or serum. Where percentages are not shown there was no attachment of PEC and only the numbers of live and dead worms are given.

Contents of wells	Time at which observations were made				
	+ 12 hours	+ 14 hours	+ 16 hours	+ 18 hours	+ 24 hours
NBL + 199	302 1; 2 D	286 1; 4 D	297 1; 11 D	306 1; 8 D	311 1; 7 D
NBL + IMS	286 1; 8 D	248 1; 24 D	300 1; 6 D	276 1; 9 D	243 1; 21 D
NBL + NMS + mouse PEC	299 1; 3 D 0%	281 1; 6 D 2%	308 1; 7 D 2%	294 1; 3 D 0%	304 1; 6 D 0.5%
NBL + mouse PEC	274 1; 2 D 2%	294 1; 3 D 1%	306 1; 6 D 0%	281 1; 9 D 2%	294 1; 8 D 1%
NBL + IMS + mouse PEC	100%	100%	100%	93%	8 D; 86%
NBL + IMS + mouse young PEC	7 D; 87%	2 D; 89%	88%	95%	1 D; 84%
NBL + IMS + mouse lactating PEC	100%	98%	96%	100%	95%
NBL + IRS + mouse PEC	3 D; 24%	2 D; 46%	4 D; 20%	8 D; 11%	3 D; 27%
NBL + IRS + rat PEC	97%	95%	86%	99%	97%

1 = number of live NBL  
D = number of dead NBL  
IMS = Immune mouse serum

NMS = Normal mouse serum  
IRS = Immune rat serum  
PEC = Peritoneal exudate cells

100:1. 275 NBL were pipetted into each of 49 wells in 25  $\mu$ l volumes. The wells were divided into 7 groups and to a proportion of these was added 25  $\mu$ l amounts of the serum IMS - Exp 6.6 or NMS, and/or 27,500 PEC in 25  $\mu$ l of medium 199. The absence of cells and/or serum was again compensated for by the addition of medium 199. The plate was sealed and incubated at 37°C. The first observations were made 3 hours after the start of incubation and remaining observations were made at intervals up to 28 hours after the commencement of incubation. The protocol and results of this experiment are summarized in Table 32.

Again, worm death was negligible in both the control and experimental wells during the time period studied. The wells containing NMS and PEC, or PEC alone, showed that little or no cell attachment to worms occurred in the absence of immune serum. The groups which had NBL incubated with immune serum and PEC from normal, young or lactating mice showed that the serum mediated attachment of cells to larvae occurred within 3 hours and that there was little increase in percentage attachment after this time.

It should be noted that in these experiments the immune serum was not heat inactivated, so the complement components C3b, C5a, C567 could have mediated some cellular adhesion.



TABLE 32 Experiment 6.20.

The percentage number of NBL covered by mouse PEC after incubation with cells and/or serum

Contents of wells	Time at which observations were made					
	+ 3 hours	+ 5 hours	+ 8 hours	+ 24 hours	+ 26 hours	+ 28 hours
NBL + 199	276 1; 0 D	294 1; 1 D	261 1; 4 D	226 1; 11 D	268 1; 3 D	298 1; 4 D
NBL + NMS + PEC	261 1; 0 D 0%	281 1; 1 D 0%	283 1; 3 D 0.6%	264 1; 1 D 0%	254 1; 2 D 1.6%	275 1; 6 D 0.4%
NBL + IMS	298 1; 2 D	261 1; 1 D	276 1; 0 D	302 1; 3 D	264 1; 1 D	279 1; 4 D
NBL + PEC	264 1; 5 D 0%	259 1; 4 D 0.8%	274 1; 1 D 0%	273 1; 4 D 0.4%	269 1; 1 D 1%	281 1; 6 D 1.4%
NBL + IMS + PEC	95%	83%	90%	93%	88%	3 D 77%
NBL + IMS + young PEC	80%	85%	88%	91%	84%	84%
NBL + IMS + lactating PEC	2 D 70%	98%	91%	1 D 89%	6 D 82%	5 D 83%

NBL shown 20 hours after incubation in medium 199 with serum and/or peritoneal exudate cells (PEC)



Plate 4. NBL in medium 199

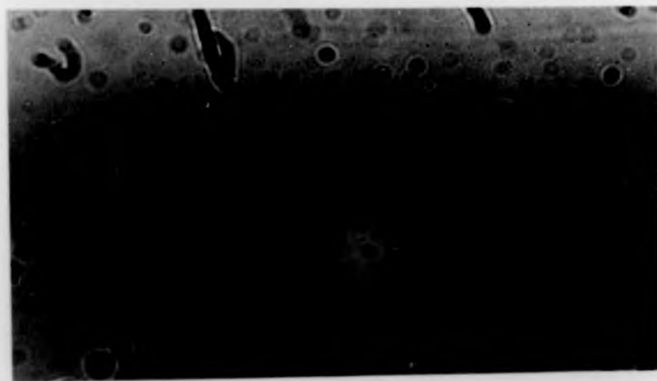


Plate 5. NBL in Immune Mouse serum



Plate 6. NBL in Normal serum with PEC from mice



Plate 7. NBL with PEC from mice

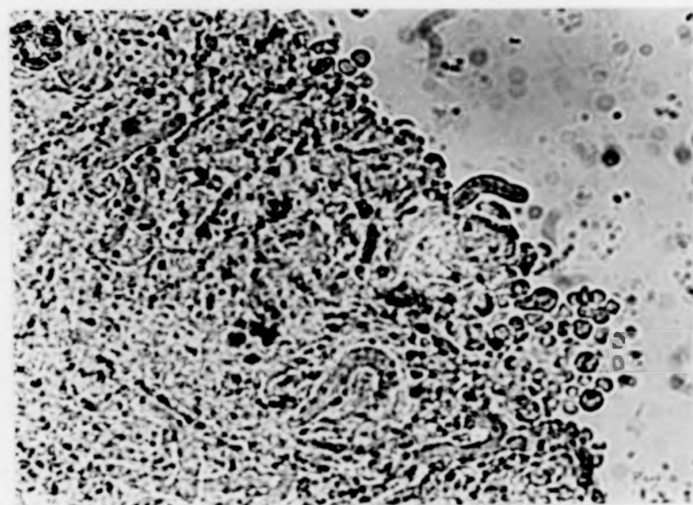


Plate 8. NBL in Immune Mouse serum with PEC from mice

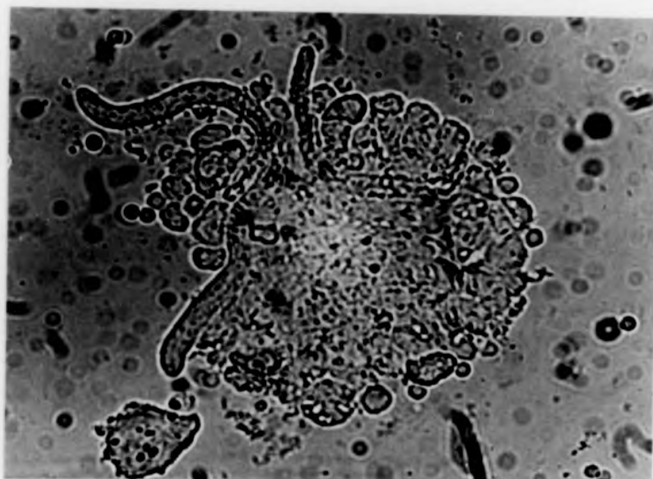


Plate 9. NBL in Immune Mouse Serum with PEC from Neonatal Mice

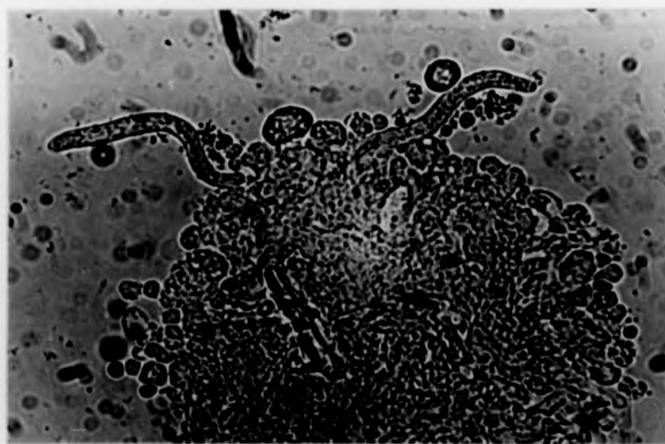


Plate 10. NBL in Immune Mouse Serum with PEC from Lactating Mice.

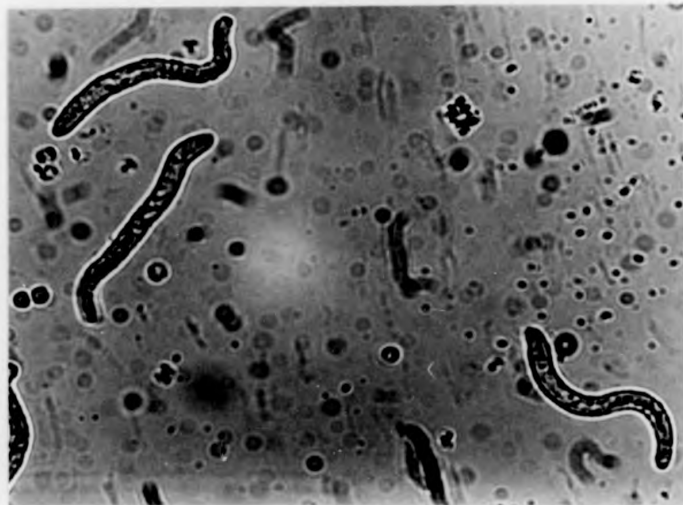


Plate 11. NBL in Immune Rat Serum and PEC from Mice.

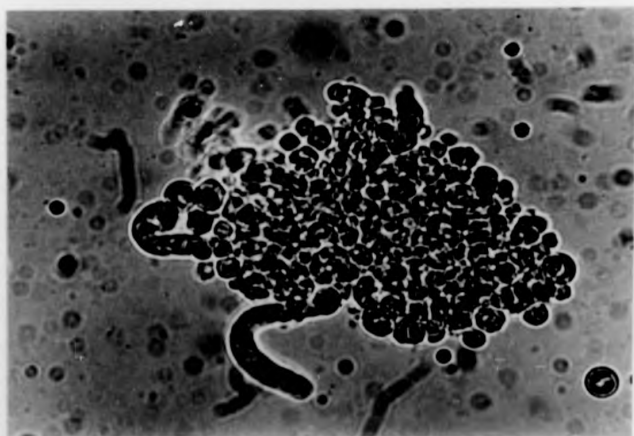


Plate 12. NBL in Immune Rat Serum and PEC from Rats.

DISCUSSION

In the experiments described in this chapter I have attempted to define the type of immune response which limits the establishment of muscle larvae derived from an i.v. injection of NBL. In experiments 5.4. and 5.5. it was shown that female T. spiralis worms from a challenge infection have a reduced fecundity but produce enough NBL to necessitate the development of an immune response, by the host, against the parenteral phase of infection.

The effector stage of the immune response against T. spiralis in mice involves two stages. First, factors mediate damage to the worms (Wakelin and Wilson, 1977a) and second myeloid derived cells (Wakelin and Wilson, 1977b) and T cells (Wakelin and Wilson, 1979) effect the expulsion of the worms.

The passive and adoptive transfers, reported in this chapter, were made against parenteral challenges of T. spiralis in mice and their effects suggest that the immune responses, which control enteral and parenteral infections, differ.

The adoptive transfers employed both sensitized SpC and MLNC. Sensitized MLNC had to be given i.p. to mice (see Chapter 3, p. 67). Dineen and Kelly (1973b) showed that 3 to 4 times as many sensitized lymphocytes had to be transferred i.p. to achieve expulsion of N. brasiliensis of a similar magnitude to that which results from the same cells given i.v. In this report only twice the number of SpC or MLNC were given i.p., as compared to the number of SpC given i.v. A double quantity of SpC given i.p. was always more effective than those given i.v. (Experiments 6.3., 6.4. and 6.5.) which suggests that the route of administration of SpC might not be as critical to their function against NBL as Dineen and Kelly (1973b) have inferred.

When SpC or MLNC were administered simultaneously i.p. to the same animal they had no inhibitory effect on the establishment of NBL, which suggests that factors suppressing the function of immune effector cells were generated as a result of mixing the two lymphoid populations, but the nature of these suppressive factors is at present unknown.

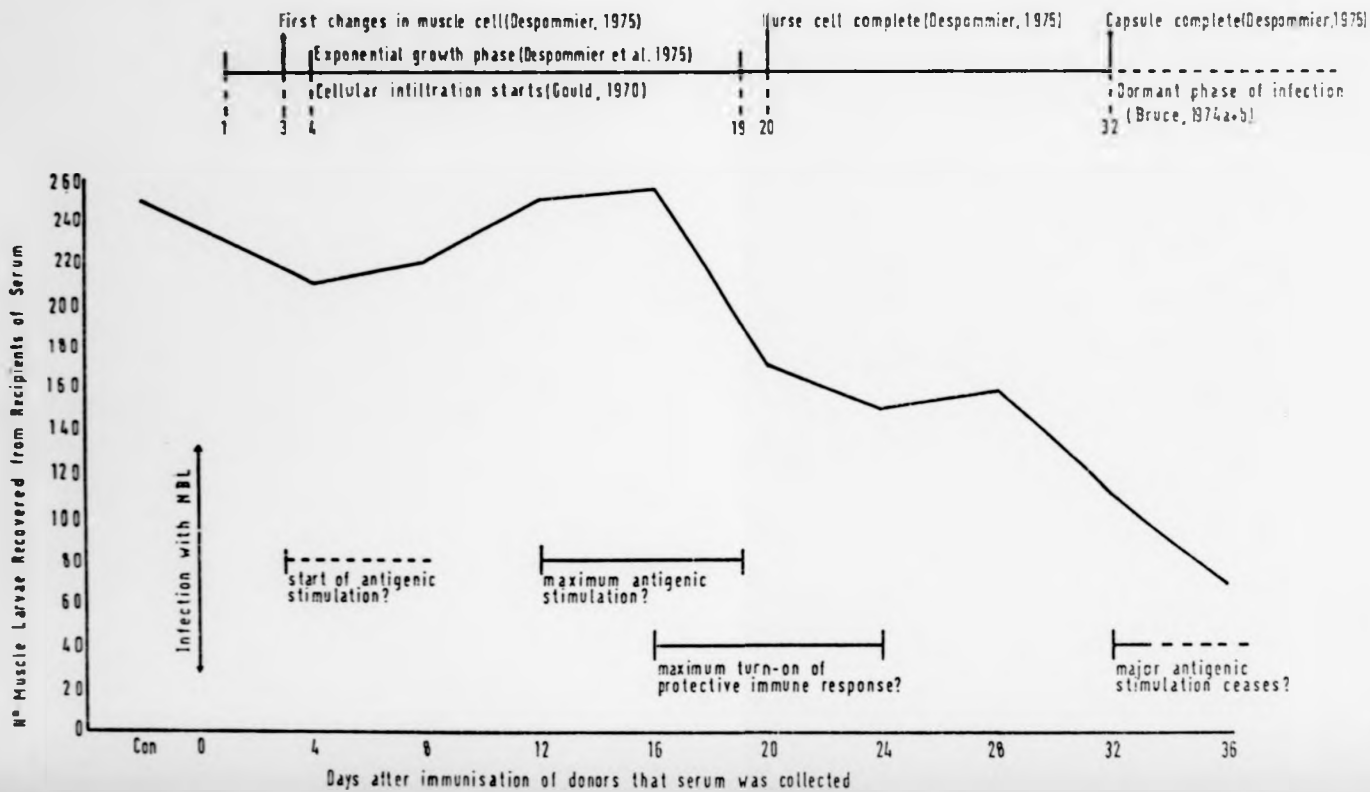
With the exception of experiment 6.1. immune serum was always more effective than sensitized cells in transferring immunity against parenteral challenge to mice. Furthermore, the results, obtained from the passive transfer experiments, leave little doubt that a serum factor plays a major role in protecting mice against a challenge of NBL. It is assumed that these serum factors are antibody, but this is not entirely proven.

Experiments 6.7. and 6.9. (iii) showed that protective factors first appeared in the serum of donor mice 17 to 20 days after a single parenteral immunization. The serum became progressively more protective with the increasing age of infection in the donor mice. This development of protective serum factors suggests that it is the latter stages in the development of muscle larvae which are the major stimulus of immunity against the establishment of muscle larvae.

The protection afforded by serum can be correlated to the developmental stages of the muscle larvae (see Figure 30). The majority of the i.v. administered NBL should have penetrated muscle cells within 24 hours (Harley and Gallicchio, 1971a), but Despomnier (1975) showed that the first changes observed in muscle cell structure did not occur until 3 days after penetration. An exponential growth phase of both the larva and the muscle cell occurred between days 4 and 19 after penetration (Despomnier, Aron and Turgeon, 1975). The nurse cell unit was complete by day 20 and a fully formed collagen capsule was evident



Fig.30 The Mean N° of Muscle Larvae Recovered from Mice Treated with Serum Taken from Donors After Increasing Periods of Parenteral Infection.



32 days after penetration (Despommier, 1975). After this the fully developed muscle larva lies dormant in its nurse cell and Bruce (1974a and b) showed that the glands associated with secretion ceased to function suggesting that no major antigenic stimulation of the host occurs after the development of the muscle larvae and capsule is complete. Maximum antigenic stimulation in a synchronized parenteral infection should, therefore, occur between 12 and 19 days after penetration, during the principle growth and development of the worms (Despommier et al., 1975). If minor antigenic stimulation occurred before this time then the major effector stage of the immune response should be activated between days 16 and 23 after penetration. It was during this time period that protective serum factors first appeared in donor mice (Experiments 6.7. and 6.9. (iii)). After this, time would be required to build up the levels of serum factors and thus cause the maximum protection which was observed 36 days after infection. It is possible that further stimulation and enhanced immunity could occur after this period as products secreted during the developmental stages of the muscle larvae would need to be cleared from the host's system.

Sensitized SpC taken from donors 6, 8 and 17 days after a single parenteral immunization had no protective capacity in recipient mice (Experiments 6.9. (i), (ii) and (iii)). It is probable that the cells concerned with protection, in these 3 experiments, were localised in the lymph nodes draining the infected muscles and had not as yet disseminated to the spleen; whereas by 17 days post immunization, antibody diffusion from the site of B cell location had probably occurred. A similarity exists with the results of Wakelin and Wilson (1977a) which showed that lymphocytes, capable of transferring protection against intestinal T. spiralis infections, appeared in the mesenteric

lymph nodes as early as 4 days after infection, but protective cells were not found in the spleen 7 days after infection.

Cellular infiltrates occur around infected muscle cells soon after larval penetration (Gould, 1970). Grove and Warren (1976) inferred that this infiltration was cell mediated and not antibody dependent. Thus it is suggested that this cellular response to muscle larvae is locally produced and is concerned with the muscle larvae once penetration has occurred. Whereas the humoral response takes longer to develop, is systemic and is probably concerned with arresting the migrating NBL, resulting from secondary infections.

It is possible that the granuloma which forms around the muscle larva, soon after penetration, is non-specific but could prevent leakage of antigens for a time, thus aiding in the delay observed in systemic antibody production. However, the granuloma could be specific, so histological studies should have been made to determine if enhanced granuloma formation occurred in mice which received cells from infected donors.

Multiple parenteral immunizations of mice yielded better protective serum than single parenteral immunization but a single full immunization with infective larvae (Experiment 6.4.) was as effective as multiple parenteral ones (Experiments 6.1. and 6.2.). This suggests that the degree of immunity produced, in donor animals, was dependent upon the amount of antigen involved rather than the number of infections given. However the question of primary and secondary stimulations are inextricably complex in living infections.

When donors were immunized by parenteral infections, their MLNC were less effective than their SpC in conferring protection to recipients (Experiment 6.5.). Both SpC and MLNC enhanced the performance of immune serum, but the latter failed to protect recipients in the absence of immune serum, in contrast to SpC which when given alone (i.p.) afforded

38% protection against challenge.

SpC and MLNC will become active cell populations either as a result of sensitization by antigen in situ or by lymphocyte traffic. In the latter case the difference between the efficacy of sensitized SpC and MLNC which were taken 5 weeks after the first of two parenteral immunizations (Experiment 6.5.) is corroborated by the work of Doenhoff, Leuchars and Rees (1976) who showed that MLNC had only two thirds of the mitotic activity of SpC when taken 1 to 5 weeks after the systemic immunization of mice with SRBC. Thus, memory T cells at least did not equilibrate evenly between the mesenteric lymph nodes and spleens of these mice. Similarly if the sensitization of SpC and MLNC in experiment 6.5. occurred by antigen in situ, then antigens leaking from infected muscle cells into the blood stream would be carried primarily to the spleen.

When donors were sensitized by full infections their MLNC were as effective as their SpC in conferring immunity, against parenteral challenge, to recipients (Experiments 6.3. and 6.4.). There should be little difference in the distribution of muscle larvae resulting from either enteral or parenteral infection as the major route of migration of NBL released from adults is via the thoracic duct lymph to the blood and thence the muscle (Harley and Gallicchio, 1971a). However there could have been a quantitative difference between the antigenic stimulation resulting from the enteral immunizations in Experiments 6.3. and 6.4. and the parenteral immunizations in Experiments 6.5. but this is not known as the muscle larval burdens of the donors were not enumerated.

Alternatively a priming of the MLNC of donor mice, in the initial stages of an enteral infection, could account for the greater efficacy of MLNC in Experiments 6.3. and 6.4. when compared with MLNC sensitized

by parenteral infections (Experiment 6.5.). 3 possibilities are postulated:-

- 1) Adult antigens could have primed the MLNC to respond more rapidly to the ensuing parenteral antigens, in which case the demarcation of stage specific immunity (James and Denham, 1975; James et al., 1977) is not as rigid as was supposed.
- 2) NBL are deposited by adult females in the vicinity of the mesenteric lymph nodes and although NBL, alone, do not promote immunity (Experiments 6.6. and 6.9.; Despommier, 1971) they could have primed the MLNC, as they are immunogenic and capable of stimulating the production of surface specific antibodies (Mackenzie et al., 1978).
- 3) Alternatively the birth fluid released, with the NBL, from the female worms could be a priming antigen.

Serum, alone, in small doses conferred protection against parenteral challenge upon recipients (Experiment 6.6.) and it is possible that the transferred cells, by themselves were effective as a result of producing antibody alone. The relative inefficiency of cells compared with serum may be due to the time lag required for the transferred immune macrophages, T cells and B cells to recognize antigen and start producing antibody by which time the majority of NBL may have penetrated muscle cells.

If the ultimate effects of cells result from antibody production then additive effects between cells and serum should be expected. Co-operative effects between these components were observed (Experiments 6.2. and 6.5.) but were significant only when low levels of protection were transferred by serum (Experiment 6.5.). It is possible that more additive effects would have been observed if lower doses of serum had

been used in conjunction with SpC or MLNC.

However, the relative contributions made by the host and the transferred cells to this complicated process of antibody synthesis cannot be estimated from the results reported in this chapter. The relative contributions of cells and serum to protection against parenteral infection can only be analysed by selective reconstitution of immune depleted hosts, thus leading to a clearer picture of the mechanisms of immunity in this system.

When immune serum was given 4 days after an i.v. injection of NBL (Experiments 6.10 and 6.11) it had no effect upon larval establishment in the muscle. Similarly to affect the larval development of N. brasiliensis antiserum has to be given before infection with larvae (Jones and Ogilvie, 1967; Greenberg, 1971; Ogilvie and Love, 1974).

The lack of efficacy of immune serum given as soon as 2 hours after an i.v. parenteral infection (Experiment 6.12.) suggests that the NBL are quick to leave the circulation and penetrate muscle cells, whereafter serum factors cannot affect them. Therefore the rapid action, necessary to prevent parenteral establishment, suggests that either serum factors alone can incapacitate NBL, possibly by blocking their sensory and penetration organs, or that these factors act in concert with complement and/or the readily available cells of the recipient, i.e. non-specific cell populations capable of being rapidly activated by antibody and complement components.

Non-specific cells such as macrophages, eosinophils, neutrophils, mast cells and basophils are myeloid derived and can take part in the effector stage of the immune response to worms after stimulation by lymphokines, antibodies or complement. The expulsion of adult N. brasiliensis and T. spiralis, from the intestine, has been shown to involve myeloid derived cells (Dineen and Kelly, 1973a; Kelly et al.,

1973 ; Wakelin and Wilson, 1977a and b) but the role of these cells in controlling parenteral infections is uncertain.

However, other experiments (6.13 (i), 13 (ii), 14, 16, 17, 19, 20) reported in this chapter point to the possibility of non-lymphoid cellular components cooperating with immune serum in the control of parenteral T. spiralis infections.

The exposure of NBL to immune serum in vitro did not, in itself, affect their motility or viability; but when NBL which had been exposed to antiserum, were injected into clean mice their infectivity was considerably reduced (Experiments 6.16. and 6.17.). It is again suggested that serum could be acting alone, by blocking the sensory and penetration organs of NBL or that the serum had rendered the NBL chemotactic (by opsonization) to some type of non-specific effector cell.

Lactating and young rats can damage N. brasiliensis worms but cannot expel them (Ogilvie and Hockley, 1968; Keller and Kiest, 1972; Dineen and Kelly, 1973a; Ogilvie and Love, 1974). The cellular step in the expulsion of N. brasiliensis is induced but cannot act in lactating rats and is not induced in young rats (Ogilvie and Parrott, 1977). Similarly, effector T cells are suppressed in lactating mice, infected with T. spiralis (Ngwenya, 1976) and neonatal mice have only a small proportion of the adult's complement of T cells (Doenhoff, Leuchars, Kerbel, Wallis and Davies, 1979). The results of Experiment 6.14. showed that antiserum could confer immunity, to parenteral T. spiralis infection, upon young (60% protection) and lactating recipients (35% protection). However as the same antiserum transferred more protection upon normal recipient mice (87% protection) it is suggested that immune serum has some inhibitory action without effector T cells, but to be more completely effective needs the co-operation of the non-specific cellular components of the host's immune response. The latter mediators

of immunity may be less readily available in young and lactating recipient mice.

The fact that immune serum conferred 60% protection in young mice and only 35% in lactating mice could simply be due to the serum to body weight ratios employed, although an attempt was made to adjust these. Alternatively there could have been a greater loss of non-specific cellular function in the lactating mice.

In Experiment 6.19. immune rat serum mediated the attachment of normal mouse PFC to 26% of the NBL in vitro, whereas in the homologous systems (rat serum with rat PEC and mouse serum with mouse PEC) over 90% of the NBL were covered with PEC. Furthermore, passively transferred immune rat serum protected only 1 out of 4 groups of mice given treatment (Experiments 6.13 (i) and (ii)). These in vivo results seem to correlate with the in vitro ones, where rat serum was much less effective than mouse serum in mediating the attachment of mouse PEC to NBL.

If protective serum factors act alone against the NBL of T. spiralis then immune rat serum should effectively protect recipient mice against challenge. Thus the results of Experiments 6.13 (i), 6.13 (ii) and 6.19. suggest that serum requires the co-operation of some non-specific cellular component in the recipient and such cells may not act as readily with heterologous serum as they do with homologous serum. Similarly immune serum taken from rats infected with N. brasiliensis could not protect mice against the homologous parasite (Ogilvie and Jones, 1971)

In Experiment 6.15. mice were treated with PVP in an attempt to inactivate their macrophages. This treatment had no effect upon the ability of immune serum to protect mice against a parenteral challenge of T. spiralis. However no independent test was performed to show that macrophage blockade had been achieved, so the results are inconclusive



with respect to the possible co-operation between macrophages and immune serum in preventing the establishment of muscle larvae.

In comparison, Chaicumpa, Jenkin and Fisher (1977) and Chaicumpa and Jenkin (1978) have shown that immunity against the infective third stage larvae of Nematospiroides dubius in mice can be effected by macrophages which have been armed by specific antibody. It is possible that this system of co-operation, between non-specific cells and immune serum, could also be involved in anti-parenteral immunity to T. spiralis.

In other studies a case has been made for the eosinophil acting as a non-specific effector cell in concert with serum in anti-parenteral immunity. McLaren et al. (1977) have shown that antibody mediated attachment of eosinophils in vitro occurs with all stages of T. spiralis and N. brasiliensis; but the in vitro studies on T. spiralis, by Kazura and Grove (1978) demonstrated that only NBL were susceptible to both attack and destruction by eosinophil enriched PEC. The process involved specific opsonic antibodies and occurred without the involvement of complement. Preabsorption of the serum with NBL or depletion of the PEC by anti-eosinophil serum prevented the killing of NBL.

The in vitro culturing of NBL with PEC, from naive rats and mice, in the presence of immune serum (Experiments 6.19 and 6.20.) did not result in the killing of NBL, probably because less than 2% of the PEC used would have been eosinophils, and cell populations in which eosinophils constitute such a small proportion do not cause the death of NBL in vitro (Kazura and Grove, 1978).

Grove et al. (1977) suggested that the treatment of mice with anti-eosinophil serum accelerated the migration and intramuscular development of NBL. The results of Experiments 6.10.-6.12., showed that immune serum cannot affect the larvae once they reach the muscle cell. The inference from these results is that in normally acquired

infections, antibodies and eosinophils affect NBL during their migration from the intestinal mucosa to the muscle.

Consideration of previously published in vitro work, and the in vivo experiments, described in this chapter would seem to lend support to the hypothesis that eosinophils together with antibody, are responsible for immune resistance against parenteral infections. However, the results of Experiment 6.6. discount this hypothesis.

Consider, single parenteral immunizations did not give rise to protective serum factors until 17-20 days after infection (Experiments 6.6. and 6.9. (iii)). The serum became progressively more potent with increasing exposure of the donors to parenteral infection and it is suggested that the latter stages of the development of muscle larvae were responsible for the major stimulation of immunity. Mackenzie, Preston and Ogilvie (1978) demonstrated that each of the 3 stages, of a T. spiralis infection, stimulated the production of antibodies which were specific for the surface antigens of that stage only. The antibodies specific to the surface of NBL were evident 15 days after a full infection in rats and peaked at 20 days, whereafter the titres fell. The production of NBL from the enteral infection given to rats by Mackenzie et al. (1978) should have started 4 days after infection (Harley and Gallicchio, 1971a). Thus these rats produced peak titres of surface directed antibodies to NBL approximately 16 days after their first exposure to the parenteral stage of the infection. However in Experiment 6.9. (iii), reported here, protective serum factors did not appear in mice until 17 days after their exposure to a single parenteral infection. Furthermore, Mackenzie et al. (1978) showed that the titres of surface directed antibodies had dropped to almost zero, 40 days after the enteral infection in rats, i.e. approximately 36 days after these rats were first exposed to the parenteral phase of infection.

Conversely, in Experiment 6.6. mouse serum bestowed maximum protection upon recipients when it was taken 36 days after a single parenteral infection of the donors.

Thus, notwithstanding the difficulties inherent in the comparison of animals of different species it is likely that these surface directed antibodies do not represent the protective factors in immune serum. Furthermore, the pattern of development of the protective factors, as shown in Experiment 6.6., suggests that the muscle larvae are responsible for inducing protective anti-parenteral immunity.

Mackenzie et al. (1978) quantitated the surface directed antibodies in rat serum by using their capacity to mediate eosinophil attachment to NBL in vitro; so either eosinophils are not the cell type involved, when immune serum protects in vivo or the mechanism of serum and eosinophil co-operation does not act against the surface of NBL.

Kazura and Grove (1978) used pooled serum from mice infected for 4 to 10 weeks with T. spiralis, this serum would presumably contain both antibodies with reactivity against the surface of NBL and protective serum factors, so their in vitro results do not facilitate elucidation of the mechanisms responsible for the inhibition of NBL.

The distinction between surface directed antibodies, which mediate eosinophil attachment and protective serum factors is complicated by the findings that a significant proportion of the protection afforded to recipients by immune serum was absorbed out by the pre-incubation of this serum with NBL in vitro (Experiment 6.18.). Further the infectivity of NBL was decreased when they were incubated in immune serum before infection (Experiment 6.16.) but their infectivity was not impaired by incubation in immune serum which had been pre-absorbed with NBL (Experiment 6.17.).

It is unlikely that the protective serum factors are inactivated by secretory products as NBL possess neither a stichosome nor hypodermal glands (Despommier, 1971; Bruce, 1974a). It is probable that these serum factors attach to the cuticle of NBL but are distinct from the surface directed antibodies, described by Mackenzie et al. (1978) as they do not mediate the attachment of eosinophils. If these protective serum factors are antibodies then it is suggested that the surface receptors of eosinophils do not have affinity for the F<sub>c</sub> portion of these protective antibodies.

If pre-absorption of protective factors from immune serum had been shown to occur only in the presence of NBL, then this specificity may have been indicative of the protective serum factors being antibody.

In conclusion, it has been shown that passive transfer of immune serum into intact mice can effectively control a challenge of T. spiralis NBL. It is tentatively suggested that the NBL are killed by antibody acting in concert with non-specific cells. Thus anti-parenteral immunity differs from that manifested against intestinal worms, as the latter requires the additional action of T lymphocytes (Wakelin and Wilson, 1979).

Further work is required to confirm whether the active serum factors are antibodies and to elucidate the mechanisms by which NBL are inhibited from establishing in muscle. This work should be facilitated by selective reconstitution of immune-depleted hosts with fractionated sera and cell populations. It should also be determined whether the model described here for investigation of the nature of acquired immunity to parenteral infections with NBL bears any relationship to the events which occur during the course of a natural infection with infective T. spiralis larvae.

## CHAPTER 7

Summary and Conclusions

1. The literature on T. spiralis and N. brasiliensis was reviewed and the immune responses elicited by the host against each of these parasites was compared. Strong immunity against challenge is expressed by rats or mice infected with either parasite. It is suggested that the format of the immune response which acts against the intestinal phase of a T. spiralis infection is similar to that which acts against N. brasiliensis in the gut.

There are two sequential phases in the process of worm expulsion.

- 1) Cytological damage to the genital and intestinal systems of these worms is probably brought about by the combined effects of locally produced secretory IgA and non-specific cells.
- 2) The actual expulsion of T. spiralis and N. brasiliensis from the gut requires the action of effector T cells with the probable co-operation of non-specific cells.

The adults are the major source of protective antigens in infections of N. brasiliensis, but T. spiralis stimulates a stage specific immune response, both enteral and parenteral phases inducing a strong resistance to that phase only.

It is suggested that the larvae of N. brasiliensis are more susceptible to the action of immune serum than the adults. In vitro studies on T. spiralis infer that parenteral immunity is manifested by the antibody mediated attachment of eosinophils to NBL. However little work was reported on the in vivo control of the parenteral establishment

of T. spiralis.

2. The time taken by mice to expel adult T. spiralis worms is a measure of the efficiency of the immune response which they generate upon infection. In Chapter 4 it was shown that NIH mice expel both primary and challenge infections of T. spiralis rapidly, whereas CBA mice respond poorly to the parasite; in terms of phenotype these strains of mice were classified as responder and non responder respectively.

The majority of the  $F_1$  progeny, obtained from the cross breeding of NIH and CBA mice, respond to T. spiralis in the manner of the NIH parenteral strain; thus the responder characteristic tends towards dominance.

The patterns of response to T. spiralis made by the progeny produced by the  $F_1$  generation backcross to the responder and non responder parenteral strains suggests that the development of resistance to T. spiralis in NIH and CBA mice is controlled by autosomal dominant alleles which are not linked to the gene coding for the colour of the mice. The number of alleles involved were not determined but the pattern of inheritance of resistance in the progeny of the backcrosses would suggest that there are few.

The activation of cell populations in the mesenteric lymph nodes of NIH mice can be correlated with the expulsion of T. spiralis from naive and sensitized hosts, whereas there was no evidence of cell activity in the mesenteric lymph nodes of the non-responder CBA mice. Furthermore it is generally accepted that the expulsion of worms from the gut is an immunological phenomenon, so it is suggested that the ability of mice to respond to T. spiralis is determined by a genetically controlled immune response.

As the immune response mounted against T. spiralis is stage specific it would have been reasonable to investigate a possible genetically determined difference in the immune response of NIH and CBA mice to the parenteral phase of infection. In this context it was noted that the mesenteric lymph nodes of NIH mice are mainly activated by the enteral phase of infection whereas splenic enlargement occurred during the parenteral phase of infection. The non-responder CBA mice showed no splenic activity suggesting that they also respond poorly to the parenteral phase of infection.

Snell and T.O. mice respond similarly to the enteral phase of a T. spiralis infection and comparison with NIH and CBA mice would classify them as average responders. As with NIH mice the pattern of activation of the mesenteric lymph nodes of Snell mice can be correlated with the expulsion of primary and challenge infections of T. spiralis and their spleens appear to respond to the parenteral phase of infection.

3. In Chapter 5 it was shown that Snell mice do not differ at the major histocompatibility loci and can be used as the donors and recipients of sensitized lymphoid cells.

After parenteral immunization both Snell and T.O. mice are strongly immune to parenteral challenge. The similarity in the degree of acquired immunity expressed against a parenteral challenge in Snell mice (79%) and T.O. mice (82%) suggests that these strains are suitable for conjunctive use in preliminary investigations into the mechanisms of parenteral immunity reported in Chapter 6.

It is suggested that the manifestation of a parenteral immune response is necessary as the production of NBL from female T. spiralis worms is not completely suppressed by the effects of enteral immunity in secondarily infected Snell mice.

4. Serum taken from Snell and T.O. donors, which have been stimulated by full or parenteral infections, confers strong immunity to recipients against a challenge of NBL. The degree of immunity transferred is dependent upon the size of infection rather than the number of infections given to the donor mice. Serum from donors given heavy immunization schedules transfers equal or even better protection than that which results from acquired immunity.

As little as 0.1 ml of immune serum can give significant protection against challenge to recipients but the protection becomes progressively better with the transfer of increasing amounts of immune serum. It is suggested that the protective serum factors are antibodies, but no direct evidence is given.

Immune serum transfers better levels of protection than sensitized, unseparated spleen cells or mesenteric lymph node cells. It is tentatively suggested that any protection afforded by cells is due ultimately to the production of antibody and the relative inefficiency of cells compared to serum may be a result of the time taken for the transferred immune macrophages, T cells and B cells to recognize the parasite's antigens and promote the production of antibody.

Protective serum factors first appear in the serum of donor mice 17-20 days after a single parenteral immunization, the serum becomes progressively more protective after this time which suggests that the latter developmental stages of muscle larvae are responsible for inducing parenteral immunity. The peak titre of protective serum factors was observed 36 days after a single parenteral immunization of donors. It is probable that the actual peak titres occur on or soon after this time. These titres should remain high for a few months whereafter a decline in the efficacy of the serum will occur as serum taken 5 months after immunization conferred only 50% of its original



protective capacity and 9 months after sensitization such serum no longer gave protection to recipients.

Spleen cells taken up to 17 days after a single parenteral immunization failed to protect recipients upon challenge, suggesting that at this time sensitized lymphocytes are still localized in the lymph nodes which drain the infected muscles of the donors. It is therefore probable that the earlier appearance of protective serum factors is a result of their diffusion from these localized lymph nodes.

The incubation of NBL in immune serum in vitro has no effect upon their motility but decreases their infectivity when inoculated into naive recipients. This again suggests that serum can either act alone by disorientating the NBL or by blocking their penetration organs or that immune serum opsonises the NBL for non-specific cells in vivo.

The protective factors in immune serum are depleted by incubation with NBL in vitro and it is probable that this occurs by the absorption of these factors onto the surface of NBL. This lends further support to the hypothesis that immune serum can opsonise NBL for non-specific cells in vivo.

Immune rat serum is less effective than immune mouse serum in conferring passive protection to mice and in opsonising NBL for normal mouse peritoneal exudate cells in vitro. This suggests that immune serum acts in concert with the host's non-specific cells and that this co-operation does not occur as readily between heterologous serum and cells as it does between homologous serum and cells.

Immune serum can confer passive protection upon young and lactating mice which suggests that the inhibition of NBL by the immune response occurs in the absence of effector T cells as young mice have only a small percentage of the adult's complement of T cells and effector T

cells are suppressed in lactating mice. This is in marked contrast to the expulsion of adult T. spiralis from the gut which requires the action of effector T cells. However immune serum confers less protection to lactating and young mice than it does to normal recipients and this again suggests that immune serum cooperates with non-specific cells which are less readily available in young and lactating mice as their production is T cell dependent.

The type of non-specific cell which contributes to the prevention of parenteral establishment is not known but previously published in vitro work suggests that it is the eosinophil. However the times of appearance and peak titres of protective serum factors in mice (reported here) do not correlate with the appearance of antibodies in rats which mediate the attachment of eosinophils to the surface of NBL. This suggests that eosinophils are not involved in the protective immune response against NBL but this can only be confirmed by the repetition of this latter work in mice or the former work in rats.

In conclusion it is suggested that developing muscle larvae induce an immune response which acts against NBL and prevents their establishment in the muscle. This immune response against NBL is mediated by serum factors which act in concert with a non-specific cell population.

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**IMMUNITY TO *TRICHINELLA SPIRALIS*. VII. RESISTANCE  
STIMULATED BY THE PARENTERAL STAGES  
OF THE INFECTION**

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## IMMUNITY TO *TRICHINELLA SPIRALIS*. VII. RESISTANCE STIMULATED BY THE PARENTERAL STAGES OF THE INFECTION

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**ABSTRACT:** In three experiments mice were given three intravenous injections of *Trichinella spiralis* newborn larvae produced in vitro. Following a challenge infection of newborn larvae the mice were shown to be on the average 85% resistant i.e., only 15% of the challenge larvae developed to encysted muscle stage larvae in these mice; when the challenge consisted of normal muscle larvae administered *per os*, the mice were 51% resistant. Neither the number nor longevity of adult worms in the intestines was affected by this immunization procedure.

Oliver-Gonzalez (1941) suggested that both intestinal adult worms and muscle larvae of *Trichinella spiralis* are immunogenic and that antibody production stimulated by the worm is, to a certain degree, specific to these two stages; this he called the "dual antibody" response.

The immunogenicity of the intestinal phase was confirmed by James and Denham (1975) working with mice. They also showed that resistance stimulated by the intestinal phase was largely restricted to the intestinal phase itself and had little or no effect on newborn larvae injected intravenously. This seemed to confirm that one side of Oliver-Gonzalez' dual antibody hypothesis could be applied to the *in vivo* situation.

The observations by Doerr and Schmidt (1930) and Matoff (1943) that muscle larvae were produced after injecting pregnant female *T. spiralis* intramuscularly led to the use of an *in vitro* culture system to collect newborn larvae and inject these intravenously (Dennis, Despommier, and Davis, 1970). This method has been used to demonstrate that the parenteral phase of the infection can generate good immunity (James and Denham, 1974; Ruitenberg and Steerenberg, 1976).

Here, we are reporting further experiments which confirm the immunogenicity of the parenteral phase and suggest that resistance stimulated by this phase does not affect the intestinal phase.

### MATERIALS AND METHODS

The London strain of *T. spiralis* was used, and the experimental host animals were female T.O.

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strain albino mice, weighing approximately 25 g each at the beginning of each experiment. The methods used to obtain viable muscle larvae by acid pepsin digestion, to infect mice, and to enumerate encysted muscle larvae and adult worms have been described previously (Denham, 1968). The method of producing newborn larvae *in vitro* was that of James and Denham (1974), which is similar to that of Dennis et al. (1970).

The statistical significance of any differences between experimental groups was analyzed using Student's *t*-test.

### EXPERIMENTAL PROCEDURE AND RESULTS

In all three experiments mice were "immunized" with three intravenous injections of newborn larvae and were challenged either with an injection of more newborn larvae or with normal infective larvae, derived from muscle digests, given *per os*.

#### Experiment 1

Twenty-five mice each received intravenous injections of 2,100, 1,600, and 1,900 newborn larvae at weekly intervals. Fifteen uninfected mice were retained to act as "challenge controls." Fourteen days later eight of the infected mice and nine of the uninfected control mice were challenged *per os* with 300 infective larvae each. On the same day seven "immunized" mice and six previously uninfected mice were challenged intravenously with approximately 2,200 newborn larvae. The remaining 10 infected mice were left to act as "immunized-unchallenged" controls. After a further 30 days all the mice were killed and the number of muscle larvae counted. The results are summarized in Table 1.

At least the first of the immunizing infections must have been of poor viability since a mean of only 571 larvae was found in the



TABLE I. Mean number of larvae found in normal and immunized mice after either intravenous injection of newborn larvae or per os challenge with infective larvae in Experiment 1.

Number of mice	Mouse group	Mean no. larvae per mouse (SE)	No. larvae attributed to challenge
10	Immunized not challenged	571 (46)	—
9	Immunized-challenged per os	49,300 ( 8,100)	48,700
8	Challenged per os	67,500 (11,200)	67,500
7	Immunized-challenged i.v.	990 (282)	425
6	Challenged i.v.	1,605 (136)	1,605

"immunized-unchallenged" mice. In the mice "immunized" and challenged with newborn larvae there was a mean of 425 more larvae than in the "immunized-unchallenged" mice and this represents 26% of the number of larvae recovered from the challenge controls. The immunized mice were, therefore, 74% resistant to a parenteral challenge infection.

There were approximately 43,700 more larvae in the immunized mice challenged per os than in the "immunized-unchallenged" mice. This is 72% of the number of larvae in the challenge controls; these mice were, therefore, only 28% resistant to this form of challenge.

#### Experiment 2

This was essentially a repeat of Experiment 1. Twenty-six mice were each injected intravenously with 2,000, 2,000, and 2,800 newborn larvae at weekly intervals. Fourteen days later 10 of these mice and 10 normal mice were challenged with approximately 100 infective larvae per os. On the same day, eight of the immunized mice and 11 normal mice were challenged intravenously with 7,200 newborn larvae each. The remaining infected mice acted as "immunized-unchallenged" controls. All the mice were killed and digested 28 days later. The results are summarized in Table II.

The immunized mice challenged per os har-

bored 8,080 more larvae than the "immunized-unchallenged" mice; this is 38% of the number of larvae in the challenge control group and these mice, therefore, were 62% resistant to this form of challenge. In mice immunized and challenged with newborn larvae there were 102 fewer larvae than in mice which had been immunized but not challenged and there was no statistically significant difference between these groups. Since the challenge controls had such a high level of infection it is reasonable to assume that the immunized mice were 100% resistant to challenge.

#### Experiment 3

This was a repeat of Experiment 2 with the addition of a group of mice, the intestines of which were examined for adult worms. Sixty-two mice were each injected with three doses of 2,000 newborn larvae at weekly intervals. Fourteen days later nine of these immunized mice and 10 normal control mice were challenged per os with 30 infective larvae each. Seven immunized mice and seven control mice were challenged by intravenous injection with 6,000 newborn larvae. The remaining mice acted as unchallenged controls. All the mice were killed and digested 28 days later and the results are shown in Table III.

The mice immunized and challenged with

TABLE II. Mean number of larvae found in normal and immunized mice after either intravenous injection of newborn larvae or per os challenge with infective larvae in Experiment 2.

Number of mice	Mouse group	Mean no. larvae per mouse (SE)	No. larvae attributed to challenge
8	Immunized not challenged	1,120 (537)	—
10	Immunized-challenged per os	11,400 ( 2,080)	8,080
10	Challenged per os	21,300 (3,950)	21,300
8	Immunized-challenged i.v.	1,220 (686)	—100
11	Challenged i.v.	5,150	5,150

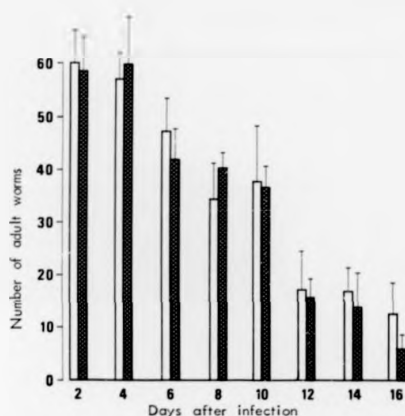


FIGURE 1. Number of adult worms found in the intestines of normal and immunized mice following *per os* infection with 100 infective larvae in Experiment 3. Key: Mean number of adult worms (with SE) recovered from intestines of normal (open columns) and "immune" (stippled columns) mice.

newborn larvae had a mean of 769 larvae more than the "immunized-unchallenged" mice which is 18% of the number of larvae in the challenge controls. Thus, these mice were 82% resistant to parenteral challenge.

The immunized mice which had been challenged *per os* with infective larvae had 4,500 more larvae than the unchallenged controls. This is 38% of the number in the challenge control mice and showed that the immunized mice were 62% resistant to this type of challenge.

Forty mice immunized as above and 40 normal mice were each challenged with 100

infective larvae; on alternate days, from days 2 to 16, five mice from each of these two groups were killed and the number of adult worms in their intestines determined. The results of these counts are shown in Fig. 1. On no day was there a statistically significant difference between the "immunized" and control mice.

#### DISCUSSION

These results and those of Ruitenbergh and Steerenberg (1976) clearly demonstrate that the parenteral phase of *T. spiralis* infections stimulates resistance against the successful establishment of a challenge infection. In the three experiments reported above, mice challenged by the intravenous inoculation of newborn larvae were 74, 100, and 82% resistant to challenge. Thus, under the conditions of these experiments, the mean figure for resistance was 85%. If mice immunized by the same procedure were challenged *per os* with normal infective larvae they were much less resistant; in the three experiments they were 28, 62, and 62% resistant, with a mean of 51% resistance. There are several possible explanations for this. In all the experiments relatively few larvae resulted from the immunization procedure and from the challenge with newborn larvae. It is conceivable that resistance stimulated by a light infection could overcome a light challenge but not the much heavier challenge arising from *per os* challenge. In support of this it is noteworthy that resistance to challenge *per os* (62%) was greater in Experiments 2 and 3 where the immunizing infection was higher and the challenge lower than in Experiment 1.

An alternative explanation is that the presence of adult worms in the intestine in some way exacerbates muscle infection. At first sight this seems most unlikely but Svet-Moldav-

TABLE III. Mean number of larvae found in normal and immunized mice after either intravenous injection of newborn larvae or *per os* challenge with infective larvae in Experiment 3.

Number of mice	Mouse group	Mean no. larvae per mouse (SE)	No. larvae attributed to challenge
6	Immunized not challenged	3,220 (224)	—
9	Immunized-challenged <i>per os</i>	7,720 (1,261)	4,500
10	Challenged <i>per os</i>	12,000 (4,000)	12,000
7	Immunized-challenged i.v.	3,990 (532)	770
7	Challenged i.v.	4,360 (453)	4,360

sky et al. (1970) and Faubert and Tanner (1971) showed that *T. spiralis* infections immunodepress mice. Aboul Atta, Colli, and Denham (unpublished) have shown, using antibody production and plaque assay, that intestinal *T. spiralis* are responsible for part of this immunodepression. As parenteral and enteral *T. spiralis* are antigenically dissimilar it is possible that the adult worm immunodepresses the host in relation to the parenteral phase.

Some preliminary experiments in our laboratory show that if newborn larvae are inoculated into mice already carrying a sterile intestinal infection a higher number of muscle larvae results. If these results can be confirmed we are left with a beautifully evolved host-parasite relationship: the host on becoming infected with intestinal *T. spiralis* would generate an immune response directed against the adult worm. However, the newborn larva is not attacked by this response during migration as it has evolved a separate antigenic identity for its offspring and the adult also immunodepresses the host to restrict the development of the response directed against the parenteral forms.

Although the observations on adult worms in Experiment 3 are not entirely conclusive they suggest that the immunity produced against muscle larvae affects neither the number nor the longevity of the intestinal phase of the infection. This, together with the previous observation that resistance stimulated by intestinal worms does not adversely affect the parenteral phase of infection (James and Denham, 1975), suggests that the dual antibody hypothesis of Oliver-Gonzalez (1941), which was proposed following an *in vitro* study of antibodies in infected rabbits, may well have a functional *in vivo* reality.

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## Effects of immune serum and cells on newborn larvae of *Trichinella spiralis*

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**Summary** Serum and cells, collected from mice immunized by infection either with newborn larvae (NBL) or *per os* with infective larvae of *Trichinella spiralis*, were passively transferred to mice which were challenged with NBL. Serum always gave very strong protection if given before challenge but not if given within 2 h after challenge. Cells from mice immunized with NBL, also gave good protection, whereas those from mice immunized *per os* did not. If NBL were incubated in serum from immunized mice their infectivity was reduced, but if the serum was pre-absorbed with NBL this effect was lost. Such absorbed serum did not confer immunity on recipient mice.

**Keywords:** *Trichinella spiralis*, immunity to *T. spiralis*, muscle larvae of *T. spiralis*

### Introduction

Many papers have been published concerning the effects of transferred serum and/or cells on the rate of expulsion of intestinal *Trichinella spiralis* (Despommier *et al.* 1977, Larsh *et al.* 1970, Love, Ogilvie & McClaren 1976). We are not aware of any such studies on the newborn *T. spiralis* larvae which migrate from the intestine to the musculature and we report here some *in vivo* and *in vitro* experiments with this phase of the infection. Newborn larvae of *T. spiralis* are born in the mucosa of the small intestine and migrate through the lymphatics to the blood and thence to the muscles where they penetrate the muscle cells and develop into infective larvae.

### Materials and methods

The London strain of *T. spiralis* was used in all the experiments. The general

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methods used were those of Denham (1968), and the newborn larvae (NBL) were collected *in vitro* from adult *T. spiralis* which has been grown in rats for 6 days (James & Denham 1974). Outbred Tuck TO albinos were used to produce serum and inbred males of the Snell strain were used as donors and recipients of cells and serum. In each experiment mice were killed and digested 28 days after challenge with NBL to determine the number of muscle larvae they harboured.

To obtain spleen cells for transfer to recipients, spleens were removed aseptically from donor Snell mice and gently pushed through a 200  $\mu\text{m}$  pore sieve into a small container of medium 199 kept on ice. The resulting suspension was washed three times with 199 in a centrifuge at 4 °C. The cells were counted in a Neubauer haemocytometer, using trypan blue as a dead cell marker, and an appropriate concentration of cells was made up for injection.

The significance of the results was assessed by Student's *t*-test. Where no *P* values are given in the tables, the differences were not significant at 0.05.

#### Experimental procedure and results

In each experiment reported below control groups received normal serum, spleen cells or both at the same rate as the experimental mice. On no occasion was there a significant difference in the larval counts between the untreated control mice and those given serum or cells and, for the sake of clarity and brevity, the results obtained from these groups are not reported in this paper.

#### EFFECTS OF SERUM AND CELLS FROM MICE IMMUNIZED WITH NEWBORN LARVAE

In experiment 1, 18 Snell and 10 TO mice were given three intravenous (i.v.) injections of 2000 NBL each at weekly intervals. One week after the third infection the mice were killed and serum was collected from all of them. The spleens were removed from the Snell mice and cell suspensions prepared. Twenty-eight 8-week-old Snell mice were randomly assigned to four groups. One group was given  $4 \times 10^7$  spleen cells i.v., another group was given 1 ml of serum intraperitoneally (i.p.), a third group was given both cells and serum and the fourth group was left untreated. The next day each of the 28 mice was challenged with 2000 NBL i.v. The results of the muscle larvae counts in this experiment are summarized in Table 1 together with those of experiments 2-4.

In experiment 2, 14 Snell mice were given 3000, 5000 and 3000 NBL each at weekly intervals. Eight TO mice were given 3000, 2000 and 2000 NBL each at weekly intervals. Cells and serum were collected 7 days later. Six Snell mice were given  $4 \times 10^7$  spleen cells i.v., five were given 1 ml of serum i.p., six received serum i.p. and spleen cells i.v. at the same rate and six were left untreated. One day later each mouse was challenged i.v. with 1500 larvae. The results of the muscle counts are shown in Table 1.

**Table 1.** Mice were given cells and/or serum from mice immunized with either newborn larvae (NBL) or infective muscle larvae (ML) of *Trichinella spiralis*. All recipients were challenged with NBL. Where *P* values are not shown they are greater than 0.05. The results of experiments 1-4

Experiment	Method of immunizing donors	Number of muscle larvae (SE) recovered from mice given:			
		Spleen cells	Serum	Spleen cells + serum	Nothing
1	3 infections NBL	222 (40)	247 (18)	262 (30)	602 (55)
		↔	↔	↔	↔
2	3 infections NBL	330 (47)	220 (66)	166 (32)	1057 (50)
		↔	↔	↔	↔
3	4 infections ML	496 (96)	2.3 (1.2)	4.3 (2)	862 (95)
		↔	↔	↔	↔
4	1 infection ML	3240 (520)	1150 (550)	1490 (530)	4680 (360)
		↔	↔	↔	↔

SE = standard error of mean.

In these two experiments both spleen cells and serum conferred resistance to challenge with NBL, but only in the second experiment were the two together more effective than each alone and even then the additive effect was insignificant. Combining the two experiments, we find that spleen cells conferred 66% immunity, serum, 69% and both together, 70%.

#### EFFECTS OF SERUM AND CELLS FROM MICE IMMUNIZED BY INFECTION WITH MUSCLE LARVAE

In experiment 3, 22 Snell mice and 18 TO mice were infected four times with 300 muscle larvae each at 14 day intervals. Serum and cells were collected 14 days after the last infection. Six Snell mice were given  $4 \times 10^7$  spleen cells *i.v.*, six were given 1.5 ml serum *i.p.*, six received both cells *i.v.* and serum *i.p.* and six were left untreated. One day later they were all challenged *i.v.* with 4000 NBL. The results of the larval counts are summarized in Table 1. In this experiment the protection afforded to mice by spleen cells was 42%. This level of adoptive immunity was less than that transferred in experiments 1 and 2, but the mice given serum were 99.7% resistant and those given serum and cells 99.5% resistant. This degree of resistance was remarkable, even taking into account the heavy immunization schedule to which the donors had been subjected.

In experiment 4, the donor mice were immunized with one infection of 300 infective larvae each, 24 days before the collection of cells and serum. All the recipient mice and the controls were challenged with 10 000 NBL each, 1 day after serum cell transfer. The results are summarized in Table 1. In this experiment, serum alone gave 75% protection, cells no substantial protection and cells plus serum, 68%.

#### THE EFFECT OF TRANSFER OF IMMUNE SERUM BEFORE AND AFTER CHALLENGE

In experiment 5, 14 Snell mice were immunized with 6000 NBL each and exsanguinated 4 weeks later. Five mice were given 1.5 ml of serum and then challenged with 2000 NBL each 24 h later at the same time. Six mice were challenged with the same number of larvae and given 1.5 ml serum 4 days later; six mice were only given the challenge infection. The results of the larval counts are summarized in Table 2. The mice given serum before challenge were only 51% resistant, but this should be related to the light immunization of the donors. The mice given serum 4 days after challenge yielded more larvae than the controls and were obviously not protected.

In experiment 6, which was essentially a repeat of experiment 5, serum was collected 1 week after the second infection from mice which had been immunized with 10 000 and 5000 NBL. The challenge infection was of 10 000 NBL and there were five mice per group. The results of the larval counts are shown in Table 2. The mice given serum before challenge were 54% resistant, whereas those given serum after challenge were as susceptible as the controls.



*Passive immunity against Trichinella spiralis larvae*

**Table 2.** The number of muscle stage *T. spiralis* recovered from mice given immune serum 24 h before or 4 days after challenge with newborn larvae compared with the same challenge in normal mice

Experiment	Number of larvae (SE) in mice given		
	Serum 24 h before challenge	Serum 4 days after challenge	No serum
5	299 (118)	880 (318)	606 (115)
	← $P = 0.05$ →		
6	1660 (236)	3540 (376)	3630 (392)
	← $P = < 0.005$ →		
	← $P = < 0.005$ →		

Where *P* values are not shown they are greater than 0.05

In experiment 7, 25 TO mice were immunized with 200 infective larvae each, 4000 NBL each 50 days later and 14 days later, 6000 NBL each. The mice were bled for serum 21 days after the last infection. Five TO mice were given 0.6 ml of this serum and 2 h later were challenged, together with 30 other mice, with 3000 NBL. Five of the 30 mice were left as challenge controls, the rest were randomly assigned to five groups of five mice and were given 0.6 ml serum each 2, 6, 24, 48 and 72 h post-challenge. All the mice were killed and digested 28 days after challenge; the results of the larval counts are shown in Table 3. The mice given

**Table 3.** The mean number of muscle stage *T. spiralis* larvae recovered from mice given 'immune' serum before and after challenge with newborn *T. spiralis* larvae

Time serum given in relation to challenge	Mean larval count (SE)	Probabilities
None	728 (84)	$\left. \begin{array}{l} 0.005 \\ 0.05 \\ 0.005 \end{array} \right\} 0.005$
2 h before	366 (42)	
2 h after	608 (122)	
6 h after	690 (35)	
24 h after	848 (54)	
48 h after	833 (67)	
72 h after	643 (49)	

Where *P* values are not given they are greater than 0.05

serum before challenge were 50% resistant, but all the groups given serum after challenge were not significantly different from the controls.

#### EFFECTS ON THEIR INFECTIVITY OF EXPOSING NBL TO IMMUNE SERUM IN VITRO

In experiment 8, two types of antiserum were used. One batch was obtained from TO mice immunized with three infections of 300 infective larvae every 3 weeks, and serum was collected 3 weeks after the last infection. The other batch was obtained from TO mice given three injections of NBL every week (3000, 4000 and 2000 NBL, respectively) and serum was collected 2 weeks after the last infection.

200 000 NBL were prepared and divided into four lots. One lot was incubated in phosphate buffered saline (PBS), a second in normal mouse serum (NMS), a third in serum from mice given infective larvae and the fourth in serum from mice given NBL. Incubation was at 37 C for 1 h. The NBL were washed twice in PBS and then injected in batches of 10 000 into normal mice. After this treatment, approximately 90% of the larvae were motile and no difference could be detected between the activity of larvae in any of the groups. The larval counts are shown in Table 4. It can be seen that there was little difference between the infectivity of NBL incubated in PBS or NMS, but that the infectivity of those incubated in serum from infected mice was considerably reduced.

In experiment 9, we attempted to absorb out the anti-NBL effect demonstrated in experiment 8. The serum used was obtained from TO mice given two infections of 300 larvae each by stomach intubation and 5000 NBL 2 weeks later; the mice were then bled 2 weeks later. This immune serum (2.5 ml) was incubated with 200 000 NBL at 37 C for 2 h. Four batches of 75 000 NBL were incubated in either PBS, NMS, immune serum or absorbed immune serum for 2 h at 37 C, washed twice in PBS and then injected into the mice at the rate of 15 000 NBL per mouse. The results of the larval counts are shown in Table 3. As in experiment 8, immune serum reduced the infectivity of NBL, but the infectivity of NBL which had been incubated in 'absorbed' immune serum was almost identical with that of NBL incubated in normal serum.

#### THE EFFECT OF ABSORBED SERUM IN VIVO

In experiment 10, 3.5 ml of the same immune serum as used in experiment 7 was incubated for 1 h with 100 000 NBL. Five mice were given 0.6 ml each of this absorbed serum and another five were given 0.6 ml of the unabsorbed serum. Two hours later, these mice, along with the five untreated animals, were challenged with 3000 NBL. The results of the muscle larva counts are summarized in Table 5. The mice given absorbed serum were not significantly different either from their controls or from mice given the unabsorbed serum. The mice given unabsorbed serum had significantly fewer larvae than the challenge controls.

**Table 4.** Larval counts in mice which had been challenged with NBL, which had been incubated *in vitro* with phosphate buffered saline, normal mouse serum, immune mouse serum or absorbed immune mouse serum

Experiment	Newborn larvae pre-treated with:			
	Phosphate buffered saline	Normal mouse serum	Immune mouse serum from: ML-infected mice	NBL-infected mice
8	4280 (520)	5250 (990)	2030 (250)	2950 (820)
		↔ $P = 0.01$	↔ $P = 0.10$	↔
	↔ $P = 0.01$	↔ $P = 0.20$	↔	↔
9	Phosphate buffered saline 6000 (600)	Normal mouse serum 6290 (820)	Immune mouse serum 2030 (310)	Absorbed immune mouse serum 6220 (310)
	↔ $P = 0.001$	↔ $P = 0.001$	↔ $P = 0.001$	↔ $P = 0.001$
	↔ $P = 0.001$	↔ $P = 0.001$	↔ $P = 0.001$	↔ $P = 0.001$

Where  $P$  values are not shown they are greater than 0.05

**Table 5.** The mean number of muscle larvae recovered from mice given immune serum and immune serum absorbed with newborn larvae

Mouse group	Mean number of worms (SE)	Probabilities
Mouse serum + challenge	366 (42)	0.1-0.2
Absorbed mouse serum + challenge	496 (118)	
Challenge only	728 (84)	0.5, 0.1 < 0.005

### Discussion

In experiments 1 and 2, in which the donor mice were immunized with light infections of NBL, both spleen cells and serum were capable of imparting partial resistance to recipient mice but there was little, or no, additive effect when they were used together. At present we have no information as to the type of cell involved in the transfer of this resistance with spleen cells and we are currently attempting to analyse this situation using methods similar to those of Crum, Despommier & McGregor (1977). In the third and fourth experiments, where the mice were immunized with ML, spleen cells were less effective than in experiments 1 and 2 and much less effective than serum taken from the same donors.

Despommier *et al.* (1977) transferred thoracic duct lymphocytes (TDL) from rats, which had been immunized with only the early intestinal phase of infection, to normal rats and obtained a more rapid expulsion of adult worms from the intestine. They found that the same cells had no effect upon NBL injected *iv.* However, in their experiments the donors were immunized with normal infections terminated 48 h later by chemotherapy and it has been shown that whilst both the intestinal and parenteral phases of the infection stimulate good immunity, this is almost entirely stage-specific (James & Denham 1975, James, Moloney & Denham 1977). One would not expect, therefore, the rats of Despommier *et al.* (1977) to be immune to NBL and it is possible that if they had immunized their rats with either full infections of NBL they would have achieved the diametrically opposite result.

In each of our experiments in which immune serum was given to mice before they were challenged with NBL, there was a significant decrease in the number of larvae recovered. Easily the best resistance was seen in the mice given serum from donors immunized with four infections with normal larvae (exp. 3, Table 1). In this experiment the mice given immune serum were 99.7% resistant to invasion of their muscle cells by NBL. This was probably because the donor mice were subjected to a much heavier immunization procedure than in any of the other experiments.

Immune serum was always effective (even if only poorly so) when it was transferred before challenge with NBL, but when it was transferred even as soon as 2 h after challenge it had no significant effect. We believe that this is because the NBL penetrate muscle cells very soon after inoculation and then inhabit a 'privileged' site where antibody can no longer reach them, and that they are susceptible to attack only in the lymph, or blood, or before they leave the intestinal mucosa.

Exposure of NBL to immune serum *in vitro* did not, in itself, affect their motility, but when NBL which had been exposed to antibody were injected into mice their infectivity was considerably reduced (Expts 8 & 9). We suggest that exposure to immune serum *in vitro* makes NBL chemotactic to some type of 'killer' cell and that this cell prevents them from infecting a muscle cell. Evidence supporting this hypothesis is provided by the finding that NBL exposed to immune serum are rapidly attacked and destroyed *in vitro* by peritoneal exudate cells from normal mice (Kazura & Grove 1978). These authors showed that NBL were destroyed *in vitro* if they were incubated with eosinophil-enriched peritoneal exudate cells in the presence of immune serum and that this reaction was abrogated by anti-eosinophil serum (AES). MacKenzie (personal communication) has shown that both macrophages and eosinophils kill NBL in the presence of immune serum and complement.

Grove, Mahmoud & Warren (1977) claimed that AES increased the number of muscle larvae which developed in a primary *T. spiralis* infection. They also claimed that AES had no effect on the expulsion of the adult worms, but as their mice were examined for adult worms at '1 week' and '2 week's' (presumably 7 and 14 days), it is possible that AES delayed expulsion for a few days without this being apparent at 14 days.

AES might increase the fecundity of *T. spiralis* as worm damage (which affects fecundity) is antibody-mediated (Love *et al.* 1976) and eosinophils may be involved in this damage. Grove *et al.* (1977) suggested that AES may facilitate the migration and intramuscular development of NBL. Our experiments 5, 6 and 7 suggest that immune serum cannot affect the larvae once they reach the muscle cell. The eosinophil and immune serum may, therefore, affect the migration of NBL from the intestinal mucosa to the muscle.

It would seem that by a combination of *in vivo* and *in vitro* studies the problem of the cause of the death of NBL under immune attack may be near solution. However, more work is needed on the effects of AES as the report of Grove *et al.* (1977) is based on a single experiment. We are attempting at present to determine whether the factor in immune serum which is involved in the death of NBL is antibody.

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