THE UNIVERSITY OF MANITOBA

MOUSE IMMUNITY TO *TRICHINELLA SPIRALIS*

BY

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ABSTRACT

Swiss Webster mice were given primary and challenge infections of 150 *Trichinella spiralis* larvae and the kinetics of intestinal worm recovery were recorded. Protocols for obtaining serum, gall bladder, bile, and intestinal lumen samples were established and the kinetics of IgA, IgG, and IgM responses in these samples were examined during primary and challenge infections. Peak titres of bile, gall bladder, and intestinal IgA, and of serum IgG coincided with worm expulsion. The effect of different levels of infection on intestinal worm recovery, muscle larvae recovery, *in vitro* larval release, female worm length, and immunoglobulin response was examined. Primary infection with 10 larvae resulted in delayed worm expulsion, a 45% increase in the muscle larvae recovery, a 42% decrease in fecundity, no difference in female worm length, and lower titres of immunoglobulin as compared to primary infection with 150 larvae. Worm fecundity was reduced up to 94% by incubation with IgA-positive gall bladder extract. Infection with 10 larvae was sufficient to prime a secondary immune response to challenge with 150 larvae in terms of more rapid worm expulsion, decreased worm fecundity and worm length, and elevated immunoglobulin titres. Dose dependent effects were observed for both primary and challenge infections with regard to worm recovery, immunoglobulin production, and peripheral

eosinophil response. The kinetics of worm expulsion and immunoglobulin production in mice infected with *Trichinella spiralis* var. *pseudospiralis* were similar to those for infection with *Trichinella spiralis* and a high degree of cross-reactivity was observed between the crude antigens from the two isolates. Serum IgG and IgM titres remained high for up to 190 days post-infection and the ability of mice to mount a secondary immune response to challenge infection as measured by faster worm expulsion and increased immunoglobulin titres was retained for this period.

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This thesis is dedicated to my two greatest accomplishments - my daughter, Nico, and my son, Sean.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

GENERAL INTRODUCTION

Trichinella spiralis is a parasitic nematode which completes its life cycle in one host. There are two main stages to the life cycle. The first is the intestinal stage which begins when the host ingests infected tissue and the digestive processes in the stomach release the encysted infective larvae (L1). The larvae are carried into the duodenum where they attach to the intestinal epithelium and undergo four molts to reach the adult stage at which time they mate. By the sixth day of infection the females produce live young and the second, or parenteral, stage begins when these newborn larvae (NBL) penetrate the intestinal epithelium and migrate through the host via connective tissue and the venous blood system to lodge primarily in striated muscle tissue (Berntzen, 1965). Once in the tissue, they occupy a striated muscle cell and become encysted infective larvae thus completing the life cycle.

Since the description of trichiniasis by Owen (1835) attempts have been made to define the nature of the disease. Natural infections of *Trichinella* were shown to confer protection to subsequent infection as measured in several host species by reduced intestinal worm recovery and prevention of establishment of muscle larvae (McCoy, 1931, 1932b; Bachman & Rodriguez-Molina, 1933; Roth, 1939; Oliver-Gonzalez, 1940; Culbertson, 1942; Fischthal, 1943) but attempts to duplicate this immunity through artificial means were not entirely successful. Bachman & Rodriguez-Molina (1933), using worm

antigen, were unable to immunize hogs. McCoy (1935) reported increased resistance to infection in rats through vaccination but not through passive transfer with immune serum. Culbertson & Kaplan (1938) achieved partial immunity in mice through passive transfer of immune rabbit serum and Culbertson (1942) immunized mice using worm antigens. Dorin (1946) produced almost complete immunity to infection in rabbits by injecting parasite antigen adsorbed onto aluminum cream and demonstrated passive transfer of immunity to rats by intraperitoneal injection of immune rabbit serum. Attempts were made to find the antigen(s) responsible for stimulating protective immunity and a wide range of antigen preparations and routes of injection were tried. Larval excretory/secretory (ES) antigens given sub-cutaneously without adjuvant (Campbell, 1955) and intraperitoneally in complete Freund's adjuvant (CFA) (Gamble, 1985), and adult ES antigens given intraperitoneally without adjuvant (Chipman, 1957) conferred immunity to subsequent infection when injected into mice as did cell-free mature muscle larva homogenates (Despommier *et al.*, 1977), large-particle fraction of muscle larva (Despommier & Lacetti, 1981), muscle larva surface antigens (Grencis *et al.*, 1986), and highly purified 37K, 48K, and 50/55K antigens from muscle larva (Silberstein & Despommier, 1984) emulsified in CFA and administered intraperitoneally.

Investigations into the mechanism of acquired immunity in the host revealed the activation of humoral, cellular, and

non-specific immune responses. As early as 1898 Brown reported the proliferation of blood and tissue eosinophils in human cases of trichiniasis. Increased mucus secretion and intestinal peristalsis in rats (McCoy, 1931) and villus atrophy and malabsorption in mice (Olson & Richardson, 1968) were the result of infections with *Trichinella*. Larsh & Race (1954) described an intestinal inflammatory response with mucosal edema and infiltration of polymorphonuclear leucocytes, plasmacytes, and macrophages. Increases in numbers of intestinal mast cells (Ruitenberg & Elgersma, 1976), basophils and eosinophils (Ogilvie *et al.*, 1980; Lindor *et al.*, 1983) have been shown. The T cell dependence of many of these mechanisms has been reported (Walls *et al.*, 1973; Manson-Smith *et al.*, 1979; Korenaga *et al.*, 1989) and enhanced accumulation of mesenteric T cells has been shown to occur in *Trichinella*-infected mice (Rose *et al.*, 1976). T and B cells have been shown to transfer immunity to the parasite (Despommier *et al.*, 1977; Wakelin & Wilson, 1979) and the involvement of B cells implied a role for antibody.

Precipitating antibody to *Trichinella* in serum was first demonstrated by Bachman (1928) using a precipitin test. Mauss (1940) found that incubation of larvae in immune serum for twelve hours resulted in the formation of precipitates around the oral cavity and reduced larval infectivity by 30%. Mauss (1940) also determined that the gamma globulin fraction of the serum was responsible for these reactions. Oliver-Gonzalez

(1941) demonstrated anti-larval and anti-adult antibodies in immune serum and achieved passive transfer of immunity to subsequent infection with the anti-adult but not the anti-larval fraction. Roth (1939) confirmed the presence of precipitating antibodies in immune serum but was not able to show reduced infectivity after incubation of larvae in the serum for two hours.

The complex nature of the immune response made it difficult to establish the relative importance of each component in expulsion and to integrate all the components into a well-defined mechanism of immunity. Most of the controversy surrounded the involvement of antibody in the expulsion process. Chandler (1939), in his theory of local immunity, hypothesized that expulsion was a result of immune action stimulated locally at the intestinal site of infection and was generally independent of circulating antibody response. Taliaferro (1940) presented a theory of general immunity, in which he hypothesized a relationship between circulating antibody and the induction of intestinal immunity. Support for Chandler's theory was found in experiments involving duodenally transplanted single sex adults in rats (Anderson & Leonard, 1940), irradiated larvae in mice (Kim, 1957), and chemically terminated infections in mice (Campbell *et al.*, 1963) which eliminated the larval parenteral stage yet conferred protection to infection. In a further study, Campbell (1965) found that the parenteral/enteral infections

do not confer any greater immunity than enteral infections alone. In support of Taliaferro's theory, Larsh (1953) and Larsh & Race (1954) reported the presence in intestinal tissue of protein material resembling a precipitate which they believed to be antibody-antigen complexes that triggered the cellular response. They argued that so-called enteral only infections are not restricted to the intestinal tissue since worms embedded in the epithelium are in contact with the circulatory system and thus stimulate parenteral immunity. Further arguments against local immunity came from Zaiman *et al.* (1954, 1955a, 1955b) who showed that immunity developed to irradiated larvae in parabiotic rats is also present in nonexposed mates which experienced no intestinal stimulation prior to infection.

The early experiments were based on the assumption that antibody to *Trichinella* was circulatory in origin. The concept of intestinal immunity underwent major revision with the description by Tomasi *et al.* (1965) of an immunological system involving the exocrine secretions that was distinct from and independent of the serum immunological system. This finding was confirmed by later experiments (Benveniste *et al.*, 1971; Kaiserlian *et al.*, 1985). The theory of a common mucosal immune system was proposed when it was discovered that specific antibodies to orally administered antigens were present in the secretions of remote mucosal tissues (Mestecky *et al.*, 1985; Czerkinsky *et al.*, 1987). Earlier experiments

demonstrated that gamma globulin A (IgA) was the predominant class of gamma globulin in human saliva, colostrum, lacrimal secretions (Chodirker & Tomasi, 1963), and nasal and bronchial washings (Remington *et al.*, 1964; Keimowitz, 1964) and differences were observed in the sedimentation coefficients (Tomasi & Zigelbaum, 1962, 1963) and immunodiffusion patterns of the IgA (Tomasi *et al.*, 1965) found in secretions and serum. Crabbé *et al.* (1965, 1968, 1969) defined the role of the intestine as a site of IgA synthesis and Tomasi *et al.* (1965) showed local synthesis of IgA in the salivary gland by fluorescent and autoradiographic methods.

It has been demonstrated that a specific anamnestic secretory IgA (sIgA) response can be induced by oral immunization with viral and bacterial antigens (Ogra & Karzon, 1969; Keren *et al.*, 1982) and that this induction is not restricted to the site of stimulation. This phenomeneon may explain the results of *Trichinella* infections in parabiotic rats where resistance to infection was found in the intestine of rats which had not been infected directly but were linked to infected rats by Thiry-Vella loops. This would attribute an important role to sIgA in *Trichinella* and other similar nematode infections but little research has been done to date in this area. Most research on antibody response to infections with *Trichinella* has focused on serum immunoglobulins (Oliver-Gonzalez, 1941; Hendricks, 1950; Sadun *et al.*, 1968; Ljunstrom, 1973; Rivera-Ortiz & Nussenzweig, 1976; Philipp *et*

al. 1981; Almond & Parkhouse, 1986). The first comprehensive study to examine the antibody content of intestinal secretions during infection with *Trichinella* was that of Crandall and Crandall (1972) in a mouse host. Using indirect fluorescent antibody staining they determined the presence of IgA in intestinal contents by the second week of primary infection and, by monitoring serum immunoglobulin levels simultaneously, concluded that it was locally produced. Kozek & Crandall (1973) detected IgA in intestinal perfusates of mice on Day 8 which increased by Day 14 of primary infection while in hyperinfected animals IgA was detected in perfusates by 24 hours and remained at higher than primary levels throughout the 11 day study. They concluded that IgA was locally produced but were unable to demonstrate that the increased IgA response in hyperinfected mice was significant. Jacqueline *et al.* (1978) incubated fecund female *Trichinella* with the intestinal secretions of normal and infected mice and rats *in vitro* and found a significant reduction of larval production only with secretions from infected animals and specifically with the IgA fraction of these secretions. Sinski *et al.* (1983) detected only IgA in the intestinal wash of mice given primary infections of 200 L1. Brown & Bruce (1989) compared the percentage of labelled intestinal IgA plasma cells in uninfected mice and mice which had been infected 5, 9, 13, or 18 days previously with *Trichinella* which were given a single injection of ^{3}H-thymidine 24 hours before examination and

found an increase in positive IgA cells in infected mice from day 5 through 18. The significance of the increase with regard to expulsion was not clarified.

Stimulation of sIgA production has been examined in other parasite/host systems. Eddie *et al.* (1971) found high levels of anti-*Salmonella typhimurium* IgA in colostrum and intestinal fluid of rabbits given oral doses of the live, but not dead, bacteria. Poulain *et al.* (1976) showed that intestinal IgA appeared before serum antibody in rats infected with *Nippostrongylus brasiliensis* but did not find an elevated response after reinfection characteristic of an anamnestic response. Infection with very low doses of larvae (83) also produced a local immune response and in this case a second infection of 3000 larvae generated a significant increase in IgA antibody in the intestinal secretions. Sinski & Holmes (1977) also found IgA in the mucosal extract of rats infected with *N. brasiliensis* and demonstrated that a 10-fold increase in the level of this immunoglobulin occurred after a challenge infection. Detection of intestinal IgA as early as Day 3 of primary infection and increased levels of the antibody after challenge were reported by Cypess *et al.* (1977) for *Heligosomoides polygyrus*-infected mice. Passive transfer of resistance in mice to *Taenia taeniaeformis* with an IgA, but not an IgG, fraction of intestinal secretion and colostrum demonstrated the protective capacity of this antibody (Lloyd & Soulsby, 1978). Snider and Underdown (1986) used an

immunoradiometric assay to measure the immunoglobulin content of intestinal secretions from mice infected with *Giardia muris*. They observed that IgA was the only detectable anti-parasite antibody throughout the seven-week course of infection, that expulsion of the parasite paralleled the appearance and increase of IgA in the secretions, and that serum IgA was much slower to develop. Intestinal IgA was also monitored in intestinal lymph of sheep infected with *Ostertagia circumcincta* (Smith *et al.*, 1987).

The contribution of bile to intestinal IgA was demonstrated in rats (Lemaître-Coelho *et al.*, 1978) and mice (Delacroix *et al.*, 1985) and the liver and biliary system were recognized as part of the mucosal immune system (Kleinman *et al.*, 1982). The presence of anti-worm IgA in bile of *N. brasiliensis*-infected rats was reported by Brown *et al.* (1981) using an enzyme-linked immunosorbent assay (ELISA), with higher levels found after reinfection, and in bile of mice infected with *Ascaris suum* even at very low parasite doses (Jeska & Stankiewicz, 1989). The ELISA, a sensitive test for antibody, has been used successfully to detect total IgA and specific anti-cholera toxin IgA antibodies in intestinal secretions (Elson *et al.*, 1984).

The objects of this study were: i) to examine the kinetics of IgA, IgG, and IgM antibody production in serum, intestinal secretions, and bile of mice during primary and challenge infections with *Trichinella* using the ELISA

technique to detect the immunoglobulins; ii) to determine if an anamnestic mucosal immune response (sIgA) is established; iii) to examine the effect of size of infection dose on antibody and worm expulsion kinetics; iv) to compare the kinetics of antibody production and worm expulsion between two strains of the parasite; v) to determine if any detrimental effects on the parasite could be attributed to IgA obtained from mucosal sources.

CHAPTER 1: ESTABLISHING EXPERIMENTAL PROTOCOLS CONCERNING THE KINETICS OF IMMUNOGLOBULIN RESPONSES IN SERUM, GALL BLADDER, AND SMALL INTESTINE OF MICE.

INTRODUCTION

The object of these experiments was to establish protocols for obtaining samples for immunoglobulin testing and for enumerating larval and adult *Trichinella* that would provide consistent and comparable results between experiments. The care and handling of mice, the techniques for infection with and counting of parasites, and the method for procuring serum have been well established. However, few previous studies addressed the question of mucosal immunity of mice to *Trichinella* infections and there was little information available regarding the timing and procedure for procuring samples of intestinal secretions. As knowledge was gained from an experiment, subsequent experiments were modified to incorporate these findings. Details of the experiments given in Materials and Methods reflect these modifications.

MATERIALS AND METHODS

Animals

Male and female Swiss Webster mice (Cr1COBS: CFW(SW)) 16-24 weeks old were used in all experiments except the first set in which male mice 8 weeks old were used. Mice were given food and water *ad libitum* until sacrifice for initial experiments. In later experiments food was removed before sacrifice at times stated in the experimental design. The design of all experiments was dependent on the number of mice of similar age and sex available.

Parasites

A strain of *Trichinella spiralis* originally obtained from pig (P1) and maintained by successive passages through Swiss Webster mice was used in all experiments. Infective larvae (L1) were collected from the muscle tissue of previously infected mice by digestion of the skinned and eviscerated carcasses in 200 mls of 1% HCl/ 1% pepsin for 2 hours at 37°C with shaking (200 rpm in a Lab Line Environ Shaker, Lab Line Instruments Inc., Melrose Park, Ill.) followed by filtering through a 180 μ then a 63 μ screen (Canadian Standard Sieve Series, Combustion Engineering Canada Inc., St. Catherine's, Ontario). Larvae were washed off the small filter with saline (0.15 M NaCl) into a 25 ml round-bottomed glass test tube. Larvae were allowed to settle and were then washed 3 times by

siphoning the saline and adding fresh saline to the tube. After the final wash, the larvae were suspended in 10 ml of saline and a small stir bar added. The tube was placed on a magnetic stirrer and the speed adjusted to give a uniform suspension. Five 0.01 ml aliquots were taken from the suspension and placed on a counting grid. The worms in each aliquot were counted under a dissecting microscope and the counts averaged. The average multiplied by 1000 gave an estimate of the total number of larvae.

Infection Procedure

For infections of 50 L1 and over, suspensions containing the desired number of worms in 0.15 - 0.20 ml of saline were made by appropriate dilution of the original suspension. Infections were delivered *per os* using a bulb-tipped needle on a 1.0 ml syringe. Throughout the infection process, individual infection volumes were collected after every fourth mouse. The worms in these volumes were counted and the average of the counts was used as the infection level for subsequent calculations. For infections of 10 L1, the worms were counted into a nine round-bottomed well glass plate under a dissecting microscope. The worms were drawn into the syringe and the well checked to ensure the removal of all worms. After delivery, the syringe was rinsed into the well of origin and the well examined under the microscope for any worms which had been retained in the syringe. If any worms were seen, they were

drawn into the syringe again and administered to the mouse in as small a volume of saline as possible.

Worm Counts

Intestine: Mice were killed by cervical dislocation and the intestine removed. After flushing, the intestine was slit open longitudinally then cut transversely into 4-5 cm pieces. These sections were placed into a large Petri dish containing warm saline and incubated at 37°C for 2-4 hours. After incubation, the tissue was examined under a dissecting microscope for attached worms, then discarded. The worms were counted by transferring small amounts of the saline to a gridded Petri dish and counting the worms in each section under a dissecting microscope.

Muscle Larvae: After removal of the intestine, each mouse was de-limbed, eviscerated, and skinned. The carcass was digested in 1% HCL/pepsin and the larvae recovered as previously described in the infection procedure. In the initial experiment, in order to monitor the first appearance of larvae in the muscle tissue, the liquid level in the test tube was reduced by siphoning to approximately 1.0 ml and the entire 1.0 ml volume was screened for larvae. Once the larvae recovered became too numerous to count in this way, the method previously described was employed to estimate total muscle larvae number.

In Vitro Larval Release

Female worms obtained from the incubation of intestinal tissue in saline were placed in fresh sterile saline at 37°C and washed in sterile saline until free of debris, confirmed under a dissecting microscope. The worms were washed in RPMI 1640 culture media (Sigma Chemicals) then placed one to a well in sterile 96-well culture plates containing 200µl of RPMI with 10% fetal calf serum (FCS) and antibiotic (100 IU/ml penicillin and 0.1 mg/ml streptomycin). Plates were placed in a 37°C/5% CO_2 incubator for 24 hours. Newborn larvae (NBL) released by the females over the 24 hour period were counted using a Nikon inverted microscope.

Female Worm Length

Female worms were removed from culture media, fixed with hot 70% EtOH, and placed in 70% EtOH with 10% glycerol. The worms were cleared by allowing the EtOH to evaporate, leaving them in a solution of pure glycerol. These worms were mounted on slides in glycerol, covered with a cover slip, and the lengths measured by tracing the worm on paper with the aid of a drawing tube attached to a Leitz microscope with an ocular micrometer. The scale was also traced. As the worms were often curled and could not be measured directly with accuracy, a piece of thread was matched to the tracing of the worm and the length of the thread measured on the scale to give worm length.

Procedures for Obtaining Samples

Serum: Mice were cervically dislocated and the skin pulled back to expose the right brachial artery. The artery was severed with a pair of scissors and the blood drawn into a siliconized pasteur pipette then dispensed into a 1.5 ml microcentrifuge tube (siliconized 5 3/4" Pasteur pipettes were prepared at least 24 hours prior to use by drawing undiluted Sigmacote (Sigma Chemicals) into the pipette with a rubber bulb, then emptying the pipette and leaving it in a vertical position to dry). After 1 hour at room temperature, the tube was ringed with a wooden applicator stick, the clot removed, and the sample allowed to settle at 4°C. The sample was centrifuged for 2 minutes in a Fisher model 235B microcentrifuge and the serum drawn off into a clean tube for storage at -70°C until testing.

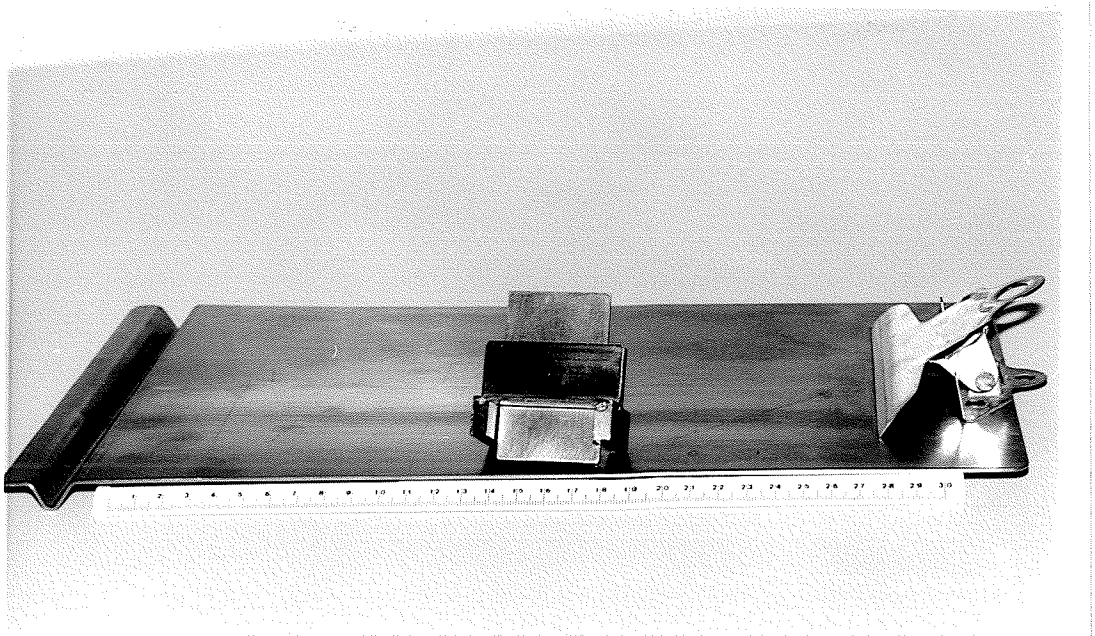
Gall Bladder: After the blood was collected, the mouse was opened from the lower abdominal region to the neck to fully expose the internal organs. The gall bladder was located and removed intact. Adhering tissue was carefully teased away and the gall bladder rinsed in phosphate-buffered saline, pH=7.4 (PBS). In initial experiments, the gall bladder was placed into a centrifuge tube and homogenized for 30 seconds in 0.5 ml of PBS using a tissue grinder. The sample was centrifuged and the supernatant removed to a clean microcentrifuge tube for storage at -70°C until testing.

Samples obtained in this manner were identified as gall bladder. In later experiments, the gall bladder was located, removed, and cleaned as described above. After rinsing in PBS, the gall bladder was blotted dry, then placed into a microcentrifuge tube and pierced. The bile was collected by carefully pressing the gall bladder then the tissue was removed from the tube and discarded. The bile sample was kept on ice until sampling was completed. The sample was stored at -70°C until testing. Samples obtained in this manner were denoted as bile.

Intestinal Lumen: After collection of serum the mouse was opened from the neck to the lower abdominal region. In the initial experiment, the small intestine was cut at the pyloric and ileocaecal junctions and removed to a large glass Petri dish where it was cut into two equal lengths. Each half was flushed with 1.0 ml of PBS delivered with a 9" Pasteur pipette. The flushed contents were collected in a 10.0 ml polypropylene test tube and the volume adjusted to 2.0 ml. After vortexing for 30 seconds the intestinal lumen sample (which from here on in will be abbreviated to IL) was centrifuged at 18,000 rpm for 15 minutes at 4°C in a Beckman model J2-21M centrifuge. The supernatant was pipetted into two 1.5 ml microcentrifuge tubes for storage at -70°C until testing. In subsequent experiments, the small intestine was clamped at the pyloric and ileocaecal junctions. The intestine

was cut above the clamp at the pyloric junction and removed from the body cavity. Scissors and tweezers were used to free the intestine from mesentery tissue when necessary. The intestine was cut below the clamp at the ileocaecal junction and washed in a trough (37.0 x 6.5 x 5.0 cms) of 37°C PBS by running it twice in each direction with gentle sideways shaking. The intestine was then placed onto paper towelling, blotted dry, and the relaxed length recorded. The intestine was transferred to a layer of damp paper towelling on a 34.3 x 22.9 cm stainless steel scraping board (Figure 1). This board was designed and built specifically for the purpose of IL and mucosal sampling. It was equipped with a fixed clamp at one end (the "top") and a round-bottomed trough (1.0 cm top width, 1.2 cm deep) running the width of the board at the other (the "bottom"). The intestine was cut into two halves and the unclamped ends placed into the trough. Each half was flushed with 0.25 ml of PBS followed by 2.5 ml of air delivered by a 3.0 ml disposable syringe with a 1 1/2" 25g needle bearing a 3.0 cm length of tygon tubing which fit tightly to the needle. The tubing was inserted into the unclamped junction end of each half and the tissue held firmly against the tubing to force the liquid and air through the length of the intestine. If liquid or air bubbles were observed leaking anywhere along the length of the intestine the tubing was removed and reinserted into the site of the leak and the procedure continued. After the injection of PBS

Figure 1. Photograph of the mucosal scraping apparatus designed for the purpose of intestinal lumen and mucosal layer sampling. A scale (in cms) is shown. The holder is tilted in order that the plate can be observed coming through the slot. When in use, the holder rests on the flat bottom and the plate is free to move vertically. Samples are collected in the trough. The narrower bottom of the trough fits the mouth of a microcentrifuge tube to allow easy transfer of material.



and air, the tubing was removed and the upper end reclamped. The intestine was placed back onto the damp paper towelling and the remaining contents removed by pressing the intestine against the towelling down the length of the intestine towards the trough end. The IL collected in the trough was transferred to a microcentrifuge tube and placed on ice until sampling was completed. Each IL sample was vortexed for 30 seconds and centrifuged for 15 minutes in a microcentrifuge. The supernatant was removed to a clean tube and stored at -70°C until testing. The precipitate was suspended in saline and checked for the presence of worms by the method described for counting intestinal worms. The number of worms found was added to the corresponding intestinal count.

A number of other modifications to the technique were tried. The first was the collection of anterior and posterior IL for separate testing to determine if a difference occurred along the length of the intestine with regard to immunoglobulin production. The second was mechanical homogenization with a Kinematica tissue grinder instead of vortexing in order to release immunoglobulin trapped in the mucus structure (Clamp, 1977; Allen *et al.*, 1984) and freezing of the sample before homogenization (Harbitz *et al.*, 1980). The third modification was to heat the sample for 30 minutes in a 56°C water bath after collection but before vortexing and to centrifuge for three minutes instead of fifteen to reduce proteolytic activity. As explained in the results, only the

third modification was adopted.

Intestinal Mucosa: For the first experiment, after flushing the contents of the intestinal lumen, the intestine was placed into a large Petri dish containing approximately 2.0 ml of PBS and cut open longitudinally. The flat end of a measuring spatula was held at a 45 degree angle and used to scrape off the mucosal layer gently. The mucosal material obtained was collected in a polypropylene test tube and the final volume brought to 5.0 ml. The tube and contents were vortexed for 30 seconds then centrifuged at 18,000 rpm for 15 minutes at 4°C. The supernatant was pipetted into microcentrifuge tubes and stored until testing. The precipitate was suspended in saline and checked for worms. The number of worms found was added to the corresponding intestinal count. In subsequent experiments, in order to control the pressure used to remove the mucus, a specially designed scraping device was manufactured for use with the previously described board which consisted of a 25 gm, rectangular (6.4 x 3.8 cms) stainless steel plate vertically positioned in a holder with a central slot just large enough to hold the plate upright but still allow free movement up and down (Figure 1). The plate was made with one round and one sharp edge. In trial scrapings using the sharp end, large amounts of healthy villi were present, while those done with the round end contained few or no villi. Therefore, the round

end was used throughout the course of subsequent experiments. After flushing, each half of the intestine was clamped to the scraping board (a slit piece of Tygon tubing was placed over the clamp to ensure that the clamp did not sever the tissue) and then cut open longitudinally. The scraping device was positioned over the exposed tissue as close to the clamp as possible and the intestine scraped by pulling the scraper slowly toward the trough end of the board. If the tissue was torn, this broken end was clamped and the process continued. The mucosal material was collected in the trough and washed into a microcentifuge tube with 0.5 ml of PBS. The sample was placed on ice until sampling was completed, then vortexed for 60 seconds. The sample was centrifuged for 15 minutes and the supernatant removed to a clean tube and stored until testing.

A modification to the above method was to scrape the portion of the intestine under the clamp as well as the rest of the length. Modifications made to the treatment of the sample were exactly as previously described for IL samples.

ELISA Procedure

Serum, intestinal lumen, mucosal, gall bladder, and bile samples were tested in duplicate for the presence of IgA, IgG, and IgM antibody by ELISA. The antigen (Ag) used for the ELISA was a crude extract of P1 muscle larvae obtained by homogenizing L1 in PBS for 30 minutes using a ground glass tissue homogenizer attached to an electric drill. The mixture

was centrifuged at 20,000 rpm (Beckman model J2-21M) for 30 minutes at 4°C and the supernatant dialyzed in 4 X 2 litres of PBS over 48 hours. The dialyzed supernatant was centrifuged as before. The protein content of the final supernatant was determined by Lowry (Lowry *et al.*, 1951) and Bio-Rad (Bio-Rad protein assay kit, Bio-Rad Laboratories, Richmond, California) protein determination methods.

Antigen was applied to the wells of Immulon II ELISA plates (Dynatech Laboratories, Chantilly, Virginia) by the addition of 100 µl of a 10 µg/ml solution of P1 Ag in 0.05 M carbonate buffer, pH=9.6 to each well. Carbonate buffer alone was used in no antigen control wells. The plates were left overnight at 4°C. The next day the plates were washed three times with washing buffer (0.05% Tween 20 in PBS) and 200 µl of blocking buffer (3% BSA in washing buffer) were added to each well. Washing buffer alone was used in the non-blocked control wells. The plates were left overnight at 4°C and washed the next day as before. Test samples were diluted in blocking buffer and 100 µl added to each well. Plates were left at room temperature for 1 hour then washed as before. Horseradish peroxidase (HRP)-coupled goat anti-mouse Ig (A, G, or M - Sigma Immunochemicals, St. Louis, Missouri) was diluted with blocking buffer according to manufacturer's instructions and 100 µl added per well. Plates were incubated at room temperature for 1 hour then washed as before. Substrate (0.04% orthophenylenediamine in 0.05 M phosphate-citrate buffer,

pH=5.0 with 0.012% hydrogen peroxide) was prepared immediately prior to use and 100 µl added to each well. Absolute reaction control wells were set up at this time with 100 µl of HRP*Ig and 100 µl of substrate. Plates were left at room temperature for 30 minutes. The reaction was stopped by the addition of 100 µl of 1N HCl and the plates read at 490 nm on a Biotech Microplate reader (model EL308) to give optical density (O.D.) values for each sample. The O.D. values for the duplicates of each sample were averaged and these average O.D. values were used directly as a measure of the titre of antibody present in the sample.

Experimental Design

Four experiments were performed to establish a protocol for sampling times, sampling methods, and infection dose levels. The first experiment (Experiment #1) was designed to examine the kinetics of worm expulsion and immunoglobulin production. In order to determine the level of immunoglobulins in unparasitized mice, two uninfected mice were sacrificed as controls on Day 0 when a primary infection of 150 L1 was given to 42 mice. Two mice were sacrificed on Days 1 and 28 and three on Days 3, 6, 9, 12, 15, and 19 post-infection. The remaining mice were challenged with 150 L1 on Day 28. Two mice were sacrificed on Day 29 and three on Days 31, 34, 37, 40, 43, and 47 post-infection. Serum, intestinal lumen, and mucosal samples were collected. Intestinal worms and muscle

larvae were counted for each mouse.

In the second experiment (Experiment #2), four uninfected mice were sacrificed as controls and 68 mice were given a primary infection of 150 L1 on Day 0. Four mice were sacrificed on Days 3, 6, 9, 12, 15, 18, 21, 28, and 56 post-infection. The remaining 32 mice were challenged with 150 L1 on Day 28 and four mice sacrificed on Days 31, 32, 33, 34, 37, 43, 49, and 84 post-infection. Serum, intestinal lumen, and mucosal samples were collected and intestinal worms counted for each mouse. Muscle larvae were counted for all challenged mice.

The third experiment (Experiment #3) was designed to examine the effect of different infection levels on worm expulsion and immunoglobulin production. Control mice were sacrificed on Days 0 and 28. Mice given primary infections of 10, 50, and 100 L1 were sacrificed on Days 6, 12, 18, and 28 post-infection while mice given a primary infection of 150 L1 were sacrificed on Days 6 and 12. The remaining mice were challenged on Day 28 with infection levels identical to the primary infection. Mice challenged with 10, 50, and 100 L1 were sacrificed on Days 31, 34, and 40 post-infection while mice challenged with 150 L1 were sacrificed on Days 34 and 40. There were four mice in each experimental group and for each time of sampling. Serum, intestinal lumen, mucosa, and gall bladder samples were collected and intestinal worms counted for all mice. Muscle larvae were counted for all challenged

mice.

Results from the initial experiment to assess the level of immunoglobulins in the gall bladder (Experiment #3) were highly variable. The volume of bile in the gall bladder varied widely from full to completely empty. As bile is released from the gall bladder when food enters the stomach and duodenum, I reasoned that bile should be retained in the gall bladder in a fasted mouse. However, since no information was available on the effect of fasting on *Trichinella* expulsion, this required investigation. In the fourth experiment (Experiment #4) thirty-two uninfected and eight previously infected mice were given 150 L1. Sixteen of the primary infected group and the eight challenged mice were sacrificed three days later while the remaining sixteen mice of the primary infected group were sacrificed six days after infection. Food was removed and shavings changed (to remove excess food in the shavings) for half of the mice in each group twelve hours before sacrifice.

RESULTS

Intestinal Worm Recovery

The design of the first two experiments was considered adequate for monitoring worm recovery until expulsion was complete. A drastic drop in recovery on Day 12 and complete expulsion by Day 15 of worms from the primary infection was observed for both Experiment #1 and Experiment #2 (Figures 2 & 3). Worms from challenge infections were completely expelled by Day 34 in Experiment #1 and this observation prompted the addition of Days 32 and 33 to the sampling schedule of Experiment #2, giving a more detailed pattern of post-challenge worm expulsion in this experiment. Expulsion of challenge infections was complete by Day 37.

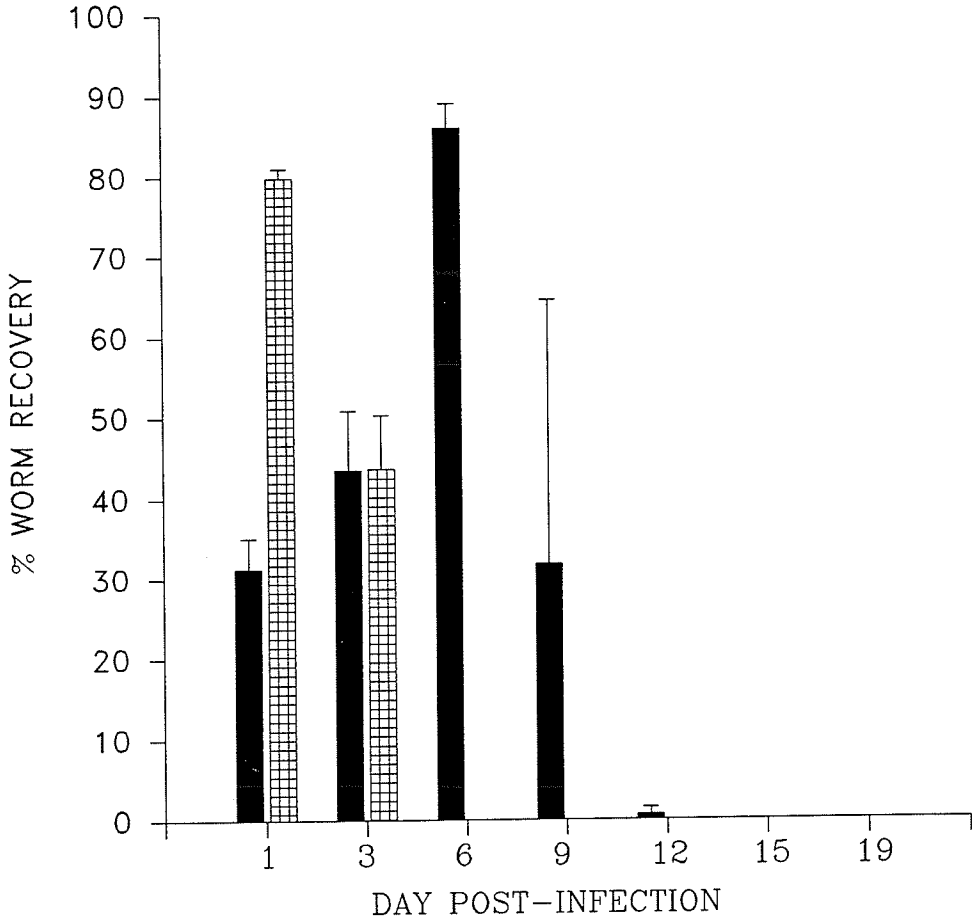
The effect of infection level on worm expulsion is summarized in Table 1. No effect on recovery was seen except for the Day 6 count for infection with 10 L1 which was significantly lower than the Day 6 recoveries for the other infection levels. However, Experiment #3 demonstrated that low level infection worm expulsion could be monitored.

There was no significant difference in worm recoveries for the fed and fasted groups in Experiment #4 (Table 2).

Muscle Larvae Recovery

No muscle larvae were observed in the primary infection until Day 15 when three larvae were found in one of the three

Figure 2. The kinetics of intestinal worm recovery for Experiment #1. Swiss Webster mice were given primary (■) and challenge (▦) infections of 150 *Trichinella spiralis* larvae. The % worm recovery is the mean of the values for the mice in each group (n=3 except Day 1 where n=2). The t-bars represent the standard deviation from the mean. Challenge infections were given 28 days after primary infection (Day 28 post-primary infection ≡ Day 0 challenge infection). The two infections are plotted side-by-side for the purpose of comparison.


100
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80
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60
50
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% WORM RECOVERY
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9
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15
19
DAY POST-INFECTION

Figure 3. The kinetics of intestinal worm recovery for Experiment #2. Swiss Webster mice were given primary (■) and challenge (▦) infections of 150 *Trichinella spiralis* larvae. The % worm recovery is the mean of the values for the mice in each group (n=4). The t-bars represent the standard deviation from the mean. Challenge infections were given 28 days after primary infection (Day 28 post-primary infection ≡ Day 0 challenge infection). The two infections are plotted side-by-side for the purpose of comparison.

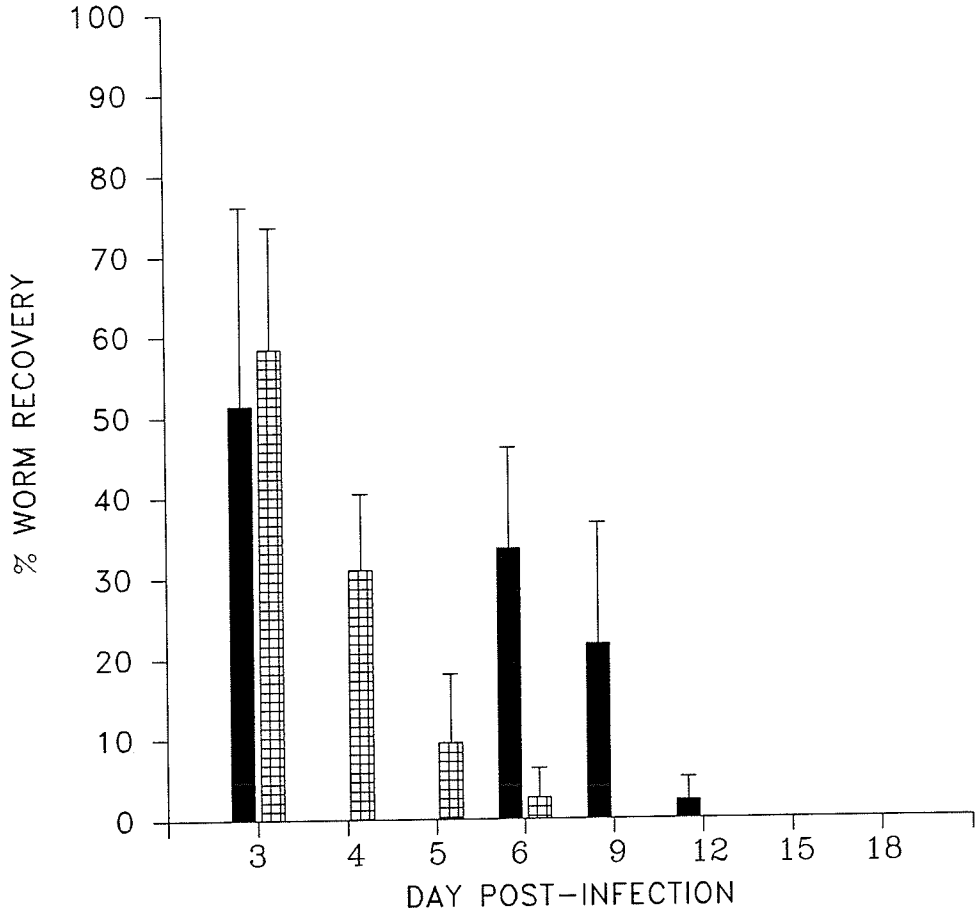

100
90
80
70
60
50
40
30
20
10
0
% WORM RECOVERY
3
4
5
6
9
12
15
18
DAY POST-INFECTION

Table 1. Intestinal worm recoveries from Swiss Webster mice given different primary and challenge infection levels of *Trichinella spiralis* larvae (L1) in Experiment #3. The mean ± standard deviation of worm recovery (expressed as % of the number of L1 given) for mice at each infection level (n=4) is shown. Challenge infection was given on Day 28 post-primary infection (Day 31 ≡ Day 3 post-challenge).

	Primary Infection Level			
Day	10L1	50L1	100L1	150L1
6	2.5 ± 4.3	30.1 ± 11.6	36.2 ± 11.5	30.9 ± 9.0

	Primary/Challenge Infection Level		
	10/10L1	50/50L1	100/100L1
31	17.5 ± 14.8	25.0 ± 18.2	16.7 ± 14.1

Table 2. Intestinal worm recoveries from fed and fasted Swiss Webster mice given primary and challenge infections of 150 *Trichinella spiralis* larvae (L1). The mean ± standard deviation of the number of worms recovered (expressed as % of the number of L1 given) from the mice in each group is shown. Challenge infection was given on Day 28 of primary infection (Day 31 ≡ Day 3 of challenge).

	Primary		Challenge
	Day 3	Day 6	Day 31
Fed	55.0% ± 15.4% (n=8)	69.2% ± 19.2% (n=8)	33.7% ± 10.1% (n=4)
Fasted (12 hrs)	51.2% ± 13.7% (n=8)	81.8% ± 37.4% (n=8)	40.0% ± 5.6% (n=4)

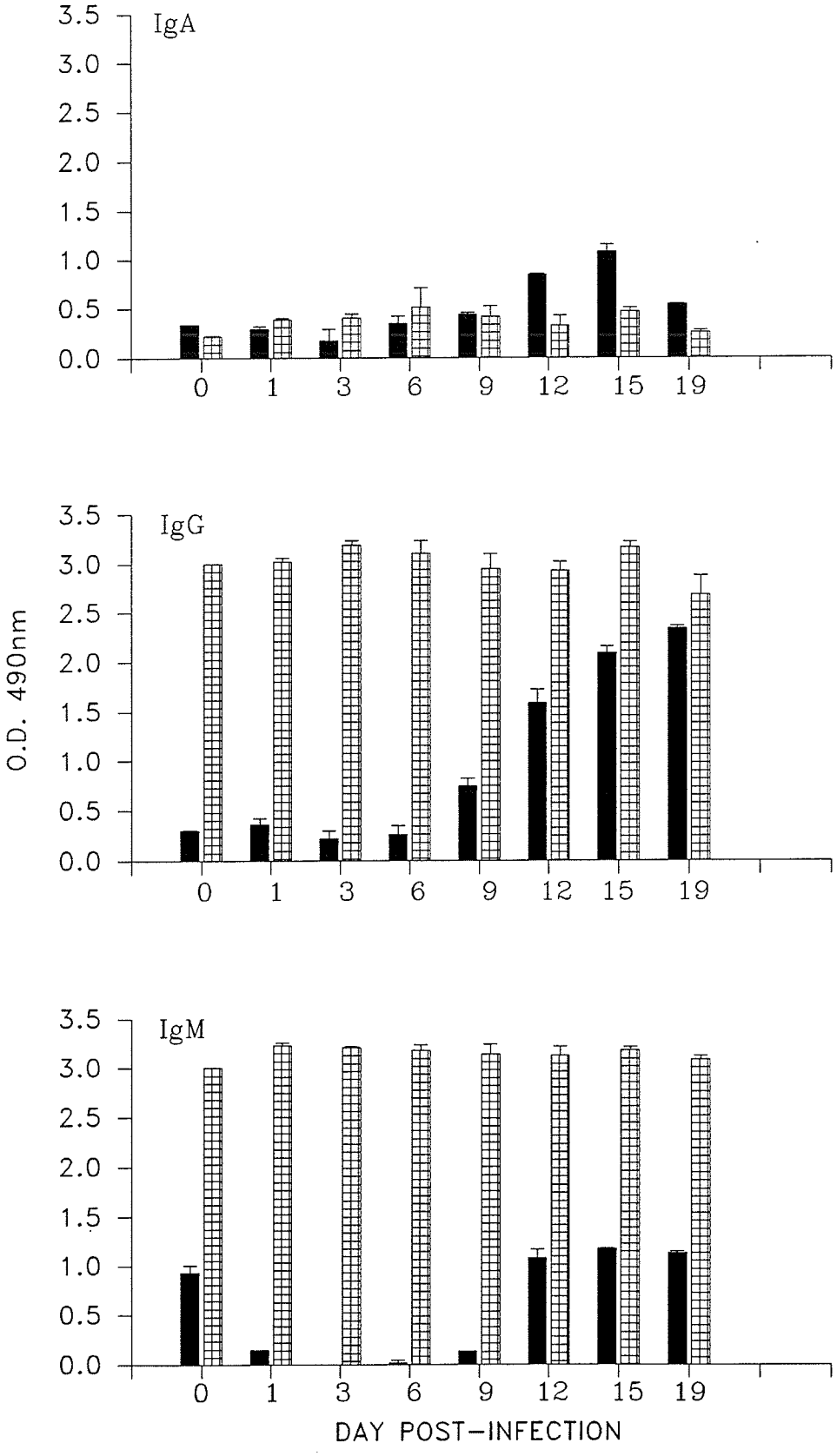
mice sacrificed. Although substantial numbers of larvae were present in all three mice (1667 ± 189) by Day 19, these values were only 1/10th the number found in mice infected 28 days or longer (16,208 ± 3179 in Experiment #1 and 17,417 ± 3909 in Experiment #2). On the basis of this result, no muscle larvae were counted for mice with infections of less than 28 days.

Serum Immunoglobulin Response

Serum samples were tested at 1:10 dilution. The serum immunoglobulin titres in Experiment #1 and Experiment #2 are shown in Figures 4 & 5. Experiment #1 control samples showed significant background titres of all immunoglobulins in the serum of uninfected mice (Figure 4 - Day 0 values). Similar elevated titres were found for the four control mice of Experiment #2 (Figure 5 - Day 0 values). These findings raised the question of the validity of using only Day 0 controls for each experiment as the background titres might change with the age of the mouse (Van Der Heijden *et al.*, 1987, 1988). In experiment #3 the differences in background immunoglobulin titres of mice from Day 0 and Day 28 were examined (Table 3). There were no significant differences found between Day 0 and Day 28 values for any sample or any immunoglobulin justifying the use of controls from Day 0 for experiments with this time span.

The kinetics of the serum immunoglobulin response were similar in Experiments #1 and #2 with IgA peaking on Day 15

Figure 4. Kinetics of the serum immunoglobulin response of Swiss Webster mice to primary (▬) and challenge (▦) infections with 150 *Trichinella spiralis* larvae (L1) for Experiment #1. Immunoglobulin titre is expressed as the mean optical density (O.D.) from enzyme-linked immunosorbent assay of serum samples (tested at 1:10 dilution) from mice in each group (n=3 except Day 1 where n=2). The t-bars represent the standard deviation from the mean. Challenge infections were given 28 days after primary infection (Day 28 post-primary infection ≡ Day 0 of challenge infection). Primary and challenge infection values are plotted side-by-side for the purpose of comparison.


IgA
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O.D. 490nm
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3.0
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1
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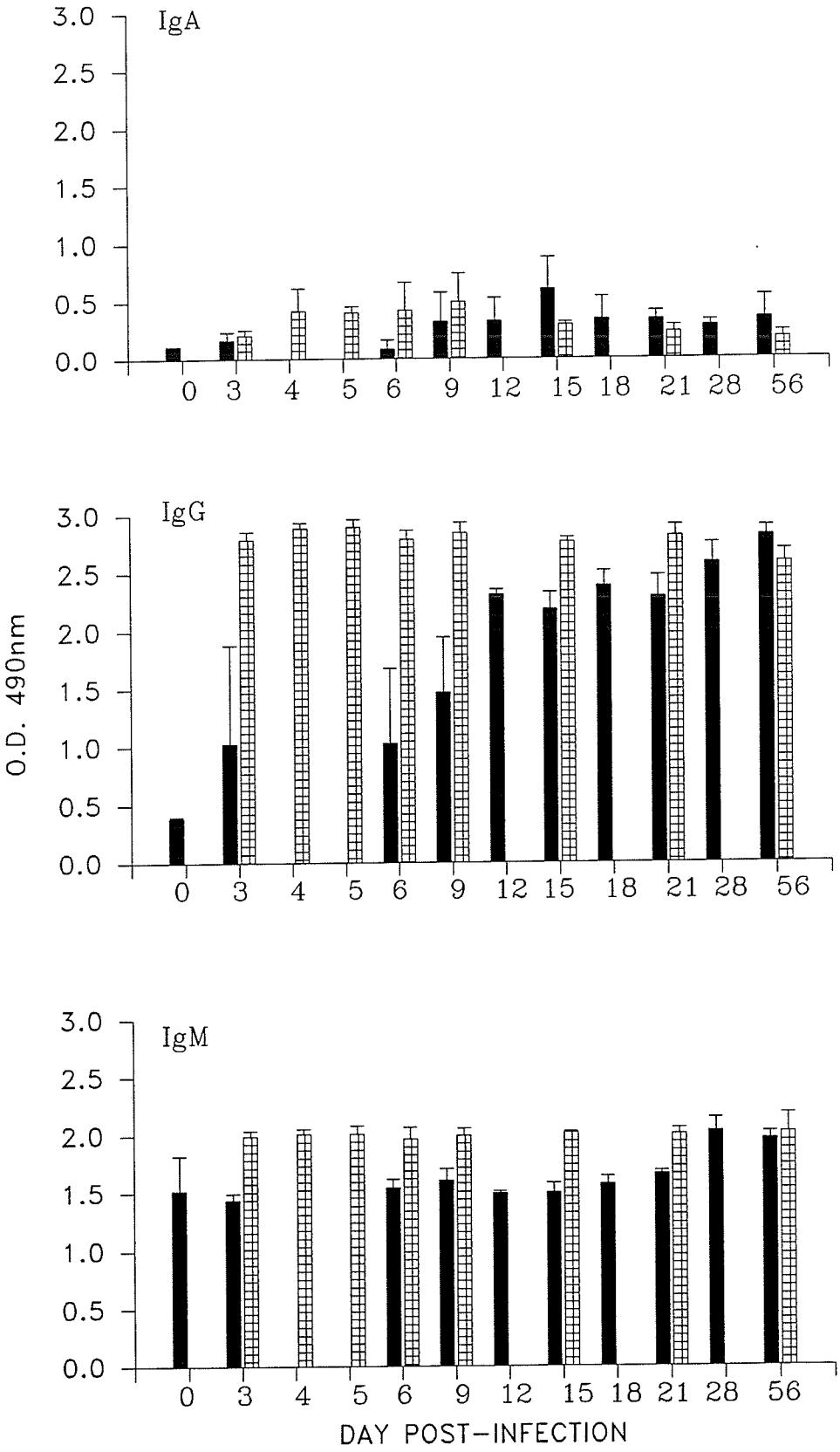
6
9
12
15
19
DAY POST-INFECTION

Figure 5. Kinetics of the serum immunoglobulin response of Swiss Webster mice to primary (■) and challenge (▦) infections with 150 *Trichinella spiralis* larvae (L1) for Experiment #2. Immunoglobulin titre is expressed as the mean optical density (O.D.) from enzyme-linked immunosorbent assay of serum samples (tested at 1:10 dilution) from mice in each group (n=4). The t-bars represent the standard deviation from the mean. Challenge infections were given 28 days after primary infection (Day 28 post-primary infection ≡ Day 0 of challenge infection). Primary and challenge infection values are plotted side-by-side for the purpose of comparison.


IgA
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1.0
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0.0
0 3 4 5 6 9 12 15 18 21 28 56
DAY POST-INFECTION

Table 3. Background immunoglobulin titres of serum, bile, and intestinal lumen (IL) samples from uninfected Swiss Webster mice. Mice were of similar ages at the start of the experiment but were sacrificed at two different times four weeks apart (Days 0 and 28). The titres are expressed as the mean ± standard deviation of the optical density values from enzyme-linked immunosorbent assay of samples from mice in each group (n=12).

	Serum IgA	Bile IgA	IL IgA
Day 0	0.258 ± 0.076	0.072 ± 0.072	0.061 ± 0.019
Day 28	0.293 ± .101	0.073 ± 0.065	0.081 ± 0.006

	Serum IgG	Bile IgG	IL IgG
Day 0	0.050 ± 0.019	0.001 ± 0.001	0.000 ± 0.000
Day 28	0.083 ± 0.073	0.001 ± 0.001	0.007 ± 0.006

	Serum IgM	Bile IgM	IL IgM
Day 0	1.686 ± 0.298	0.006 ± 0.006	0.000 ± 0.000
Day 28	1.673 ± 0.198	0.001 ± 0.001	0.001 ± 0.001

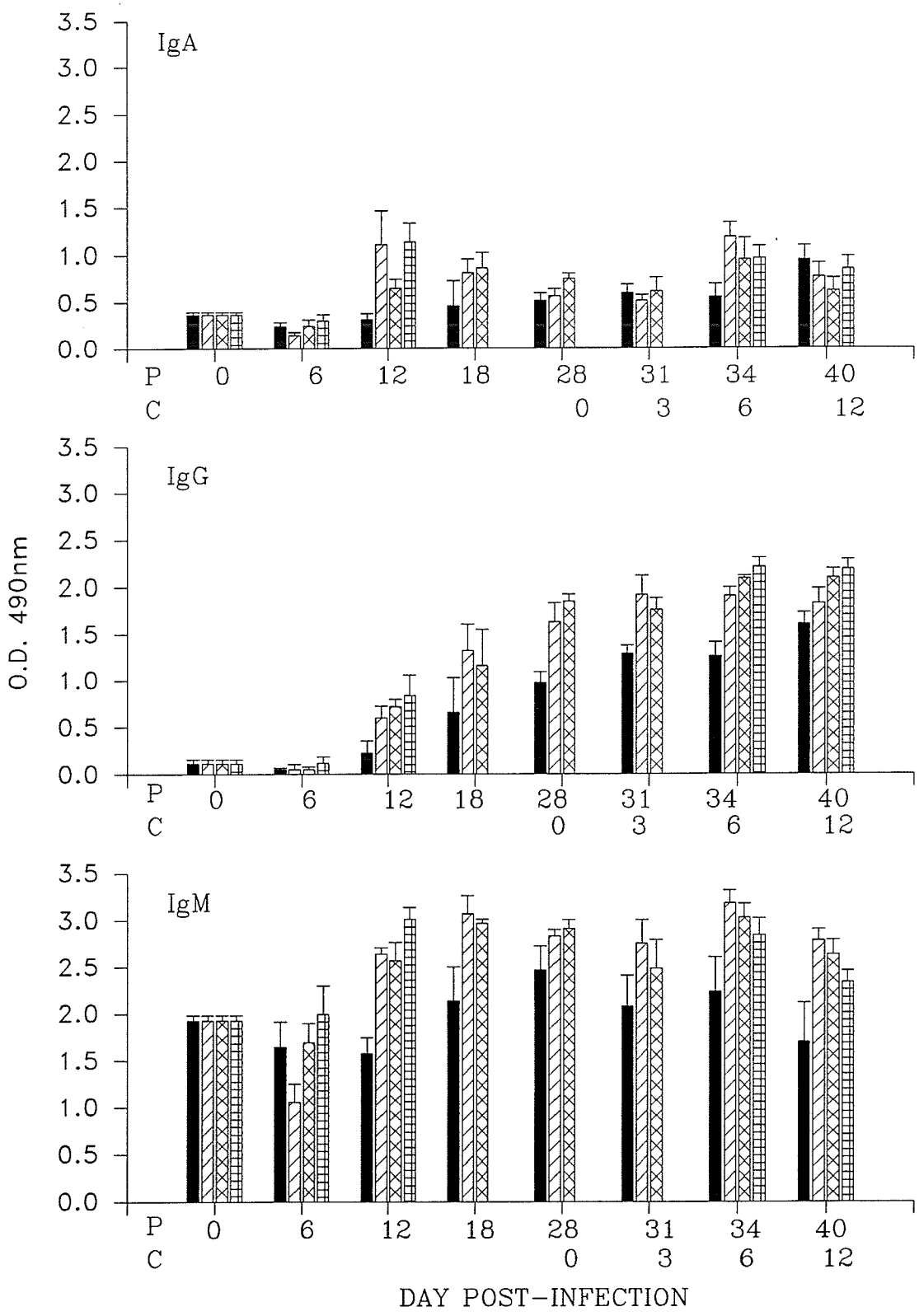
post-infection with no increase after challenge. Serum IgG and IgM increased steadily through primary infection and reached a plateau by Day 28 which was maintained for the duration of the experiments. Variation of infection level did not affect the pattern of immunoglobulin response but the degree of response was consistently lower for infection with 10 L1 than for the three higher infection levels (Figure 6). However, the experiment served the purpose of demonstrating that production of immunoglobulin can be monitored even at a very low infection level.

Intestinal Lumen Immunoglobulin Response

Intestinal lumen (IL) samples from Experiment #1 were tested undiluted and from Experiment #2 at 1:2 dilution. The volume of sample available was the parameter which initially set these dilution values. Duplicate samples of 100 μl for three different immunoglobulins required 600 μl of sample. Experiment #1 IL samples were collected in 2.0 ml of PBS which provided ample volume for testing. Experiment #2 samples were collected in 0.5 ml of PBS which was too small a volume for testing undiluted. However, at 1:2 dilution there was a sufficient quantity for testing as well as leaving a reserve for re-testing. The low ELISA values obtained at these dilutions confirmed the need for testing IL samples at low dilutions.

The immunoglobulin titres of IL samples taken from the

Figure 6. Infection level of *Trichinella* and immunoglobulin kinetics of serum. Swiss Webster mice were given primary (P)/challenge (C) infections of 10/10 (■), 50/50 (▨), 100/100 (▩), and 150/150 (▦) *Trichinella spiralis* larvae (L1) with the challenge infections given on Day 28 post-infection. Serum samples were taken and tested for the presence of IgA, IgG, and IgM immunoglobulins by enzyme-linked immunosorbent assay (ELISA). The optical densities obtained from the ELISA were used as a measure of immunoglobulin titre. The mean of the optical densities recorded for the samples in each group (n=4) was calculated and plotted against the post-infection sampling day. The t-bars represent the standard deviation from the mean.


IgA
IgG
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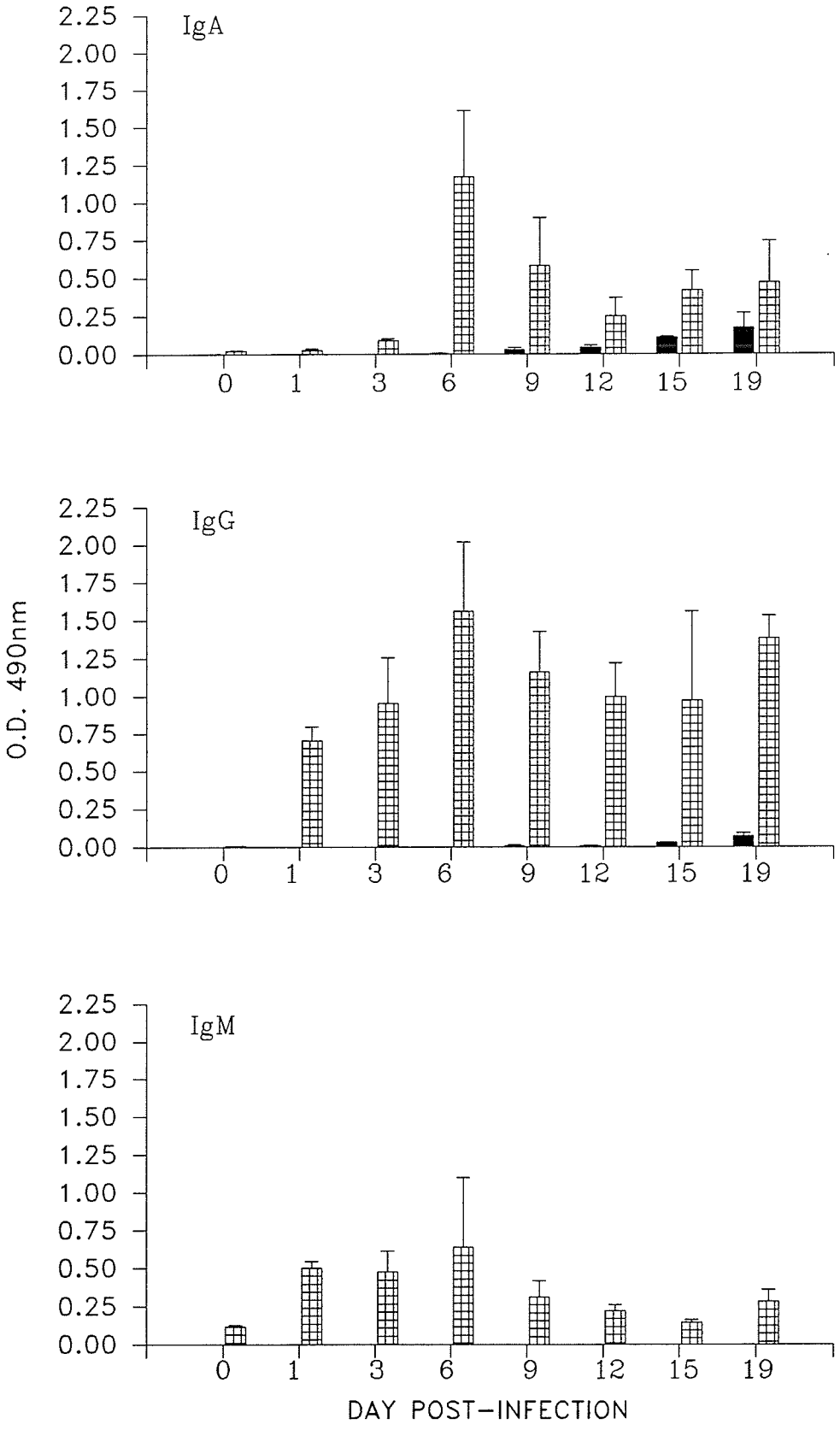
P 0 6 12 18 28 31 34 40
C 0 3 6 12
DAY POST-INFECTION

Figure 7. Kinetics of the intestinal lumen immunoglobulin response of Swiss Webster mice to primary (▬) and challenge (▦) infections with 150 *Trichinella spiralis* larvae (L1) for Experiment #1. Immunoglobulin titre is expressed as the mean optical density (O.D.) from enzyme-linked immunosorbent assay of intestinal lumen samples from mice in each group (n=3 except Day 1 where n=2). The t-bars represent the standard deviation from the mean. Challenge infections were given 28 days after primary infection (Day 28 post-primary infection ≡ Day 0 of challenge infection). Primary and challenge infection values are plotted side-by-side for the purpose of comparison.


IgA
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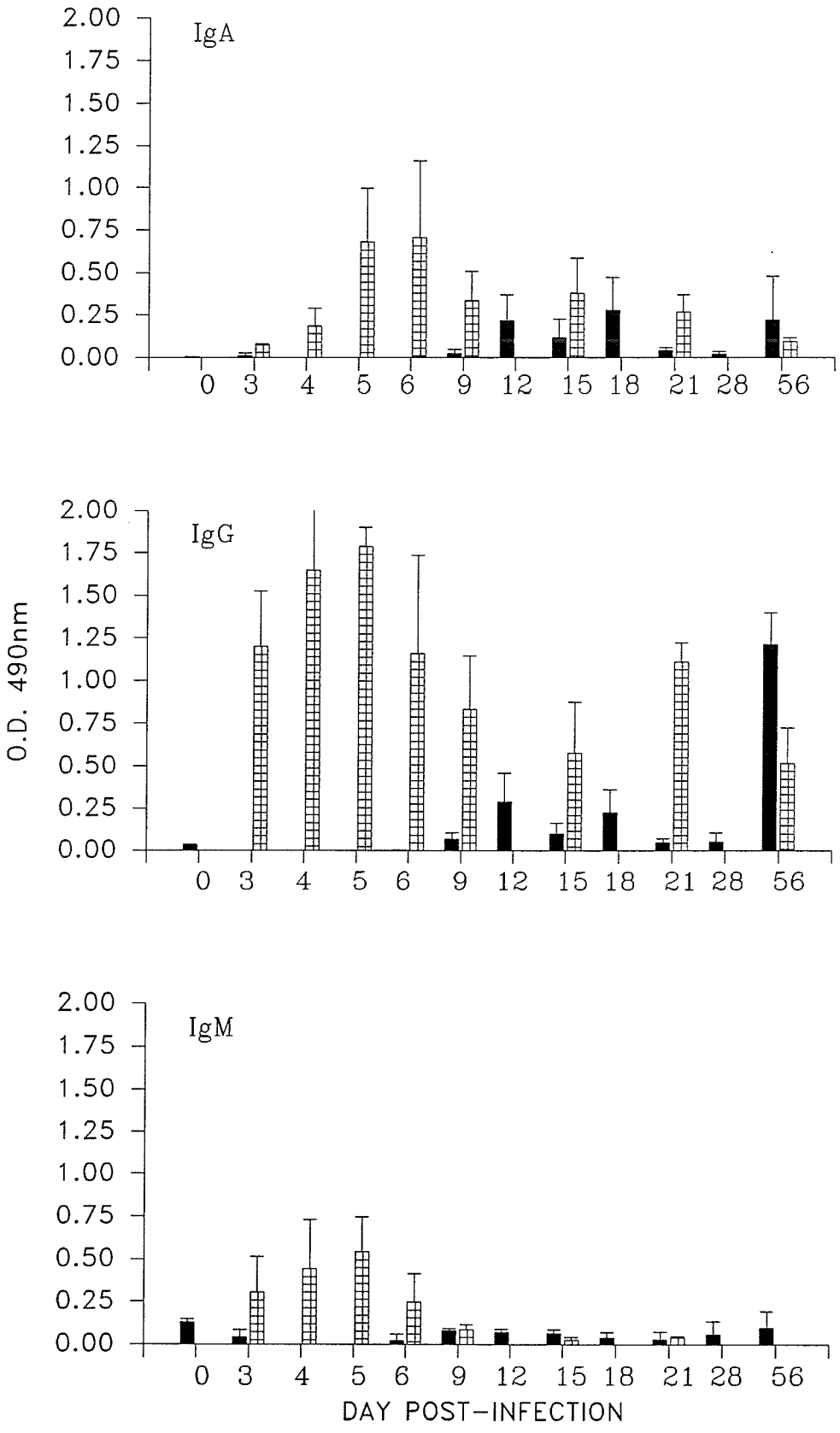
DAY POST-INFECTION

Figure 8. Kinetics of the intestinal lumen immunoglobulin response of Swiss Webster mice to primary (■) and challenge (▦) infections with 150 *Trichinella spiralis* larvae (L1) for Experiment #2. Immunoglobulin titre is expressed as the mean optical density (O.D.) from enzyme-linked immunosorbent assay of intestinal lumen samples from mice in each group (n=4). The t-bars represent the standard deviation from the mean. Challenge infections were given 28 days after primary infection (Day 28 post-primary infection ≡ Day 0 of challenge infection). Primary and challenge infection values are plotted side-by-side for the purpose of comparison.


IgA
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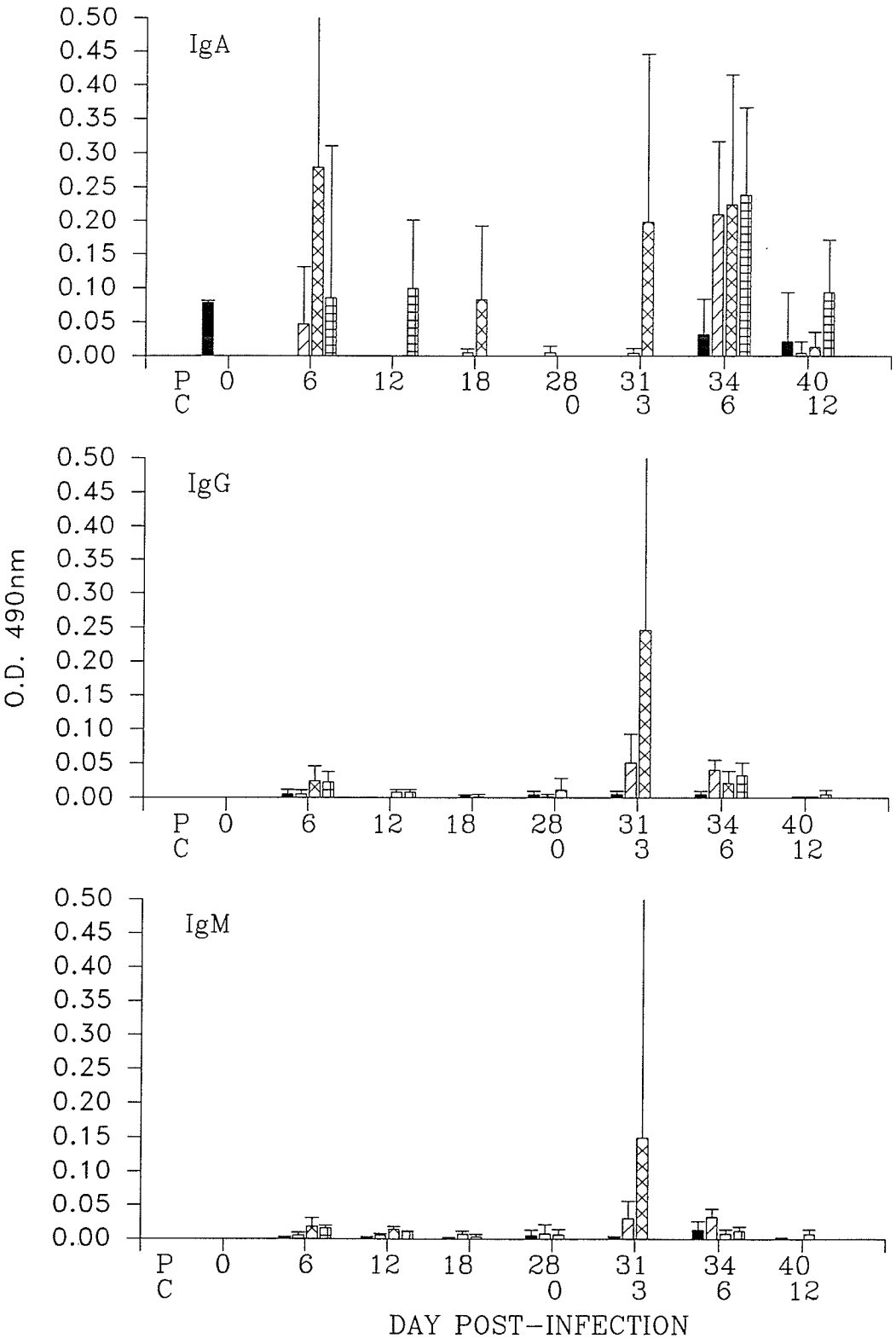
O.D. 490nm
0 3 4 5 6 9 12 15 18 21 28 56
DAY POST-INFECTION

two experiments are shown in Figures 7 & 8. Background titres were extremely low and there was no significant difference in these titres between Day 0 and Day 28 (Table 3). Immunoglobulin in the lumen did not appear until Day 9 post-infection when IgA and IgG appeared in Experiment #1 and IgA, IgG, and IgM in Experiment #2. Primary infection titres remained low for all immunoglobulins. Challenge infection produced substantial increases in the titres of all three immunoglobulins with peak values reached by Day 34. IgG titres remained relatively high while IgA decreased and IgM fell to background.

Intestinal lumen immunoglobulin response in Experiment #3 (Figure 9) was markedly different in the titres of IgG and IgM. Except for large peaks on Day 31, low titres of these two antibodies were recorded in contrast to the high titres observed in the first two experiments. Contamination of the sample by blood in the earlier experiments would explain this discrepancy. Care was taken in Experiment #2 to prevent contamination by blood but, as IgG and IgM titres remained high for Experiment #2, and were low in all subsequent experiments, it was assumed that some of these samples were contaminated with host blood. IgA was high for infection with 50, 100, and 150 L1 by Day 6 but did not appear at all in primary infection samples of mice given an infection dose of 10 L1. After challenge infection, elevated IgA titres were observed by Day 31 for infection with 100 L1 and by Day 34 for

Figure 9. Infection level of *Trichinella* and immunoglobulin kinetics in the intestinal lumen. Swiss Webster mice were given primary (P)/challenge (C) infections of 10/10 ( ), 50/50 ( ), 100/100 ( ), and 150/150 ( ) *Trichinella spiralis* larvae (L1) with the challenge infections given on Day 28 post-infection. Intestinal lumen samples were taken and tested for the presence of IgA, IgG, and IgM immunoglobulins by enzyme-linked immunosorbent assay (ELISA). The optical densities obtained from the ELISA were used as a measure of immunoglobulin titre. The mean of the optical densities recorded for the samples in each group (n=4) was calculated and plotted against the post-infection sampling day. The t-bars represent the standard deviation from the mean.


IgA
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P 0 6 12 18 28 31 34 40
C 0 3 6 12
DAY POST-INFECTION

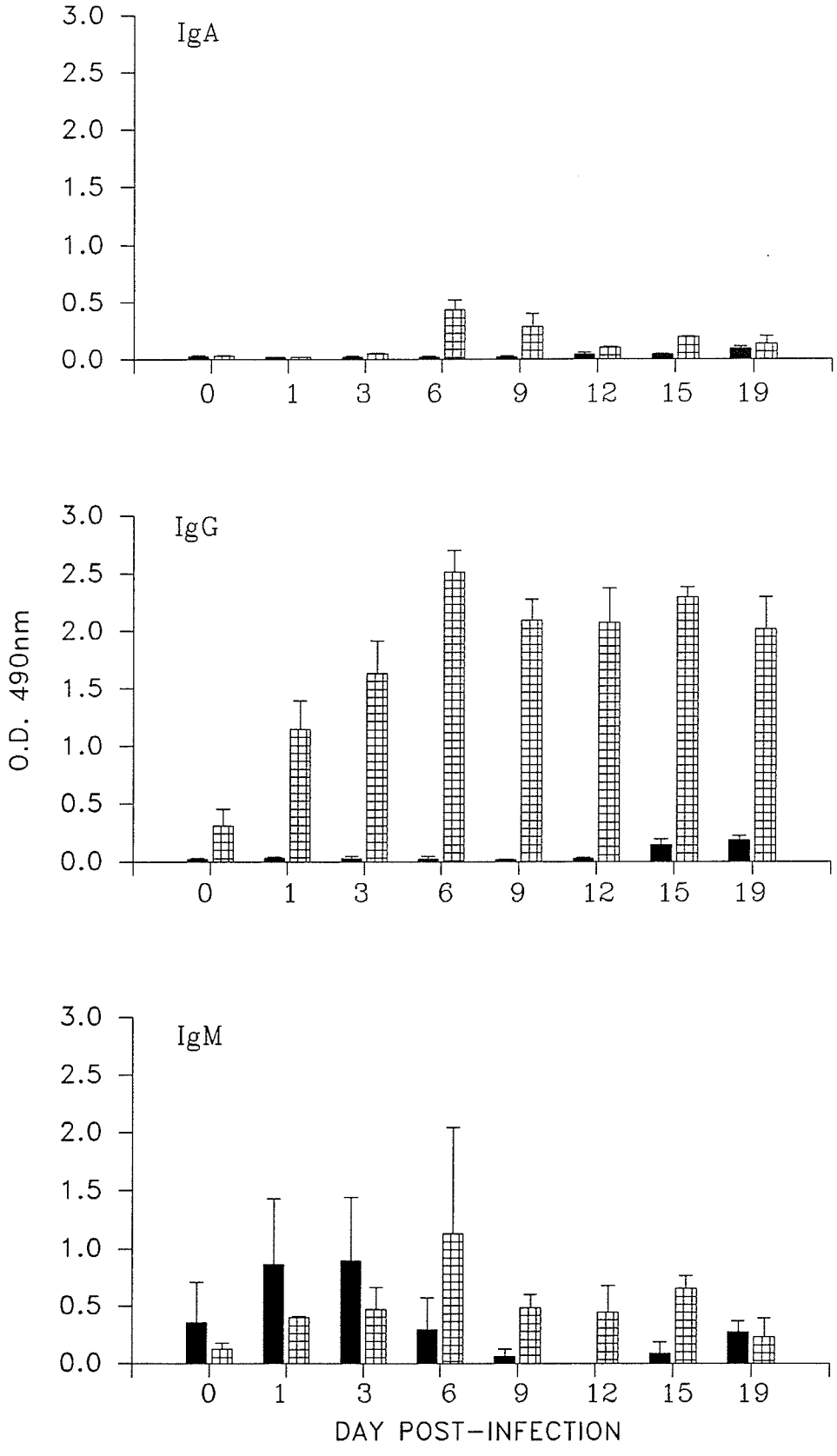
all infection levels. The IgA titre in the group infected with 10 L1 remained well below that produced by the higher infections. Day 40 saw a sharp drop in IgA for all infection levels indicative of a short-lived response possibly coupled to the absence of the parasite at this time.

New information was obtained from the modifications of the sampling techniques. In the first experiment, the intestine was removed without clamping and this resulted in the loss of some of the lumen material. Addition of the clamps ensured that nothing exited or entered the lumen until the intestine was on the board. The flushing of the intestine without washing or removing mesentery tissue resulted in contamination of the contents by blood and the volume used to flush the gut was greater than necessary for the task. There were no differences between samples taken from the anterior and posterior halves of the lumen so this method of collection was discontinued. Freezing, thawing, and homogenizing did not increase nor decrease the recovery of immunoglobulin in IL samples. Heating the sample and reducing centrifugation time also did not alter the results but in this case were retained in the method to lessen the chance of proteolytic degradation.

Mucosal Immunoglobulin Response

Mucosal samples were tested undiluted for Experiment #1 (Figure 10) and at 1:2 for Experiment #2 (Figure 11) for the same reasons outlined for intestinal lumen samples. Patterns

Figure 10. Kinetics of the mucosal immunoglobulin response of Swiss Webster mice to primary (■) and challenge (▦) infections with 150 *Trichinella spiralis* larvae (L1) for Experiment #1. Immunoglobulin titre is expressed as the mean optical density (O.D.) from enzyme-linked immunosorbent assay of mucosal samples from mice in each group (n=3 except Day 1 where n=2). The t-bars represent the standard deviation from the mean. Challenge infections were given 28 days after primary infection (Day 28 post-primary infection ≡ Day 0 of challenge infection). Primary and challenge infection values are plotted side-by-side for the purpose of comparison.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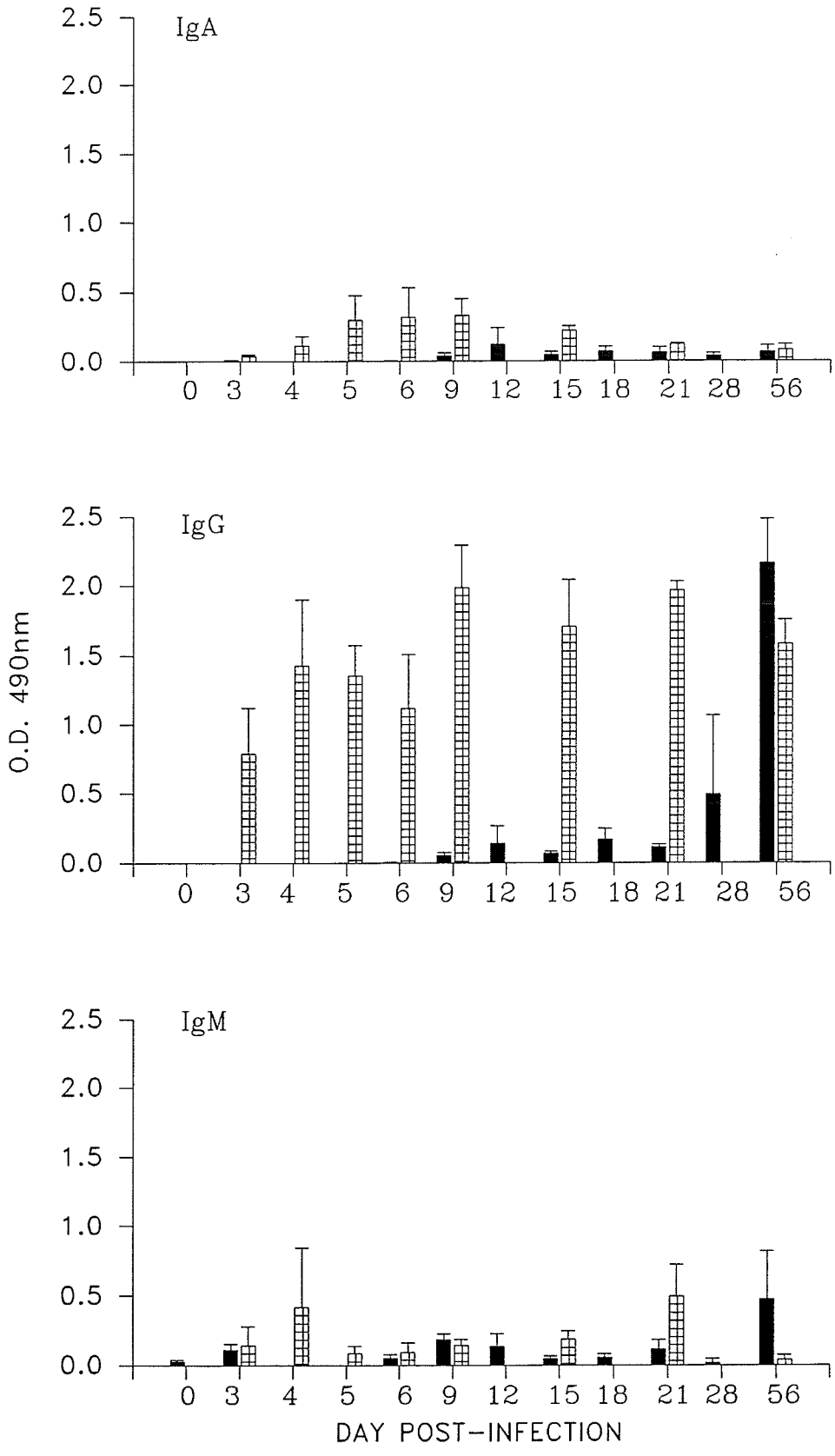
DAY POST-INFECTION

Figure 11. Kinetics of the mucosal immunoglobulin response of Swiss Webster mice to primary (■) and challenge (▦) infections with 150 *Trichinella spiralis* larvae (L1) for Experiment #2. Immunoglobulin titre is expressed as the mean optical density (O.D.) from enzyme-linked immunosorbent assay of mucosal samples from mice in each group (n=4). The t-bars represent the standard deviation from the mean. Challenge infections were given 28 days after primary infection (Day 28 post-primary infection ≡ Day 0 of challenge infection). Primary and challenge infection values are plotted side-by-side for the purpose of comparison.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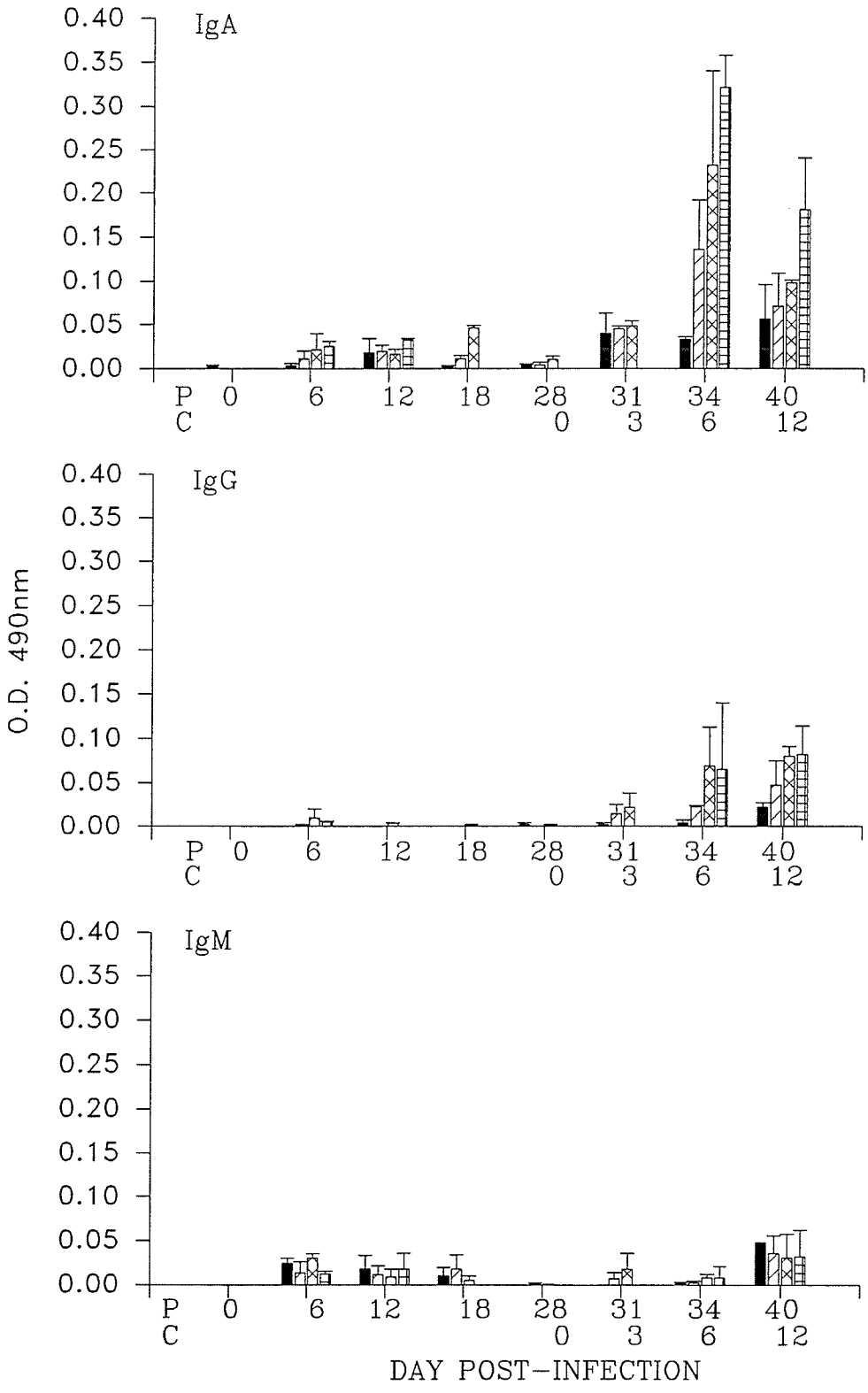
0 3 4 5 6 9 12 15 18 21 28 56
DAY POST-INFECTION

Figure 12. Infection level of *Trichinella* and immunoglobulin kinetics in the mucosa. Swiss Webster mice were given primary (P)/challenge (C) infections of 10/10 (■), 50/50 (▨), 100/100 (▩), and 150/150 (▦) *Trichinella spiralis* larvae (L1) with the challenge infections given on Day 28 post-infection. Mucosal samples were taken and tested for the presence of IgA, IgG, and IgM immunoglobulins by enzyme-linked immunosorbent assay (ELISA). The optical densities obtained from the ELISA were used as a measure of immunoglobulin titre. The mean of the optical densities recorded for the samples in each group (n=4) was calculated and plotted against the post-infection sampling day. The t-bars represent the standard deviation from the mean.


IgA
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O.D. 490nm
P 0 6 12 18 28 31 34 40
C 0 3 6 12
DAY POST-INFECTION

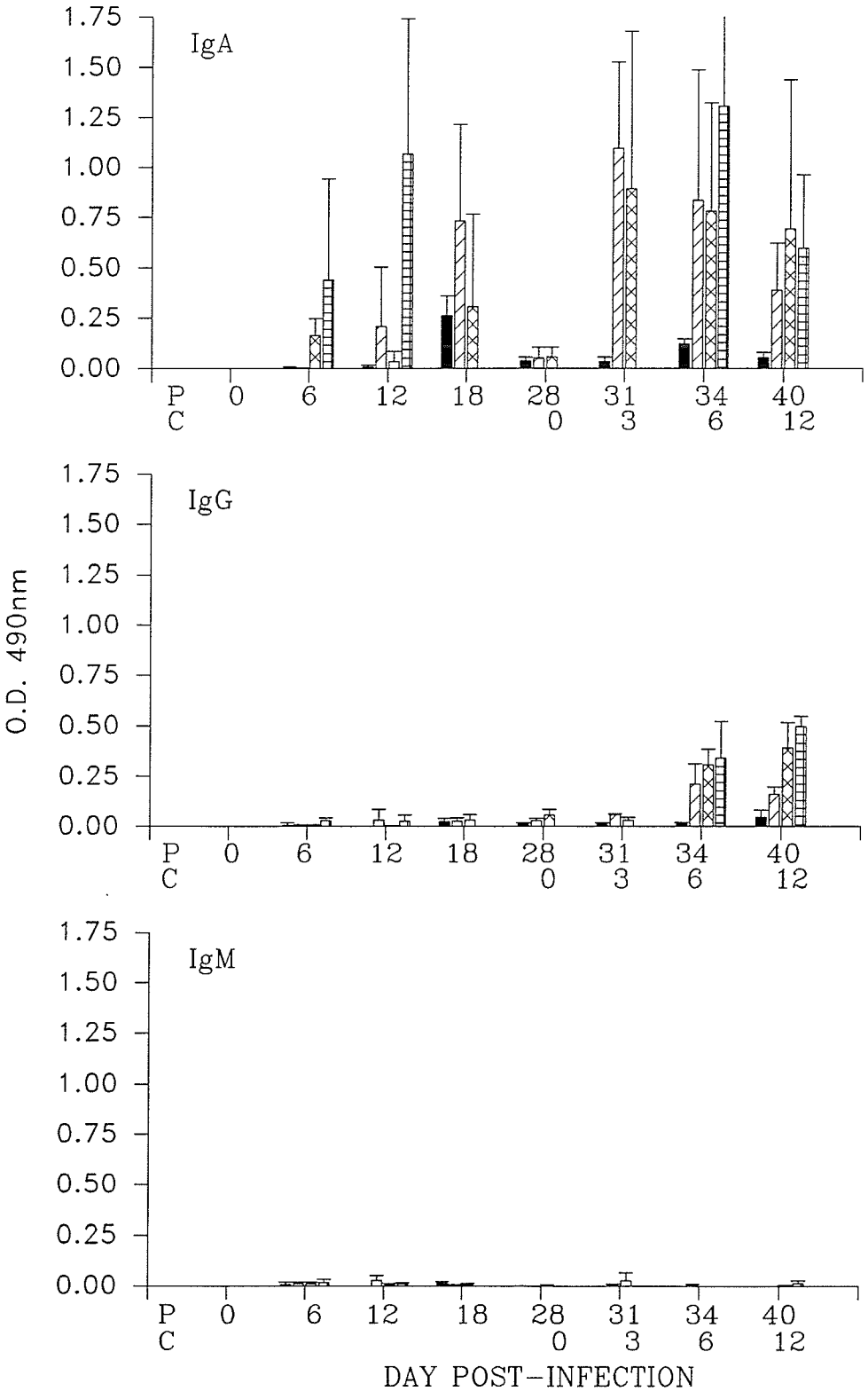
of IgA, IgG, and IgM immunoglobulin responses were similar to results for the IL samples for these experiments as well as Experiment #3 (Figure 12).

Scraping of the mucosal layer with a hand-held spatula in the first experiment produced large quantities of healthy villi, indicating that the scraping removed cells from the epithelium. Replacement of the spatula with the scraper ensured that each mucosal sample was collected in a reproducible manner with a constant pressure and that only mucus and sloughed tissue, not healthy villi, were removed. Despite this improvement in technique, the titres of IgG and IgM immunoglobulin fell only slightly in Experiment #2. The titres were far lower in Experiment #3. Modifications aimed at increasing the amount of immunoglobulin recovered from the mucosa were unsuccessful, with the titres of all immunoglobulins remaining more or less constant. As mucosal sampling was time-consuming and the immunoglobulin kinetics from mucosal samples were similar to those obtained from IL samples, the collection of mucosal samples was discontinued.

Gall Bladder Immunoglobulin Response

Gall bladder (GB) samples from Experiment #3 were tested at 1:5 dilution (Figure 13). Despite the variability of the data, a pattern of immunoglobulin response was obtained. Background titres of IgA, IgG, and IgM in GB samples were near zero. Titres of IgA increased by Day 6 for infections of 100

Figure 13. Infection level of *Trichinella* and immunoglobulin kinetics in the gall bladder. Swiss Webster mice were given primary (P)/challenge (C) infections of 10/10 (), 50/50 (), 100/100 (), and 150/150 () *Trichinella spiralis* larvae (L1) with the challenge infections given on Day 28 post-infection. Gall bladder samples were taken and tested for the presence of IgA, IgG, and IgM immunoglobulins by enzyme-linked immunosorbent assay (ELISA). The optical densities obtained from the ELISA were used as a measure of immunoglobulin titre. The mean of the optical densities recorded for the samples in each group (n=4) was calculated and plotted against the post-infection sampling day. The t-bars represent the standard deviation from the mean.


IgA
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28 0
31 3
34 6
40 12
DAY POST-INFECTION

and 150 L1, by Day 12 for infections of 50 L1, and by Day 18 for infections of 10 L1. Titres of IgA declined to near background by Day 28 for all infection levels. Elevated titres of IgA occurred by the third day after challenge infection for mice given infections of 50 and 100 L1 (there were no mice with an infection level of 150 L1 tested on this day). There was a slight increase in the titre of IgA in GB samples from mice given an infection of 10 L1 by Day 34 but the value was much lower than those for the higher infection levels. Gall bladder IgG did not increase until Day 34 post-infection and the titre of IgG was still increasing on the last day of the experiment for all infection levels. Very low titres of IgM were detected for all infection levels with no increases observed during primary or challenge infections.

Fasting for twelve hours enhanced both the number of mice with bile in the gall bladder and the amount of bile recovered. Based on this finding and the worm recovery data, fasting was incorporated in the experimental design of subsequent experiments. There were two additional benefits. Firstly, the intestinal lumen was easier to flush as there was less food present, reducing handling time for this sample. Secondly, there was little lipid in the serum which facilitated collection and testing.

DISCUSSION

The establishment of protocols for a study on the kinetics of immunoglobulin production in mucosal secretions is a difficult task. Mucosal secretions, unlike serum, cannot easily be collected from the same mouse over a period of time. Elson *et al.* (1984) describe a technique to obtain intestinal secretions "without harm" to mice by administration of a lavage solution followed by an injection of pilocarpine, which causes rapid expulsion of gut contents, but this technique could not be used to study rates of parasite expulsion from the intestine. Elson *et al.* (1984) also report considerable variation in the total IgA recovered by this method in the same group of mice sampled on different occasions. Clearly, this method of collecting intestinal contents still has problems with reproducibility. Bile has been collected in a number of experiments by bile duct cannulation in rats (Andrew & Hall, 1982a) and mice (Delacroix *et al.*, 1985) but this requires surgery and the effects on the immune system of the animal and on the parasite are unknown. A more promising technique for monitoring intestinal levels of immunoglobulins was recently reported by deVos & Dick (1991). They isolated coproantibodies from faecal pellets of *Trichinella*-infected mice and detected parasite-specific IgA and IgG antibodies. This method eliminated the necessity of sacrificing the animal to obtain an intestinal sample, allowed sampling from the same

individual over a period of time, and reduced the number of mice needed for a long-term experiment. There was also no interference with the physiology of the mouse or with the course of the parasite infection as there is with serum sampling. Differences in the kinetics of the intestinal immunoglobulin response in faecal monitoring as opposed to intestinal flushing were not investigated.

These initial experiments to examine the timing of worm expulsion and antibody appearance allowed a redesign of subsequent experiments to select "key" days which were most important to test my hypothesis. I concluded that three day intervals were sufficient to monitor immunoglobulins in primary infections but due to the more rapid response following challenge, sampling on the first three days following challenge was necessary.

The choice of Day 28 for challenge was based on an estimate of the amount of time required to completely clear the primary infection from the intestine to ensure that worm counts after challenge did not include any stragglers from the primary infection. In these experiments, worms were consistently cleared from the intestine at least thirteen days (usually earlier) before challenge. On the basis of these results, challenge infections were given on Day 28 in all subsequent experiments.

My results confirmed the need for proper controls. High background serum IgM was found consistently but, aside from

Crandall & Crandall (1972), who mention background titres briefly in their report, there is little information on this aspect of the host immune response to intestinal parasites. Without assessing the background IgM titre, one could conclude that high IgM is a result of *Trichinella* infection. Recent experiments in this laboratory demonstrated antibodies in the intestine specific for phosphorylcholine (PC) which is an antigen produced by normal intestinal flora. This is likely the source of the high background titre as PC antigen is also produced by *Trichinella*. Blanking ELISA plates with PBS and running control samples simultaneously with experimental samples allows the determination of the absolute level of immunoglobulin pre-and post-infection and provides for a more accurate comparison of results between experiments.

In these initial experiments, serum samples were tested at 1:10 dilution. While this dilution proved adequate for monitoring the IgA response, IgG and IgM titres were too high to be accurately assessed at this dilution, often reaching values near those for the absolute reaction of enzyme/substrate included in each test. In order to determine the true kinetics of the response of these immunoglobulins and to properly assess the effect of infection level on the immunoglobulin response, it was necessary to test high value samples at increasing dilutions in subsequent experiments.

Treatment of mucosal samples is another highly variable aspect of the protocol. Although *Trichinella* establishes

primarily in the anterior half of the intestine, no difference in the immunoglobulin production of the two halves was observed. Mechanical homogenization of the mucus to release trapped immunoglobulin (Allen *et al.*, 1984) was also ineffective in increasing *Trichinella*-specific antibodies although this process may have increased total immunoglobulin which was not tested in this experiment. Harbitz *et al.* (1980) stated that solubilization of mucus must be accomplished before determination of IgA in the sample can be made and that use of reducing or denaturing agents alters the native conformation of IgA. Harbitz *et al.* (1980) proposed a method of treatment for sputum samples to release immunoglobulin which involved freezing, thawing at 4°C, and stirring in phosphate buffer. This method did not increase the immunoglobulin recovery in my study. The proteolytic nature of the intestinal secretions is well known. Protease inhibitors have been used to prevent degradation in intestinal samples by addition after collection (Elson *et al.*, 1984) and by adding the inhibitors to the fluid used to flush the gut (deVos & Dick, 1991). In my experiments, rapid handling of samples, heating at 56°C, freezing the sample at -70°C, and keeping the sample on ice when thawed for ELISA testing were the methods employed to reduce proteolytic degradation. This may have resulted in lowered IgG and IgM levels due to protein degradation, but, as secretory IgA is resistant to proteolysis due to its structure (Mestecky & McGhee, 1987), results

concerning IgA are more reliable.

In initial experiments with bile samples, there were unexpectedly high levels of IgG. In an attempt to clarify this situation, a more specific IgG antibody was used in the ELISA to test the samples, specifically anti-IgGF(ab)$_2$*HRP (DeVos, Danell, & Dick, 1992). The IgG readings dropped sharply, so the whole molecule antiserum used in initial experiments was reacting with non-IgG immunoglobulins to give excessively high readings. This likely also explains the high intestinal lumen and mucosal readings in the second experiment where care was taken to prevent contamination by blood.

These initial experiments served to establish the timing and techniques required for reproducible and comparable results from further studies on the immune response of mice to infection with *Trichinella*. In summary, experiments would be timed to maximize the number of mice of similar age and sex available. These mice would be divided equally into experimental groups with one control group sacrificed on Day 0. Mice would be infected *per os* on Day 0 and sampling would be done every three days during the primary infection through Day 18 at which time expulsion is complete and antibody levels are elevated. Sampling would be done on Day 28 in order to assess antibody levels immediately prior to challenge. Mice would be challenged on Day 28 and sampling would occur on Days 29, 30, 31, 34, 37, 40, 43, and 46. Intestinal worms would be counted would be done for each mouse and muscle larvae counted

for Day 28 and all post-challenge mice. *In vitro* larval release would be assessed on Days 6 and 34 and the lengths of the female worms used in these assays measured. Serum, intestinal lumen, bile, and faecal samples would be obtained and immunoglobulin titres would be determined in the samples using ELISA, with end point dilutions determined in all cases.

CHAPTER 2: EFFECT OF INFECTION LEVEL ON THE KINETICS OF WORM RECOVERY AND IMMUNOGLOBULIN RESPONSE.

INTRODUCTION

The stimulation of non-specific host immune responses following intestinal parasite infections makes the evaluation of the contribution of parasite-specific immunoglobulin to host defence difficult as seperation of the two processes is not easily accomplished. Suppression of immunoglobulin production in mice by treatment with anti-μ antibodies has been described (Lawton *et al.*, 1972) and there is evidence that anti-μ-treated mice still possess the ability to reject *N. brasiliensis* (Jacobsen *et al.*, 1977). Treatment with cortisone suppresses inflammation and Larsh & Race (1975) found persistence of *Trichinella* infection in the absence of inflammation in cortisone-injected mice. Treatment with cortisone, however, affects the humoral immunoglobulin response as well. Campbell (1968) tested two steroidal (hydrocortisone and dexamethasone) against two non-steroidal (indomethacin and phenylbutazone) anti-inflammatory drugs in *T. spiralis* and *Trichuris muris*-infected mice and found suppression of worm loss only in the mice given the steroidal compounds.

Larsh (1963) stated that the degree of intestinal damage resulting in disease symptoms is correlated to the size of the infection dose in mice and Larsh & Race (1975) associated size of challenge infection with degree of inflammation. This led to the consideration of reduced infection level as an

alternate method of reducing inflammation but little information was available on the effect of small infections on the kinetics of worm expulsion and immunoglobulin production. Bell *et al.* (1984) found that reduction of infection dose abolished genetic differences in expulsion rates in inbred mice. Wakelin & Lloyd (1976) found recovery of a challenge infection of 500 L1 on Day 7 post-challenge was 0% in NIH mice given a primary infection of 45 L1 and 13.9% in mice given a primary infection of 10 L1 compared to previously uninfected mice in which recovery was 48.3% but no information on immunoglobulin production was recorded. Smith *et al.* (1987) and Jeska & Stankiewicz (1989) measured increases in immunoglobulin for low level infections of *Ostertagia circumcincta* in sheep and *Ascaris suum* in mice, respectively, but there has been no record to date regarding immunoglobulin production in low level *Trichinella* infections in mice.

Stunting of female worms has been reported by Rappaport and Wells (1951) and Grencis *et al.* (1986) in challenge infections and Culbertson (1942) found immunity to further muscle invasion in previously-infected mice. Culbertson (1942) and Campbell (1955) observed reduced intestinal adult worm recoveries and muscle larvae recoveries in parenterally vaccinated mice as compared to nonvaccinated controls and Campbell (1955) also noted stunting of female worms following vaccination. Direct antibody effects on *Trichinella* were demonstrated by Mauss (1940) and Oliver-Gonzalez (1941) who

observed the formation of precipitating antibody with larvae incubated *in vitro* in immune serum with subsequent reduction in larval infectivity. Jacqueline *et al.* (1978) found reduced larval production in fecund female *Trichinella* incubated *in vitro* in intestinal secretions from previously infected mice but not in intestinal secretions from uninfected mice and further identified the active fraction of the secretions as IgA. I hypothesize that antibody contributes to immunity during challenge infections by interfering with worm growth during the molting stages that take place in the intestine, thus impairing adult development, function, and survival, and by reducing the number of newborn larvae which survive after birth and during migration. If my hypothesis is correct then a dose-dependent effect on antibody production should result in a dose-dependent effect on worm length and *in vitro* larval release.

The objectives of these experiments were i) to determine if the number of larvae in the infection dose altered the host immune response to infection with regard to timing and intensity; ii) to determine the lowest number of larvae needed to prime an immune response to challenge with low and high infection levels; iii) to examine the effect of infection level on number of muscle larvae produced, *in vitro* larval release, and female worm length.

MATERIALS AND METHODS

A large scale experiment was designed to investigate the effect of infection level on worm expulsion and immunoglobulin production. An infection of 10 L1 was chosen based on the results of the initial experiment described in Chapter 1 which demonstrated that worm recovery could be monitored and immunoglobulins could be detected in samples taken from mice infected at this level. As few differences were observed between infections of 50, 100, and 150 L1, the highest level was chosen to increase the low/high infection ratio in order to maximize any differences in infection kinetics. Eight mice given 10 L1 and eight given 150 L1 were sacrificed on each of Days 3, 6, 12, 18, and 28. Eight mice with primary/challenge infections of 10/10 L1, eight with 10/150 L1, and eight with 150/150 L1 were sacrificed on each of Days 29, 30, 31, 34, 40, and 46. Serum, intestinal lumen, and bile samples were taken and blood smears made for the purpose of examining the peripheral eosinophil response (Appendix 1). Intestinal worms were counted for each mouse and muscle larvae were counted for Day 28 and all post-challenge mice.

A second experiment was designed to examine the effect of infection level on larval production and female worm length. To ensure the recovery of sufficient numbers of female worms, the number of mice in each group was set according to the recovery of worms at each infection level as determined in the

initial experiments (Chapter 1). Thirty-two mice with a primary infection of 10 L1, sixteen with 150 L1, sixty with primary/challenge infections of 10/10 L1, sixteen with 10/150 L1, and twenty-eight with 150/150 L1 were sacrificed on the sixth day following the last infection. Female worms were recovered from the intestines of mice in each group for the *in vitro* larval release assay and the length of each worm was determined after completion of the larval assay.

RESULTS

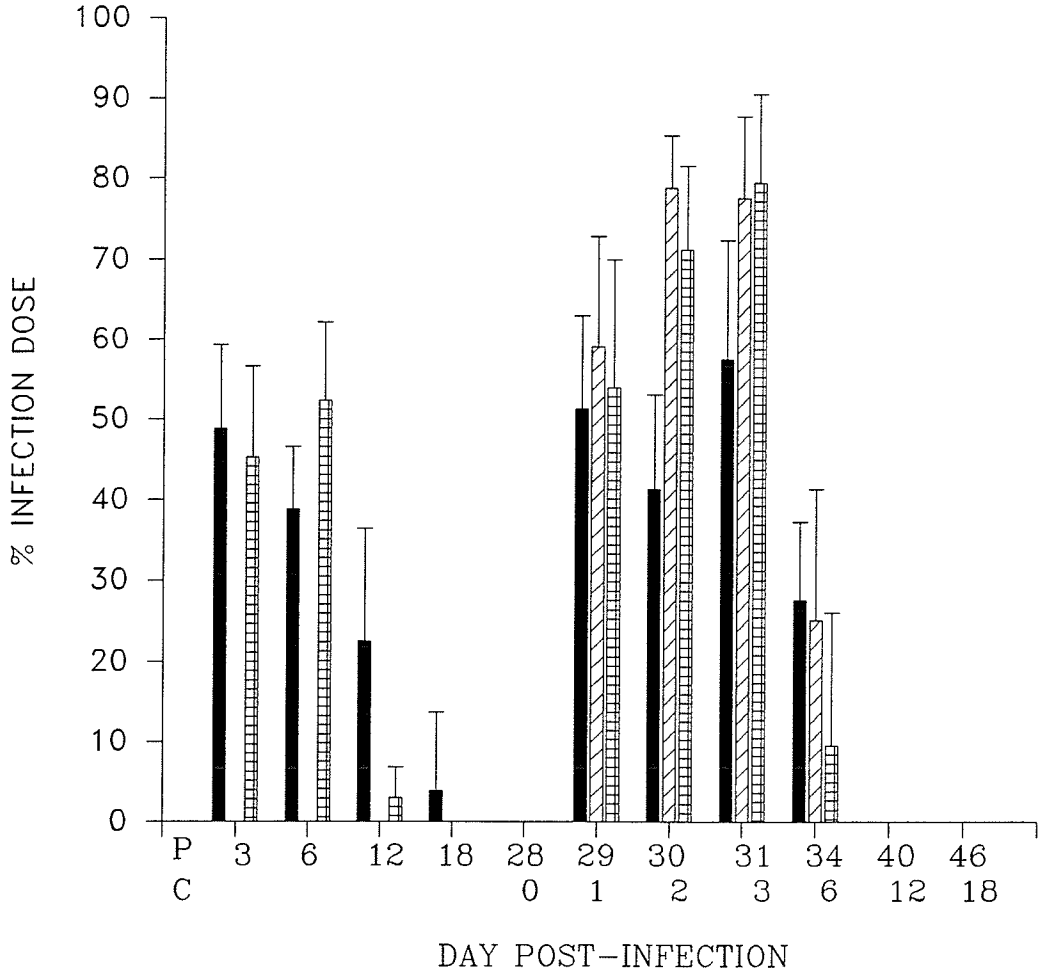
Effect of Infection Level on Intestinal Worm Recovery

The percent recovery of worms from the primary infection of 10 L1 was similar to that of 150 L1 for Days 3 & 6 (Figure 14). A higher percentage of worms was found on Days 12 and 18 for infection with 10 L1. Percent recovery of intestinal worms following a challenge infection were lower for 10/10 L1 than 150/150 L1 for Days 29, 30, & 31 but higher on Day 34 with complete expulsion by Day 40 post-infection for all infection levels. Mice given primary infections of 10 L1 and challenged with 150 L1 recovered in a similar pattern to the 150/150 L1 group for Days 29, 30, & 31 but closer to the 10/10 L1 group on Day 34. Significant differences (Student's t-test; $P \leq 0.05$) were recorded for Day 6 10 L1 vs Day 34 10/10 L1 and Day 6 150 L1 vs Day 34 150/150 L1 . There was no significant difference between the values for 150/150 L1 vs 10/150 L1.

Effect of Infection Level on Muscle Larvae Recovery

Total muscle larvae recovery was determined for all Day 28 and post-challenge mice. The total number of larvae was divided by the infection dose to give a value representing the total larval production per worm of the primary infection. For infection with 10 L1, the value was 163.9 ± 98.8 (n=103), significantly different ($P \leq 0.05$) from the value for 150 L1 which was 113.3 ± 50.3 (n=56).

Figure 14. Effect of infection level of *Trichinella* on intestinal worm recovery. Swiss Webster mice were given primary infections (P) of 10 (■) and 150 (▦) *Trichinella spiralis* larvae (L1). Mice given primary infections of 10 L1 were challenged (C) with 10 (■) or 150 (▨) L1 on Day 28 post-infection. Mice given primary infections of 150 L1 were challenged with 150 L1 (▦) on Day 28. The number of worms recovered from the intestine was expressed as percent of the infection dose and and the mean for each group of mice (n=8) was plotted against the day of sampling. The t-bars represent the standard deviation from the mean.


100
90
80
70
60
50
40
30
20
10
0
% INFECTION DOSE
P C
3
6
12
18
28 0
29 1
30 2
31 3
34 6
40 12
46 18
DAY POST-INFECTION

Effect of Infection Level on *In Vitro* Larval Release

The total number of newborn larvae (NBL) produced for each infection level was divided by the number of female worms tested at that level to give the average number of NBL per worm (Table 4). The differences in the number of NBL produced were significant ($P \leq 0.05$) among all infection levels except 10/- L1 vs 10/150 L1. Values for primary infections were higher than the values for the corresponding challenge infections. The value for the 10/150 L1 group was lower than the value for 150/- L1 but higher than that of the 150/150 L1 group. *In vitro* larval release following challenge was lowest in the 150/150 L1 group followed by the 10/10 L1 group and highest in the 10/150 group though this value was still lower than for the two primary infection levels. Recalculation of the data excluding the worms which produced no larvae was done to examine the relationship among larvae-producing worms (worms producing no larvae were 29.7% of the 10/- group, 37.3% of the 10/10 group, 1.9% of the 10/150 group, 22.6% of the 150/150 group, and 4.2% of the 150/- group). This recalculation established a significant difference between the values for infection with 10/- L1 and 10/150 L1 and abolished the significance of the difference between the values for infection with 10/10 L1 and 10/150 L1.

Table 4. Effect of infection level on *in vitro* larval release and female worm length. Swiss Webster mice were infected with different levels of *Trichinella spiralis* larvae (L1). Adult female *Trichinella* were recovered from the intestine six days after primary and challenge infection and cultured *in vitro* for 24 hours. The number of larvae released was determined for each female worm and the mean ± standard deviation calculated for each infection level. Female worms from each group were pooled, fixed, mounted on slides, and the lengths measured. The mean ± standard deviation of the lengths were calculated for each infection level.

	Infection Level				
	Primary		Challenge		
	10 L1	150 L1	10/10 L1	10/150 L1	150/150 L1
In vitro larval release					
[a]	42.5 ± 53.1 [1](n=64)	72.8 ± 42.6 (n=119)	18.6 ± 20.8 (n=59)	33.4 ± 14.0 (n=54)	8.8 ± 8.6 (n= 31)
[b]	60.5 ± 54.1 (n=45)	76.0 ± 40.6 (n=114)	29.7 ± 19.1 (n=37)	34.1 ± 13.4 (n=53)	11.3 ± 8.2 (n=24)
Female worm length in mms					
	2.37 ± 0.35 (n=31)	2.32 ± 0.19 (n=69)	1.69 ± 0.21 (n=21)	1.51 ± 0.15 (n=22)	1.44 ± 0.10 (n=15)

[a] Values including worms which produced no larvae.

[b] Values not including worms which produced no larvae.

[1] The number (n) of females per infection level is shown below the larval release and worm length values. Female worms lost or damaged in the fixing and mounting procedures account for the lower number of individuals available for measurement of length.

Effect of Infection Level on Female Worm Length

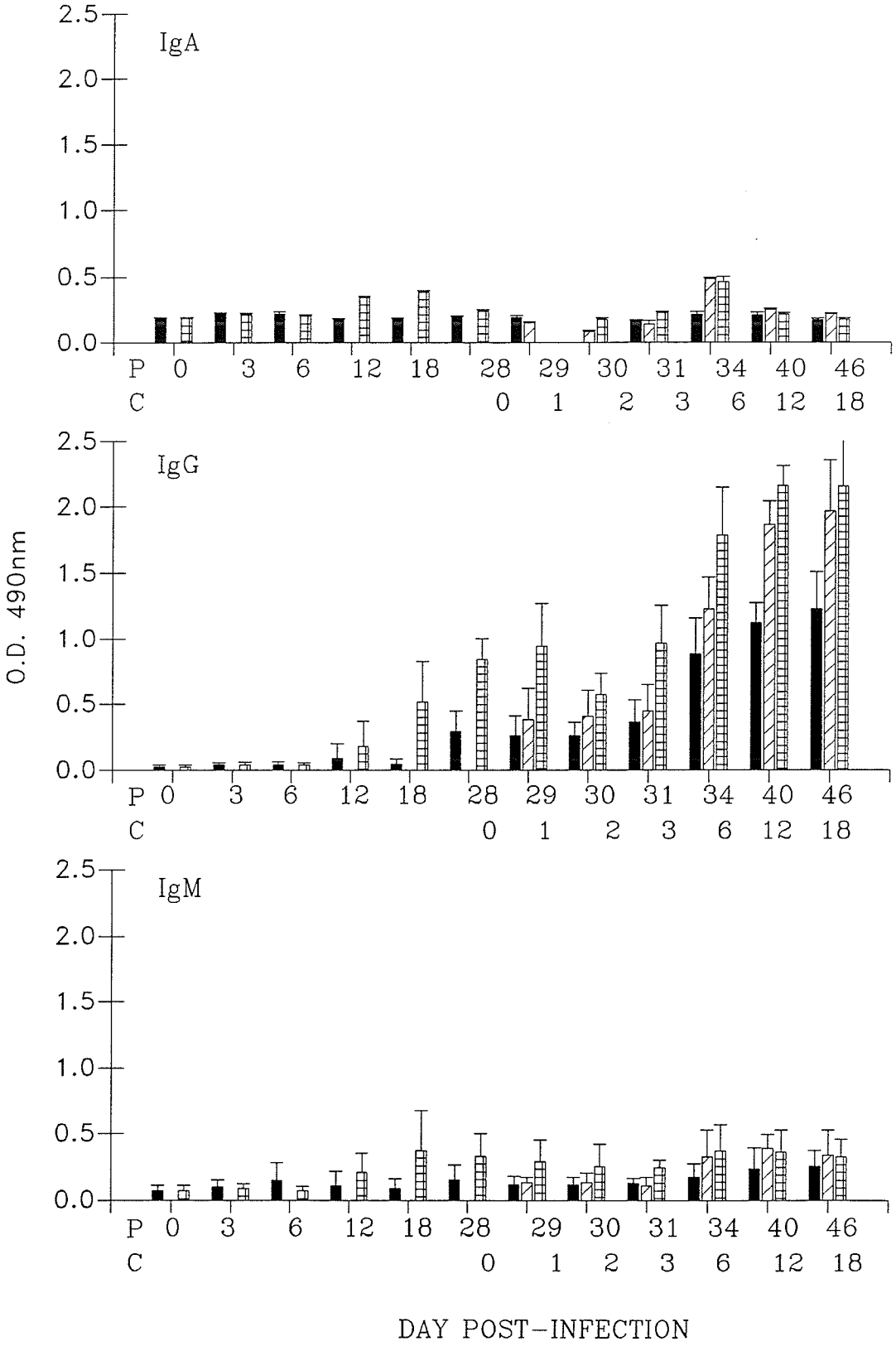
Female worms recovered from fecundity experiments were measured and the means and standard deviations are given in Table 4. In all cases, the worms from challenge infections were significantly smaller than those from primary infections ($P \leq 0.05$). Worms from primary infections of 10 L1 and 150 L1 were the same length. The worms from infections of 10/10 L1 were larger than those for 10/150 L1 and 150/150 L1 groups. No significant difference was observed between the 10/150 L1 and 150/150 L1 groups.

Effect of Infection Level on Immunoglobulin Response

Serum Immunoglobulin: Little change was observed in serum IgA titres at the lower infection level throughout primary and challenge infection (Figure 15). The primary response following a 150 L1 infection increased by Day 12 and peaked slightly higher on Day 18, but declined by Day 28. A peak on Day 6 challenge was observed for 10/150 L1 and 150/150 L1, but the titre fell by Day 40. Overall, there was little serum IgA response.

Serum IgG titres began to increase by Day 12 of the primary infection and rose steadily until testing ended on Day 46. The titres were consistently higher for the higher infections over the infections with 10 L1 and 10/10 L1 and for the 150/150 L1 group over the 10/150 L1 group.

Figure 15. Effect of infection level of *Trichinella* on kinetics of the immunoglobulin response in serum. Swiss Webster mice were given primary infections (P) of 10 (■) and 150 (▦) *Trichinella spiralis* larvae (L1). Mice given primary infections of 10 L1 were challenged (C) with 10 (■) or 150 (▨) L1 and mice given primary infections of 150 L1 were challenged with 150 L1 (▦) on Day 28 post-infection. Serum samples were tested by enzyme-linked immunosorbent assay at 1:10 dilution for IgA and 1:1000 dilution for IgG/IgM. The resulting optical densities were used as a measure of immunoglobulin titre. The mean optical densities of the samples from mice in each group (n=8) were calculated and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.


IgA
IgG
IgM
2.5
2.0
1.5
1.0
0.5
0.0
O.D. 490nm
P 0 3 6 12 18 28 29 30 31 34 40 46
C 0 1 2 3 6 12 18
DAY POST-INFECTION

Serum IgM titres increased by Day 12 of the primary infection and maintained a steady level with a slight increase seen by Day 34. Although titres for the higher infection levels were consistently above those for the low level infection there were no significant differences.

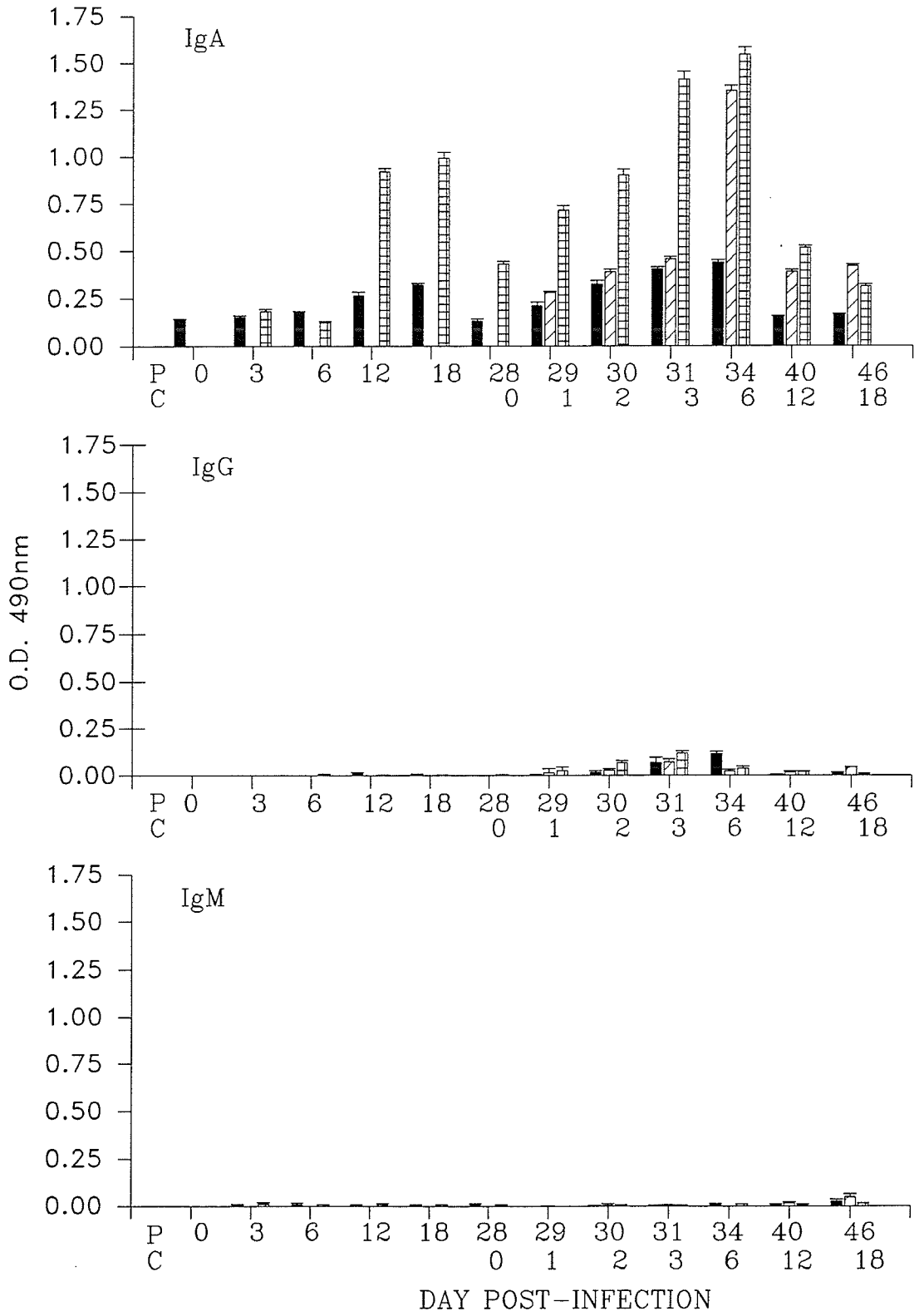
Bile Immunoglobulin: IgA titres increased to significantly higher levels for 150 L1 than for 10 L1 by Day 12 (Figure 16). Titres peaked by Day 18 and fell to near background by Day 28. Following a challenge infection, there was an increase by Day 1, again higher for the 150 L1 group, with peaks on Day 34 post-infection and a rapid decrease by Day 40. The 10/150 L1 titres were comparable to 10/10 L1 values for Days 29, 30, & 31 but rose to 150/150 L1 titres for Days 34, 40, & 46.

Little detectable IgG was observed for any infection level during primary and challenge infections. Slightly higher titres of IgG were observed for Days 30 and 31 at all infection levels and on Day 34 there was a higher titre of IgG in infections with 10/10 L1 than the larger infections, but these titres fell by Day 40.

No significant amount of IgM was observed for any infection level for primary or challenge infection.

Intestinal Lumen Immunoglobulin: An increase in intestinal lumen (IL) IgA was observed by Day 6 of primary

Figure 16. Effect of infection level of *Trichinella* on kinetics of the immunoglobulin response in bile. Swiss Webster mice were given primary (P) infections of 10 (■) and 150 (▦) *Trichinella spiralis* larvae (L1). Mice given primary infections of 10 L1 were challenged (C) with 10 (■) or 150 (▨) L1 and mice given primary infections of 150 L1 were challenged with 150 L1 (▦) on Day 28 post-infection. Bile samples were tested by enzyme-linked immunosorbent assay at 1:250 dilution. The resulting optical densities were used as a measure of immunoglobulin titre. The mean optical densities of the samples from mice in each group (n=8) were calculated and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.

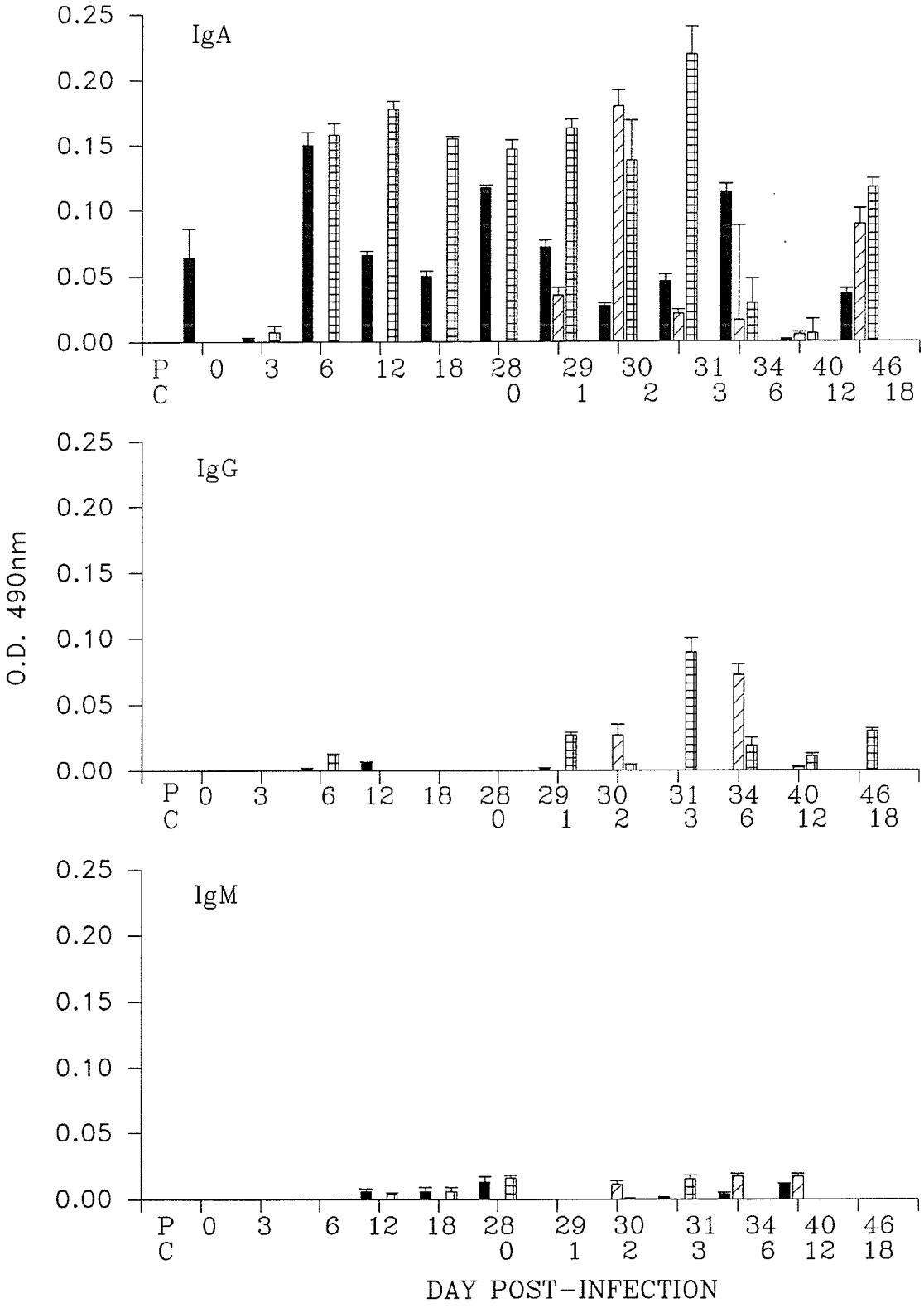

IgA
IgG
IgM
O.D. 490nm
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
P C 0 3 6 12 18 28 0 29 1 30 2 31 3 34 6 40 12 46 18
DAY POST-INFECTION

infection with little difference in the intensity of the response between infections of 10 L1 and 150 L1 at this time (Figure 17). By Day 12 the titre resulting from an infection with 10 L1 had fallen while increasing for infection with 150 L1. Titres of IgA remained fairly steady at the higher infection level and, while fluctuating, were detectable throughout the primary infection for the 10 L1 group. Challenge titres reached a peak at Day 31 for an infection level of 150 L1 and Day 34 for an infection level of 10 L1. These titres dropped on Day 40 then rose slightly on Day 46.

A low IgG response was observed during the primary infection for infections of 10 L1 and 150 L1. Titres of IgG rose after challenge with 150 L1, peaking on Day 31 for the 150/150 L1 group and on Day 34 for the 10/150 L1 group followed by a decline in titre to background by Day 40. No IgG was detected for the low level challenge groups.

Very low titres of IgM appeared by Day 28 for both infection levels but dropped to zero by Day 29. Low titres again appeared by Day 30 for infections with 10/150 L1, Day 31 for 150/150 L1, and Day 34 for 10/10 L1, but these titres dropped to zero by Day 46.

Figure 17. Effect of infection level of *Trichinella* on kinetics of the immunoglobulin response in the intestinal lumen. Swiss Webster mice were given primary (P) infections of 10 (■) and 150 (▦) *Trichinella spiralis* larvae (L1). Mice given primary infections of 10 L1 were challenged (C) with 10 (■) or 150 (▨) L1 and mice given primary infections of 150 L1 were challenged with 150 L1 (▦) on Day 28 post-infection. Intestinal lumen samples were tested by enzyme-linked immunosorbent assay. The resulting optical densities were used as a measure of immunoglobulin titre. The mean optical densities of the samples from mice in each group (n=8) were calculated and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.


IgA
IgG
IgM
0.25
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O.D. 490nm
P C
0
3
6
12
18
28 0
29 1
30 2
31 3
34 6
40 12
46 18
DAY POST-INFECTION

DISCUSSION

Antibody production and worm expulsion data from these experiments demonstrated a strong secondary immune response as evidenced by the presence of IgA antibodies in bile and intestinal lumen and IgG antibodies in serum. Worm expulsion was closely associated with peak titres of the IgA antibodies in both primary and challenge infections. The rates of worm expulsion in these experiments were comparable to those reported by other researchers. Culbertson (1942) found 50% worm recovery on Day 7 of primary infection and 5% recovery on Day 7 of challenge using infections of 100 and 175, respectively. Rappaport & Wells (1951) found worms through Day 20 of a primary infection of 300 L1 but a similar size challenge infection was completely expelled by Day 10. Primary expulsion began on Day 8 and was complete by Day 14 for infection with 200 L1 (Sinski *et al.*, 1983), 500 L1 (Alizadeh & Wakelin, 1982) and 450 L1 (Kennedy, 1980) and in the latter study, expulsion following a challenge infection began on Day 6 and was complete by Day 10. On the other hand, Crandall & Crandall (1972) reported 25% of infection dose still present on Day 15 of a primary infection.

Wakelin & Lloyd (1976) reported that primary infections of 10, 45, 90, and 120 L1 were capable of providing substantial to complete immunity in NIH mice challenged with 500 L1 as measured by intestinal worm counts on Day 7 post-

challenge. A similar result was found in my study with a primary infection of 10 L1 or 150 L1 resulting in complete expulsion of a challenge infection by Day 12 post-challenge. Primary infection with 10 L1 was sufficient to prime the host to expel a challenge of 150 L1 as quickly as a primary infection of 150 L1. Bell *et al.* (1984) used various infection levels to study the effect of genetics on *T. spiralis* expulsion in mice and reported that rejection was fastest at doses of 50 - 100 muscle larvae and that infections of less than 300 reduced the genetic effect on expulsion, but they did not examine challenge infections. Wassom *et al.* (1984) also used different infection levels to study genetic control of expulsion and observed that doses of 100-200 L1 were expelled more quickly than doses of 400-600 L1 but again did not investigate the response following challenge.

Khamboonruang (1971) studied the effect of different infection doses of *T. spiralis* in Swiss Albino mice (4-8 weeks old) on total muscle larvae recovered and reported averages of 500 larvae for infections of 2-20 L1 and 250 for infections of 25-200 L1. This finding is similar to my results in which lower infection levels yielded higher muscle larvae per worm of the infection dose (163.9 larvae for infection with 10 L1 and 113.3 larvae for infection with 150 L1). Although the larval recoveries in my experiment were lower than those of Khambooruang (1971) who used similar infection levels, they are higher than those reported by Corba and Spaldonova (1973)

who recovered an average of 90 muscle larvae per worm of an infection dose of 300 L1 given to white (strain unspecified) mice. McCoy (1932a) also reported higher larvae production per worm in lower infection levels in rats.

While recovery of muscle larvae is commonly used as a measure of fecundity, the newborn larvae are exposed to both intestinal and serum immune defences. *In vitro* larval release assays, on the other hand, measure fecundity after exposure to the secretory immune system only. Reduced fecundity following challenge infection has been reported (Kennedy, 1980). Wassom *et al.*(1984), investigating the genetic influence on the reduction of fecundity, found reduced fecundity in worms recovered from challenge infections given to all strains of mice tested. Despommier *et al.* (1977) and Gamble (1985) found reduced fecundity of adult female worms recovered from mice immunized intraperitoneally with crude larval antigen before infection. However, no data has been published to date on the effect of infection dose on worm fecundity. My experiments reveal a dose dependent effect on fecundity. Primary infection with 10 L1 yielded values of NBL release significantly different from values for primary infection with 150 L1. This appears to contradict the information from muscle larvae recoveries where numbers of muscle larvae per worm of infection were larger for low infection levels. However, the *in vitro* larval release assay measured production of larvae over a 24 hour period while muscle larvae recovery measured

production over the entire course of the primary infection. Total production per worm may be increased by the length of time the adult exists in the intestine which was longer for primary infection at the lower level. Also, there may be a major role for the serum immune defence system in the prevention of establishment of muscle larvae as infection at the higher level stimulated a faster primary response. Primary infection with 10 L1 significantly reduced the fecundity of worms from a challenge of 10 L1 and 150 L1 but not as much as a primary infection of 150 L1 did for a challenge of 150 L1 indicating that the higher level of infection was more effective at priming an anti-fecundity immune response. The larval release values following challenge also demonstrate a dose dependent response with 150/150 L1 > 10/10 L1 > 10/150 L1.

Rappaport & Wells (1951) reported stunting of female worms in challenge infections of 150 L1 with a reduction in average length from 3.12 mm in primary infection to 1.96 mm in challenge. Grencis *et al.* (1986) recorded female worm lengths of 2.7 mm in control mice and 1.97/2.27 mm in immunized mice. Although these values are higher than those recorded here (2.42 mm for primary of 150 L1 and 1.44 mm for challenge), the difference in the percent reduction of length is similar.

The kinetics of antibody response is more difficult to confirm except for serum values as there is no information available specifically for *T. spiralis* infections in the

mouse. Kozek & Crandall (1973) reported predominantly IgA in intestinal perfusates of infected mice detectable by Day 8 and increased by Day 14 of a primary infection, and detectable by 24 hours after challenge but could not show a significant increase after challenge using an indirect fluorescence assay. Sinski *et al.* (1983) reported an increase in the IgA in intestinal washes of *Trichinella*-infected mice by Day 9 of infection, but total IgA, not specific anti-*Trichinella* IgA, was measured and no measurement was made for challenge infection. Serum IgG and IgA increased by Day 14 and, as observed in this study, IgA declined by Day 24 while IgG stayed high. Andrew and Hall (1982b) demonstrated immunological memory in biliary IgA in rats injected via Peyer's patches with sheep red blood cells or killed *Brucella abortus* organisms. Doses insufficient to raise biliary IgA levels were given as the priming dose and when similar doses were given in challenge injections as long as one year later, more rapid and higher levels of IgA were monitored in the bile.

Crandall & Crandall (1972) reported serum IgG and IgA by Day 11 of primary infection with a secondary IgG response but no consistent increase in IgA after challenge. Serum IgM antibody results were also similar to those in this study with a high background level, an increase after the first week of primary infection with a peak at Day 18, and a slight secondary response on Day 34.

A dose dependent effect was observed in these experiments for worm expulsion, muscle larvae burden, *in vitro* larval release, worm stunting, and the time and intensity of immunoglobulin production, especially IgA. Dose dependent effects were not observed for challenge levels of serum IgM and IgG when tested at 1:10 dilution but became apparent when the end point dilution of 1:1000 was used with titres consistently higher for 150/150 L1 and lowest for 10/10 L1 with 10/150 L1 titres between these values.

A low primary infection of 10 L1 effectively primed the immune response. This low infection level plus a challenge infection may be a method of studying the effect of immunoglobulin on worm expulsion without the complications of debilitating inflammation and genetic predisposition.

CHAPTER 3. COMPARISON OF THE KINETICS OF THE IMMUNE RESPONSE OF MICE TO TWO ISOLATES OF *TRICHINELLA* KNOWN TO INDUCE DIFFERENT RESPONSES IN MICE.

INTRODUCTION

Trichinella spiralis var. *pseudospiralis* was first found in a wild carnivore by Garkavi (1972) who, on the basis of morphological differences and the lack of a cyst surrounding the muscle larvae, identified it as a new species of *Trichinella*. Although reproductive isolation from *T. spiralis* was demonstrated, gene flow between *T. spiralis* var. *pseudospiralis* and other strains of *Trichinella* is possible (Dick & Chadee, 1983). This interbreeding capability, along with the lack of clear cut morphological, biochemical, and immunological differences, led to the more conservative classification of isolates for *T. spiralis* var. *pseudospiralis* (Dick, 1983). Nevertheless, many differences in the host response to infection with *T. spiralis* and *T. spiralis* var. *pseudospiralis* have been shown with regard to worm expulsion (Kramar *et al.*, 1981; Palmas *et al.*, 1985), larval production (Kramar *et al.*, 1981; Stewart *et al*, 1985), degree of inflammation produced (Przyjalkowski *et al.*, 1983; Stewart *et al.*, 1985), and cellular immune response (Palmas *et al.*, 1985).

One aspect of host immunity to the two strains of the parasite which has not been compared is immunoglobulin production. This is accompanied by a singular lack of studies on immunoglobulin production in infections with *T. spiralis* var. *pseudospiralis*. If differences in the kinetics of worm

expulsion for infection with *T. spiralis* var. *pseudospiralis* are or are not parallelled by differences in the kinetics of immunoglobulin production, this information would be useful in the debate surrounding the *Trichinella* species question as well as the debate surrounding the contribution of immunoglobulin to the expulsion of the parasite.

This experiment was designed i) to examine the kinetics of worm expulsion and immunoglobulin production for primary and challenge infections with *T. spiralis* var. *pseudospiralis*; ii) to compare the results with those obtained for *T. spiralis* in previous experiments;iii) to investigate antigenic differences between the two strains by determining the degree of cross-reactivity of antibody in samples obtained from *T. spiralis* var. *pseudospiralis*-infected mice to P1 antigen.

MATERIALS AND METHODS

Parasites

A strain of *Trichinella spiralis* var. *pseudospiralis* maintained in the laboratory by successive passages through mice as described for *T. spiralis* (Chapter 1) was used. A crude antigen extract derived from the muscle larvae of this parasite (Tps) was made as described for P1 antigen (Chapter 1) for use in ELISA testing.

Experimental Design

Eighty-four mice were infected with 150 *T. spiralis* var. *pseudospiralis* L1. Eight mice were sacrificed on each of Days 9, 12, and 18 while twelve mice were sacrificed on each of Days 6, 31, 34, 37, and 40 with challenge of 150 L1 given on Day 28. Serum, bile, and intestinal lumen samples were obtained for each mouse. Intestinal worm counts were done for each mouse and muscle larvae counts for all mice sacrificed on or after Day 28.

RESULTS

Kinetics of Worm Recovery

Intestinal Worm Recovery: Over 50% of the infection dose was recovered on Day 6 (Figure 18) and 25% on Day 9. Expulsion of the primary infection was complete by Day 12. There was a very rapid loss of challenge infection worms with only 12.5% of infection recovered on Day 31 and complete expulsion by Day 34 (Day 6 of the challenge infection).

Muscle Larvae Recovery: The average muscle larvae recovery per worm of infection dose for the sixty post-Day 28 mice was 41.2 ± 21.4.

Kinetics of the Immunoglobulin Response

Serum Immunoglobulin: Serum IgA titre peaked by Day 12 in the primary infection (Figure 19). This titre was the same on Day 18 but had declined by Day 31. By Day 34, the titre had reached a value greater than the primary peak and this value was maintained up to Day 40 post-infection. This peak titre was less than half of that seen for serum IgG and IgM.

Serum IgG levels began to increase by Day 12 and increased steadily until Day 31. This peak titre was maintained throughout the remainder of the testing period.

Figure 18. Kinetics of intestinal worm recovery for primary (■) and challenge (▦) infections with *Trichinella spiralis* var. *pseudospiralis*. Swiss Webster mice were given primary and challenge infections of 150 *T. spiralis* var. *pseudospiralis* larvae. Challenge infection was given on Day 28 post-infection (Day 28 post-infection ≡ Day 0 challenge infection). Worm recovery was expressed as the percent of infection dose. Mean recoveries were calculated for each group of mice (n=8 for Days 9, 12, & 18 and n=12 for Days 6, 31, 34, 37, & 40 post-infection) and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.

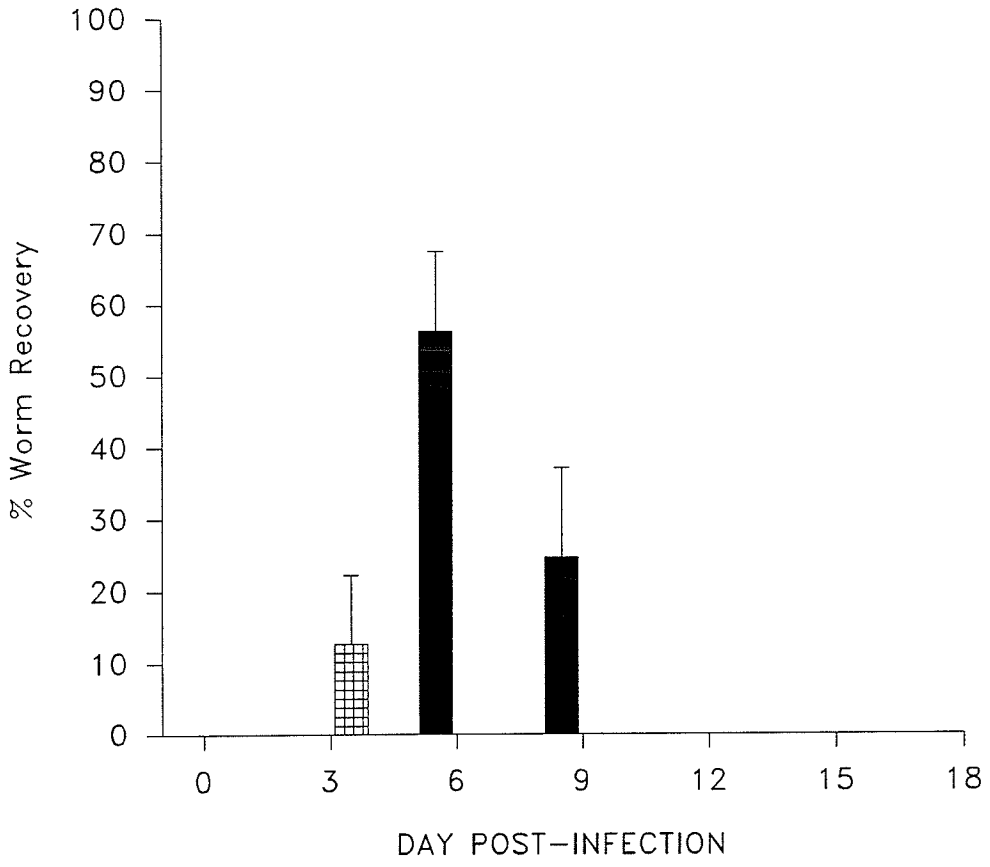
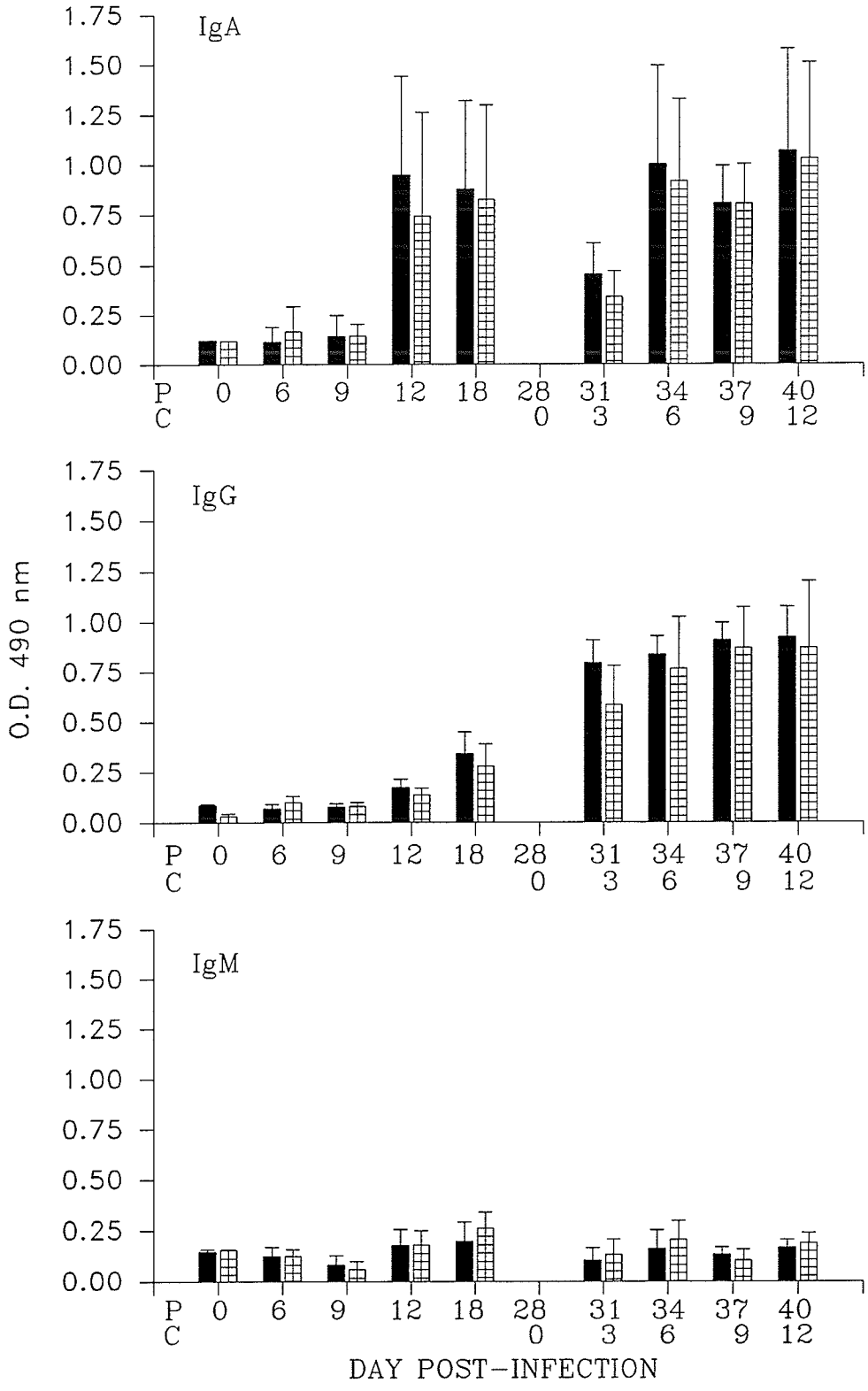

100
90
80
70
60
50
40
30
20
10
0
% Worm Recovery
0
3
6
9
12
15
18
DAY POST-INFECTION

Figure 19. Kinetics of the serum immunoglobulin response for primary (P) and challenge (C) infections with *Trichinella spiralis* var. *pseudospiralis* (Tps) and cross-reactivity of serum samples with *Trichinella spiralis* antigen (P1). Swiss Webster mice were given primary and challenge infections of 150 *T. spiralis* var. *pseudospiralis* larvae. Challenge infection was given on Day 28 post-infection. Serum samples were tested by enzyme-linked immunosorbent assay at 1:10 dilution for IgA and 1:100 dilution for IgG/IgM against Tps antigen (■) and P1 antigen (▦). Optical density was used as a measure of immunoglobulin titre. The mean optical density was calculated for each group of mice (n=8 for Days 9, 12, & 18 and n=12 for Days 6, 31, 34, 37, & 40 post-infection) and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.


IgA
IgG
IgM
O.D. 490 nm
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
P 0 6 9 12 18 28 31 34 37 40
C 0 3 6 9 12
DAY POST-INFECTION

There was a measureable background titre of IgM. The titre decreased slightly by Day 9, then began to increase. A peak titre was reached by Day 18. There was no significant change in titre following challenge.

Bile Immunoglobulin: A low background titre of IgA was present in the bile of uninfected mice (Figure 20). In infected mice a primary peak titre was reached by Day 9 but this titre had decreased by Day 18. By Day 31 a titre higher than the primary peak value had been reached and was maintained throughout the remainder of the experiment with a slight increase on Day 37 post-infection.

There was no detectable bile IgG throughout the primary infection. The titre of this immunoglobulin became detectable by Day 31, peaked on Day 37, and was decreasing by Day 40 post-infection. The peak titre of IgG was less than half of the value obtained for IgA.

Titres of IgM in the bile were close to zero for both primary and challenge infections.

Intestinal Lumen Immunoglobulin: A substantial background titre of IgA was found in the control mice (Figure 21). The IgA titre was lower than background on Day 6 post-infection, started to increase by Day 9, and peaked by Day 12 before falling to background by Day 18. After challenge the IL IgA titre increased by Day 31 and peaked on Day 37 post-infection.

Figure 20. Kinetics of the bile immunoglobulin response for primary (P) and challenge (C) infections with *Trichinella spiralis* var. *pseudospiralis* (Tps) and cross-reactivity of bile samples with *Trichinella spiralis* antigen (P1). Swiss Webster mice were given primary and challenge infections of 150 *T. spiralis* var. *pseudospiralis* larvae. Challenge infection was given on Day 28 post-infection. Bile samples were tested by enzyme-linked immunosorbent assay at 1:250 dilution against Tps antigen (■) and P1 antigen (▦). Optical density was used as a measure of immunoglobulin titre. The mean optical density was calculated for each group of mice (n=8 for Days 9, 12, & 18 and n=12 for Days 6, 31, 34, 37, & 40 post-infection) and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.

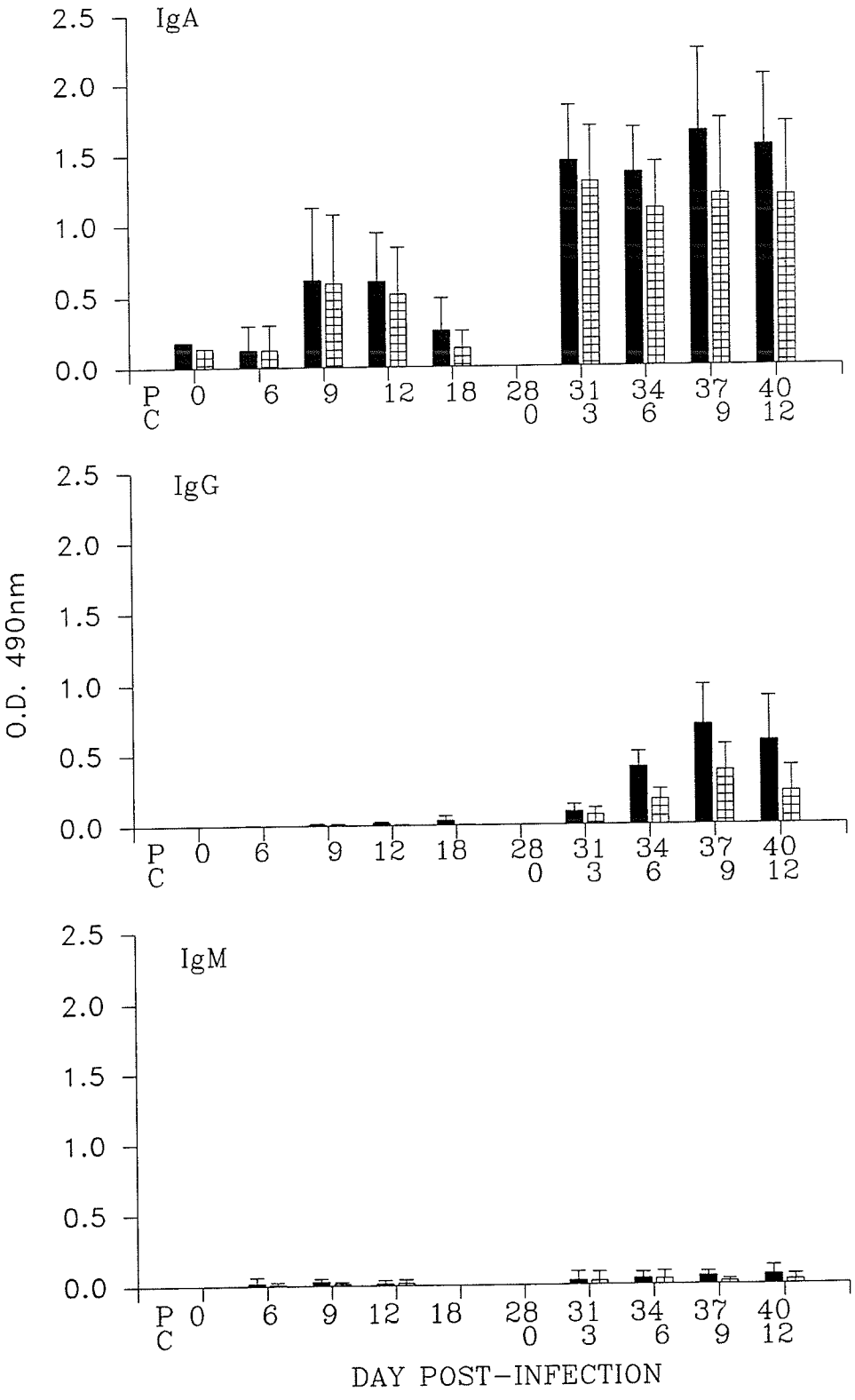
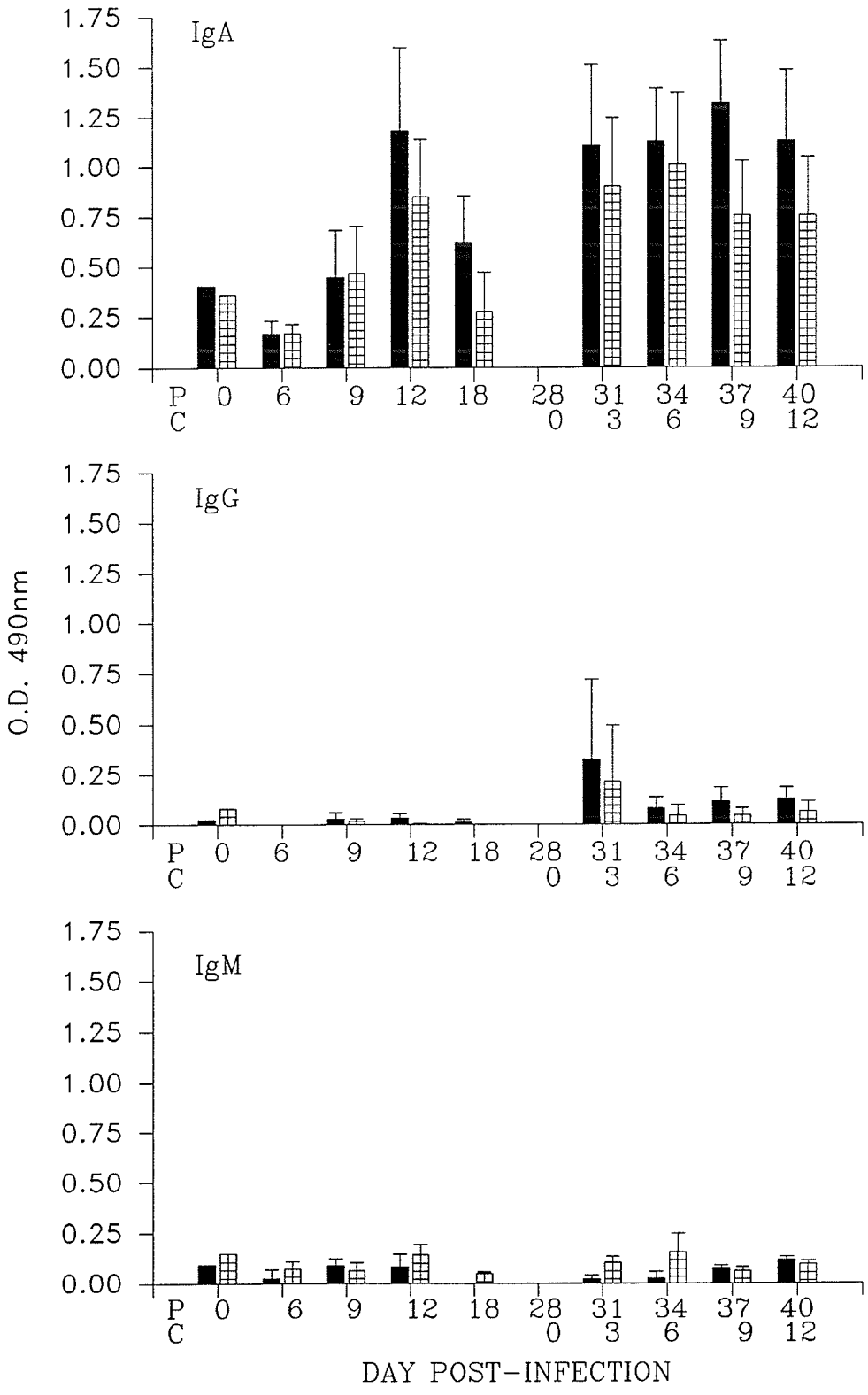

IgA
IgG
IgM
2.5
2.0
1.5
1.0
0.5
0.0
O.D. 490nm
P C
0
6
9
12
18
28 0
31 3
34 6
37 9
40 12
DAY POST-INFECTION

Figure 21. Kinetics of the intestinal lumen immunoglobulin response for primary (P) and challenge (C) infections with *Trichinella spiralis* var. *pseudospiralis* (Tps) and cross-reactivity of bile samples with *Trichinella spiralis* antigen (P1). Swiss Webster mice were given primary and challenge infections of 150 *T. spiralis* var. *pseudospiralis* larvae. Challenge infection was given on Day 28 post-infection. Intestinal lumen samples were tested by enzyme-linked immunosorbent assay against Tps antigen (■) and P1 antigen (▦). Optical density was used as a measure of immunoglobulin titre. The mean optical density was calculated for each group of mice (n=8 for Days 9, 12, & 18 and n=12 for Days 6, 31, 34, 37, & 40 post-infection) and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.


IgA
IgG
IgM
O.D. 490nm
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
P C
0
6
9
12
18
28 0
31 3
34 6
37 9
40 12
DAY POST-INFECTION

Very low titres of IL IgG were seen in primary infection. An increase was monitored on Day 31 but this value had fallen by Day 34. Detectable titres were obtained throughout the remainder of the experiment but the absolute values were well below those obtained for IgA.

A low background titre of IgM was observed. IgM titres declined on Day 6 of primary infection then increased on Day 9 to the background titre. Challenge values were below background on Days 31 and 34 then rose on Day 37 and reached a value slightly higher than background by Day 40 post-infection.

Cross-Reactivity of *T. spiralis* var. *pseudospiralis* Samples with P1 Antigen

Serum Immunoglobulin: A comparison of immunoglobulin responses of serum samples obtained from mice infected with *T. spiralis* var. *pseudospiralis* to Tps and P1 antigens was made (Figure 19). The values obtained for serum immunoglobulin versus P1 and Tps antigens, although consistently higher for Tps, showed no significant differences throughout primary and challenge infections with the exception of the Day 12 serum IgG value which was significantly higher for Tps antigen ($P \leq 0.05$).

Bile Immunoglobulin: There were no significant

differences in the values obtained for bile samples when P1 and Tps antigens were used although values were again consistently higher for the homologous antigen (Figure 20).

Intestinal Lumen Immunoglobulin: There were no significant differences in the values obtained for IL samples against P1 and Tps antigens although the values for Tps antigen were consistently higher (Figure 21).

DISCUSSION

Kramar *et al.* (1981) recovered significantly fewer adult worms from the intestines of CD-1 Swiss white mice infected with 250 *T. spiralis* var. *pseudospiralis* larvae than with 250 *T. spiralis* on Days 7, 9, 11, and 13 post-infection. Palmas *et al.* (1985), however, found slower expulsion of *T. spiralis* var. *pseudospiralis* than *T. spiralis* in NIH and B10G mice using infection doses of 300-350 larvae. The difference could be due to the strain of mice used in the two experiments. In this experiment, expulsion of 150 *T. spiralis* var. *pseudospiralis* L1 was complete by Day 12 of primary infection and by Day 6 post-challenge. This was more rapid than the expulsion rate for *T. spiralis* found in previous experiments where worms from a primary infection were still present on Day 12 and from a challenge infection on Day 6 post-challenge. This result supports the work of Kramar *et al.* (1981), although the time of complete expulsion is earlier in my experiments. As both experiments employed Swiss white mice, the difference in rate of expulsion cannot be attributed to mouse strain. However, Kramar *et al.* (1981) used mice 8 - 12 weeks of age where I used mice of 16 - 21 weeks of age. This age difference accelerated the expulsion of *T. spiralis* in NIH mice (Wakelin & Lloyd, 1976) by 1 - 2 days. Lower per worm larval production by *T. spiralis* var. *pseudospiralis* than *T. spiralis* was reported by Kramar *et al.* (1981) (28.3 and 80.6,

respectively) and a similar result was obtained here (41.2 and 113.3, respectively).

The kinetics of immunoglobulin production are strikingly similar for infection with the two strains of the parasite. Serum IgA response to *T. spiralis* var. *pseudospiralis* appears to be much stronger with more definite primary and secondary peaks, but the timing of peak titre response does not differ from peak titre found in mice infected with *T. spiralis*. Serum IgG and serum IgM kinetics are the same, however, the end point for infection with *T. spiralis* var. *pseudospiralis* was found to be 1:100 dilution compared to 1:1000 for *T. spiralis*. Bile IgA and IgM production are identical for the two parasite strains. Bile IgG response after challenge infection is stronger for infection with *T. spiralis* var. *pseudospiralis* than was seen for *T. spiralis*, but the kinetics of the response remain the same. There was little difference in the strength and pattern of the IL immunoglobulin response between the two strains of parasite.

The high degree of cross-reactivity of immunoglobulin raised by *T. spiralis* var. *pseudospiralis* to P1 antigen signifies little antigenic difference between the crude antigen extracts of the two strains. This finding is in accord with that of Ermolin & Efremov (1974) who found no differences in somatic extracts of the two strains using immunodiffusion, immunoelectrophoresis, and disc electrophoresis. Palmas *et al.* (1985) also reported extensive cross-reactivity between the

two strains of the parasite when infected by or injected with antigen from one strain and challenged with an infection of the heterologous strain. Almond *et al.* (1986) analyzed surface antigens and secretions of *T. spiralis* var. *pseudospiralis* and *T. spiralis* by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). They found a mixture of common and strain-specific proteins but were not able to correlate any antigenic differences with differences in the pathology caused by the two strains. Wassom *et al.* (1988) also found common and strain-specific antigens between *T. spiralis* var. *pseudospiralis* and *T. spiralis* and reported that mesenteric lymph node cell proliferation could be induced by heterologous antigen but it was always strongest with the homologous antigen. They concluded that immunological differences between the isolates could be attributed to differential expression of functionally relevant antigens.

In conclusion, the difference in the kinetics of worm expulsion between *T. spiralis* var. *pseudospiralis* and *T. spiralis* coupled with no detectable difference in the kinetics of immunoglobulin production suggest that antibodies are not responsible for the more rapid expulsion of *T. spiralis* var. *pseudospiralis* in Swiss Webster mice. The high degree of cross-reactivity in the samples from *T. spiralis* var. *pseudospiralis*-infected mice with antigen from *T. spiralis* suggest that the majority of antigenic determinants in the crude extracts of the two isolates are common.

CHAPTER 4. LONGEVITY OF THE IMMUNE RESPONSE OF MICE TO INFECTION WITH *TRICHINELLA SPIRALIS*.

INTRODUCTION

Acquired immunity is the ability to generate a more rapid immune response upon second exposure to an antigen. A desirable facet of acquired immunity is to retain this ability for a long period of time between first and second exposure. The ability of mice to mount an anamnestic immune response to *Trichinella spiralis* as expressed in accelerated worm expulsion and increased antibody production in serum and secretions has been demonstrated in previous chapters. The longevity of this response in mice with regard to more rapid worm expulsion has been examined with varying degrees of success. Rappaport & Wells (1951) found no difference in worm recoveries from unimmunized mice and mice immunized with 100 *T. spiralis* larvae 5 or 14 weeks previously until the 17th day after infection with 800 L1. Wakelin & Lloyd (1976) found a substantial reduction of worm recovery in NIH mice immunized with 450 L1 and challenged with 500 L1 after six weeks. Grove *et al.* (1977a) challenged mice given primary infections of 150 L1 3, 7, 13, 20, 29, and 39 weeks later with 500 L1 and found resistance to reinfection at three weeks. This resistance disappeared at 7 & 13 weeks but returned at 20 weeks and remained through the 39 week testing period.

The duration of the anamnestic nature of the immunoglobulin response to *Trichinella* has not been examined to date. The purpose of this experiment was to examine the

worm recovery and immunoglobulin levels in serum and secretions of mice challenged with 150 L1 three and six months after a primary infection with 150 L1.

MATERIALS AND METHODS

Serum Collection

For the purpose of long term examination of serum antibody, individual mice had to be sampled repeatedly at specified times during the experiment. The mice were restrained in a plexiglass cage and the tail held taut. The ventral caudal vein was partially severed by a firm, quick slash with a scalpel blade and drops of blood were collected in a microcentrifuge tube. The blood sample was then treated as previously described (Chapter 1).

Experimental Design

Twenty mice were infected with 150 L1. Twelve mice were challenged with 150 L1 on Day 84 and eight on Day 168. Mice were sacrificed six days after the challenge infection and intestinal worm and muscle larvae counted. Serum, gall bladder, and intestinal lumen (IL) samples were obtained. Serum samples were also taken on Days 18, 28, 56, and 112. To ensure that the mice were not immunologically impaired by blood loss, no more than twelve drops ($\approx$ 0.5 ml) were taken at any one time and sampling intervals were well-spaced to allow the mouse to replenish its blood volume. Care was also taken not to draw a blood sample near the time of challenge in order not to interfere with the normal response to reinfection.

RESULTS

Intestinal Worm Recovery

The recovery of worms from the intestines of mice challenged on Day 84 and sacrificed on Day 90 was 5.3%. The recovery of worms challenged on Day 168 post-infection and sacrificed on Day 174 was 28.8%.

Immunoglobulin Response

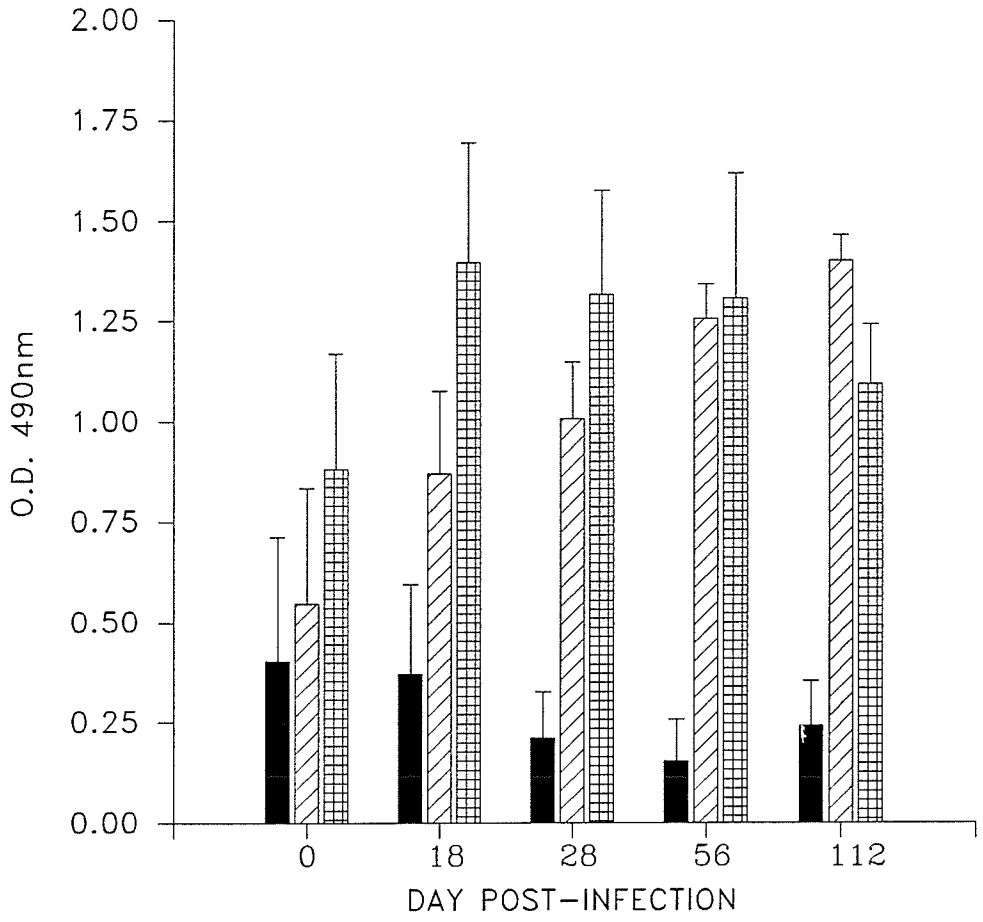
The immunoglobulin titres of serum, gall bladder, and intestinal lumen samples taken on Day 6 post-challenge are given in Table 5. No significant differences were observed between the values obtained at three months and six months for any immunoglobulin or any sample. The ability to generate an anamnestic immunoglobulin response had not decreased in this space of time.

Serum IgA was at background titre by Day 28 post-infection and decreased on Days 28 and 56 with a slight increase on Day 112 (Figure 22). Serum IgG increased steadily throughout the experimental period. Serum IgM showed a peak on Day 18 but remained above background titres for Days 28, 56, and 112.

Table 5. Immunoglobulin titres from mice infected and challenged 84 & 168 days post-primary infection. Swiss Webster mice were given primary infections of 150 *Trichinella spiralis* larvae (L1) and challenged 84 or 168 later. Mice were sacrificed six days following challenge infections (Day 90 and Day 174 post-infection) and serum, gall bladder, and intestinal lumen samples taken. Immunoglobulin titre is expressed as the mean ± standard deviation of the optical density of the samples from mice in each group as determined by enzyme-linked immunosorbent assay.

	Day 90 (n=12)	Day 174 (n=6)
Serum (1:10)		
IgA	0.489 ± 0.185	0.833 ± 0.161
IgG	2.097 ± 0.473	2.484 ± 0.095
IgM	2.797 ± 0.321	2.516 ± 0.249
Gall Bladder (1:5)		
IgA	1.085 ± 0.557	1.108 ± 0.048
IgG	0.313 ± 0.101	0.226 ± 0.189
IgM	0.046 ± 0.010	0.038 ± 0.003
Intestinal lumen (1:2)		
IgA	0.390 ± 0.148	0.432 ± 0.185
IgG	0.079 ± 0.036	0.211 ± 0.100
IgM	0.010 ± 0.002	0.019 ± 0.017

Figure 22. Kinetics of the serum immunoglobulin response of Swiss Webster mice for long term primary infection with *Trichinella spiralis*. Mice were infected with 150 *T. spiralis* larvae and serum samples taken on Days 0, 18, 28, 56, & 112 post-infection. Serum samples were tested at 1:10 dilution by enzyme-linked immunosorbent assay for the presence of IgA (▬), IgG (▨), & IgM (▦) antibodies. Optical density was used as a measure of immunoglobulin titre. The mean optical densities were calculated for each group of mice (n=20 for Days 0, 18, 28, & 56 and n=8 for Day 112) and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.


2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
O.D. 490nm
0
18
28
56
112
DAY POST-INFECTION

DISCUSSION

Results of the long term kinetics study demonstrated the durability of the serum IgG and IgM response and emphasized the weakness of the serum IgA response. Oliver-Gonzalez (1941) monitored anti-larval precipitating antibodies in the serum of mice through Day 70 of a primary infection and found that the titre began to rise by Day 25, peaked by Day 45, and remained at this peak until Day 65 when a decrease in titre was recorded. Rivera-Ortiz & Nussenzweig (1976) monitored serum IgG in several mouse strains for fourteen weeks following infection with 200 L1 and found steadily increasing levels in DBA/1 and LAF/1, similar to the results in this experiment. The two other strains, AKR and SJL showed peak IgG titres at 8 weeks which then declined steadily until the end of the testing period. Jungery & Ogilvie (1982) also monitored serum from two strains of mice for precipitating antibodies and antibodies that mediate eosinophil adherence through 90 days of a primary infection with *Trichinella*. Titres for both types of antibody rose on Day 25 for C_3H mice. Eosinophil-mediating antibody rose to a peak titre on Day 56 and began to decline on Day 75 while precipitating antibody rose steadily through the 90 day period. In NIH mice, both antibodies peaked on Day 50 after which precipitating antibody began to decline while eosinophil-mediating antibody continued to increase through Day 70 when testing was stopped for this strain.

Although the immunoglobulin type of each antibody was not clarified, IgE was eliminated as the serum samples had been heated to 56°C before testing which destroys the ability of IgE antibodies to bind to cells.

The serum and secretions of C57BL/6 and BALB/c mice infected with *Giardia muris* were monitored over a seven week period and serum of BALB/c mice over an eighteen week period by Snider & Underdown (1986). In both strains of mice, serum IgA and IgG increased steadily in titre through seven weeks and remained high for up to ten weeks following clearance of the parasite in BALB/c mice. The difference in the serum IgA level found for *G. muris* and that for *T. spiralis* in this study may be the duration of the infection which continues for seven weeks for the former compared to two weeks for the latter. No IgM was detected in the *G. muris*-infected mice. In intestinal secretions, only IgA was detected with titres elevated by Day 7, peaking by Day 21, and remaining high for the seven week period.

Grove *et al.* (1977a) found lower resistance to reinfection with 500 *Trichinella* L1 in Swiss albino mice infected 13 weeks previously than in mice infected 20 or 29 weeks previously. In this experiment, the opposite result was found, with resistance to reinfection greater in mice infected 12 weeks previously than those infected 24 weeks before challenge. The difference in the size of the challenge infection may account for the discrepancy. Rappaport & Wells

(1951) found that challenge of 800 L1 36 days and 3.5 months after initial infection showed little difference in expulsion rate while challenge of 300 L1 given on Day 35 was rapidly expelled. Unfortunately, they did not include a group given a challenge of 300 L1 after 3.5 months in their experiment so no conclusions can be drawn regarding rapid expulsion of the smaller dose after the longer time. Wakelin & Lloyd (1976) reported rapid expulsion of 500 *Trichinella* L1 from NIH mice infected 42 days previously with 450 L1.

It is difficult to compare my results with other published information due to the large number of variables including strain of mouse used, size of primary and challenge infections, and timing of infections. However, a general conclusion can be drawn that acquired immunity to *Trichinella* is retained in mice for a considerable length of time, up to 39 weeks as reported by Grove *et al*. (1977a). More interesting is the immunoglobulin response monitored in this experiment. In all samples and for all immunoglobulins, secondary responses similar to those recorded following challenge on Day 28 were seen following challenge after 84 and 168 days. The worm expulsion rate, however, decreased in this experiment using mice challenged on Day 168 post-infection despite little change in antibody production, suggesting that antibody level and expulsion rate are not closely interrelated.

CHAPTER 5. EFFECT OF GALL BLADDER EXTRACT ON *IN VITRO* LARVAL RELEASE.

INTRODUCTION

Acquired immunity to *Trichinella* resulted in accelerated worm expulsion, decreased larval production, and reduced worm growth (Chapter 2). These effects were paralleled by increased levels of bile, serum, and intestinal lumen antibodies but the direct contribution of these antibodies to the detrimental effects on the worms was not determined. Jacqueline *et al.* (1978) demonstrated that the IgA fraction of intestinal secretions as well as serum IgM and IgG from parasitized mice reduced *in vitro* larval production when added to the culture media and also demonstrated an *in vitro* effect of sIgA from bile on *Trichinella* (Jacqueline *et al.*, 1981).

The object of this experiment was to investigate the effect of gall bladder extracts from uninfected and infected mice on the larval production of worms from a primary infection.

MATERIALS AND METHODS

Parasites

Female *Trichinella* were recovered from the intestines of mice infected six days previously with 150 L1. The worms were prepared for *in vitro* larval release as described in Chapter 1.

Preparation of Gall Bladder Extract

Gall bladder samples from previous experiments which were IgA-positive and IgG/IgM-negative to P1 antigen were pooled, lyophilized, and reconstituted in double-distilled water which was added gradually until all crystals were dissolved (positive GB). Gall bladder samples from uninfected controls which were IgA/IgG/IgM-negative to P1 antigen were prepared similarily (negative GB). Protein concentrations were determined and the volumes adjusted to ensure solutions of identical protein concentration (900 μg/ml). Further dilutions were made in PBS where necessary.

Experimental Design

Adult female *Trichinella* were divided into five groups of thirty (Groups I, IIA, IIB, IIIA, and IIIB). Group I was the control group which was cultured without any additions to the culture media. Group IIA had 22.5 μg of negative GB protein in 25 μl added to the culture media of each female while Group

IIB had 4.5 μg in 25 μl added. Group IIIA had 22.5 μg of positive GB protein in 25 μl added to the culture media of each female while Group IIIB had 4.5 μg in 25 μl added. The *in vitro* larval release assay was otherwise performed as previously described (Chapter 1).

RESULTS

Larval production in the low protein negative GB group was significantly higher than for the control group while production in the low protein positive GB value was not significantly different from that of the control (Table 6). Both high protein GB samples reduced larval production significantly but the difference between these two groups was also significant. Group IIA had 52.7% of control larval production while Group IIIA reduced larval production to 5.7% of control.

In the control and both low protein groups, female worms in the wells were highly motile during the counting procedure. Group IIA worms were lethargic and granular material was seen in the media. Group IIIA worms were virtually stationary and clumps of granular material were observed, often attached to the worms. The nature of this granular material was unknown.

Table 6. Effect of gall bladder extracts on *in vitro* larval release. Swiss Webster mice were given primary infections of 150 *Trichinella spiralis* larvae and sacrificed six days later. Adult female worms were recovered from the intestines of the mice and cultured for 24 hours in media alone (Group I), media containing high (Group IIA) or low (Group IIB) protein content IgA-negative gall bladder extract, or media containing high (Group IIIA) or low (Group IIIB) protein content IgA-positive gall bladder extract. The mean ± standard deviation of the larvae released per female in each group was calculated and values analyzed by t-test (*=significantly different from control value at $P \leq 0.05$).

Group I	Group IIA	GroupIIB	Group IIIA	Group IIIB
control	high -ve	low -ve	high +ve	low +ve
84.35 ± 27.02	44.45* ± 27.38	103.37* ± 29.18	4.81* ± 21.58	90.93 ± 30.21
(n=26)	(n=29)	(n=30)	(n=27)	(n=28)

DISCUSSION

The reduction in the larval production of worms incubated with the high protein negative GB sample is a suggestion that normal bile can non-specifically interact with the parasite and cause reduction of larval production. It is possible that the high bile salt concentration was responsible for impairment of the worms. If only salt concentration or non-specific reactions were responsible for reduction of larval production then no difference in the values would be expected between Groups IIA and IIIA. However, larval production is further reduced in the high protein positive GB group indicating a role for IgA antibody in the enhancement of larval reduction.

Jacqueline *et al.* (1978) found larval numbers reduced by 16% when the IgA fraction of intestinal secretions from immunized mice was added to the culture media. The reductions achieved in this experiment were far greater using GB samples. Also, Jacqueline *et al.* (1978) found no impairment of function in secretions from unimmunized mice while I found a significant reduction of larval production with GB samples from uninfected mice. Further investigation of this situation is necessary to resolve the discrepancies. In future experiments, I would use purified bile IgA rather than whole gall bladder extract to eliminate the complication of bile salt presence.

GENERAL DISCUSSION

This section is a presentation of the general principles of immunity and relates their relationship to helminth infections. The ultimate purpose behind the investigation of immunity to any pathogen is prevention. Acquired immunity is the development of specific memory to foreign antigen that is responsible for a more rapid and heightened antibody response to second contact with the antigen. The purpose of vaccination against disease is to generate acquired immunity by first exposing the immune system to a harmless form of the pathogen so that any exposure to the actual pathogen will trigger a strong secondary-type immune response which will minimize the effects of the pathogen. Acquired immunity to *Trichinella* is achieved after a primary infection. It is desirable to have a vaccine that would stimulate the same or greater immunity to *Trichinella* as a primary infection. As *Trichinella* first contacts the mucosal immune system, it is this system that must be primed by vaccination. In order to achieve this goal, a full understanding is required of i) what immune mechanisms are present in the mucosa, ii) which of these mechanisms can be primed for a secondary-type response, and iii) which of the mechanisms which can be activated are important in immunity to *Trichinella*.

While the third point is still an ongoing investigation, the first two points can be answered with an overview of mucosal immunity which is a complex network of specific and non-specific factors and cells. The first line of defence is

found in the secretions which bathe all surfaces exposed to the external environment and is aimed at the prevention of microbial establishment or adhesion of foreign antigen. Tears, saliva, urine, and nasal secretions contain enzymes such as lysozyme, lactoperoxidase, and lactoferrin, potent antimicrobial and proteolytic factors which are non-specific by nature. Mucus secreted by goblet cells overlying the mucosal epithelium consists of a variety of glycoproteins which form a fluid film over the epithelium that is credited with the prevention of bacterial and viral adhesion and inhibition of absorption of soluble antigens. Foreign substances trapped in the mucus are subject to removal by mechanical means such as coughing, sneezing, ciliary action, and intestinal peristalsis. Goblet cell secretion is stimulated by antigen-antibody complexes (Walker *et al.*, 1977) and there is a structural relationship between sIgA and mucus glycoproteins by which IgA can anchor antigen in mucus leaving it vulnerable to removal or proteolysis (Clamp, 1977). Antibodies, primarily sIgA, are also found in mucosal secretions and are produced by plasma cells in the lamina propria. SIgA binds to bacteria, viruses, and soluble antigens preventing adhesion to the epithelium.

Also found in the lamina propria are macrophages, eosinophils, basophils, and mast cells which, when appropriately stimulated by antibody, release a variety of mediators including histamine, serotonin, prostaglandins, and

leukotrienes as well as peroxidases and super oxides. T and B cells residing in the lamina propria underlying the mucosal epithelium originate in specialized lymphoid follicles called Peyer's patches which are found throughout the small intestine. The epithelial cells overlying the follicle are cuboidal, and there are reduced numbers of goblet cells and the presence of a specialized antigen-sampling cell called an M cell (Owen, 1977). Particulate antigens are taken up by the M cell and delivered to macrophage and dendritic cells beneath the epithelial layer where the antigen is processed and presented to T cells present in the follicle. These T cells proliferate and stimulate B cells located in a germinal centre in the follicle which in turn results in the proliferation of antigen-specific but immature B cells. These immature B cells and T cells leave the follicle and enter the lymphatic circulation. After passing through the mesenteric lymph nodes and thoracic duct they enter the bloodstream which carries them to distant mucosal sites where they home, settling in the lamina propria as mature cells. The common origin of mucosal plasma cells means that antigen presented at a mucosal site distant to its first appearance will still be recognized and cause antibody production.

If humoral antibody is important in the protection of a host to intestinal parasites such as *Trichinella*, then a secondary type of response should occur during a challenge infection along with accelerated worm expulsion. In

experiments conducted in this study there was a strong secondary response for bile and intestinal lumen IgA and for serum IgG when mice were infected and challenged with 150 *T. spiralis* L1. The time of initiation of worm expulsion was closely associated with the peak titre of these antibodies for both primary and challenge infections. These results are comparable to results obtained by other researchers. Culbertson (1942), using primary infection of 100 L1 and challenge with 175 L1, found 50% recovery on Day 7 primary and 5% recovery on Day 7 challenge. Rappaport & Wells (1951) found worms through Day 20 of a primary infection of 300 L1, but the challenge infection was completely expelled by Day 10. Primary expulsion began on Day 8 and was complete by Day 14 for infection doses of 500 L1 (Alizadeh & Wakelin, 1982) and 450 L1 (Kennedy, 1980) and, in the latter study, expulsion during a challenge infection began on Day 6 and was completed by Day 10. Sinski *et al.*(1983), using infections of 200 L1, also found expulsion of a primary infection was started by Day 8 and completed by Day 14. There was a significant increase of total IgA in the lumenal wash on Day 9 that persisted through Day 24. Specific anti-*Trichinella* IgA was not examined. Total IgA is not a good estimator of specific antibody due to the presence of anti-phosphorylcholine IgA. Significant increases in serum IgG and IgA were noted on Day 14 with the IgG titre staying high and the IgA titre returning to background by Day 24. Parasite-specific antibodies were demonstrated for serum

IgG by immunofluorescence. Crandall & Crandall (1972), using radial immunodiffusion, detected serum IgG by Day 11 of a primary infection which showed a definite secondary response after challenge. Serum IgA was detectable by Day 11 - 15 but results were inconsistent after challenge with increases in titre in some mice and no change in others. IgM antibodies were present in uninfected mice and this background titre increased in the first week after infection then remained high with no increase after challenge, findings which are corroborated by my study. Expulsion of worms from a primary infection yielded recoveries of 50% on Days 7 & 11, but contrary to previously mentioned results, where expulsion was complete by Day 14, 25% of the infection dose of 200 L1 was still present on Day 15. Serum IgG and IgA responses similar to those demonstrated by Crandall & Crandall (1972) were found by Kozek & Crandall (1973) who also reported that IgA predominated in intestinal perfusates detectable by Day 11.

Given that mucosal IgA and serum IgG were temporally relationed to worm expulsion, the role of these antibodies in worm expulsion requires clarification. The first consideration is the source of target antigen(s). Mucosal IgA is coincident with the adult stage of the parasite and serum IgG with the migrating larval stage. This relationship is correlative, as shown by Sinski & Holmes (1977) who associated specific anti-worm IgA antibodies with expulsion of adult *N. brasiliensis* and the elevation of serum IgG, which occurred after

expulsion, with the migratory larval stages in rats. Sinski & Holmes (1977) also demonstrated greater binding capacity of hyperimmune serum IgG to the immature stages of *N. brasiliensis* than the adult stage. Philip *et al.* (1981) followed the primary serum antibody response to *Trichinella* surface proteins and reported stage-specific antibodies to infective larval, adult, and newborn larval surface antigens and suggested that the host produced new antibodies to changing parasite surface antigens. Qualitative differences in larval and adult worm antigens were reported by Oliver-Gonzalez (1941, 1963), Ortega-Pierres *et al.* (1984), Philipp *et al.* (1980), and Parkhouse & Almond (1985). James & Denham (1975) reported that immunity generated by the intestinal stage of *T. spiralis* in mice is only effective for the intestinal phase. In a second study, James *et al.* (1977) showed that intravenous injection of NBL caused an 85% reduction in the establishment of larvae given in a subsequent intravenous challenge, a 51% reduction in establishment of larvae resulting from a *per os* infection of muscle larvae, but no effect on the number or longevity of adult worms. Denham (1966) found that a single infection of *Trichinella* terminated after 48 hours did not produce significant immunity to an unterminated challenge infection in mice but immunity was produced by primary infections of 3 or 4 days. The larval stages apparently do not prime the host to expel the adults. Campbell *et al.* (1963) had previously shown that preadult

worms could induce immunity to challenge through repeated infections terminated after one day by thiabendazole but the difference in these findings could be due to multiple versus a single infection. Accelerated expulsion and reduced fecundity were produced by injection of purified larval antigens (Silberstein & Despommier, 1985). Transplantation of adult worms to the intestine of a naive host produced immunity to subsequent challenge equal to that produced by oral administration of muscle larvae, evidence that exposure to intestinal larval stages is not necessary to produce immunity (Kennedy *et al.*, 1979). Kennedy *et al.* (1979) proposed the existence of antigenic similarities between adult and larval stages to explain their findings. Bell *et al.* (1979) reported that the muscle larvae and adult stages both cause a reduction in adult worm recovery and loss of female worm fecundity but that the immune response to pre-adults is stronger against fecundity while the immune response to adults is directed at worm expulsion. Further investigation using parabiotic rats (Bell & McGregor, 1980) revealed that at low to moderate infections two stimuli are required to produce rapid expulsion (RE). One stimulus is transferred to the parabiotic mate; the other is not. But the transferable stimulus, induced by the preadult stage, will not generate RE unless the intestine has been exposed to the other stimulus, namely exposure to adult worms.

Knowing the source of the target antigens, the next

consideration is the role of the antigen-antibody reaction in immunity to intestinal parasites. Oliver-Gonzalez (1963) concluded that antigen/antibody complexes enhance the formation of acquired immunity. He determined that injection of an adult-antigen/antibody complex obtained from the incubation of adult worms in immune serum until precipitates formed produced a higher degree of protection to subsequent infection than injection of antigen or immune serum alone. The increase in mucus production caused by antigen-antibody complexes aids in the exclusion of larvae from the epithelium by trapping of the worms in mucus (Lee & Ogilvie, 1981). Mice subjected to anti-μ injections showed loss of serum IgM and reduction of serum IgG1, IgG2, IgA, and reaginic antibody but retained their capacity to expel *N. brasiliensis* (Jacobson *et al.*, 1977). No attempt was made to determine if IgA antibodies were present in the intestinal environment, the argument justifying this was that anti-μ treatment had been shown to deplete mice of all intestinal plasma cells (Lawton *et al.*, 1972). It was concluded that antibody was not a requisite for expulsion. A completely different conclusion was reached by Grove & Warren (1976) who used niridazole to suppress the cellular responses of mice while leaving humoral antibodies intact. No significant differences were found in control and niridazole-treated animals infected with 280 *Trichinella* L1 for adult worm recovery, duration of infection, or muscle larvae numbers indicating that humoral factors alone could

transfer immunity. Significant reduction of inflammation around the muscle larvae was observed, verifying the success of the niridazole treatment in suppressing cellular functions. Campbell (1968) administered steroidal and non-steroidal anti-inflammatory drugs to mice infected with *T. spiralis* or *Trichuris muris* and recorded suppression of worm loss with the steroidal but not the non-steroidal compounds. As non-steroidal compounds do not suppress humoral immunoglobulin as well as steroids, it would appear that worm loss following steroidal injection is more likely due to immunosuppression than a reduction of inflammation.

Contradictory results have also been reported for the effectiveness of T and B cell populations at transferring immunity. Despommier *et al.* (1977) found enriched populations of immune B cells more effective than T cells at promoting *Trichinella* expulsion in rats. They proposed that expulsion is mediated in the intestine by local IgA antibodies while T cells function as "helpers". In experiments performed by Wakelin & Wilson (1979), T and B cell fractions from mesenteric lymph nodes (MLN) were effective in conferring protection to *Trichinella* in mice as measured by accelerated worm expulsion and depressed fecundity. Treatment of the fraction with anti-Thy 1.2 abolished the ability of B cells to transfer immunity. When non-immune T cells were added to the B cell fraction, the anti-fecundity but not the expulsion activity returned. It was concluded that T cells were

responsible for changing the environment causing worm loss and the contribution of antibody to the process was minor.

Infected mice treated with an antihistamine to suppress inflammation had prolonged infections (Campbell *et al.*, 1963). Larsh & Race (1975) correlated acute intestinal inflammation with worm expulsion and persistence of worms with an absence of inflammation. They attributed the expulsion process to reactions between antigen-sensitive T cells and antigen followed by non-specific inflammatory reactions. Larsh & Race (1975) did not attempt to reconcile these findings with the results of earlier experiments (Larsh, 1953) where *Trichinella* administered intracaecally promoted acquired immunity equal to that of an oral infection. Larsh (1953) concluded that a primary antibody response and a secondary cellular response cooperated in the expulsion process, a conclusion reached in another set of experiments (Larsh & Race, 1954). A cooperative effect was also reported by Love *et al.* (1976) in their experiments involving transfer of immunity by antiserum or cells. In one experiment, cells alone conferred greater immunity while in another, antiserum was more effective, but the best immunity was achieved by a combination of serum and cells.

Depletion of T cells by thymectomy (Ruitenberg, 1973; Walls *et al.*, 1973) or by administration of anti-lymphocyte serum (ALS) (Ruitenberg, 1973; Machnicka, 1972) resulted in prolonged adult survival and increased muscle larvae number.

This effect is more likely due to the lack of T cell stimulation of B cells than to the lack of direct T cell action on the parasites. Elson *et al.* (1979) and Bond *et al.* (1987) reported that T cells were necessary for murine IgA synthesis. Korenaga *et al.* (1989) isolated 0X8- 022- T-helper cells from the thoracic duct lymph (TDL) of *Trichinella*-infected rats and found them responsive to both adult and larval antigens *in vitro*. These T-helper cells were generated in the intestine and appeared in mesenteric lymph nodes (MLN) and efferent lymph by Day 3 of infection. Draining lymph for 48 hours did not remove the cells and it was concluded that a resident cell population existed in the intestine. These T-helper cells possess B cell differentiation and antibody secretion functions.

Worm expulsion and reduction of worm fecundity are commonly used as parameters of acquired immunity. The involvement of antibody in the expulsion process was discussed above. The involvement of antibody in reduction of fecundity *in vitro* was demonstrated in my study, where significantly lower NBL production was found for worms incubated in high protein gall bladder extracts from positive anti-*Trichinella* IgA samples (5.7 % of control). Significant reduction was also found in negative specific IgA samples but not to the same degree (52.7 % of control). Jacqueline *et al.* (1978) found a reduction in *in vitro* larval release with intestinal samples containing IgA and reported a similar effect with serum IgM

and IgG. Another frequently reported facet of challenge infections is "stunting" or reduced worm length. As was seen in my results, worm length was significantly less for challenge infections even when primary infection was 10 L1 and challenge 150 L1.

Antibodies also have the capacity to stimulate protective functions of cells. Proliferation of eosinophils is a common feature of *Trichinella* infections (Brown, 1898; Ismail & Tanner, 1972; Basten *et al.*, 1970; Basten & Beeson, 1970) and there is evidence for antibody involvement in eosinophil activation. Kazura & Grove (1978) described antibody-dependent eosinophil-mediated destruction of *Trichinella* NBL in mice but did not identify the immunoglobulin class responsible. Ortega-Pierres *et al.* (1984) produced an IgG1-secreting monoclonal antibody which was capable of promoting eosinophil-based destruction of NBL *in vitro* and *in vivo*. Penttila *et al.* (1984) found antibodies incapable of producing eosinophil-mediated destruction of larvae *in vitro* but were using *Nematospiroides dubius*. Van der Vorst *et al.* (1988) determined the number of intestinal eosinophils with IgE and IgA on their surface in *Hymenolepis diminuta*-infected mice and found IgE more prominent on primary infection cells and IgA on secondary infection cells. In both the primary and secondary infections labelled eosinophils were most abundant in the parasite's preferred habitat, the second quarter of the small intestine. Binding of IgA and IgG to blood eosinophils was described by

Abu-Ghazaleh *et al.* (1989) and it was noted that sIgA-binding was the most potent signal for eosinophil degranulation.

The importance of immunoglobulins in immunity to *Trichinella* was examined by infection of genetic strains of mice which were designated as high or low antibody producers (Perrudet-Badoux *et al.*, 1975; 1978; Ruitenberg *et al.*, 1980; Jungery & Ogilvie, 1982; Wakelin *et al.*, 1986) and, in general, the high antibody producers were more capable of resisting infection than low antibody producers and were more responsive to immunization against the worm. Almond & Parkhouse (1986) examined the humoral response of a resistant (NIH) strain and a susceptible (C3H) strain of mice to infection with *Trichinella* and found that the resistant mice produced serum IgA antibody to surface antigens of adult worms while the susceptible strain did not. They suggested that there was a correlation between the presence of the antibody and the expulsion of the parasite. Bell *et al.* (1984) found that at infection levels below 300 L1, the phenotypic variations in strong, intermediate, and weak responder mice begin to disappear to the point where strong responders to 400 L1 become weak responders to 50 L1. This dose dependent effect on genetic responsiveness to infection was confirmed by Wassom *et al.* (1984) but with opposite results. They found that with infections of 100-200 L1, H-2^q and H-2^f strains of mice expelled *Trichinella* more quickly than the H-2^k strain. This H-2-controlled effect was reduced with infections of 400 L1

and eliminated with infections of 500-600 L1.

The dose dependency of the immune response of mice to *Trichinella* infection has been well documented with regard to expulsion kinetics. My experiments provide evidence that immunoglobulin kinetics are also dose dependent. Infections of 10 L1 consistently resulted in slower and weaker immunoglobulin responses than infections of 150 L1 for both serum and mucosal secretions. Infection level also affected peripheral blood eosinophilia as observed in this study (Appendix 1) with greater numbers of eosinophils present at the higher infection level. This corroborates the study by Ismail & Tanner (1972) who reported a linear relationship between infection dose and number of eosinophils present at peak response for *Trichinella*-infected rats.

Future research on the immunoglobulin response of mice to infection with *Trichinella* should include kinetics studies in several strains of mice to establish the genetic basis of response and studies using larger infection doses to examine the relationship between immunoglobulin production and expulsion of heavy infections.

The experiment involving infection with *T. spiralis* var. *pseudospiralis* was designed to investigate worm expulsion and immunoglobulin kinetics in a strain of the parasite which displayed differences from *T. spiralis*. These differences included being physically smaller and lacking a capsule around the muscle larvae typical of other *Trichinella* isolates

(Garkavi, 1972), being expelled more slowly from the host (Palmas *et al.*, 1985), being capable of infecting avian hosts, and producing more NBL at 42°C than at 37°C. If differences were observed in immunoglobulin kinetics as well it would provide more evidence for an important function for antibody in immunity to the parasite, particularly if prolonged worm survival was paralleled by lower mucosal immunoglobulin levels. However, worms of this strain did not remain in the intestine longer in my experiments and were completely gone by Day 6 challenge, thus corroborating the study of Kramer *et al.* (1981). Reports of antigenic differences between the two strains (Almond *et al.*, 1986; Wassom *et al.*, 1988) prompted examination of the cross-reactivity between them by testing samples raised through infection with *T. spiralis* var. *pseudospiralis* with P1 antigen. Although reaction with the homologous antigen was generally higher, no significant differences were observed.

In conclusion, the experiments reported here support the involvement of antibody in the immune response to *Trichinella*. Secondary immunoglobulin responses were seen for IgA in bile and intestinal lumen secretions and for IgG in serum. The appearance of these antibodies in primary and challenge infections correlated with the kinetics of worm expulsion. Small primary infections were capable of priming the host to produce a secondary immune response when challenged with a higher level infection, to reject higher challenge infections

at an accelerated rate, and to reduce worm size and fecundity following challenge. A dose dependent effect was observed on immunoglobulin production in the serum and secretions of infected mice. A direct effect of worm-specific anti-IgA from gall bladder was demonstrated in the reduction of *in vitro* larval release. The ability of mice to mount a secondary immunoglobulin response to *Trichinella* infection was found to be retained for at least six months.

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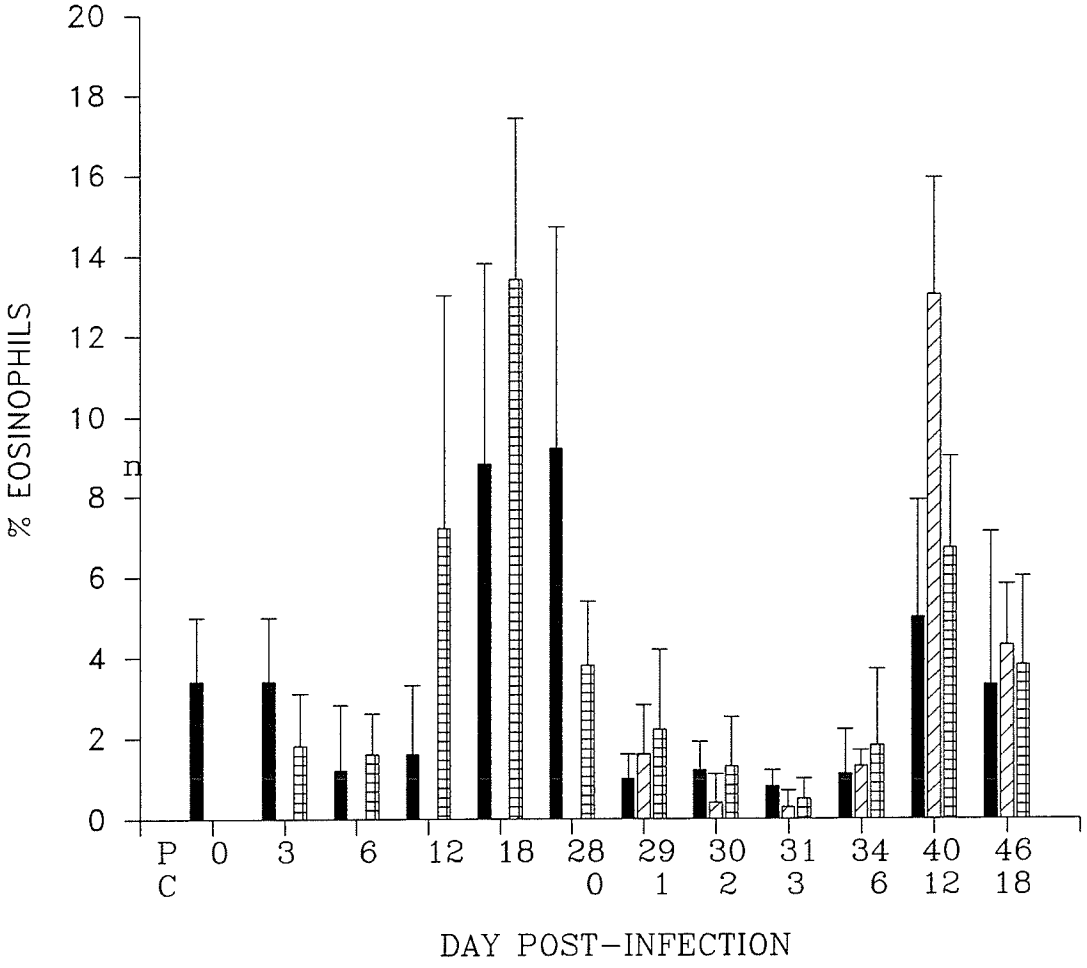
Appendix I. Effect of infection level on the peripheral eosinophil response of Swiss Webster mice during primary and challenge infections with *Trichinella spiralis*.

Mice were given primary (P) infections of 10 (■) or 150 (▦) *Trichinella spiralis* larvae (L1). Mice given primary infections of 10 L1 were challenged (C) with 10 (■) or 150 L1 (▨) and mice given primary infections of 150 L1 were challenged with 150 L1 (▦) on Day 28 post-infection. Blood samples were taken on Days 0, 3, 6, 12, 18, 28, 29, 30, 31, 34, 40, & 46 post-infection and two blood smears made for each mouse. The blood smears were air-dried, stained for three minutes in Wright's stain, rinsed gently with tap water, dried, and examined under the 40X objective of a Leitz microscope. Cell counts were made by starting at a random point of the smear and advancing one field at a time systematically to ensure no area was missed or repeated until one hundred white blood cells had been counted. Eosinophil numbers were expressed as percent of white blood cell count. The two slides made for each mouse were counted twice. The four counts per mouse were averaged and the mean of these averages calculated for each group of mice (n=8) and plotted against the day of sampling. The t-bars represent the standard deviation from the mean.

A background of 3.4% was found in uninfected (Day 0) mice. This percentage was the same on Days 3 and 6. Following infection with 150 L1, the eosinophil response rose to 8% by Day 12 and peaked at 13% on Day 18 before declining to near background by Day 28. Following challenge with 150 L1, no increase was seen until Day 40 when the level peaked at 7%

then began to decrease by Day 46. Infection with 10 L1 did not produce an increase in circulating eosinophils until Day 18, peaking on Day 28 at 9% but at background on Day 29. A peak of 5% was seen on Day 40 following challenge with 10 L1 and a drop in level was observed by Day 46. In the 10/150 L1 group, there was a 13% peak on Day 40, similar to the Day 12 peak in a primary infection with 150 L1.

The pattern of eosinophil response seen in this study is similar to those reported by other researchers (Ismail & Tanner, 1972; Grove *et al.*, 1977b; Lin & Olson, 1973; Basten *et al.*, 1970) with initial response for a moderate infection dose by Day 12 and a peak response between Days 18 and 21. The dose dependency demonstrated here was also seen by Ismail & Tanner (1972) in rats who also reported a faster response in a moderate infection dose than in a low or high (not performed in this study) infection. As there was no examination of eosinophil response after challenge infection, the values reported in this study could not be compared. The observation that the eosinophil level following challenge with 150 L1 was the same for previously uninfected mice as those given an initial infection of 10 L1 and higher than that of mice given an initial infection of 150 L1 implies that an initial low infection does not ready the host for a strong secondary response to a larger infection.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