STUDIES ON THE TRANSFER OF IMMUNITY FROM MOTHER TO OFFSPRING IN MICE INFECTED WITH *TRICHINELLA SPIRALIS* (NEMATODA)

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by

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I

to my Mother

P R E F A C E

This study is the result of research performed by the author in the Department of Zoology, University of Glasgow from 1979 to 1984.

Experimental results of this research are presented in four sections. The Protocols, Tables, Figures and Plates referred to in the text are grouped at the end of each section and are numbered according to each section.

The reference section includes all the references cited throughout.

An Appendix containing detailed data from section 1 is also included.

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SUMMARY

The transfer of protection from mother to offspring during *Trichinella spiralis* infection, was studied in the NIH strain of mice.

The establishment and the course of infection of *T. spiralis* were investigated in naive infant mice suckling naive mothers. Mice to the age of four weeks at the time of infection were less susceptible to infection in that the numbers of *T. spiralis* established was considerably lower than in adult mice: infections in mice at one week of age were highly variable. With two week old mice more consistent infections were achieved. The distribution/localization of intestinal *T. spiralis* in young mice to three weeks of age was different from that in adult mice in that a much larger proportion of the worms are located in the posterior half of the gut.

Young suckling mice were immunocompetent in expelling a burden of intestinal *T. spiralis*, although they were a little slower of clearing the gut of adult worms than adult mice. Apart from being less susceptible to intestinal infection than adult mice, young NIH mice appeared to be adequate host animals in that the fecundity of the worms was unimpaired in these animals.

Immunofluorescence studies of naive infants suckling naive mothers showed the presence of a few immunoglobulin-containing cells in the lamina propria of the infant small intestine. Both IgA and IgM-containing cells were detectable from the sixth day of age, with traces of IgM present in the enterocytes. Immunoglobulin-containing cells were not observed in the intraepithelial position. However, there is a possibility that these Ig-containing cells were of maternal origin and as such offered the infant potential protection. IgG was visualized clearly in the infant gut tissues at a very young age, but with few cells observed and very little

IgG could be visualized in enterocytes. None of the immunoglobulins were visualized in or onthe brushborder.Using the enzyme linked immunosorbent assay (ELISA) young mice, infected at 1 week of age, were shown to be able to produce detectable amounts of IgM and IgG antibodies in their sera within 14 days of T. spiralis infection.

It was shown that maternal protection to T. spiralis infection in the infant is entirely an endowment conveyed through the milk. This study also clarified that this protection is wholly protection against the establishment of infective and developing larvae in the gut and is not protection involving early expulsion of an established infection. Protection of the order of a 75% reduction in establishment of T. spiralis in the infant mouse was observed and a considerable degree of protection was maintained for at least one week after suckling had ceased.

A primary infection of as short a period as 9 days in lactating mothers resulted in considerable protection to suckling infants. A period of suckling milk from infected/immune mothers as short as 6 h. conferred a considerable degree of protection.

It was confirmed that in lactating adult mice, there was a considerable traffic of lymphoblasts from the gut associated lymphoid tissues to the mammary gland. Attempts to trace and quantify the maternal cellular endowment in the milk, and possible internalisation of such cells to the infant tissues, using ^{125}I-radiolabelled cells did not yield the desired information due to the technical problem of liberation of radiolabel from cells and its rapid uptake by gut tissues.

In lactating NIH mice a considerable population of immunoglobulin-containing cells were observed in the tissues of the mammary gland and in the secreted milk in the ducts with IgA-containing cells the most common of the Ig-containing cells examined. In T. spiralis infected lactating mice there was a statistically significantly greater number of immunoglobulin-containing cells of IgA, IgM and IgG in the mammary gland during

the mid-lactation period. Furthermore, a considerable cellular endowment from the mother was observed. The numbers of lymphocytes and macrophages delivered to the infants was of order of 1.1×10^5 and 3.0×10^5 cells per ml respectively.

In adult non-lactating mice the occurrence of detectable amounts of antibodies to *T. spiralis* larval antigen in the serum of a primary infection was observed by day 5-post-infection for IgM and consistently by day 15 for IgG antibodies; these antibodies were also observed during secondary infections. IgA antibodies were not detected. In infected lactating mice, the serum antibody titres of both IgG and IgM were lower in comparison to non-lactating mice.

In milk from infected mice IgG antibody levels were 1:120 in a secondary infection and 1:30 in a primary infection of 17 days duration. IgM antibodies were just detectable at a titre of 1:15 in both primary and secondary infections, and IgA antibodies, undetectable in sera of adult mice, were just detectable in the milk of infected mice. When total immunoglobulin levels were assessed by Radial Immunodiffusion (RID), milk from infected mice was shown to contain approximately 2-3 times the amounts of IgG and IgA present in the milk from uninfected mice, with only minute amounts of IgM. IgA levels in the milk of infected mice were 0.88 mg/ml and IgG levels 0.63 mg/ml with less than 0.1 mg/ml of IgM. All of the above measurements, which indicate clearly the immunocompetence of infected lactating mice, are believed to be the first quantified evidence of the immunologic endowment in milk.

The protective capacity of the milk was assessed by feeding 0.3 ml of immune milk to infants by oral intubation over a period of 24 hours. Significant protection was observed.

In mice suckling infected/immune mothers all of the components listed in the maternal milk were detected in the milk in the stomachs of infants. The presence of viable maternal cells was also assessed. Maternal leucocytes could not be detected in the lumen of the small intestine of infants suckling immune mothers, and cells were not visualized attached to the brush border or in the intraepithelial positions. Likewise maternal immunoglobulins were not visualized in the lumen or brush border of the small intestine of suckling infants. In naive infants suckling immune mothers IgG antibodies were detectable in the sera from the eighth day of age, at a titre in excess of 1:100 until the nineteenth day of age.

Thereactivity of the antibodies detected in sera and milk to worm components and hence the occurrence and location of worm antigens were examined by immunofluorescence on sections of infective larvae. Antibodies to the presumed secretory product of the stichosome (oesophageal gland) were demonstrated for IgG and IgM, as early as day 12 of a primary infection for IgG. IgA antibodies to the stichosome secretions were not observed. IgA, IgG and IgM antibodies to the surface of the cuticle or to adherent material were demonstrated and also antibodies of all three classes to the material contained within the lumen of the intestine of the worm. Thus the three major areas of the worm associated with possible secretory or excretory antigens were shown to be reactive for antibodies produced by adult mice.

The postulate is made that one or all of these sites are affected in some way by maternal antibodies located in the intestine of the suckling infant, with the consequence of rendering the infective larvae less able to establish within the tissues of the small intestine of the infant.

GENERAL INTRODUCTION

The Parasite:

Trichinella spiralis is a nematode parasite which is able to infect a wide range of hosts including man. It has attracted the attention of clinicians and researchers for 150 years. Richard Owen (1835) first described and named the parasite *Trichinella spiralis* in a discussion of the observation made by the student James Paget in 1835. The life cycle of the parasite has been reviewed in great detail by Villella (1970) and the host - parasite relationship and disease by Gould (1970). *T. spiralis* is unique among parasitic nematodes in that all the stages of its life cycle occur within a single host animal. In its life cycle, sequentially it is an inter-and intra-cellular parasite of the epithelium of the small intestine as an infective, and developing larva and as an adult. The newborn larvae are conveyed around the body in the lymphatic fluid and the bloodstream and travel along connective tissue until they penetrate a striated muscle cell, and the developing infective larva and 'resting' infective larva is an intracellular parasite of striated muscle. The parasitized muscle cell is surrounded by a capsule of host material. Infective larvae are ingested by a new host animal and are freed from the host capsule in the acid-pepsin environment of the stomach. The infective larvae, so released, pass into the small intestine and invade and establish in the epithelial layer.

Infections in the rat and mouse have served as useful models to study immunological aspects of the host-parasite relationship. The pathological and immunological reactions to *T. spiralis* observed in these animals resemble to some degree those of man. Recently in Glasgow, many workers have established a system to study *T. spiralis* expulsion in NIH and Balb/C mice

(Wakelin and Lloyd, 1976 a & b ; Bruce and Wakelin, 1977; Manson-Smith, Bruce, Rose and Parrott, 1979 a; Manson-Smith, Bruce and Parrott, 1979b; Kennedy, 1979, 1980a), and a lot of information regarding the host immune response against the intestinal phase of this parasite has been determined, although the mechanisms of the expulsion of T. spiralis from the gut of mice are not fully understood.

The strain of T. spiralis used in this study is known as the London strain which was isolated from an outbreak of human trichinosis in Penrith, England in 1939, and which has been maintained in rodents and rabbits.

The immune response to intestinal nematodes:

Over the last 50 years, reports concerned with immunity to T. spiralis have given conflicting results, some investigators have succeeded in transferring passive immunity to T. spiralis in terms of accelerated worm expulsion, by transferring serum from immune to naive animals (Culberston and Kaplan, 1938; Culbertson, 1942; Hendricks, 1953; Mills and Kent, 1965; Wakelin and Lloyd, 1976b; Love, Ogilvie and McLaren, 1976); while others have failed to show an acceleration of worm expulsion after passive transfer (McCoy and Bond, 1941; Larsh, Goulson and Weatherly, 1964a; Denham, 1969; Larsh, Goulson, Weatherly and Chaffee, 1970; Gore, Burger and Sadun, 1974). Transfer of immunity with lymphoid cells has been reported in mice using lymph node and peritoneal exudate cells (Larsh, Goulson and Weatherly, 1964a&b; Larsh, Race, Goulson and Weatherly, 1966); and Larsh, Goulson, Weatherly and Chaffee (1969) also noted that spleen cells taken from artificially immunized donors could transfer an immunity operative against adult worms; however, Staroniewicz (1971) was unable to transfer immunity with lymph node,

spleen and peritoneal exudate cells taken from actively or artificially immunized mice.

Immunity to the intestinal phase of Nippostrongylus brasiliensis has been extensively studied in rats and mice, and shows several similarities to the studies involving T. spiralis in mice and rats. Sarles and Taliaferro (1936) and Chandler (1938) demonstrated that rats can be passively immunized against N. brasiliensis infection with antiserum from immune donors. However, Ogilvie and Jones (1968) noted that the capacity of pools of antisera to cause worm expulsion from recipient rats varies enormously. The circulating antibodies which were found to be most effective in passive immunity were in the IgG class, and in particular, IgG_1 (Jones, Edwards and Ogilvie, 1970). It has been suggested that the function of antibodies is not primarily to expel, but to damage the worms, rendering them susceptible to other components of the immune response (Ogilvie and Love, 1974). However, there is no direct evidence that antibodies can effectively damage N. brasiliensis, and comparable apparent damage can be induced in worms by in vitro culture in the absence of antibody (Love, Ogilvie and McLaren, 1975). The existence of a component in addition to antibodies in the immune expulsion of N. brasiliensis was first suggested in studies on worms from neonately infected rats, which do not expel within the normal period of time; and worms recovered from these rats had the characteristics of 'damaged' worms (Ogilvie and Hockley, 1968; Jones and Ogilvie, 1971). A similar situation was found to occur in lactating rats (Kelly and Ogilvie, 1972), which are also unable to expel N. brasiliensis (Connan, 1970). The antibody response of young rats to N. brasiliensis infection is apparently normal although somewhat slower

to develop, and serum from young infected rats has been used to transfer immunity (Jarrett,Urquhart and Douthwaite,1969). The reaginic antibody response has been shown also to be normal in young rats (Jarrett et al., 1969). Young rats given immune mesenteric node cells from adult animals expelled a larval infection, but no demonstrable immunity to N.brasiliensis was conveyed by the transfer of mesenteric node cells from young infected rats to adult rats (Dineen and Kelly, 1973). Ogilvie and Love (1974) concluded that in a primary infection with N. brasiliensis antibodies first damaged the worms, making them susceptible to the expulsive effect of the second step involving the collaborative action of lymphocytes and bone marrow derived cells.

A key feature in the development of our knowledge of parasite immunology was the evidence that immunological responsiveness to the intestinal phase of T. spiralis infection was T-cell dependent (Walls, Carter, Leuchars and Davies, 1973).

The immune response of T. spiralis has been studied by Larsh and his co-workers over a long period of time (see Larsh and Race, 1975). They proposed a hypothesis to explain expulsion of T. spiralis, mainly derived from histological work on the mouse, that immunity of mice against the adult worms has a cell-mediated basis, that a specific delayed hypersensitivity is produced in response to an allergen within or released by the infective larvae or artificial sensitization, and upon challenge infection they proposed that the delayed hypersensitivity response caused allergic inflammation due to injury of cells and tissues of the small intestine with the result that biochemical changes occur to produce unfavourable conditions that lead to worm expulsion. They cited

the following events: a mild infiltrate of polymorphonuclear leucocytes was evident by day 4 post-infection increasing by day 6, and on day 8 the mucosa and lamina propria were highly inflamed and contained large numbers of polymorphonuclear cells, lymphocytes and plasma cells. Expulsion of the worms occurred between 11 and 14 days after infection, and on day 14 lymphocytes, monocytes and plasma cells were still present in large numbers. In challenge infections a similar though accelerated sequence of intestinal inflammation was observed. They referred to this phenomenon as allergic inflammation. Walls et al. (1973), working with mice, noted a mild inflammatory infiltrate in the submucosa between days 3 and 10, consisting of neutrophils, eosinophils, lymphocytes and macrophages. Inflammatory changes in the wall of the small intestine were negligible.

A similar sequence of events to that in T. spiralis infection of the mouse was observed in N. brasiliensis infection of the rat by Taliaferro and Sarles (1939) who described a mixed inflammatory reaction of plasma cells, lymphocytes, mononuclear cells, macrophages, eosinophils and, in particular, noted the occurrence of large numbers of connective tissue basophils and globular leucocytes.

Uber, Roth and Levy (1980) demonstrated an increased number of mast cells in the mucosa of the small intestine of mice infected with N.brasiliensis. The increase in intestinal mast cells has been much studied and although a temporal association between the mast cell rise and expulsion has been reported, there is a disagreement over the precise timing of the rise (Murray, 1972; Ogilvie and Love, 1974). However, the exact role of mast cells in the immune response to intestinal nematodes is still unclear. The concept that delayed hypersensitivity responses and effects provides

adequate explanation of immune expulsion is not substantiated when the phenomenon of rapid expulsion is considered. In rats following a primary infection, the very large majority of a challenge infection are removed from the intestine within 24 h. This was first noted by McCoy (1940). Russell and Castro (1979) and Lee and Ogilvie (1981a & b) noted that such rapid expulsion persisted in rats for a considerable period of time. In mice, as noted by Bruce and Wakelin (1977) and by Ottaway, Bruce and Parrott (1983), the period in which rapid expulsion occurs is confined to within one week of the expulsion of a primary infection. Although the components of immediate hypersensitivity reactions are present in that numerous mast cells (Brown, Bruce, Manson-Smith and Parrott, 1981) and IgE antibodies (Perrudet-Badoux, Binaghi and Boussac-Aron 1976) are present, drugs blocking the release of amines or their activity do not prevent the rapid expulsion. Apparent trapping of infecting larvae in lumenal mucus is observed (Lee and Ogilvie 1981 a & b) but the mechanisms of this response have not been determined.

The mechanism of eosinophil- and neutrophil mediated damage to T. spiralis has been an active area of study. The importance of the eosinophil as an effector of resistance against T. spiralis was demonstrated in a series of in vitro studies. Kazura and Grove (1978) reported that eosinophils obtained from the peritoneal cavities of T. spiralis or Schistosoma mansoni - infected mice were capable of killing newborn larvae by an antibody-dependent mechanism. MacKenzie, Jungery, Taylor and Ogilvie (1980) have demonstrated that rat neutrophils also exert potent cytotoxic activity against newborn and possibly

infective larvae. Miller and Nawa (1979) demonstrated that goblet-cell hyperplasia occurs in N. brasiliensis infection and that this is a thymus-dependent phenomenon.

Mesenteric lymph node cells (MLN) and thoracic duct lymphocytes (TDL) have been used extensively in the analysis of resistance to helminth infection in the laboratory models. Kelly and Dineen (1972) and Dineen, Kelly and Love (1973) demonstrated that the expulsion of N.brasiliensis from the rat intestine can be accelerated by the adoptive transfer of immune MLN cells; while Ogilvie, Love, Jarra and Brown (1977) and Nawa and Miller (1978) observed similar events by using immune thoracic duct lymphocytes. Resistance to infection with Trichostrongylus colubriformis can be transferred between syngeneic guinea-pigs by lymphoid cell suspensions from immune donors (Dineen and Wagland, 1966). MLN cells were also found to transfer immunity against T. spiralis in mice (Wakelin and Wilson, 1977), and rats (Love, Ogilvie and McLaren, 1976). Selby and Wakelin (1973) have shown that immunity to Trichuris muris can also be transferred by serum or cells from immune donors; and the expulsion is thymus dependent (Wakelin and Selby, 1974). Crum, Despommier and McGregor,(1977) used TDL to transfer immunity to T. spiralis, and have shown in another study that adoptive transfer of immunity is accomplished more efficiently with a population of purified B-cells from thoracic duct lymphocytes than T-cells. In all cases that have been adequately studied, it has been shown that immunity to parasitic helminths is thymus-dependent and transferable adoptively with T-cell populations (Mitchell, 1980)

The localization of immunologically-active cells may be important in the onset of intestinal expulsion of worms. The host's initial contact with the parasite is in the gut; and the majority of the studies have thus assessed

lymphocyte traffic in this site. In rats infected with either T. spiralis or N. brasiliensis, larger numbers of radioactively-labelled TDL lymphoblasts accumulate in the small intestine than in naive animals (Love and Ogilvie, 1977), and in NIH mice, similar findings were reported by Rose, Parrott and Bruce, (1976a) who demonstrated that mesenteric lymphoblasts and particularly mesenteric T-lymphoblasts homed to the small intestine in infected mice and the enhanced accumulation occurred 2 and 4 days after infection. However, this localization was antigen independent; blast cells activated by unrelated stimuli were as capable of localizing in the intestine in increased numbers as were cells from donors infected with the parasite. Further study by Manson-Smith, Bruce, Rose and Parrott (1979a) showed that in NIH mice, the majority of T. spiralis worms are located in the anterior half of the small intestine early in the enteral phase of infection and, enhanced localization of mesenteric lymphoblasts and mesenteric T-lymphoblasts are most prominent in the anterior region of the small intestine. In Balb/C mice,worms resided primarily in the distal small intestine, and the enhanced localization of lymphoblasts was demonstrable only in this area.

The reasons for the deficiency in expulsive capacity in neonatal and lactating rats is not known, but there is evidence, however, that the cellular component which expels the worms once they are damaged by antibody from mature rats, is either deficient or defective in immature rats (Keller and Keist, 1972; Dineen and Kelly, 1972, 1973),reviewed by Ogilvie and Love,(1974).

The antigen preparations derived from whole parasites or their

secretations have been documented in many reviews (Soulsby, 1966; Thorson, 1970; Silverman, 1970; Ogilvie and Worms, 1976; Clegg and Smith, 1978; Ogilvie and de Savingy, 1982).

Studies on the antigenic composition of T. spiralis larvae have shown a multiplicity of somatic and metabolic antigens. Norman and Sadun (1959); Tanner and Gregory (1961), Tanner (1962,1963) and Catty (1969), have detected at least sixteen components. The nature and the immunological relevance of these antigens is mostly unknown since the majority of the experiments have been performed with rather crude extracts. The origin of functional antigens is not fully known, but a preponderance of evidence indicates that metabolic antigens are important (Ewert and Olson, 1961; Mills and Kent, 1965; Tanner and Gregory, 1961; Jacqueline, Vernes and Biguet, 1978). Characterization of crude nematode-secreted products suggests that these are usually simple mixtures of relatively few antigens compared to the multiplicity of antigens present in crude whole worm preparations, thus simplifying purification and standardization (de Savigny, 1975; Jenkins and Wakelin, 1977). It is known that secreted antigens of the infective T. spiralis larvae induce high levels of protection when injected into mice (Campbell, 1955; Tronchin, Dutoit, Vernes and Biguet (1979). Despommier (1974a) was successful in vaccinating mice against reinfection with T. spiralis by parenteral injection of secretory/excretory antigens from muscle larvae. These antigens were subsequently found to originate from the stichosome, the oesophageal gland of the parasite (Despommier and Müller, 1976) and in particular were found to be associated with the stichocyte granules, secreted as soluble products into the host during the first 30 hours of the enteral phase of infection (Despommier, 1974b). These

granules were found to be capable of inducing a high level of protection against an oral challenge of infection using very low doses of antigen (Despommier, Campbell and Blair, 1977a). Recently, the soluble portion of a cell-free preparation rich in antigens and subcellular particles, including the stichocyte secretory granules, has been characterized regarding the proteins and antigens (Despommier and Laccetti, 1981a). The antigens in this preparation (i.e. S_3 fraction) were further purified by immunoaffinity chromatography and characterized with respect to their molecular weight, isoelectric point, carbohydrate content and ability to induce protection in mice (Despommier and Laccetti, 1981b). Silberstein and Despommier (1984), reported on purification of T. spiralis antigens employing immunoaffinity chromatography with monoclonal antibodies. This strategy has led to the isolation of three antigens. Two of these, which induce protection in mice, are secreted proteins and one of these (48K) conferred a level of protection comparable to that elicited by exposure to an entire infection.

It should be made clear that the antigens being studied by these authors are antigens produced by the infective larvae at or shortly after invasion and although shown to be protective these same antigens may not be involved in expulsion of adult T. spiralis from the gut. As Oliver-Gonzalez (1941) pointed out there are different antibodies reactive to infective larvae and adult worms.

Studies by Phillip, Parkhouse and Ogilvie (1980), Parkhouse, Phillip and Ogilvie (1981) and Phillip, Taylor, Parkhouse and Ogilvie (1981) on the isolation of immunogenic surface proteins from T. spiralis which are known to be shed in vitro, have indicated the presence of genus,

species and stage specific antigens within the antigen mosaic of the surface of T. spiralis.

Transfer of immunity from mother to offspring:

As Tomasi (1979) in his introduction to the Immunology of Breast Milk, emphasises, the transmission of immunity from mothers to their offspring is of such fundamental importance in biology and medicine that it has been investigated extensively from the early years of the study of immune phenomena. He notes that one of the earliest observations on the transmission of immunity to newborns was that of Bollinger in 1877 who determined that vaccination against cowpox during pregnancy gave rise to newborn lambs which were immune, and that similar observations were made by Burckhardt in 1879 in human infants born of mothers vaccinated against vaccinia during late pregnancy. There was, however, a considerable debate in the early literature regarding the mechanisms of transfer, whether it took place in utero or after birth as a result of the ingestion of colostrum and milk. The publication of Ehrlich (1892) clearly demonstrated that in mice passive immunity could be acquired via the placenta and through the milk after birth. Breed (1914) was one of the first to report that bovine colostral milk contained a large number of leucocytes. The clinical significance of colostrum in the immunity of the newborn animal was clearly demonstrated by Smith and Little (1922) in showing that nearly 75% of newborn calves deprived of colostrum died of Escherichia coli septicaemia and that infection could be prevented by a single ingestion of colostrum. Thus both medical and veterinary scientists have long recognized the importance of immunity,

or at least protection,acquired after birth from the mother.

Brambell (1970) thoroughly reviewed investigations carried out up to 1968 on the transmission of passive immunity from mother to young and concluded that neonatal mammals are dependent upon a passive immunity mediated by antibody transmission from the mother before and/or after birth (see also review by Sterzl and Silverstein, 1967). Subsequently many aspects of the immunobiology of lactation including the significance of antibodies in colostrum and milk, the types and number of cells in mammary exosecretions and their immunological role and functional capacity, the fate of lacteal immunoglobulins and/or maternal cells in the neonatal gut, have been reviewed by Lascelles and McDowell (1974); Beer, Billingham and Head (1974); Head (1977); Head and Beer (1978); Butler (1979); Ogra and Dayton (1979); Newby, Stokes and Bourne (1982) and McClelland (1982). Thus although it has long been known that the mammary gland secretions contain leucocytes as well as antibodies, detailed analyses of their significance have been undertaken only in the last three decades.

Maternal transfer of immunity in parasitic infections:

The transmission of immunity from mother to offspring in parasitic infections was examined in rats for the first time by Culbertson (1938, 1939) who noted that transmission of immunity to Trypanosoma lewisi can be effected through the colostrum and milk although the possibility of the passage of antibody in utero was not ruled out. This finding was confirmed by Kolodny (1939) and Miles (1972) using Trypanosoma cruzi in rats and mice respectively.

In rodent malaria, passive transfer of protection from mothers to their young has been demonstrated by Bruce-Chwatt (1954); Bruce-Chwatt and Gibson (1956); Terry, (1956); Desowitz (1973); Palmer (1978); Orjih, Cochrane and Nussenzweig (1981) utilising Plasmodium berghei in mice. These investigators concluded that maternal antibody was being transferred through the milk and that very little immunity was transferred in utero. However, according to a review by Cox (1975), antibodies received from the mother affect subsequent infections in various ways; if too little antibody is present, protection is minimal, if too much is present, the young animal either does not have an opportunity to acquire an immunity because all the parasites with which it subsequently becomes infected are immediately destroyed, or its own immune response is inhibited by passively acquired antibodies.(Jacobsen, Zuckerman & Greenblatt, 1978).

Stevens and Frank (1978) using Giardia muris, showed that infant mice are resistant to infection by orally administered cysts when suckling immune milk. Using the same model, Andrews and Hewlett (1981) demonstrated in milk from immune mothers the presence of specific IgA and IgG antibodies which might be the mediators of the protection of the suckling infants either by interfering with the excystment process of the cysts or by influencing the capacity to attach to the enterocytes of the intestine.

In cestode infections, antibodies involved in colostral transfer of immunity have been examined in detail in the rodent/Taenia taeniaeformis system. Musoke, Williams, Leid and Williams (1975) demonstrated passive transfer of immunity to neonates with oral administration of colostral IgA immunoglobulins from rats infected with T. taeniaformis. Lloyd and Soulsby(1978) also showed that IgA immunoglobulins are involved in the passive transfer of immunity via the colostrum of mice.

Colostral transfer of immunity has been demonstrated against a number of other cestodes, viz. Hymenolepis nana in mice (Larsh, 1942, 1944), Taenia ovis in lambs (Rickard and Arundel, 1974) and Taenia hydatigena in lambs (Gemmell, Blundell-Hasell and MacNamara, 1969). Also Lloyd and Soulsby (1976) demonstrated that newborn calves fed immune serum or colostral immunoglobulins, which were induced by local injection of the mammary gland of a cow with oncospheresof T. saginata, were protected against this infection.

In Schistosomiasis in man, a delayed hypersensitivity reaction to Schistosoma mansoni antigen was demonstrated in uninfected children born to infected mothers (Camus, Carlier, Bina, Borojevic, Prata and Capron, 1976). Circulating antigens, antibodies and immune complexes in milk from mothers infected with S.mansoni were also demonstrated by Santoro, Borojevic, Bout, Tachon, Bina and Capron (1977), and all may be implicated in the development of delayed hypersensitivity in naive children. These authors also considered the possibility of the induction of tolerance in children experiencing schistosome antigens in the milk.

In nematode infections, studies by Chaicumpa, Jenkin and Rowley (1976) and Shubber, Lloyd and Soulsby (1981), using Nematospiroides dubius in mice, showed that the young of immunized females were protected against infection through a colostral transfer of immunity. In Nippostrongylus brasiliensis infection in rats, Jones and Ogilvie (1967), and Greenberg (1971) demonstrated the occurrence of passive transfer of protective immunity from immune mothers to their infants and concluded that the mother's milk constitutes the main source of resistence to infection. In pigs, Smith and Herbert (1976) investigated passive transfer of immunity to Hyostrongylus rubidus and showed that offspring which had suckled infecte mothers had a demonstrable serum agglutinin titre 7 days after birth, and suggested that passively transferred agglutinating antibodies, mainly

of IgG class, were associated with the protection in these piglets. Kelley and Nayak (1965) showed that immunity against Ascaris suum infections in sows can be transferred via the colostrum to their young, and that passive immunity can be transferred to non-sensitized piglets by parenteral administration of immune serum.

Several workers have observed the transfer of immunity to Trichinella spiralis from immune mothers to their offspring. Mauss (1940) demonstrated that the offspring of T. spiralis infected rats, rabbits and hamsters were less susceptible to infection with the parasite than were the offspring of uninfected controls. Culberston (1943) showed that the transmission of immunity in rats was mediated through the milk suckled by their young, and little or no transmission of immunity through the placenta was thought to occur. Duckett, Denham and Nelson (1972) reported that protection against the intestinal phase of T. spiralis was associated with suckling milk from previously infected mothers and that the protection was lost at the time of weaning. Perry (1974) confirmed these findings and further demonstrated the presence of agglutinating T. spiralis antibodies in the sera of infected mothers.

Apart from the considerations by Cox (1975) and by Santoro et al (1977) of the possible disadvantages of milk antibodies, the issue of the induction of tolerance to parasitic infections is not a subject which has been investigated thoroughly by many authors, and certainly not with the comprehension of the induction of IgE tolerance in rats following suckling as shown by Jarrett and Hall (1979). It is probably also true to say that the concept of the transfer of transient protection to parasitic infections to the suckling infant as distinct from the transfer of immunity is not one which has been analysed comprehensively.

The objective of the present study:

It is evident from the review of the literature, that the passive transfer of immunity from mothers to their offspring, and the effect of this transfer on infection in the neonates have proved important to the development of our understanding of immunological competence. However, most of the details regarding this phenomenon are still unclear. The main objective of the present work was to try to determine the mechanisms by which protection is afforded to infant mice using the T. spiralis/mouse system as a model of infection. In all experiments reported, NIH mice were used as experimental hosts. This strain of mice has been extensively used in Glasgow University in both immunological and parasitological work and proved to be an excellent model.

The approach to this investigation included four areas of study:

(1) population studies in young mice i.e. the establishment and course of infection of T. spiralis in young NIH mice; the protection afforded by infected mothers; the required duration of infection in mothers before protection was afforded to suckling infants; the quantification of milk components and the required duration of suckling; the issue of long term protection, and the issue of the induction of tolerance following the ingestion of protective components in the milk.

(2) The traffic of lymphocytes in mice; and the possible uptake and role of maternal lymphocytes in suckling infants.

(3) Immunoglobulin-containing cells in the milk and in the infant gut and the sites of maternal immunoglobulins in the infant by immunofluorescence; and the location of antigens in the infective larvae, also by immunofluorescence.

(4) A study of the amounts of IgG, IgM and IgA antibodies and immunoglobulins in sera of infected mothers; in maternal milk, and in milk taken from the gut of infants; to determine the uptake of immunoglobulins from the intestine of infants; the immunocompetence of infants in terms of antibody production against infection.

Each of the above aspects is dealt with in a separate section with an introduction to each of these sections. The results of each topic are discussed fully in each section and comprehensive integrations of the significant salient findings is undertaken in the General Discussion.

GENERAL MATERIALS AND METHODS

This section contains descriptions of the materials and methods which apply widely throughout the thesis; descriptions of special techniques used only in portions of this study are given in appropriate sections.

Experimental animals and their maintenance

The inbred N1H strain of mouse only was used in this study. Stock mice were obtained from Anglia Laboratory Animals, Huntingdon, England.

In an effort to define and standardise the quality and immunological history of Laboratory animals, the Medical Research Council, Laboratory Animals Centre (LAS), Carshalton, Surrey, grades mice from various suppliers according to a scale of five categories given in "Register of Accredited Breeders and Recognised Suppliers" (Medical Research Council, 1976). According to this scale, all the mice used were rated at three stars, that is that they are free from all coccidia, Hexamita muris; Giardia muris, all cestodes, all arthropods, all nematodes except Aspiculuris tetraptera and Syphacia obvelata, and certain micro-organisms.

All mice arrived at 6-8 weeks of age and were quarantined for 2 weeks before use.

Mice were caged in groups of 6 or less in polythene cages, 33 x 15 x 13 cm (M_2 Cage, North Kent Plastic Cages Limited (NKP), Dartford, Kent), in groups of 8 or less in cages 38 x 15 x 13 cm (M_3 cage, NKP), or 15 or less in cages 38 x 25 x 18 cm (RM_2 Cage, NKP). While in quarantine, the mice were caged in groups of 50 in 56 x 38 x 18 cm cages (RC1 Cage, NKP). Mice were fed on diet (Oxoid 41B) ad libitum and given tap water in drip bottles for drinking. They were kept at 20-22°C on an artificial light/dark cycle maintained automatically at 12 hours light from

0.800 h to 20.00h with no day light. Sawdust litter was changed twice weekly.

The maintenance of breeding mice (pregnant and lactating).

Adult male and female mice were mated over a 14 day period in a ratio of two males to six females to ensure that progeny were born with a minimal time spread. In some experiments when more breeding mice were required a ratio of six males to twenty females were mated in a large cage 45 x 28 x 18 cm (MB, Cage NKP). At the end of 2 weeks, the males were removed and pregnant mice were housed in separate cages (M_2 Cage NKP) which were supplied at this stage with nesting material composed of sawdust or wood shavings which covered the floor of each cage to a depth of about 1 cm, and some cotton wool (Hospital grey non-absorbent). Pregnant females were transferred at this stage to allow them time to prepare themselves for parturition in stable conditions. Great care was taken not to disturb them more than necessary.

They were maintained on Oxoid Breeding Diet (Oxoid Ltd., London) during the later part of pregnancy and throughout the lactation period. It was found that "Litter success" was greater on the breeding diet. As Oxoid describes it, it is high in protein and vitamin E and fully supplemented with other vitamins and minerals. Cages were cleaned twice a week except in the first week after parturition. This would minimise disturbance to mothers and their infants. All mice were born and reared in a windowless room with the temperature kept at about ($21^{\circ} \pm 1^{\circ}C$) and twelve hour light/dark cycle. All litters were weaned 21 days post-partum. Only mice which were actively lactating and suckling litters of five or more infants were used in the experiments, which were conducted during different stages through the entire period of lactation.

Some experiments were carried out during pregnancy and after weaning. For mothers day "0" is the day of parturition, day "1" the day after parturition, and so on. In the discussion and tables presented in this thesis, "Lactation" is taken as a period from day 1 to day 21 inclusive; early lactation represents the period between day 1 - day 6 inclusive; mid-lactation is the period between day 7 - day 13 inclusive; and the term late lactation refers to day 14 - day 21 inclusive. Because of the logistics of obtaining the desired number of lactating females at the appropriate stage of lactation, twice the number of mice required were bred to ensure that lactating mice were obtained on a designated day. In experiments the number of progeny per female was adjusted (e.g. 5-8 infants per mother in different experiments) in order to reduce the variability of experimental design, the extra infants being removed soon after birth. In other experiments, the exchange of litters between different mothers under different treatments was undertaken. This delicate process was accomplished successfully soon after birth, by removing two mothers of a pair to clean mouse cages, transferring the litters between the original cages and replacing the mothers in the boxes from which they had been removed, also drops of maternal urine were collected, and the new infants were contaminated with that urine before replacing the mothers in the cage so the recognition of foreigness by the mothers would be diminished. During all the process disposable polythene gloves were used, thus the degree of environmental change and stress to the mothers was minimal. Successful fostering resulted in suckling of the new litters within two hours.

Fostering was carried out at different times after parturition. For most of the experiments fostering was performed within one day of

parturition, this was the most delicate process to achieve successfully, with about 85% success. Other experiments determined that fostering was undertaken at various times up to 2 weeks of age.

Trichinella spiralis.

The strain of T.spiralis used was originally derived from the London School of Hygiene and Tropical Medicine, several years previously, and was maintained by serial re-infections as stock infections in mice of various strains including NIH and Balb/c.

Recovery of infective larvae.

Muscle stage larvae (infective larvae) were recovered by digestion of muscle tissue from stock mice which had been previously infected for at least one month. Mice were killed by cervical dislocation, skinned, eviscerated, and macerated with digestion fluid in a blender ("Atomix", Measuring and Scientific Equipment Ltd., Crawley, Sussex) for one minute. The digest fluid consisted of 0.85% W/V NaCl, 0.5% v/v concentrated HCl (specific gravity: 1.18) and 0.5% w/v Pepsin Powder (1 Anson unit/g) (BDH Chemicals Ltd., Poole, England) dissolved in tap water. One litre of digestion fluid was used per mouse. The digestion was carried out for 2-5 hours at 38°C and agitated gently by bubbling air through the mixture or by providing constant stirring. At the end of this period, undigested sediment was filtered off on a coarse sieve of mesh size "250 µm" (Endecott (Filters) Ltd., London), into graduated cylinders (1 litre size) and allowed to stand at room temperature. The larvae were washed by at least three sedimentations in 0.85% NaCl solution. The volume of the suspension of larvae was adjusted to 100 ml, and the concentration of larvae measured using a

worm-counting chamber (Hawksley, England). After allowing the larvae to sediment into a small volume, excess fluid was drawn off and a solution of 0.2% agar (BDH) in 0.85% saline was added to make a final concentration of 1500 larvae per ml (i.e. 450 larvae/0.3 ml). Any other concentrations of larvae required for different experiments were done by adding more agar solution.

Since T.spiralis larvae sediment rapidly in water, the addition of a dilute agar solution prevents sedimentation and reduces the variability between doses of larvae at inoculation. All adult mice and infant mice were infected with larvae by stomach intubation using a 1 ml syringe and a blunted needle (of various lengths). The time of day at which mice were infected can affect the distribution of the resultant population of worms (Sukhdeo, 1978), therefore, infection of mice was done at the same time of day i.e. mid-afternoon.

Recovery of adult worms:

Adult worms were recovered from the intestines of mice by means of a modified Baermann technique. The mice were killed, the small intestine was removed, opened longitudinally in warm Hank's balanced salts solution pH(6.9 - 7.2), and placed into a bag of nylon gauze in a 10 x 3 cm glass tube, containing warm (38^{o}C) Hanks balanced salts solution, such that the piece of gut was just covered in fluid. This was incubated for 4 hours in a water bath at 38^{o}C, during which time the worms migrated out of the mucosa through the gauze into the tube from which they were finally collected. At the end of incubation, the bag containing the piece of gut was discarded, the worms were transferred to a 5 cm petri dish and counted under a dissecting microscope. Adult worms remained alive during this period and were therefore in good condition for subsequent direct examination and counting. The distribution of worms

in the small intestine was determined by dividing the small intestine into two portions of equal length. Generally the total number of intestinal worms was recorded. For one experiment, the separate totals of male and female worms were recorded.

In experiments requiring assessment of the intestinal worm burden earlier than day 5 post-infection, juvenile L4 or early stage adults were recovered by this method and recorded.

Hanks Balanced salts solution
preparation: . pH 6.9-7.2, Osmolarity (296-315).

phenol red indicator:

2 gm phenol red dissolved in approximately 100 ml N/20 NaOH, made up to 1 litre with deionised H_2O and N/20 NaOH to give a final pH of 7.00., put in bottles of 200 ml, and frozen.

To make 10 litre of Hanks:

Stock 1

Dissolve in 1.8 litre of deionised H_2O

168 gm NaCl
8 gm KCl
2 gm KH_2PO_4
4 gm $Na_2HPO_4 . 12\ H_2O$
+200 ml phenol Red

Stock 2

Dissolve in 2 litres of deionised H_2O

3.7 gm $CaCl_2 . 2H_2O$
2 gm $MgCl_2 . 6H_2O$

For 10 litres of Hanks:

1.1 litre of Stock 1
1.1 litre of Stock 2
25 ml 2N NaOH
7.8 litre deionised H_2O

P.B.S. (Phosphate Buffered Saline) Ph 7.2 (0.15 M)

42.5 gm NaCl (sodium chloride)
5.35 gm Na_2HPO_4 anhydrous (Disodium hydrogen orthophosphate)
1.95 gm $NaH_2PO_4 . 2H_2O$ (Sodium dihydrogen phosphate)

Make up to 5 litres with distilled water

T.spiralis antigen preparation.

A soluble extract was obtained from more than 800,000 infective larvae of T.spiralis. Encapsulated muscle stage larvae were removed from more than 30 adult N1H mice (12-14 weeks old) which had been previously infected with approximately 450 T.spiralis larvae for at least one month. All larvae were digested in 0.5% acid pepsin solution for 2-5 hours (see recovery of infective larvae). They were washed in 10 changes of phosphate buffered saline (P.B.S.) pH 7.2. The larvae which were microscopically free from adherent host tissue were homogenized in sterile PBS in a glass homogenizer (Uni-Form Homogenizer/tissue grinder "all glass", Jencons Scientific Ltd., Bedfordshire, England) immersed in an ice bath until no intact worms remained. The degree of homogenization was checked by microscopic observation. Subsequently, the suspension of homogenized larvae was allowed to solubilise by stirring at $4^{o}C$ overnight, using a Rota mixer (Taab Laboratories, Emmer Green, Reading, England). In order to obtain the soluble antigens, the extract was centrifuged at 18 000 g for one hour at 4 ^{o}C (MSE Centrifuge, Angle Head Rotor, 8 x 50 ml No.69181, max-speed 18 000 RPM, 38000g), after which the pellet was discarded. Prior to use, the protein concentration of the supernatant (14 mg/ml) was measured using a Bio-Rad Protein Assay, (Bio-Rad Laboratories, Richmond, Calif.). The Bio-Rad protein standard (lyophilized bovine gamma globulin or bovine albumin sealed under nitrogen, with concentration of approximately 1.4 mg/ml) was employed as a reference protein. The antigen was aliquoted in small volumes (30 µl) and stored at $-20^{o}C$ until used.

Serum collections from adult and infant mice.

Blood from naive (uninfected) and infected adult mice, was collected

from the tail, and the serum prepared following the method of Gray (1978). Each mouse was warmed under a lamp for approximately two minutes, in order to get dilation of the veins. A small cut was made near the end of each tail, and a 10 cm glass capillary tube (BDH, Product No.32124, BDH Chemicals Ltd., Poole, England) was filled to approximately 8 cm with blood, which was allowed to clot for about 2 hours at room temperature (21 ± 1°C). After this, the empty end was sealed in a flame, and as the air inside cooled, the serum around the clot moved to fill the contracted space, breaking off from the clot. The other end of the capillary free of serum was broken off and the clot pulled out leaving the clean serum inside the capillary. This method yields about 45-40% of the blood volume as serum which was very clean inside the capillary tubes. However this method was not practical to get sufficient amounts of blood from the tails of infant mice (e.g. 1 week and 2 weeks of age), and for this reason, the infant mice were put under ether anaesthesia and the blood was obtained by cardiac puncture, placed in Eppendorf tubes and incubated at room temperature for one hour, then the clot was ringed with a sterile applicator stick, and the tubes stored at 4°C overnight. Infant serum was collected by centrifugation at 350 g for 10 minutes at 4°C (MSE, chilspin centrifuge) to bring down free RBC's. The serum was drawn off and aliquoted in small volumes. All sera from adult and infant mice were stored at -20°C until used.

Mouse Milking Techniques:

Design of the milking device.

The apparatus used for collecting milk from lactating mice is shown in Plate M & M 1 and is based basically on the method described by Nagasawa (1979). It consisted of a stainless steel catheter (19 gauge Luer-Lok

disposable needle) with a small rubber tube acting as a teat cup fitted into the top of the needle so that it protruded approximately 4 mm above the rim, an angled glass tube connected to a wider plastic tube which contained a small vent approximately 2 mm in diameter used to control the vacuum, and a collection tube. The catheter was inserted into the collection tube through a solid rubber bung. A 10 ml graduated glass centrifuge tube was used for collecting the milk. In order to get the milk near the bottom of the tube a delicate rubber tube was attached to the end of the catheter.

Milk Collection from adult lactating mice.

Before milking, and in order to build up the milk in the mammary glands of lactating mice at different stages of lactation, the infants were separated from their mothers for 2-3 hours (infants were placed in new cages with numbers, matching the cage numbers of their original mothers).

For the milking process to be carried out, each mother was anaesthetized by intraperitoneal injection of 62.5 mg/kgm body weight of sodium pentabarbitol "Sagital" (May & Baker Ltd., Dagenham, England), followed by subcutaneous injection of 6.25 U/kg body weight of oxytocin (10 unit/ml, Sandoz Product Ltd., Feltham, Middlesex), Nagasawa,(1979). The injection of oxytocin was given several minutes prior to milking for the purpose of causing milk let down. Oxytocin was necessary to secure adequate yields of milk.

Prior to every milking, the ventral surface of each mouse was carefully washed with 70% alcohol in order to eliminate any possible contaminating materials. The mammary glands to be milked were warmed

by cotton moistened with warm water, and then the milk collection was initiated. A simple water aspirator was used to create a vacuum. The entire nipple of one mammary gland was drawn inside the teat cup and when the nipple was well seated, one finger was placed over the vent to adjust the vacuum and to create a pulsating effect which was continued until most of the milk was removed from the gland. The device was applied to each nipple until all glands had been milked. Usually the milk started to flow within one or two minutes after application of suction. Milk could be collected most effectively by proper adjustment of the position of the nipple in the teat cup.

After being milked, the mice were immediately returned to their infants. Yields of 0.1 - 0.2 ml of milk per mouse at a single milking were achieved on occasion. Variation in milk yields between mice was considerable. Milk yield in NIH mice was higher during mid and late lactation than in early lactation. On some occasions mice were successfully milked twice a day. The time necessary for milking was between 20 to 25 mins.

Immediately after milking, milk samples were centrifuged at 350 g for 10 minutes at $4^{o}C$ and the upper layer of fat was discarded, then the milk suspensions were transferred to new tubes and centrifuged at 40000 g for 2 hours at $4^{o}C$ using an MSE Prepspin50 Ultracentrifuge (Angle Titanium Rotor, 10 x 10). After centrifugation the clear supernatant layer (whey) was removed carefully using a syringe with a long needle. The whey was aliquoted in small volumes and stored in $-20^{o}C$ until used.

Milk collection from infant stomachs.

Suckling infants from litters born to mothers at different periods of lactation (i.e. early, mid and late lactation) were killed with ether

anesthesia and the stomach from each infant was removed by transection of the cardiac and pyloric sphincters. After being rinsed in 0.9% NaCl, each stomach was opened longitudinally, and the milk curds were transferred with a clean small metal spatula to a 10 ml centrifuge tube (which contained 1 ml saline) held in ice bath. The volume of milk curds was determined by displacement. The clotted milk was carefully homogenized with the 1 ml of saline with vigorous stirring and was first centrifuged at 350 g for 10 minutes at $4^{o}C$, after which the lipid supernatant layer was discarded and then the suspension was transferred to a new tube, and centrifuged to 40000 g for 2 hours at $4^{o}C$. The resultant clear supernatant (whey) was removed and concentrated 5 fold by minicon (B15 Minicon concentrator, Amicon Corp ., Lexington, Mass. U.S.A.). All samples were stored in small volumes at - $20^{o}C$ until used.

Statistical analysis:

All statistical comparisons were made using a "Tektronix 31" programmable calculator (Tektronix Inc., Beaverton, Oregon, U.S.A.). Unless otherwise stated, comparison of mean values were made using Student's t-test. Variance - ratio (F) tests were performed to ensure the use of the correct form of t-test. A probability of $P < 0.05$ was taken as indicating a significant difference between two means.

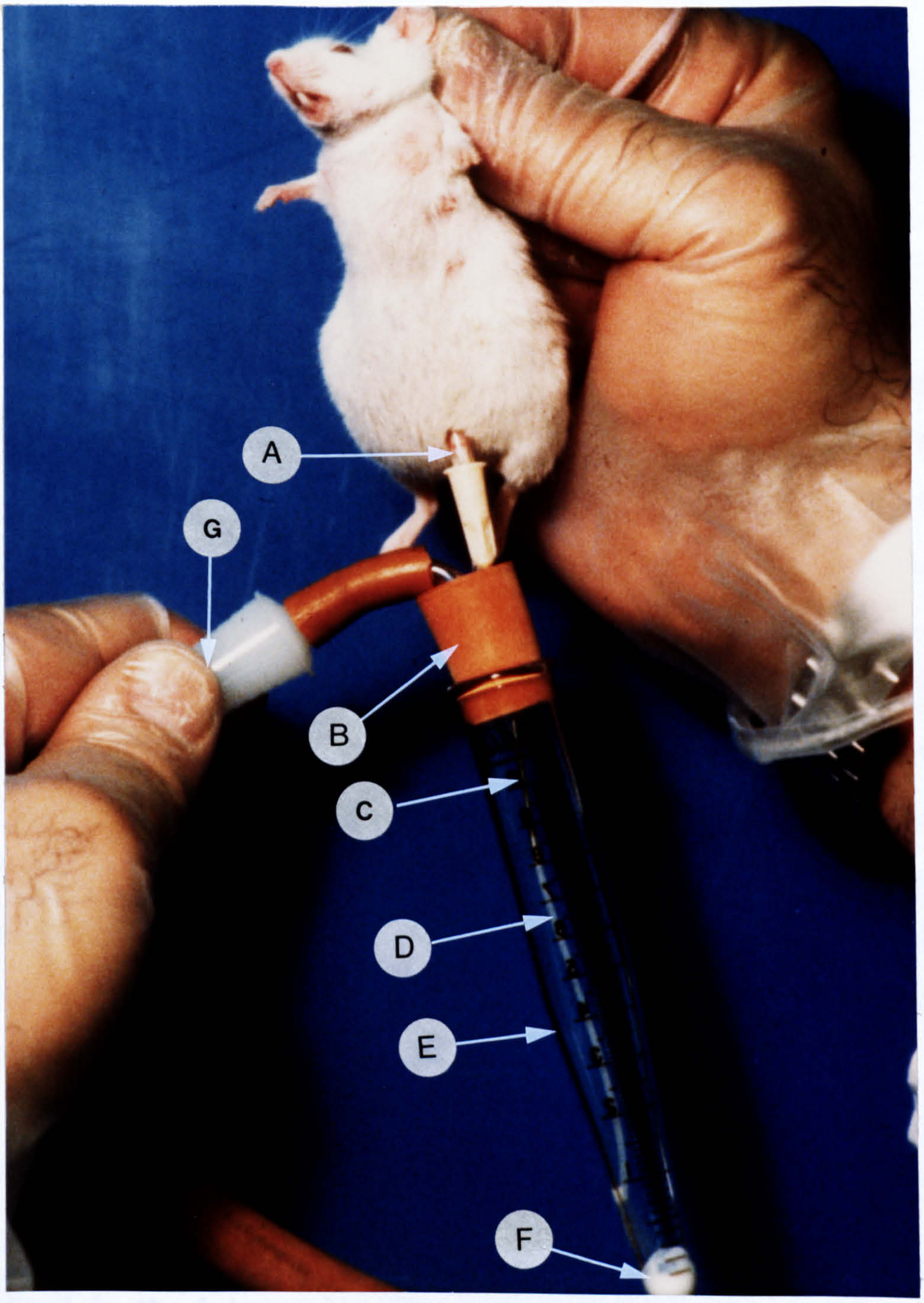


Plate M & M1:

Apparatus for collection of milk from mice.

A: teat cup

B: solid rubber bung

C: stainless steel catheter

D: collection tube

E: glass tube

F: milk

G: vent for adjusting vacuum.

SECTION I

STUDIES ON POPULATIONS OF TRICHINELLA SPIRALIS IN ADULT AND INFANT NIH MICE

1.1 Introduction.

Previous studies in laboratories in the University of Glasgow (Bruce and Wakelin, 1977; Rose, Parrott and Bruce, 1976a & b; Manson-Smith, Bruce, Rose and Parrott, 1979a; Kennedy, 1979, 1980a,b) have used the NIH strain of mouse along with the Balb/c strain and on occasion other strains of mice. In the hands of these authors, the NIH strain was shown to be highly immunocompetent against the intestinal phase of Trichinella spiralis infection, both in terms of the expulsion of adult worms from the gut in a primary infection and in terms of developing a high degree of immunity to challenge. Additionally as an inbred strain of mouse, studies of adoptive transfer and of lymphocyte traffic had been performed and had resulted in a considerable body of knowledge concerning the immunological responses to intestinal infection with Trichinella spiralis.

The NIH strain of mouse has been used throughout this study, and the initial task was to determine whether any alterations in this strain of mouse or in the strain of parasite maintained in the Zoology department had occurred in the intervening years, alterations which would be manifest phenotypically as changes in the percentage of an inoculum of parasites which established within the intestine of the mice, the areas in which the parasite localized in the intestine and the duration/expulsion of a primary infection. Equally this investigator's skills would be established.

Following this elementary work, this study documents the establishment and duration of infection of Trichinella spiralis in infant mice, the influence of age of the young mice, the conferment of protection from mothers to naive infants, the route of conferment of such protection, and quantification of these events.

1.2 Materials and Methods.

1.2.1 Mice.

Adult mice of inbred NIH strain (Anglia Laboratory, England) were used throughout. Unless otherwise stated they were 8-10 weeks old at the beginning of an experiment. All adult mice were killed by cervical dislocation. The infant mice used were bred in the departmental animal rooms. Weaning was at 21 days post-parturition. Infant mice at 1 week, 2 weeks and 3 weeks of age were killed by ether anaethesia, while 4 weeks old mice were killed by cervical dislocation.

1.2.2 T.spiralis infection.

For recovery of T.spiralis infective larvae from stock mice, see General Materials and Methods. Infection of adult and infant mice was achieved by oral intubation. In the case of infant mice a shorter blunt needle was used. The volume of inoculum for all mice was 0.3 ml except for 1 week old mice which were inoculated with 0.1 ml. Mice were always infected at the same time of day i.e. mid-afternoon, and killed at various times after infection. Adult worms were recovered from infected mice using a simplified Baermann apparatus (see General Materials and Methods). The distribution of worms was ascertained by recovering worms from two segments of the gut (anterior half and posterior half).

1.2.3 Collection of immune milk.

Immune milk was collected from lactating mothers which had been given a primary and secondary infection of T.spiralis larvae. Milk was taken during the mid-lactation period (see General Materials and Methods), and was fed to suckling infants by oral intubation.

1.2.4 T.spiralis antigen.

A soluble extract was obtained from infective larvae of T.spiralis

(for details, see General Materials and Methods). The protein concentration (14 mg/ml) was measured using the Bio-Rad Method. The antigen was stored at -20°C until used.

1.3 Experimental Design and Results.

1.3.1 Establishment, Localization and expulsion of Trichinella spiralis in a primary infection in adult NIH mice.

Adult female NIH mice (14-16 weeks old) were divided into three groups of six mice.- group "A" was infected with approximately 50 infective T.spiralis larvae, group "B" was infected with approximately 150 larvae, and group "C" was infected with approximately 450 larvae. Following infection (within an hour of administration) samples of the inoculum were recounted to determine accurately the numbers of viable larvae administered. Four days after infection, all mice were killed to ascertain the number and distribution of worms in the gut.

In these primary infections (Table 1.1), about 50% of the infective dose (52.4% in group "A", 55.5% in group "B", 52.8% in group "C") was found to have become established in the small intestine. It showed also that T. spiralis localized predominantly in the anterior part of the small intestine. In group "A" 74.4% of the worms which established were localized in the anterior part, with only 25.6% localized in the posterior part, while in group "B" 73.6% localized in the anterior part, with only 26.4% in the posterior part, and in group "C" the localization was 82.4% anteriorly and 17.6% posteriorly. The different doses of infection (50 larvae in group "A", 150 larvae in group "B" and 450 larvae in group "C") did not result in any alteration to the % of worms which established or of the localization of the worms in the small intestine. In those inocula which were of the same batch of infective larvae at different dilutions, the number of larvae administered in 0.3 ml was

slightly higher than originally estimated. The viability of the infective larvae which were given to all groups was high. In group "A" only 1.3% of the actual dose was non viable, in group "B" 0.99% and in group "C" 0.78%. This experiment has been repeated six times with similar results (see table APX 1.1 - Appendix).

A number of experiments was carried out in which mice were infected and killed at intervals in order to determine the time at which expulsion occurred. In no experiment was a recovery of more than 60% of the inoculum achieved. The number of worms fell sharply after day 8 and elimination was complete by day 12 after infection. As in the previous experiment, the results (Table 1.2, and Table APX 1.2, and Figure 1.1 which is a graphical presentation of the data of group "C" in Table 1.2) showed that Trichinella spiralis localized initially in the anterior part of the small intestine, but even before worm loss ensues there is a movement of worms posteriorly by day 6 post-infection, although the numbers do not show a significant change in the two groups given the larger infections. By day 8 post-infection it is clear that worms are being lost from the anterior small intestine. The experimental protocol of group "A" where the mice were given a dose of approximately 50 infective larvae, was repeated five times (see Table APX 1.2), and the data show that in all these experiments, the expulsion was well underway by day 8 post-infection and was completed by day 12. In the groups of mice given the larger doses of 150 and 450 larvae expulsion was completed by day 12 or by day 14 post-infection. These experiments confirmed that the strain of T.spiralis and the NIH strain of mouse did not show significant alteration from those used by other workers in particular Manson-Smith et al (1979a).

1.3.2 Establishment of Trichinella spiralis in infant mice.

Young mice of 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks of age born to naive mothers (no previous infection of T. spiralis) were infected with approximately 50 or 100 infective larvae. The infections were initiated in different groups of mice at different periods with different batches of infective larvae, and are shown as experiments 1-9 in Table 1.3. For each experiment a group of 5 adult mice were also infected and these serve as controls both for the infectivity of each batch of infective larvae and as references for the establishment and localization of the worms in the intestine of infant mice in comparison to adult mice. Five mice from each treatment were killed on day 4 post-infection, and the number of worms established in the anterior and posterior parts of the small intestine was recorded and also expressed as a %.

The results show that young mice do provide an environment in which the parasite establishes, although the level of infection was always lower than that seen in adult mice and usually significantly so; it was also noted that in both 1 week and 2 weeks old mice, the percentage of worm establishment in the posterior part of the small intestine was always higher than in the anterior part. In 1 week old mice infected with 50 or 100 larvae, all the adult worms recovered were located in the posterior part of the small intestine. In 2 weeks old mice the percentage of worms established in the posterior part of the small intestine was much greater than in the anterior part. (The percentage in the posterior part ranged between 71% - 80% in all four experiments). However in 3 weeks and 4 weeks and 5 weeks old mice the percentage of worms established in the anterior part increased considerably. In 3 weeks old, it ranged between 40% - 89% in the five experiments; in 4 weeks old mice it ranged between 63% - 82.8% in three experiments; and in 5 weeks old mice 90.9% of the

worms established in the anterior half of the small intestine. It is noticeable that there is marked variation in the intestinal distribution of T. spiralis during the period of weaning (compare experiments 5 and 6 for 3 weeks old mice). Later experiments examine this period carefully.

1.3.3 The course of primary infection of Trichinella spiralis in infant mice.

Infant mice of different ages (1, 2, 3 & 4 weeks of age) born to naive mothers were infected with different doses of T. spiralis larvae (50, 100, 200) and five or six mice were killed from each group on days 4, 6, 8, 10, 12 & 14 post-infection in order to determine the time at which expulsion occurred. The results (Tables 1.4, 1.5, 1.6 and Figures 1.2 and 1.3; see also Table APX 1.3) showed that whereas in adult mice expulsion of adult worms was in evidence by day 8 post-infection and was virtually completed by day 12, in infants expulsion was underway by day 8 post-infection in some groups or by day 10 post-infection in most groups. In addition to the differences in onset of expulsion in young mice, it is also clear that elimination of the primary intestinal infection is somewhat delayed with a small population of adult worms remaining until day 14 post-infection. These results also confirmed differences in intestinal distribution of T. spiralis in adult and infant mice. While most worms were located in the anterior half of the small intestine in adult mice, in 1 week old mice all worms established in the posterior small intestine. In two weeks old mice the majority of worms established in the posterior portion of the small intestine regardless of the size of inoculum. In mice three weeks of age at infection, there is variation in the anterior/posterior establishment with a different result in Table 1.4 from that in Tables 1.5 and 1.6. The relatively large standard deviations of the means for these 3 weeks old mice is a

further indication of instability in the host/parasite relationship at this age.

In order to determine the influence of the sex of the infants on the establishment and the expulsion of the worms, the course of infection in male and female infants was recorded from groups of 2 weeks and 3 weeks old mice infected with 100 or 200 T. spiralis larvae (Tables 1.7 and 1.8). There was no significant difference in establishment or expulsion in these groups of mice. The data in Table 1.8 concerning the naive and infected status of the mothers of the infants will be referred to later in this section.

These experiments show that young mice harbour fewer worms than adult mice. They are however, immunocompetent in terms of expelling a burden of adult Trichinella spiralis from the gut although the onset of expulsion is slightly delayed and elimination of the primary worm burden is also slightly delayed. The sex of the infant mice has no influence on the number of worms establishing, their location in the gut or on the immunocompetence of the mice.

1.3.4 The effect of immunity in mothers on the establishment and expulsion of intestinal worms in infants.

A number of experiments were carried out to determine the effect of immunity in mothers on the establishment and expulsion of the worms from their infants. Two groups of adult female NIH mice, 8-10 weeks of age at initial infection were used. One group was infected with 350 viable T. spiralis larvae, and three weeks later another dose of 200 viable larvae was given. A week after the second infection, all mice from this group (immune mothers) and the age matched naive group were mated. The infants were allowed to suckle their mothers, and

when they were 2 weeks old, they were infected with 200 viable larvae. On days 2, 4, 6, 8 and 10 post-infection, subgroups of 5-9 young mice were killed and the intestinal worm burdens were determined and recorded in Tables 1.8, 1.9 and Figure 1.4, (see also Table APX 1.4). The results in Table 1.9 and in Figure 1.4 show that in young mice suckling their immune mothers there was a significant reduction in the number of T. spiralis establishing in the small intestine in comparison to their naive counterparts. On day 2 the percentage of establishment in infants born to immune mothers was 12.31% compared with 20.11% in those infants born to naive mothers which was highly significant ($P < 0.001$). There was no real reduction in the number of the worms recovered between day 2 and day 4 post-infection in both groups indicating that the protective mechanisms operated on the process of establishment. Expulsion of the worms in the infants suckling their naive mothers was in evidence during the period 6 and 8 days post-infection ($P < 0.001$). In infants suckling their immune mothers, there was a significant reduction also between days 4 and 6 post-infection, indicating that there is a slightly earlier onset of expulsion in young mice suckling their immune mothers. The conclusion from this experiment is that in infants born of and suckling immune mothers there is a degree of protection conferred from the immune mothers. This protection is manifest primarily as a reduction in the numbers of infective larvae establishing in the intestine of young mice. The data recorded in Table 1.8 for 2 weeks and 3 weeks old mice suckling their naive or immune mothers confirms this finding.

1.3.5 Duration of protective immunity gained from mothers, and the susceptibility to infection at different ages.

The design for this experiment is shown in protocol 1.1. The results which are an accumulation of data from seven different experiments are

recorded in Table 1.10 and Figure 1.5, as the means of worm burdens recovered on day 6 post-infection. (For details of each experiment, see also Tables APX 1. 5, APX 1.6, APX 1.7, APX 1.8, APX 1.9, APX 1.10, APX 1.11 and APX 1.12). Firstly these data confirm that susceptibility to infection, determined as numbers of worms establishing in naive infants suckling naive mothers increases with age through weeks 1-4 at the time of infection. Protection against establishment of T. spiralis is evident in mice born to and suckling immune mothers up to four weeks of age at the time of infection, i.e. at least one week after weaning. The difference in the worm burdens between the infants (1 week, 10 days, 2, 3 and 4 weeks of age) born to immune mothers and those born to naive mothers was statistically significant. Expressed as a percentage the reduction in the number of worms established in infants born to immune mothers compared with infants born to naive mothers was very marked, 67% in 1 week old, 72.4% in 10 days old, 62.4% in 2 weeks old, 62.4% in 3 weeks old, and 42.4% in 4 weeks old mice, which means that the protective factors remain effective for at least one week after weaning. By the age of 6 weeks at the time of infection, the number of worms established in mice born to immune mothers in comparison with those born to naive mothers was statistically not significantly different.

1.3.6 The significance of suckling in the transfer of protection to young mice and the influence of infection and immunization of the mothers.

(a) Infection with T. spiralis larvae:

To determine whether in NIH mice the suckling of immune mothers rather than being the progeny of immune mothers is the event which confers protection to infant mice, a number of fostering experiments were carried out. The experimental design is shown in Protocol 1.2.

Two groups of 8 weeks old female NIH mice were used. Group A was given two infections of 200 infective larvae of T. spiralis at intervals of six weeks and group B was left uninfected. Seven weeks after the second infection, the mice from both groups were mated and as soon after parturition as possible, all litters were adjusted to 8 infants per mother and usually this was performed on day 1 postpartum. The litters from one set of immune mothers were transferred to naive mothers, and the litters from naive mothers were fostered by immune mothers. Litters of other sets were left to suckle their naive or infected mothers. All infants were infected at 2 weeks of age with 200 infective larvae of T. spiralis. From each group 18 infants (3 from each of 6 litters) were killed on days 6 and 8 post-infection, and 12 infants (2 from each of 6 litters) were killed on day 10 post-infection and the burdens of adult worms in the intestines of the infants determined. The results of this experiment are shown in Table 1.11 and Table APX 1.13. Examining the worm populations on day 6 post-infection, the difference between groups 1 and 4 was highly significant. There was a marked reduction in worm establishment in litters suckling immune mothers (groups 1 and 3) compared with those suckling naive mothers (groups 2 and 4), also the litters which were born to naive mothers and immediately fostered by immune mothers (group 3) had significantly fewer worms than did the mice in the control (group 4) - $P < 0.001$. The fact that the litters in group 2 which were born to immune mothers but fostered to naive mothers had an established burden similar to that of group 4 which were born of and suckling naive mothers, is further evidence that there is no protective immunity transferred across the placenta and also indicates that the small amount of colostrum which the infants received from their mothers did not have a long lasting protective effect. The results also

show that the expulsion of the worms was not enhanced in those litters suckling immune mothers (groups 1 and 3). In group 1, statistical analysis did not indicate a significant difference between day 6 and day 8 post-infection, but there was a statistically significant difference ($P < 0.01$) between day 8 and day 10 post-infection. In group 3, there was only significant difference between day 6 and 10 post-infection ($P < 0.001$). The difficulty of revealing a statistically significant difference with these small populations over a short period of time was not one which it was possible to resolve as it would have involved larger numbers of litters than the animal accommodation could provide. In contrast, analysis of group 2 and group 4, does reveal significant differences in the larger populations of worms between days 6, 8 and 10 post-infection (in group 2 $P<0.001$ between days 6 and 8, $P < 0.05$ between days 8 and 10 post-infection; in group 4, $P < 0.001$ between both days 6 and 8, and 8 and 10 post-infection). However, if the worm burdens are expressed as percentages of the burden on day 6 post-infection, it is evident that there is no clear cut pattern which would have indicated a significantly different rate of expulsion between infants suckling naive or immune mothers over the four day period.

	Day 6 P.I.	Day 8 P.I.	Day 10 P.I.
Group 1	100	81	30
Group 3	100	79	48
Group 2	100	52	35
Group 4	100	59	29

The possibility of a slightly earlier onset to expulsion i.e. day 4 to 6 P.I. as shown in Table 1.9 was not examined in this experiment.

(b) Immunization with T. spiralis antigen and adjuvant.
(Experiment No.1):

This experiment was designed as shown in Protocol 1.3 to determine whether mice immunized with Trichinella spiralis larval antigen in complete Freund's adjuvant would confer protection to their infants in a similar way as did the mice which had been exposed to a whole infection with T. spiralis. Adult female NIH mice 6-8 weeks old were divided into two groups, the mice in one group received an injection of 200 µg crude larval antigen in 200 µℓ mixed 1:1 with complete Freund's adjuvant (for antigen preparation, see General Materials and Methods). This dose was given to each mouse as 100 µg intramuscularly and 100 µg subcutaneously. Four weeks after the first injection, these mice received a second injection of 100 µg of antigen without adjuvant by the same routes. All litters were infected at 2 weeks of age with 200 T. spiralis infective larvae. For worm recovery from the small intestine, twelve infants (three from each of four litters) were killed on day 6 and 8 post-infection and eight infants (two from each of four litters) were killed on day 10 post-infection.

The results of this experiment are recorded in Table 1.12, (see also Table APX 1.14), and are similar to those of the previous experiment (see Table 1.11). The data showed a marked reduction in the number of worms established 6 days after infection in litters which were suckling their immune mothers or fostered by immune mothers in comparison to litters which suckled their naive natural or fostered mothers ($P < 0.001$ between groups 1 and 2, $P < 0.001$ between groups 3 and 4). These data confirm that protective immunity is passed to the infant through the milk. In addition this experiment shows that the transferrable protective immunity in the adult mice can be elicited by a peripherally

initiated experience of T. spiralis larval antigens in addition to the gut and somatic elicited response of a whole infection.

(Experiment No.2):

This experiment was designed to determine whether the protection which passed to the infant mice from their mothers who were immunized with T. spiralis larval antigen + complete Freund's adjuvant as shown in the previous experiment, would last until the infant mice reached the adult stage i.e. eight weeks of age. Five groups of adult female NIH mice (6-8 weeks old) were used, mice in groups 1 and 2 (see Table 1.13) were immunized with T. spiralis larval antigen + complete Freund's adjuvant as mentioned in the previous experiment, while mice in group 3 were injected with adjuvant + P.B.S only, and both group 4 and group 5 were left without treatment.

At parturition litters were adjusted to eight infants per mother. Litters from groups1, 3 and 4 were infected at two weeks of age with 200 T. spiralis infective larvae, while the litters in groups 2 and 5 were left until they reached eight weeks of age when they were infected with 200 T. spiralis larvae. Of the groups infected at 2 weeks old three mice from each group (i.e. 15 mice) were killed on days 6 and 8 post-infection, and 2 mice from each group (i.e. 10 mice) were killed on day 10 post-infection, and in 8 weeks old mice, three mice from each group (i.e. 12 mice) were killed on days 6 and 8 post-infection and 2 mice from each group (i.e. 8 mice) were killed on day 10 post-infection. The numbers recorded are the means of the worms recovered from the whole of the small intestine.

The results of this experiment are shown in Table 1.13 and reveal a marked reduction ($P < 0.001$) in the number of worms established 6 days

after infection in litters which suckled their immune mothers for 2 weeks only (group 1) in comparison to those which suckled their naive mothers (group 4), which confirms the finding in the previous experiment. There was no real difference between the number of worms established on day 6 after infection between group 3 and group 4 which showed that the treatment of the mothers with adjuvant alone had no effect in terms of establishment of the worms in their litters. However in the mice which were infected at 8 weeks of age, there was no difference between the number of worms established on day 6 post-infection in group 2 which were born to immune mothers and group 5 which were born to naive mothers indicating that as with whole infections the protection in these litters was of short term duration.

1.3.7 T. spiralis infection in young mice.

Transfer of protection to suckling infants and the duration of immunizing infection in mothers.

To determine the influence of the duration of infection in the mothers on their ability to transfer protective immunity to their offspring via the milk, a number of experiments was carried out as shown in Protocol 1.4. Pregnant and lactating mothers were infected a few days before or after parturition and their infants were infected at 2 weeks of age. The critical timing is that of days of maternal infection before the infants were infected. In group A mothers were infected seven days pre-parturition, i.e. 21 days before their infants were exposed to infection at 2 weeks of age. In groups B, C, D and E, the mothers had been infected for 11, 9, 7 and 4 days respectively prior to infection of their infants. The analysis of the results recorded in Tables 1.14, 1.15 and 1.16, shows highly significant protection in young mice in groups A and B i.e. 21 days of maternal infection in group A, and 11 days of

maternal infection in group B ($P < 0.001$) and considerable protection afforded to young mice from mothers infected for only 9 days - group C ($P < 0.01$). However mothers infected for 4 days (E) and 7 days (D) show populations of worms approximately similar to the numbers established in groups F_1 and F_3. The lack of any additional protection in group A infants in comparison to group B infants is further evidence of the absence of any significant transfer of protection via the placenta. The conclusion from these results is that an infection of between 9-11 days in the lactating mothers is the minimum requirement to elicit a protective response which is transferable via the milk to the infants.

The results shown in Table 1.17 indicate that the protection in groups A and B which occurred at 2 weeks of age was not maintained as immunity into adulthood. When litters from these groups were infected at 8-9 weeks of age, the number of worms established on day 6 post-infection shows no statistically significant difference from that of the controls.

1.3.8 The duration of intake of immune milk and protection in infant mice.

A number of experiments were carried out to determine the effect of duration of intake of immune milk on the establishment of *T. spiralis* in infant mice - Protocol 1.5. Adult female NIH mice (8-10 weeks old) were given a primary infection of 350 larvae of *T. spiralis*, followed after 3 weeks with another infection of 200 larvae. A week later these mice together with the control group of adult naive females were mated. At parturition, all litters were adjusted to 6 infants per litter, and were left to suckle their mothers for two weeks, some litters were then exchanged between immune mothers and naive mothers, and others were left with their original mothers to serve as controls. After fostering, with suckling confirmed by observation, all infants were infected with

200 T. spiralis larvae. Group 1 was infected after 6 hours of fostering, group 2 at 12 hours after fostering, group 3 at one day after fostering, group 4 at 3 days after fostering, and group 5 at 5 days after fostering. All mice were killed 6 days after infection for worm recovery from the small intestine.

The results of these experiments are recorded in Tables 1.18 and 1.19. (see also Tables APX 1.15, APX 1.16, APX 1.17, APX 1.18, APX 1.19 and APX 1.20). The mean worm burden of each litter (i.e. 6 infants) and of both litters are shown within each group. In treatment A, infants born to naive mothers and fostered at 2 weeks of age to immune mothers for 6 hours, 12 hours, 1 day, 3 days and 5 days before infection, there was a clear reduction ($P < 0.001$) in the number of worms established in comparison to the numbers of worms established in treatment D which consisted of infants suckling their own naive mothers between all groups (Table 1.18), indicating that a period of suckling of as short a time as 6 hours conferred protection to the infants. The results also showed that there was no statistically significant difference between treatment "A" and treatment "C" in which the infants suckled their own immune mothers, regardless of the time of fostering, indicating that suckling immune milk for as short a period as 12 hours conferred an equivalent degree of protection to infants as two weeks of suckling immune milk. In this experiment there was an insufficient number of litters to permit the comparison at 6 hours of suckling. In the treatment involving transfer from immune to naive mothers (treatment B), the protection conferred to the infants persisted for a considerable time. However in comparing treatment "B" with treatment "C" (infants suckling their own immune mothers) there would appear to be some waning of the degree of protection. The experiment with 6 hours of fostering has been repeated to confirm and enhance the results of this experiment, and is recorded in Table 1.19. This experiment confirmed that suckling immune

milk for as short a period as 6-8 hours conferred a considerable degree of protection against the establishment of a burden of intestinal T. spiralis.

1.3.9 Protection in infant mice given a defined quantity of immune milk.

8-10 week old adult female NIH mice were divided into two populations. One was given a primary infection of 350 T. spiralis larvae, followed after three weeks by another infection of 200 larvae (immune population), the other population was left uninfected. Both populations were mated, and after parturition the litters were adjusted to five infants per mother. All litters continued to suckle their mothers until two weeks of age. At that time (mid-lactation) ten immune mothers were milked (see General Materials and Methods). Due to the small quantity of milk which can be extracted from each mother over a period of 20-25 minutes of milking, the milk was pooled. Three groups of infants at 2 weeks of age born of and suckling their naive mothers were used as experimental groups (groups A, B and C, Table 1.20). In group "A" five infants removed temporarily from their naive mothers were fed by oral intubation with two doses each of 0.15 ml of immune milk at a 24 hour interval, while infants in group "B" were given two doses each of 0.15 ml of immune milk mixed 1:1 with P.B.S, at a 24 hour interval. The third group of infants group "C" were given similar doses of P.B.S only. Eight hours after receiving the second dose, all three groups were infected with 200 larvae of T. spiralis, and the worm populations were counted on day 6 post-infection.

The results show that there was a reduction in the mean population of the worms established in group "A", which were given neat immune milk,

compared with the number of worms established in group "C" which were given P.B.S only ($P < 0.02$). Infants in group "B" which were given diluted immune milk showed no significant reduction in worm establishment compared to group "C".

The milk from the immune mothers was assayed for the presence of antibodies against T. spiralis and for quantitative determination of different immuno-globulins, and also the cellular contents; these will be dealt with in the later sections.

1.3.10 In vivo fecundity of T. spiralis in primary infection in infant mice and adult mice.

To determine whether the clearly documented protective effect of suckling immune mothers had an effect on the development and reproductive performance of the reduced burden of established worms, the following experiment was designed to investigate the reproductive capacity of adult T. spiralis in young mice which are partly protected by suckling immune mothers. The fecundity of T. spiralis worms, which can be defined as the number of larvae produced per female worm per unit time in in vitro work, or more significantly as the number of larvae produced per female worm per infection, was determined in primary infections in infant mice (2 weeks old) which were born to naive or immune mothers, and in adult mice. As shown in protocol 1.6, three groups of mice were used. Group "I" consisted of fourteen infants at 2 weeks of age born to immune mothers, group"II"- fourteen infants at 2 weeks of age born to naive mothers, and group "III" thirteen adult female mice (8-10 weeks old). All three groups were infected with 200 T. spiralis larvae from the same batch of larvae on the same day. Six days after the infection, the numbers of adult female and male worms was determined in each of six mice from each of

groups I and II and five of group III (Table 1.21). To determine the number of muscle larvae produced in each treatment, eight mice from group I, II and III were killed on day 35 post-infection, the carcases were digested in pepsin/HCl and the numbers of infective larvae determined (Table 1.22).

Table 1.21 shows a reduced number of female worms established in group I (2 weeks old mice born to immune mothers) compared to group II (2 weeks old mice born to naive mothers), the statistical difference is highly significant ($P < 0.001$). In both groups I and II the numbers of female worms was much smaller than the number of female worms in adult mice (group III) $P < 0.001$. The ratio of female - male worms is highly variable between mice.

Table 1.22, shows the number of larvae established in the muscles of mice from each treatment after 35 days of infection. The high standard deviation about the mean number of larvae established in each group is a feature that has been noted by many authors, and demands a large group to minimize the effect of a large standard deviation. Meaningful statistical analysis of the larval data demanded that the raw data of larval burdens be converted by division by the mean number of adult females for the particular treatment. The converted data is shown in Table 1.22. One way analysis of variance on the converted data reveals a statistically significant difference between groups 1 and 3 indicating that the young mice suckling immune milk and harbouring a smaller burden of adult worms provide a more favourable host for T. spiralis at least in terms of the fecundity of the intestinal female worms. The conclusion can be made that there is no significant impairment of fecundity of T. spiralis in mice suckling immune milk. However further studies of the effects on population fecundities would be necessary before further conclusions could be made.

1.3.11 The induction of tolerance by infection early in life.

To clarify the degree of induction of immunity or the possibility of the induction of tolerance of a primary infection early in life, an experiment in which mice were given a primary infection at 1 week, 2 weeks, or 3 weeks of age followed by a challenge infection at 10 weeks of age was undertaken. The results recorded in Table 1.23 show convincingly that a primary infection at these ages induces strong immunity to a second infection. There is thus no evidence of the induction of tolerance by infections of the size used throughout this study.

Mice which received the primary infection protocol as in the above experiment and which produced litters at approximately 12-14 weeks of age, conferred an equivalent degree of protection to their offspring as did mice receiving a primary and/or secondary infection as adults.

1.4 Discussion

Earlier work in Glasgow, Wakelin and Lloyd (1976a), Bruce and Wakelin (1977), Kennedy (1980a) and in particular Manson-Smith, Bruce, Rose and Parrott (1979a) had determined, in studies of adult mice of the NIH strain, that in a primary infection of Trichinella spiralis, most of the infective larvae and subsequently the adult worms establish themselves in the anterior half of the small intestine. The adult worms are expelled from the small intestine during the period from the eighth to the twelfth day of infection. In a careful study these latter authors noted that the earliest sign of expulsion of T. spiralis in these mice was a movement from the anterior to the posterior region of the small intestine which was just underway by the sixth day of infection.

Manson-Smith et al (1979a) also noted that the pattern of location and expulsion of T. spiralis in NIH mice, was very different from that in the Balb/c strain of mice, in which the majority of worms localized initially in the posterior half of the intestine and the period of expulsion was from the fourteenth to the eighteenth day of a primary infection.

In addition, Kennedy, Wakelin and Wilson (1979) noted that T. spiralis which were transplanted into various regions of the small intestine of naive mice had little or no capacity to move anteriorly but were not inconvenienced in terms of growth or fecundity in being located in a posterior position in NIH mice.

The initial task in this study was to determine that the strain of T. spiralis used in the department of Zoology and the strain of NIH mice purchased from Anglia Laboratories had not altered in these basic aspects since the completion of the earlier studies and that in the hands of the present investigator similar results would be forthcoming.

The data recorded in Tables 1.1 and 1.2 and in Figure 1.1 confirm that in adult NIH mice an establishment in excess of 50% of the inoculum may be achieved, and that the majority of the worms localize in the anterior half of the small intestine. The percentage of the inoculum which establishes is not conditional on the numbers of infective larvae present in a standard volume of the inoculum, given a range of 50-450 larvae per inoculum. This range within the infective dose does not influence the anterior/posterior localization of the worms.

The data in Table 1.2 (and Figure 1.1) confirm the observation of the earlier workers that expulsion of a primary infection of T. spiralis in NIH mice occurs between day 8 and 12 post-infection. In the observation recorded here there is a slightly earlier completion of expulsion in mice given the relatively small infection of 53 larvae together with an earlier onset of expulsion, i.e. between 6 and 8 days post-infection. In the groups infected with 154 and 466 larvae, although the expulsion between day 8 and 12 is rapid, a small population of worms remain to be expelled between day 12 and 14 post-infection. These results merely confirm the earlier reported observations of primary infections of T. spiralis in NIH mice.

The distribution/localization of T. spiralis in the small intestine of various species of hosts or in strains of the same species of host is a subject which has given rise to some confusion and even controversy in the past. Thus in adult mice an anterior localization was noted in inbred Swiss mice by Larsh and Hendricks (1949), whereas a posterior localization in outbred ICI mice and in inbred agouti CBA mice was recorded by Denham (1968). However in young Swiss mice (Larsh and Hendricks, 1949) a posterior distribution was observed whereas in young albino Sharpe and Dohme mice (Campbell, 1967) an anterior distribution was noted. In rats Larsh and Hendricks (1949) recorded that

there was a marked shift towards a posterior localization in young rats and Dick and Silver (1980) confirmed that in suckling Sprague-Dawley rats, T. spiralis was located more posteriorly than in adult rats of the same strain. Several obvious features have been suggested by these authors to explain variation in site of localization e.g. variation in infection procedure, variation in strain of the parasite, variation in intestinal transit time.

Sukhdeo and Croll (1981) tested a number of possible causes and were able to show that in Swiss mice intestinal motility and the volume of the inoculum were critical factors in determining the site of establishment within the small intestine.

Studies of T. spiralis in young animals have not been extensive. Larsh and Hendricks (1949) noted a posterior localization in young Swiss mice and in young rats. Bass and Olson (1965) noted a much smaller establishment and posterior localization in young mice, Campbell (1967) observed an anterior localization in young Sharp and Dohme mice, and Duckett, Denham and Nelson (1972) showed varying establishment in mice of different ages.

In the present study it was a prerequisite of further experimentation that the establishment, localization and expulsion in young NIH mice be documented thoroughly. The data recorded in Tables 1.3 to 1.8 and 1.10 and in Figures 1.2 and 1.3 reveal the following features. Infection of very young mice, one week and two weeks of age, results in the establishment of much smaller burdens of worms than in adult mice, this was always the case in 1 week old mice, if less consistently so in 2 week old mice. This agrees with the quantitative study by Bass and Olson (1965). In these 1 week old and two week old mice, the worms establish entirely on very largely in the posterior half of the small intestine. In three

week old mice the percentage of worms establishing is larger and with considerable variation in site of location although predominantly anterior. In four and five week old mice the number of worms establishing increases and approaches the numbers establishing in adult mice together with a confirmed anterior location. Given a constant volume of inoculum (0.3 ml) for all the mice apart from the 1 week old groups which received only 0.1 ml, these studies reveal an age related susceptibility to infection and an age related basis to location of the worms in the intestine. Clearly the factors of gut architecture, diet and physiological features such as intestinal motility would be components of these age related phenomena. Investigation of these features was not undertaken.

Despite the marked differences in susceptibility and location of T. spiralis in young mice in comparison to adult mice, the data reveals that young NIH mice show very little in the way of immunological incompetence in terms of expelling a primary infection of different sizes. The onset of expulsion was slightly delayed in 2 and 3 week old mice and there was also a tendency for a slight delay in the completion of expulsion. Evaluation of the immunological competence of 1 week old mice in expelling a burden of worms is plagued by the problem of dealing with very small populations with very high standard deviations and firm conclusions can not be made. It is evident also that the sex of the young mice has no influence on establishment, location and expulsion of a primary infection.

Comparative detailed studies on T. spiralis are absent from the literature. However these studies do not contradict earlier studies and do complement the reports of Larsh and Hendricks (1949) and more particularly those of Duckett et al (1972).

However all of these studies result in conclusions which are in marked contrast to the conclusions pertaining to the immunological

competence of young rats subjected to infection with Nippostrongylus brasiliensis. From the work of Ogilvie and Jones (1971), Jarrett and Urquhart (1971) and Murray (1972) it is well established that whereas adult rats expel a moderate to large worm burden, young rats (less than 6 weeks of age) fail to expel a burden of less than 200 worms, and reject a burden of 200 - 250 worms more slowly than adult rats. Jarrett and Urquhart (1971) suggested that in addition to a possible threshold requirement of worm antigen the indicated deficiency in young rats was probably a quantitative deficiency in production of antibody. Clearly further comparison between the two host parasite systems in young animals is invalid at this stage.

Young mice are known to be highly competent immunologically in several respects. In terms of production of antibody Auerbach (1972) records that several strains of mice can produce antibodies against sheep red blood cells injected in the first week of life, and Crabbé, Nash, Bazin, Eyssen and Heremans (1970) showed that immunoglobulin containing/ binding cells were present in the gut associated lymphoid cells from an early age, and the number of these cells increased until 4-6 weeks of age in C_3H strain of mice. In another vein, Gazdar, Beitzel and Talal (1971) showed in eight strains of mice that cell mediated responses causing the regression of tumours resulting from a murine sacroma virus, matures during the period of the twelfth to twenty first day after birth. However some components of the intestinal immunological spectrum are missing. As Ferguson and Parrott (1972) demonstrated in CBA and in Balb/c strain mice, germinal centres in the Peyer's patches appear only after 5 weeks of age, and villus intraepithelial lymphocytes and lamina propria lymphocytes and plasma cells first appear only in the third week after birth. Whereas Chanana, Schaedeli, Hess and Cottier (1973) showed that thymus-

dependent lymphocytes predominated in gut-associated lymphoid tissue in the first week of life, Ferguson and Parrott (1972) indicated that the majority of lymphocytes in the gut tissue of mature mice are thymus-independent. However the probable significance of thymus-dependent lymphocytes which accumulate rapidly in the areas of the small intestine of adult mice harbouring T. spiralis was demonstrated by Rose, Parrott and Bruce (1976a) and it is well established that an absence or depletion of T-cells renders mice incapable of mounting an effective immunological response (Walls, Carter, Leuchars and Davies, 1973). Such studies on young mice subjected to parasitic infections have not been undertaken.

The data recorded in Tables 1.9, 1.13 and in Figure 1.4 confirms the observations of Duckett et al (1972) and Perry (1974) that protective immunity to T. spiralis in suckling mice is gained from infected and immunized mothers via the milk. In this study it is shown that protection is manifest as a 50% - 75% reduction in the establishment of adult T. spiralis in the small intestine of suckling mice with no other significant feature such as consistently earlier expulsion involved. Young mice gained protection from their mothers throughout the suckling period and a degree of protection was maintained for at least one week after the cessation of suckling (Table 1.10).

It is justified to conclude from the data that protection results in reduced establishment of pre-adult T. spiralis, with the earliest recorded observations of establishment being at 2 days post-infection (Table 1.19). It has long been a source of minor irritation to investigators of intestinal T. spiralis infection that recovery of the developing larval stages from the gut tissue earlier than 48 hours post infection gives inconsistent yields of worms unless extremely laborious techniques such as microscopical examination of the lumenal contents and mucosal scrapings of the entire

small intestine are used. Another drawback to such a technique is that it is not possible to determine whether the worms observed were alive at the time of autopsy. Accordingly day 2 post-infection is the earliest time at which quantitatively consistent recoveries of established worm can be determined with methods which are appropriate to handling moderately large numbers of animals.

The observation that partial protection against establishment of adult worms in young mice persists for at least one week after suckling ceases (Table 1.10),demands the conclusion that (a) the protective components derived from the milk persist on the lumenal surface of the intestinal epithelium in sufficient quantities to inhibit penetration by the infecting worms and function essentially in the lumen: or (b) such components are absorbed, persist in sufficient quantities and act in the intra- or inter-cellular location or are secreted to the lumenal surface, cr both (a) and (b). Whatever the precise mechanisms involved in the protection, they are only partially protective. Complete protection was not achieved in any experiment; a 50% - 75% protection was the usual observation,Perry (1974) recorded a reduction of approximately 40%.

Detailed studies with the objectives of comprehension of the expulsive mechanisms at challenge in immune rats have been carried out by Lee and Ogilvie (1981a,b) and by Russel and Castro (1979) and Castro, Hessel and Whalen (1979). Lee and Ogilvie (1981 a,b) noted that about 30-40% of infecting larvae penetrate and succeed in establishing within the epithelium of challenged animals, the remaining larvae are trapped in intestinal mucus, fail to penetrate the mucosa and are removed from the gut by peristalsis; <u>in vitro</u> studies showed that trapping in mucus is enhanced by preincubation of larvae in fresh control serum and in fresh or inactivated immune serum suggesting that activation by complement

or equally of adherence of antibodies to the worm's cuticle may be a component of the protective mechanism. These authors might also have considered that binding of antibodies to the worm secretions may play a part in protection. The work of Castro and his co-workers indicates that in rapid expulsion in immune rats many of the pathophysiological changes observed during expulsion of a primary infection are not in evidence. However challenge does result in a marked and rapid alteration in fluid flux across the mucosa leading to net fluid secretion which may interfere with survival and/or establishment. Obviously such mechanisms, if they exist in suckling mice and operating in concert, could have a deleterious effect on the establishment of the infecting larvae but less obviously on the development of larvae which succeed in penetrating the mucosa. Although the result in terms of the larvae establishing in mice suckling immune mothers is similar to the outcome of establishment of a challenge infection in immune rats, and both are manifestations of a rapid expulsion phenomenon, it would be unwise to draw further parallels as to the mechanisms involved in the two situations until such time as considerably more detailed information is available. One feature however demands further consideration, namely that a considerable number of worms do establish in the mucosa, and develop for several days in immune rats before early expulsion and for the normal period of time for a primary infection in suckling mice which are protected to some degree. It is known that the phenomenon of rapid expulsion does exist in previously infected mice but only for a short period. Thus Bruce and Wakelin (1977), Alizadeh and Wakelin (1982) and Ottaway, Bruce and Parrott, (1983) working with NIH mice indicated that an extremely effective rapid expulsion existed in mice in the period of a dew days immediately following the expulsion of a primary infection. Bell and

MacGregor (1980) showed that in other strains of mice, such rapid expulsion could persist for several days. Apart from this relatively short period, immunity in mice usually takes the form of a slightly reduced burden of worms existing in the usual location for a period of six to eight days before expulsion is completed (Kennedy, 1980a). Fatunmbi (1978) showed that deleterious effects operated on the worms as early as two days post-infection, with evidence of lack of growth and near cessation of production of ova.

It does remain therefore that in animals which are partially protected at the phase of penetration and establishment within the mucosa, worms which do succeed in establishing in the inter-and intra-epithelial position and feeding primarily intra-epithelially (Wright, 1979) are not seriously inconvenienced for several days in animals which are, in generally accepted terms, immune to challenge. This is also true of the worms which do establish in "immune" rats which rapidly expel the majority of the challenge infection. Larvae which do succeed in establishing are not apparently inconvenienced at all in suckling mice which are protected against establishment to a considerable degree, until such time as the whole expulsive process is effected.

It is clear from the results that transferable protection is elicited in the mothers both by multiple whole infection (Tables 1.9 - 1.11) and by immunization with a crude T. spiralis antigen in Freund's complete adjuvant. Of much greater significance and interest it is shown that transferable protection is elicited in a primary infection of quite short duration (Tables 1.14 - 1.17). Thus in mice experiencing an infection of T.spiralis for the first time in late pregnancy or even only during lactation a protective transferable response is produced and conveyed via the milk to suckling infants. An experience of only 9-11 days is the minimum requirement.

Thetime of onset of this protective response or at least the manifestation of this transferable response invites comparison with the development of the expulsive response in non-lactating adults undergoing a primary infection of T. spiralis. In such mice the period of 9 - 11 days post-infection is the time of maximal rate of expulsion of a primary infection. Testing the immune status of lactating and non-lactating mice Duckett et al. (1972) observed that in immune mice challenged when lactating the expulsion of a challenge infection is greatly reduced in comparison to non-lactating immune mice. Ngwenya (1977) noted that in both previously infected and in primary infection adult mice expulsion of T. spiralis was less efficient during lactation. Related studies on Nematospiroides dubius in mice - (Shubber, Lloyd and Soulsby, 1981),Nippostrongylus brasiliensis in rats - (Connan, 1970; Dineen and Kelly, 1972), Trichostrongylus colubriformis in guinea pigs - (O'Sullivan, 1974), and on Trichuris muris in mice - (Selby and Wakelin, 1975) all produced evidence that lactation resulted in considerable impairment of the expulsive response from the intestine. Dineen and Kelly (1972) concluded that in lactating rats infected with N. brasiliensis the defect in expulsion was due to the failure of sensitized lymphocytes to differentiate to effector cells.

From these reports it might have been presumed that lactating mice would be unlikely to mount an early and profound transferable response. However when the observations of Rose, Parrott and Bruce (1978) are taken into account, namely that additional circulating mesenteric lymphoblasts accumulate in the intestine of adult mice and also localise in the mammary gland during lactation, such a presumption based largely on worm population studies may be seen to be erroneous. This aspect of cellular responses will be discussed in detail later.

The results recorded in Tables 1.18 and 1.19 reveal the extremely significant finding that suckling mice gain protection from infected adult mice by suckling for as short a period as six hours. Obviously only a relatively small volume of milk is consumed in that period, and therefore a relatively small amount of the components affording protection against establishment. This small amount of material and the shortness of the timescale suggest that the protective mechanisms are probably at the lumenal surface or within the epithelial cells. There are no comparable studies of such events in the area of passive transfer of immunity in primary infection with helminth parasites. A study of Giardia muris in mice by Andrews and Hewlett (1981) yielded information concerning protection derived from the milk which was manifest after three days of suckling; this study will be referred to in detail later. Further quantification of the amount of the components in milk which afforded protection to suckling infants was determined by feeding a known volume of immune milk to naive two week old mice. The data in Table 1.20 shows a statistically significant protection in young mice which were fed a total of 0.3 ml of immune milk over a 24 hour period prior to infection. In further studies, recorded in detail in Section 4, it is shown that this volume of milk would contain approximately 0.264 mg of IgA, 0.02 mg of IgM and 0.188 mg of IgG, and that antibodies to T. spiralis infective larvae are present in each of these classes of immunoglobulin.

The final experiments in this part of the study were designed to clarify the points made earlier that young mice although less susceptible to infection with T. spiralis are nevertheless suitable hosts for the adult worms which do succeed in establishing in the intestine and that this statement is equally valid for young mice partially protected by suckling immune milk. The data and the analysis of the data in Tables 1.21 and 1.22 show quite clearly that young mice are perfectly suitable

hosts for established adult T. spiralis in that the fecundity of the adult female worms is not impaired, and indeed that in young mice partially protected by suckling immune milk the fecundity of the established worms is increased. However until this experiment is confirmed by an experiment of similar design in which identical adult worm burdens in young and adult mice are established, it is not possible to exclude the factors of availability of space/sites and it is therefore improper to make further conclusions at this stage.

Prolonged discussion of the literature as to the probable or possible mechanisms of the expulsive process and of transferable immunity seems inappropriate at this stage of the dissertation, the point having been made already that profound speculation as to the mechanisms of immunity are not justified on the evidence of population data. Discussion of these aspects will be pursued in the general discussion.

1.5 Summary

1. T. spiralis infection in young mice has been investigated. There were marked differences in susceptibility and location of the worms in young suckling NIH mice in comparison to adult mice. Protection in infants was shown to be protection involving markedly reduced establishment and did not involve enhanced expulsion of the established burden of worms, indeed the onset of expulsion was slightly delayed in 2 and 3 week old mice and was followed by a slight delay in completion of expulsion.

2. The sex of young mice has been shown to have no influence on establishment, location and expulsion of a primary infection.

3. Transferable protection was elicited in mothers both by whole infection or immunization. As confirmed by fostering experiments, this protection was transferred via the milk, and this part of the study confirms the earlier work of Duckett et al (1972) and Perry (1974).

4. Protection against worm establishment was maintained for at least one week after weaning, and did not afford long term protection, induction of immunity or tolerance.

5. Transferable protection was elicited in a primary infection of only 9-11 days in mothers.

6. Suckling milk from infected adult mice for only 6-8 hours conferred protection.

7. Although less susceptible to infection with T. spiralis, young mice including those partially protected by suckling immune milk, have been found to be suitable hosts for the adult worms which succeed in establishing in the intestine. The fecundity of adult female worms was not impaired in these mice.

Table 1.1: Number of adult Trichinella spiralis recovered from the small intestine of adult NIH mice after 4 days of a primary infection.

	Total number of worms recovered								
*Estimated inoculum →	GROUP (A) 50 larvae			GROUP (B) 150 larvae			GROUP (C) 450 larvae		
Location of worms in the small intestine	Ant	Post	Total	Ant	Post	Total	Ant	Post	Total
	16	7	23	69	20	89	174	56	230
	18	4	22	47	23	70	166	30	196
	15	13	28	78	15	93	228	39	267
	27	2	29	66	19	85	199	46	245
	27	5	32	60	35	95	194	60	254
	14	9	23	47	20	67	213	20	233
Mean	19.5	6.7	26.2	61.2	22	83.2	195.7	41.8	237.5
±s.d.	±5.96	±3.93	±4.07	±12.41	±6.87	±11.91	±23.28	±15.31	±24.48
% of establishment of estimated inoculum			52.4%			55.5%			52.8%

*The actual number of larvae given to each group in 0.3 ml dose, as follows:

Group "A" 60 ± 7 viable larvae, 0.8 non-viable

Group "B" 162 ± 2.3 " " 1.6 ± 0.5 "

Group "C" 462 ±12 " " 3.6 ± 1.8 "

Ant = anterior part of the small intestine

Post = posterior part of the small intestine

Total = total number of worms recovered from both anterior and posterior parts.

s.d. = standard deviation

Table 1.2 Expulsion of primary infection of Trichinella spiralis from adult NIH mice (8-11 weeks old).

Group	No. of T.spiralis larvae (infective dose) mean ± s.d.	Number of T.spiralis recovered from the small intestine mean ± s.d. of (5 mice) Days post-infection						Statistical significance P.value	
			4 p.l	6 p.l	8 p.l	10 p.l	12 p.l	14 p.l	day 6 vs 8	day 8 vs 10
A	53±3	A P T	23.4±5.18 4.2±2.49 27.6±7.27	16.6±5.32 8.4±3.51 25 ±2.74	9.6±2.51 7.2±2.39 16.8±4.27	0.6±1.34 0.2±0.45 0.8±1.79	0	0	P<0.01	P<0.00
% of establishment			52.1%	47.2%	31.7%	1.5%				
B	154±2	A P T	N.D.	27.4±8.44 10.8±2.59 38.2±10.42	15.2±7.5 11 4±5.59 26.6±8.2	2.6±3.58 2.2±3.03 4.8±6.57	0.4±0.89 1.8±3.03 2.2±3.89	0	N.S.	P<0.01
% of establishment				24.8%	17.3%	3.1%	1.4%			
C	466±6.6	A P T	196±48.91 80.8±43.07 276.8± 36.4	171.6±40.3 92 ±29.52 263.6±39.55	151±58.18 93.6±28.31 244.6±58.95	30.4±35.13 72.8±49.83 103.2±59.28	0 36.2±31.41 36.2±31.41	0	N.S.	P < 0.01
% of establishment			59.0%	56.6%	52.5%	22.1%	7.8%			

A : Anterior part of the small intestine P : posterior part of the small intestine

T : Total worms recovered ND : not done

s.d.: standard deviation N.S.. : not significant

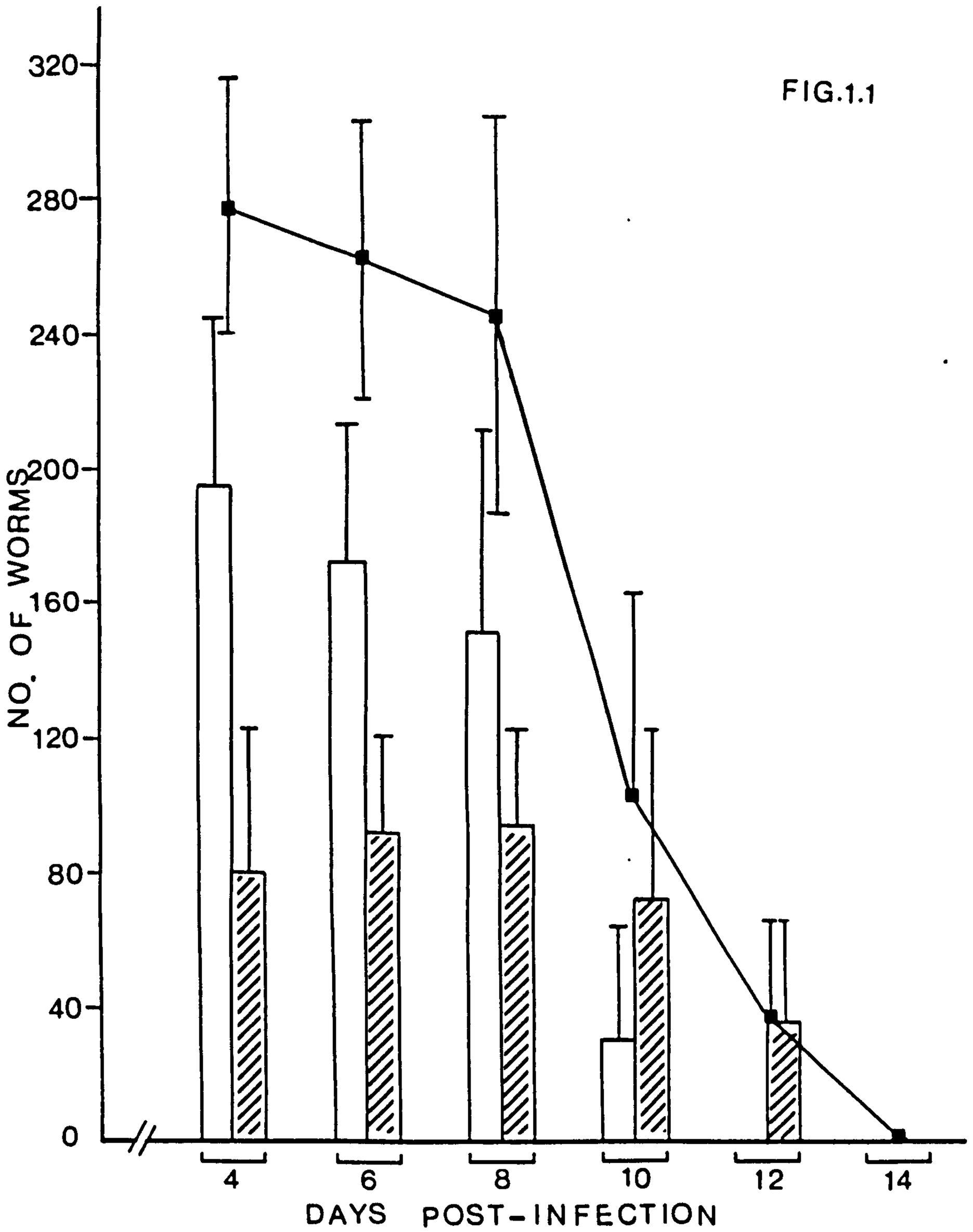


FIG.1.1

Figure 1.1.

The course of a primary infection of Trichinella spiralis in adult NIH mice

Mean number of worms recovered ± s.d. from the small intestine of groups of 5 mice. inoculum 200 T. spiralis larvae.

anterior small intestine

posterior small intestine

Table 1.3 Establishment of Trichinella spiralis in infant mice

Inoculum	Exp. No.	Age of infants at infection (weeks)	Number of worms established in the small intestine on day 4 p.i (Mean±s.d) of 5 mice						Recovery expressed as % in anterior and posterior halves of the small intestine at day 4 post infection.			
			Infant mice			*Adult mice (control)			Infant mice		Adult mice	
			A	P	T	A	P	T	A	P	A	P
50	1	1 week	0	0.3±0.52	0.3±0.52	15.6±3.58	7 ±2.45	22.6±3.43	0	100	69	31
		2 weeks	1.5±1.52	6 ±3.74	7.5±5.17	"	"	"	20	80	"	"
		3 weeks	9 ±2.97	2.2±2.56	11.2±3.66	"	"	"	80.4	19.6	"	"
		4 weeks	12 ±4.69	2.5±2.59	14.5±3.08	"	"	"	82.8	17.2	"	"
		5 weeks	17 ±5.4	1.7±0.82	18.7±5.32	"	"	"	90.9	9.1	"	"
	2	2 weeks	1.9±2.41	5.4±5.13	7.3±6.99	17.6±3.05	9.4±3.97	27 ±3.87	26	74	65	35
		3 weeks	11 ±6.29	2.7±2.5	13.7±7.74	"	"	"	80	20	"	"
	3	2 weeks	2 ±1.8	8 ±3.97	10 ±3.87	23 ±2.61	7.5±3.67	30.5±3.56	20	80	75	25
		3 weeks	10 ±4.28	2 ±1.45	12 ±4.5	"	"	"	83	17	"	"
	4	2 weeks	23 ±1.8	5.7±2.83	8 ±3	15.6±1.67	4.2±1.3	19.8±0.84	29	71	79	21
	5	3 weeks	5.1±4.01	7.6±3.24	12.7±5.48	18.6±3.36	5 ±4	23.6±1.14	40	60	79	21
	6	3 weeks	14.6±3.87	1.8±1.57	16.4±3.41	14.6±4.72	6 ±3.08	20.6±2.07	89	11	71	29
	7	4 weeks	9.7±4.53	4.5±2.2	14.2±5.15	20.2±5.54	7.2±2.86	27.4±3.78	68	32	74	26
	8	4 weeks	9.3±4.64	5.4±4.96	14.7±7.72	18.6±3.36	5 ±4	23.6±1.14	63	37	79	21
100	9	1 week	0	12.4±6.18	12.4±6.18	40.4±7.89	11.6±5.98	52 ±6.32	0	100	78	22

A = Anterior part of the small intestine
P = posterior part of the small intestine
T = Total worms recovered
s.d. = standard deviation
* = 10 - 14 weeks old

Table 1.4 The course of infection of Trichinella spiralis in infant mice
Expulsion of a primary infection of T. spiralis from infant mice after infection with 50 larvae.

Group			Worm burden recovered (mean ± s.d) 5-6 mice per group						statistical significance P value	
			Days post-infection							
			4 p.i	6 p.i	8 p.i	10 p.i	12 p.i	14 p.i	day 6 vs 8	day 8 vs 10
1	Infant mice infected at 2 weeks of age	A	1.55±1.33	2.33±1.75	2.4±1.9	1.25±1.28	0.6±0.7	0		
		P	4 ±2.45	4.33±2.94	5.6±2.67	1.88±1.13	1.6±0.7	0.25±0.5		
		T	5.55±2.92	6.66±3.14	8 ±3.23	3.13±1.64	2.2±0.92	0.25±0.5	N.S.	P< 0.02
	Adult mice (control)	A	16.4 ±2.7	14.8 ±2.38	9.2±5.93	3.2 ±2.39	0	0.2 ±0.45		
		P	3.8 ±2.58	3.2 ±3.83	3.4±1.51	1.4 ±1.67	0.2±0.45	0		
		T	20.2 ±0.84	18 ±4.12	12.6±5.68	4.6 ±3.84	0.2±0.45	0.2 ±0.45	N.S.	P< 0.05
2	Infant mice infected at 3 weeks of age	A	8.55±3.09	9.66±4.45	11.33±3.88	8.4 ±3.84	0.2±0.45			
		P	3.33±2.35	2.33±2.42	2.33±1.5	2.8 ±1.48	0	0		
		T	11.88±4.54	12 ±4.77	13.66±3.98	11.2 ±3.83	0.2±0.45		N.S.	N.S.
	Adult mice (control)	A	17.8 ±1.64	9.4 ±3.28	1.2 ±1.64	0				
		P	3.6 ±2.3	4.8 ±2.38	2.4 ±1.51	0.2 ±0.45		0		
		T	21.4 ±3.13	14.2 ±1.92	3.6 ±3.13	0.2 ±0.45	0		P< 0.001	N.S.
3	Infant mice infected at 4 weeks of age	A	N.D.	3.4 ±2.3	2.8 ±2.17	1.6 ±1.52	1 ±0.7	2.4 ± 2.88		
		P		4.2 ±0.45	2.6 ±1.14	3.6 ±1.67	0.8 ±0.84	1.2 ± 1.64		
		T		7.6 ±2.3	5.4 ±1.95	5.2 ±1.3	1.8 ±1.09	3.6 ± 4.33	N.S.	N.S.
	Adult mice (control)	A	N.D.	6.2 ±1.3	2.6 ±1.82	0.4 ±0.55	0.4 ±0.89			
		P		4.6 ±2.79	4.8 ±1.48	0.4 ±0.55	0			
		T		10.8 ±2.59	7.4 ±2.7	0.8 ±0.84	0.4 ±0.89	0	N.S.	P < 0.01

A = anterior part of the small intestine
P = posterior part of the small intestine
T = Total worms recovered
N.D. = Not done
s.d. = standard deviation
N.S. = not significant

Table 1.5: The course of infection of Trichinella spiralis in infant mice. Expulsion of a primary infection of T. spiralis from infant mice after infection with 100 larvae.

Group			Worm burden recovered (mean ± s.d) 5-6 mice in each group						Statistical significance	
			Days post-infection						P-value	
			4 p.i.	6 p.i.	8 p.i.	10 p.i.	12 p.i.	14 p.i.	day 6 vs 8	day 8 vs 10
1	Infant mice infected at 1 week of age	A		0	0	0	0	0		
		P	N.D.	4.2 ± 2.95	3 ±2.34	2.8 ±1.48	3 ± 2.83	2.2± 1.48		
		T		4.2 ± 2.95	3 ±2.34	2.8 ±1.48	3 ± 2.83	2.2± 1.48	N.S.	N.S.
	Adult mice (control)	A		20.66± 2.94	6.4 ±4.16	0.4 ±0.55				
		P	N.D.	6 ± 3.69	5 ±2.55	1.6 ±1.14	0	0		
		T		26.66±2.94	11.4 ±5.32	2 ±1.22			P < 0.001	P < 0.01
2	Infant mice infected at 2 weeks of age	A	6.33±3.08	7.66±4.23	3.66±2.87	2.33±2.58	1.4± 2.07	0.2± 0.45		
		P	14.5 ±2.88	16.33±7.79	8.66±5.68	6 ±4.33	0.4± 0.89	0		
		T	20.83±2.48	24 ±11.37	12.33±7.42	8.33±5.78	1.8± 2.17	0.2± 0.45	N.S.	N.S.
	Adult mice (control)	A	28.6 ±11.5							
		P	7 ± 1.73			N.D.				
		T	35.6 ±11.95							
3	Infant mice infected at 3 weeks of age	A	18.16± 6.24	13.66 ±5.85	5.6 ±4.04	7 ±11.83	2 ±1.41	0		
		P	6.83± 5.95	11.83 ±4.49	10.6 ±2.07	2.6 ± 4.77	1.6 ±2.07			
		T	25 ±11.35	25.5 ±7.03	16.2 ±4.87	9.6 ±16.53	3.6 ±3.05		P < 0.05	N.S.
	Adult mice (control)	A	31 ± 3.94	28.4 ±7.92	13.8 ±8.53	0.6 ± 0.55		0.2± 0.45		
		P	8.6 ± 3.91	9 ±3.08	18.2 ±7.82	9.2 ± 4.82	0	0		
		T	39.6 ± 6.58	37.4 ±8.79	32.2 ±1.64	9.8 ± 4.71		0.2± 0.45	N.S.	P < 0.001
4	Infant mice infected at 4 weeks of age	A		8.6 ±6.54	7.4 ±4.28	1.8 ± 1.92	1.8 ±3.49	0.2± 0.45		
		P	N.D.	6 ±4.18	6.6 ±1.52	5.6 ± 2.3	1.4 ±2.61	0.2± 0.45	N.S.	P < 0.02
		T		14.6 ±2.88	14 ±2.91	7.4 ± 3.51	3.2 ±6.09	0.4± 0.89		
	Adult mice (control)	A		19.4 ±3.85	6.2 ±3.63	0.2 ± 0.45				
		P	N.D.	6.4 ±2.7	4.6 ±2.07	1.2 ± 0.84	0	0		
		T		25.8 ±1.64	10.8 ±3.83	1.4 ± 0.89			P < 0.001	P < 0.001

A = Anterior part of the small intestine P = Posterior part of the small intestine T = Total worms recovered N.D.=not done
s.d. = standard deviation N.S. = not significant

Table 1.6 The course of infection of Trichinella spiralis in infant mice. Expulsion of a primary infection of T.spiralis from infant mice after infection with 200 larvae.

Group			Worm burden recovered (Mean ± s.d.) 5-6 mice in each group						Statistical significance	
			Days post-infection						P Value	
			4 p.i	6p.i.	8 p.i.	10 p.i.	12 p.i.	14 p.i.	day 6 vs 8	day 8 vs 10
1	Infant mice infected at 2 weeks of age	A	15.4±10.94	10.2± 2.95	7.4± 7.09	3.8± 4.76	1.4± 0.89	0.8± 0.84	N.S.	P<0.05
		P	32.2±10.18	37.2±12.81	22.8± 7.19	8.0± 9.16	5.4± 4.83	6.6± 4.72		
		T	47.6±15.17	47.4±14.89	30.2± 9.36	11.8±13.84	6.8± 4.32	7.4± 4.83		
	Adult mice (control)	A	41.2± 8.07	52.6± 7.92	26.6± 8.99	0.6± 1.34	0	0	P<0.01	P<0.01
		P	12.6± 3.43	8.4± 2.7	9.6± 6.54	2.2± 1.79				
		T	53.8± 6.91	61.0± 5.61	36.2±11.09	2.8± 3.03				
2	Infant mice infected at 4 weeks of age	A	N.D.	13.8± 4.15	9.2± 4.32	1.4± 1.67	0	0.4± 0.55		
		P		17.0±11.66	9.2± 3.27	1.4± 1.67		0.4± 0.55		
		T		30.8±13.32	18.4± 4.72	2.8± 3.27		0.8± 1.09		
	Adult mice (control)	A	N.D.	36.8± 5.26	2.6± 2.41	0.4± 0.89	0	0		
		P		17.6± 7.23	5.0± 1.73	0.8± 0.84				
		T		54.4± 7.13	7.6± 3.65	1.2± 1.3				

A = anterior part of the small intestine
P = posterior part of the small intestine
T = total worms recovered from anterior and posterior parts of the small intestine
N.S. = not significant
N.D. = not done
s.d. = standard deviation

FIG.1.2

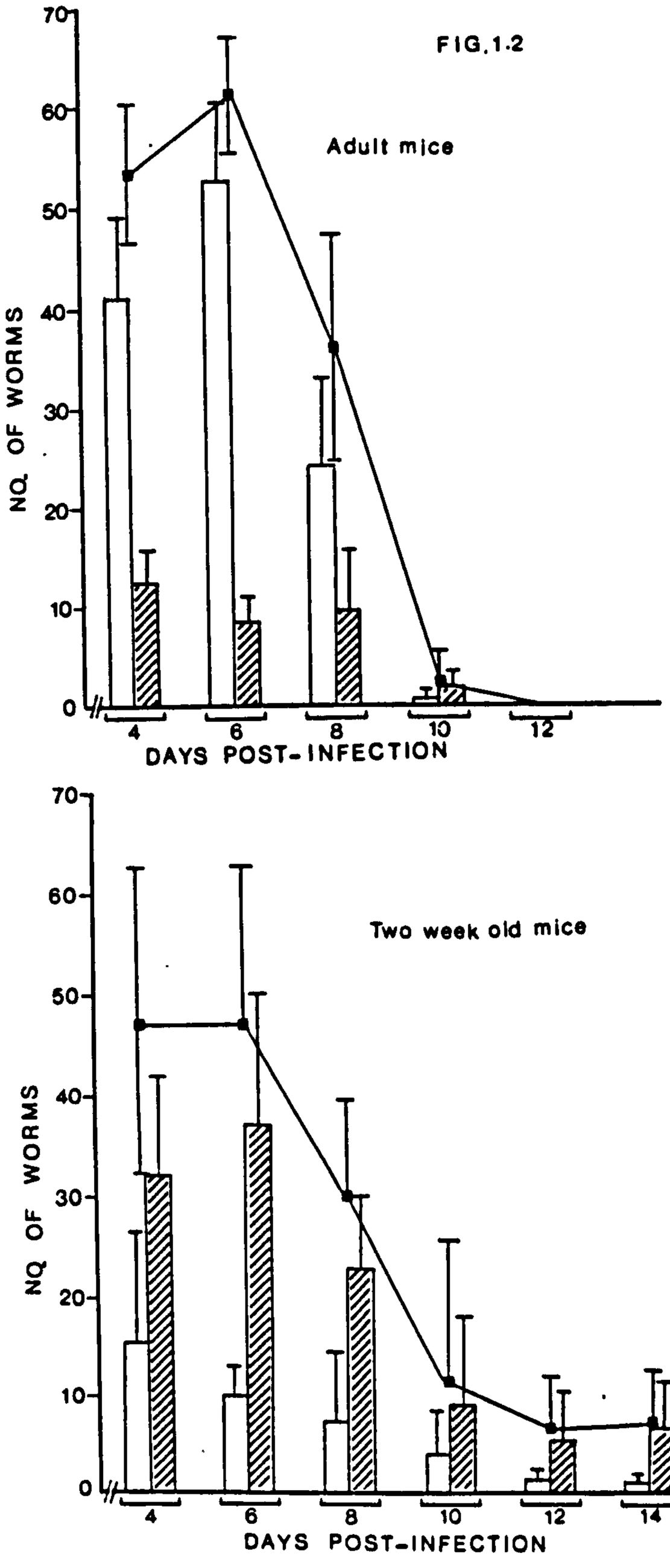


Figure 1.2 The course of a primary infection of Trichinella spiralis in adult and infant (2 week old) NIH mice.

mean number of worms recovered ± s.d. from the small intestine of groups of 5-6 mice; inoculum 200 T. spiralis larvae

anterior small intestine

posterior small intestine

FIG.1.3

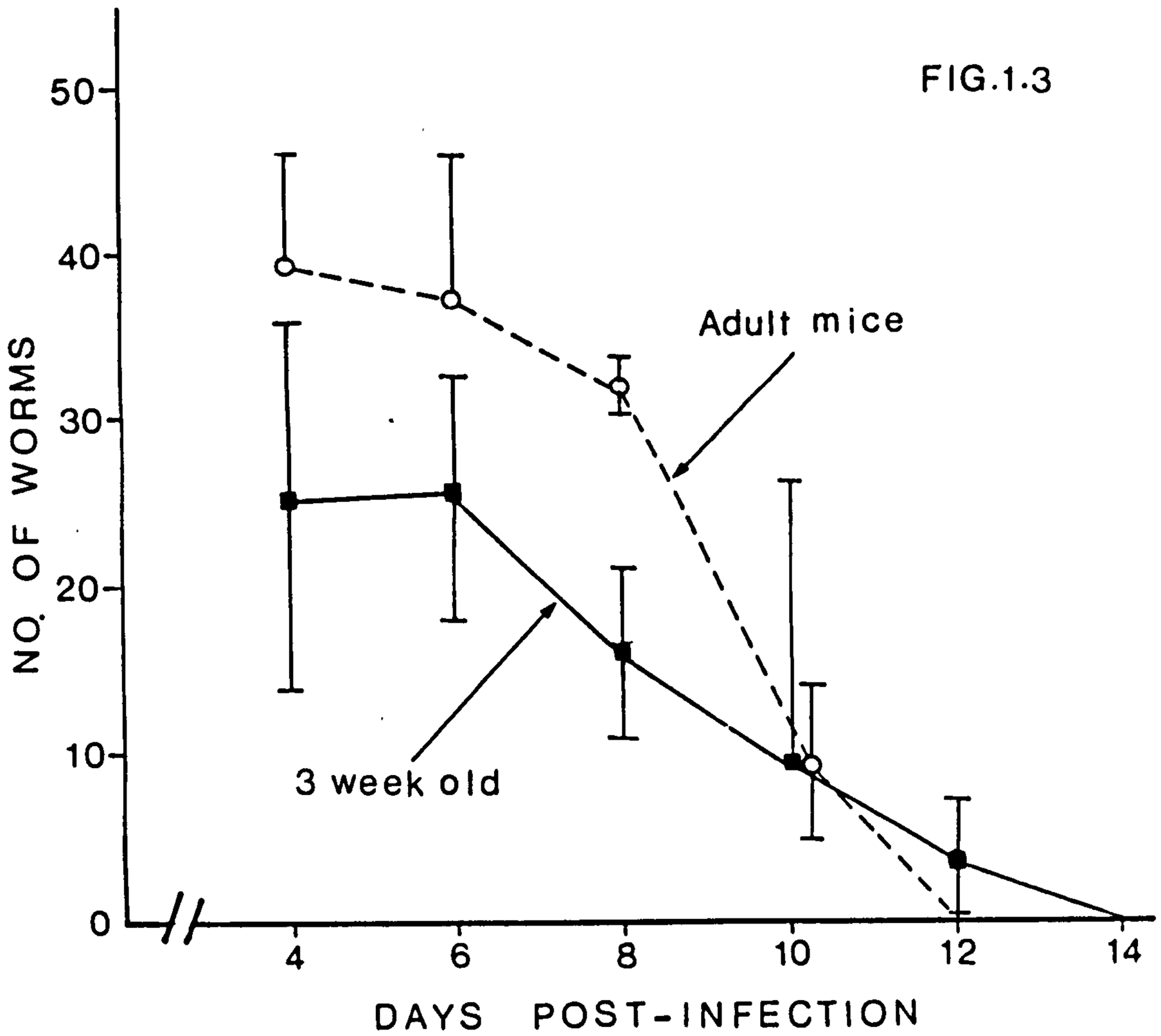


Figure 1.3.

The course of a primary infection of Trichinella spiralis in adult and infant (3 weeks old) NIH mice
Mean ± s.d of 5-6 mice per group; inoculum 100 larvae.

Table 1.7 Establishment and course of primary infection of Trichinella spiralis in male and female infant mice.

Age	Group	Θ Inoculum	Mice Sex	Worm burden recovered (Mean* ± s.d) Days post-infection 4 p.i	6 p.i	8 p.i	10 p.i	12 p.i	14 p.i	Statistical significance P Value day 6vs8	day 8vs10
Infant mice infected at 2 wks of age	1	100	Male	21 ±3.6	23.66 ±11.5	15 ±9.53	9.66 ±5.5	2.4 ±2.64	0.33 ±0.57	N.S	N.S
			Female	21 ±1.73	24.33 ±13.79	9.66 ±5.03	7 ±5.29	1.5 ±2.12	0	N.S	N.S
	2	200	Male	44 ±8.48	53 ±12.72	27.5 ±13.43	24 ±15.55	N.D	3.5 ±2.12	N.S	N.S
			Female	50 ±20.07	43.66 ±17.61	32 ± 8.54	3.66 ±3.78	8 ±3.9	8.66 ±5.13	N.S	P < 0.01
Infant mice infected at 3 wks of age	3	100	Male	22.33 ±11.59	24.66 ±10.78	15.33 ± 4.93	21.5 ±24.74	4.33 ±3.21	0	N.S	N.S
			Female	27.66 ±12.89	26.33 ± 2.3	17.5 ± 6.36	1.66 ± 2.08	2.5 ±3.53	0	N.S	P < 0.05
	4	200	Male	52.33 ±15.56	53 ±16.96	23 ±25.7	2 ± 2.8	5.5 ±6.36	14.5 ±2.12	N.S	N.S
			Female	50 ± 9.89	53.33 ±17.78	21.5 ±17.67	11.66 ± 9.5	13.33 ±8.02	N.D	N.S	N.S

* Each value represent a mean of 2 or 3 mice Θ Number of estimated viable infective larvae.

Statistical significance between male and female in each group N.S = Not significant

Group	4 p.i	6 p.i
1	N.S	N.S
2	N.S	N.S
3	N.S	N.S
4	N.S	N.S

Table 1.8 Establishment and course of primary infection of Trichinella spiralis in male and female infant mice (inoculum 200 infective larvae)

		Worm burden recovered on day 6 post-infection (*mean ±s.d.) of 6 mice											
		2 weeks old						3 weeks old					
		Born of immune Suckling immune			Born of naive Suckling naive			Born of immune Suckling immune			Born of naive Suckling naive		
Mice	Sex	A	P	T	A	P	T	A	P	T	A	P	T
		4	7	11	19	26	45	10	5	15	29	16	45
		2	18	20	10	33	43	4	2	6	21	15	36
		5	15	20	15	39	54	9	3	12	35	21	56
Male		4	11	15	8	24	32	7	15	22	36	8	44
		7	10	17	14	35	49	18	4	22	14	25	39
		5	12	17	11	34	45	15	3	18	17	12	29
	*Mean	4.5	12.2	16.7	12.8	31.8	44.6	10.5	5.33	15.83	25.3	16.2	41.5
	±s.d.	±1.64	±3.87	±3.39	±3.97	±5.71	±7.34	±5.17	±4.84	±6.21	±9.35	±6.11	±9.18
		9	16	25	17	26	43	5	5	10	35	6	41
		2	8	10	16	39	55	0	8	8	39	9	48
		3	17	20	10	32	42	18	8	26	16	9	25
Female		6	5	11	10	25	35	16	5	21	8	34	42
		5	6	11	11	27	38	6	12	18	15	12	27
		4	14	18	9	34	43	7	7	14	24	16	40
	*Mean	4.8	11	15.8	12.2	30.5	42.7	8.7	7.5	16.2	22.8	14.3	37.1
	±s.d.	±2.48	±5.29	±6.11	±3.43	±5.47	±6.83	±6.92	±2.59	±6.82	±12.15	±10.21	±9.11

A = Anterior part of the small intestine
P = Posterior part of the small intestine
T = Total worms recovered
s.d.= standard deviation
* = Means not significantly different between males and females in all groups.

Table 1.9 Trichinella spiralis infection in young mice.

Effect of immunity in mothers on the establishment and expulsion in 2 weeks old mice

Infant infected* with 200 larvae at 2 weeks of age	Worm burden recovered (Mean ± s.d)[x]					Statistical significance P.value within each group (Days)			
	Days post-infection								
	2 p.i	4 p.i	6 p.i	8 p.i	10 p.i	2 vs 4	4 vs 6	6 vs 8	8 vs 10
(Born of naive	40.83 ±5.27	46 ± 9.65	43.55 ± 7.02	24.66 ±10.87	15.5 ± 5.82	N.S.	N.S.	P<0.001	N.S
(Suckling naive									
% establishment	20.11%	22.66%	21.34%	12.14%	7.63%				
(Born of immune Ø	25	26.4	17.33	10.44	5.66	N.S	P<0.01	P<0.05	N.S
(Suckling immune	± 3.8	± 3.64	± 4.89	± 6.72	±6.88				
% establishment	12.31%	12.8%	8.37%	5.14%	2.78%				
Statistical significance (P.value) between groups	P<0.001	P<0.01	P<0.001	P<0.01	P<0.05				

* Actual dose of infection = 203 ± 2

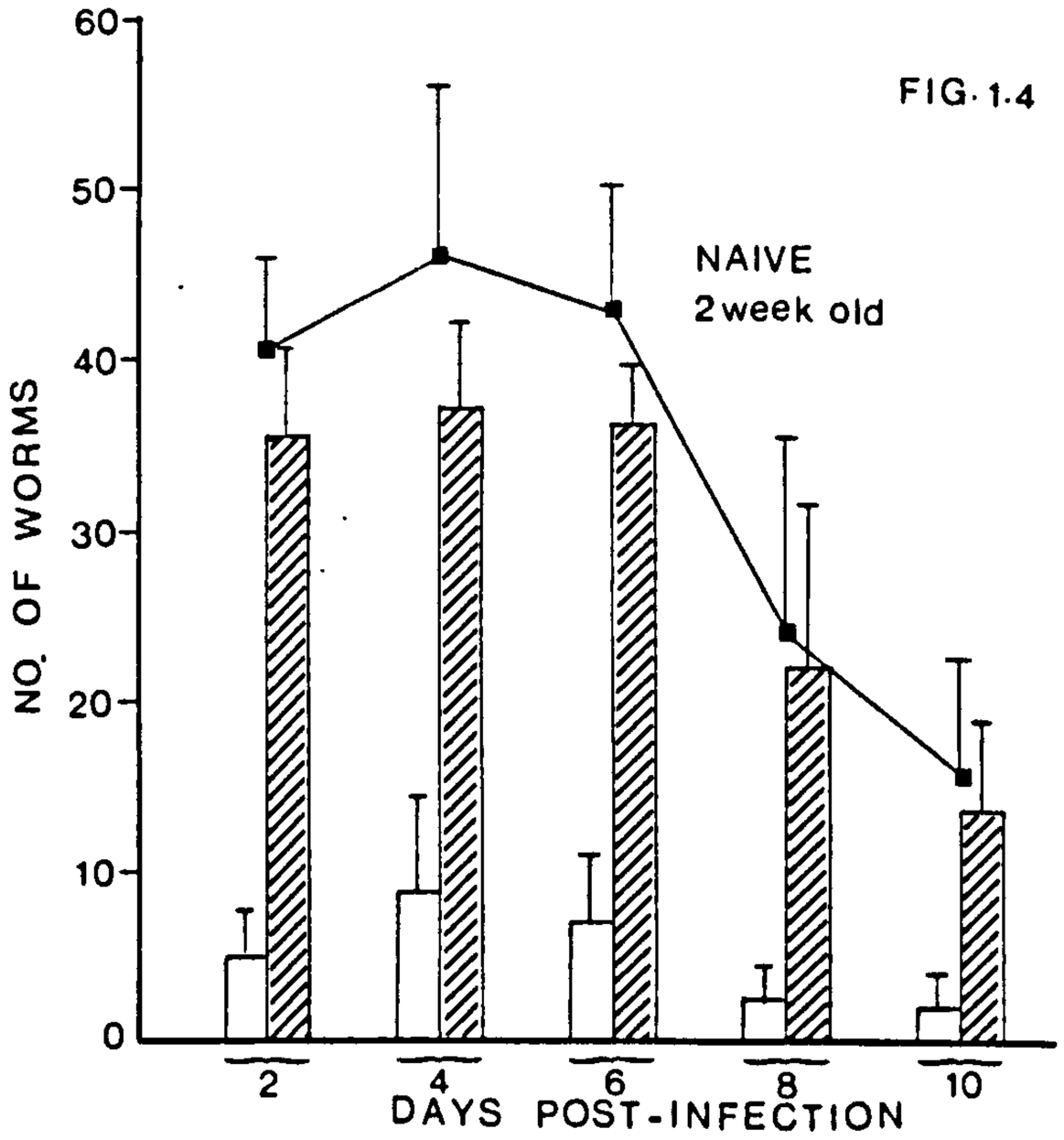
Ø Immune mothers were given a primary infection of 350 larvae of T.spiralis followed after 3 weeks with a secondary infection of 200 larvae.

x Each figure represent a mean of 5-9 mice

s.d. Standard deviation

N.S. Not significant

FIG. 1.4



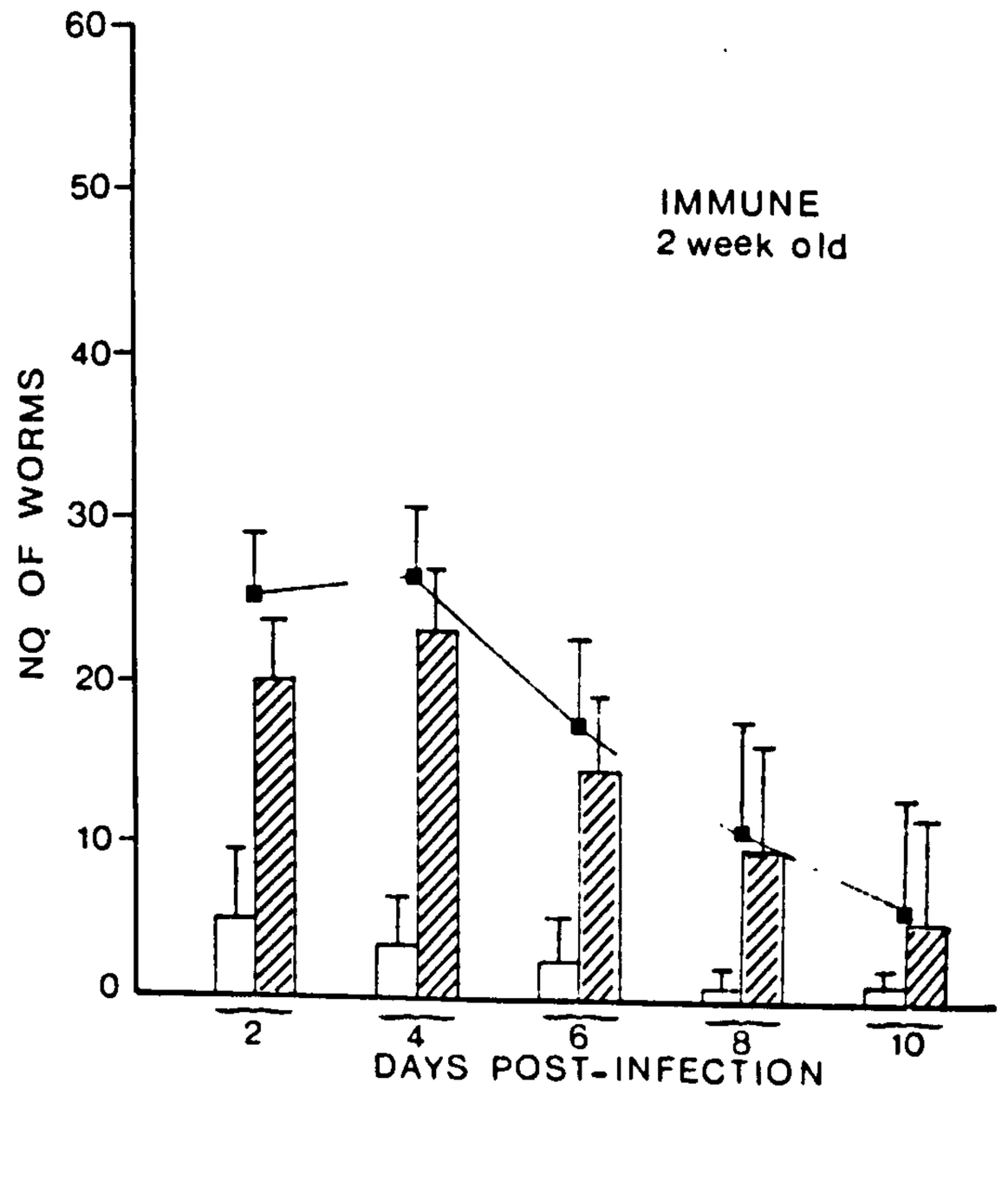


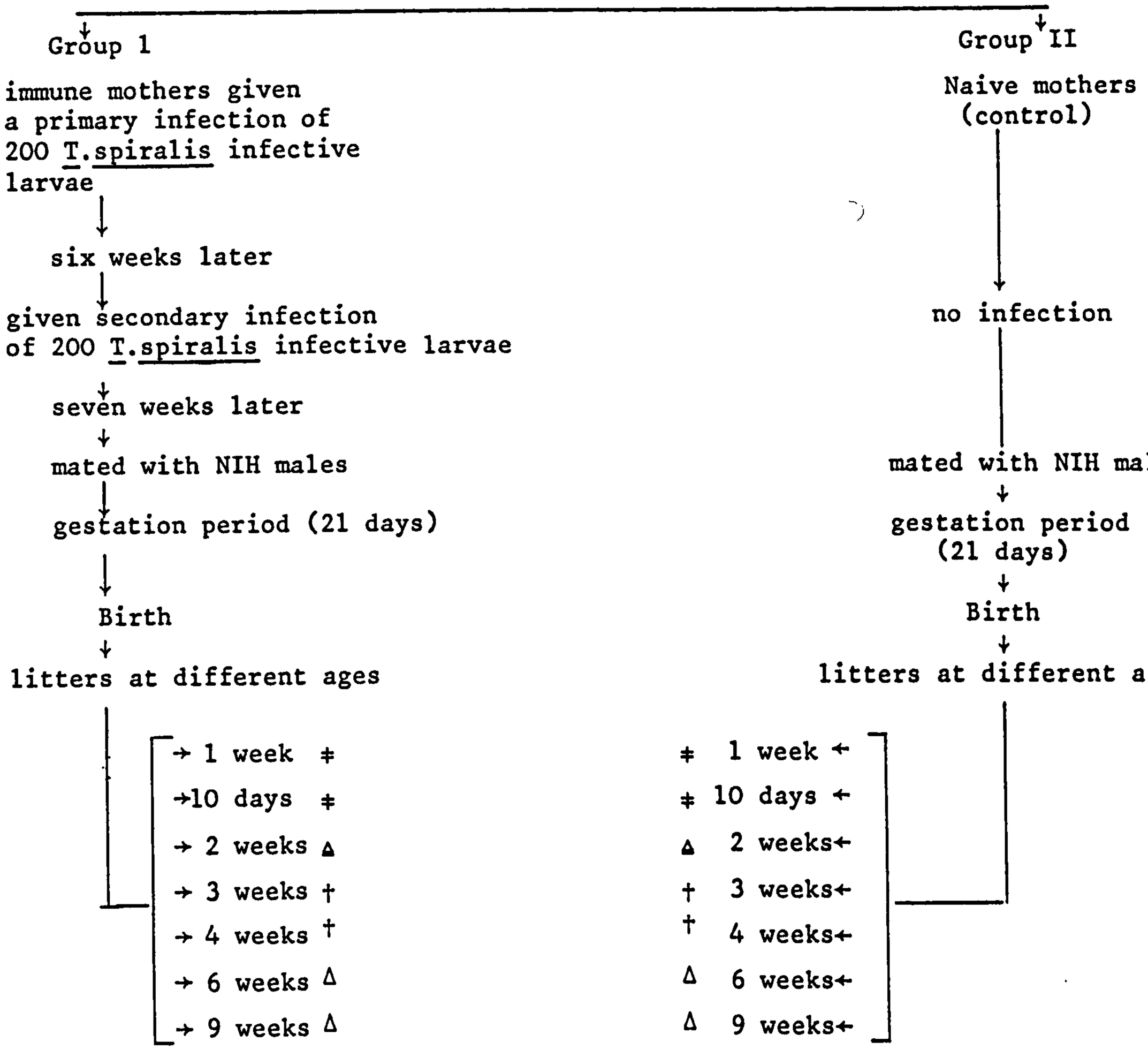
Figure 1.4. Establishment and course of a primary infection of Trichinella spiralis in two week old mice suckling naive or immune mothers.

■——■ mean number of worms recovered ± s.d. from the small intestine of groups of 5-9 mice; inoculum 200 T. spiralis larvae.

□ anterior small intestine

▨ posterior small intestine

Protoool 1.1 For the duration of protective immunity gained from immune mothers.
Adult Female NIH mice (6-8 weeks old)



Θ In 1 week old, 3 litters from each group (each litter with 5 infants, except one with 6 infants)
In 10 days old, 3 " " " " (each litter with 5 infants)
In 2 weeks old, 3 " " " " (each litter with 6 infants, except one with 4 infants)
In 3 & 4 weeks old, 6 litters from each group (each litter with 8 infants
In 6 & 9 weeks old, 5 litters from each group (each litter with 8 infants

≠ 5 mice killed on days 6,8 & 10 p.i.
▲ 6 mice killed on days 6 & 8, 4 mice killed on day 10 p.i.
†18 mice killed on days 6 & 8, 12 mice killed on day 10 p.i.
Δ15 mice killed on days 6 and 8, 10 mice killed on day 10 p.i.

Table 1.10 Trichinella spiralis infection in young mice.
Duration of protective immunity gained from mothers

Group	Infant mice	Worm burden (Mean ± s.d)+ recovered at day 6 Post-infection Age at time of infection 1 week	10 days	2 weeks	*3 weeks	*4 weeks	*6 weeks	*9 weeks
1	Born of naive Suckling naive	24.8 ±8.44	46.4 ±2.3	44.7 ±5.24	42.22 ±12.41	52.66 ±9.74	51.53 ±8.52	55.4 ±9.78
2	Born of immune Suckling immune	8.22 ±5.06	12.8 ±9.88	16.8 ±3.97	15.88 ±5.79	30.33 ±10.39	47.46 ±5.93	57.73 ±8.04
	Adult (control)	59.6 ±7.23	64 ±10.1	57.4 ±5.68	62 ±4.85	60.75 ±5.19	59.6 ±8.96	N.D
	Statistical significance P.value (group 1 vs group 2)	P<0.01	P<0.001	P<0.001	P<0.001	P<0.001	N.S.	N.S.

* Weaned after 21 days lactation.
Infecting dose = 200 T.spiralis larvae

\+ Each value represent a mean of 5 mice (1 week and 10 days old) 6 mice (2 weeks old) 15 mice (6 and 9 weeks old) 18 mice (3 and 4 weeks old).

N.D. Not done
s.d. Standard deviation
N.S. Not significant

FIG.1.5

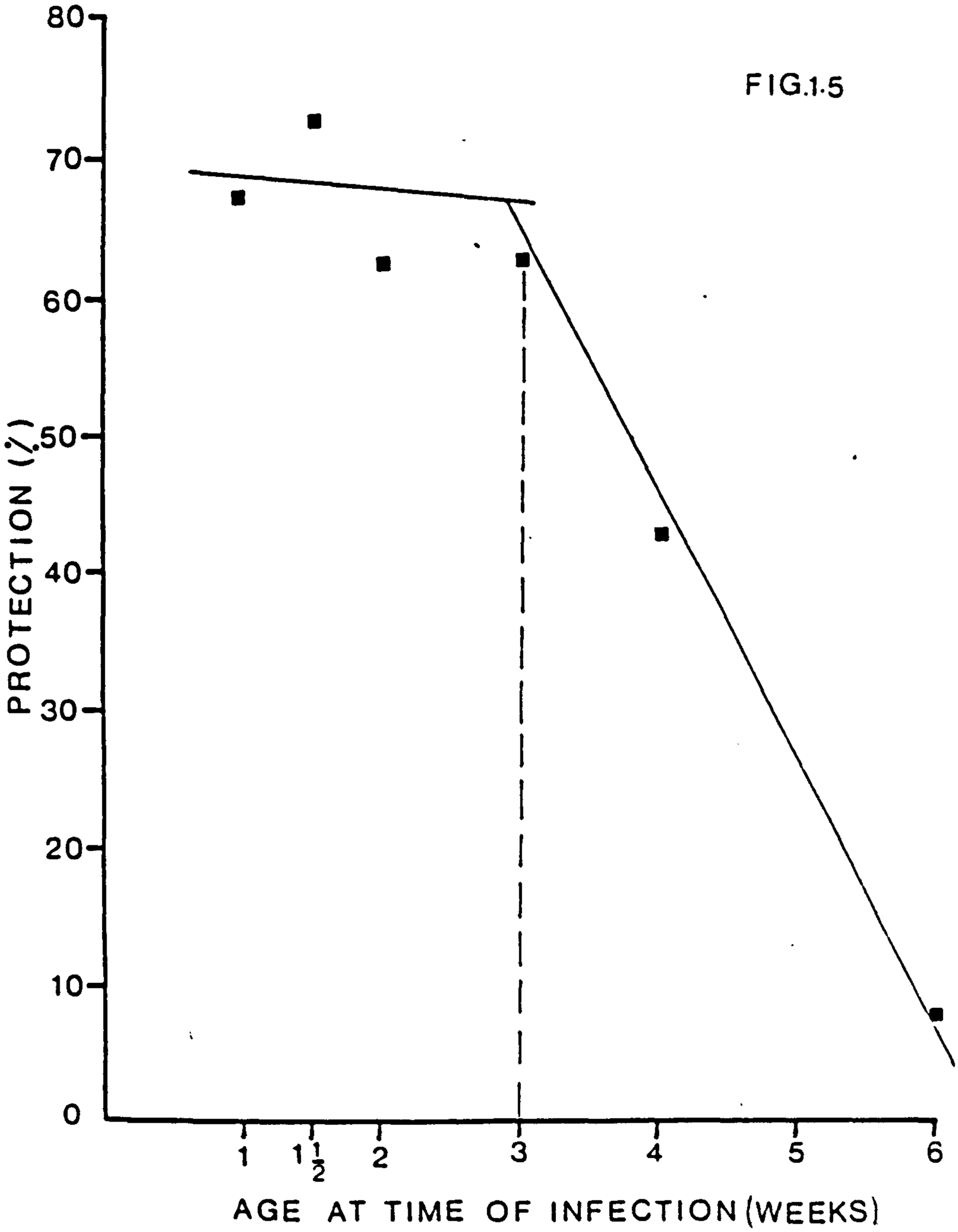
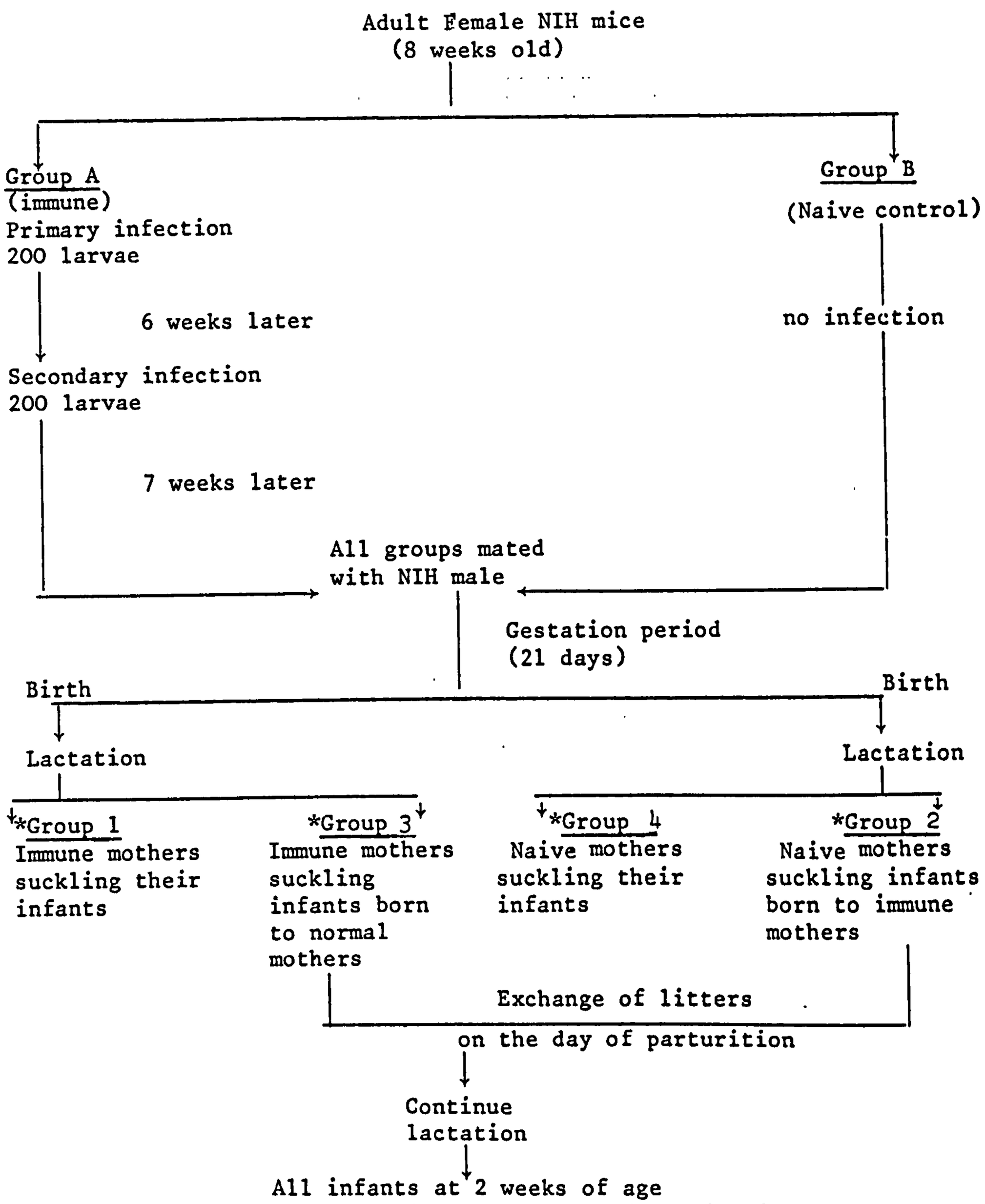


Figure 1.5.

The degree of protection in young mice suckling or ex-suckling mothers immune to Trichinella spiralis. Data derived from Table 1.10

$$\text{protection} = 100 - \frac{\text{mean no of worms at day 6 post-infection in mice suckling immune mothers}}{\text{mean no of worms at day 6 post-infection in mice suckling naive mothers.}}$$

Protocol 1.2: Infection and Fostering



*Groups 1,2,3 & 4. Each group had 6 litters, each litter had 8 infants.
3 infants from each litter were killed on days 6 and 8 post-infection
2 infants from each litter were killed on day 10 post-infection.

Table 1.11 Trichinella spiralis infection in young mice. Route of protection from immune mothers
Nature of immunization: Mothers infected with 200 larvae 16 and 10 weeks before parturition.

	Infant infected with 200 larvae at 2 weeks of age	*Mean intestinal worm count ± s.d. Day 6 p.i	Day 8 p.i	Day 10 p.i.	Statistical significance P. value within each group (days) 6 vs 8	8 vs 10	6 vs 10
1	Born of immune Suckling immune	14.33 ± 4.89	11.61 ± 7.01	4.33 ± 6.06	N.S.	P<0.01	P<0.001
2	Born of immune Suckling naive	44.94 ± 7.12	23.55 ± 8.24	15.66 ± 9.84	P<0.001	P<0.05	P<0.001
3	Born of naive Suckling immune	16.16 ± 6.64	12.83 ± 8.49	7.83 ± 6.79	N.S.	N.S.	P 0.001
4	Born of naive Suckling naive	43.22 ± 8.93	25.38 ± 9.4	12.33 ± 6.27	P<0.001	P<0.001	P<0.001

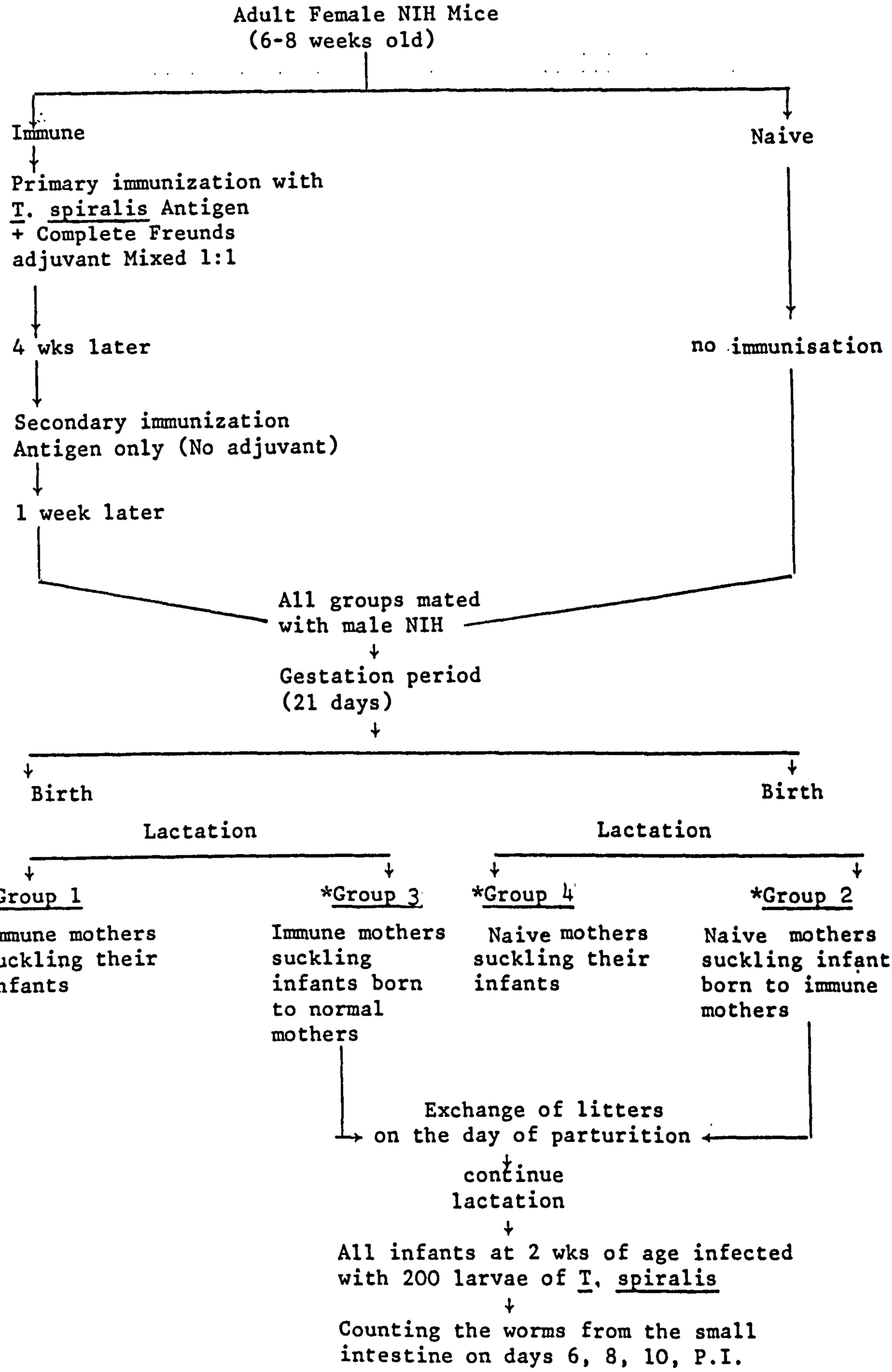
*Each value represents a mean of 18 or 12 mice

s.d. Standard deviation
N.S. Not signififant

Statistical significance between groups at day 6 p.i.

Groups	P. value
1 vs 2	P<0.001
1 vs 3	N.S.
1 vs 4	P<0.001
2 vs 3	P<0.001
2 vs 4	N.S.
3 vs 4	P<0.001

Protocol 1.3; Immunization and Fostering



*Groups 1,2,3 & 4. Each group had 6 litters, each litter had 8 infants.
3 infants from each litter were killed on days 6 & 8 post-infection
2 infants from each litter were killed on day 10 post-infection.

Table 1.12 Trichinella spiralis infection in young mice. Route of protection from immune mothers.

Nature of immunization: Mothers immunized with saline soluble antigen in complete Freund's adjuvant 8 and 4 weeks before parturition.

Groups	Infants infected with 200 larvae at 2 weeks of age	*Mean intestinal worm count (± s.d.)			Statistical significance between groups	
		Days post-infection			P.value day 6	
		6 p.i	8 p.i	10 p.i		
1	Born of immune Suckling immune	23.41±7.3	17.25±6.18	7.37±7.26	Group 1 vs 2 Group 1 vs 3 Group 1 vs 4	P<0.001 N.S P<0.001
2	Born of immune Suckling naive	47 ± 8.44	33.41±13.94	19.87±11.61	Group 2 vs 3 Group 2 vs 4	P<0.001 N.S.
3	Born of naive Suckling immune	22.33±7.49	19.33±7.03	10.12±8.35	Group 3 vs 4	P<0.001
4	Born of naive Suckling naive	45.58± 8.77	29.85±11.03	16.87±7.91		

* Each mean represents 12 mice (days 6 and 8) and 8 mice (day 10)

N.S. not significant

s.d. standard deviation

Table 1.13 Trichinella spiralis infection in young mice. Route of protection from immune mothers.

Nature of immunization: Mothers immunized with saline soluble antigen and complete Freund's adjuvant 8 and 4 weeks before parturition.

Groups	Treatment of Mothers	Mice infected with 200 T.spiralis larvae	Mice age when infected	*Mean intestinal worm count ± s.d. Days post-infection 6 p.i		8 p.i		10 p.i
1	Immunized with antigen + adjuvant	Born of immune Suckling immune	2 weeks	24.06±7.37	N.S	18.86±6.67	P<0.001	5.3±6.21
2	"	"	8 weeks	58.16±5.25	P<0.001	39.58±7.53	P<0.001	7.75±4.13
3	Injected with adjuvant + P.B.S only (control 1)	Born of naive Suckling naive	2 weeks	54.26±9.26	P<0.001	29.6±12.23	P<0.001	13 ±6.23
4	No treatment (control 2)	Born of naive Suckling naive	2 weeks	52.4±9.43	P<0.001	26.73±10.34	P<0.01	13.7±7.36
5	"	"	8 weeks	55.91±5.98	P<0.001	42.5±8.27	P<0.001	8.5±4.75

* Means of 15 mice on days 6 and 8 p.i. and of 10 mice on day 10 p.i. (2 weeks old)
Means of 12 mice on days 6 and 8 p.i. and of 8 mice on day 10 p.i. (8 weeks old).

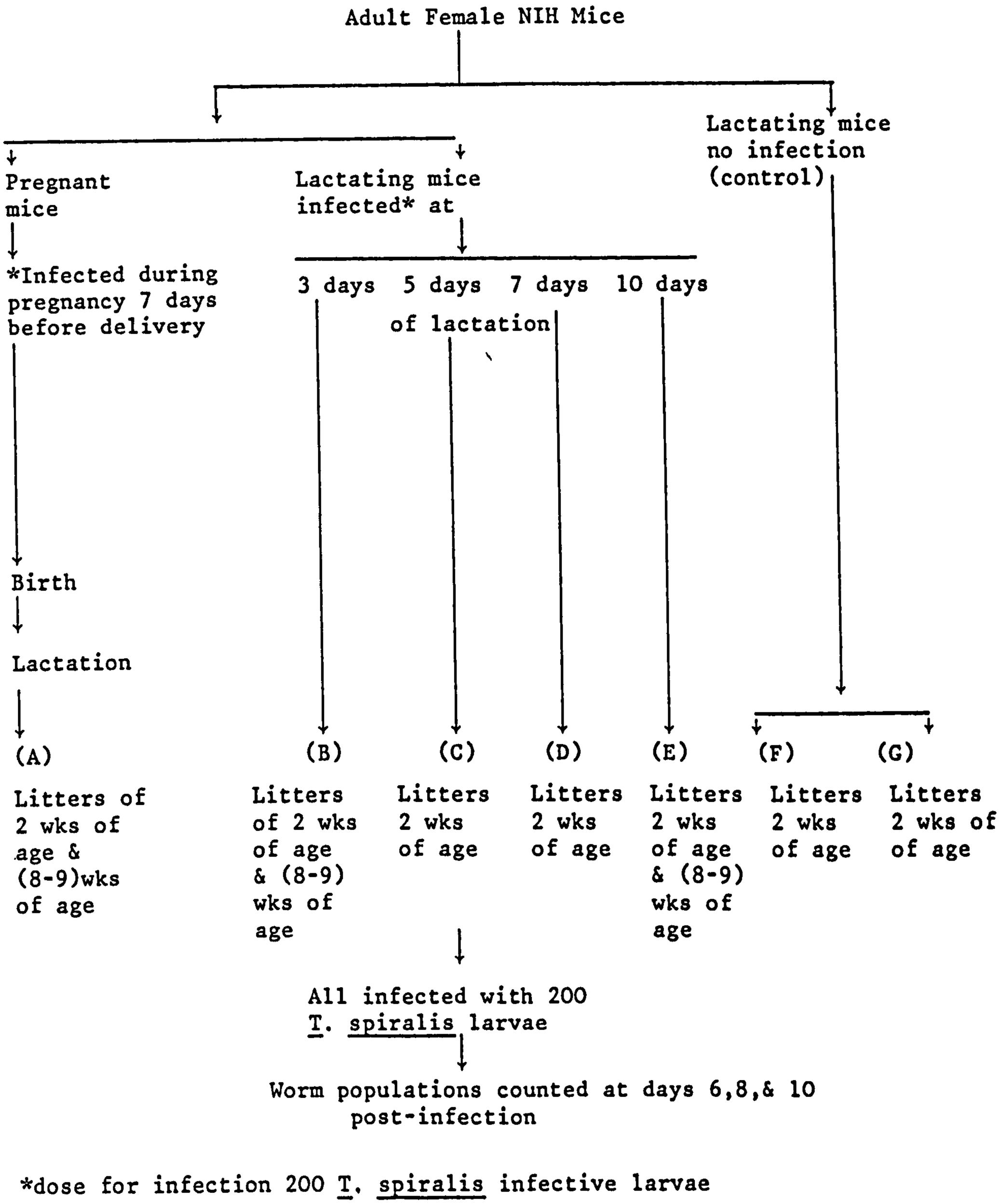
s.d. standard deviation

Statistical significance between groups on day 6 p.i.

Group 1 vs 3 = P<0.001

Group 1 vs 4 = P<0.001

Protocol 1.4: Experimental design for the effect of pre-and post-natal infections of NIH mice to their offspring



*dose for infection 200 T. spiralis infective larvae

Table 1.14 *Trichinella spiralis* infection in young mice.

Transfer of protection to suckling infants.
Duration of immunizing infection in mothers.

Groups	Mother treatment	Infants infected with 200 larvae at 2 weeks of age and suckling their mothers — Mean worm burden (± s.d) — Days Post-infection									Statistical significance between groups on day 6 post-infection
		6 p.i (n = 18)			8 p.i (n = 18)			10 p.i (n = 12)			P.value
		A	P	T	A	P	T	A	P	T	
A	Mothers infected with 200 larvae 7 days pre-parturition (i.e. 21 days of infection)	7.11 ±4.68	20.16 ±5	27.27 ±7.54	5.38 ±2.22	15.12 ±5.66	20.5 ± 6.67	1.25 ±1.71	7.75 ±5.62	9 ±6.66	A vs F_1 P<0.001
B	Mothers infected with 200 larvae 3 days post-parturition (i.e. 11 days of infection)	7.55 ±4.44	15.33 ±5.81	22.88 ±8.87	4.61 ±2.22	15.83 ±7.85	20.44 ±8.91	2.16 ±2.28	14.6 ±5.8	16.66 ±7.3	B vs F_1 P<0.001
E	Mothers infected with 200 larvae 10 days post-parturition (i.e. 4 days of infection)	11.77 ±4.55	30.11 ±11.45	41.88 ±14.59	9.33 ±5.65	27.16 ±8.97	36.5 ±12.25	1.5 ±1.44	21 ±12.6	22.5 ±13.6	E vs F_1 N.S.
F_1	Uninfected mothers (control)	10.46 ±5.85	32.79 ±9.89	43.25 ±11.45	4.42 ±3.67	15.87 ±5.7	20.29 ±7.46	3.37 ±3.26	12.62 ±5.4	15.99 ±7.16	

A = Anterior part of the small intestine
P = Posterior part of the small intestine
T = Total worms recovered
n = number of mice used
s.d. = Standard deviation
N.S. = Not significant

Table 1.15 *Trichinella spiralis* infection in young mice.

Transfer of protection to suckling infants.
Duration of immunizing infection in mothers.

Groups	Mothers treatment	Infants infected with 200 larvae at 2 weeks of age and suckling their mothers								
		Mean worm burden (±s.d.)								
		Days post-infection								
		6 p.i. (n=12)			8 p.i. (n = 12)			10 p.i. (n = 7)		
		A	P	T	A	P	T	A	P	T
C	Mothers infected with 200 larvae 5 days post-parturition (i.e. 9 days of infection)	7.91 ± 3.12	28.41 ± 10.92	36.33 ± 12.51	4.66 ± 4.09	21.25 ±11.35	25.91 ±11.75	1.57 ±1.51	12 ± 6.45	13.57 ± 6.95
F_2	Uninfected mothers (control)	(n=12)			(n=12)			(n=8)		
		14.58 ± 4.38	31.83 ± 8.08	46.41 ± 9.98	4.75 ±3.49	16.66 ± 5.21	21.41 ± 6.84	3.37 ±3.38	11.25 ±5.97	14.62 ±8.36
	Statistical significance (P.value)	P<0.01								

A = Anterior part of the small intestine
P = Posterior part of the small intestine
T = Total worms recovered
n = Number of mice used
s.d = Standard deviation

Table 1.16 *Trichinella spiralis* infection in young mice.

Transfer of protection to suckling infants.
Duration of immunizing infection in mothers.

Groups	Mothers Treatment	Infants infected with 200 larvae at 2 weeks of age and suckling their mothers								
		Mean worm burden (± s.d)								
		Days post-infection								
		6 (n=12)			8 (n=12)			10 (n=8)		
		A	P	T	A	P	T	A	P	T
D	Mothers infected with 200 larvae 7 days post-parturition (i.e. 7 days of infection)	10.75 ± 6.19	27.66 ±10.46	38.41 ±15.88	5 ±4.18	16.91 ±7.57	21.91 ±9.57	3 ±3.29	11.12 ±6.27	14.12 ±8.77
F_3	Uninfected mothers (Control)	6.33 ±3.91	33.75 ±11.71	40.08 ±12.36	4.08 ±3.96	15.08 ±6.29	19.61 ±8.18	3.37 ±3.38	13.62 ±5.29	17 ±6.05
	Statistical significance (P.value)			N.S.						

A = Anterior part of the small intestine
P = Posterior part of the small intestine
T = Total worms recovered
n = number of mice used
s.d = Standard deviation
N.S = not significant

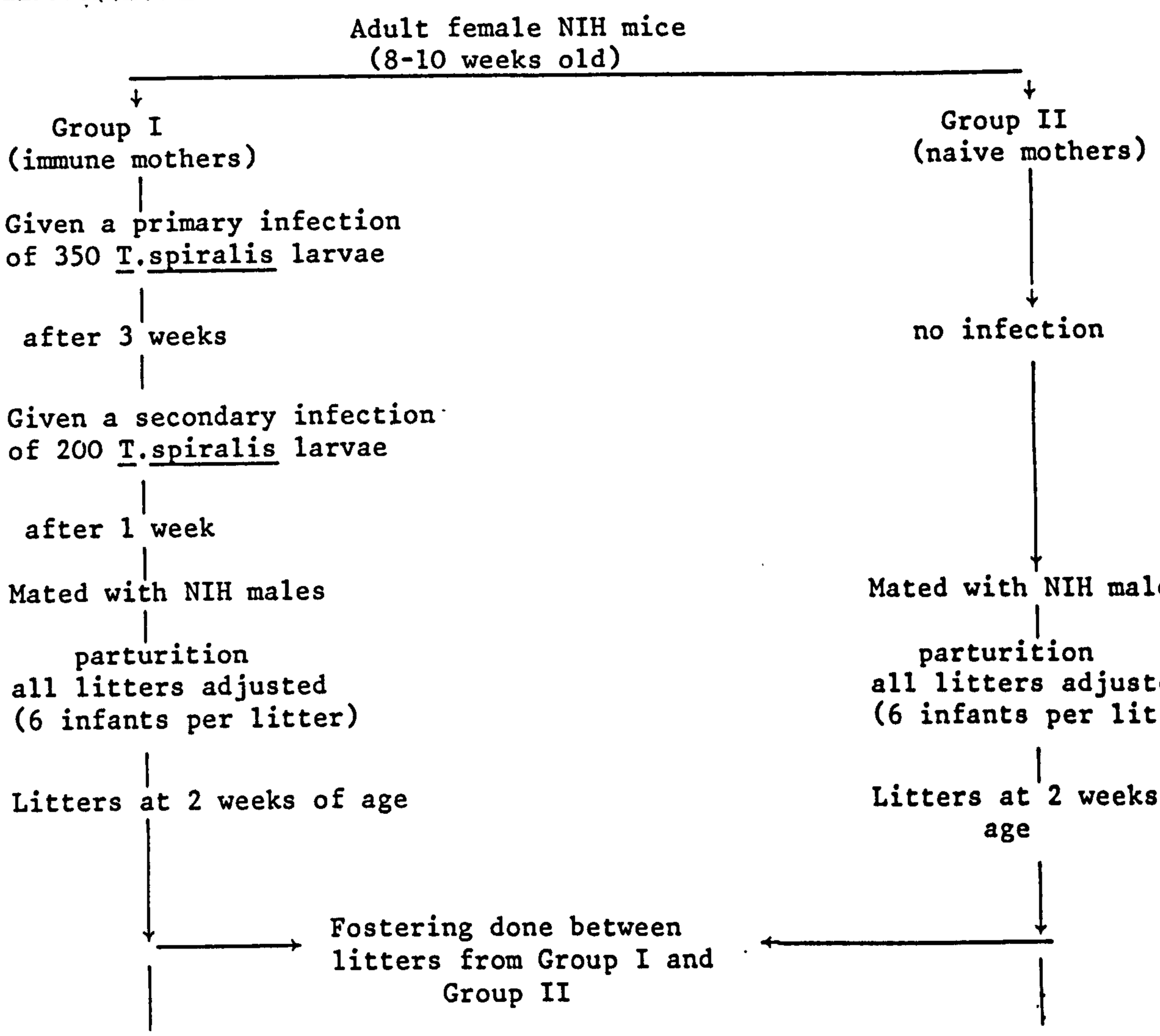
Table 1.17 *Trichinella spiralis* infection in young mice.

Transfer of protection to suckling infants.
Duration of immunizing infection in mothers.

Groups	Mothers treatment	Mice suckled their mothers for 3 weeks and infected with 200 larvae at 8-9 weeks of age. Mean worm burden (± s.d). Days post-infection									Statistical significance between groups on day 6 p.i (p values)
		6 p.i (n=9)			8 p.i. (n=9)			10 p.i (n=6)			
		A	P	T	A	P	T	A	P	T	
A	Mothers infected with 200 larvae 7 days pre-parturition	42.33 ±6.89	17.67 ±5.41	60 ±4.95	25 ±12.72	21.77 ±9.01	46.77 ±11.84	3.66 ±1.63	5.17 ±7.63	8.83 ±7.88	A vs G N.S
B	Mothers infected with 200 larvae 3 days post-parturition	39.77 ±9.4	17.88 ±8.85	57.66 ±8.35	26 ±17.19	12.55 ±7.95	38.55 ±13.88	4.5 ±3.08	4.66 ±3.61	9.16 ±5.19	B vs G N.S
E	Mothers infected with 200 larvae 10 days post-parturition	33.11 ±8.97	20.11 ±5.06	53.22 ±10.57	24.88 ±8.43	10.77 ±6.55	35.66 ±12.43	7.5 ±9.16	3.16 ±2.71	10.66 ±8.93	E vs G N.S
G	Uninfected mothers (control)	47.33 ±13.04	13.33 ±7.82	60.66 ±11.22	18.88 ±9.94	19.88 ±7.44	38.77 ±11.79	1.67 ±2.66	3.83 ±4.16	5.5 ±6.59	

A = Anterior part of the small intestine
P = Posterior part of the small intestine
T = Total worms recovered
n = number of mice used
s.d = Standard deviation
N.S = not significant

Protocol 1,5



10 litters used (litters number)		10 litters used (litters number)
1, 2	(Group 1), 6 hours of fostering	1, 2
3, 4	(Group 2), 12 hours of fostering	3, 4
5, 6	(Group 3), 1 day of fostering	5, 6
7, 8	(Group 4), 3 days of fostering	7, 8
9, 10	(Group 5), 5 days of fostering	9, 10

After fostering all litters infected with 200 T. spiralis larvae, killing and counting the worms on day 6 post-infection.

The control mice for each group:

1. Two litters (6 infants each) of 2 weeks old mice suckling their immune mothers, Treatment C.
2. Two litters (6 infants each) of 2 weeks old mice suckling their naive mothers, Treatment D.
3. Five adult mice (10-12 weeks old) for worm establishment, for each infection.

*Actual dose of infection: 205.25 ± 2.21 (Group 1), 202.00 ± 3.6 (Group 2)
203.16 ± 4.11 (Group 3), 207.33 ± 1.52(Group 4)
207.66 ± 3.51 (Group 5).

Table 1.18 Trichinella spiralis infection in young mice.
The duration of intake of immune milk and protection in 2 weeks old mice given 200 larvae

Time of fostering before infection in Treat.A & B	Worms recovered on day 6 p.i (Mean ± s.d)											
	Treatment "A"			Treatment "B"			Treatment "C"			Treatment "D"		
	n=6 1st litter	n=6 2nd litter	n=12 Both	n=6 1st litter	n=6 2nd litter	n=12 Both	n=6 1st litter	n=6 2nd litter	n=12 Both	n=6 1st litter	n=6 2nd litter	n=12 Both
6 hrs (Group 1)	21.83 ± 7.08	24.66 ± 4.17	23.25 ± 5.73	22.83 ± 8.03	17.66 ± 5.61	20.25 ± 7.14	Not Done			40.33 ± 9.00	not done	40.33 ± 9.00
12 hrs (Group 2)	24 ± 7.16	23.16 ± 6.17	23.58 ± 6.39	29.83 ±11.37	22.5 ±10.36	26.16 ±11.06	19.83 ± 3.54	not done	Θ19.83 ± 3.54	37.66 ± 9.02	*37.75 ± 3.5	37.7 ± 7.02
1 - day (Group 3)	25 ± 4.81	15.5 ±4.84	20.25 ±6.77	35.33 ±6.31	26.5 ±7.52	30.91 ±8.07	17.5 ±3.61	18.83 ±5.23	18.16 ±4.34	38.66 ±7.96	*41.75 ±2.21	39.9 ±6.27
3-day (Group 4)	11.5 ±4.67	13.33 ±4.63	12.41 ±4.54	23.83 ±4.16	24.5 ±6.15	24.16 ±5.02	13.83 ±4.26	8.83 ±6.52	11.33 ±5.87	34.5 ±5.12	34.33 ±2.66	34.41 ±3.89
5 - day (Group 5)	13.5 ±4.89	15.5 ±8.24	14.5 ±6.54	26.33 ±5.71	25.5 ±7.96	25.91 ±6.62	17 ±7.26	18.16 ±3.43	17.58 ±5.45	35.5 ±2.81	29 ±5.02	32.25 ±5.15

n = number of mice used * = n = 4 Θ = n = 6 s.d. Standard deviation

Treatment A = 2 wks infants born to naive mothers and suckling immune mothers for specified periods
" B = 2 wks " " " immune " " " naive " " " "
" C = 2 wks " suckling their immune mothers throughout
" D = 2 wks " " " naive " "

Statistical significance (P.value) : Treatment "A" vs "C" not significant in all groups
Treatment "A" vs "D" $P < 0.001$ in all groups

Table 1.19 *Trichinella spiralis* infection in young mice. The duration of intake of immune milk and protection in 2 weeks old mice given 200 larvae.

Time of fostering before infection	Worms recovered on day 6 p.i. (Mean ± s.d)											
	Treatment "A"			Treatment "B"			Treatment "C"			Treatment "D"		
	1st litter n=5	2nd litter n=7	Both n=12	1st litter n=5	2nd litter n=4	Both n=9	1st litter n=7	2nd litter n=6	Both n=13	1st litter n=7	2nd litter n=6	Both n=13
6 - 8 hours (Group 1)	35.2 ±10.75	34.42 ±11.27	34.75 ±10.55	33.4 ± 5.85	32.75 ±10.24	33.11 ± 7.52	28.71 ± 5.31	26 ± 6.22	27.46 ± 5.68	49.85 ±19.17	51 ±8.59	50.38 ± 14.66
Statistical significance (P.value)	A vs D P<0.01 A vs B N.S. A vs C P<0.05 B vs C N.S. B vs D P<0.01 C vs D P<0.001											

Treatments

A 2 wks infants born to naive mothers and suckling immune mothers for 6-8 hours only before infection of the infants with 200 larvae.

B 2 wks infants born to immune mothers and suckling naive mothers for 6-8 hours only before infection of the infants with 200 larvae.

C 2 wks infants born to and suckling their immune mothers before infection of the infants with 200 larvae.

D 2 wks infants born to and suckling their naive mothers before infection with 200 larvae.

E Adult mice infected with 200 larvae as control for establishment.

(A = anterior part of the small intestine
P = posterior part of the small intestine
T = total worms recoveredfrom both parts
s.d = standard deviation
n = number of mice used

Treatment "E" Worms recovered on day 6 p.i (mean ± s.d) n = 4			
	A	P	T
mean ±s.d	58 ± 7.61	22.77 ± 9.06	80.75 ± 6.55

Table 1.20 — Trichinella spiralis infection in young mice
Feeding of naive infants (2 weeks old) with quantified immune milk* before infection with 200 larvae.

Groups	Infant mice	No. of infants in each group	Worms recovered on day 6 p.i (Mean ± s.d) Location in the small intesting			Statistical significance between groups (P.value)
			Anterior	Posterior	Total	
A	2 weeks old mice born of naive mothers given two doses of neat immune milk (no dilution) 0.15 ml each in 24 hour interval	5	13	32	45	A vs C P<0.02
			3	21	24	A vs B N.S
			6	19	25	B vs C N.S
			10	25	35	
			3	36	39	
			Mean 7 ±s.d ±4.41	26.6 ± 7.23	33.6 ± 9.04	
B	2 weeks old mice born of naive mothers given two doses of diluted immune milk mixed 1:1 with P.B.S 0.15 ml each in 24 hour interval	5	7	47	54	
			20	26	46	
			10	24	34	
			2	43	45	
			6	22	28	
			Mean 9 ± s.d ±6.78	32.4 ±11.67	41.4 ±10.33	
C	2 weeks old mice born of naive mothers given two doses of P.B.S, 0.15 ml each in 24 hour interval (as control)	4	11	32	43	
			2	57	59	
			32	47	79	
			16	40	56	
			Mean 15.25 ±s.d ±12.58	44 ±10.61	59.25 ±14.88	

* Immune milk was collected during the mid-lactation period from mothers which had been infected with 350 larvae as a primary infection, followed after 3 weeks with 200 larvae as secondary infection.

s.d standard deviation

N.S not significant

Protocol 1.6: The fecundity of T. spiralis in primary infections in infant mice (2 weeks old) and adult mice

Infant Mice (2 weeks old)

Group I

Born to immune* mothers n=14

Group II

Born to naive (uninfected) mothers n=14

Adult mice (8-10 weeks old)

Group III

Naive (uninfected) n=13

All mice infected with 200 larvae (same day with same batch of larvae)

6 days post-infection

6 mice from group I)
6 mice from group II) were killed to determine the
5 mice from group III) number of adult male and female
) T. spiralis established in the small intestine

35 days post-infection

8 mice from group I)
8 mice from group II) were killed to determine the
8 mice from group III) number of muscle larvae per each mouse.

* Mothers were given a primary and secondary infection of 200 T. spiralis larvae 3 weeks apart. A week after the last infection all mice were mated.

Table 1.21: Trichinella spiralis infection in young mice. The numbers of male and female adult T. spiralis in the small intestine of infant mice (2 weeks old) and adult mice.

Groups		Number of worms established on day 6 post-infection (Mean ± s.d.)		
		Female	Male	Male & Female (Total)
I	2 weeks old mice born to immune mothers (n=6)	11	3	14
		11	3	14
		16	1	17
		10	1	11
		16	3	19
		17	4	21
	Mean ± s.d.	13.5 ±3.15	2.5 ±1.22	16.0 ±3.69
II	2 weeks old mice born to naive mothers (n=6)	19	7	26
		21	8	29
		29	7	36
		21	2	23
		32	15	47
		25	9	34
	Mean ± s.d.	24.5 ±5.13	8.0 ±4.19	32.5 ±8.59
III	Adult mice 8-10 weeks old naive (control) (n=5)	29	9	38
		33	11	44
		36	5	41
		41	17	61
		29	7	36
	Mean ± s.d.	33.6 ±5.08	9.8 ±4.6	44 ±9.97

Statistical significance between the numbers of Female T. spiralis established in different groups. (Analysis of Variance)

Group I vs II P<0.001 (t = 4.263 d.f.=14)
Group II vs III P<0.001 (t = 4.176 d.f.=14)
Group I vs III P<0.001 (t = 9.22 d.f.=14)

Table 1.22: Trichinella spiralis infection in young mice. The number of T. spiralis larvae recovered from the muscles of infant mice (2 weeks old at time of infection) and adult mice, 35 days post-infection.

	Muscle larvae recovery (Mean ± s.d.)		
	Infant mice		Adult mice
Groups	Born to immune (I) mothers	Born to naive (II) mothers	(III)
	2666	4500	5500
	3500	5000	5833
	3666	6000	6166
	4500	6166	6833
	4666	7666	8166
	5000	8166	8500
	5500	9000	9166
	6166	9500	10166
Mean	4458	6999.75	7541.25
±s.d.	±1140.05	±1851.58	±1703.62
Number of T.spiralis adult Female worms established on day 6 post-infection Mean ± s.d. (Ref.Table 1)	13.5 ±3.15	24.5 ±5.13	33.6 ±5.08

In order to do statistical analysis (one way analyses of variance) each figure in table 2 has been divided by the corresponding mean number of Female T. spiralis. The results as follows:

Group I	Group II	Group III
197.48	183.67	163.69
259.26	204.08	173.60
271.56	244.90	183.51
333.33	251.67	203.36
345,63	312.90	243.04
370.37	333.31	252.98
407.41	367.35	272.79
456.74	387.76	302.56
330.22	285.70	224.44
±84.44	±75.58	±50.70

Analysis of variance: Group I vs II N.S. (t=1.24 d.f. 21)
Group II vs III N.S. (t=1.71 d.f. 21)
Group I vs III P<0.01(t=2.94 d.f. 21)

N.S. = not significant s.d. = standard deviation

Table 1.23: Induction of protection or tolerance in NIH mice by infection of T. spiralis at an early age

Treatment, challenged at 10 weeks of age following	Worm burden - mean ± standard deviation of 5 mice Days Challenge Infection				Statistical significance (P value) Primary vs naive on	
	Day 4	Day 6	Day 8	Day 10	Day 4	Day 8
Primary at 1 week old	106.2 ±20.6	18.6 ±16.9	0	0	P < 0.01	P < 0.001
Primary at 2 weeks old	138.4 ±54.26	14.4 ±8.38	0.2 ±0.44	0	N.S.	P < 0.001
Primary at 3 weeks old	89 ±25.9	12.8 ± 9.14	0.2 ±0.44	1 ±0.81	P < 0.001	P < 0.001
Naive control	170.6 ±16.04	128.0 ±17.63	47.2 ±7.36	27.6 ±20.5		

Primary infections: 1 week old - 200 T. spiralis;

2 and 3 weeks old - 400 T. spiralis

Challenge infection: 400 T. spiralis

N.S. = Not significant

SECTION 2

THE TRAFFIC OF LYMPHOCYTES IN LACTATING MICE AND THE LOCALIZATION OF LYMPHOCYTES FED ORALLY TO SUCKLING MICE.

2.1 Introduction:

The main objective of following the traffic of lymphoblasts to the small intestine is to throw some light on the immunological responses to and mechanisms of expulsion of T. spiralis. Cells from mesenteric lymph nodes (MLN) and thoracic duct lymph (TDL) of infected animals have been shown to be able to transfer immunity against nematode parasites of the gut (Wakelin, 1975; Wakelin and Lloyd, 1976b; Ogilvie, Love, Jarra and Brown, 1977; Manson-Smith, Bruce and Parrott, 1979b). These cell populations during infection contain a large number of activated blasts which readily take up isotopically labelled DNA precursors or analogues of DNA precursors. Such cells are known to have a propensity to extravasate into the mucosal layer of the small intestine (Gowans and Knight, 1964; Hall, Parry and Smith, 1972; Parrott and Ferguson, 1974). Immunoblasts could therefore make close, if not direct, contact with parasites in the gut tissue, although contact between such cells and the surface of intestinal nematodes in vivo has never been reported. Equally they are likely to initiate/control other components of the immunological response.

Transfer of immunity to nematode infection has been studied extensively in laboratory models. Thus the expulsion of Nippostrongylus brasiliensis from the rat intestine can be accelerated by the transfer of immune MLN cells (Keller and Keist, 1972; Kelly and Dineen, 1972; Dineen, Ogilvie and Kelly, 1973; Dineen, Kelly and Love, 1973) or immune TDL (Ogilvie et al., 1977; Nawa and Miller, 1978), and in rats and mice immunity to

Trichinella spiralis can be transferred with MLN cells (Love, Ogilvie and McLaren, 1976; Wakelin and Wilson, 1977; Grencis and Wakelin, 1982) or TDL (Crum, Despommier and McGregor, 1977).

A technique that has been used frequently to trace the traffic of cells of the gut-associated lymphoid tissues (GALT) to the site where they are presumed to function, namely the lamina propria, is to incubate cells from the mesenteric lymph node or thoracic duct lymph with the DNA analogue 5-iodo-2-deoxyuridine radio-labelled with ^{125}iodine ^{125}(I-UdR) and to determine their subsequent location following intravenous injection, by gamma counting of appropriate tissues.

During intestinal infection with T. spiralis in the mouse (Rose et al., 1976 a & b; Manson-Smith et al., 1979a; Ottaway, Manson-Smith, Bruce and Parrott, 1980; Ottaway, Bruce and Parrott, 1983) and T. spiralis and Nippostrongylus brasiliensis in the rat (Love and Ogilvie, 1977) the enteral phase of the infection is associated with enhanced localization of mesenteric lymphoblasts in the small intestine, indicating that the infected tissue is more attractive to or retentive of blast cells. It has been found that in T. spiralis infected NIH strain mice the enhanced localization in the small intestine of radio-labelled blasts from infected donors is greatest in recipients infected 2-4 days previously (Rose et al., 1976a; Manson-Smith et al., 1979a; Ottaway et al., 1980). These authors showed that day 4-donors produced a large number of cells and that day 4-recipients produced maximum accumulation of MLN in the small intestine.

In naive uninfected mice, both T. and B blasts home to the gut mucosa (Guy-Grand, Griscelli and Vassalli, 1974; Parrott, Rose, Sless, de Freitas and Bruce, 1976; Sprent, 1976). However, in NIH mice infected with

T. spiralis, the majority of cells which accumulate in the infected small intestine early in infection are T lymphoblasts (Rose et al., 1976a; Manson-Smith et al., 1979a), and the number of B blasts from MLN suspensionsmigrating to the gut is the same in infected as in normal controls (Rose et al., 1976a). It has also been shown that a population of functional T lymphocytes is necessary for expulsion of T. spiralis and for the onset of crypt hyperplasia and of villous atrophy (Manson-Smith et al., 1979b). It seems clear that the homing of lymphoblasts to the intestine is not an antigen driven event (Halstead and Hall, 1972; Moore and Hall, 1972; Parrott and Ferguson, 1974; Guy-Grand et al., 1974). Experiments have shown that this localization of blast cells occurs in unsuckled newborn rats and in a graft of foetal gut where no such antigenic stimulation is occurring (Halstead and Hall, 1972; Moore and Hall, 1972). Furthermore Rose et al., (1976a & b) have demonstrated the absence of antigen specificity in two ways. Firstly by the enhanced localization in the infected gut of lymphoblasts from uninfected donors, and secondly by the observation that even peripheral lymphoblasts sensitized to the contact sensitizer oxazalone · accumulate in the small intestine of infected recipients, although they do not do so in normal intestines.

The observation that IgA antibodies,readily detected in the colostrum of swine,(Bohl, Gupta, Olquin and Saif, 1972) and rabbit,(Montgomery, Cohn and Lally, 1973) had specificity for antigen fed orally, while IgA antibodies of the same specificity were absent in serum, suggested that gut stimulated lymphoblasts had migrated to the mammary gland. Many investigators have demonstrated the presence of leucocytes in colostrum and milk, including both T and B lymphocytes (Diaz-Jouanen and Williams,

1974; Goldblum, Ahlstedt, Carlsson, Hanson, Jodal, Lidin-Janson and Sohl-Akerlund, 1975; Parmely, Beer and Billingham, 1976; Ogra and Ogra, 1978). One tissue source of IgA-producing lymphocytes in the milk is the gut-associated tissues (Goldblum et al., 1975). Experiments with isotopically labelled lymphoblasts involving mesenteric lymphoblasts and thoracic duct lymphoblasts have shown that these cells do migrate to the mammary gland (Roux,McWilliams, Phillips-Quagliata, Weisz-Carrington and Lamm, 1977; Love and Ogilvie, 1977; Rose, Parrott and Bruce, 1978). Roux et al. (1977) showed that normal mesenteric lymph node (MLN) lymphoblasts as compared to peripheral (axillary, inguinal and cervical) node (PN) blasts will preferentially seek the mammary gland after intravenous transfer into lactating recipients, which indicates that MLN blasts have greater tendency to home to the mammary gland than do PN blasts; moreover, the homing cells were shown to be committed to the production of IgA while still in the mesenteric lymph node, and within a day of reaching the mammary gland 90% of cells of donor origin were IgA - secreting plasma cells. The experiments of Love and Ogilvie (1977), (see also considerations of Ogilvie and Parrott, 1977), were prompted by observations that expulsion of nematodes such as Trichinella spiralis and Nippostrongylus brasiliensis is delayed in lactating animals, and it was postulated that this delay could be a consequence of appropriately primed cells failing to reach the gut because of diversion to the mammary gland. This postulate was not however fulfilled; Rose et al (1978) and Parrott (1979) showed that lactation did not divert gut-derived cells from the intestine, in fact there was a larger accumulation of cells in the small intestine of lactating mice reflecting the greatly increased size of the gut during lactation. They also found a substantial amount of

radioactivity located in the mammary glands of lactating mice, and this was confirmed by the finding of labelled cells by auto-radiography; no significant amounts of radioactivity were detected in the mammary glands of virgin mice.

It has been shown that colostrum and milk from most mammals are essentially suspensions of viable cells in a highly nutritive medium (Beer, Billingham and Head, 1974). Apart from a small component of alveolar and ductal epithelial cells in various stages of disintegration, other cellular elements namely macrophages and lymphocytes were represented in considerable numbers (Head, 1977). There is increasing evidence (Ogra, Weintraub and Ogra, 1977) that milk lymphocytes may be able to gain access to the newborn's tissue via transit of intestinal epithelium; also Beer and his colleagues had provided evidence that cell-mediated competence is transferred directly from mother to newborn during suckling (Beer et al., 1974; Beer and Billingham, 1975; Head, Beer and Billingham, 1977).

In the present study, the localization of lymphoblasts within regions of the small intestine during the course of T. spiralis infection, and the migration of intravenously injected lymphoblasts to the mammary glands of lactating and virgin mice have been studied for the confirmation of the earlier reports and as a base for the new investigation involving the localization of labelled adult mesenteric cells administered orally to suckling infants.

2.2 Materials and Methods:

2.2.1 Treatment of donors:

Mesenteric lymph nodes (MLN) were removed from mice which had been

infected with T. spiralis for 4 days. In experiments involving lactating and suckling mice, MLN were taken either from non-lactating or lactating mice which had been suckling their infants.

2.2.2 Donor cell suspensions:

Mesenteric lymph node cells (MLNC) were gently dispersed by teasing with forceps and scalpel in RPMI-1640 medium containing 20 mM Hepes buffer (RPMI) and 2% foetal calf serum (Gibco-Biocult Ltd., Paisley, Scotland). The cell suspensions were filtered through stainless steel mesh, were washed thoroughly and clumps of dead cells removed by suction. The suspension was then pelleted by centrifugation (250 g for 10 minutes, twice). The number of viable cells was determined by their ability to exclude 0.1% trypan blue. All cell suspensions were prepared at room temperature.

2.2.3 In vitro labelling of lymphocytes:

To label cells undergoing DNA synthesis, mesenteric lymph node cells were incubated with the radiolabelled analogue of thymidine ^{125}I-5-iodo-2'-deoxyuridine (^{125}I-UdR), specific activity 185 GBq/mmol(5 Ci/mg), (Radiochemical Centre, Amersham, England). Incubations of 10^7 viable cells per ml were carried out in RPMI with 5% foetal calf serum containing 18.5 KBq (0.5 μCi) per 10^7 viable cells for 60 minutes at 37°C in a gently shaking water bath. In order to label small lymphocytes as distinct from lymphoblasts, cells were incubated in radiolabelled sodium chromate (Na_2 ^{51}Cr O_4). Radioactive chromium enters cells and binds to intracellular protein with a subsequent very low loss from living lymphocytes. A further advantage of ^{51}Cr is that after release from cells it is excreted rapidly

(Ford, 1978). The suspensions were incubated at a concentration of 50 μCi ^{51}Cr (sodium chromate ^{51}Cr, Radiochemical Centre, Amersham), per 5×10^7 cells per ml for 30 minutes at 37° C in a gently shaking water bath. After labelling, the cells were washed three times in RPMI and their viability assessed with 0.1% trypan blue. Recipients were injected via a lateral tail vein with $1.5 - 2.5 \times 10^7$ viable cells in 0.2 ml from a suspension in which at least 85% of cells were viable. Duplicate doses were retained to determine the amount of injected radioactivity. In experiments involving suckling infants, labelled cells (dose $4 \times 10^6/5$ μl per infant unless otherwise stated) were fed to the recipients by oral intubation. In double-label experiments, cells were incubated with either ^{125}I-UdR or ^{51}Cr (combined doses of 2×10^6 ^{125}I-UdR + 2×10^6 ^{51}Cr/μℓ) and fed to the infants by oral intubations. In experiments with free isotopes, 5 μℓ doses of both ^{125}I-UdR and ^{51}Cr containing (0.4 - 0.8 μCi/ml of ^{125}I-UdR + 60-80 μCi/ml of ^{51}Cr) were fed to infants. Actual doses of labelled cells and free isotopes in each experiment are recorded in the tables of results.

2.2.4 Treatment of recipients:

Animals were either left untreated (i.e. not infected) or infected with approximately 450 T. spiralis viable larvae 4 days before receiving labelled MLN cells. Virgin mice and lactating mice were injected with labelled cells from syngeneic donors. Suckling infants were fed orally with labelled cells or free isotope.

2.2.5 In vivo distribution of labelled cells:

At various times, usually 20 or 24 hours after cell transfer, the

recipients were killed with ether, and their organs were removed and placed in small vials for counting. A variety of lymphoid and non-lymphoid tissues was removed as follows: peripheral (axillary or brachial or inguinal) and mesenteric lymph nodes, Peyer's patches, small intestine either entire or divided into anterior and posterior halves, caecum, large intestine, spleen, liver, lungs and mammary glands. In experiments involving suckling infants, the stomach (in some cases the stomach wall and the stomach contents were taken separately), thymus and limbs were also removed. The radioactivity in each organ was measured by counting for one minute in a gamma-counter (Gamma Set 500, ICN). The one-minute background counts were measured for each experiment and were subtracted from the organ counts. In the double-label experiments, simultaneous counts were performed on two channels measuring either ^{125}I-UdR or ^{51}Cr. The amount of radioactivity was expressed as a percentage of the injected dose recovered from the various organs.

2.2.6 Statistical analysis:

The significance of differences between experimental and control groups or between different groups was assayed using Student's t-test.

2.3 Results:

2.3.1 The localization of mesenteric lymphoblasts in the small intestine of naive (uninfected) and T. spiralis infected adult NIH mice.

The localization of ^{125}I-UdR-labelled MLN blasts in mice 4 days after receiving T. spiralis infection (i.e. day 4 recipients) 20 hours after their intravenous injection is shown in Table 2.1 and Figure 2.1. The data in Table 2.1 shows that there was a considerably greater accumulation of mesenteric blasts in the anterior small intestine than in the posterior small intestine in both naive and infected mice. In

comparison to naive recipients a significant increase in the % injected dose was found in the anterior small intestine. The results of this experiment show also an increase of localization in mesenteric lymph nodes of infected mice, although it is not statistically significant. In the spleen the increase was significant. The localization of cells in the caecum and large intestine was apparently depressed in infected mice although it was not statistically significant from the localization of uninfected mice. It is notable that the total amount of radioactivity recovered from all organs was greater (although not statistically singificant) in infected mice and the difference can be accounted for by the greater accumulation of cells in the intestine of infected mice. The conclusion from this experiment is the confirmation that the presence of T. spiralis in the gut results in an enhancement of mesenteric lymphoblast localization 4 days after infection particularly in the anterior portion of the small intestine. This enhancement would appear to be mediated by some alteration to the small intestine making it more attractive or retentive for mesenteric blast cells. This study confirms the work of Rose et al.(1976a) and of Manson-Smith et al.(1979a).

2.3.2 The localization of mesenteric lymphocytes in virgin (non-lactating) and lactating NIH mice.

The intestines of lactating mice are much larger than the intestines of virgin mice, the results of Table 2.2 show an approximate 2 to 2½ fold increase in the weight of the intestine during lactation.. This factor will be borne in mind when evaluating lymphoblast localization.

To determine whether there is competition between the mammary gland and the gut for the same population of cells, the distribution of labelled

MLN blasts in lactating and virgin mice was compared (Table 2.3, Figure 2.2). In this experiment ^{125}I-UdR-labelled mesenteric lymphoblasts were prepared from donors which had been infected with T. spiralis 4 days previously as described in the previous experiment, and injected into a group of lactating (7-13 days post-partum) and virgin mice. Twenty hours after the injection of labelled cells, the mice were killed and organs prepared for counting in the usual way. The results show that there was a considerable localization of radioactivity in the mammary glands of lactating mice. The increase in radiolabel was of the order of 11 fold, although the glands were increased by only 4 fold in wet weight at this stage of lactation (Table 2.2). There was an increased accumulation of radioactivity in the small intestine of lactating mice especially in the anterior half ($P < 0.02$), although this did not reflect the arithmetic increase in the mass of the gut during lactation, the weight weight of which increased 2 - 3 fold during mid-lactation (see Table 2.2). Contrary to the slight elevation in the mesenteric lymph nodes, there was statistically significantly decreased localization in the large intestine and liver.

Parrott (1979) drew attention to the fact that in experiments with tissues such as the mammary glands with a very high fluid content, it would be desirable that an isotope without the elution (removal of adsorbed substance by washing) problems associated with ^{125}I-UdR be used. Rannie and Donald (1977) had stressed that tritiated compounds also have the disadvantage of large-scale elution which may lead to gross overestimates of cell concentration. In their opinion ^{51}Cr is the most useful isotope for quantitative studies to such sites. Also Rose et al.(1976a) noted

that the distribution of ^{51}Cr-labelled mesenteric lymph node cells was very different from that of ^{125}I-UdR-labelled blast cells. For these reasons, ^{51}Cr, which labels all lymphocytes, and ^{125}I-UdR were used to label populations of cells from donors.

In the experiment recorded in Table 2.4, mesenteric lymph node cells were prepared from non-lactating mice and injected into virgin and lactating recipients at different stages of lactation. Twenty hours after the injection of the two populations of labelled cells (1.44×10^7 ^{125}I-UdR + 1.56×10^7 ^{51}Cr MLN cells in 0.2 ml dose per mouse), the mice were killed and their organs were prepared for counting. The results show that there are substantial amounts of radioactivity associated with ^{125}I-UdR in the mammary glands of lactating mice at all stages of lactation. There was a 34 fold increase in the amount of radioactivity in the mammary gland in group 2, a 71 fold increase in group 3 and a 50 fold increase in group 4. In the small intestine (mostly the anterior half), more radioactivity with ^{125}I-UdR was found in lactating mice in comparison to virgin mice. In the large intestine of the lactating mice, there was an increase in the amount of radioactivity in contradistinction to the previous experiment. There was no significant increase in the amount of radioactivity in the MLN of lactating mice, although such an increase was observed in the previous experiment and the result for the spleen was likewise reversed.

With ^{51}Cr-labelling, there was a considerable localization of radioactivity in the mammary glands of lactating mice at all stages of lactation with increase of approximately 35-40 fold. Some increase in the amount of radioactivity was also shown in the anterior parts of the

small intestine in all lactating groups. The large traffic of small lymphocytes labelled with ^{51}Cr to the spleen and liver is well demonstrated in virgin mice. There is a consistent but statistically non-significant decrease in % injected label in the spleens of lactating mice, and no significant increase in the livers. These results might suggest that diversion from the spleen to the mammary gland has occurred.

In the previous two experiments (Tables 2.3 and 2.4), suckling infants were removed from their mothers at the time of injection of radiolabelled cells to prevent possible loss of label via the milk. The experiment recorded in Table 2.5 was conducted in the same way as the previous ones, but in this case the injected mothers were allowed to continue to suckle their infants. The mesenteric lymph node cells were collected from non-lactating donors, and the labelled dose (1.00×10^7 ^{125}I-UdR + 1.4×10^7 ^{51}Cr-labelled MLN cells) was given to six virgin mice and eight lactating mice (suckling 5 infants each at 2 days post partum) 24 hours before killing. The results show as before that there was a considerable increase in radio-label in the mammary glands of lactating mothers with both ^{125}I-UdR and ^{51}Cr-labelling.

A substantial amount of radioactivity with ^{125}I-UdR was found in the stomachs of the infants, with a small amount recorded from the small intestine of the infants but not from the other organs which were tested. Very small amounts of ^{51}Cr were also recovered from the stomach and small intestine of these infants.

2.3.3 Oral intubation of labelled mesenteric lymphocytes to suckling infants:

To determine the localization of ^{125}I-UdR and ^{51}Cr-labelled MLN cells

in suckling infants, a number of experiments were carried out with different times of tissue recovery (3,4,6,20,24,48 and 72 hour). The MLN cell suspensions were composed either of live cells or dead cells (suspensions heated to 56°C for 20 minutes before incubation) or additionally free isotopes ^{125}I-UdR or ^{51}Cr were administered. Donors were either lactating or non-lactating mice. The age of suckling infants ranged between 48 hours and 11 days. The doses containing MLN cells (either live or dead) labelled with either ^{125}I-UdR or ^{51}Cr and/or free isotopes were administered as 5 or 10 µl doses by oral intubation. The small intestine, large intestine, spleen, liver, lung, stomach, thymus, MLN and limbs (essentially the bone marrow) were taken variously from suckling infants in different experiments for radioactivity counting. In some experiments, the stomach was divided into stomach wall and stomach contents and the parts were counted separately. The result of these experiments are given in Tables 2.6, 2.7, 2.8, 2.9, 2.10. The data presented in Tables 2.6, 2.7 and 2.8 show that a substantial amount of radioactivity (^{125}I-UdR-label) was found in the infant's stomachs irrespective of the considerable variation observed between experiments. Thus with mice aged between 6 and 11 days of age, the % of injected dose detected at 24 hours in the whole stomach varied as 6.75%, 7.93% and 16.35%, within the corresponding experiments at 6 hours (Tables 2.7 and 2.8), 11.69% and 54.46% recovered after administration of the same inocula. As shown in Table 2.8 the preponderance of activity recorded in the stomachs was present in the stomach contents and the reduction from 6 hours to 24 hours may be attributed to the passage of milk from the stomach. The reduction in this period of time in the amount of recovered ^{125}I-UdR

in the small intestine is considerably less. In the experiment recorded in Table 2.7 there is no reduction in the intestinal label from 6-24 hours, whereas in the experiment recorded in Table 2.8 there is a reduction approaching 50% between 6 and 24 hours. In the experiments of Tables 2.6 and 2.7 the whole stomach and small and large intestine contain approximately 88% of the label recovered from the organs examined, with small amounts only from the spleen, liver, lungs and mesenteric lymph nodes of the young mice. However, the presence of label in these other organs indicates the uptake of labelled cells or the uptake of freed isotope liberated from cells in the stomach and/or intestine. In Table 2.8 where the limbs of the young mice, containing a considerable part of the bone marrow of these animals were tested in addition, c.17% of the ^{125}I recorded is detected in the limbs after 24 hours, indicating a considerable uptake and retention of label. The presence of the almost identical amounts of the injected dose of ^{125}I in the limbs after 3 hours and 6 hours indicates a rapid uptake from the stomach and/or intestine of the infants.

The data concerning ^{51}Cr-labelled cells in Table 2.7 and 2.8 show very clearly that there is very little uptake into the tissues and retention of labelled cells or freed isotope label via the gut. With reference to the amount of label detectable at 24 hours, it is clear that few cells are retained in the stomach by this period. At 6 hours, there is a significant amount present in the stomach, nearly all in the stomach contents, which was slightly lower than the amount detectable at 3 hours. However, even at 3 hours after administration of ^{51}Cr-labelled cells only 25% of the label was detectable in the stomach, with 70 +% detected in the intestine. This finding indicates a very rapid passage of entire cells or freed isotope

through the stomach, with little in the way of binding or uptake of the ^{51}Cr label or of labelled cells by the stomach tissue. Progressively from 3-24 hours the amount of detectable label in the large intestine increases as seen particularly in Table 2.8 to the extent that by 24 hours virtually all of the injected label is detectable in the small and large intestine. Whether it remains associated with entire cells, or is freed and becomes attached to the surface of epithelial cells, but is not internalized to any degree cannot be deduced from these data. It is somewhat surprising that so little of the injected dose has been voided in the faecal mass by 24 hours.

The interpretation and speculation prompted from these experiments apart from the non-internalization of freed ^{51}Cr or of the entry of ^{51}Cr-labelled lymphocytes, is that possibly lymphoblasts labelled with ^{125}I-UdR, (a) remain longer in the stomach than do small lymphocytes, (b) enter the epithelial tissues of the alimentary tract and establish within the body tissues or (c) that label freed from whole or disrupted cells remains in the stomach for some period or (d) freed iodine is internalized and retained in the body tissues to some degree.

To examine these possibilities an experiment utilizing ^{125}I-UdR-labelled lymphoblasts and free ^{125}I-UdR was conducted and the amounts of radiolabel detected at 4, 20, 48 and 72 hours after administration are recorded in Table 2.9. At 4 hours it is quite clear that the amounts of isotope in various organs of the infant body are virtually identical following the inoculum of labelled cells or free isotope, indicating that of the amounts absorbed and distributed around the body virtually all the detectable isotope was taken in as free isotope irrespective of the

original form at inoculation. At the subsequent time intervals there is a progressive reduction in the totals of isotope recovered but with little in the way of variation between cellular and free administration. Perhaps the most interesting additional observation is that of the relatively large amount of isotope detectable in the stomach and particularly in the stomach contents after 3 days. This might point to the physiological feature of a recirculating pool of iodine, possibly via the saliva, in the stomach, as it is very difficult to conceive of ingesta remaining in the stomach for this length of time. The previous experiment showing the absence of ^{51}Cr in the stomach contents at 24 hours would seem to prove this point adequately.

The final experiment in this series involved the oral intubation of infants with live and dead cells labelled with ^{125}I-UdR or ^{51}Cr and also of the free isotopes. The results recorded in Table 2.10 show the radiolabel detected at 6 hours after administration. With the ^{51}Cr-label the only noteworthy point apart from the higher total recovery of label with the dead cells, is the possibility of a slower passage of dead cells through the stomach. As in the previous experiments there is good evidence that very little ^{51}Cr or ^{51}Cr-labelled cells are internalized and retained in the tissues with the only positive detection being in the bone marrow of the limbs.

With the ^{125}I-UdR-labelled cells, there is also a higher degree of recovery of the radiolabel with the dead cell suspension and also an indication of slower passage of cells or of freed label through the stomach. In addition the amounts recovered are higher in all of the tissues excepting the spleen indicating perhaps a slower release of radiolabel from the dead cells, a slower uptake of iodine and therefore

slower excretion. However, in additional experiments (data not reported in detail) recovery at 20, 24 and 28 hours did not reveal these features of larger amounts and larger retention of detected label from the dead cell inocula.

2.4 Discussion:

In the present study the influence of a current intestinal infection of T. spiralis has been used to confirm the altered migration and retention of lymphoblasts to the small intestine in NIH mice after cell transfer, which findings agree with those of Rose et al. (1976a), Manson-Smith et al. (1979a), and Ottaway et al. (1980). It seems that the enhancement of localization of lymphoblasts in infected small intestines is not antigen-specific. Rose et al. (1976a) found that cells from normal donors migrated to the gut of 4 day infected recipients as readily as cells from infected donors. This finding indicates that the migrating cells are not recruited or retained in the gut as a direct consequence of specific priming to the antigen of T. spiralis. This absence of antigenic specificity was also observed by Love and Ogilvie (1977) who observed that intestinal parasites including T. spiralis cause non-specific accumulation of ^{125}I-UdR-labelled rat thoracic duct cells in the small gut. According to these results, it would seem that the most likely explanation for the accumulation of lymphoblasts produced by T. spiralis is a non-specific mechanism.

Most studies of localization of lymphoblasts in the small intestine have been concerned with potential IgA forming B blasts (Gowans and Knight, 1964; Halstead and Hall, 1972; Hall, Parry and Smith, 1972; Guy-Grand

et al. 1974); however, the studies of Rose et al. (1976a & b), Manson-Smith et al. (1979a) have demonstrated that T-lymphoblasts obtained from the mesenteric lymph nodes are also able to migrate to the lamina propria of the small intestine, they showed moreover that mesenteric T-lymphoblasts are more efficient at discriminating between normal and infected gut than unseparated cells. Their studies indicated also that the infection with T. spiralis causes the intestinal retention of mesenteric blasts which would not normally localize in any tissue, and that this increased retention consists primarily of mesenteric T lymphoblasts.

It is difficult to assess the functional significance of lymphoblast migration to the small intestine in relation to T. spiralis expulsion. The evidence provided by Rose et al. (1976a) and Manson-Smith et al. (1979a), and the present observations, show a temporal relationship between lymphoblast migration to parasitized gut on day 2 and 4 post-infection prior to the commencement of the expulsion of worms on day 8 (see Section 1). This suggests that the migrating cells may be involved with the expulsion process. Expulsion of T. spiralis has been found to be T-dependent (Walls et al. 1973), also Manson-Smith et al. (1979b) presented strong evidence that the morphological changes which accompany the intestinal phase of T. spiralis infection in NIH mice are a consequence of a local delayed-type hypersensitivity reaction, in that they are abolished in T-cell deprived mice and immediately restored by the adoptive transfer of primed mesenteric T-lymphoblasts. In NIH mice, the first effects on adult T. spiralis are evident on day 6 as a decrease in production of eggs by the ovary (Fatunmbi, 1978), which closely follows the peak of localization of the lymphoblasts in the gut (Rose et al. 1976a), and the detection of cells capable of

transferring immunity (Wakelin and Wilson, 1977). It is thus tempting to suggest a direct action of lymphocytes. However, there is no *in vivo* evidence to support the suggestion that lymphocytes can directly affect *T.spiralis*. Although it has been shown that immunity to parasitic helminths is thymus-dependent and transferable adoptively with T-cell populations (Mitchell, 1980), and whereas there are many descriptive accounts of T-cells involvement, there have been relatively few analyses of the kinetics of the response or of the nature of function of the cells concerned. T-cells could be involved primarily in anti-worm antibody responses or in initiating and amplifying inflammatory responses. In the case of *T.spiralis*, immunity against the intestinal phase is expressed most strikingly in worm expulsion, and the T-cells appear to have a role in it, perhaps not against the worm itself but their action may be in stimulating the action of other agents. These considerations are most relevant to this study given the evidence that lymphoblasts accumulate in the mammary gland during lactation, and with the probability of lymphocyte migration to the milk as detailed by Head (1977), the interesting possibility of a role for maternal lymphocytes in the lumen or tissues of the infant gut was selected for examination. Initially it was necessary to confirm the findings of Rose *et al.*(1978) and Love and Ogilvie (1977) that lymphoblasts accumulate in the mammary gland during lactation, and in the experiments recorded in this section it is confirmed that there is a significant accumulation of labelled lymphoblasts and lymphocytes in the lactating mammary gland. Although Rose *et al.* (1978) concluded that lymphoblasts were not diverted from the gut to the mammary gland, the data from the present experiments indicates that there may be a degree of diversion. This postulate is arrived at when the amount of the cell traffic

to the lactating gut is related to the mass of the gut tissue. It is observed that the increase in cellular localization in the larger gut of lactating mice is not a pro-rata increase.

From Table 2.2 it is obvious that there is an increase in weight of the small intestine, - taking the weights for the anterior and posterior small intestine together. In other data (ref.Table 3.1) the mean weight of virgin mice is recorded as 24.75 g and in supplementary data - not recorded in detail, the mean weight of lactating mice was measured as 30.20 g.

Thus:	Virgin	Lactating	
Wt. of small intestine (Table 2.2)	1.26 g	2.92 g	
Mean wt. of mice	24.75 g	30.20 g.	
∴ SI as %	5.09%	9.67%	
From Table 2.3			
observed injected dose	2.364	2.978	
expected injected dose (pro-rata weight)		4.54	Discrepancy - 1.57 (34.6%)
From Table 2.4			
(mid-lactation data)			
observed injected dose	5.94	6.99	
expected injected dose (pro-rata weight)		11.43	- 4.44 (38.8%)

It would therefore appear that there are fewer cells localising in the small intestine of lactating mice. Such a shortfall may well comprise part of the population of lymphoblasts localising in the mammary gland during lactation.

It has been suggested that the link between the gut and the mammary gland is an example of a universal linkage between all mucosal immune systems, and the term "common mucosal system" has been used to suggest this relationship (Bienenstock, 1974). Roux et al. (1977) demonstrated that mesenteric lymphoblasts already committed to IgA synthesis can home to the mammary gland late in pregnancy and throughout lactation, but not to the resting mammary gland. These authors also provide evidence that the ability to home to both mammary gland and small intestine is a property of mesenteric node blast cells but not resting small lymphocytes. This might explain the data presented in this study (Tables 2.4 and 2.5) which show the very low recovery of radioactivity when ^{51}Cr was used in comparison to ^{125}I-UdR in these organs. Although antigen drawn homing of lymphoblasts has been shown by Pierce and Gowans (1975) who demonstrated that IgA lymphoblasts were drawn to sites of antigen location in the gut, Rose et al. (1976a) demonstrated that T. spiralis induced homing of lymphoblasts to the gut was not necessarily worm antigen drawn. Antigen, however, is unlikely to play a direct role in homing of (GALT) cells to the mammary gland since this organ appears not to contain enteric antigens adsorbed from the gut (Montgomery, Rosner and Cohn, 1974; Goldblum et al. 1975). Although in the present study the migration behaviour of separated populations of T and B cells to the mammary gland was not examined, many investigators have demonstrated the association of both T and B lymphocytes with the mammary gland (Diaz-Jouanen and Williams, 1974; Parmely, Beer and Billingham, 1976; Ogra and Ogra 1978).

The localization of lymphoblasts in the mammary gland with subsequent exudation into the milk or secretion of antibodies might provide effective

mechanisms for protection of the suckling young. As shown from the work of Bohl et al. (1972) in swine, and Montgomery et al. (1974) in rabbits, these blasts are stimulated as a result of intestinal antigenic stimulation and the milk might as a result have antibodies with specificity for intestinal organisms potentially pathogenic for the infants. Alternatively, primed cells may migrate into the milk and possibly function within the infants as suggested by the studies of Ogra, Weintraub and Ogra (1977), Beer et al. (1974), Beer and Billingham (1975), and Head et al. (1977) which indicated that ingested lymphocytes entered the intestinal tissues and conferred cell-mediated immunological responsiveness to the suckling infants. In the present study when ^{125}I-UdR blast-injected mothers were allowed to continue suckling their infants (see Table 2.5) radioactivity was detected in the suckling infants 24 hours later. This was present mainly in the stomach and not equally distributed between the stomach and intestine. Parrott (1979) described a similar pattern when oxazolone blast-injected mothers were allowed to continue to suckle their young. As will be shown in Section 3, the numbers of lymphocytes present in the milk are relatively small and for the purpose of quantification of labelled cells localization, it was necessary to feed infants with relatively large numbers of labelled cells. The oral intubation of ^{125}I-UdR-labelled mesenteric lymph node blasts into suckling infants (Table 2.6) confirmed the enhanced presence of radioactivity in the stomachs of infant mice as did the natural suckling. The results also show a substantial amount of radioactivity in the stomach contents as distinct from the stomach wall, and also the presence of a considerable amount of radiolabel in the small intestine.

The first significant problem, however, is that the amount of ^{125}I-radiolabel detectable in the stomach contents remains quite high over a considerable period of time, much larger than would be predicted for the passage of ingesta. Oral intubation of ^{125}I-UdR-labelled dead cells or of free isotope yielded the same feature of a relatively high localization of radiolabel in the stomach contents. Although relatively small amounts of radiolabel following live cells intubation were detected in the usual group of organs examined - spleen, liver, lungs, which might indicate a low degree of internalization, equivalent amounts were detectable in infants fed with free ^{125}I-UdR, indicating that virtually all of the internally detected isotope was absorbed as free isotope from the stomach and/or intestine. The relatively small amounts of radiolabel detected internally in these organs also suggest a relatively low degree of retention of the ^{125}I-UdR. However, when the limbs containing much of the infant's bone marrow were examined, very much larger amounts of radiolabel were detected in these tissues indicating a considerable degree of retention/storage or incorporation of the thymidine analogue in synthesising tissues. This latter event would seem unlikely according to the consideration concerning reutilisation of ^{125}I-UdR discussed by Rose et al. (1976a). The only conclusion which can be derived from these results is that a very considerable amount of ^{125}I is freed from the cell suspensions and is internalized and recirculated by some route involving the stomach, and obviously the use of ^{125}I-UdR as a marker for studying the possible uptake of relatively small number of maternal immunocytes by the infant gut is precluded.

The use of ^{51}Cr-labelled cells for these purposes was investigated

more from the point of view of evaluating the uptake of free label from the gut than with a conviction that this label could be used to assess cell internalization.

Since (^{51}Cr)sodium chromate labelled all types of lymphocytes indiscriminately if unevenly - the more cytoplasm (lymphoblasts) the greater the degree of cell labelling, it would have to be the case that relatively large numbers of cells would have to be internalized - even if mostly lymphoblasts, before statistically significant numbers could be detected. In fact only very small amounts of ^{51}Cr radiolabel were detected in the internal organs of the infant mice, with equivalent amounts detected following the administration of live or dead cells or free labelled sodium chromate. Thus quantification of cell internalization by these methods was not possible, but the conclusion at this stage would have to be that from the data recorded, there is no suggestion of the internalization of cells.

In view of these technical difficulties encountered, this approach to the investigation was terminated.

2.5 Summary:

1. The localization of ^{125}I-UdR-labelled mesenteric lymph node cells in the small intestine of NIH mice has been examined. Compared with naive mice, enhancement of the localization of mesenteric lymphoblasts in the small intestine of mice undergoing enteric infection with *T. spiralis* was evident.

2. Lymphoblasts from the mesenteric lymph nodes of NIH mice home to the mammary gland of syngeneic recipients during different lactation periods. Homing does not occur in virgin mice. The recovery of radioactivity in the mammary gland and small intestine was greater in lactating mice.

3. Allowing suckling infants to remain with their mothers after injection of the mothers with radiolabelled cells revealed the presence of radiolabel in the infants alimentary tract. Oral intubation of infants with ^{125}I-UdR-labelled live or dead cells yields equivalent amounts of radiolabel in the gut and internal organs with considerable retention of radiolabel in the stomach contents and in the limbs. That the presence of radiolabel in the internal organs is a function of the uptake of freed ^{125}I-UdR is confirmed by the feeding of free ^{125}I-UdR to the infants. The method is therefore unsuitable to the investigation of internalization of ^{125}I-UdR-labelled cells.

Use of ^{51}Cr Sodium Chromate - labelling all types of lymphocytes, while not presenting a problem of uptake of free isotope, yielded very small amounts of radiolabel in the infants internal organs and tissues. The amounts recorded were too small to allow identification of a cell bound component of isotope which would indicate internalization of maternal cells from the gut.

Table 2.1: The 20 hour localization of ^{125}I-UdR-labelled mesenteric lymphoblasts in the small intestine of naive or infected NIH mice (4 days after infection with T. spiralis).

	Mean % injected dose ± s.d.		
	Naive Uninfected (n=5)	Infected (n=5)	Statistical significance (P.value)
Peripheral lymph node	0.101 ±0.035	0.127 ±0.035	N.S.
Mesenteric lymph node	0.338 ±0.073	0.405 ±0.105	N.S.
Peyer's patches	0.235 ±0.048	0.237 ±0.103	N.S.
Small intestine (anterior)	3.130 ±0.431	4.053 ±0.601	<0.05
Small intestine (posterior)	1.796 ±0.294	2.207 ±0.541	N.S.
Caecum	0.836 ±0.217	0.819 ±0.272	N.S.
Large intestine	1.381 ±0.403	1.292 ±0.391	N.S.
Spleen	1.101 ±0.240	1.531 ±0.157	<0.02
Liver	1.410 ±0.353	1.352 ±0.308	N.S.
Lung	0.440 ±0.102	0.528 ±0.051	N.S.
Total small intestine	4.926 ±0.696	6.261 ±1.111	N.S.
Total recovery (all organs)	10.768 ±1.519	12.553 ±1.667	N.S.

Dose 0.2 ml containing 2×10^7 MLN cells taken from 4 day-infected donors.

n = number of mice used

s.d. = standard deviation

N.S. = not significant

Total recovery = mean ±s.d. of totals of individual mice.

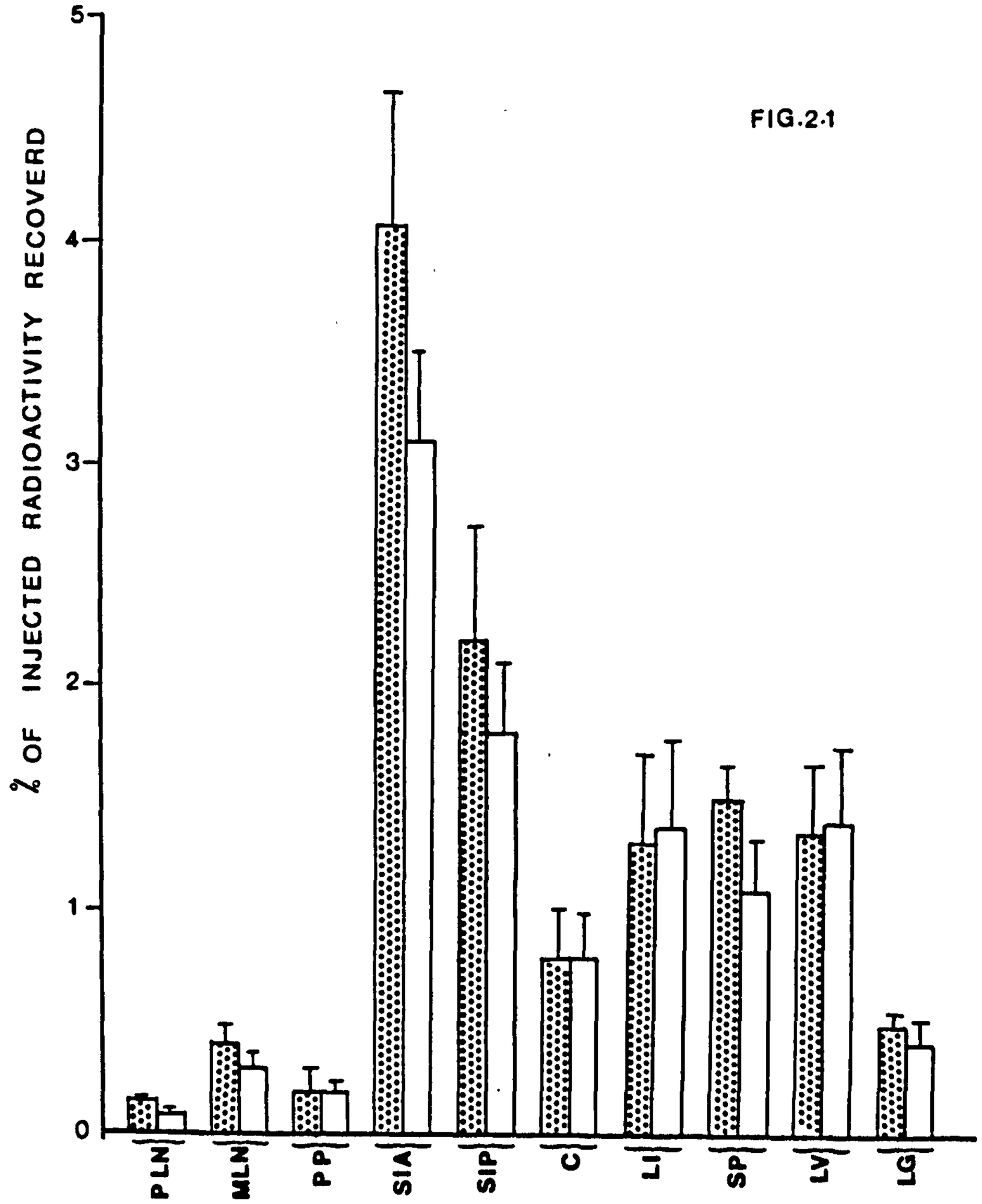


FIG.2.1

Figure 2.1. Distribution of radioactivity in infected ▩ and naive □ N1H mice. 20 hours after injection of ^{125}IUdR-labelled lymphoblasts - Each value represents the mean for 5 mice. Standard deviations are indicated by vertical lines.

PLN : Peripheral lymph nodes.
MLN : Mesenteric lymph nodes
PP : Peyer's patches
SIA : Small intestine (anterior)
SIP : Small intestine (posterior)
C : Caecum
LI : Large intestine
SP : Spleen
LV : Liver
LG : Lung

Table 2.2 Weights of mammary glands and small intestine in virgin and lactating mice

Mice	No. of mice	Organ	Mean *wet weight (gm) ± s.d.	Mean +dry weight (gm) ± s.d.
Virgin	16	●Mammary gland	0.64 ± 0.18	0.45 ± 0.15
		Anterior small intestine	0.79 ± 0.09	0.17 ± 0.02
		Posterior small intestine	0.47 ± 0.07	0.13 ± 0.03
Lactating (early lactation)	6	●Mammary gland	2.34 ± 0.49	0.65 ± 0.13
		Anterior small intestine	1.89 ± 0.12	0.33 ± 0.03
		Posterior small intestine	1.12 ± 0.09	0.19 ± 0.02
Lactating (mid-lactation)	4	●Mammary gland	2.58 ± 0.57	0.80 ± 0.15
		Anterior small intestine	1.83 ± 0.23	0.35 ± 0.05
		Posterior small intestine	1.09 ± 0.27	0.20 ± 0.05
Lactating (late-lactation)	6	●Mammary gland	3.35 ± 0.66	1.02 ± 0.22
		Anterior small intestine	2.19 ± 0.13	0.38 ± 0.02
		Posterior small intestine	1.19 ± 0.15	0.21 ± 0.03

*The small intestine was split longitudinally, washed in saline, dried on filter paper.
+After washing as above the small intestine was dried for 24 hours at 110°C.
●Combined weights of all ten mammary glands per mouse.
s.d. Standard deviation.

The weights of all the mammary glands and portions of the small intestine of the lactating mice in comparison to virgin mice were statistically significantly different at $P < 0.02$ or less.

Table 2.3 The 20 hour localization of ^{125}I-UdR-labelled mesenteric lymphoblasts in mammary glands and small intestine of NIH virgin and lactating mice at the mid-lactation period (7-13 days post-partum).

	Mean % injected dose ± s.d.		
	Virgin (n=4)	Lactating (n=5)	P. Value
Mammary gland	0.161 ± 0.066	1.757 ± 0.310	<0.001
Mesenteric lymph node	0.102 ± 0.035	0.172 ± 0.023	<0.02
Peyer's patches	0.112 ± 0.039	0.122 ± 0.039	N.S.
Small intestine (anterior)	1.324 ± 0.233	1.728 ± 0.169	<0.02
Small intestine (posterior)	1.039 ± 0.178	1.250 ± 0.176	N.S.
Caecum	0.426 ± 0.079	0.579 ± 0.195	N.S.
Large intestine	0.643 ± 0.076	0.455 ± 0.093	<0.02
Spleen	0.580 ± 0.113	0.568 ± 0.154	N.S.
Liver	0.507 ± 0.065	0.338 ± 0.061	<0.01
Lung	0.118 ± 0.051	0.071 ± 0.035	N.S.
Recovery whole small intestine	2.362 ± 0.357	2.978 ± 0.306	<0.05
Total recovery	5.011 ± 0.533	7.041 ± 0.762	<0.01

Donors infected with 400 T. spiralis larvae for 4 days.
The number of suckling infants per female was adjusted to 7.
These were removed at the time of injection.
Dose:- 0.2 ml containing 2×10^7 MLN cells (taken from 8 infected donors).
n = number of mice used
s.d. = standard deviation
N.S. = not significant
Total recovery = Mean ± s.d. of totals of individual mice.

FIG.2.2

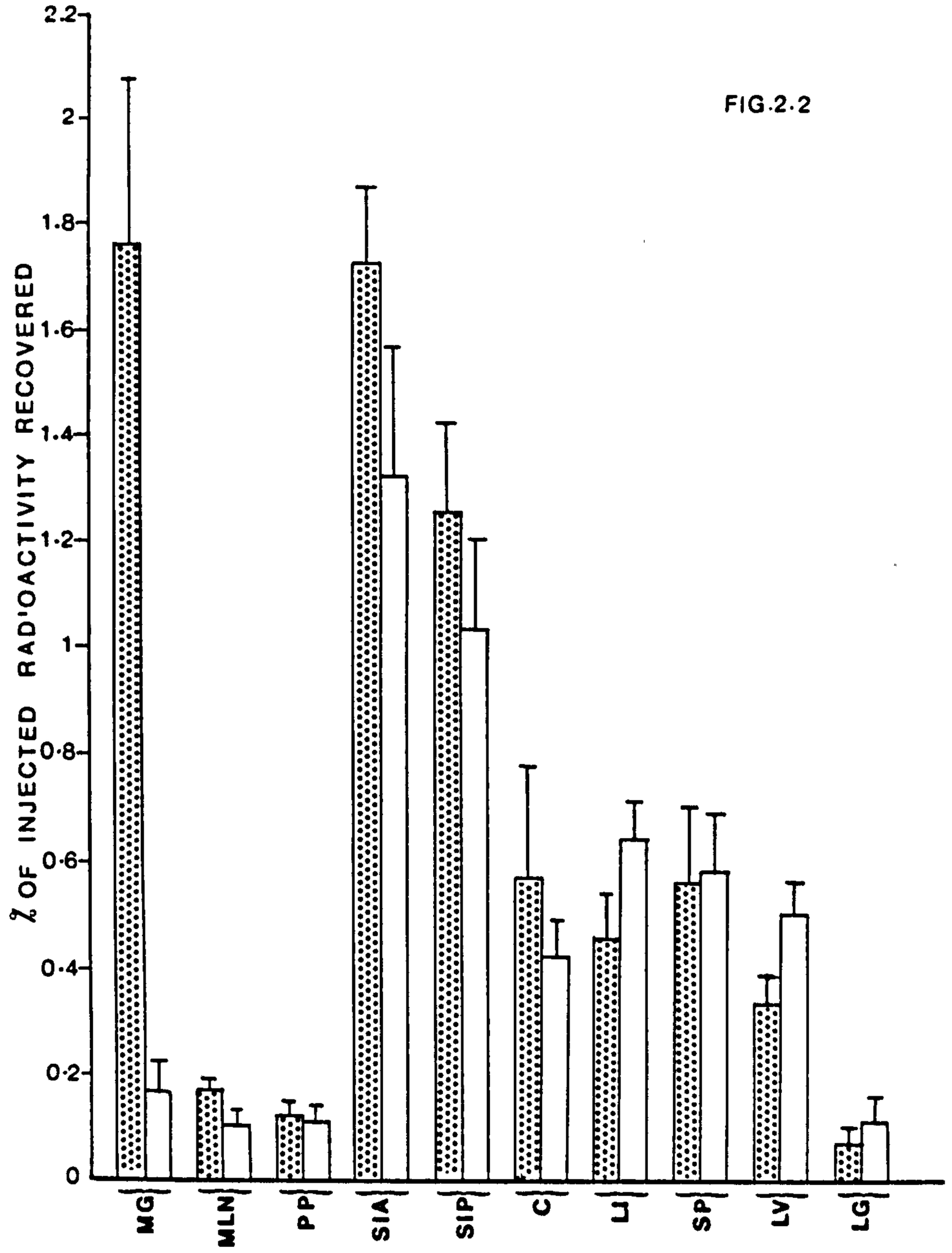


Figure 2.2 Distribution of radioactivity in lactating ▩ and nonlactating □ mice, 20 hours after the injection of ^{125}IUdR - labelled lymphoblasts. Each value represents the mean for 5 mice (lactating)and 4 mice (non-lactating). Standard deviations are represented by vertical lines.

M.G. = Mammary gland
MLN = Mesenteric lymph nodes.
PP = Peyer's patches
SIA = Small intestine (anterior)
SIP = Small intestine (posterior)
C = Caecum
Li = Large intestine
SP = Spleen
LV = Liver
LG = Lung

Table 2.4: 20 hour recovered radioactivity in virgin and lactating mammary glands and other organs of adult mice following injection of ^{125}I-UdR and ^{51}Cr-labelled - mesenteric lymph node cells from non-lactating mice.

		Mean% injected dose ± s.d.											
Recip. Group	Isotopes	Mammary gland	Mesent. lymph nodes	Peyer's patches	Small intest. (ant.)	Small intest. (post.)	Caecum	Large Intest.	Spleen	Liver	Lungs	Small intest. (entire)	Total recovery
virgin (non-lactating) Group 1	^{125}I-UdR	0.12 ±0.10	0.63 ±0.26	0.45 ±0.20	3.72 ±0.73	2.22 ±0.57	0.85 ±0.20	0.66 ±0.09	1.96 ±1.14	1.80 ±0.61	0.51 ±0.31	5.94 ±1.30	12.92 ±0.88
	^{51}Cr	0.07 ±0.11	7.29 ±3.24	1.76 ±0.81	0.78 ±0.11	0.63 ±0.28	0.76 ±0.37	0.49 ±0.17	17.18 ±6.12	26.65 ±5.67	2.22 ±0.96	1.41 ±0.36	57.83 ±3.29
lactating (4-5 days) Group 2	^{125}I-UdR	4.06 ±0.71	0.66 ±0.08	0.40 ±0.10	4.31 ±0.27	2.64 ±0.22	0.72 ±0.24	0.80 ±0.24	1.32 ±0.19	1.57 ±0.16	0.28 ±0.06	6.95 ±0.46	16.76 ±1.29
	^{51}Cr	2.88 ±1.08	6.68 ±1.40	1.51 ±0.32	1.05 ±0.14	0.75 ±0.10	0.55 ±0.21	0.34 ±0.1	14.27 ±0.43	29.25 ±2.10	1.55 ±0.18	1.81 ±0.13	58.83 ±2.66
lactating (9-14 days) Group 3	^{125}I-UdR	8.52 ±4.36	0.71 ±0.19	0.19 ±0.07	4.55 ±0.45	2.44 ±0.30	0.55 ±0.09	1.03 ±0.22	0.94 ±0.22	1.44 ±0.27	0.29 ±0.09	6.99 ±0.70	20.66 ±4.99
	^{51}Cr	2.29 ±0.65	8.35 ±1.06	1.21 ±0.17	1.07 ±0.13	0.50 ±0.10	0.34 ±0.07	0.68 ±0.22	13.38 ±1.08	26.38 ±1.11	1.51 ±0.33	1.57 ±0.20	55.71 ±2.88
lactating (18 days) Group 4	^{125}I-UdR	6.00 ±6.10	0.49 ±0.13	0.20 ±0.04	4.04 ±0.25	2.30 ±0.38	0.57 ±0.08	1.03 ±0.19	1.33 ±0.48	1.16 ±0.23	0.35 ±0.07	6.34 ±0.39	17.47 ±6.6
	^{51}Cr	2.79 ±1.67	7.26 ±1.87	1.35 ±0.19	1.04 ±0.11	0.58 ±0.09	0.39 ±0.08	0.71 ±0.25	13.37 ±2.09	27.26 ±1.27	1.51 ±0.36	1.62 ±0.14	56.66 ±3.14

Dose: 0.2 ml containing 1.44×10^7 ^{125}I-UdR + 1.56×10^7 ^{51}Cr-labelled MLN cells injected 20 hours before killing.
Suckling infants were removed from the lactating mothers at the time of injection of labelled cells.
Donors: 15 non-lactating mice.
Total recovery: Mean ± s.d. of totals of individual mice. s.d. = standard deviation.

Table 2.5: 24 hour recovered radioactivity in virgin and lactating mammary glands and other organs of adult mice, and the stomach and small intestine of infant mice following injection of ^{125}I-UdR and ^{51}Cr-labelled mesenteric lymph node cells into the adult mice.

	Mean % injected dose ± s.d.					
	Virgin (non-lactating) 6 mice		Lactating 8 mice 2 days post partum		5 infants suckling their injected mothers	
	^{125}I-UdR	^{51}Cr	^{125}I-UdR	^{51}Cr	^{125}I-UdR	^{51}Cr
Mammary glands	0	0.005 ±0.006	1.397 ±0.435	0.763 ±0.868		
Mesenteric lymph nodes	0.406 ±0.222	4.613 ±0.404	0.392 ±0.124	5.454 ±1.328		
Peyer's patches	0.192 ±0.055	1.137 ±0.184	0.118 ±0.070	1.184 ±0.201		
Small intestine (anterior)	3.265 ±0.222	0.872 ±0.094	3.505 ±0.227	1.059 ±0.274	(small intestine entire) 0.0569 ±0.0335	0.0392 ±0.0025
Small intestine (posterior)	2.022 ±0.303	0.512 ±0.107	1.883 ±0.243	0.647 ±0.196		
Caecum	0.406 ±0.089	0.381 ±0.058	0.328 ±0.108	0.290 ±0.035		
Large intestine	0.730 ±0.184	0.605 ±0.069	0.500 ±0.156	0.582 ±0.161	0	0
Spleen	1.377 ±0.424	13.007 ±1.355	1.158 ±0.180	10.041 ±1.058	0	0
Liver	1.783 ±0.215	26.501 ±4.003	1.865 ±0.315	29.918 ±2.20	0	0
Lung	0.432 ±0.145	1.721 ±0.201	0.301 ±0.071	1.699 ±0.193	0	0
Stomach (infants)					0.7643 ±0.1744	0.0026 ±0.002

Dose: 0.2 ml containing 1.00×10^7 ^{125}I-UdR + 1.4×10^7 ^{51}Cr-labelled MLN cells. The lactating mice each suckled 5 infants.
Donors: 14 non-lactating mice.
s.d. = Standard deviation.

Table 2.6: 24 hour recovered radioactivity in organs of infant mice following oral intubation of ^{125}I-UdR-labelled mesenteric lymph node cells.

	Mean % injected dose ± s.d. (n = 7)	
Stomach	6.753	±1.150
Small intestine	1.154	±0.227
Large intestine	0.445	±0.144
Spleen	0.091	±0.079
Liver	0.349	±0.189
Lung	0.159	±0.072
Mesenteric lymph node	* 0.586	±0.139
Thymus	0.175	0.059

* MLN Mean ± s.d. (Mice 1-4 = 0 mice 5 - 7 = 0.586 ± 0.139)

Donors: 5 NIH Female non-lactating mice

Recipients: 7 NIH infants (10 days old)

Dose: 10 µl containing 8×10^6 cells

n = number of mice used

s.d. = standard deviation

Total recovery: Mean ± s.d. of totals of individual mice.

Table 2.7: 6 and 24 hour recovered radioactivity in the organs of infant mice following oral intubation of ^{125}I-UdR- and ^{51}Cr-labelled mesenteric lymph node cells.

	Mean % injected does ± s.d.					
Isotope	^{51}Cr			^{125}I-UdR		
Age of recipient	8 days old	2 days old	6-7 days old	8 days old	2 days old	6-7 days old
Time of Recovery	6 h	6 h	24 h	6 h	6 h	24 h
Stomach	2.56 ±2.45	1.07 ±0.07	0.42 ±0.30	11.69 ±3.41	4.58 ±1.12	7.93 ±1.38
Small intestine	51.61 ±17.31	29.72 ±1.37	60.71 ±12.83	2.99 ±2.54	0.86 ±0.70	3.00 ±0.67
Large intestine	1.61 ±0.49	1.89 ±0.21	3.60 ±0.38	0.47 ±0.46	0	0.25 ±0.16
Spleen	0	0.03 ±0.01	0.03 ±0.03	0.36 ±0.18	0.70 ±0.30	0.51 ±0.84
Liver	0.06 ±0.09	0.48 ±0.01	0.08 ±0.04	1.39 ±0.76	2.16 ±0.42	0.60 ±0.34
Lung	0.01 ±0.03	0.05 ±0.09	0.01 ±0.03	0.63 ±0.54	0.33 ±0.55	0.07 ±0.30

Donors: 4 lactating NIH mice

Recipients: 6 infants aged 8 days
6 infants aged 6-7 days
2 infants aged 2 days

Dose: 5 µl containing 1×10^6 ^{51}Cr + 1×10^6 ^{125}I-UdR-labelled cells

s.d. = standard deviation

Table 2.8: 3, 6 and 24 hour recovered radioactivity in infant mice following oral intubation of ^{125}I-UdR and ^{51}Cr-labelled mesenteric lymph node cells.

	Mean % injected dose ± s.d.					
	^{51}Cr			^{125}I-UdR		
	3 h	6 h	24 h	3 h	6 h	24 h
Stomach wall	1.01 ±0.88	0.41 ±0.40	0	2.83 ±0.87	2.77 ±0.99	1.83 ±1.16
Stomach contents	24.66 ±14.44	16.86 ±13.66	0.04 ±0.02	59.54 ±20.94	51.69 ±25.36	14.52 ±4.06
Small intestine	73.85 ±15.16	83.52 ±10.89	86.19 ±10.25	6.52 ±4.34	6.32 ±2.07	3.84 ±1.54
Large intestine	4.57 ±1.99	10.60 ±4.48	14.91 ±3.51	0.35 ±0.30	0.86 ±0.54	1.61 ±0.84
Spleen	0	0	0	0.22 ±0.15	0.93 ±1.14	1.06 ±1.42
Liver	0.04 ±0.01	0.03 ±0.02	0.06 ±0.02	1.50 ±0.69	1.66 ±0.61	1.80 ±1.25
Lungs	0	0	0	0.58 ±0.37	1.20 ±0.87	0.65 ±0.63
Thymus	0	0	0	0.42 ±0.29	0.82 ±0.63	1.19 ±1.61
MLN	0.03 ±0.05	0	0	0	0	0.82 ±0.87
Limbs	0.14 ±0.04	0.46 ±0.19	0.41 ±0.06	4.07 ±1.44	5.86 ±1.71	5.39 ±1.28
Total recovery	104.32 ±13.17	111.92 ±12.01	101.52 ±12.72	76.23 ±17.12	72.56 ±24.39	32.72 ±8.57

Donors: 10 NIH non-lactating female mice.
Recipients: 23 NIH infants (11 days old)
8 at 3 hours, 7 at 6 hours and 8 at 24 hours.
Dose: 5 µl containing 2×10^6 ^{125}I-UdR + 2×10^6 ^{51}Cr-labelled cells.
s.d. = standard deviation.

Total recovery = mean ± s.d. of totals of individual mice.

Table 2.9: 4, 20, 48 and 72 hour recovered radioactivity in infant mice following oral intubation of ^{125}I-UdR-labelled mesenteric lymph node cells and free isotope.

Time (hour)	Cells/ free isotopes	mean % injected dose ± s.d. Stomach wall	Stomach contents	Small intestine	Large intestine	Spleen	Liver	Lung	Thymus	Limbs	Total Recovery
4	Cells	0.84 ±0.74	11.31 ±7.89	1.92 ±0.97	0.25 ±0.25	0.22 ±0.22	1.70 ±0.63	0.91 ±0.61	0.30 ±0.38	4.20 ±1.87	21.71 ±10.54
	Free isotope	1.49 ±1.21	10.53 ±3.92	2.21 ±0.78	0.47 ±0.47	0	1.67 ±0.68	0.85 ±0.63	0	5.74 ±1.77	23.21 ±8.18
20	Cells	0.52 ±0.48	5.88 ±1.76	1.84 ±0.75	0	0	0.96 ±0.57	0.51 ±0.34	0	2.63 ±0.87	12.68 ±3.83
	Free isotope	0.68 ±0.52	8.88 ±1.97	2.10 ±0.30	0.62 ±0.91	0	0.70 ±0.33	0	0	2.69 ±0.45	16.12 ±3.02
48	Cells	0.41 ±0.50	3.46 ±1.82	1.38 ±0.67	0	0	0.55 ±0.40	0.44 ±0.55	0	2.24 ±0.47	8.48 ±3.17
	Free isotope	0 *	5.12 ±1.10	1.31 ±0.55	0	0	0.23 ±0.29	0	0	1.79 ±0.30	9.09 ±1.63
72	Cells	0.73 ±1.11	1.69 ±0.88	1.40 ±0.63	0.251 ±0.25	0	0	0	0	1.75 ±0.74	6.69 ±3.18
	Free isotope	0	3.14 ±1.75	1.01 ±0.65	0	0	0	0	0	1.46 ±0.36	6.12 ±2.52

* Where 4 or more recipients showed no activity, means are recorded as 0.
Donors: 8 NIH lactating mice.
Recipients: 56 NIH infants 2 - 4 days old (7 litters of 8) - 14 per time of recovery.
Dose: 5 µl containing 2×10^6 ^{125}I-UdR-labelled cells or 5 µl free ^{125}I-UdR (0.4 µci/ml).
Total recovery: Mean ± s.d. of totals of individual mice.
s.d. = standard deviation.

Table 2.10: 6 hour recovered radioactivity in infant mice following oral intubation of ^{125}I-UdR and ^{51}Cr-labelled mesenteric lymph node cells, and free isotope.

	Mean % of injected dose ± s.d. (n=6)					
	^{51}Cr			^{125}I-UdR		
	Live cells	Dead cells	Free isotope	Live cells	Dead cells	Free isotope
Stomach wall	0.10 ±0.09	0.73 ±0.49	0.21 ±0.29	1.24 ±0.53	4.31 ±2.27	1.69 ±1.61
Stomach content	1.84 ±1.81	10.09 ±4.63	6.07 ±2.49	12.51 ±2.92	30.03 ±13.79	16.09 ±3.39
Small intestine	54.40 ±4.19	66.75 ±15.33	51.19 ±16.22	2.46 ±0.46	6.15 ±2.06	1.63 ±0.34
Large intestine	3.64 ±2.27	6.08 ±2.84	4.08 ±0.91	0.64 ±0.30	0.73 ±0.13	1.89 ±1.36
Spleen	0	0	0	0.64 ±0.14	0.12 ±0.08	0.12 ±0.05
Liver	0	0	0.04 ±0.01	0.68 ±0.23	1.37 ±0.29	0.62 ±0.30
Lungs	0	0	0	0.52 ±0.13	0.61 ±0.14	0.41 ±0.14
Thymus	0	0	0	0.43 ±0.16	0.62 ±0.15	0.38 ±0.12
Limbs	0.24 ±0.17	0.43 ±0.30	0.24 ±0.07	3.93 ±1.40	5.13 ±1.23	3.57 ±1.88
Total Recovery	60.22 ±5.13	84.08 ±17.08	61.83 ±16.08	23.05 ±4.97	49.07 ±12.18	26.4 ±5.00

Donors: 10 NIH nonlactating mice.
Recipients: NIH infants (9-10 days old) 3 litters of 6, - 2 from each litter receiving different inocula.

Dose: 5 µl containing 2 × 10^6 ^{125}I-UdR + 2 × 10^6 ^{51}Cr-labelled cells or

5 µl of free isotope (^{51}Cr 80 µci/ml, ^{125}I-UdR 0.8 µci/ml).

n = number of infant mice.

Total recovery: mean s.d. of totals of individual mice.

s.d. = standard deviation.

SECTION 3. IMMUNOHISTOLOGY OF MATERNAL AND INFANT MICE, AND OF TRICHINELLA SPIRALIS LARVAE.

3.1 Introduction.

The morphology and the fine structure of the mammary gland in mice, rats and other animals have been demonstrated by many authors (Helminen and Ericsson, 1968, 1971; Pitelka, Hamamoto, Duafala and Nemanic, 1973; Topper and Oka, 1974; Pitelka and Hamamoto, 1977; Wooding, 1977; Lascelles and Lee, 1978; Burwen and Pitelka, 1980). In their studies on rat lactating mammary gland Helminen and Ericsson (1968) noted that the bulk of the tissue consists of tightly packed alveoli with high, basophilic secretory epithelium, and lumens containing a finely granular material and vacuoles corresponding to dissolved droplets of neutral fat, but they did not observe cells in the lumens. Their studies showed that mast cells, plasma cells, macrophages and different types of white blood cells were present in small amounts in the extraalveolar spaces. Cells of the lymphocyte-plasma series are only occasionally seen in tissue sections of normal lactating mammary glands (Mayer and Klein, 1961; Feldman, 1961). In contrast the involuting gland contains numerous lymphocytes (Mayer and Klein, 1961; Lee and Lascelles, 1969a&b). Electron microscopic studies on lactating and involuting mammary tissue also showed lymphocytes to be usually located adjacent to the basal surface of the glandular epithelium or inbetween the midportions of two epithelial cells (Helminen and Ericsson, 1968; Reid and Chandler, 1973; Lascelles and Lee, 1978).

Helminen and Ericsson (1971) observed macrophage-like cells with typical vacuolar cytoplasmic structure in normal rat lactating mammary gland from day 2 of lactation onward. Mayberry (1964) used trypan blue stain to label macrophages within lactating and involuting mammary

tissues of mice and also within the resting mammary tissue of nulliparous animals, and noted that the number of macrophages in lactating mammary tissue is extremely low, and remains so until the fifth day of weaning. Richards and Benson (1971) also used trypan blue to label macrophages in the lactating and involuting glands of the rat. Their observations were generally in accord with those of Mayberry (1964) for the lactating gland. Electron microscopic studies have confirmed the presence of macrophages in mammary tissue, and their increase in number during involution (Helminen and Ericsson, 1968; Richards and Benson, 1971). These cells are seen in the interalveolar areas sometimes between myoepithelial and epithelial cells and also in the alveolar lumen, which appeared to have taken up milk protein droplets (Lascelles and Lee 1978).

There has been considerable controversy regarding the identification and significance of the different cell types in normal milk. Based on morphological grounds, as many as 12 different types of cells have been described. These cells fall into two major groups, namely, polymorphonuclear leukocytes and mononuclear cells, the latter comprising lymphocytes, monocytes and epithelial cells of various description. In milk from infected glands, neutrophils are numerous, whereas in milk from uninfected glands mononuclear cells are usually more numerous than neutrophils (for references, see Lascelles and Lee, 1978).

Antibodies in mammary secretions are either derived from the blood by selective transfer and/or passive transudation, or are produced locally by plasma cells in the mammary tissue (Tomasi and Bienenstock, 1968). There are marked species differences in the contribution of locally produced immunoglobulin to mammary secretions; while most of the immunoglobulin in human mammary secretion is produced locally (Hanson, 1961), immunoglobulin in ovine mammary secretion is mainly

transported from serum (Richards and Marrack 1962; Mackenzie and Lascelles, 1968).

Immunoglobulin-containing cells have been demonstrated by immunocytochemical methods in the mammary glands of mice (Weisz-Carrington, Roux and Lamm, 1977); rats (Lee, Ladds and Watson, 1978; Lee, Ladds, Watson and Goddard, 1979); pigs (Brown, Bourne and Denny, 1975); rabbits (Hurlimann and Lichaa, 1976; Lee, Carey-Hanly and Knight, 1977; Lee & Carey-Hanly, 1978), and humans (McCarty, Grant, Georgiade, Wilkinson, Graham, Ferguson, McCarty and Seigler, 1981; McCarty, Sasso, Budwit, Georgiade and Seigler, 1982).

Morphological studies of the mouse mammary gland by Weisz-Carrington et al. (1977) showed that in virgin females and males only a few plasma cells and little in the way of immunoglobulins were found intraepithelially, but during pregnancy and at the beginning of lactation, these authors noticed an increase in the number of plasma cells, especially IgA containing cells, which continued during lactation. They also found that cells making IgG and IgM were much rarer compared with those producing IgA. Similar findings in rats were noted by Lee et al. (1978). These authors found an overwhelming predominance of IgA containing cells over IgG or IgM cells during the entire period from late pregnancy through lactation to involution.

The predominance of the IgA class of antibodies in mucosal secretion is well documented, and is known to have a protective role against infection of local mucosa (Lamm, 1976; Tomasi, 1976). The protective role of maternal colostrum and milk has been studied by many authors (Lee and Lascelles, 1970; Goldman and Smith, 1973; Allardyce, Sherman, McClelland, Marwick, Simpson and Laidlaw, 1974; Montgomery, Rosner and Cohn, 1974; Goldblum, Ahlstedt, Carlson, Hanson

Jodal, Lidin-Janson and Sohl-Akerlund, 1975; Bohl and Saif, 1975; Ahlstedt, Carlson, Hanson and Goldblum, 1975; Stoliar, Kaniecki-Green, Pelly, Klaus and Carpenter, 1976). The newborn infant has an immature secretory immune system but is able to benefit from antibodies obtained passively in maternal milk, IgA immunoglobulins therefore must act in the offspring's gastrointestinal tract and exert their beneficial effects locally in the gut since IgA is not absorbed at least in humans (Ammann and Stiehm, 1966) and in mice (Guyer, Koshland and Knopf, 1976). Studies in pigs showed that IgA antibodies are not absorbed by the piglet but play an important role in the intestinal defences (Kohler and Bohl, 1966; Wilson and Svendsen, 1971). A concept of a common mucosal immunologic system has resulted from studies done on a variety of species. Bohl, Gupta, Olquin and Saif (1972) were the first to demonstrate that antibodies in the mammary gland occurred as a consequence of oral immunization of pigs with transmissible gastroenteritis virus, and that IgA was the predominant immunoglobulin class of antibody in the milk. Similar results have been obtained by oral immunization of women with live E. coli 083 (Goldblum et al. 1975), of rabbits with DNP conjugated to killed pneumococci (Montgomery et al. 1974), and of rats with formalin killed Streptococcus mutans (Michalek, McGhee, Mestecky, Arnold and Bozzo, 1976). Other observations (Mata and Urrutia, 1971; Allardyce, et al. 1974; Holmgren, Hanson, Carlson, Lindblad and Rahimtoola, 1976; Stoliar et al, 1976) also support the idea that the various specificities of milk secretory IgA reflect prior antigenic encounters in the intestinal tract. The result of colonization experiments in pregnant women suggest that lymphoid cells in gut-associated lymphoid tissue home to the mammary gland where they produce IgA antibodies to intestinal microorganisms (Goldblum et al.1975).

The milk from normal rats contains several distinguishable nucleated cell types, as well as many anucleate cell bodies (Head, 1977; Head and Beer, 1978). These authors found that approximately 90% of the nucleated cells are macrophages, and the remaining 10% are lymphocytes, neutrophils and plasma cells. Human and rodent studies suggest that cell-mediated immune responses can be transferred from mother's milk to the neonates. In the human, for instance, tuberculin sensitivity can be passed to the infants by breast-feeding (Mohr 1972, 1973; Schlesinger and Covelli, 1977). Human milk contains lymphocytes that respond to several different B & T lymphocyte antigens and mitogens (Diaz-Jouanen and Williams, 1974; Parmely, Beer and Billingham, 1976). T & B lymphocyte functions have also been defined in the colostrum by their ability to form sheep red cell rosettes and to synthesize immunoglobulin (Murillo and Goldman, 1970; Ahlstedt et al.,1975; Parmely et al.,1976). Bovine and canine milk also contain lymphocytes that respond to a variety of mitogens and antigens (Smith and Schultz, 1977).

In rodents, cell-mediated immune responses can be transferred to the newborn directly via the milk. Beer, Billingham and Head (1974) have reported that the immunological activity of rats can be altered by their receiving immunocompetent cells from the milk. Head, Beer and Billingham (1977) have shown that congenitally athymic nude mice may receive T lymphocytes from their mothers, and resistance to tumour growth could also be transferred via milk from a foster mother to susceptible allogeneic neonates Seelig and Billingham (1980)
have found that many of the Fischer rat node cells introduced into surgically isolated segments of FI hybrid ileum crossed the intestinal wall within 24-48 hours of inoculation. On the other hand, many

studies have failed to substantiate any such transfer of cellular immunity from the mother to the neonates via the milk. In their study employing the same rat strains as those used by Beer et al (1974), Silvers and Poole (1975) could not find any evidence that fostering mice or rats on allogenic mothers could alter infant survival or immunological incompetence when transplanted with skin grafts of the same genotype as their surrogate mothers. When stained smears of milk taken from the stomachs of 12 hour old neonatal rats were examined, no viable cells could be found, suggesting that maternal immuno-competent cells do not survive any length of time in the newborn's digestive tract (Silvers and Poole, 1975). This finding is in complete contrast to the observations of Head, Beer and Billingham (1977) and Head and Beer (1978) who found intact nucleated lymphoid cells including macrophages, lymphocytes, neutrophils and plasma cells within the stomach contents of rats and mice from birth to 14 days. Steinmuller (1961) has studied the survival times of adult skin grafts on suckling rats at various times after birth and found that Brown-Norway rats nursed by their own mothers did not attain the ability to reject a Lewis skin graft in a comparable fashion to the adult until 10 days of age, suggesting that if the infants had received immunocompetent cells from their mother's milk, they should have been able to reject the graft acutely much earlier. Slobodian, Carlson and Wegmann (1979) have found, using radiolabelling techniques, that killer T lymphocytes administered intra-orally to neonatal mice (0-5 days old) did not cross into the infant's gastrointestinal tract, nor did intra-orally administered leukemic cells, which normally resulted in high infant mortality when given intraperitoneally, cross the gut wall or result in increased infant mortality. Furthermore, by using chromosomally

marked foster mothers, Trentin, Gallagher and Priest (1977) could find no evidence of chromosomally marked (maternally derived) cells in the gastrointestinal wall of suckling non-marked mice of 14 or 21 days of age. However the study of these authors was restricted to the latter stages of suckling by which time the immune system of mice may be competent and dependence upon a maternal endowment of immuno-competent cells (or other components) would seem unnecessary. Miller (1981) by using [^{3}H] Thymidine has demonstrated that isotope-labelled cells present in the colostrum and milk ingested by newborn and young mice do not cross the epithelial walls of the gut in the neonatal period ranging from 1 hour to 14 days.

Fluorescent antibody methods have been employed in studies with T. spiralis by many investigators. In addition to a better understanding of the immune response of the host, these investigations have led to the development of an indirect fluorescent antibody (IFA) test for antibodies to T. spiralis. In an early study, Jackson (1959) reported that precipitates, formed around anal and oral orifices of living T. spiralis larvae, stained brightly after exposure to antisera labelled with fluorescein isothiocyanate. Labzoffsky, Kuitenen, Morrissey and Hamvas(1959) found that cuticles of T. spiralis, mechanically freed from internal organs, reacted specifically with antisera of fluorescent antibody tests. Sadun, Anderson and Williams (1962) described an IFA test in which whole fixed larvae were used as antigen. Sulzer (1965) showed that cuticles of T. spiralis larvae, with their internal organs removed by peptic digestion could be used as antigen and that this antigen was relatively stable on storage. The test as described by Sadun et al (1962) and modified by Sulzer (1965) was performed in test tubes, and it was shown by Sulzer and Kagan (1967)

that multiple washes of sensitized antigen were required. Later, Wegesa, Sulzer and Orden (1971) prepared a stable cuticular slide antigen for use in IFA test for trichinosis. In their method, frozen sections of cuticles of T. spiralis larvae following prolonged peptic digestion and embedded in Tissue-Tek OCT embedding medium were used. Cryostat sections of either tongue or diaphragm in which T. spiralis larvae were present or on cryostat sections of T. spiralis larvae, were used in immunofluorescent studies by Ruitenberg, Ljungström, Steerenberg and Buys (1975). These authors detected specific antibodies in sera of experimentally infected mice 14 days after infection, whereas with the tube test antibodies were detected on day 24 postinfection and consistently thereafter. In addition to the cuticle, internal structures of T. spiralis larvae (musculature, cell membranes of the digestive tract, stichocytes) exhibited specific fluorescence (Jackson, 1959; Ljungström, 1974). Extensive analysis of antigens from the stichosome of T. spiralis has been carried out by Despommier and colleagues using chromatographic and other techniques. Granules have been isolated from the stichosome of larval T. spiralis using differential centrifugation and centrifugation in a sucrose gradient. These granules contain antigens which can be detected in culture medium (Despommier and Muller, 1970, a & b, 1976) and have been used to protect mice against infection using very low doses of antigen (Despommier, Campbell and Blair 1977a). Also soluble antigens, probably derived from the stichosome,isolated from a large particle fraction of T. spiralis larvae by isoelectric focussing were shown to immunize against challenge with the parasite (Despommier and Laccetti, 1981 a & b). Other recent studies on nematode antigens have focussed on cuticular components, for example, the works of Phillip, Parkhouse and Ogilvie

(1980) and Phillip, Taylor, Parkhouse and Ogilvie (1981), and Parkhouse, Phillip and Ogilvie (1981), on the isolation of immunogenic surface proteins from T. spiralis which are known to be shed in vitro. Although these antigens occur in all stages of the parasite, it is not known whether they are of importance in eliciting the immune responses which expel the worms from the gut.

Little information is available on the changes of immunocyte populations in the mouse mammary gland during the period from pregnancy through parturition and lactation to involution of the gland. In this study an attempt has been made to gain insights into the origin of immunoglobulins in the milk by studying the distribution of immunglobulins and immunoglobulin-containing cells in the mouse mammary tissues. In T. spiralis infection changes in the immunocyte populations of the mouse mammary gland were investigated at different stages of pregnancy and lactation and after-weaning in relation to the response to the antigenic stimulation of a whole infection in the small intestine before parturition.

In view of the possible significance of the cellular component of the maternal immunologic endowment in the milk to the suckling neonates, the cellular aspects of maternal milk and milk recovered from infant's stomachs were also investigated.

In this study the immunofluorescent technique was also employed to investigate the occurrence of antibodies specific to T. spiralis larvae, and the location of the antigens in the worms.

3.2 Materials and methods:

3.2.1 Animals:

NIH females and male mice, 8 weeks of age at the commencement

of study were fed on Oxoid breeding diet.

3.2.2 Pregnancy, lactation and neonates:

Pregnancy was confirmed by two methods. First, the females were weighed daily and once a gain in weight of 10% was noticeable, they were considered to be in the first week of pregnancy (see Table 3.1). Secondly, by the observation of vaginal plugs after mating. The day following the observation of the plug was counted as day 1 of gestation. Upon delivery, the males were removed and the females were kept with their litters. The uteri of the test mice sacrificed at intervals before parturition were examined to confirm pregnancy. To minimise variation in the development of the mammary gland, each litter size was adjusted to 6-8 infants per mother. All mice were weaned at 21 days old. Mothers and infants were used during different stages of lactation.

3.2.3 T. spiralis infection for mammary gland studies:

Immune mothers were given a primary infection of 400 T. spiralis larvae, followed after 3 weeks by a secondary infection of 200 larvae. The last infection was given approximately one week before mating. These mice are referred to as infected/immune mice.

3.2.4 Preparation of tissues for immunofluorescence and staining:

(a) Mammary gland:

Mice were sacrificed during pregnancy approximately 1 week or 2 weeks prepartum, during lactation at early lactation 1-7 days, mid-lactation 8-13 days, and late-lactation 14-21 days; and 1 week and 2 weeks post-weaning. All mice were etherized and sacrificed by

cervical dislocation. For removal of the mammary glands, a ventral midline incision was made, and the glands were freed from the underlying fascia. The glands were then separated from the overlying skin with fine scissors, embedded in Tissue-Tek 11 OCT compound (Lab-Tek Products Naperville, U.S.A.) and frozen at -20°C. Frozen serial sections, 6 to 8 µm thick were cut in a Slee cryostat (South London Electrical Equipment Co., Ltd., London) at -20°C on to alcohol cleaned microscopic slides. Sections from the midportion of the gland were fixed for 10 minutes in ethanol and some with acetone at room temperature, and stored at -20°C until used. Parts of the mammary gland tissue were fixed in buffered 10% formalin or Bouin fixative or Carnoy fluid, and stained with Haemalum and Eosin (H & E) stain or Methyl green pyronin (Unna Pappenheim)stain. These were used for tissue orientation and for general morphology.

Unna-Pappenheim (Methyl Green Pyronin)Stain

To prepare the stain:

50 ml of solution 1 mixed with 30 ml of solution 2 and make up to 100 ml with distilled water.

Dilute this mixture to 300 ml with distilled water and keep refridgerated.

Solution 1	Solution 2
0.5% Aqueous Methyl Green·Heat to mix, then cool, store on an equal volume of chloroform - this removes the methyl violet and leaves pure methyl green floating on the top. To separate off the methyl green, place the mixture in a separating funnel and drain off the chloroform (violet) part. Keep the green part.	0.5% Aqueous Pyronin Y Heat to mix, until dissolved. Cool. Freshly made.

Staining

1. Bring sections through xylene and alcohol to water.
2. Stain slides for 10-20 minutes.
3. Rinse briefly in distilled water, drain slides and place on blotting paper.
4. 2 rinses in acetone.
5. Place in xylene for at least 5 minutes.
6. Mount in D.P.X.

Haemalum and eosin stain. Mayers Haemalum and eosin (H & E) 0.1% haematoxylin in 5% aluminium potassium sulphate.

1. Bring sections to water.
2. Stain in 0.1% haemalum for 3 min.
3. Rinse in tap water.
4. Dehydrate to 90% alcohol.
5. 0.2% alcoholic eosin for 1 min.
6. Absolute alcohol 1 min.
7. Clear in xylene and mount in D.P.X.

(b) Tissues from infant mice:

Stomachs from infants which had suckled their mothers for periods of 12 hours to 2 weeks were removed and sectioned and stained in the same way as the mammary glands. Small intestinal tissue taken at the same periods was removed, divided into anterior and posterior halves, and frozen sections from the mid- portions of each half processed as described for the mammary gland.

3.2.5 Collection of cells from milk:

Milk was collected from groups of 5 or 6 immune mothers, each

suckling 6-8 infants during the early and mid-lactation periods. All samples of milk were defatted by centrifugation (250 g for 10 minutes at 4°C). The sedimented cells were washed three times by centrifugation with Eagle's minimal essential medium (MEM) and resuspended in a specific volume using the same medium. The total number of cells was determined in a hemocytometer. Differential cell counts were performed on cytocentrifuge preparations stained with Giemsa stain. Viability of cells was determined by their ability to exclude 0.2% trypan blue in P.B.S.

Giemsa stain

1 in 10 dilution of Giemsa stain (BDH) in Giemsa buffer.

Giemsa buffer PH 7.4

3.0 g Na_2HPO_4

0.6 g KH_2PO_4

made up to one litre with distilled water.

Procedure

1. Fix smear in methanol for 2 min and air dry.
2. Stain in 1 in 10 Giemsa for 40 min.
3. Rinse in Giemsa buffer for a few seconds.
4. Air dry and examine directly, or,
5. Air dry and mount in DPX.

3.2.6 T. spiralis larvae:

T. spiralis larvae were prepared after digestion of mice which had been infected for not less than one month (see General Materials and Methods). Using a Pasteur capillary pipette, a few hundred infective larvae were inserted into a small cavity made in semisolid

OCT compound and left to solidify at -20°C. Serial sections 4-6 μ thick were cut in a Slee cryostat. The sections were attached to a clean glass slide, allowed to air dry and fixed in absolute ethanol for 10 minutes, and stored at -20°C until used.

3.2.7 Serum collection:

Pooled sera from mice during primary infection were collected, (for details see General Materials and Methods). Naive sera were collected from uninfected mice. These sera were used during immuno-fluorescent staining of T. spiralis larvae.

3.2.8 Antisera used:

Fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG (FC fragment, heavy chain specific), and Tetramethylrhodamine isothiocyanate (TRITC) conjugated goat anti-mouse IgM (Mμ chain specific), were obtained from Cappel Laboratories Inc., Cochranville, Pa ., U.S.A. FITC - conjugated goat anti-mouse IgA (FC) was obtained from Nordic Immunological Laboratories, Tilburg, The Netherlands. These conjugates were reported by the manufacturers to be specific following testing by immunoelectrophoresis.

The conjugates were reconstituted with the indicated amount of cold sterile distilled water, and centrifuged (250 g for 10 minutes) to remove insoluble particles and then divided into small aliquots (30 μℓ) which were frozen and stored in the dark at -20°C. Repeated thawing and freezing were avoided. As required, aliquots were thawed slowly at room temperature in the dark and used to prepare working dilutions by adding the required amount of cold sterile phosphate buffered saline (PBS) PH 7.2.

3.2.9 Staining procedure for immunofluorescence:

(a) Direct method for the mammary gland and infant small intestine:

Sections on slides were thawed at room temperature (21-22°C) and rehydrated with PBS PH 7.2 for 2 minutes. They were incubated with a drop of the relevant conjugated antisera in a moist chamber at room temperature for 30 minutes. Following this, they were individually rinsed in PBS before being washed 3 times, ten minutes per wash in PBS with continuous stirring at room temperature. The surplus PBS was blotted carefully from each slide with paper tissue, and sections were mounted in glycerol/PBS 50:50 and sealed with TEXPEN (Marktex resist ink). In each test known positives (sections of spleen) were used. The working dilution of the conjugated antisera most commonly used was 1:40 for FITC and 1:20 for TRITC. With these dilutions immunoglobulin containing cells were easily recognized. The specificity of the reaction was controlled by (a) blocking with unconjugated specific antiserum, obtained from Cappel Laboratories, prior to incubation with conjugated antiserum. (b) Treatment of sections with normal goat serum, obtained from the Scottish Antibody Production Unit, Scotland, for 30 minutes prior to incubation with conjugated antiserum. (c) Use of sections without conjugates i.e. PBS only throughout the test, blotted and mounted in the same way as the conjugated sections.

(b) Indirect method for T. spiralis larvae:

A double layer technique was used. Test serum, at different dilutions up to 1:1024, to cover the section was applied with a Pasteur pipette. The slides were then placed in a humid chamber and left at room temperature for 30 minutes. They were then rinsed individually with PBS, and placed in a suitable carrier in a bath of PBS with

continuous stirring, they were washed 3 times 10 minutes each, then drained and drops of the relevant conjugated anti-sera of 1:50 dilution were applied to the sections. (Each slide was removed singly from the bath, and the conjugate applied before taking out the next slide so the test areas were not allowed to dry out). The slides were left for a further 30 minutes in a humid container at room temperature, and then washed again three times in PBS, each of ten minutes, mounted and sealed as described earlier. For controls, the sections were treated (a) with sera from naive mice followed by conjugate or (b) with the conjugate alone or (c) with PBS only (no serum on conjugate).

3.2.10 Microscopy for immunofluorescence:

Slides were examined with a Leitz Ortholux II microscope using incident light fluorescence (Ploem system) with an HBO 50 high-pressure mercury vapour lamp. For fluorescein (FITC) reactions, 2 x KP490 (exciting), TK 510 (dichroic mirror) and K515 (suppressing) filters were used, and for rhodamine (TRITC), 2 mm BG36 and S546 (exciting), TK 580 (dichroic mirror) and K580 (suppressing) filters were used. Colour photomicrographs of representative fields were taken using Kodak Ektachrome 200 ASA film, which was developed as for 400 ASA. Colour or black and white prints were made.

3.2.11 Cell counts:

For mammary gland sections, the method used was to count the number of fluorescing cells in 100 fields. The fields were selected at random and observed with a 50 x objective and 10 x eye piece lens. The cells counted were those which showed clearly defined cytoplasmic staining. Statistical analysis was done using Student's t-test. In

some sections distinctly granulated mast cells fluoresced with conjugate for IgG. These cells were excluded from the counts. Few immunoglobulin-containing cells were seen in sections of infant small intestine; no counts were made of these cells.

3.3 Results.

3.3.1 Histology and immunohistology of the mammary gland:

Histological examination of mammary glands during late pregnancy and lactation was undertaken initially on sections of tissue of Bouin or formalin fixed material embedded in wax and stained with haemalum and eosin. Representative photomicrographs of these studies are Plates 3.1.A. and 3.1.B. which demonstrate the essential morphology of the mammary gland during lactation. The alveolar and duct epithelium is relatively thin consisting of a single layer of cuboidal cells lying on a narrow connective tissue interstitium; capillaries and areas of loose connective tissue associated with adipose tissue are dispersed within the mammary gland. Such an area of loose interstititium is visible to the left of the capillary in plate 3.1.A. In properly fixed tissue, the components of the milk in the ducts are evident as finely granular material with profiles of lipid droplets occurring frequently. Many of the epithelial cells contain lipid droplets in the apical margins giving them a distinctly vacuolated appearance. Within the interstitium lymphoid cells are readily visible and occur quite frequently whereas macrophages are less frequent and less easily identified as most of them are either closely applied to the base of the epithelium or they occur in intra-epithelíal positions in which they resemble closely the epithelial cells in histological appearance and with some degree of vacuolation.

Both lymphoid cells and macrophages together with sloughed epithelial cells are identified in the secreted milk, Plate 3.1.B , particularly in the ducts rather than in smaller alveoli. Such secreted cells were not uniformly distributed throughout the mammary gland. In any section it was always the case that there were small areas of alveolar ducts which contained an abundance of lumenal cells as demonstrated in Plate 3.1.B.

Polymorphonuclear cells were observed only very infrequently. However, mast cells occurred frequently in areas of loose connective tissue and adipose tissue (Plate 3.2). These were most readily demonstrated using the methyl green/pyronin stain of Unna Pappenheim. Such mast cells were also observed in numbers in association with the occasional aggregates of lymphoid cells which occur infrequently in the mammary gland.

Examination of the mammary gland with fluorescein or rhodamine isothiocyanate conjugates of anti-mouse immunoglobulins on frozen sections post-fixed in ethanol was undertaken to demonstrate the occurrence of immunoglobulins and immunoglobulin-containing cells in the mammary gland tissues and in secreted milk. For all batches of conjugated antisera, the positive and negative responses were determined initially on sections of mouse spleen. Pre-incubation with unconjugated mouse antisera effectively blocked binding of conjugated antisera and yielded no measurable fluorescence. A very faint halo associated with lumenal lipid droplets could be observed as shown in Plate 3.3.A. In such blocked sections, or sections treated with conjugated antisera only or in sections treated with PBS only or in sections treated with normal goat serum, potentially interfering autofluorescence was not observed.

The only autofluorescence which occurred was associated with small granules or fragments which produced orange/yellow fluorescence when excited with UV of 490 nm.

In most of the sections the free lipid droplets and granular material of the secreted milk in the lumens of the alveoli were absent having been washed out in the incubation process. However with very careful washing and incubation the secreted material was occasionally retained in the lumen and showed some fluorescence when incubated with low dilution antisera (Plate 3.3.B). IgM and IgA could also be visualized in this fashion. Results were inconsistent and unreliable and an assessment of free immunoglobulins in the milk was not made using these methods.

Treatment with the higher dilutions of $\frac{1}{20}$ and $\frac{1}{40}$ of the conjugated antisera yielded consistent and reproducible results in terms of clearly distinguishable immunoglobulin-containing cells. IgG containing cells were observed in the interstitial connective tissue and extruding into the lumen of the ducts but were to be observed most frequently in the basal intraepithelial position or closely applied to the base of the epithelia. Plate 3.4.A. shows three such cells. Only structures in which a nuclear and cytoplasmic component could be identified were recorded as immunoglobulin-containing cells. Two only of the cells shown in Plate 3.4.A. are in a common focal plane and show these features. A faint staining through the epithelial cells indicated a non-lymphoid cell occurrence of small amounts of IgG.

IgA containing cells, demonstrated with fluorescein conjugated goat anti-mouse IgA, fluoresced moderately with $\frac{1}{20}$ and $\frac{1}{40}$ dilutions of the antisera. IgA cells occurred more frequently than IgG cells, and were observed most commonly in the interstitium and basally in the

epithelium. Occasionally IgA cells were observed in the alveolar lumen as shown in Plate 3.4.B. There was no detectable IgA in the epithelial cells.

IgM containing cells, detected with rhodamine conjugated goat anti-mouse IgM, had a predominantly interstitial or basal location. These cells occurred occasionally in groups as shown in Plate 3.5.A. but were usually isolated. As with IgG there was faint staining of the cytoplasm of the epithelial cells and a notable feature was the moderate staining, as a halo, associated with lipid droplets which were on the point of being extruded into the lumen of the alveoli.

The mast cells, either in the aggregates of lymphoid cells or in the interstitium showed marked fluorescence with the conjugated anti-sera to IgG. As far as could be determined, the fluorescence was limited to the granules of the mast cells as shown in Plate 3.5.B.

3.3.2 Immunocyte populations in the mammary gland.

(a) During pregnancy:

During mid and late periods of pregnancy, immunoglobulin-containing cells were distributed in the connective tissues throughout the mammary gland; most of the cells observed were solitary. The numbers of IgA and IgM cells increased with advancing pregnancy (Table 3.2, Fig.3.1). IgG containing cells were not seen during mid-pregnancy, and at all stages, IgA cells were more numerous than IgM or IgG cells. The number of IgA cells and the degree of staining increased significantly at late pregnancy prior to lactation. The intensity of staining in cells was considerably lower during lactation. Although the number of immunoglobulin-containing cells of all classes in mammary gland tissue from infected mice was found to be higher than those from naive mice (Table 3.2),

there is no statistically significant difference between these two groups at this stage.

(b) <u>During lactation</u>:

As noted in section 2, the development of the mammary glands continues during lactation. It increases c.5 fold towards the late-lactation period in comparison to the mammary glands from virgin females (see Table 2.2). Immunofluorescent staining showed that the mammary glands from both naive and infected mice contained large numbers of immunoglobulin-containing cells of all three classes, and the populations of these cells increased as lactation progressed (Table 3.2). There was a marked increase in IgA cell numbers from late pregnancy through all stages of lactation. In infected mice, the increase in IgA cells between early-lactation and mid-lactation was statistically significant ($p < 0.05$). At all stages of lactation in both naive and infected mice, IgA containing cells were the predominant type in mammary gland tissues with approximately twice as many IgA cells as IgM cells and approximately $2\frac{1}{2}$ times as many IgA cells as IgG cells (Table 3.2). The numbers of IgA cells peaked during late-lactation. At all stages of lactation there was a greater number of IgA cells in infected animals. However, the only period at which there was a statistically greater number of IgA cells in infected animals was during mid-lactation.

IgM containing cells were mostly solitary. The number of these cells increased also during lactation ($p < 0.05$ in naive mice between late-pregnancy and early-lactation), and peaked during mid or late-lactation ($p < 0.05$ between early and mid-lactation in infected mice; $p < 0.001$ between early and mid-lactation in naive mice).

The number of IgG cells within the sections of the mammary glands was less than that of IgA or IgM cells. As with IgM cells, the number of IgG cells reached a peak during mid-lactation with a statistically significantly greater number of IgG cells in infected mice in comparison to naive mice at this stage.

(c) After weaning:

With reference to Table 3.2, it is clear that there is a rapid decline in the numbers of immunoglobulin-containing cells in the mammary glands of both naive and infected mice. The reduction was most evident in the case of IgG cells.

3.3.3 Total and differential counts of cellular components in the milk.

(a) Maternal Milk:

Further to the histological studies which have demonstrated the presence of epithelial cells macrophages and lymphoid cells, some of which at least contain immunoglobulins, in the milk within the mammary glands, quantitative observations of the cellular component of milk were undertaken.

Volumes of milk were obtained directly from the mammary gland principally during the mid-lactation period. This stage was selected for two reasons:

(1) useful volumes of milk were much more easily obtained from lactating mice in this period than from the earlier period of lactation and

(2) the short time fostering studies were associated with this period of nursing. It was therefore desirable that as much information concerning the potential maternal endowment of this period be obtained.

To quantitate the total cells in milk, counts were made using the improved Neubaer haemocytometer. The milk samples were taken from 5 or 6 mice at early and mid-lactation periods from naive and infected 'immune' mice. The cell counts were as follows:

Milk taken at	Number of cells per ml	
	Naive mice	Immune mice
Early-lactation	0.57 - 0.63 x 10^6/ml	0.48 - 0.52 x 10^6/ml
Mid-lactation	0.70 - 0.76 x 10^6/ml	0.64 - 0.72 x 10^6/ml

These counts indicate a slight increase in the number of cells present from early to mid-lactation. However, immune mice showed slightly lower numbers of cells in the milk. Attempts to determine the viability of these cells by trypan blue exclusion was an unrewarding and fruitless task for the following reasons. In these preparations there was a considerable background of particulate milk material which took up the stain and there was a large number of dead cells in all the preparations, most of these cells were epithelial cells. Increased centrifugation and washing did not alter the viability counts for the better, and decreased centrifugation/washing left more particulate matter in the preparations. An additional factor which should be taken into account is the time which is taken to obtain reasonable volumes of milk from the mice (not less than 30 minutes per mouse), during which time the milk was being agitated quite vigorously and could not be maintained at a very low temperature. Neither feature would enhance cell viability.

Differential cell counts which could be performed on preparations

which were subject to minimal unfavourable procedures other than the collection procedure and defatting were carried out. Cytocentrifuge preparations were made using diluted defatted milk, which permitted the dispersal and removal of most of the particulate milk, and were fixed in methanol and treated with Giemsa stain. The defatting procedure was a critical feature and the handling of slides had to be carried out very carefully to prevent detachment of the cells during staining and mounting. Detached cells could be determined as ghost marks. Slides in which more than 5% of the cells had detached were discarded.

The differential cell counts of the milk from naive mice are recorded in Table 3.3, and for milk from infected 'immune' mice in Table 3.4. Macrophages were the predominant cell and together with lymphoid cells accounted for approximately 60% of all the cells. This is a very much higher figure than was predicted from the histological observations of cells in the milk ducts in the mammary gland. Subsequent counting of the cells in the milk in histological sections of the mammary gland indicated that about 85% of the cells would be identified as epithelial cells. A notable feature of the cells classified as epithelial cells in the milk cytocentrifuge preparations is that virtually all of them were concluded to be dead cells. In observation, the nuclei were of uniform deep pink colour with a fine granular appearance and the cytoplasm a lighter pink colour with a coarse grained or fibrous appearance. They were of identical appearance to disrupted epithelial cells. The assumption is made that a large proportion of the epithelial cells had denatured or disintegrated beyond recognition before or during the making of the preparations. However, between the naive and infected preparations there was not a significant difference

in the epithelial cell populations, and on the assumption therefore that cell death was of similar proportions in both naive and infected preparations, it can be presumed that the samples are comparable in respect of the other cells. Thus the populations of macrophages and of total lymphocytes show marginally statistically significant increases between naive and infected mothers.

The instability of, and the presence of background material in the smear or cytocentrifuge preparations rendered efforts to determine the numbers of cells which contained immunoglobulin by immunofluorescence unsatisfactory. Very few cells would remain attached to the slides during the prolonged series of incubations and washes, and quantitation was therefore not possible. Considerable background fluorescence from streaks of particulate material also rendered the preparations unsatisfactory. In preparations in which a few cells remained, it was possible to observe an occasional cell which stained for IgG or IgM or IgA. All the cell types - lymphoid, macrophage and epithelial as identified under brightfield illumination, were observed to show some fluorescent staining properties.

(b) Milk from infant stomachs:

Examination of the stomachs of infant mice suckling either naive or infected mothers from 12 hours of age to 2 weeks of age revealed a component of intact and apparently viable lymphoid cells and macrophages as seen in routinely prepared histological specimens (Plate 3.6.A). These cells were of the same size, structure and staining properties as cells so identified in the mammary gland and milk preparations. Other cellular structures were tentatively identified as degenerating mammary gland epithelial cells. These apparently

viable cells were distributed throughout the partially congealed milk although an impression was gained that rather more cells were located peripherally. In most of the preparations some shrinkage away from the stomach epithelium had occurred, and in no case were any macrophages or lymphoid cells observed to be closely applied to or integral to the lumenal epithelium.

In an effort to quantify the numbers of the viable cells in the milk in the infant stomachs, the congealed contents of the stomachs were removed and resuspended in normal saline with vigorous stirring and agitation, and centrifuged (250 g) to pellet the cells separately. However the partially congealed or flocculated milk did not disperse sufficiently to yield a population of free cells which could be quantified or assessed for viability.

Frozen sections of infant stomachs post-fixed in ethanol and then treated with conjugated anti-sera as detailed in the section on mammary glands were unsuccessful in determining the presence of immunoglobulin containing cells in the contents of the stomach lumen. In these preparations it was not possible to retain the sectioned milk material on slides due presumably to the poor adhesive qualities of the lipid bearing material.

3.3.4 Immunohistology of infant's small intestine:

Histological examination of the small intestine of infant mice suckling naive or infected mothers from 2 days to 2 weeks of age indicated that there were very few cells present in the lamina propria early in age with a small increase in the number of cells by 2 weeks of age.

Cryostat sections post-fixed in ethanol were incubated with

fluorescein or rhodamine conjugated goat anti-mouse immunoglobulins as in the studies of the mammary glands. Plates 3.6.B, 3.7.A & B show the location of IgA, IgG and IgM in the infant intestinal tissue. Fluorescence with all conjugates was weak even with very low dilutions, but at low dilutions the definition and intensity of the staining was very poor. However, at higher dilutions the intensity of staining was not reduced and definition was greatly improved. With all conjugates distinct immunoglobulin-containing cells could just be visualised in the lamina propria of the intestine by day 6. IgA-containing cells (Plate 3.6.B) were very few in number and stained very weakly. IgM-containing cells (Plate 3.7.B) occurred more frequently and earlier than IgA cells and were more readily visible. In nearly all the sections examined there was some fluorescence located basally in the epithelial cells. IgG-containing cells occurred only infrequently in the lamina propria and also in the submucosal areas. A considerable degree of staining for IgG was associated with the central ducts of the lamina propria and with the submucosal spaces between the base of the crypts and the muscle layers presumably in association with draining ducts. The epithelial cells did not show any discrete staining for IgG.

In none of the sections examined was there any suggestion of a layer of fluorescing material associated with the brush border or of notable fluorescence in the apical regions of the epithelial cells.

3.3.5 Immunohistological studies of Trichinella spiralis larvae:

T. spiralis has the elongate vermiform structure of most nematodes, with the mouth at the extreme anterior tip and an alimentary tract consisting of a cuticle-lined oesophagus leading to an intestine with an anus near the posterior tip. The stichosome, a column of large glandular cells with the oesophagus embedded at the margin occupies most

of the anterior two-thirds of the mature infective larvae. Each stichocyte has a system of canaliculi opening into the oesophagus, the function of these is to discharge the secretory products of the stichocyte, the product being present in the stichocytes as secretory granules. In the posterior third of the body, the stichosome and the oesophagus are replaced by the intestine and primordial gonad. The lumen of the intestine of the worm is likely to contain some of the secretion product of the stichosome as well as digestive enzymes of the worm and together comprise part of the excretory materials which may be antigenic.

The binding of specific antibodies to components of the infective stage of T.spiralis utilized cryostat sections post-fixed in ethanol. The tissue and cytological structure of the larvae is well preserved by this method. Plate 3.8 shows transverse sections of T. spiralis infective larvae, and two of these sections are represented diagramatically in thc adjacent Figure 3.2.

Frozen sections post-fixed in ethanol were incubated with serum from mice infected with T. spiralis. When incubated subsequently with fluorescein conjugated goat anti-mouse antisera, fluorescence associated with most of the worm components was detectable. Plate 3.9 shows two sections of infective larvae incubated with $^{1}/_{200}$ mouse antisera from a 21 day primary infection, and $^{1}/_{50}$ conjugated goat anti-mouse IgG. The feature of the worm cell membranes, particularly those of the hypodermal tissue muscle and primordial gonad together with their basal laminas, fluorescing prominently was one noted with all the conjugates. It is notable also that the only part of the cuticle to show fluorescence is the extreme outer surface. In this plate prominent fluorescence is not evident in the stichocyte but is noted in the lumenal contents of the intestine of the worm. Sections incubated with more diluted mouse

antisera ($\frac{1}{512}$) from a 28 day primary infection (Plate 3.10.A), show a well defined if weak IgG fluorescence on the surface of the cuticle, and a strong fluorescence, disseminated or particulate, in stichocytes, with no fluorescence in the worm's intestinal contents in this particular section or in the oesophagus. The disparity of the staining of stichocyte contents is elucidated with reference to Plate 3.10.B. which shows a longitudinal section of the stichosome region of an infective larva. In this section through approximately ten stichocytes there are three patches of prominent if weak fluorescence indicating that only some of the stichocytes contain antigens which have elicited strong antibody responses. This particular preparation utilizing antisera from mice with only a 12 days primary infection indicates that certain stichocyte material is highly antigenic and elicits an early antibody response. No other components in this section show significant fluorescence. Staining of other components of the infective larvae was evident when sera from older infections were used. The development of IgG antibody components is recorded in Table 3.5. and shows that antibody to components other than stichocyte contents cannot be visualized until day 15 post-infection. In the period days 15-19 post-infection there is a marked and sustained development of detectable antibody responses to most of the worm components. It is particularly noteworthy that apart from the extreme outer membraneous part and surface of the cuticle there is no detectable fluorescence to the cortical and median layers of the cuticle. It was also most noticeable that while very weak or weak fluorescence could be detected in association with all other cellular components of the infective larvae, the strongest and most prominent fluorescence was associated with the membranes and the basal laminas of the hypodermal and muscle tissue

as shown particularly in Plate 3.9.

Treatment of sections of infective larvae with mouse antisera taken on days 12, 15, and 21 post-primary infection followed by fluorescein conjugated goat anti-mouse IgA, indicated that antibodies were first detectable on day 15, particularly on the surface of the cuticle (Table 3.6). By day 21 post-infection, there was prominent if weak fluorescence at a titre of 1:512 on the cuticle surface and associated with the membranes and basal laminas of the hypodermal, muscle and primordial gonad tissue. The contents of the worm intestine also fluoresced weakly. There was no fluorescence of stichocyte contents at any stage. The fluorescence of the surface of the cuticle was notably patchy indicating perhaps that irregularly adherent surface material was responsible for some of the cuticular fluorescence (Plate 3.11.A). Very weak non specific IgA binding with sera obtained from naive mice was observed in dilutions of up to 1:200 (Plate 3.11.B), but was confined to the cells of the muscle, hypodermis, primordial gonad and to a lesser extent of the intestine. The cuticle and stichocytes did not show any non-specific IgA binding. Fluorescence of non-specific IgA binding was extinguished at dilutions greater than 1:200 naive sera.

Treatment of sections with mouse antisera and rhodamine conjugated goat anti-mouse IgM is illustrated in Plate 3.12. As with the previous treatments the peripheral parts of the hypodermal cells, muscle cells and cells of the primordial gonad together with the respective basal laminas are clearly identified albeit with weak fluorescence. There is minimal fluorescence on the surface of the cuticle and occasional weak fluorescence within the stichocytes. Very weak oesophageal and intestinal fluorescence was observed only occasionally. The development

of IgM antibodies is recorded in Table 3.6. There is no detectable antibody prior to day 15 post-infection, with clear if weak fluorescence of the previously cited components thereafter. When sera from naive mice were used, there was little or no fluorescence of the rhodamine conjugates indicating virtually no non-specific binding of IgM antibodies.

Owing to the relatively large volumes required to flood numerous sections a single test only was performed with the fluid component of milk from infected mice. In this test for IgG antibodies, reactivity to all the aforementioned worm components was observed, indicating that the antibodies detectable in sera are also secreted into the milk.

3.4 Discussion:

The possible roles of the cells in the milk is not one which was addressed with exhaustive effort until fairly recently, and no less than Brambell (1970) focussed his attention almost entirely on the antibody component of milk. However, a number of aspects of the occurrence of leucocytes in milk have received some attention, not the least being the source of the cells.

It is now well established that leucocytes increase significantly in the mammary gland during development and lactation, and that many cells are present in the secreted milk. Breed (1914) was one of the first to quantify the number of cells present in cows milk. Smith and Goldman (1968) studied the cells of human colostrum, and Seelig and Beer (1978) studied the transepithelial migration of leucocytes in the mammary gland of lactating rats. Helminen and Ericsson (1968) studying the lactating mammary gland in rats distinguished macrophages and lymphocytes (referred to as 'pale' cells when observed with the light microscope) as an integral part of the wall of the alveolus. At the E.M.

level these authors clarified the nature of the 'pale' cells as lymphocytes containing small arrays of ribosomes.

The results of this section, indicating the significant increase of leucocytes in the mammary gland during late pregnancy place NIH mice firmly in accord with the published work of Weisz-Carrington, Roux and Lamm,(1977) on CAF mice, of Lee, Ladds and Watson (1978) on rats, and of Brown, Bourne and Denny (1975) on sows. The possible significance of the leucocyte population in the mammary gland was indicated by Bohl, Gupta, Olquin and Saif (1972) who showed that IgA antibody to transmissible gastro-enteritis (TGE) virus appeared in the milk of sows following infection in the gut. They postulated that the link between the gut and the mammary gland must involve the traffic of primed lymphocytes from the gut to the mammary gland on the basis of IgA being synthesized locally. Bohl and Saif (1975) extended this work in the sow and conclusions concerning lymphocyte traffic to the mammary gland was confirmed for man (Goldblum et al., 1975), rats (Michalek, McGhee, Mestecky, Arnold and Bozzo, 1976), and mice (Roux, McWilliams, Phillips-Quagliata, Weisz-Carrington and Lamm, 1977). This latter work showed that, in the mouse, lymphocytes from the mesenteric lymph node home to the mammary gland during late pregnancy and during lactation. They also indicated that the majority of the cells were IgA precursor cells. In NIH mice infected with T. spiralis Rose, Parrott and Bruce (1978) showed that mesenteric lymphoblasts, some of which would be primed to gut located T. spiralis, accumulated in the mammary gland during lactation, despite the presence of a highly damaging concurrent infection in the tissue of the small intestine. These observations invited the

consideration that translocation and/or differentiation of lymphoblasts in the mammary gland could lead to a component of cell mediated immunity being involved in the maternal endowment of protection to the neonates during suckling.

Until now there has been no information as to the composition and dynamics of the immunocyte population in the mammary gland in lactating T. spiralis infected animals. The determination of the population of lymphocytes in the mammary gland of lactating rats was recorded by Seelig, Holt and Beer (1979). These authors showed that there was a rapid increase in the numbers of lymphocytes in the late stages of pregnancy and also during early lactation with cells present basally and in the intraepithelial position in the alveoli. Lascelles and Lee (1978) concluded that phagocytic activity,evidenced as uptake of lipid droplets, was commonplace in milk macrophages. Weisz-Carrington et al., (1977) described the occurrence and location of immunoglobulins in CAF mice with respect to the mammary gland. They indicated that there was a marked increase in the number of immunoglobulin-containing cells from the virgin state, through pregnancy and into lactation with the maximal number of plasma cells parallelling the development of the glandular epithelium.

In the present study, the observations of Seelig et al., (1979) on rats and Weisz-Carrington et al., (1977) on mice are confirmed for NIH mice in that in naive lactating mice there is a marked and significant increase in the numbers of immunoglobulin-containing cells of A, G and M classes. The largest population is that of IgA-containing cells while proportionately the largest increase during lactation is that of IgG-

containing cells. The major interest in this work however, is the finding that in infected lactating mice there are statistically significantly greater numbers of IgG, IgM and IgA-containing cells in the mammary glands this being most prominent in the mid-lactation period. The potentially greater conferment of a cellular component or of liberated immunoglobulins in the exosecretion of the gland is obvious. Correlation of these observations with the documented total immunoglobulins in the milk will be considered in the general discussion.

As in the reports of Weisz-Carrington et al.(1977), the majority of the immunoglobulin-containing cells are located basally to the alveolar epithelium but with a notable number of immunoglobulin-containing cells in the intraepithelial position and extruded into the alveolar lumen. With the notable exception of mast cells in the interstitial tissue, which fluoresced brilliantly with the IgG conjugate, virtually all the other cells in the mammary gland which contained immunoglobulin were identified as lymphoid cells. Of the immunoglobulin-containing cells which were on the point of being extruded into the alveolar lumen, or the few which were wholly extruded there was no predominance of immunoglobulin class. Both IgA and IgG-containing cells were observed quite frequently in the migratory positions. IgM-cells were infrequently observed intraepithellialy or in the alveolar lumens.

The presence of a notable number of fully granulated mast cells in the connective tissue of the murine mammary gland is a subject which invites comprehensive study. The absence of any marked change in the numbers, position or state of granulation of such mast cells in T.spiralis infected mice in comparison to naive mice suggested however that an

easily determined relationship of T. spiralis infection and mast cells would not be forthcoming and further, given the observations of similar degrees of IgG binding/fluorescence in both naive and infected animals, it was decided not to pursue this area of enquiry. It must be presumed that the mast cells in the mammary gland of naive animals, occupying their usual sub-epithelial position, would have a function during local pathogenic assault, perhaps associated with the process of involution, at which time they were noted to increase in numbers in sheep (Lee and Lascelles 1969a). To have developed the different assays and undertake prolonged studies of the mast cell population in the mammary gland would have precluded many of the other aspects of the present work.

As was noted in the results with reference to Plates 3.1 and 3.2, which comprise sections of routinely prepared histological specimens, the predominant cell type in the milk is the epithelial cell. The next most common cell is the macrophage with relatively few lymphoid cells. In the cryostat sections very few of these extruded cells could be/were retained on the slides, but in the tissues, significant amounts of fluorescence were not observed in macrophages or in epithelial cells at the usual dilutions of conjugates (1:40). However, in treatments utilizing very low dilutions of conjugate (1:8), (Ref. Plate 3.3.B), the amounts of immunoglobulins which were present in tissue macrophages or, more interestingly, in intact alveolar epithelium were very small although considerable amounts were detectable in the secreted milk, suggesting that epithelial translocation of free immunoglobulin is a rapid process which does not involve storage of immunoglobulin in the epithelial cells or in pre-exudate macrophages. The larger cell

populations in infected animals did not show any displacement in location or frequency of alveolar location.

The consistent observation of an apparent lipid droplet association of immunoglobulins, particularly IgM, is an interesting and perhaps significant finding if it can be proved and traced within the infant gut. For the present the possible artifactual nature of the lipid droplet halo could not be resolved. Should it be possible to determine immunoglobulin/lipid droplet affinity in these mice through the gut passage, it would provide a simple and extremely efficient mode of uptake in the neonate gut via the lipid phagocytosis of the microfold type cell of the Peyer's Patches or the similar particle absorption facility of intestinal epithelial cells in very young mice for at least two weeks.

The increase in numbers of immunoglobulin-containing cells recorded in this study follow much the same pattern, even if quantitatively the results are divergent, as was reported by Weisz-Carrington et al. (1977) for mice and by Lee et al. (1977) for rabbits. The essential difference in this study is the recording of immunoglobulin-containing cells in the milk. Interestingly if the photomicrographs of Weisz-Carrington et al. (1977) are examined carefully, it is obvious that they show paralumenal and possibly intra-lumenal immunoglobulin-containing cells.

The results recording the immunoglobulin-containing cells during the involution period post-weaning indicate a rapid decline, which correlates with the observations of Weisz-Carrington et al. (1977), and Lee, Laddsand Watson (1978) for mice and rats. Routine histologic examination of the involuting gland was not undertaken and so the

observations of several authors, as reviewed by Lascelles and Lee (1978), of massive cellular infiltrations in the involuting gland can not be commented upon.

The observations as to the number of cells which can be detected in released milk suggests that the NIH mouse is not atypical. The mean milk cell count reported for mice by Head and Beer (1978) was 2.19×10^6 cells/ml in comparison to the 0.73×10^6/ml recorded in this study for naive mice. However, these cell counts require to be treated with great circumspection. The counts of Head and Beer (1978) are reported to be counts of leucocytes whereas the counts in this study are of total cells which on differential counting were shown to be approximately 60% leucocytes. It was not possible to assess viability of the cells easily, for example with Trypan blue exclusion, but it was certainly the case that the vast majority of the epithelial cells were dead cells.

The profile of cells which were recovered from milk was obviously very different from that of cells observed in the lumens of alveoli in histological preparations, and the conclusion was made that a very large proportion of the sloughed epithelial cells which were visible in the alveoli did not survive the period of storage in the mammary gland and/or the milking process. The disintegration rate for epithelial cells could not be determined. It must be considered that a proportion of the macrophages and lymphoid cells also do not survive transmammary passage. Of those which were vizualised in Giemsa stained milk preparations very few of the cells identified as macrophages or lymphoid cells were thought to be other than viable at the time of preparation.

One particular feature to note is that very few of the lymphoid cells had the appearance or could be identified as plasma cells. As such, all were classified as lymphocytes, although many of the large lymphocytes had a considerable body of cytoplasm; these latter cells were concluded to be late stage lymphoblasts.

Given the differential cell counts i.e. leucocytes approximately 60% of total, it would seem appropriate to adjust the figures for NIH mouse milk from approximately 0.73×10^6 cells/ml to an approximate figure of 0.44×10^6 leucocytes/ml for naive mice at mid-lactation, and 0.41×10^6 leucocytes/ml for milk from infected/immune mice at mid-lactation.

Perhaps the most interesting point about thesedata is the lack of any increase in the numbers of cells in the milk of infected mice in contradistinction to the considerably greater number of immunoglobulin-containing cells in the mammary gland of infected mice. However, it is clear that there is a significant increase in the percentage of the milk cells which were classified as lymphocytes. If one extrapolates the percentage figures from Tables 3.3 and 3.4 with the total cell counts the approximate number of lymphocytes in naive milk is revealed as 0.91×10^5 lymphocytes/ml, and for infected/immune milk as 0.11×10^6 lymphocytes/ml. Neither figure can be regarded as indicating an enormous population of lymphoid cells being offered to the suckling infant. Consideration such as these have not been noted in the literature, e.g. Head and Beer (1978) consider that there is a very much larger lymphocyte endowment.

The macrophage population in the milk is larger than that of the

lymphocytes with approximately half of the total milk cells being designated as macrophages. The cells identified as of the macrophage line in the interstitium were not observed to contain large inclusions, indicating perhaps a non-phagocytosing history at this stage. On the other hand cells emigrating through the epithelium seemed to acquire lipid droplets as they were emerging into the lumen, and virtually all the cells observed in the lumen exhibited phagocytosed lipid droplets and presumably also phagocytosed particulate matter. These observations are in agreement with those of Seelig, Holt and Beer (1979). The phagocytosing macrophages of the milk are referred to as foamy macrophages by some authors (Smith and Goldman, 1968; Holman, 1974; Lawton and Shortbridge, 1977; see also Newby, Stokes and Bourne, 1982). A few eosinophils were observed in the connective tissue of the mammary gland of some mice, but neither eosinophils or polymorphs were observed in the secreted milk, either in histological section or in collected milk. Thus the mammary glands of all the animals which were examined were presumed to be free of local damage or infection which might have involved a local inflammatory response.

Attempts to determine the proportion of the cells in milk which were involved with synthesis or transport of immunoglobulins did not proceed to a satisfactory conclusion. The reasons for this have been described in the results section. Apart from lymphoid cells which were shown to contain immunoglobulins of the three classes, it was also the case that some macrophages were also observed to fluoresce/contain all three classes of immunoglobulin. The presumption must be made that the macrophages had phagocytosed liberated immunoglobulin in the milk and might therefore

perform the function, accidentally or otherwise, of transporting immunoglobulin. This concept was also promulgated by Pittard, Polmar and Fanaroff (1977). Possible defence/immunological activity has been demonstrated for both macrophages and lymphocytes in the milk by several authors. Thus Ho and Lawton (1978) concluded that milk macrophages, even after absorbing milk lipid, maintained some phagocytic and bacteriocidal properties. Parmely, Beer and Billingham (1976) demonstrated that milk lymphocytes will transform in vitro with mitogenic stimulation, and Ogra and Ogra (1978) also demonstrated reactivity of milk lymphocytes.

The issue of viability, survival and possible uptake of maternal milk lymphocytes in the suckling infant is one which has raised considerable controversy in recent years. Kmetz, Dunne and Schultz (1970) reported that in suckling rabbits, bovine leucocytes migrated into the intestinal epithelium. Beer, Billingham and Head (1974 and 1975), and Head, Beer and Billingham (1977) concluded that the lymphocyte component in milk conferred some protection to the suckling infant and induced favourable or unfavourable competence to grafts and tumours. They indicated that lymphocytes remained viable in the passage through the stomach of the suckling mouse and that maternal lymphocytes could then cross the infants intestinal epithelium, establish within the tissues and thereby confer competence to the young animal. Silvers and Poole (1975) however, concluded from their fostering experiments that cell mediated immunologic competence could not be transferred via the milk, and that maternal leucocytes could not survive for a suitable period of time in the neonates alimentary tract. Trentin, Gallagher and Priest (1977) using

chromosomally marked cells also concluded that gut transepithelial migration did not occur in the suckling infant mouse at 14 and 21 days of age, and Slobodian, Carlson and Wegmann (1979) also concluded that T-lymphocytes did not enter the infant's gut epithelium. However, Schlesinger and Covelli (1977) produced good evidence that tuberculin reactivity could be observed in suckling babies which probably indicated the uptake of T-lymphocytes from the mothers milk. Head and Beer (1978) maintained that maternal leucocytes remained viable in the suckling mice and rats, and quoted studies on rats by Manville and Lloyd (1932) which showed that the pH of the stomach of rats is 6.6 at birth and does not fall below 5.0 until nearly 3 weeks post-partum, and of Luckey, Mende and Pleasants (1954) who commented upon the high buffering capacity of rat milk which may be an additional protective measure for cells, and of Tatematsu, Takahashi, Tsuda, Hirose, Furihata and Sugimura (1975) who reported that little or no peptic activity can be detected in the stomachs of rats until they were more than two weeks old.

The observations of the present study indicate the presence of apparently viable maternal leucocytes in the stomachs of suckling infants through at least the first two weeks of suckling. Attempts to determine the viability of these cells in vitro were unsuccessful with few cells being adequately freed from the semi-congealed mass of milk in the stomach. Attempts to register the continued immuno-globulin content of these maternal leucocytes was therefore thwarted, as were the efforts involving labelled conjugate fluorescence on cryostat sections of the infant stomach. In this latter case it was impossible to maintain the section of stomach milk on the slides due

presumably to the high free lipid content of the ingesta. In the sections in which the stomach tissue could be maintained on the slide there was no evidence of absorbed immunoglobulins in the stomach epithelium or submucosa.

Sections of infant small intestine also did not demonstrate the presence of leucocytes in the lumen. Most frequently the lumen contents had detached during the incubation, but even in sections where some ingesta remained close to the epithelial surfaces, cells were not observed. However, personal communications from Bruce and Manson-Smith indicate that in infant mice which were artificially given large numbers of maternal mesenteric lymphocytes by oral intubation, considerable populations of these maternal lymphocytes could be recovered intact from the infant small intestine, or as viewed by scanning electron microscopy would have been judged to be intact and probably viable cells. It would therefore seem reasonable to support the views of Head, Ogra and colleagues in concluding that maternal leucocytes or at least a reasonable percentage of them remain viable as far as the infants small intestine.

In the studies on the small intestine of the infants, epithelial attached or intraepithelial leucocytes were not observed. Had they been so, it might have suggested maternal leucocytes in transit. A well defined layer of immunoglobulins on the epithelial surface of the intestinal cells was not observed, nor was there any evidence of notable or discrete amounts of immunoglobulin in the epithelial cells. Thus it is not possible to add to knowledge of the possible mechanisms of uptake of maternal immunoglobulins by the neonates. It was intended

to pursue this area of investigation utilizing peroxidase labelled worm antigen at the electron microscopic level but it was not possible to devote sufficient time to this proposal.

It is quite clear however, that there is discrete cellular localization of IgA and IgM immunoglobulins within the submucosa of the infant small intestine. These were first observed at 6 days of age. The fluorescence obtained was always very weak and the number of cells observed were few in number and of very inconsistent distribution. In contrast IgG was more readily observed in the submucosa but rarely of a cellular localization. The central and basal draining ducts of the villi and the crypts showed quite marked localization of IgG. While much more regular in occurrence, not all villi or areas of the sections exhibited the same degree of fluorescence but it was not possible to determine any regional morphology which might explain the patchy occurrence of the IgG. Obviously it is not possible, given the limited scope of this part of the investigation to state categorically that the observed immunoglobulins whether duct related or cell localized in the infant intestine are of maternal or infant origin. However the observations of Crabbé et al.(1970) are extremely pertinent. These authors recorded that in C3H mice the first immunoglobulin-containing cells to colonise the infant small intestine were of the IgA class at 10 days of age followed by small numbers of IgM and IgG containing-cells intermittently from day 13. Perhaps the observations in NIH mice indicating a very much earlier localisation of cells reflects on a strain difference of the species. Alternatively the observations of these cells in NIH mice might indicate translocation of maternal cells into the infant

tissue associated with a possibly larger endowment of maternal milk lymphocytes. It was not possible to determine convincingly the nature of all of the fluorescing cells in the infant intestine but a proportion of them were tentatively identified as lymphoid cells.

Given the observations of Crabbé et al. (1970) of Halliday (1955a,b, 1957), and Brambell (1970) on the near zero ability of very young animals to synthesise immunoglobulins and the capacity of young mice to take up maternal immunoglobulins, it would be difficult to conceive that the IgG which is so clearly observed in the draining ducts and interstitium of the NIH infant intestine is not of maternal origin.

For other species Vaerman and Heremans (1969) had noted that IgM containing cells were the only type present in the intestine of 6 day old dogs. Allen and Porter (1977) studying pigs recorded that intestinal IgM-containing cells were observable by 2 days of age, a few IgA-containing cells by 4-5 days of age and IgG-containing cells by 9-10 days of age. In humans Perkkiö and Savilahti (1980) noted that Ig-containing cells were not observed before 12 days of age but very interestingly they noted that, as in the present study, intercellular and duct (capillary) related IgG was observed. Regrettably, however, this paper by Perkkiö and Savilahti did not even record whether the infants were breast fed. It must be recalled that with the very different gut closure times of these species from each other and/or from mice that little in the way of a concensus conclusion, other than that IgM-containing cells are the first to appear, can be arrived at.

Immunofluorescence which is successfully used for diagnosis in many parasitic diseases (Houba, 1980) has one particular advantage over most

other assay systems in that intact organisms or sections can be used as the substrate. Given that the worm components remain unaltered, immunofluorescence can be used to identify the antigenic components of parasites, as well as demonstrating host responses. Hogarth-Scott (1966) used this technique on the second stage larvae of Toxocara canis and Toxocara cati and demonstrated the occurrence of precipitates at the oral, excretory pore and the anal orifices of these larvae, which were shown to be of specific antibody nature, thus indicating the presence and possible significance of the antigenicity of metabolic components of the worm. In this study the infective larvae only of T. spiralis were used to determine the antigenic sites and the antibodies which reacted to them. This was not because they are the most readily obtainable stage of T. spiralis or that they are the predominant stage in the life of the parasite - in any natural infection with T. spiralis, over 90% of the life of the parasite is spent as the mature infective stage. The logic for selecting this stage for fluorescence studies emerged from the conclusions of Section 1 where it was determined that the only protection afforded by the mother to the infant was protection involving the non-establishment of the infective larvae in the intestinal niche within the infant. Thus the protective components should indicate reactive sites within the infective larvae.

It would be predicted that the key features of the worm which should show antigenicity and therefore possibly be subject to damage, alteration or neutralization by the components of the host response would be the cuticle, with or without secreted components, the secretion products of the stichosome or the cells and product of the worm's intestine which

might be affected by ingested host materials. It was equally predictable that virtually every visible component of the worm would show some fluorescence/antigenicity. Many authors working with nematodes had observed that worm muscle and cuticle showed antigenicity eg. Jackson (1959) and Ljungström (1974) working with T. spiralis and Smith, Quinn, Bruce and Girdwood (1982) working with Toxocara canis and Toxascaris leonina. In this latter study these authors had demonstrated cross-reactivity of many worm components.

In the present study, it is shown that there are antibodies of the IgG, IgA and IgM classes to several components of the worm. Most prominently and consistently the membranes and basal laminas of the lateral hypodermal cords, and of the single layer of the longitudinal muscle showed the strongest fluorescence with conjugates for IgG, IgA and IgM antibodies. The membranes of the primordial gonad cells also fluoresced prominently. All these structures yielded some fluorescence when incubated with relatively low dilution naive sera, thus indicating non-specific binding of immunoglobulin. These same structures are frequently involved in cross reactions with antisera to other parasitic infections as exemplified by Smith et al. (1982).

Antibody to the cuticle was demonstrable in all three classes of immunoglobulin even if only very weak fluorescence was observed with IgM. With IgG and IgA, infection specific antibody was present by day 15 post-primary infection. By infection specific it is meant that fluorescence was obtained with titres well in excess of weak fluorescence obtained with naive sera. With IgA no naive sera fluorescence was detected on the cuticle. Of particular note is the

fact that cuticular fluorescence was confined to the extreme outer margin of the cuticle i.e. the outer membraneous component and of material adherent to the outer surface. Patchiness indicating accumulation of externally attached material was particularly in evidence with IgA. The absence of fluorescence from the main body of the cuticle would seem to indicate that the external material was not a component of the cuticle which was slowly released at the surface. The concept of membrane or externally attached antigens in T. spiralis is not a new one, having been particularly well demonstrated by Despommier, Kajima and Wostmann (1967) when ferritin conjugated antibodies were shown to adhere to the outer surface of the cuticle of T. spiralis. The origin of these antigens remains obscure, although several studies have demonstrated the presence of stage specific cuticular antigens in T. spiralis and other nematodes (Phillip et al., 1980, 1981; Parkhouse et al., 1981; Maizels, Phillip and Ogilvie, 1982).

The metabolic nature of such cuticular surface antigens was demonstrated clearly by Klaver-Wesseling, Vetter and Visser (1978) in larvae of Ancylostoma caninum, and subsequently by Smith, Quinn, Kusel and Girdwood (1981) for Toxocara larvae. These authors showed that cuticle surface antigen could be detected on viable larvae if incubation was performed at low temperatures (2°C) but were not detectable if incubation was carried out at 37°C indicating that the antigenswere readily shed and detached from the surface of the cuticle. It was also observed by Despommier et al., (1967) that the ferritin conjugated antibodies which bound to the cuticle surface would also bind with accumulation of secretions/excretions at the oral and anal orifices of T. spiralis.

Thus the source and/or mechanism of release of the surface antigens is not resolved. Furthermore no-one has been able to demonstrate that antibodies bound to the cuticle surface impair the functioning of the infective larvae of any species although cell mediated (eg. eosinophil) killing of newborn larvae subjected to antibodies has been demonstrated in vitro (Kazura and Grove, 1978; Mackenzie, Jungery, Taylor and Ogilvie, 1980). Such observations, however, revealing of possible mechanisms of anti-nematode activity, are clearly not relevant as explanations of non-establishment of infective T. spiralis in naive infant mice.

The antigenicity of some components of the secretions of the stichosome has also been well documented, particularly by Despommier and colleagues (Despommier and Muller 1976; Despommier et al., 1977a; Despommier and Laccetti, 1981 a & b). These authors showed that extracts of stichocytes conferred good immunity in mice and pigs and that the most antigenic component was derived from the α granules of the stichocyte. As Bruce (1974) and Despommier (1974b)noted, the granule profile of the stichosome altered very rapidly with development in the intestine. Within 12 hours few of the highly antigenic α granules were to be observed, being replaced almost entirely by continued production of β granules. In the present study it is demonstrated very clearly that the first detectable antibodies (IgG) in a primary infection of T. spiralis in NIH mice are raised against stichocyte material and are in evidence by day 12 post-primary infection, some days before antibodies to any other worm components are observed. Crandall and Crandall (1972) also noted that serum IgG and IgM levels were elevated in the second week of infection of T. spiralis in mice and both IgG_1 and IgG_2 classes of antibodies

exhibited specificity for stichocytes. They also showed that IgM and IgA classes of antibodies showed more affinity for the various membranes of the larvae. In the present study IgA antibody binding to the stichocytes could not be demonstrated, suggesting therefore that the antigenicity of the stichocytes was different to that of the cuticle surface membrane or adhesions. The exact nature and function of the stichocyte secretions are not known although Bruce (1967) and Despommier (1974b) suggested that they were enzymatic and when released from the worm via the mouth or the anus might function as tissue digesting enzymes affording the worm extra-corporeal digestion; Bruce (1967) demonstrated the presence of aminopeptidases in the stichocytes.

There was very little in the way of antibody binding to the cells of the intestine of the worm, and the lumenal contents of the intestine were an unreliable source of antigens for fluorescence, - the fine material comprising the contents frequently detaching during incubation. When present, strong fluorescence of IgG antibodies was observed, with weaker fluorescence of IgA antibodies and very weak fluorescence of IgM antibodies. The IgA antibodies to gut contents, although weak, indicate a difference in antigenicity between stichocyte material (no reaction) and gut contents.

It is conceivable therefore that antibodies to materials secreted by the worm, to be used in penetrating and feeding on epithelia could neutralize the enzymatic function of these secretions and render establishment difficult. An alternative or additional possibility is that antibodies binding to the surface of sensory receptors such as the amphids might render them inefficient and also result in reduced

establishment. From the point of view of comprehending the partial protection conferred by mother to young via the milk, the recent developments in determining the stage specificity of cuticular antigens of T. spiralis (Phillip et al., 1980, 1981; Parkhouse et al., 1981; Maizels et al., 1982) has once more focussed attention on the significant antigenicities of different stages of this nematode. It should be recalled that Oliver-Gonzalez (1941) first reported that two types of antibodies are produced by T. spiralis infected rabbits,one group acting on infective larvae and a second acting on adults.

Thus it is now possible to conceive that the endowment in the milk of infected/immune mothers contains antibodies which operate in the lumen of the infant small intestine, or at the epithelial lumen surface, or within the epithelial cells to impair the efficiency of the penetration/establishment process of the infective larvae, or any two or three of these sites. If however, larvae do succeed in establishing within the epithelial habitat, then rapid development and alteration of the cuticular and stichocyte antigens would render them less susceptible to the relatively small amounts of antibodies derived from the mother which are present in the tissues of the infant. Thus development to adulthood would be relatively unimpaired and expulsion would not occur until the infant's endogenous reactions were forthcoming.

3.5 Summary.

In the mammary gland of both naive and T. spiralis infected mice lymphocytes and macrophages occur frequently in the connective tissue and in the intraepithelial position in alveoli. A considerable number

of the lymphoid cells were shown to contain either immunoglobulin A or M or G with IgA-containing cells being two to three times commoner than IgM or IgG-containing cells. In T. spiralis infected mice there was a significant increase in the numbers of immunoglobulin-containing cells in the mammary gland particularly during the mid-lactation period.

Lymphocytes, some of which contained immunoglobulins were present in the milk. IgG-containing lymphocytes were more abundant than IgA-containing cells. Macrophages are also present in the milk and constitute approximately 45% of the total leucocytes in the milk. Most of these macrophages contained phagocytosed lipid droplets and some of them also contained immunoglobulins. The milk of infected mice contained a slightly greater number of lymphocytes than did naive milk. The leucocyte endowment of the milk of infected mice was calculated as 1.1×10^5 lymphoid cells and 3.0×10^5 macrophages per ml. Both lymphocytes and macrophages, apparently intact and viable at the time of fixation were observed in histological sections of the stomachs of suckling mice. They were not visualized in the small intestine of suckling infants. Neither leucocyte was observed attached to or penetrating the infant gut epithelial tissues.

Immunoglobulin-containing cells were observed in the lamina propria and submucosa of infant mice from the sixth day of age. IgA and IgM-containing lymphoid cells were just detectable. The amounts of IgG were considerably greater, but with few IgG-containing cells. IgG was readily detectable in ducts associated with the central lacteal drainage channels of the lamina propria and with ducts in the submucosa. This is thought to be the earliest recorded occurrence of Ig-containing cells in the gut of infant mice.

IgG, IgA and IgM antibodies to several components of the infective larva of T. spiralis were present in the sera of adult mice. They were also detected but not studied comprehensively in milk from infected mice. In sera, IgM antibodies (max. titre 1:400) reacted with most of the components of the worm including the three components of the worm, - the cuticle, stichosome and intestine which were most likely to be associated with excretory/secretory metabolic antigens. With reference to these structures IgG antibodies (to 1:512) were recorded on the surface of the cuticle, the contents of some stichocytes and to the contents of the lumen of the larval intestine. IgA antibodies (to 1:512) reacted with the cuticle surface and accumulations of material on the surface, to 1:256 to intestinal lumen contents but with no reactivity to stichocyte materials. This would indicate a different antigenic identity for the stichocyte material - presumably the particular secretory granules present in some stichocytes. IgG antibody to the stichocyte material was the first detectable response and was recorded on day 12 of a primary infection.

Table 3.1; Weight increase of Female NIH mice after mating and during pregnancy.

	Weight in g			
	Mouse	Days after mating		
		1	7	14
	1	23.6	25.6	34.05
	2	25.5	26.4	32.55
	3	23.8	29.3	31.8
	4	25.4	27.5	35.05
	5	25.7	27.6	31.65
	6	24.3	28.00	33.45
	7	23.5	29.35	34.00
	8	25.9	27.6	34.09
	9	26.00	26.8	33.04
	10	24.8	27.2	33.7
	11	23.9	28.4	33.75
	12	25.35	28.2	30.7
	13	24.35	27.5	30.05
	14	25.6	26.2	29.05
	15	23.65	27.3	30.6
Mean ± s.d.		24.75 ± 0.92	27.53 ± 1.04	32.5 ± 1.76
% of weight increase			11.23%	31.31%

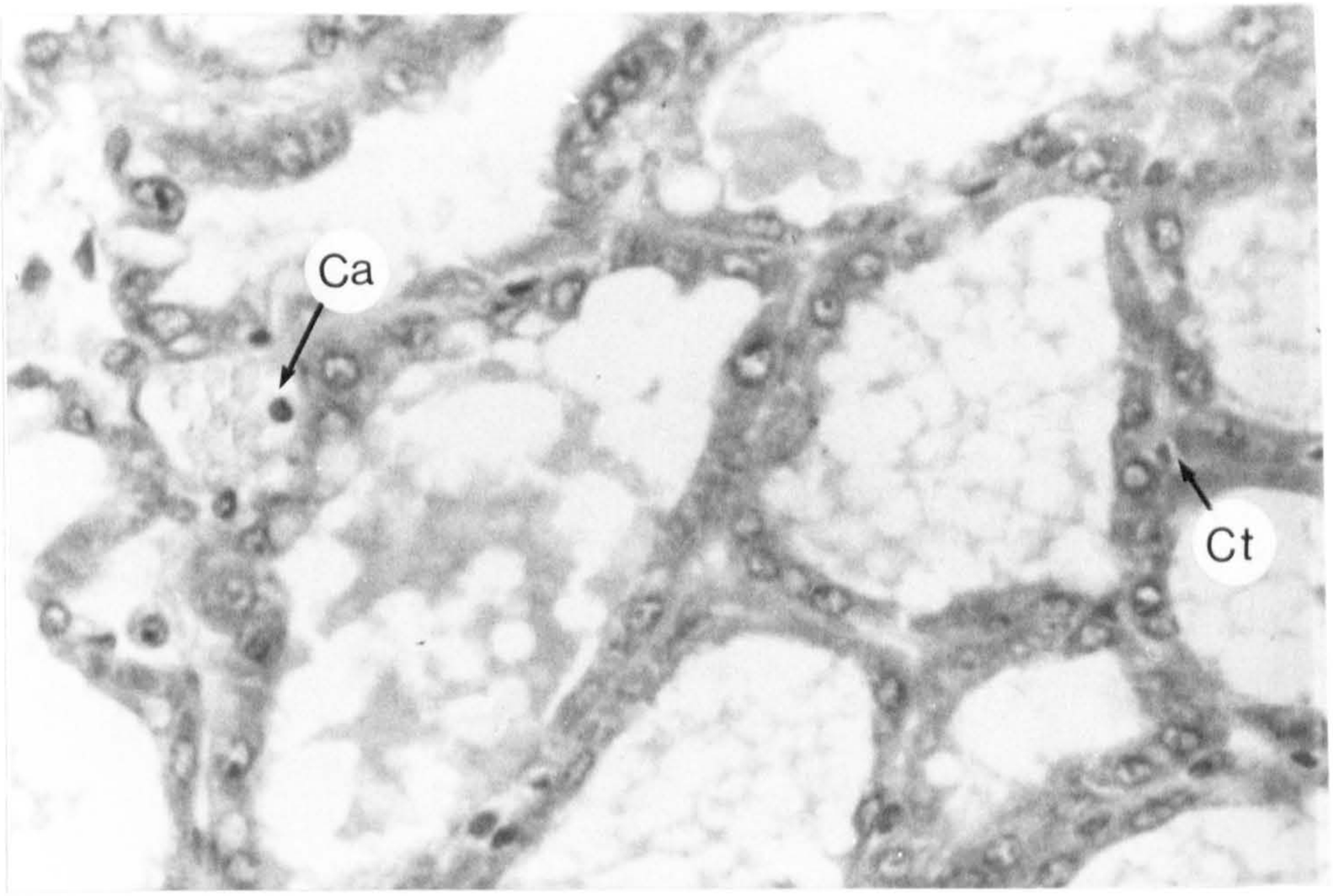


Plate 3.1.A: Lactating mammary gland (7 days). Bouin fixation. Wax section. H & E stain-magn. X500.
This section shows the cuboidal alveolar epithelium with the lumen of the ducts filled with milk. The section contains a capillary (CA) with lymphoid cells which are also visible in the connective tissue (Ct). The milk solids appear as fine particulate matter and lipid droplets are visible as clear circular profiles in the ducts and in the apical regions of the epithelial cells.

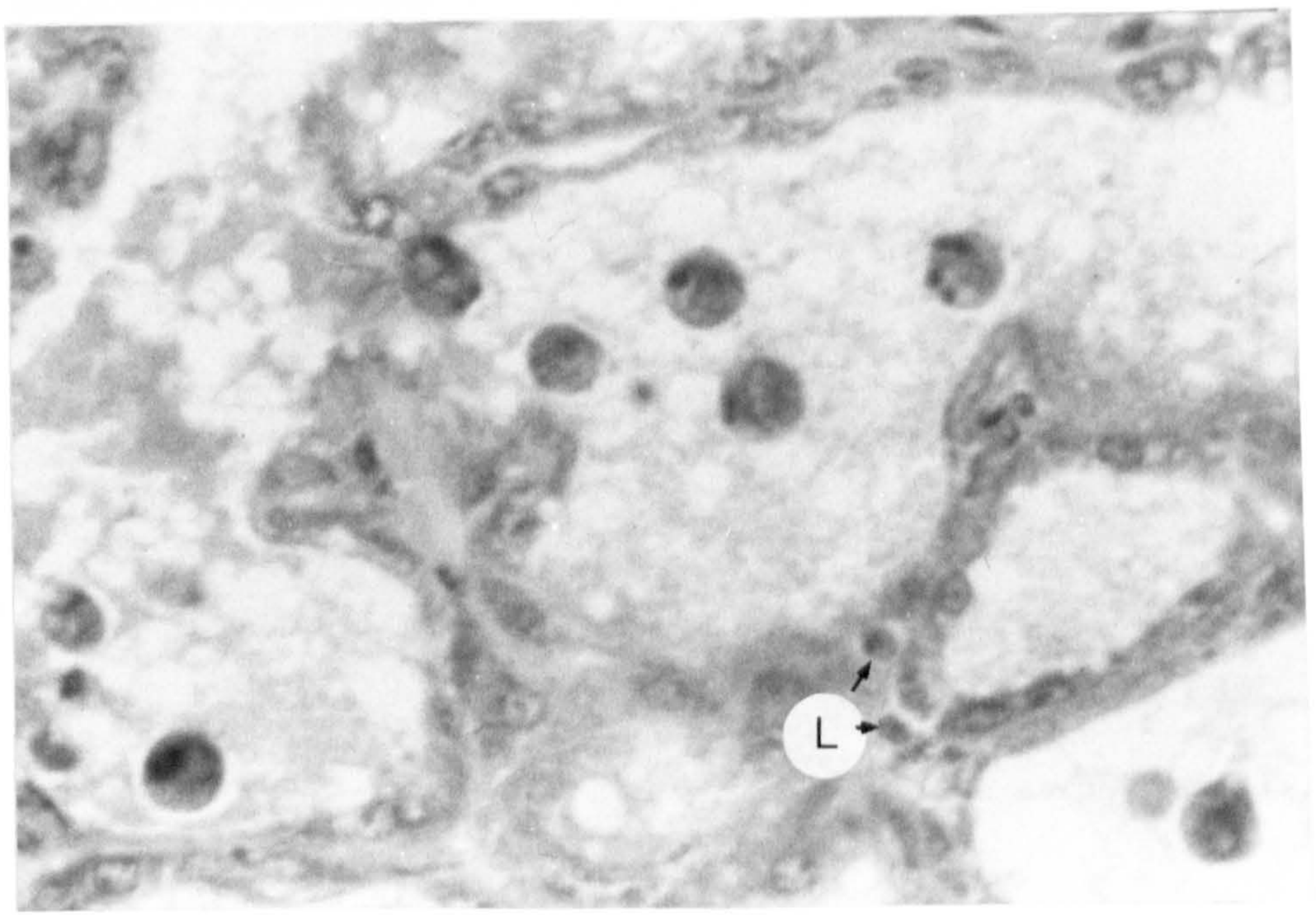


Plate 3.1.B: Lactating mammary gland (7 days). Bouin fixation. Wax section. H & E stain. Magn. X580.
Most of the cells visible in the lumen of alveoli are sloughed epithelial cells characterized by pyknotic nuclei and a slightly vacuolar appearance. To the lower right are a vacuolated macrophage and a lymphoid cell. Several lymphoid cells (L) are present in the subepithelial layer of connective tissue.

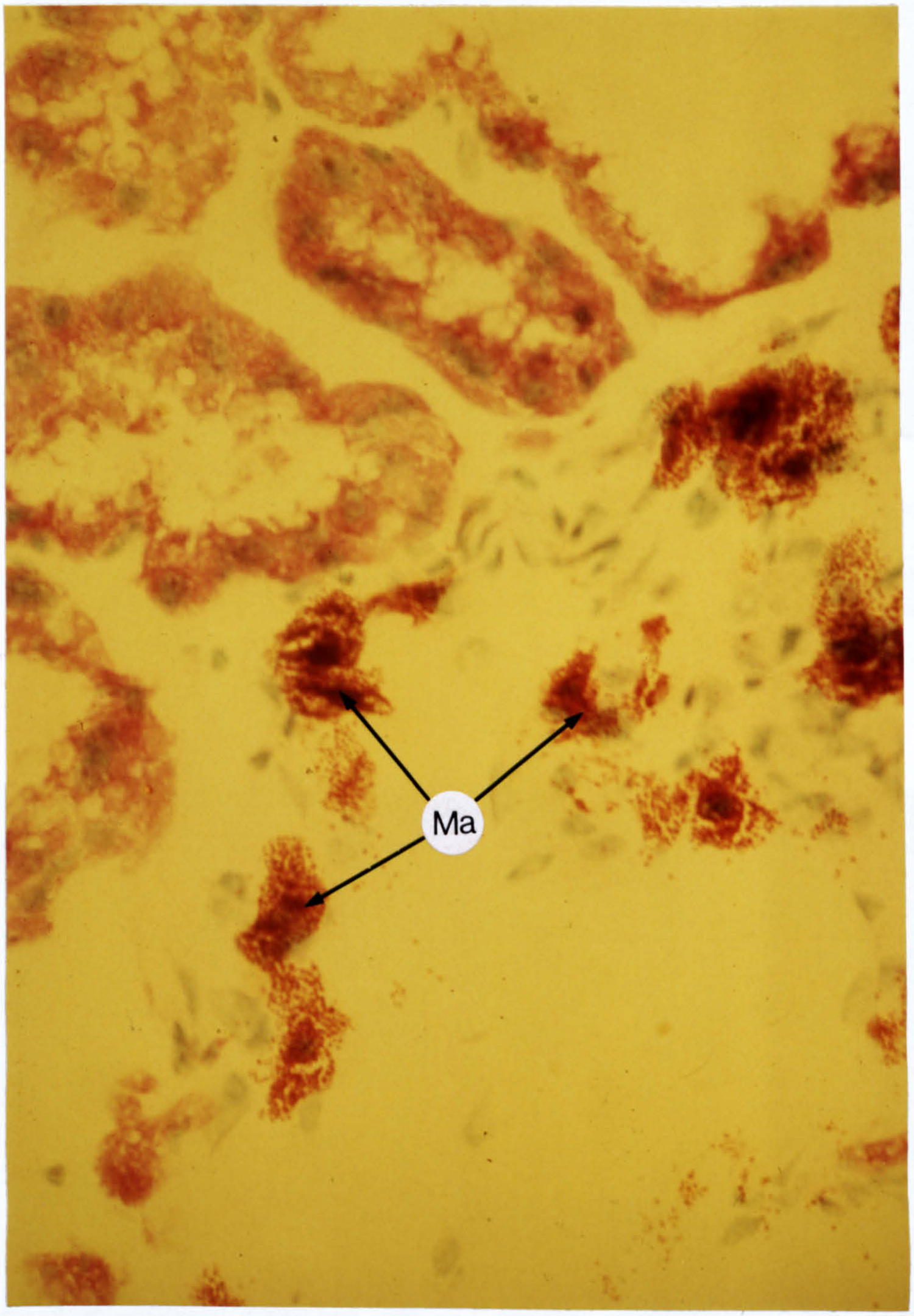


Plate 3.2:

Lactating mammary gland (9 days) from T.spiralis infected mouse. Wax section. Unna Pappenheim stain. Magn.X800. Mast cells (Ma) in the connective and adipose tissue.

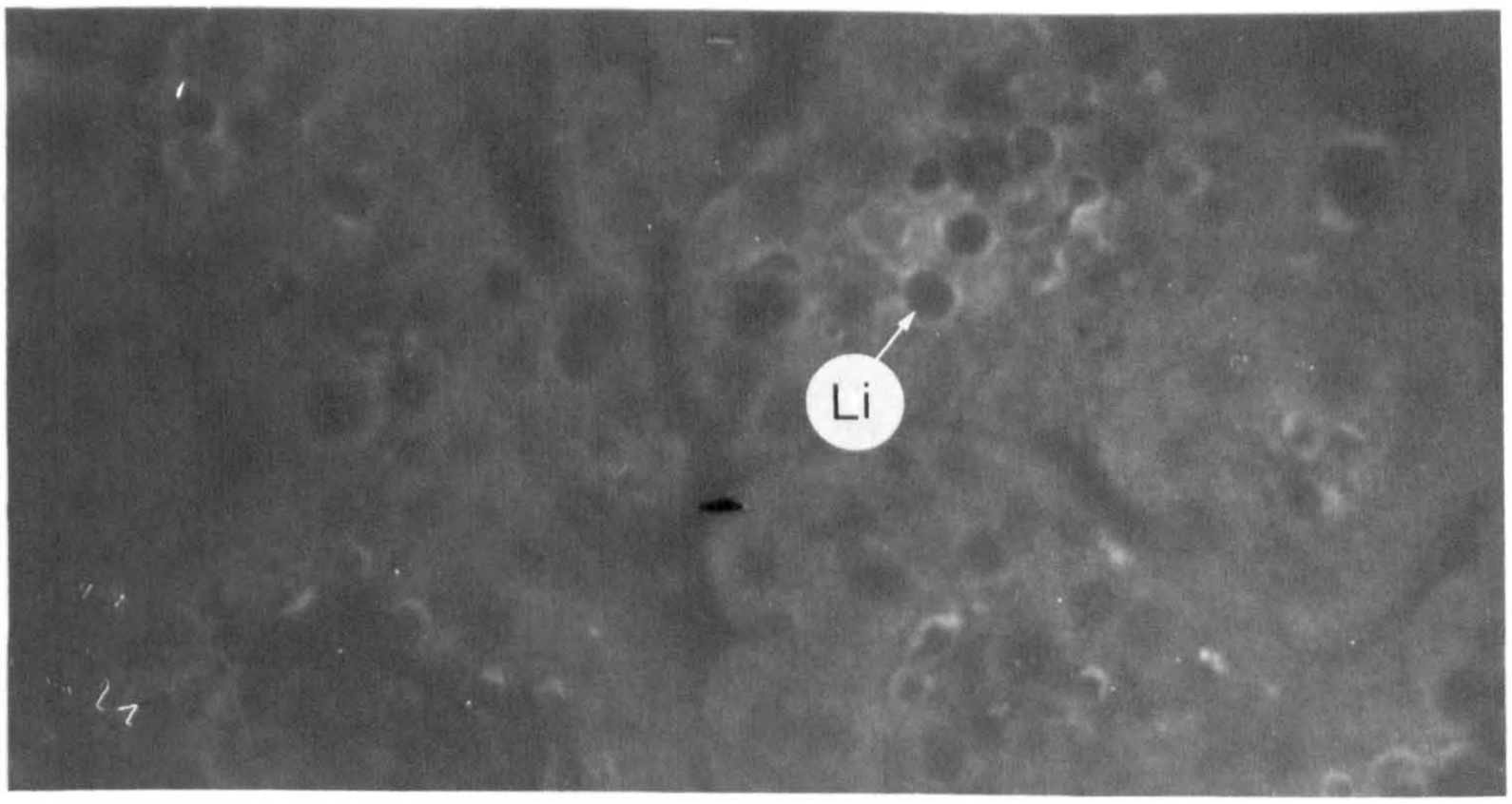


Plate 3.3.A: Lactating mammary gland (10 days) from naive NIH mouse. Frozen section, pre-incubated with unconjugated goat anti-mouse IgG, followed by FITC conjugated goat anti-mouse IgG. Magn. X 500. A very fiant halo effect is visible around the droplets of lipid (Li) within the alveolar epithelial cells and in the lumen of the alveoli.

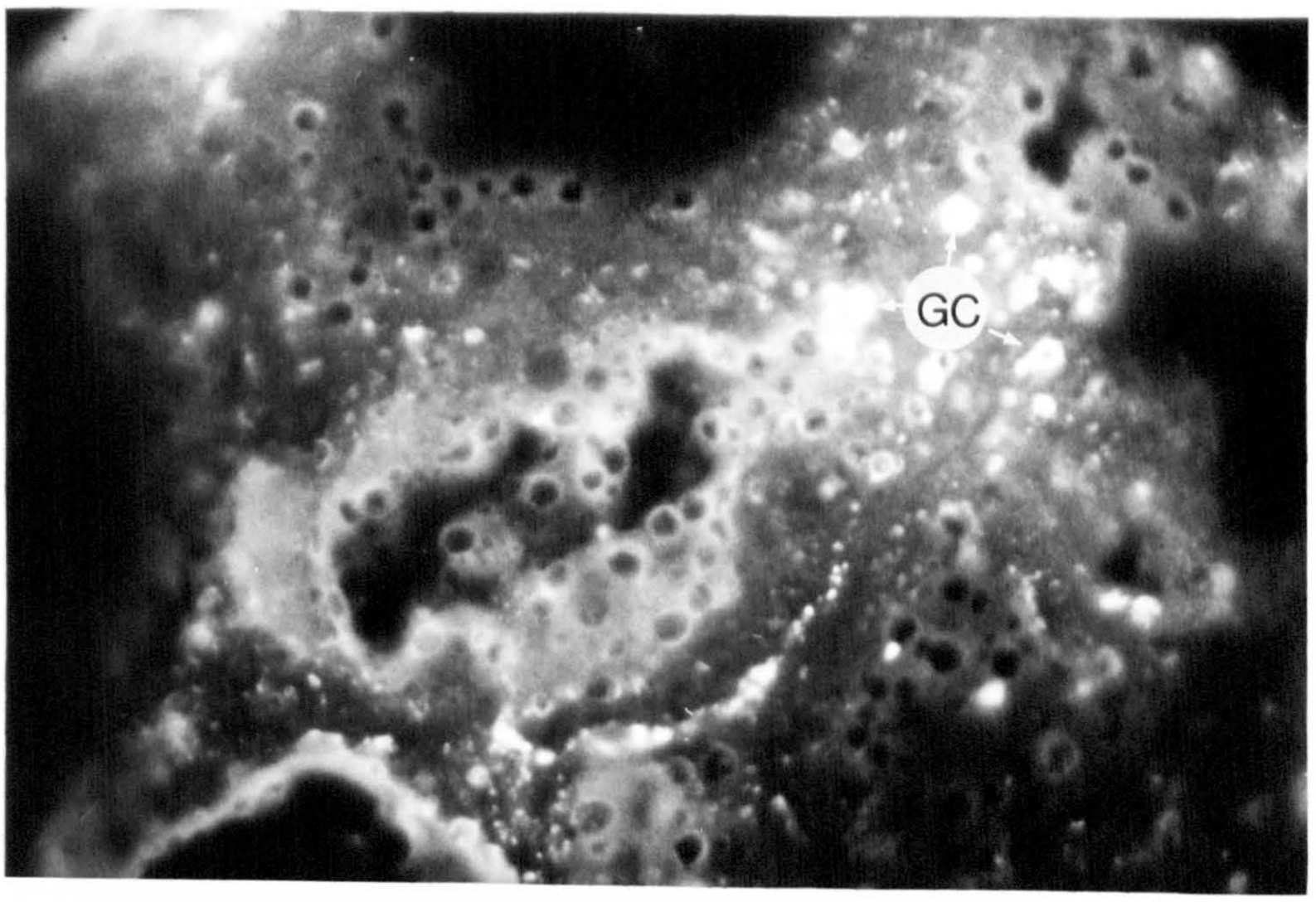


Plate 3.3.B: Lactating mammary gland (16 days) from T.spiralis infected NIH mouse. Frozen section, incubated with 1/8 dilution of FITC conjugated goat anti-mouse IgG. magn. x 400.
A number of strongly fluorescing cells (GC) situated basally to the alveolar epithelium and within the connective tissue are in evidence. In the centrally placed alveolus there is moderate fluorescence associated with the fine particulate material of the milk and with the lumenal border of the epithelial cells. One of the adjacent alveoli shows a fluorescing margin . The two other alveoli in the field contain no milk material (presumed to have washed out) and show no significant fluorescence.

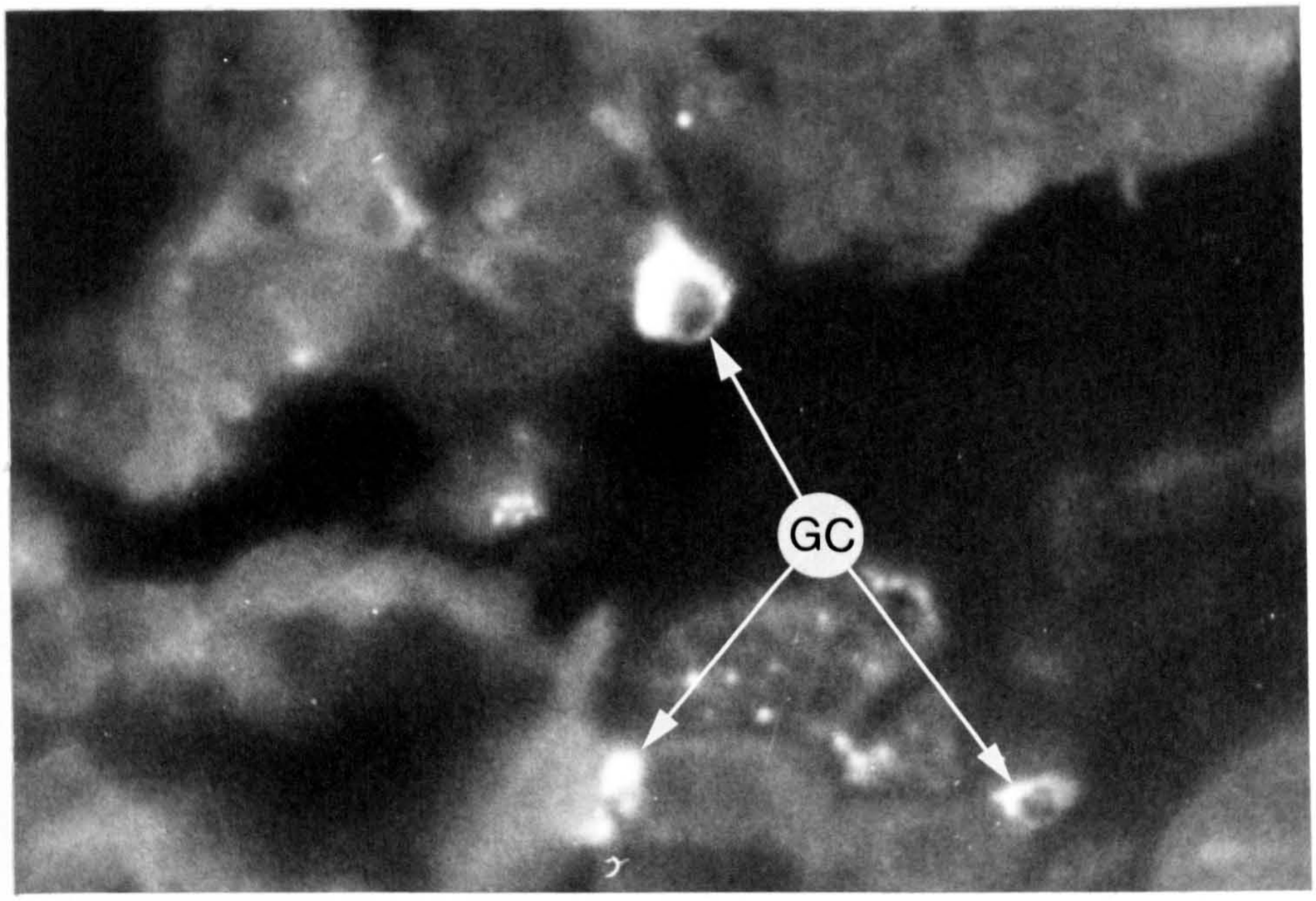


Plate 3.4.A: Lactating mammary gland (10 days) from naive NIH mouse. Frozen section, acetone fixation - incubated with 1/20 dilution of FITC conjugated goat anti-mouse IgG. Magn. x 650.
Strongly fluorescing IgG containing cells (GC) located in the intraepithelial/basal position.

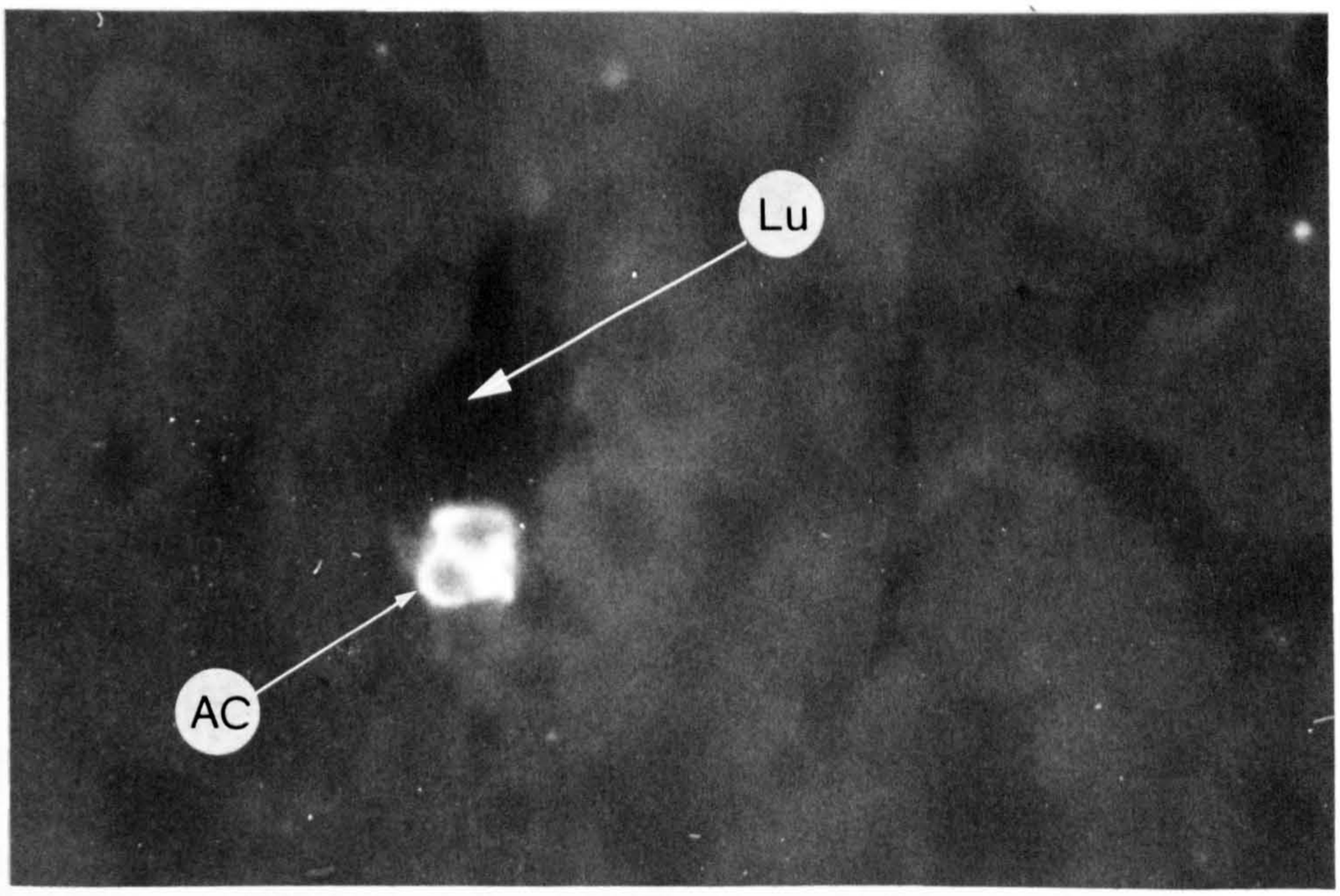


Plate 3.4.B: Lactating mammary gland (10 days) from naive NIH mouse. Frozen section, incubated with 1/40 dilution of FITC conjugated goat anti-mouse IgA. magn X 650.
Two strongly fluorescing IgA containing cells (AC) extruding from the epithelial margin into the lumen of an alveolus (Lu). There is no evidence of connective tissue or epithelial cell fluorescence.

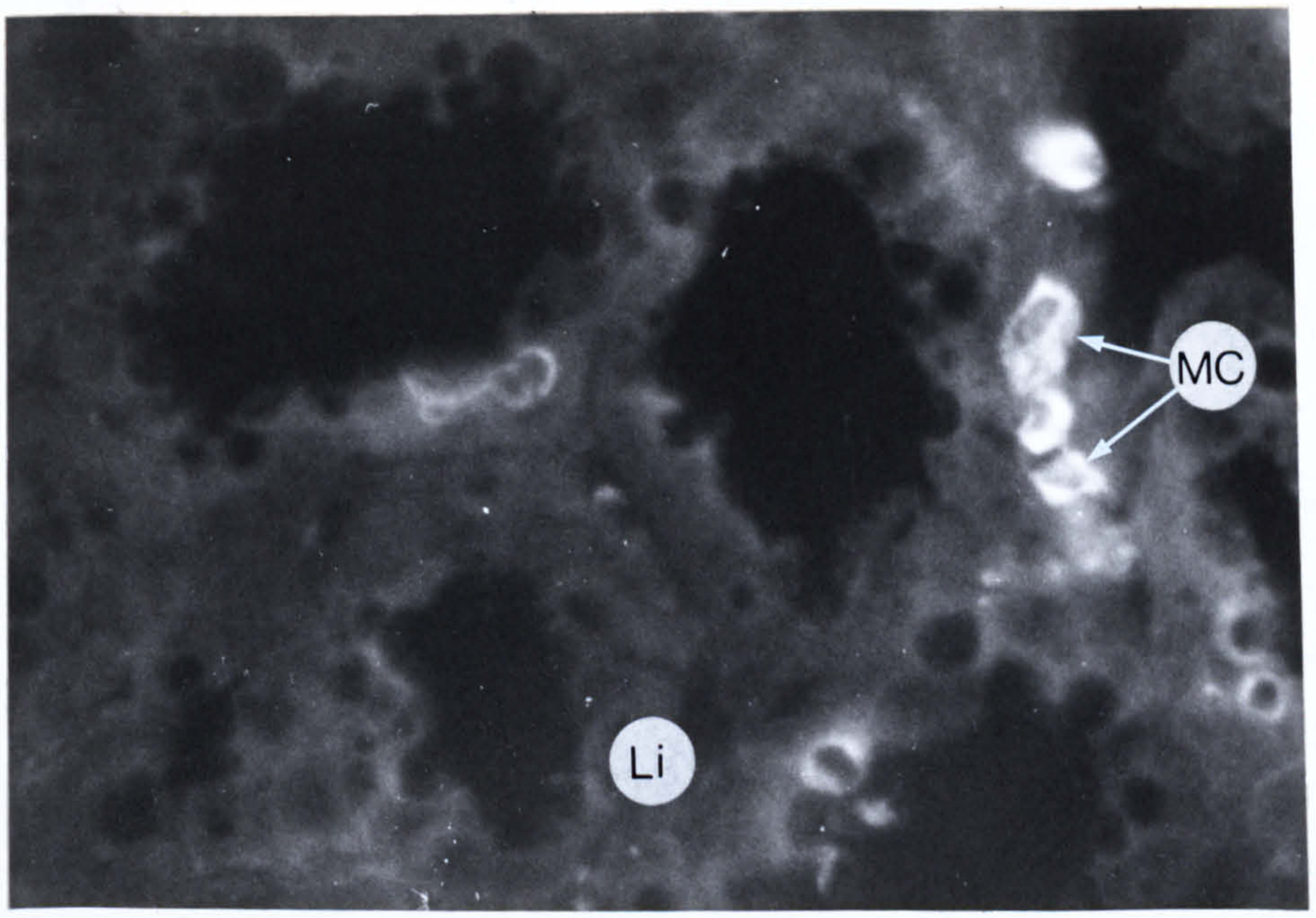


Plate 3.5.A: Lactating mammary gland (3 days) from a T.spiralis infected NIH mouse. Frozen section, incubated eith 1/40 rhodamine conjugated goat anti-mouse IgM. Magn X 450. Strongly staining cells (Mc) located within connective tissue and basally within alveolar epithelium. A very common feature in these preparations is the strong halo staining of some lipid droplets (Li) which are on the point of being extruded into the lumen of the alveolus.

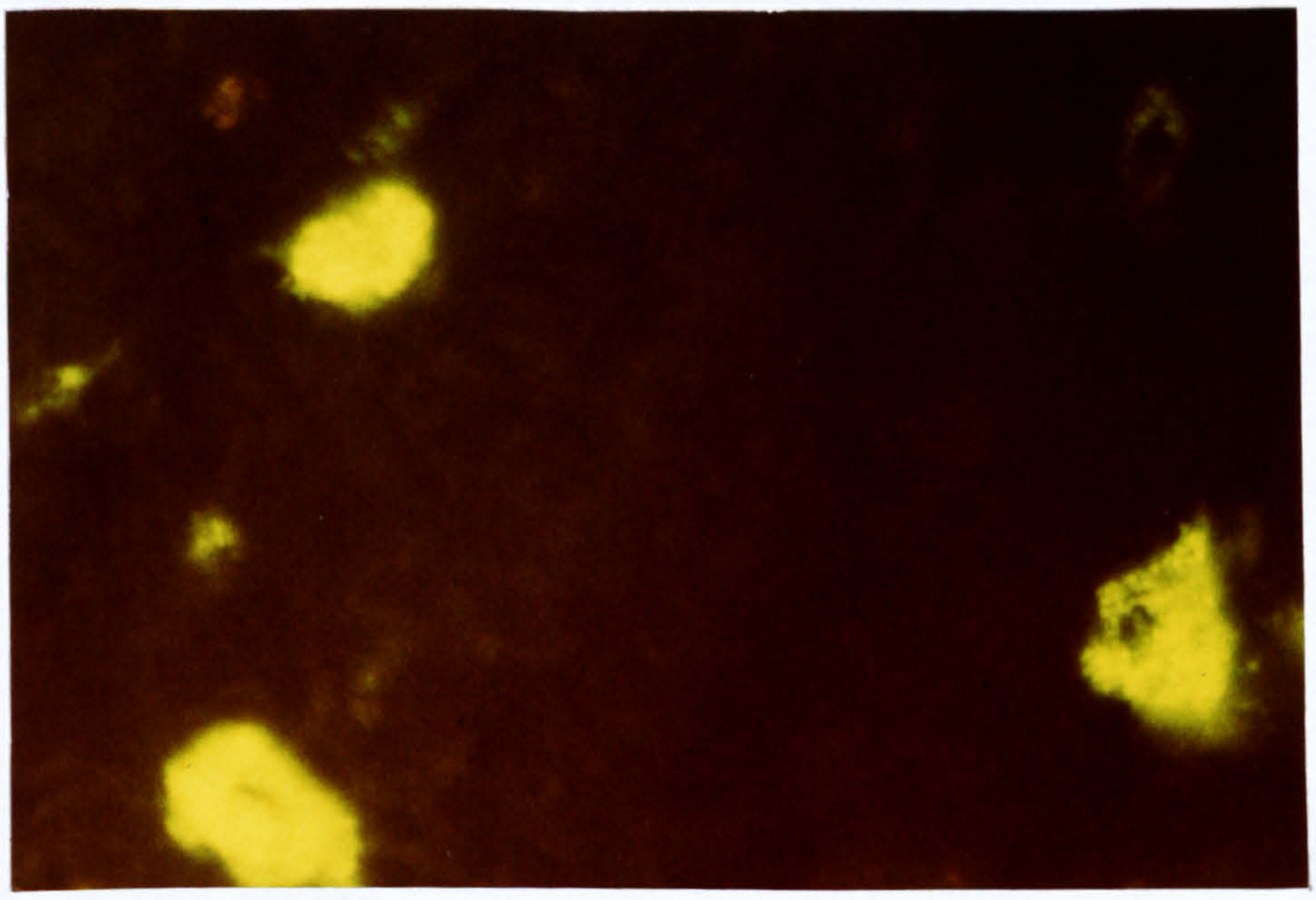


Plate 3.5.B: Mammary gland from naive NIH mouse during late pregnancy. Frozen section incubated with 1/40 dilution of FITC conjugated goat anti-mouse IgG. Magn X 700. Mast cells within the lymph nodes associated with the mammary gland. The one to the right shows the strongly fluorescing granules clearly.

Table 3.2: Number of IgA, IgM and IgG - containing cells per 100 fields in the mammary glands of naive and infected/ immune mice during pregnancy, lactation and after weaning.

	Number of immunoglobulin containing cells(*Mean ±s.d.) Stage of gestation or lactation						
Mouse condition	Pregnancy		Lactation			Post-weaning	
	Mid	Late	Early	Mid	Late	1 week	2 weeks
IgA							
Infected/ immune	8.3 ±1.23	14.98 ±3.69	38.99 ±3.83	48.29 ±4.47	56.64 ±8.11	26.3 ±3.21	9.23 ±2.08
Naive	4.48 ±1.66	10.23 ±0.09	33.53 ±0.9	39.14 ±4.03	47.9 ±5.75	20.34 ±1.9	6.72 ±5.30
Statistical significance (P value)	N.S.	N.S.	N.S.	P<0.01	N.S.	N.S.	N.S.
IgM							
Infected/ immune	7.66 ±2.88	12.52 ±2.88	19.66 ±5.22	27.22 ±1.85	23.01 ±4.24	10.95 ±5.3	1.94 ±1.33
Naive	3.45 ±0.88	9.58 ±0.82	15.38 ±2.17	18.3 ±2.26	25.99 ±5.71	14.42 ±4.55	N.D.
Statistical significance (P value)	N.S.	N.S.	N.S.	P<0.01	N.S.	N.S.	-
IgG							
Infected/ immune	N.D.	6.5 ±2.12	9.01 ±3.06	18.61 ±2.79	12.94 ±5.47	4.49 ±1.21	N.D.
Naive	N.D.	5.92 ±1.48	6.11 ±1.25	13.39 ±0.64	8.9 ±2.36	3.64 ±2.41	N.D.
Statistical significance (P value)	-	N.S.	N.S.	P<0.05	N.S.	N.S.	-

Fields observed with 50 x objective, 10 x eye piece.
*Means of 2 mice during pregnancy and after weaning, and 3-8 mice during lactation. Primary infection 4 weeks and secondary infection 1 week before mating.

N.D. = Not detected.
N.S. = Not significant.
s.d. = Standard deviation.

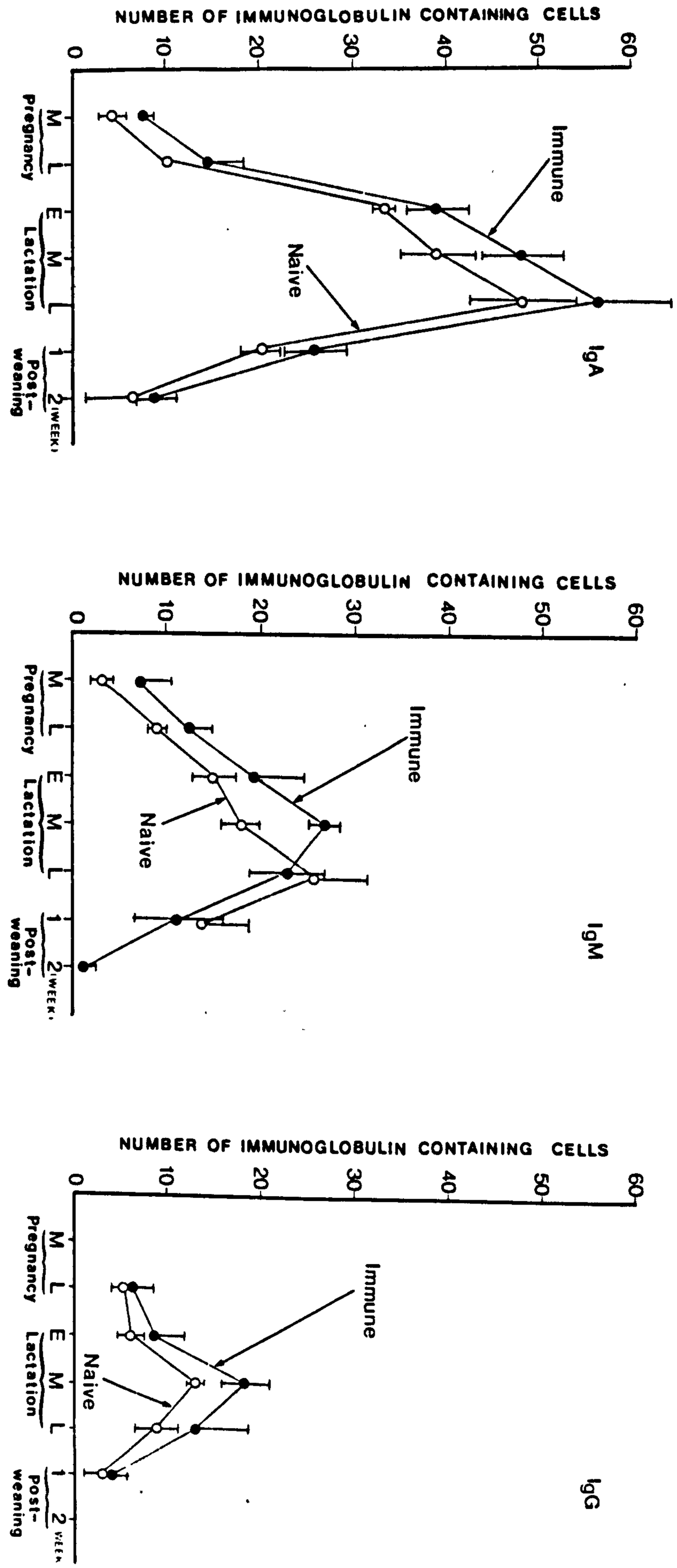


Figure 3.1: Number of IgA, IgM and IgG - containing cells per 100 fields (Mean ± s.d.) in mammary glands of naive and immune mice during pregnancy, lactation and after weaning.
Stage of lactation: E = Early, M = Mid, L = Late.

Table 3.3: Cells in naive maternal milk, at mid-lactation, (pooled from six mice). Cytocentrifuge preparations stained with Giemsa stain.

Subsamples of 100 cells examined	Number of cells					
	Macrophages	Lymphoid cells Small	Large	Total	Epithelial cells	Unidentified cells/ bodies
1	45	5	4	9	38	8
2	49	11	3	14	29	8
3	43	9	4	13	33	11
4	50	9	7	16	16	18
5	40	8	5	13	32	15
6	42	5	4	9	38	11
7	48	10	5	15	35	2
8	45	8	3	11	30	14
Mean	45.25	8.13	4.38	12.5	31.38	10.88
±s.d.	±3.54	±2.17	±1.30	±2.62	±7.05	±4.97

Table 3.4: Cells in infected/immune maternal milk at mid-lactation (pooled from 5 mice) - cytocentrifuge preparations stained with Giemsa stain.

Subsamples of 100 cells examined	Number of cells					
	Macrophages	Lymphoid cells Small	Large	Total	Epithelial Cells	Unidentified cells/bodies
1	53	9	3	12	25	10
2	50	10	10	20	25	5
3	46	13	5	18	31	5
4	53	11	7	18	24	5
5	44	14	10	24	28	4
6	59	8	5	13	25	3
7	58	8	5	13	25	4
8	51	6	7	13	29	7
Mean ± s.d.	51.78 ± 5.23	9.88 ± 2.7	6.5 ± 2.51	16.38 ± 4.31	26.5 ± 2.51	5.38 ± 2.2
Statistical significance (P-value) vs naive in Table 3.3.	P<0.02	N.S.	N.S.	P<0.05	N.S.	

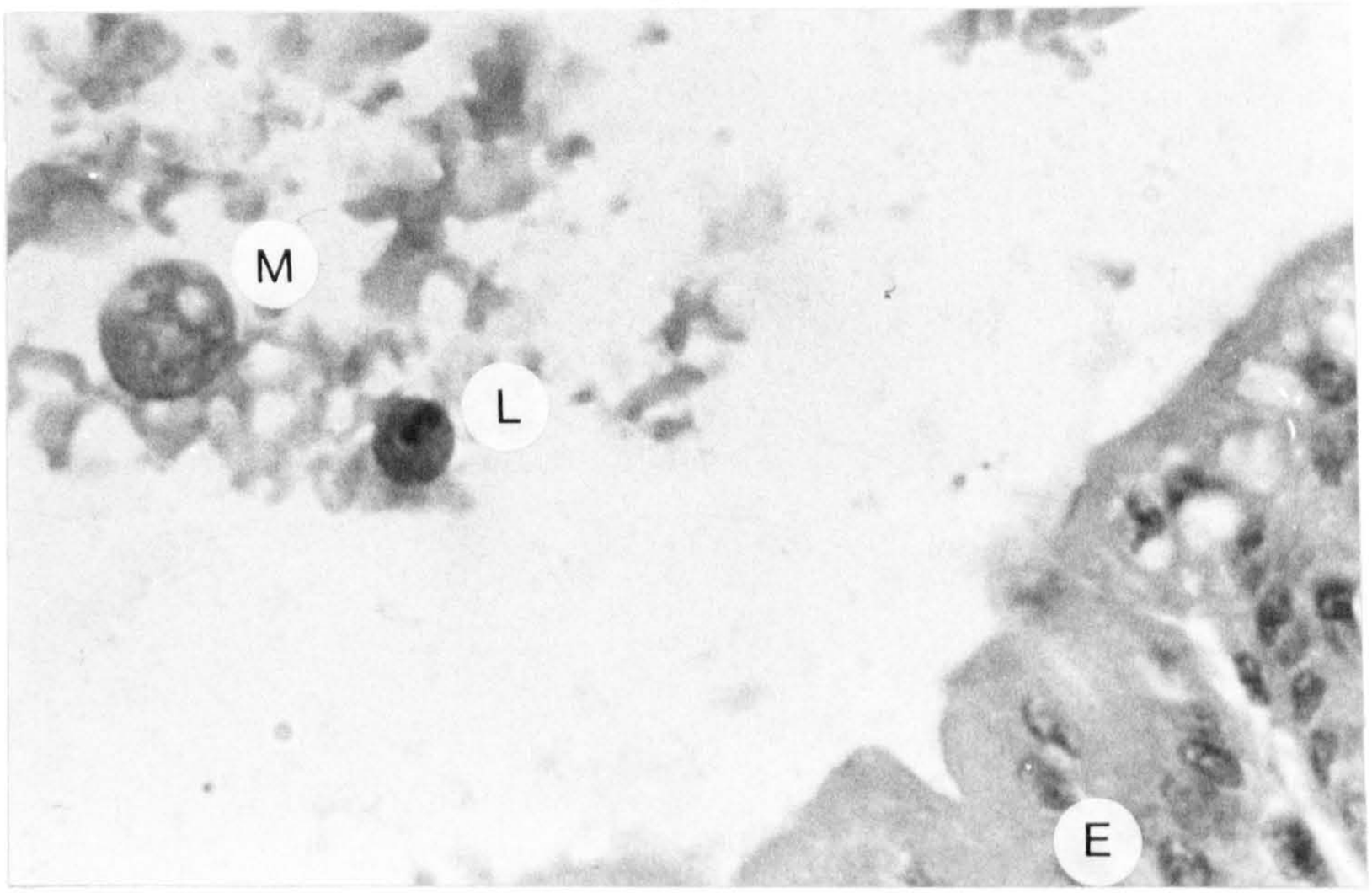


Plate 3.6.A: Two day old infant stomach. Carnoy fixation. Wax section. H & E stain. Magn. X 600. Macrophage (M) and lymphocyte (L), presumed to be of maternal origin, in the milk contents of the lumen of the stomach. Stomach epithelium labelled (E).

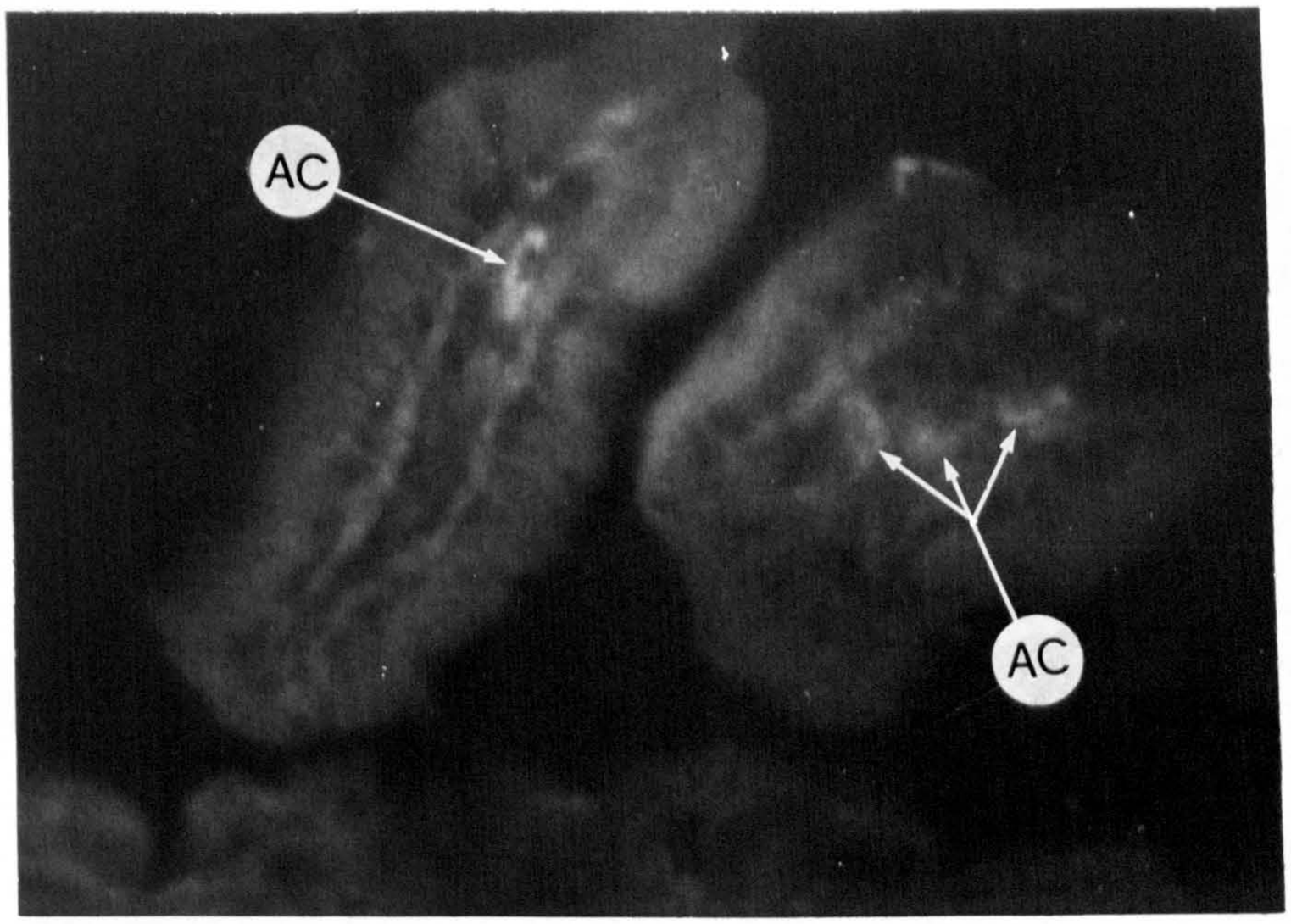


Plate 3.6.B: Section of part of the small intestine of a 9 day old NIH mouse suckling immune mother. Frozen section incubated with 1/100 dilution of FITC conjugated goat anti-mouse IgA. Magn. X.470. Weakly staining cells (AC) within the lamina propria of the villi. There is no distinguishable fluorescence on the brush border or within the epithelial cells.

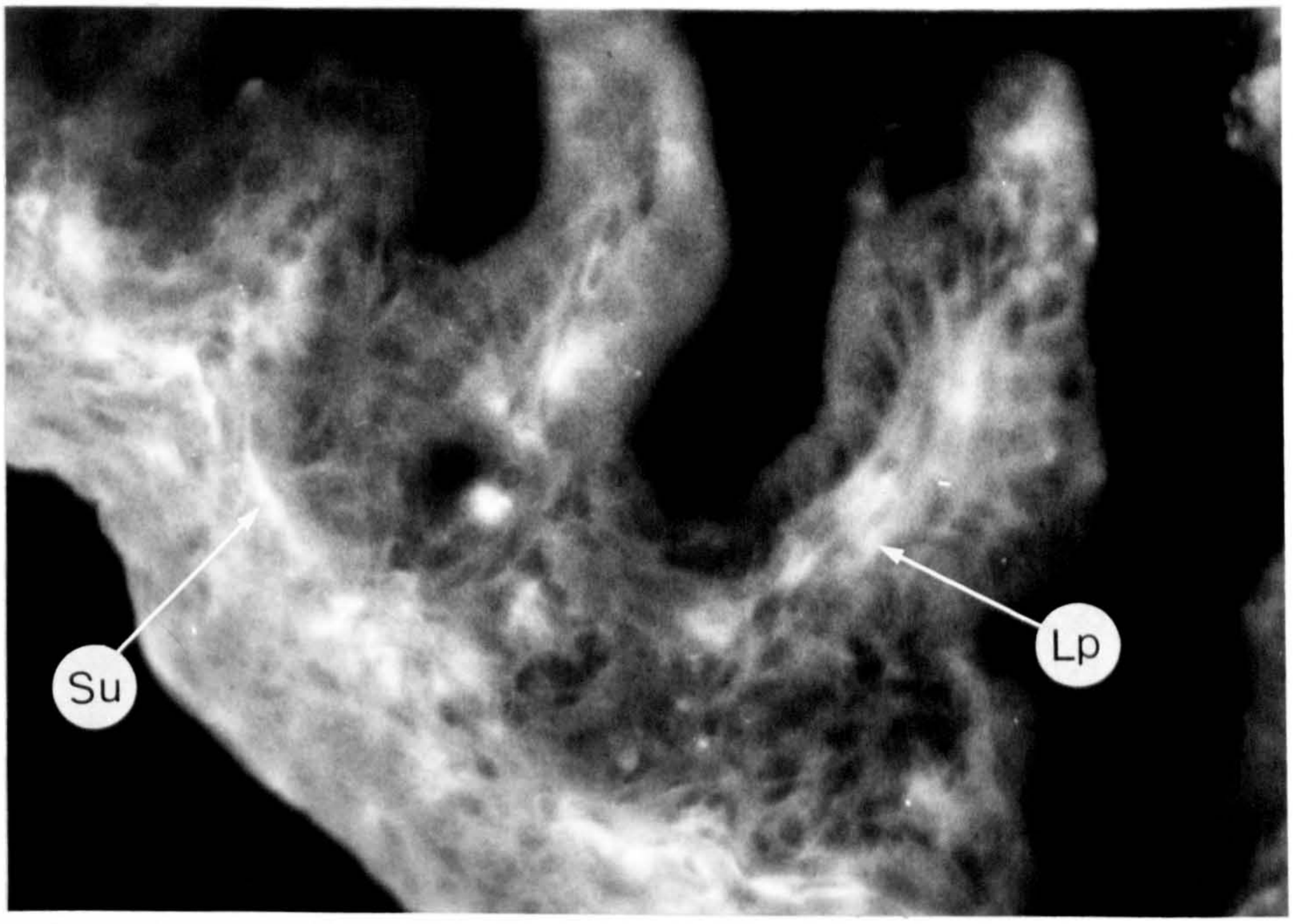


Plate 3.7.A: Transverse section of part of the small intestine of a 9 day old NIH mouse suckling an immune mother. Frozen section, incubated with 1/40 FITC conjugated goat anti-mouse IgG. Magn. X 480.
Weak to moderate fluorescence in the lamina propria and submucosal regions of the gut tissue. Very few discrete staining cells in evidence. Most fluorescence apparently associated with central ducts in the lamina propria (Lp) and in ducts in sub-mucosa (Su).

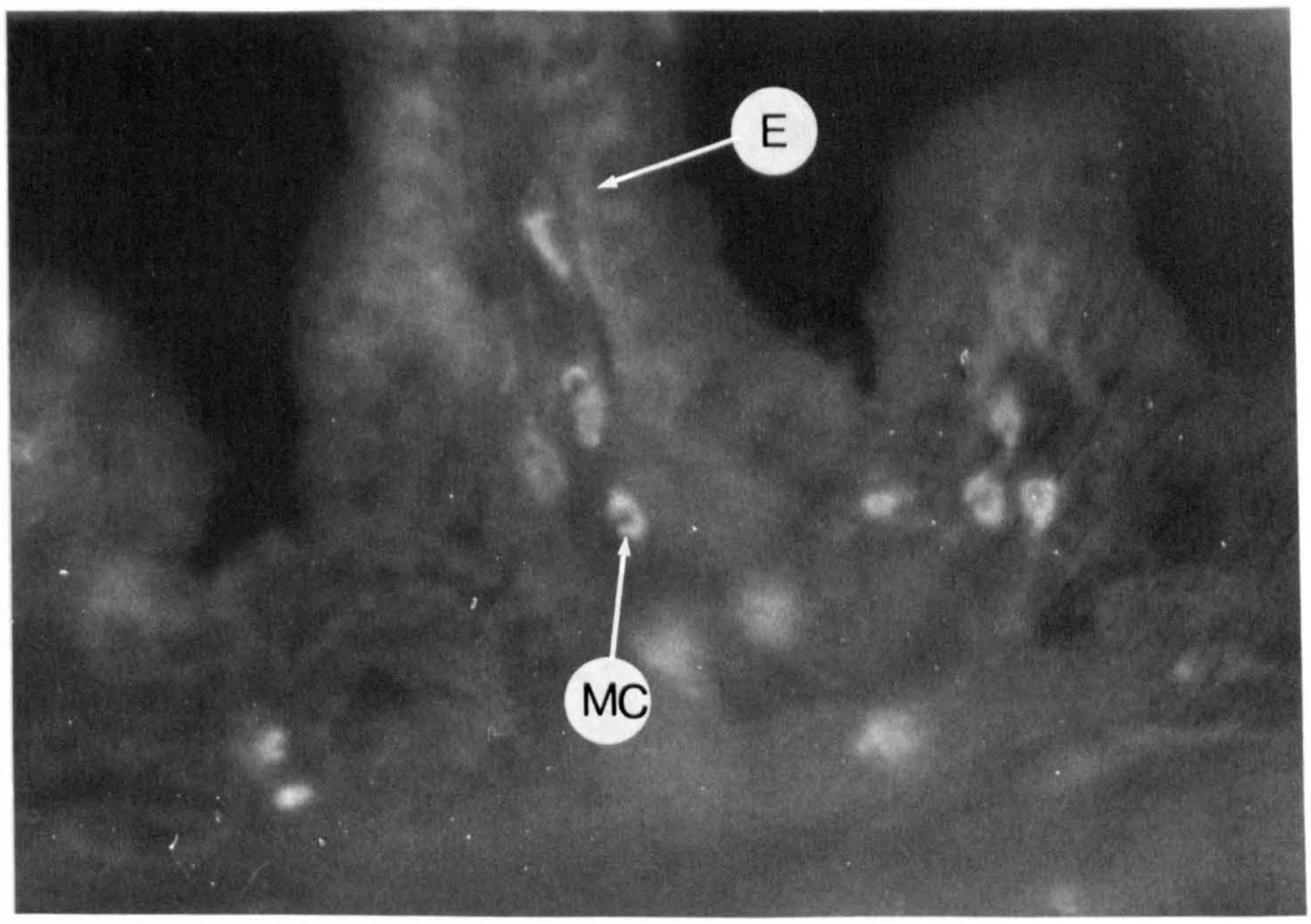


Plate 3.7.B: Transverse section of part of the small intestine of a 9 day old NIH mouse suckling an immune mother. Frozen section, incubated with 1/160 dilution of rhodamine conjugated goat anti-mouse IgM. Magn. X 470. Moderately staining IgM containing cells (MC) in the lamina propria and submucosal area of the villi. There is a suggestion of basally located IgM in the epithelial cells (E).

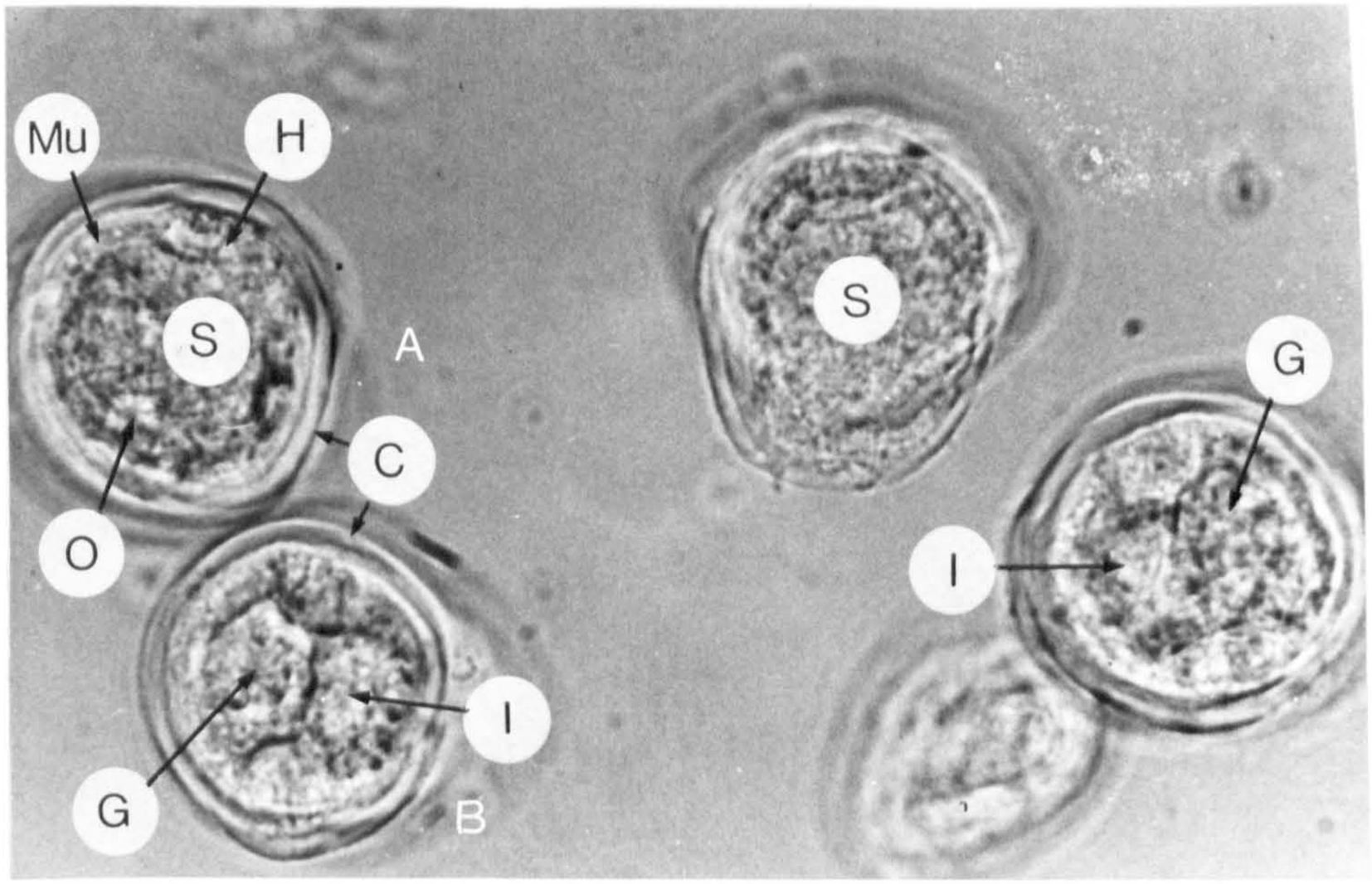


Plate 3.8: Transverse sections of infective larvae of Trichinella spiralis. Frozen sections phase contrast - Magn. X 500.

C - cuticle, H - hypodermal cords, G - primordial gonad,

I - intestine, Mu - muscle, O - oesophagus, S - stichocyte.

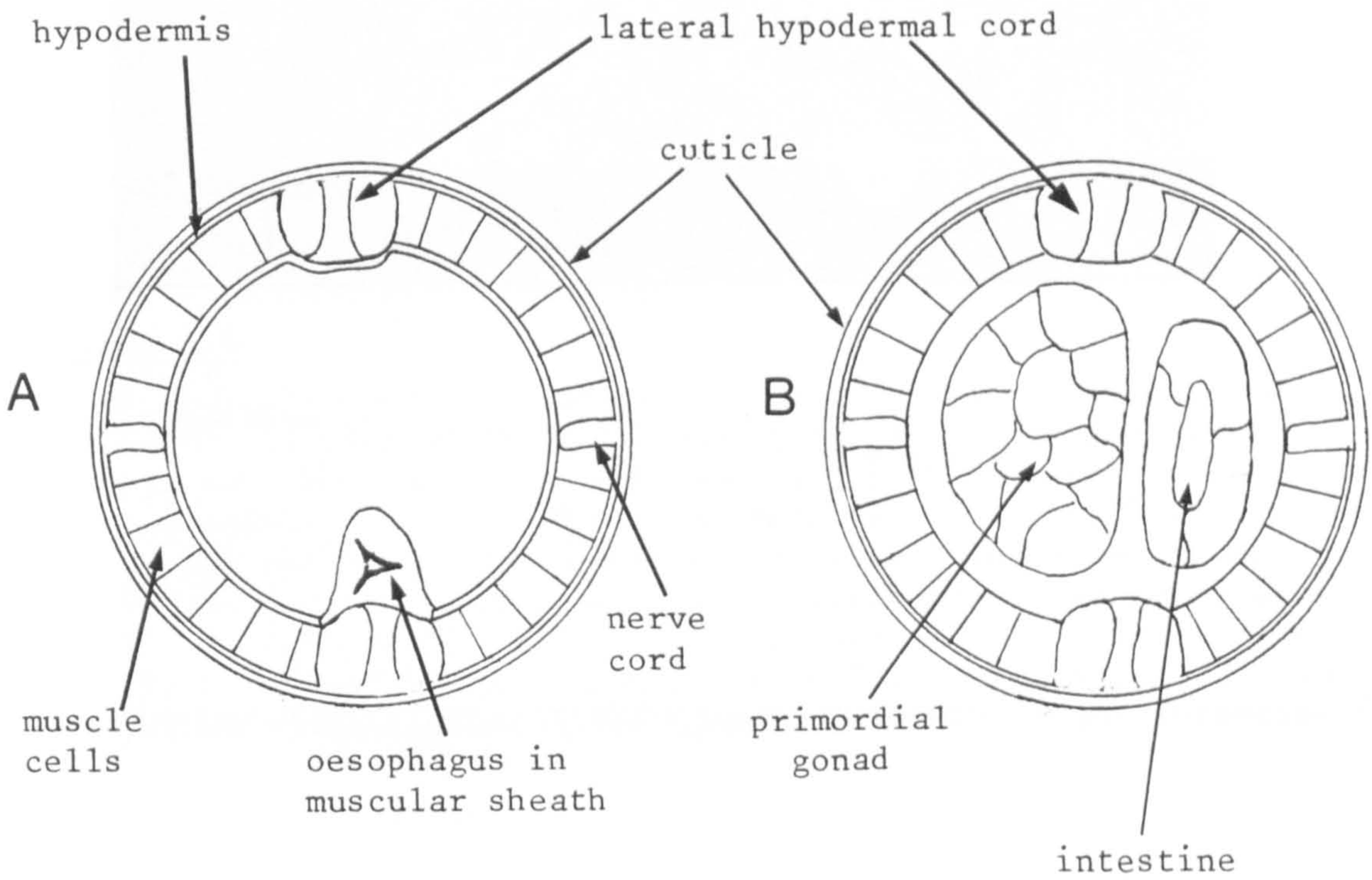


Figure 3.2: Diagramatic drawing of the sections A and B shown in plate 3.8.A.

A - section through region of stichosome.

B - section through region of intestine and gonad.

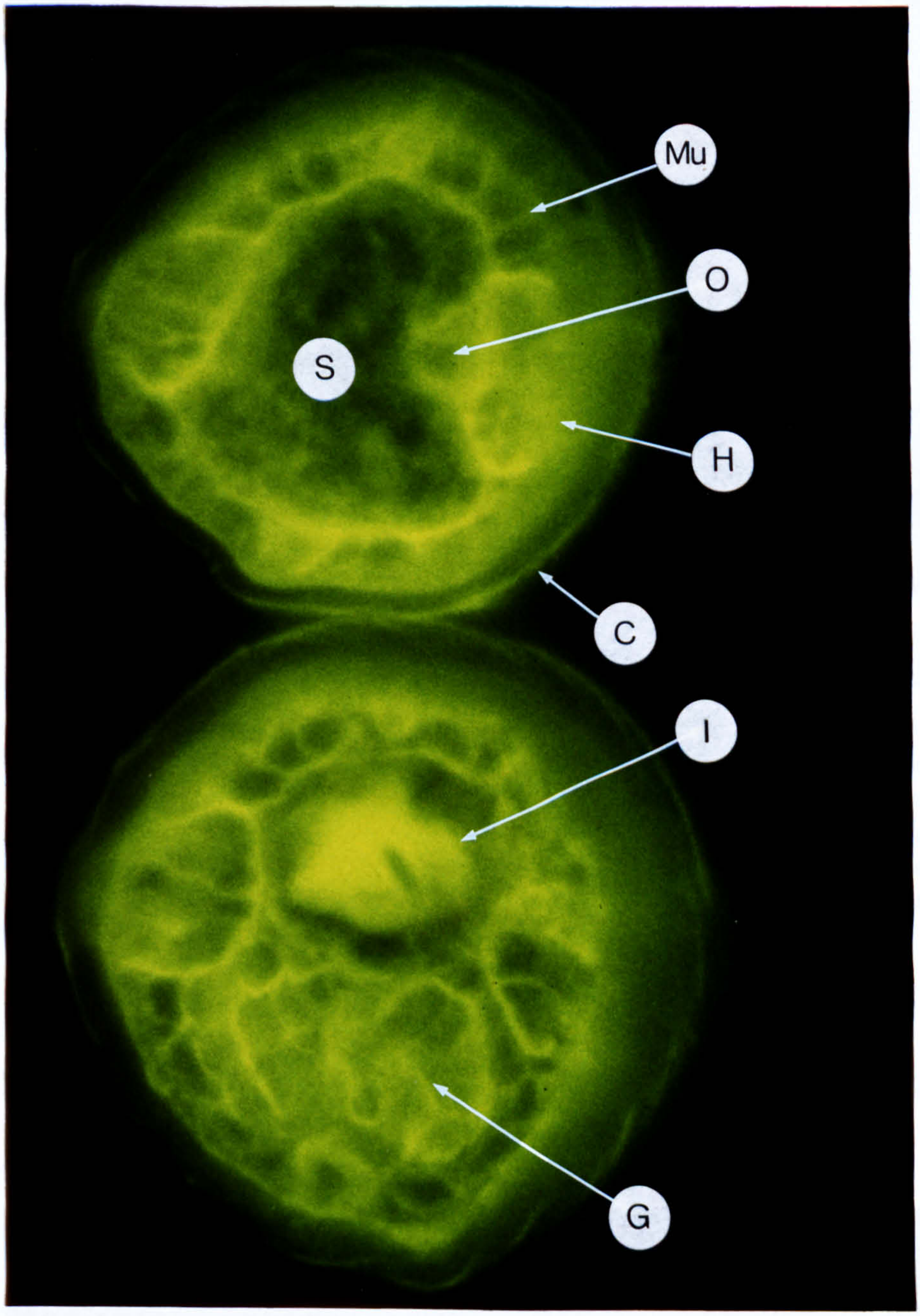


Plate 3.9.

T.spiralis infective larvae. Frozen sections, incubated with 1/200 dilution of antisera from T.spiralis infected mice (21 days post-primary infection) and 1/50 dilution of FITC conjugated goat anti-mouse IgG Magn. X 1300. Clear fluorescence on the surface of the cuticle (C) and peripherally in the hypodermal cords (H), and muscle tissue (Mu), and oesophagus (O). The stichocyte (S) shows small patches of weak fluorescence. There is weak fluorescence in the cells of the gonad (G), and strong fluorescence of the lumenal contents of the intestine (I).

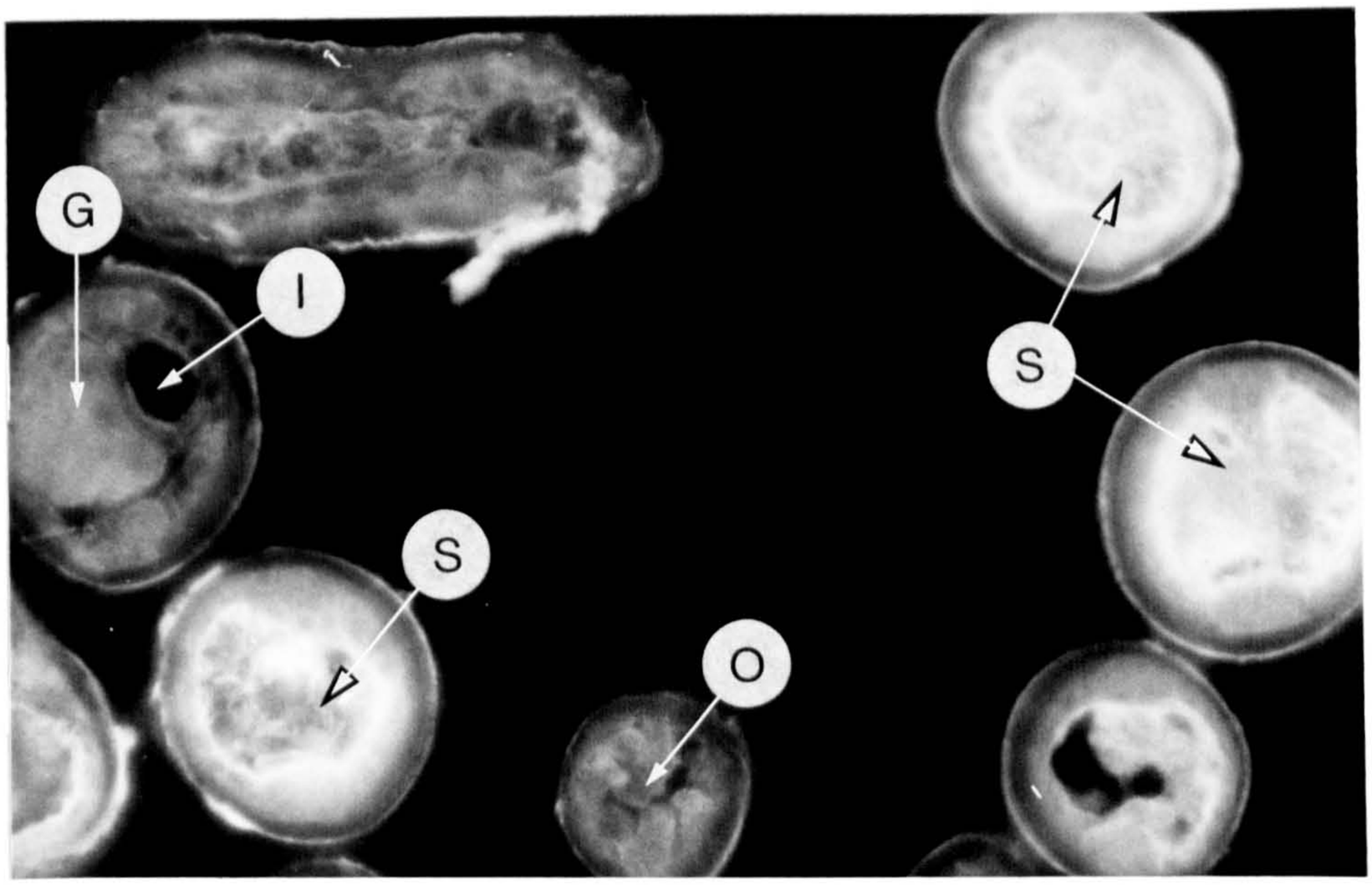


Plate 3.10.A: T. spiralis infective larvae. Frozen sections. incubated with 1/512 dilution of antisera from T. spiralis infected mice (28 days post-primary infection) and 1/50 dilution of FITC conjugated goat anti-mouse IgG - Magn. X 450. Clearly defined IgG fluorescence on the surface of the cuticle and peripherally on cells of the hypodermal cords and muscles. Weak fluorescence in the cells of the gonad (G). Strong fluorescence - disseminated or particulate in stichocytes (S). No fluorescence in the empty worm intestine (I) or anterior oesophagus (O).

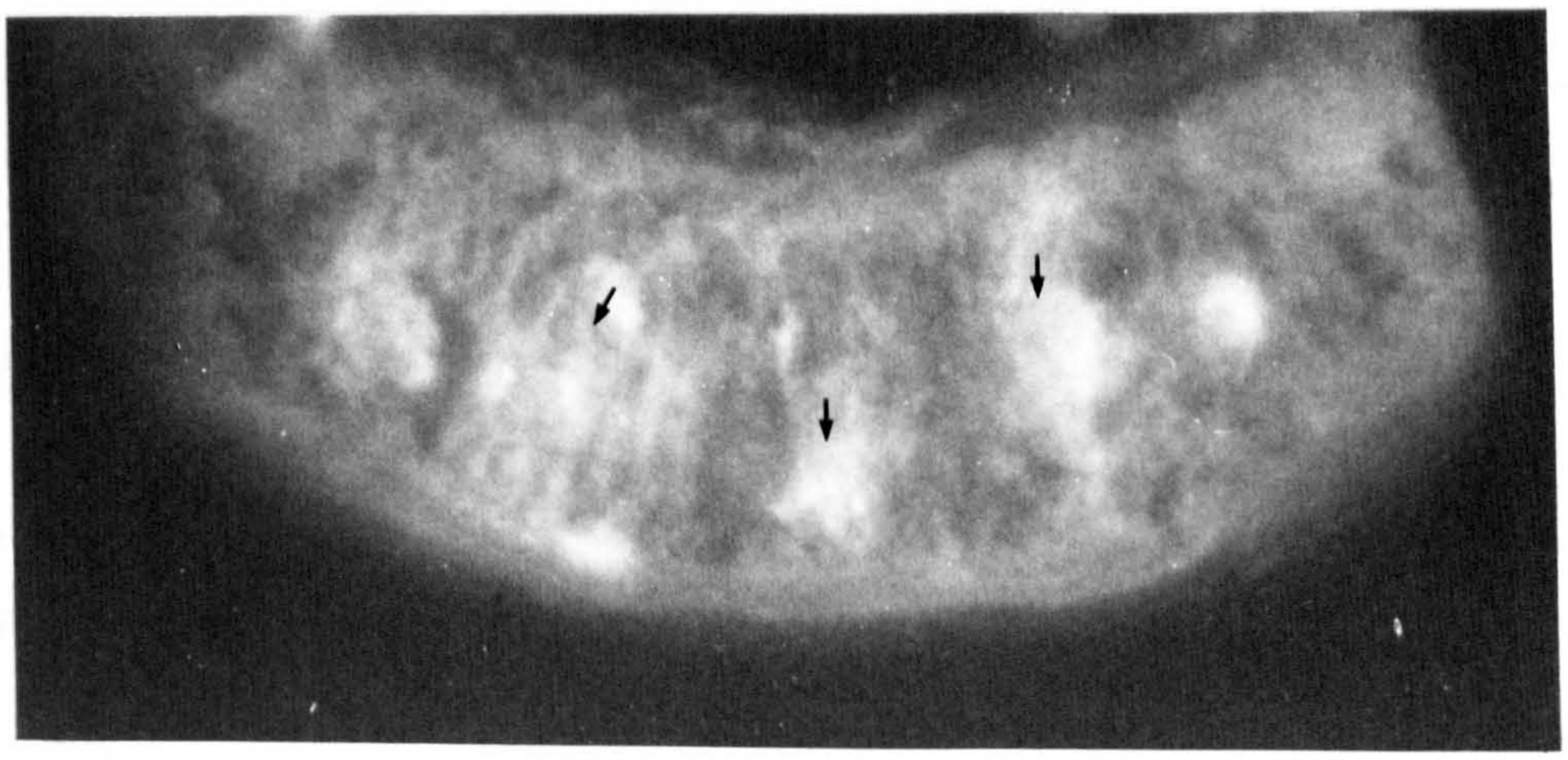


Plate 3.10.B: Longitudinal section of T. spiralis infective larvae. Frozen section, incubated with 1/512 dilution of antisera from T. spiralis infected mice (12 days post-primary infection) and 1/50 dilution of FITC conjugated goat anti-mouse IgG. Magn. X 625. Weak fluorescence of aggregates in the occasional stichocyte only (arrows).

Table 3.5: Indirect fluorescent antibody test (IFAT) for IgG antibodies to T. spiralis infective larvae

T. spiralis components	IgG antibody (titres) in pooled sera from primary infection					
	Days post-infection					
	12	15	17	19	21	28
Cuticle surface	-	+ (1:256)	+ (1:512)	+ (1:512)	+ (1:512)	+ (1:512)
Cuticle matrix	-	-	-	-	-	-
Hypodermal cells	-	- (1:512)	± (1:512)	± (1:512)	± (1:512)	± (1:512)
Hypodermal membrane (and basal lamina)	-		± (1.512)	++ (1:512)	+++ (1:512)	+++ (1:512)
Muscle	-	± (1:512)	± (1:512)	± (1:512)	± (1:512)	± (1:512)
Muscle membranes (and basal lamina)	-	+ (1:512)	++ (1:512)	++ (1:512)	+++ (1:512)	+++ (1:512)
Stichocytes	+ (1:512)	++ (1:512)	++ (1:512)	++ (1:512)	++ (1:512)	++ (1:512)
Oesophagus	-	+ (1:512)	+ (1:512)	+ (1:512)	+ (1:512)	+ (1:512)
Intestine (Cells	-	± (1:512)	± (1:512)	± (1:512)	± (1:512)	± (1:512)
Intestine (Lumen	-	-	+ (1:512)	+ (1:512)	++ (1:512)	++ (1:512)
Primordial gonad	-	+ (1:512)	+ (1:512)	+ (1:512)	+ (1:512)	+ (1:512)

Day 5 and day 10 post-primary infection sera = no fluorescence.
± very weak + weak ++ moderate +++ strong

All sections examined with dilution of antisera ranged from 1:64 - 1:1024 and conjugate dilutions of 1:50.

Table 3.6: Indirect fluorescent antibody test (IFAT) for IgA and IgM antibodies to T. spiralis infective larvae.

	IgA and IgM antibodies (titres) in pooled sera from primary infections					
T. spiralis components	IgA Days post-infection			IgM Days post-infection		
	12	15	21	12	15	21
Cuticle surface	-	+ (1:256)	+ (1:512)	-	± (1:256)	± (1:400)
Cuticle matrix	-	-	-	-	-	-
Hypodermal Cells	-	± (1:256)	± (1:512)	-	± (1:256)	± (1:400)
Hypodermal membrane (and basal lamina)	-	± (1:256)	+ (1:512)	-	± (1:256)	+ (1:400)
Muscle	-	± (1:256)	± (1:512)	-	± (1:256)	± (1:400)
Muscle membranes (and basal lamina)	-	± (1:256)	+ (1:512)	-	± (1:256)	+ (1:400)
Stichocytes	-	-	-	-	± (1:256)	+ (1:400)
Oesophagus	-	± (1:256)	± (1:256)	-	± (1:256)	-
Intestine (Cells	-	-	-	-	-	-
Intestine (Lumen	-	± (1:256)	± (1:256)	-	± (1:256)	± (1:400)
Primordial gonad	-	± (1:256)	+ (1:512)	-	± (1:256)	+ (1:400)

Day 5 and day 10 post-primary infection sera = no fluorescence.

± very weak + weak ++ moderate +++ strong

All sections examined with dilution of antisera ranged from 1:64 - 1:1024 and conjugate dilutions of 1:50.

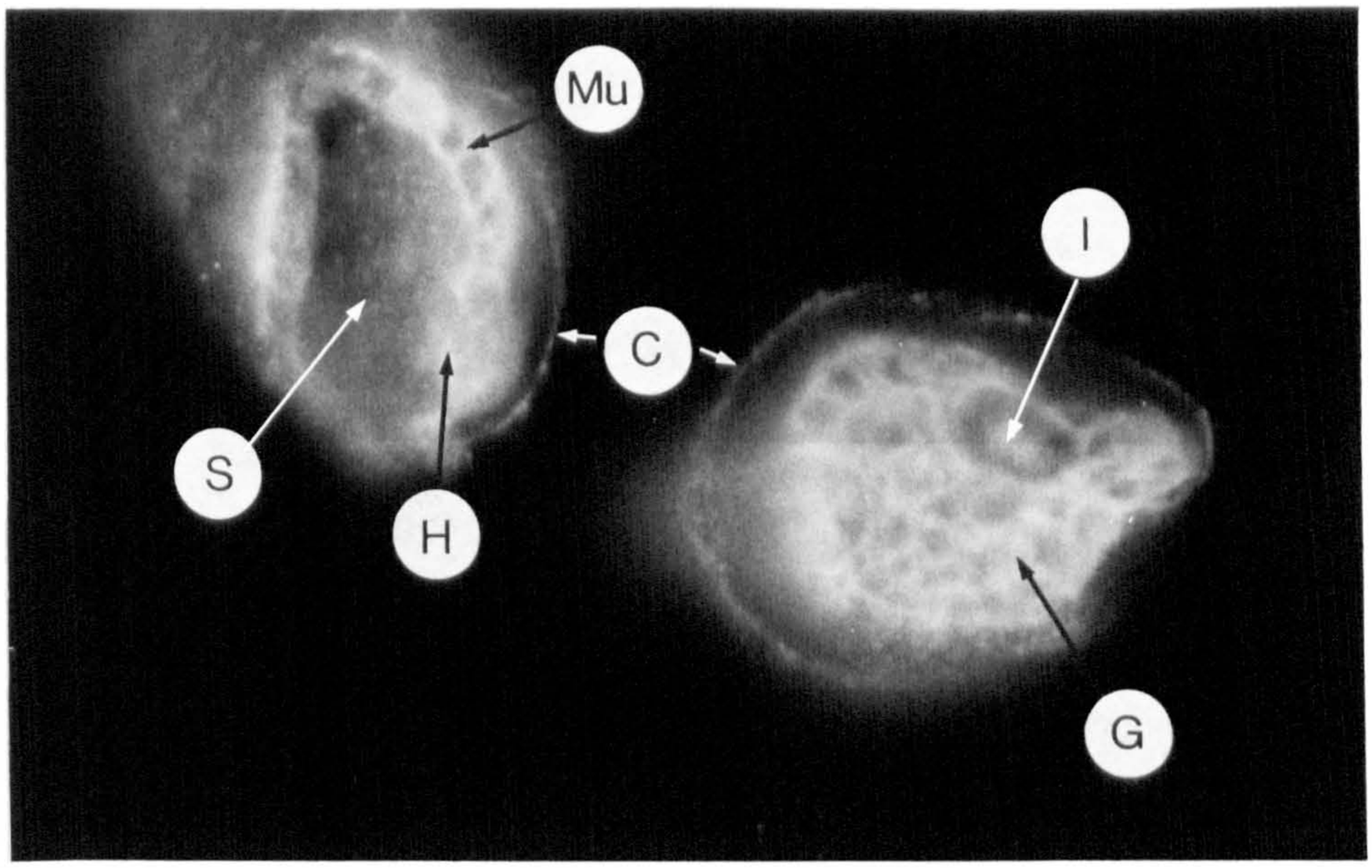


Plate 3.11.A: T. spiralis infective larvae. Frozen sections, incubated with 1/100 dilution of antisera from T. spiralis infected mice (21 days post-primary infection) and 1/50 dilution of FITC conjugated goat anti-mouse IgA. Magn. X 630. Weak fluorescence on the surface of the cuticle (C), with stronger fluorescence peripherally on cells of the hypodermal cords (H), muscles (Mu)and gonads (G), and weakly in the lumen of the intestine (I). No fluorescence in the stichocyte.

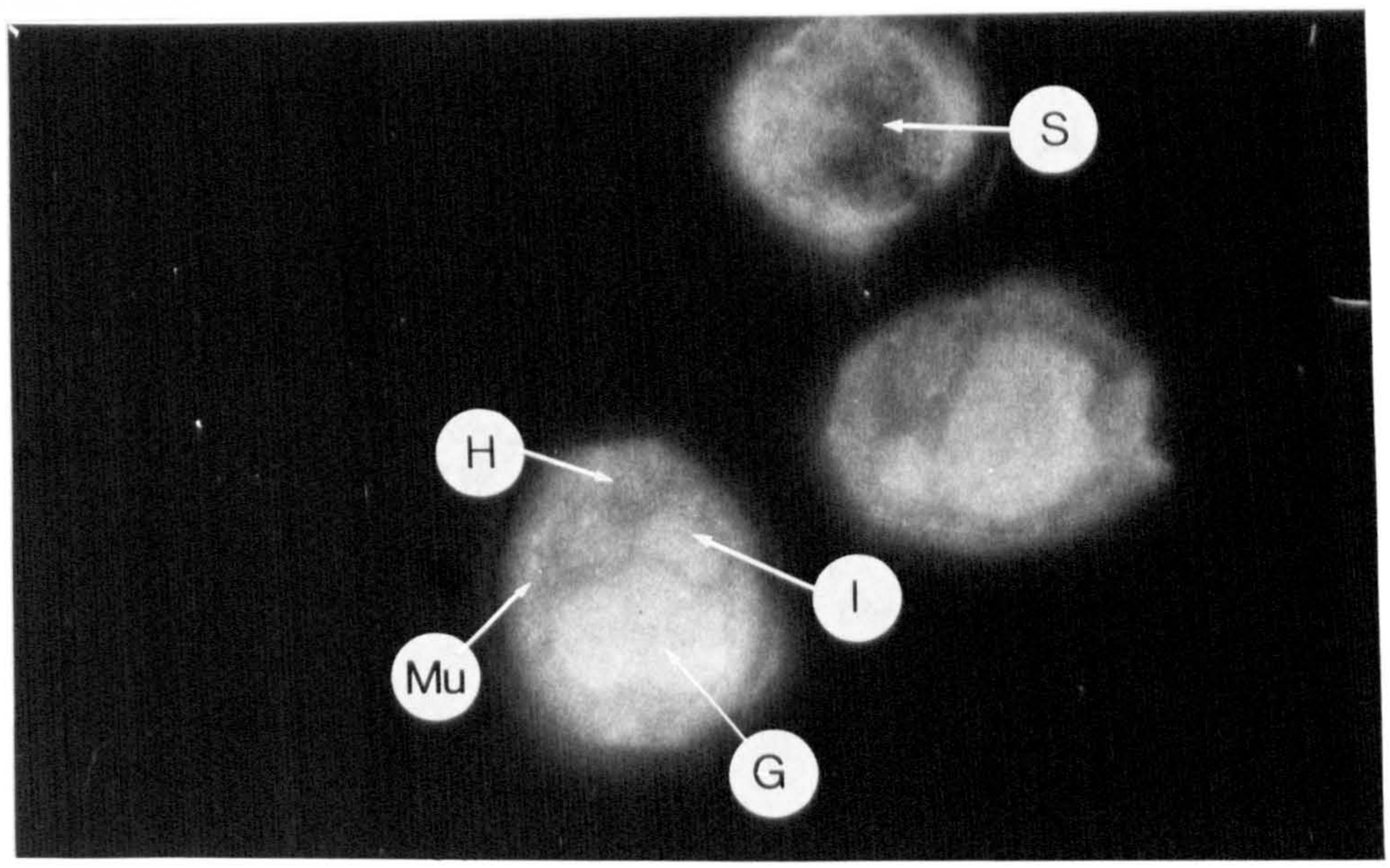


Plate 3.11.B: T. spiralis infective larvae. Frozen sections, incubated with 1/200 dilution of normal serum (taken from naive mice), and 1/50 dilution of FITC conjugated goat anti-mouse IgA. Magn. X 435. Very weak fluorescence in hypodermal cord cells (H) and muscle cells (Mu) and in the primordial gonad (G) and intestine (I). Virtually no fluorescence in the cuticle or in the stichocyte (S).

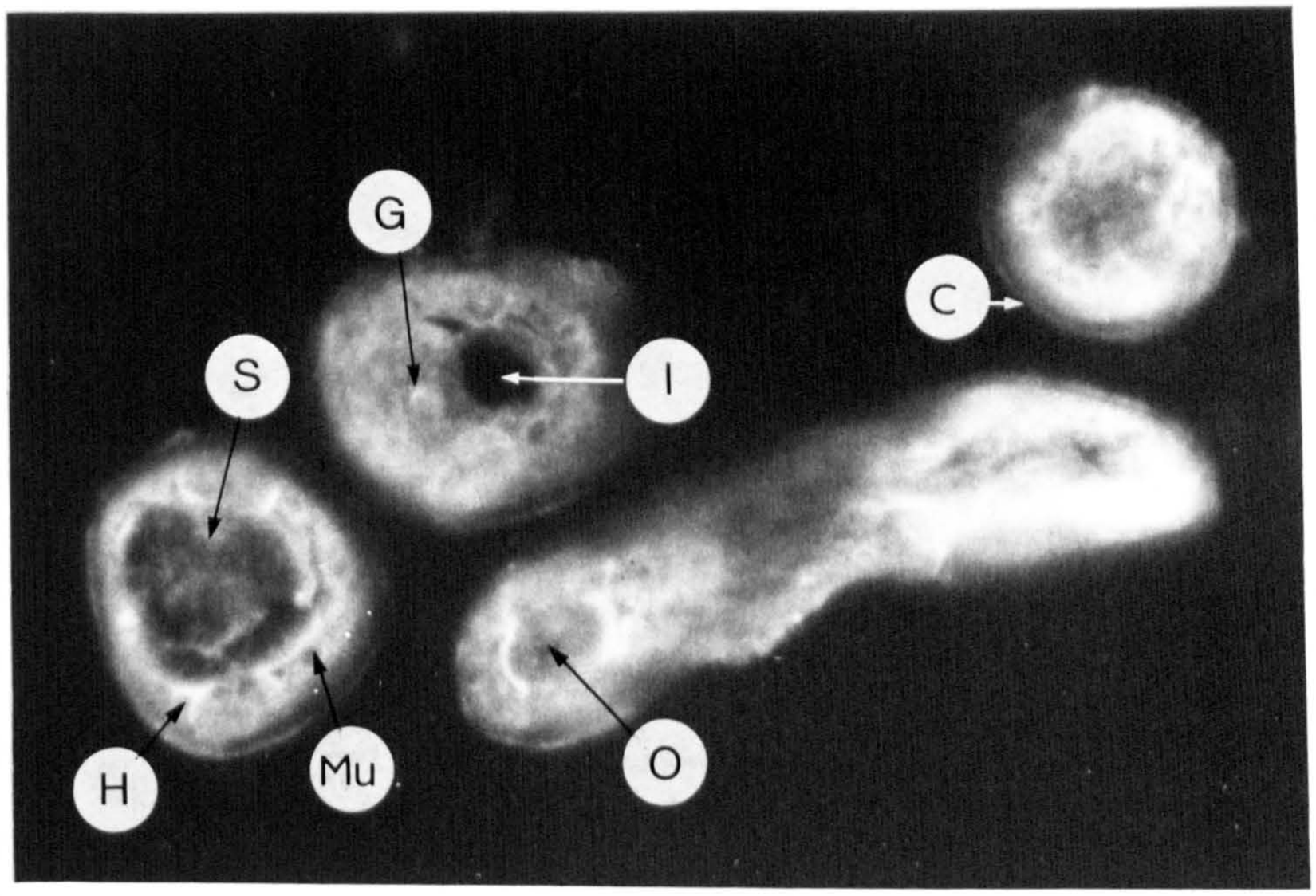


Plate 3.12.

T.spiralis infective larvae. Frozen sections, incubated with 1/400 dilution of antisera from T.spiralis infected mice (21 days post-primary infection) and 1/50 dilution of rhodamine conjugated goat anti-mouse IgM - Magn. X 440. Weak staining of the periphery of the hypodermal cords (H) and muscles (MU) and of the gonad tissue (G). There are traces of staining on the surface of the cuticle (C) and in the stichocyte (S). No staining of the intestine or its contents (I) or of the anterior oesophagus (O).

SECTION 4. Antibodies against T. spiralis and immunoglobulins in Sera and Milk in adult and infant mice and circulating antigen in sera of adult mice.

4.1 Introduction:

The practical and clinical significance of passive transfer of immunity from mother to offspring is related primarily to two factors. First, there is the need for neonates to be protected against infections until they become immunocompetent and develop their own immune responses; the second is the interference of passively acquired maternal antibodies with vaccination procedures in the young animal.

According to the differences in the route of passive transfer of immunity to offspring, Butler (1973) has divided animals into three groups. Man and rabbit belong to group I, in which the passive transfer is primarily *in utero*, and the secretory IgA in colostrum is typically not absorbed after ingestion by the nursing infant (Ammann and Stiehm, 1966; Guyer, Koshland and Knopf, 1976), but acts locally in the intestinal tract to protect against infections. Group II includes animals such as dog, rat, mouse, which have a mixed transfer - both *in utero* and via the milk of approximately equal significance. Mammals including horse, pig, sheep and cow are in group III; these species lack transplacental transfer of immunoglobulins and the colostral antibodies play an extremely important role in providing antibodies and protection for the infants.

It is known that humoral immunity in mammals which are infected with animal parasites can be passively transferred from mother to offspring via the colostrum or milk (Culbertson, 1938, 1939, 1943; Kelley and

and Nayak, 1965; Greenberg, 1971; Duckett et al., 1972; Perry, 1974; Smith and Herbert, 1976; Lloyd and Soulsby, 1978; Andrews and Hewlett, 1981). Antibodies involved in colostral transfer of immunity have been examined in detail in the Taenia taeniaeformis system (Musoke, Williams, Leid and Williams, 1975; Lloyd and Soulsby, 1978); these workers showed that IgA immunoglobulins are involved in the passive transfer of immunity via the colostrum of rats and mice. In rats, Musoke et al. (1975), noted also that colostral IgG immunoglobulins are protective when administered intravenously, but their participation in colostral immunization was insignificant. In nematode infections, Smith and Herbert (1976) concluded that colostrally transferred agglutinating antibodies, mainly of the IgG class, are associated with protection against Hyostrongulus rubidus in piglets suckling infected mothers. Duckett et al. (1972) provided evidence that maternal milk was the route by which some protection to T. spiralis was transferred to suckling mice. Perry (1974) confirmed this route of transfer and showed the presence of agglutinating antibodies in the sera of lactating T. spiralis infected mothers. Also a protective role of IgA and IgG antibodies of maternal origin against infection with Giardia muris in suckling mice has been suggested by Andrews and Hewlett (1981).

The enzyme linked immunosorbent assay (ELISA) described by Engvall and Perlmann (1971) has received extensive attention with respect to its possible use for the diagnosis of parasitic and other diseases, and numerous reviews have appeared in the literature in the past ten years (Engvall and Perlmann, 1971, 1972; Engvall and Carlsson, 1976; Bout Dugimont, Farag and Capron, 1976; Voller, Bartlett and Bidwell, 1976; Voller, Bidwell and Bartlett, 1976; WHO, 1976; Bullock and Walls, 1977;

Ruitenberg and Van Knapen, 1976, 1977a; Voller, Bartlett and Bidwell, 1978 a & b). The evidence indicates that ELISA has a sensitivity somewhat similar to radioimmunoassay which has been used experimentally for the diagnosis of some parasitic infections (Voller, Bidwell, Bartlett and Edwards, 1977). The original ELISA technique utilized polystryene tubes 'Macro-ELISA' (Engvall and Perlmann, 1971), but a modification utilizing microtiter plates 'Micro-ELISA' was logically suggested which required smaller amounts of reactants (Voller, Bidwell and Bartlett, 1976; Saunders, Clinard, Bartlett and Saunders, 1977).

Significant work utilizing ELISA has been done on the veterinary aspects of T. spiralis infection (Ruitenberg, Steerenberg, Brosi and Buys, 1974 and 1976; Ruitenberg, Van Knapen and Vermeulen, 1976; Ruitenberg and Van Knapen 1976; Van Knapen, Framstad and Ruitenberg, 1976; Ruitenberg and Van Knapen, 1977 a & b; Ruitenberg, Van Amstel, Brosi and Steerenberg, 1977; Van Knapen, Franchimont, Ruitenberg, Baldelli, Bradley, Gibson, Gottal, Henriksen, Kohler, Skorgaard, Solué and Taylor, 1980). It was recognized at an early stage that ELISA was ideally suited to screening pigs for Trichinella infection, and Ruitenberg and his colleagues set up a large programme for this purpose. They found that ELISA was more sensitive and reliable than conventional diagnostic methods. Engvall and Ljungstrom (1975) first established the method with sera from infected people. Using crude antigen prepared from T. spiralis muscle larvae, these workers demonstrated the presence of class specific antibody in human sera a week or two after infection, and they noted that ELISA was a more sensitive test than immunofluorescence.

The humoral response of the host plays a significant role in the immunologic phenomena in T. spiralis infection. Only a few studies

concerning the serum immunoglobulin levels in laboratory animals infected with T. spiralis are known. Perrudet-Badoux, Binaghi and Boussac-Aron (1976) demonstrated a marked increase in the levels of IgE and IgG_1 (IgR) immunoglobulins, and a moderate increase in the level of IgM immunoglobulin in rat sera infected with T. spiralis. It has also been reported that conventional mice infected with T. spiralis contain higher levels of serum IgG and IgA immunoglobulins than germfree mice, with a similar level for IgM immunoglobulin (. Przyjalkowski, Golinska and Bany, 1976).

There is a lack of data in the literature concerning immunoglobulin levels in mouse colostrum and milk, and there is no report of the quantity of milk immunoglobulin of different classes in mice after the infection with T. spiralis. However, studies in rats (Michaleck, Rahman and McGhee, 1975; McGhee, Michaleck and Ghanata, 1975) and many other mammals (Tomasi, 1976) have demonstrated the levels of IgA, IgG and IgM immunoglobulin in lacteal secretions.

Knowledge of immunoglobulin class, and antibody,concerned in passive immunization in T. spiralis infection, would contribute to overall understanding of immunity in nematode infection.

Soluble antigens have been detected in the sera of subjects carrying certain parasitic diseases. Berggren and Weller (1967); Gold, Rosen and Weller (1969); Capron, Bigeut, Tran Van Ky and Moschetto (1969); Madwar and Voller (1975); Santoro, Capron, Joseph, Rousseaux-Prevost and Capron (1978) have demonstrated circulating antigen in the sera of animals infected with schistosomes. Phillips and Draper (1975) detected circulating immune complexes in human sera during schistosomiasis, whilst Houba, Koech, Sturrock, Butterworth, Kusel and Mahmoud (1976)

demonstrated the appearance of specific soluble antigens in baboons infected with Schistosoma mansoni. In human sera circulating filarial antigen has been detected (Kaliraj, Ghirnikar and Harinath, 1979; Kaliraj, Kharat, Ghirnikar and Harinath, 1981). Genitau, Verroust, Smith, Morel-Maroger, Saimot and Coulaud (1977) demonstrated the presence of circulating immune complexes in human sera, but they were not able to detect the presence of free T. spiralis antigen in the sera.

In the present investigation the possibility of using ELISA for the detection of antibodies in sera and milk to a crude antigen prepared from T. spiralis larvae has been tested. The experiments described in this study were designed to demonstrate the presence of immunoglobulin class of antibodies produced in sera and milk of adult mice, lactating mice, and in sera of infant mice against T. spiralis infection. The question of whether antibodies were passively transferred via the milk to offspring, and the possibility of uptake of these antibodies by the suckling neonates, and the production of antibodies by these mice were also investigated. The concentration of different classes of immunoglobulin in sera and milk from infected and naive mothers and infants, and the occurrence of soluble antigen and circulating antibodies in sera from adult mice after infection with T. spiralis were also examined.

4.2 Enzyme Linked Immunosorbent Assay (ELISA) of Serum and Milk Antibodies.

4.2.1 Materials and Methods.

(a) Antigen:

The crude soluble antigens of T. spiralis muscle larvae were prepared, aliquoted and stored as described in General Materials and Methods. A final concentration of 5 µg/ml was used for coating the wells in all ELISA plates.

(b) T. spiralis infection/immunization:

Unless otherwise stated, adult mice were given a primary infection of 400 T. spiralis infective larvae, followed after 4 weeks by a secondary infection of 200 larvae. The second infection was given a week before mating.

Immunization with T. spiralis larval antigen + Freund's complete adjuvant was carried out in adult mice as described in Section 1, Protocol 1.3.

Infants born to naive mothers were infected when one week of age with a primary infection of 100 T. spiralis larvae.

(c) Collection of milk from adult and infant mice:

Maternal milk was collected from naive mice or infected mice as indicated in the design of each experiment, during different periods of lactation. Milk was collected from 5-6 mothers at each specific day or period of lactation and pooled.

Suckling infants born to naive or infected mothers were killed at different periods of lactation, their stomachs were removed, and the

milk collected individually from 5-6 mice then pooled. All milk samples were stored at - 20°C in Eppendorf tubes so that each sample was thawed only once before use. (For detail on milk collection, see General Materials and Methods).

(d) Collection of sera from adult and infant mice:

Blood was collected from the tail of each adult mouse, and the serum prepared following the method of Gray (1978) as described in General Materials and Methods.

Single or pooled sera (from 6 mice) were collected from adult or lactating mice at various times during primary and secondary infections as indicated in each experiment.

Sera of 6 adult mice during secondary infection were pooled to serve as a positive control, and the serum of naive mice was used as a negative reference control to be used in each test.

Sera from infants born to either naive or infected mothers were obtained after anaesthesia by cardiac puncture (see General Materials and Methods) and recovered from batches of 5-6 infants at intervals between the 6th and 19th day of age. Sera were also collected from infants on days 8, 11 and 14 following infection with 100 T. spiralis larvae.

(e) ELISA assay:

A micro-enzyme-linked-immunosorbent assay (Micro-Elisa) as described by Voller, Bidwell and Bartlett (1976) was used to assess antibodies against T. spiralis in milk and serum. The technique consisted of the following procedures: saline soluble antigen prepared from T. spiralis larvae was diluted to a concentration of 5 µg/ml in

0.05 M carbonate buffer P.H 9.6 (coating buffer), and 150 µl of antigen was used to coat each well of the microtiter plates (Dynatech, Billingshurst, England). The plates were covered and kept in a humid chamber overnight at room temperature (20-22°C) and then rinsed three times for three minutes each in the washing solution (0.9% NaCl - 0.05% Tween 20 solution which was the standard washing solution in this procedure) to remove any unadsorbed antigen. Serial dilutions of sera or milk to be tested were diluted in incubation buffer by doubling dilutions and 150 µl was added to each well. Following incubation for two hours at room temperature, the wells were rinsed again with washing solution, washed three times - three minutes each time, to remove any unreacted serum or milk proteins. After washing, the wells were treated with 150 µl per well of horseradish peroxidase conjugated goat anti-mouse IgG or IgM or IgA (Cappel Laboratories Inc. U.S.A.) to assess the antibody activity in each immunoglobulin class. These conjugates were incubated for three hours at room temperature. After three washes, three minutes each in washing solution, 150 µl of freshly made peroxidase substrate (Ortho-Phenylene-Diamine, OPD) was added to each well. The plates were then incubated for 20 minutes at room temperature in darkness. The reaction was stopped by adding 150 µl per well of the reaction inhibitor $4M\ H_2\ SO_4 \equiv 8\ N\ H_2\ SO_4$. The enzyme product was assessed visually and spectrophotometrically. Positive reactions produced a yellow colour, and the end point was taken as the last dilution of serum or milk that gave a visually distinct colour reaction. The readings which are recorded in the results were determined using a spectrophotometer (Titerteck Multiskan, Flow), with a 492 nm filter recommended for perioxidase. Print out results were recorded in units of optical density (O.D.).

(f) Conjugates: preparation/dilution:

The following conjugates were used during the assay:

1. Peroxidase conjugated IgG fraction goat anti-mouse IgA (Alpha chain specific)
2. Peroxidase conjugated IgG fraction goat anti-mouse IgG (FC fragment, Gamma chain specific)
3. Peroxidase conjugated IgG fraction goat anti-mouse IgM (Mμ chain specific).

For each conjugate 2 ml of sterile distilled water was added and aliquots of 50 μl were made and stored at -20°C until used. The conjugates were diluted in incubation buffer and the working dilution for each conjugate was determined after preliminary tests as 1:1000 for anti-mouse IgG, 1:500 for anti-mouse IgM and 1:250 for anti-mouse IgA. These concentrations of conjugates gave the maximum absorbance when tested with a known positive sera. These tests also showed that there was no specific adherence of conjugates to antigen in the absence of serum, and the conjugates did not adhere non-specifically to the plates.

(g) Control reactions for ELISA:

The following control treatments were employed:

(a) Substrate control - antigen-coated wells containing only substrate, i.e. no antisera, no conjugate.

(b) Conjugate control - antigen-coated wells to which conjugate was added, followed by substrate i.e. no antisera.

(c) Negative serum control i.e. regular procedure using serum from naive mice.

(d) Positive serum control i.e. regular procedure using serum from infected mice.

The pooled positive control sera were used in each run to assess the reproducibility of test treatments, and a pooled negative sera to check for non-specific background reaction. The substrate and conjugate controls yielded negligible optical density (O.D.) values.

(h) Buffers for ELISA:

1. Coating Buffer 0.05M carbonate PH 9.6

1.59 g $Na_2 CO_3$

2.93 g $NaH CO_3$

dissolved in 1 litre distilled H_2O

2. Incubation Buffer - PBS - 0.05% Tween 20 PH 7.4

17.0 g NaCl

2.56 g $Na_2 HPO_4$

0.312 g $Na H_2 PO_4 \cdot 2H_2O$

dissolved in 2 litres distilled H_2O

Take 900 ml from above solution and add 0.45 ml Tween 20 and use this mixture for incubation.

3. Washing Solution - 0.9% NaCl - 0.05% Tween 20

9 g NaCl

0.5 ml Tween 20

dissolved in 1 litre distilled H_2O

Peroxidase Substrate (Working Solution)

(For 30 ml, enough to coat 2 plates)

15 ml distilled H_2O

7.41 ml Citric acid (0.1 M)

7.59 ml $Na_2 HPO_4$ (0.2 M)

0.3 ml Stock (OPD)

3 µl 30% H_2O_2 (Hydrogen Peroxide 30% $^{W}/_{v}$)

<u>OPD Working Solution</u> 10 mg/ml prepared freshly

30 mg of (O-phenylene-Diamine $C_6H_4(NH_2)_2$

\+ 3 ml absolute Methanol and kept in the dark.

<u>Reaction Inhibitor</u> 4 M H_2SO_4 (≡ 8N H_2SO_4)

4.2.2 <u>Experimental design and results:</u>

(a) <u>The determination of antigen concentration to be used for ELISA:</u>

A crude saline extract of <u>T. spiralis</u> muscle larvae was used as antigen to coat the wells in all ELISA tests throughout this study. To determine the effect of different concentrations of this antigen on antibody activity during <u>T. spiralis</u> infection in adult mice, tests were run in which four different antigen concentrations ranging from 0.7 µg/ml to 7 µg/ml were assayed. Pooled infected sera from 6 mice during primary or secondary infections, and naive sera collected from uninfected mice were used with each antigen concentration and assayed for the presence of IgG antibody against <u>T. spiralis</u> infection. All sera were diluted in a doubling dilution starting with 1:100 and finishing with 1:12800. The results to 1:6400 are recorded in Table 4.1 (see also plate 4.1). The positivity of the reaction was determined by using the criterion that any reading of optical density (O.D. value) which was greater than twice the mean value of the naive control would be regarded as positive, and the titre was calculated accordingly. This criterion will be discussed later. The results in Table 4.1 show that with sera from a 26 day primary infection, the titre which is regarded as the last dilution giving a positive reaction was the 1:800 dilution of the infected sera with all four antigen concentrations. With secondary infection sera, the titre had risen to 1:6400 with three antigen concentrations, and to 1:3200 in the other. Thus the result

of this experiment showed no major difference in antibody activity using the different concentrations of crude larval antigen. A similar conclusion was drawn from the result shown in Table 4.2 when milk at early and mid-lactation was collected from six infected or 6 naive mice as a control and used for detecting IgG antibody activity. In this experiment, three dilutions 2, 5 and 10 µg/ml of crude larval antigen were used. Using the same criterion mentioned earlier for discrimination between positive and negative reactions, the results showed that in all three concentrations of antigen, clear positivity was evident in 1:15 dilution of immune milk, but it was evident that the OD values with 5 µg antigen were considerably higher than with 2 µg antigen. Thus it was decided to use an antigen concentration of 5 µg/ml throughout the subsequent tests for both sera and milk. In this regard it is perhaps worth mentioning that this concentration, i.e. 5 µg/ml, of crude larval antigen has been used in ELISA tests for detection of class specific antibody in human trichinosis (Van Knapen, Franchimont, Verdonk, Stumpf and Undeutsch, 1982) and for detection of T. spiralis infection in pigs (Ruitenberg and Van Knapen, 1977b).

(b) Setting the positive/negative discrimination level:

The discrimination between positive and negative is a crucial element in the design of all assays for antibody activity. In most ELISA runs used in this study, the titration method was applied in which serum or milk under test was serially diluted to the point (titre) at which the antibody activity can no longer be detected. The titres are reported as serum or milk dilutions and expressed as ratios. This method has both advantages and disadvantages (de Savigny and Voller, 1980); among the obvious advantages is that the titres are highly

quantitative measures of relative antibody activity, and the visual readings can be used also. However, the titres can be inaccurate and variable because ELISA dose-response curves flatten towards the titre end point.

To evaluate this issue in the present study, sera from primary infection mice of 21 and 33 days of infection, and naive sera were tested for IgG and IgM antibodies and the results assessed as dose-response curves. The data are recorded in Figures 4.1 and 4.2. The serum dilutions were 1:25 - 1:800 for both classes. The slope of the dose response curves was flattened with increasing dilutions of IgG antibody as seen in Figure 4.1. With IgM antibody at the dilutions tested there is much less flattening of the slope of the curve for the positive sera. Thus with these data for IgG antibody, the opportunity for demonstrating a precise and reproducible point of determination of positivity is restricted to a level of twice the mean of the negative controls. The reasons for such discrepancy are not altogether clear. However, in exhaustive testing of anti-Toxocara antibody responses, de Savigny and Voller (1980) noted that a dose response curve can be altered by the presence/absence of responses in the other classes of immunoglobulins. These authors also noted that antibody affinities of different sera cannot be assumed to be identical. Given the observations of Figures 4.1 and 4.2 and the considerations detailed by de Savigny and Voller (1980), it was concluded that the determination of positivity in the current series of experiments would be set at twice the mean value of the negative controls which scheme is recommended by de Savigny and Voller (1980) for the routine use of Microelisa determinations in testing for antibody responses in parasitic disease.

(c) Serum antibody against T. spiralis:

(i) Antibodies in adult mice.

A number of experiments have been carried out to determine the presence of antibody of different classes against T. spiralis infection. Pooled sera from infected adult mice, taken at intervals after primary or secondary infection, were tested for antibodies of IgG, IgM and IgA classes using class specific conjugates. Normal sera collected from naive mice was included in all experiments as a negative control. The results obtained are shown in Tables 4.3, 4.4 and 4.5, and Figures 4.3, 4.4 and 4.5. One method by which the development and consistency of the antibody response can be demonstrated easily relies on the assumption that, other components being equal, the strength of the reaction - measured as the OD value - at a standard titre/dilution is a reasonable demonstration of the amount of antibody present in the sample. This should be independent of problems of specific affinity but may not be independent of antigen blocking by other species of immunoglobulin. From this point of view the chronological development of IgG and IgM antibodies is demonstrated in Figure 4.3. Sera were taken from batches of mice at intervals during a primary infection and tested at a dilution of 1:100. IgG antibody was clearly positive on day 15 post-infection and continued to rise steadily to the day 35-40 period. IgM antibody was just positive as early as day 5 post-infection and then followed an erratic course with peak responses at day 15 and the period around day 30 post-infection.

With reference to Table 4.3 which records the O.D. readings of two sets of determinations of the same sera at different starting dilutions, it can be seen that a positive IgG antibody response at 1:50 dilution is detectable at day 10 of a primary infection. There is a relatively

even increase in titre after this time, with the lowest titre recorded after day 24 post-infection being 1:200.

IgM antibodies are detectable at a titre of 1:25 by day 5 post-infection and do not fall below 1:200 after day 15 post-infection (Table 4.4).

For both these classes of antibodies, detectable titres are in evidence in sera taken from mice 47 days after a secondary infection with titres of 1:50 to 1:100 for IgM and titres in excess of 1:400 for IgG antibodies.

The microelisa test has been criticised frequently by many authors engaged in serodiagnosis of parasitic disease particularly on the issue of reproducibility under different conditions and additionally even with respect to inherent differences between individual microelisa plates or batches of plates. While these difficulties can be overcome to a considerable degree by always running known positive sera and known negative sera on each plate, plate/antigen binding affinities can obscure low levels of antibody present in the test sera. This would be detrimental to the present investigation in which it was desirable to determine the earliest duration of infection which would produce measurable antibodies which might explain some aspects of maternal/infant transfer of protection.

To evaluate the reproducibility and therefore the reliability of the system particularly in regard to the detection of low titre responses, a number of tests were conducted on the same batch of serum samples. The infected sera were collected from groups of 5-6 mice during a primary infection on days 5, 10, 15, 18, 21, 24, 27, 30, 33, 36, 40 and 46 post-infection, and on day 47 post-secondary infection. The results plotted as maximum titre of positive reaction, are recorded

in Figures 4.4 and 4.5. The results for IgG antibody in Figure 4.4 show that there is a reasonable degree of reproducibility in sera containing antibodies with titres of 1:50 or more. Thus with the sera of day 15, two positive responses from three tests are recorded, whereas with the sera of day 10, only one positive response (1:50) is recorded from three tests.

From these data it is clear that accuracy of the results spans approximately two serial dilutions, and that investigation of low titre serum antibody responses requires that several assessments of a test sample be undertaken. Nonetheless it is clear that detectable antibody responses are present at day 10 post-infection with consistent results recorded thereafter. These show that there is a rapid increase in antibody titre to about 1:400 by day 27 post-infection which is maintained thereafter during primary infection until day 46 post-infection. Thus from a different aspect the findings of Figure 4.3 are confirmed. In a secondary infection these levels are maintained until day 47 post-secondary at least.

The results of the repeated assessments of IgM antibodies are recorded in Figure 4.5. With the same critical evaluation it is clear that at low titres of serum antibodies the microelisa system has an inconsistency over 3 or 4 dilutions, which probably explains the apparently erratic course of development recorded in Figure 4.3. However positivity was demonstrated on days 5 and 10 of a primary infection with a very rapid increase to peak levels by day 15-18 post-primary infection. There is a suggestion of a waning of the IgM antibody response by day 36-40 period, which is confirmed by low levels at 47 days post-secondary infection.

All the samples of serum which have been tested for IgG and IgM

antibodies with results recorded in Tables 4.1 - 4.4 and Figures 4.1 - 4.5, were also tested for IgA antibodies. The results of some of these tests are presented in Table 4.5. Using the criterion of positivity as being twice the mean of the negative controls, IgA antibody could not be detected in the sera of adult mice. As with the results to be presented in Table 4.6 for serum antibody levels in lactating mice, it would have been possible to declare positive IgA antibody levels in these tests had the criterion for positivity been the mean +2 × s.d. of the naive mice, which criterion has been proposed by some authors. One of the major problems of the IgA antibody results with microelisa is the relatively very high O.D. values of the negative sera which might be masking some slight production of anti- T. spiralis antibodies in the sera of infected animals.

(ii) Antibodies in lactating mice:

Following the series of experiments to determine the nature of antibody responses in adult mice and the reliability of the microelisa system, it was obvious that from the point of view of the main objective of this dissertation it was necessary to determine the levels of serum antibodies in lactating mice, particularly during the mid-lactation period, which was associated with the critical fostering experiments.

For the determination of IgG and IgM antibodies at the mid-lactation period two batches of mice which had received different infection protocols, both in terms of age and of duration of infection were used. One batch of 6 mice received a primary infection of 400 T. spiralis infective larvae 5 weeks before mating, and a secondary infection of 200 larvae one week before mating. These mice were therefore approximatley 35-40 days post-secondary infection at the time

of recovery of sera at mid-lactation. The second group of 6 mice received a primary infection of 200 T. spiralis larvae approximately 7 days before parturition. These mice were therefore of approximately 16-17 days post-infection at the time of recovery of sera at mid-lactation.

For the investigation for possible IgA antibodies present in the sera during lactation, two infection protocols were used. One batch of 6 mice was infected approximately one week before parturition with 200 T. spiralis larvae and sera were recovered from these mice during the early lactation period, that is approximately 11 days post-infection. Another batch of 6 mice was infected approximately 5 weeks before parturition, and sera recovered during mid-lactation, that is approximately 40-45 days post-infection.

From the data recorded in Table 4.6 it is clear that with respect to IgG antibodies the lactating mice present with positive serum antibody levels. As with the non-lactating adult mice referred to in earlier results, a primary infection of 16-17 days duration yields a positive result of low titre. The sera from the mice with a secondary infection yielding higher titres. Repeat runs of these sera confirmed these data. IgM antibodies show the inverted pattern of higher titres in 16-17 day primary than in the 35 day post-secondary infection.

The results for IgA indicate that antibodies are not detectable using the criteria of positivity as twice the mean of the negative controls.

(iii) Serum antibody levels in adult and lactating mice following immunization:

Sera from adult and lactating mice which had been subjected to

primary and secondary immunization with T. spiralis larval antigen (see General Materials and Methods) in combination with Freund's complete adjuvant, (according to protocol 1.3, section 1), were collected at different days after primary immunization. Then one week after secondary immunization, these mice were mated and sera were collected during the last days of pregnancy i.e. 19 days post-secondary immunization. The results of this experiment are recorded in Table 4.7, which show that IgM antibodies were first detected on day 5 post-immunization at low dilution (1:50). However, the titre had risen to 1:100 on day 10, and was maintained at this level during pregnancy and lactation in doubly immunized mice. IgG antibody was first observed in low dilution (1:25) on day 10 post-immunization with a sharp rise by day 20. A titre of at least 1:800 was evident during pregnancy and lactation. Repeat testing yielded similar O.D. values.

(d) Antibodies in milk:

(i) Maternal milk:

Milk was recovered from infected and naive mice at early, mid, and late lactation periods (for milk recovery, see General Materials and Methods). Infected mice were given a dose of 400 T. spiralis larvae as a primary infection, followed after 4 weeks with a secondary dose of 200 larvae which was given one week prior to mating. After milk centrifugation to separate the fat and particulate material, the milk supernatant was tested for the presence of antibodies.

Table 4.8 records data for IgG, IgM and IgA antibodies in milk. Applying the same criterion of positivity, IgG antibodies were present at titres of 1:30 during early and mid-lactation, and at 1:60 in late lactation. IgM antibody was detected at low dilution (1:15) only during late lactation, and IgA antibody was detected at 1:15 in mid-

lactation. Several repetitions of these tests on different batches of milk from different groups of mice yielded almost identical results. A summary of all of the results of the tests on batches of milk are recorded in Table 4.9. Colostral supernatant was obtained in very small quantities from one batch of mice only, and showed IgG antibody at a titre of 1:15. From this table it can be concluded that low to moderate titres of IgG antibody to T. spiralis are to be found in the milk of infected mice at all stages of lactation. IgM and IgA antibodies are to be found at low titres inconsistently in mid-lactation or late lactation milk. IgA antibody in colostrum was not determined.

(ii) Antibodies in milk in infant stomachs:

Batches of milk were collected from the stomachs of 5-6 infants (see General Materials and Methods) during different lactation periods and tested for the presence of IgG, IgM and IgA antibodies. The results of one experiment recorded in Table 4.10 show that IgG antibody was present during early and mid-lactation periods at 1:20 to 1:30 dilution. IgM positivity in milk ranged from 1:5 to 1:15 during the same lactation periods. IgA antibody was observed at a low level (1:10) during mid-lactation and was not detected in milk from the late lactation period. The results of repetitions of these experiments using additional batches of milk recovered from the stomachs of infants are additionally recorded in Table 4.11. These additional experiments reveal inconsistent detection of low levels of IgG and IgM antibodies.

(e) Antibodies in the sera of naive infants suckling immune mothers:

To gain insight as to the possible uptake and retention of antibodies from the milk by infant mice, sera were recovered from batches of 5-6 infant mice at intervals between the 6th and 19th days

of age. The mice were born of infected/immune mothers and suckled their own mothers until the sera were collected. Sera taken at days 6 and 12 from naive infants suckling naive mothers were used as controls.

Tested by the microelisa method as used in the previous studies, IgM and IgA antibodies could not be detected at titres of 1:25 and 1:10 respectively. The observed highest measurements of IgG antibodies are presented as a graph of Log 2 antibody titre against age in Figure 4.6. From the negative result at day 6 of age it can be concluded that there is no endowment of IgG antibody via the transplacental route, nor of a significant uptake and/or retention of antibody from the milk. By day 8, IgG antibody at a titre of 1:200 is detectable increasing to 1:800 by day 10 and falling thereafter to 1:100 on day 19.

(f) Antibodies in sera of infected infants:

These experiments were undertaken to determine the capacity of young mice to produce antibodies to intestinal T. spiralis. Infant mice suckling their naive mothers were infected at one week of age with 100 T. spiralis infective larvae, and sera were collected 8, 11 and 14 days after infection from different batches of 6 young mice (2 from each of 3 litters on each day), and tested by microelisa. Similar batches of naive infants were used as controls.

IgA antibody was not detected. For IgG and IgM classes of antibody, a maximum positive response at a titre of 1:50 for IgG antibody and 1:200 for IgM antibody was detectable 14 days after infection. These titres were observed consistently for IgM antibodies in repetitions on the same batch of serum, but not for IgG antibody. The development of the response at titres of 1:50 is recorded in Figure 4.7. The development of the IgM antibody response indicates

a steady increase whereas there is a considerably slower response for IgG antibody followed by a more rapid increase.

Table 4.12 summarizes the maximum antibody levels of all the experiments undertaken in this part of the study. In the sera of non-lactating adult infected mice, both IgG and IgM antibodies are present at high titres, and at low to moderate titres in primary infection in lactating mice. IgA antibodies were not detected in sera. In milk recovered from infected adult mice IgG antibody could be detected at moderately high levels, while IgM and also IgA could be detected at low titres. With milk recovered from the stomachs of infant mice suckling infected/immune mothers, titres of the three classes, almost identical to that of the milk from the mothers, were recorded. Only IgG antibodies could be detected in the sera of naive infants suckling infected mothers and it must be concluded that only IgG antibody is taken up and/or retained by the infants. Infant mice have detectable IgG and IgM antibodies in their sera after 14 days of infection and therefore exhibit immunological competence.

4.3 Radial immunodiffusion assay (R.I.D.) of milk and serum immunoglobulins:

4.3.1 Materials and methods:

T. spiralis infection, and the collection of milk and sera from adult, lactating and infant mice was carried out as described in the Materials and Methods for ELISA assay.

R.I.D. Assay:

Immunoglobulin levels in milk and sera from naive and infected mice were quantified using the single radial immunodiffusion technique

of Mancini, Carbonara and Heremans (1965). Quantitative radial immunodiffusion plates for the determination of mouse IgG and IgM were purchased from Miles Laboratories Ltd., Slough, England; R.I.D. plates for IgA were obtained from Meloy Laboratories Inc., Springfield, Virginia, U.S.A. Reference standards of mouse immunoglobulins were also obtained from the same sources and were used to plot standard curves from which the concentrations of test samples were calculated. The techniques used in these plates were those described by the manufacturer; serial dilutions of reference standards including 100% 'no dilution', 50% '1:2 dilution', 25% '1:4 dilution', and other further dilutions were prepared by mixing thoroughly in sterile saline. Wells in each plate were filled with 5 µl of standard reference using a 0-20 µl Finpipette. It was obvious that valid results were dependent on consistency and accuracy during this step. The remaining wells in each plate were filled with 5 µl of supernatant from milk or with sera. After filling the wells, the plates were incubated in a moist box in an upright position and left undisturbed for 48 hours at 37°C. The diameter of the precipitin rings developed by the standards and test samples were measured using a measuring magnifier with a graticule calibrated in millimeters (Polaron Equipment Ltd, Watford, England). Such a viewer was used by placing it on the back of the plate and viewing the rings against a dark background with light coming from the top or sides. The diameters of the precipitin rings were squared and the reference line plotted on linear graph paper, which was then used to determine the concentrations of immunoglobulins in the test samples in mg/ml.

Although greater accuracy can be obtained when unknown samples are run in duplicate in a single plate, this was not always practical due to

the large number of samples to be tested and the limited number of plates purchased for each class of immunoglobulin.

4.3.2 Results:

Plate 4.2 shows the form of result obtained when testing for total IgA in sera and milk obtained from naive and infected animals. The standard of consistency and reproducibility of repeat samples is well illustrated. The determination of the amounts of total IgA, IgG and IgM are recorded in Figures 4.8, 4.9 and 4.10. The majority of the milk samples were taken from mice at mid-lactation and from the stomachs of naive infants suckling mice during the mid-lactation period. Adult mouse sera were taken from naive or 21 day infected mice. As before the mid-lactation period was chosen for extensive study on the basis that the most critical fostering studies were focussed on this period.

The results for milk from this period are recorded in Table 4.13 along with the data for naive and infected sera. The levels of IgA in naive maternal milk during the mid-lactation period ranged from 0.12 - 0.46 mg/ml, and it can be seen that there is a marked increase in IgA levels in infected mice which ranged from 0.7 - 1.14 mg/ml. In infant mice suckling naive or infected mothers there was a very much lower concentration of IgA in milk recovered from their stomachs. Notwithstanding these low levels there is evidence of an increased amount of total IgA in the infants suckling immune milk. The levels of IgG in maternal milk also show a marked (c.2 fold) increase in infected animals. In milk from infants' stomachs, as with IgA there is an increase in the amount of IgG, in this case about 3 fold, in infants suckling infected mothers. The levels of IgM in maternal milk are low, considerably less than 0.1 mg/ml in both naive and infected animals. The amount of IgM in the stomachs of

infant mice suckling naive mothers was too low to be measured by this method, however, the small amount that could be detected in infants suckling infected mothers was the same as that recovered in the maternal milk.

In sera collected from naive and 21 day infected adult mice there was a significant amount of IgA present and an indication of slightly increased IgA in infected mice. For IgG there was an enormous increase in the amount in infected sera, whereas there appeared to be no significant increase in the amount of IgM.

4.4 Detection of soluble antibodies and antigen in infected sera.

4.4.1 Materials and Methods:

(a) T. spiralis infection and serum collection:

Sera were recovered from 6 mice given a primary infection of 450 T. spiralis infective larvae, at day 5 and approximately weekly thereafter until day 82 post-primary infection. Another group of 6 mice were given a second infection of 350 T. spiralis larvae on day 35 post-primary infection, and sera was collected from these mice 5 days post-secondary infection and weekly thereafter. Sera were collected from the mice by capillary suction from the tail individually and stored as single sera, or the sera from the 6 mice were pooled. (For details on T. spiralis infection, and serum collection, see General Materials and Methods).

(b) Antigen:

T. spiralis larval antigen (protein concentration 14 mg/ml), as described in General Materials and Methods was used throughout this study. This antigen was designated as (Lag).

(c) Rabbit antisera:

Crude saline extract antigen of the infective larvae of T. spiralis (Lag) was used for the production of rabbit anti-T.spiralis sera. A New Zealand White X Sandy Lop rabbit 8-10 months old, 2½-3 Kg in weight was used. Immunization was carried out according to the protocol of Smith and Herbert (1976) as follows: the antigen (Lag) was emulsified ultrasonically (MSE, ultrasonicator) for 20 seconds with an equal volume of Freund's complete adjuvant (FCA) in a small beaker held in an ice bath. To check the quality of the mixture, it was tested by putting one drop on the surface of cool water, and if no spread over the surface was observed, the mixture was properly emulsified. A total of 1.2 ml of the emulsion (Lag/FCA) was injected into multiple sites. Two injections, each 0.3 ml were given subcutaneously under the loose skin of the neck and flank, and another two injections each of 0.3 ml were given intra-muscularly into the thigh muscles. Five weeks later a booster dose of T. spiralis larval antigen with no adjuvant was given into multiple sites subcutaneously and intramuscularly (approximately 0.1 ml each injection). The rabbit was bled from a marginal vein of an ear 10-14 days after challenge. The blood was collected and left at room temperature (21-22°C) for one hour, then the clot was ringed with a sterile applicator stick and stored at 4°C overnight. Serum was obtained after centrifugation (350 g for 10 minutes at 4°C) and was designated Anti - T.spiralis Larval serum (ALaS). The serum was aliquoted in small volumes and stored at -20°C until used. The serum antibodies were subjected to three different tests, Ouchterlony immunodiffusion, Counter immunoelectrophoresis (C.I.E.) and immunoelectrophoresis. All tests were done in duplicate, and carried out as follows:

(d) Ouchterlony immunodiffusion:

1% of agarose (BDH) was prepared by melting 0.1 g of agarose in 10 ml of P.B.S pH7.2 in a boiling water bath for 2 minutes. 7 ml of agarose was poured onto 3 x 2 inch slides held on a level surface and following solidification of the gel (approximately 20 minutes), wells were punched out. The central well was filled with Lag, and the outer wells filled with rabbit test sera (ALaS) or rabbit normal sera. Slides were placed in a humid chamber in a fridge for 24 hours incubation, and if precipitin lines were visible, the gel was stained with Amido Black 10 B.

(e) Immunoelectrophoresis:

1% of agarose (BDH) was prepared by melting 0.1 g of agarose in 10 ml of veronal acetate buffer pH8.6 in a boiling water bath for 2 minutes. Two wells were filled with Lag and the slide was placed in the electrophoresis tank which contained tank buffer, and then connected from each side by a filter paper wick (Whatman 3 mm Chromotography paper). The electrophoresis was continued for one hour using a current of 15 mA per inch width slide (i.e. 30 mA for 3 x 2 inch slide) at 10°C. After the electrophoresis, the trough in the agar was removed and filled with ALaS. Incubation was carried out overnight in a humid chamber at 10°C. Following the appearance of bands, the gel was stained with Amido Black 10B.

(f) Single counter current immunoelectrophoresis (C.I.E) and double counter current immunoelectrophoresis (D.C.I.E.).

The principle for counter immunoelectrophoresis has been mentioned by many authors (Corkill, 1977; Thompson, 1977; Roitt, 1977). C.I.E.

and D.C.I.E. were performed on 3 x 2 inch glass slides using 1% agarose (prepared by melting 0.1 g agarose in 5 ml veronal acetate buffer + 5 ml distilled water) with veronal acetate tank buffer. The agar was poured on to level slides and left to solidify for 20 minutes. The C.I.E. pattern as shown in plate 4.3 was used to test the positivity of rabbit anti-serum. In this test Lag was placed in cathodal wells, and ALaS was placed in anodal wells. In D.C.I.E, it is possible to arrange the test wells so that antigen and antibody in a test sample may be detected simultaneously with either of the patterns shown in Figure 4.11. Wells 3 mm in diameter were punched into an agar gel. In the first pattern (the method of Phillips and Draper, 1975) there are three wells in a straight line set 5 mm apart(Figure 4.11.A). In the second pattern (Figure 4.11.B) there are two pairs of wells, each well of a pair being set 5 mm apart, with one pair of wells positioned below the other and off-set. 6 µl of test serum from mice was placed in two central wells in pattern B, and in the central well in pattern A. Anti T. spiralis larval serum (ALaS) was placed in the anodal well, and T. spiralis larval antigen (Lag) placed in the cathodal well. In most cases four samples were run on each slide. The slides were placed in a Shandon tank containing tank buffer, the buffer - agarose gel connection was made by paper wicks (Whatman 3mm Chromotography paper),and electrophoresed at a constant current of 15 mA per slide for one hour at 10°C. After electrophoresis the slides were washed in normal saline and allowed to remain thereafter at room temperature (21-22°C) in PBS pH 7.2 for 24-48 hoursnormally, or if bands were not clearly defined, the slides were maintained in several changes of PBS with 0.2% sodium azide for several days until the clarity of the precipitin bands improved. The slides were then dried using an electric hot fan, and

stained with Amido Black 10 B, and decolourized in 7% acetic acid until the precipitation bands were clearly observed. The slides were dried at room temperature and examined for precipitin bands with the aid of a viewer (Thousand and One Lamp Ltd., London). A positive reaction was recorded if single or multiple precipitin bands were present between inner wells containing the test serum and the outer wells containing (Lag) or (ALaS). The criteria used were those of Thompson (1977) as detailed in Figure 4.11,A.

(g) Buffers and stains for counter current immunoelectrophoresis, gel diffusion and immunoelectrophoresis:

Veronal Acetate Buffer pH 8.6

Sodium barbitone	48.9 g
Sodium acetate	32.35 g
HCl	300 ml 0.1 N

Made up to 5 litres with distilled water.

Tank Buffer:

Veronal acetate buffer	800 ml	(4 parts)
Distilled water	200 ml	(1 part)

Amido Black Stain

Amido Black 10 B	2 g
Glacial acetic acid	12 ml
Absolute ethanol	20 ml

Made up to 100 ml with distilled water.

4.4.2 RESULTS

(a) Testing for antibody activity in rabbit sera:

In the immunodiffusion study prominent bands were produced indicating the presence of antibody in the test sera (plate 4.3). Normal rabbit sera did not produce any bands.

In the study using C.I.E. with ALaS in the anodal wells tested against Lag in the cathodal wells, precipitin bands were also visible (plate 4.3). Using immunoelectrophoresis, the reaction between Lag placed in the outer wells and ALaS in the trough produced several precipitin arcs as shown in plate 4.3.

The results from these three tests indicated the positivity of rabbit anti T.spiralis anti-sera which were used throughout the present investigation.

(b) Circulating antigen and antibodies by D.C.I.E.

Initial tests involved predicted strong positive test serum taken from mice 26 days post-secondary infection. Serum taken from naive mice was tested and showed no reactions, and was therefore taken to be a negative control. An additional control test was to run test sera alone - without antigen in the cathodal wells. These did not show any bands using pattern 'A'. A preliminary experiment using only Lag and ALaS in the outer wells showed that a precipitin reaction about the central well occurred, which was due presumably to the reaction between ALaS and Lag which had migrated and reacted near the central well. For this reason pattern 'B' was employed, as any direct interaction between ALaS and Lag would produce precipitin lines which would be lying between the upper and lower central wells at an angle and would be distinct from those which were produced when either test serum and ALaS or test serum and Lag reacted.

The reactivity of the mouse test sera (26 days post-secondary) and antigen was tested following neutralisation. Equal volumes of test serum and antigen were mixed and left overnight at 4°C. The solution was then centrifuged at 20,000 g at 4°C for 10 min. to sediment complexes and the supernatant tested on DCIE. No precipitin bands between the test supernatant and Lag were observed, indicating that all of the reacting antibodies in the test serum were neutralised by Lag at the concentrations used.

A band indicating soluble antigen was first detected on day 12 post-infection in a single serum and in pooled sera (plate 4.4 A & B, Table 4.14). Soluble antigen was also present in the pooled sera on day 19 post-infection, but not detected in single serum samples. On day 26 post-infection, no soluble antigen precipitin bands could be detected in single or pooled sera (plate 4.4 A & B). On day 40 post-infection a band was observed in a single mouse serum from four tested (plate 4.5A). No antigen precipitin bands were observed from days 47 - 82 post-primary infection.

Antibody in the test sera was first observed as a very weak band on day 26 post-infection (plate 4.4A, Arrow) in one individual serum only. However, on day 33 post-primary infection bands were observed in the single sera tested (plate 4.4C). By day 40 post-infection, two bands could be detected in three out of four single sera (plate 4.5A). Other tests with pooled sera showed that by day 47 post-infection two strong bands were visible in all sera, and these persisted until the termination of the primary infection on day 82 post-infection.

In the group of mice which were given a secondary infection on day 35 of the primary infection, circulating soluble antigen was detected on day 5 post-secondary infection in pooled sera (plate 4.5B),

but on day 12 post-infection and thereafter soluble antigen was not detected. A detectable antibody precipitation band in the test sera was present 5 days post-secondary infection, and was of approximately the same intensity as the day 40 post-primary infection, and remained without apparent increased intensity until two heavier bands were observed in preparations of day 40 post-secondary infection. Antibody bands were detectable until day 82 post-secondary infection at the termination of the experiment.

4.5 Discussion.

In comparison to the immunofluorescent technique, ELISA was shown to be more sensitive in detecting T. spiralis infection (Ruitenberg et al., 1974; Engvall and Ljungström, 1975; Van Knapen, Framstad and Ruitenberg, 1976). However, a valid serodiagnostic assay must have both acceptable sensitivity and negligible cross reactivity with other parasites. In nematode infections, the specificity of ELISA was shown to be high by Carroll,Karthigasu and Grove,(1981) who demonstrated the absence of significant cross reactivity between Strongyloides ratti and Ascaris suum using crude saline extract antigens. Cross reactivity due to the presence of antigens common to the species of filaria and T. spiralis was reported by Engvall and Ljungström (1975), which might suggest that purification of T. spiralis antigens would be desirable. In view of many reports (de Savigny, 1975; de Savigny and Tizard, 1977; de Savigny, Voller and Woodruff, 1979; Smith, Quinn, Bruce and Girdwood, 1982) which demonstrated the high sensitivity and specificity of Execretory-Secretory (ES) Toxocara antigen in the ELISA assay, a preliminary experiment conducted during the course of this investigation

but not reported in detail here, indicated no enhanced sensitivity when T. spiralis E.S antigen (protein concentration 200-300 µg/ml) was used with both sera and milk in comparison to crude larval antigen. Since it was not intended to examine the aspect of parasite specificity in this study, the considerable effort in producing large volumes of excretory-secretory T.spiralis antigens was not undertaken, and crude saline extract antigen was used throughout.

The present study confirmed the findings of other workers (Engvall and Ljungström, 1975; Van Knapen et al., 1982) that ELISA is a sensitive test in detecting class specific antibodies. Results presented here show that IgM antibody was first detected in sera of infected mice by day 5 post-primary infection (Table 4.4). The positivity of IgG antibody in sera was evident on day 10 or 15 post-infection (Table 4.3). The resultsalso show that the levels of IgG antibody increased steadily throughout the entire period of primary infection to the day 35-40 post-infection period and IgM showed a steady increase after day 15 post-infection with a maximum positivity observed around day 30 post-infection. Although during secondary infection both antibodies were demonstrated in infected sera, the level of IgG antibody was much higher than IgM. Engvall and Ljungström (1975) demonstrated the presence of specific IgG and IgM antibodies in trichinosis patients even after 11 months of infection by ELISA, although immunofluorescence yielded negative results.

IgA antibody was not detectable in sera from mice with a primary or secondary infection. Crandall and Crandall (1972) using IFAT demonstrated antibodies against T. spiralis in mouse IgG_1, IgG_2, IgM

and IgA immunoglobulin classes by day 11-15 post-infection. Low levels of IgA antibody to T. spiralis have been demonstrated in man (Engvall and Ljungström, 1975) using ELISA; also in human sera, IgA antibody was detectable up to 3 months post-infection although no correlation with either specific IgG or IgM was demonstrated (Van Knapen et al., 1982). With reference to Table 4.5, the dilemma concerning the positive/negative discrimination is well illustrated. In Table 4.5A, had the point of positivity been taken as the mean of the negative control plus twice the standard deviation, a positivity of 1:10 on day 33 and at 1:40 on day 46 would have been suggested but with no other positive result recorded. In Table 4.5B on the other hand a positivity at 1:40 on day 10, 1:160 on day 21, 1:320 on day 28 and 1:160 on day 26 of a secondary infection would have been indicated. It is concluded that such profound inconsistency substantiates the decision to adopt as the criterion of positivity a value of twice the mean negative control value. The undetectable IgA antibody in infected sera used in the present investigation, may be due to competition between antibodies of different affinities and/or avidities; Engvall and Ljungström (1975) noted the decrease in the amount of IgA antibodies at a time when the amount of IgM antibodies is maximal.

The total immunoglobulin levels in sera of naive and infected mice revealed the presence of considerable amounts of G, M and A immunoglobulins, with a very marked increase in the amount of circulating IgG during infection and a slight elevation in IgA. There was no significant increase in circulating IgM during infection. It is notable that in naive sera the amount of IgA is approximately 1.5 mg/ml which is

considerably higher than the other two classes, and this feature may be responsible for the higher O.D values which were always obtained with naive sera tested for IgA antibodies. As a part explanation of the efficiency of the test, in the preliminary testing there was an insignificant degree of adherence of serum IgA antibodies to plates which were not coated with antigen, and so non-antigen binding to the plates can be ruled out. Therefore the assumption of non-specific immunoglobulin binding to the crude saline antigen is the only explanation of these high negative values.

As in the case with whole infection, immunization of mice with T. spiralis antigen and adjuvant yielded a detectable amount of both IgG and IgM antibodies from sera of these animals subjected to this treatment, during both primary and secondary immunization (Table 4.7). These antibodies were also present during lactation with a higher titre of IgG than IgM antibody. No determinationsof IgA antibodies were attempted on the sera from these immunized mice.

The present study demonstrated the presence of IgG and IgM antibodies in sera collected from lactating mice during primary and secondary infection, although the antibody titres were lower than in non-lactating adult mice. IgA antibody was not detected in sera of lactating mice at any time. It has been reported in several experimental models that lactation was associated with the suppression of the expulsive and protective immune response against parasitic infections (Connan, 1970, 1972; Dineen and Kelly, 1972; O'Sullivan, 1974; Selby and Wakelin, 1975; Shubber, Lloyd and Soulsby, 1981). Although the mechanism whereby lactation affects immunity is not well understood, and many explanations of this defect have been suggested including the failure of sensitized

lymphocytes to differentiate to effector cells (Dineen and Kelly, 1972) or the presence of lactogenic hormones (Kelly and Dineen, 1973; Ngwenya, 1976), it is quite clear that the mice used in this study are capable of producing antibodies against the infection with T. spiralis and passively transferring them to their suckling infants.

Quantification of the amounts of antibody present in milk based on the titre of the antibodies must be seen to be an issue of relativity between different classes of antibody but much more problematically between stages of lactation, let alone between different batches of milk. Thus while it is perhaps reasonable to conclude that there is more IgG antibody in milk from infected/immune mothers than IgM and IgA antibodies at all stages of lactation, an apparent increase or decrease in the titre of antibody present in milk at different stages may be simply a reflection on the actual volumes of milk being secreted. In relation to the fostering experiments, virtually all of these were undertaken in the mid-lactation period and attention can therefore be focussed primarily on that period. With reference to Table 4.9, however, it is noted that considerable variation in the degree of positivity (titre) of one batch or of several batches of milk is quite marked, and whereas there is consistent detection of IgG antibodies at low to moderate titres, there is inconsistent detection of IgM and IgA antibodies at low titres. The issue of the positive/negative discrimination point is again highlighted when considering the results in Table 4.8. With reference to IgA, had the criterion for positivity been the mean + twice the standard deviation of the naive O.D values, there would have been a consistent recording of IgA antibody titre of not less than 1:10

for early, mid and late lactation milk. However, the probable absurdity of early lactation infected milk being recorded at a titre of 1:320 while not showing any marked decline in the OD values makes the point forcefully that as with any test, which is being operated at its lower limit, considerable care should be taken in an evaluation of the results.

Were the results for IgM and IgA antibodies to be expressed as dose response curves, both would be very flat and demonstrate the requirement of a rigorous determination of discrimination. The results as shown in Table 4.9 are, for the purposes of this dissertation, taken to indicate that there are low to moderate levels of IgG antibody and very low levels of IgM and IgA antibodies present in the milk of infected/immune mothers, and low to very low levels of IgG and IgM antibodies in primary infection milk. When these observations are put alongside those of the determinations of total immunoglobulins in mid-lactation milk as shown in Table 4.13, with, in particular, the marked elevation of total IgA in infected milk, it is quite clear that IgA antibodies must comprise a very small component of the total immunoglobulins in secreted milk from infected mothers. Conversely therefore, of the very notable increase in the amount of IgA immunoglobulin secreted in the milk by infected mothers, very little of it is directed at T. spiralis and it must be presumed that the majority of IgA synthesizing cells directed from the T.spiralis insulted gut circulation to the mammary gland are not primed to T. spiralis infective larval antigens.

It is clear that a very small fraction only of the IgM is secreted into the milk although there is some slight evidence of an increased amount in infected animals. The amount of IgG although similar in amount

to that of IgA is but a small fraction of the very large amount of IgG in systemic circulation in infected mice. Halsey, Johnson and Cebra (1980) demonstrated that the mammary gland can efficiently remove dimeric IgA from the serum, and this indicates that some of the IgA observed in milk might be primarily derived from extra-mammary sites by antibody-producing cells that are abundant in the gut lamina propria. In an extensive study on IgA in mouse milk by Halsey, Mitchell, Meyer and Cebra (1982) IgA was shown to be efficiently transferred from the serum of lactating mice into the milk, and the rate of disappearance from the serum of polymeric IgA was observed to increase dramatically during lactation suggesting that this immunoglobulin might be derived at least in part from the serum. The sites of synthesis of milk IgA (local vs extra-mammary gland) were also evaluated by these authors by determining the extent of dilution of isotopically labelled serum IgA during transport through the mammary gland into the milk, and showed that early in lactation the majority of the IgA in mouse milk appeared to be derived from distant sites and transferred via the blood to the mammary gland. However, their work demonstrated that by day 8 of lactation the isotopically labelled polymeric IgA in milk was significantly diluted by the IgA synthesized in the mammary gland. IgG was not diluted by local synthesis indicating that this immunoglobulin was exclusively serum derived. Other studies on IgG in milk arrived at similar conclusions; Mink and Benner (1979) reported that serum IgG_1 and IgG_2 levels decrease by about 50% during lactation in mice, and this might be due to transudation into the milk. IgG in lacteal secretions could be transported from serum into milk as shown

from studies in cows (Brandon, Watson and Lascelles, 1971). It has been found that the acinar epithelial cells of the mammary gland possess specific receptors for these immunoglobulins (Sasaki, Larson and Nelson, 1977). In rat milk, IgG and IgM derived from the circulation, are allowed access to the milk by means of some selection process within the mammary gland (Head, 1977). McGhee et al.(1975) noted an increase of IgG_{2a} in rat colostrum and milk between day 0 and day 19 of lactation. In the present study, a very low level of IgM in milk could be detected in infected mice. This immunoglobulin was not detected in colostrum or milk from rats (McGhee et al., 1975).

Thus the present study, in classifying and quantifying the antibodies to T.spiralis present in infected mothers extends considerably the observations of Duckett et al, (1972) and Perry (1974) who showed the protective benefits of milk to the offspring and the presence of antibodies in the sera of lactating adult mice respectively.

The tracing of antibodies in the congealed milk in the stomach of the infants may be seen as evidence that the antibodies are not denatured in the stomach. As recorded in section 3 - dealing with the presence of viable maternal cells in the stomach contents, it should be recalled that in young rodents the physiological conditions in the stomach are not dominated by hydrochloric acid and peptic activity and the immunoglobulinsare not therefore subjected to severe acidification or proteolytic activity.

It is quite clear from the results listed in the summary Table 4.12 and documented in Figure 4.6 that maternal IgG antibodies are taken up by young mice within the first two weeks of life. It was rather surprising in fact that detectable IgG antibody was not in evidence in

mouse sera before and at day 6 of age. However, there is a rapid uptake and retention of antibody after this with a high of 1:800 on day 10 of age. IgM and IgA antibodies were not detected in the sera of infant mice suckling immune mothers.

Brambell's review (1970) recorded that, before and during the development of its own immune system, the young mouse or rat is dependent upon a supply of maternal antibodies for immunological protection. Although a small amount of immunoglobulin is transmitted from mother to foetus prenataly, it was shown that the majority of the immuno-globulins found in the blood of suckling mice is acquired from maternal colostrum and milk during the 16 day period after birth. The transport process in the young mouse was examined by feeding the γ-globulin fraction in the circulation. The studies showed that IgG is selectively absorbed and that the amount which can be absorbed at any one time is limited. To explain these findings, Brambell envisaged active transport of IgG molecules across the intestinal mucosa by means of specific receptors (Brambell, 1966). The fate of ingested maternal IgG has been studied most extensively especially in rats and mice which have a well-documented receptor-mediated mechanism for the transepithelial translocation of IgG (Brambell, 1966; Jones and Waldmann, 1972; Rodewald, 1973; Jones, 1976; Guyer, Koshland and Knopf, 1976; Morris and Morris, 1974, 1976; Morris, 1975; Borthistle, Kubo, Brown and Grey, 1977; Nagura, Nakane and Brown, 1978). In rats, for example, the site at which the postnatal absorption of immunoglobulin occurs has been localized to the proximal third of the small intestine (Rodewald, 1973), and is transferred through the cells into the circulation intact. By contrast, the distal

small intestine nonselectively absorbs IgG and many other kinds of macromolecules but does not transport them across the epithelium (Rodewald, 1973; Kraehenbuhl and Campiche, 1969; Nagura et al., 1978).

Nagura et al., (1978) working with suckling rats, noted that maternal IgA binds to the outer portion of infant jejeunal microvilli but significant amounts were not vizualized internally in the enterocytes; maternal IgG attached primarily to the lower portion of jejeunal microvilli, was internalized and transported across the enterocytes in vesicles as proposed by Brambell (1970). Morris (1958) noted that the immunological "gate" was closed by day 16 in mice. This observation would not be challenged by the present results which indicate a decline in serum IgG antibodies in young mice before and after day 16 of age.

Results presented in this study record the production of IgM and IgG antibody responses against T. spiralis antigens. These were detectable in the serum by day 14 after a single infection of T. spiralis larvae given to infant mice at one week of age. There was no indication of the presence of IgA antibody in these sera (Figure 4.7).

In investigation in mammalian species dealing with ontogeny of individual immunoglobulin classes, the production of IgM was reported to precede the synthesis of IgG in humans (Gitlin and Biasucci, 1969), bovids (Schultz, Dunne and Heist, 1973), guinea pigs (Davie, Paul, Asofsky and Warren, 1974), or IgM synthesis occurred simultaneously with that of IgG as shown in foetal lambs (Silverstein, Thorbecke, Kraner and Lukes, 1963), and in germ free mice (Asofsky, 1965). Chapman, Johnson and Cooper (1974) studied the synthesis of IgM and IgG in pig foetuses by immunofluorescence and demonstrated the early formation of

IgM which was followed later by formation of IgG. Relatively fewer data exist on the ontogeny of IgA. However, studies on IgA formation in guinea pigs showed that the onset was later than the formation of IgM and IgG (Davie et al., 1974).

It has been suggested (Dineen and Kelly, 1973) that the defect in baby rats is a failure of division and/or maturation of lymphoid cells involved in the immune response against parasites. However, the detection of IgM and IgG antibody in infant mice used in this study indicates the competence of these infants. A study by Brown, Charley-Poulain and Pery (1981) on baby rats infected with Nippostrongylus brasiliensis at 2 and 4 weeks of age, demonstrated the presence of IgA and IgG antibody responses against worm antigens, However, these authors did not study the response of IgM class of antibody in their baby rats.

The maternal antibodies,especially IgG,might have a role to play in preventing establishment of the worms in the small intestine of the infants. The observations of Despommier and Muller (1976) showed that the functional antibodies to stichosome secretions of infective T. spiralis were IgG antibodies. Such a feature might indicate that protection against T. spiralis was similar to that which occurs against Giardia muris as reported by Andrews and Hewlett (1981). These authors found that mouse immune milk contains specific IgA and IgG antibodies against this parasite, and their study suggested that these antibodies might be the mediators of the protection of the infants, either by interfering with the excystment process of the cysts (an irrelevant factor for T. spiralis artificial infection) or by influencing the capacity to attach to the enterocytes.

Studies on Taenia taeniaeformis in rats (Musoke et al., 1975) showed that colostral IgG_1 and IgG_2 immunoglobulins were protective when administered intravenously as 1 mg doses, but this dose was not protective when administered orally; their participation in colostral immunization was therefore doubtful.

Lloyd and Soulsby (1978) studying T. taeniaeformis in young mice noted that removal of IgG from adult serum resulted in no protection being afforded to the infants when fed with the serum, whereas removal of IgA from the colostrum rendered the colostrum ineffective in protection. Removal of IgM and IgG from colostrum had no effect on the protective effect of colostrum. As in the present study, protection involved non establishment although with these tapeworm infections a period of two weeks elapsed between infections and examination of the liver where the metacestodes settle. It is not possible therefore to deduce where the protective effect may operate.

Although immunity can be transferred with immune serum it has proved difficult to correlate protection with circulating antibody levels and attention has been focussed on antibody present at the mucosal surface. Despite the fact that IgA is the dominant class of immunoglobulin in intestinal secretions (reviewed by Murray, 1972; Rowley, 1977), specific antiworm IgA has been identified only rarely, and only in one case has this been shown to be a significant element of protective immunity; IgA is known to be effective in the passive transfer of immunity from mothers to offspring against the invasive larvae of Taenia taeniaeformis, but other classes of antibody like IgG are also involved (Musoke et al., 1975; Lloyd and Soulsby, 1978). It has also

been suggested that IgA is involved in the expulsion of T. spiralis from the adult rat (Despommier, McGregor, Crum and Carter, 1977b);these authors have shown that adoptive transfer of immunity is accomplished more efficiently with a population of purified B-cells rather than T-cells from thoracic duct lymph (TDL). The majority of dividing B-cells in TDL of rats contain IgA and display IgA determinants at the cell surface (Williams and Gowans, 1975). These observations, and the fact that milk of immune mothers protects infant mice against T.spiralis (Duckett et al., 1972; Perry, 1974; see Section 1) led Despommier et al., (1977b) to postulate that acquired resistance to the parasite may be mediated by antibodies of the IgA class despite the earlier report of Despommier and Muller (1976) that IgG antibodies were the components functional against stichosome antigens. Despommier et al., (1977b)hypothesised that the poor ability of serum from immune donors to expel adult T. spiralis in normal recipients may be due to the fact that only IgA antibodies which are involved may be locally produced and/or not present in sufficient quantities in the systemic blood circulation, consequently further evaluation of the role of IgA in the immune response to nematodes is needed. The possible confusion of these hypotheses of expulsion of adult worms being mediated by IgA antibodies, where IgG antibodies may be functional against the stichosome secretions of the infective larvae require clarification. Although the work of Lloyd and Soulsby (1978) suggests a protective role for IgA, these authors could not define the mode of action of IgA antibodies against oncospheres of taenioid cestodes within the intestine of the host. In other infections, IgA is known to be effective in virus neutralization and in precipitation and

agglutination reactions (Tomasi, 1976), and it is suggested that IgA antibodies have a bacteriolytic effect in the presence of complement and lysozyme (Hill and Porter, 1974) while IgA antibody is reported to fix complement (Williams, Slaney, Price and Challacombe, 1976). IgA antibodies are capable of inhibiting the adherence of bacteria to the epithelial mucosa (Williams and Gibbons, 1972; Fubara and Freter, 1973) and in this way are protective against enteric bacterial infection in the mouse. Such a function of milk secretory IgA antibodies transferred from the mother to the infant would be of particular significance to the neonate. However, whether IgA, IgG or IgM antibodies act against T. spiralis in a way similar to that in bacterial infections in the gut has yet to be elucidated.

The experiments utilizing double counter current immunoelectrophoresis demonstrate the possible use of this test for serodiagnostic purposes of T. spiralis infection in that false positives using naive sera were not observed. However its usefulness in the present investigation is severely limited in that the detection of antibody responses early in infection were not observed, and it was not used subsequently to address the main objectives of this dissertation. However, the detection of circulating antigen in infected mice in the period c. 12-19 days post-primary infection, and of the period c.5 days post-secondary infection, is of general interest.

Counter immunoelectrophoresis, known also as counter current immunoelectrophoresis or immunoelectroosmophoresis(Corkhill, 1977) has been used for the identification of various bacterial and viral antigens (Dajani, 1973). It has also been used for the detection of

soluble antigens and antibodies in parasitic infections. With this technique Kaliraj, Ghirinkar and Harinath (1979) demonstrated the presence of circulating filarial antigen; while Seitz (1975) reported the presence of malarial antigen and antibodies in sera of mice and rats. In Schistosomiasis Hirata, Takamori and Tsutsumi (1977) were able to demonstrate the presence of circulating antigen in rabbits infected with Schistosoma japonicum. Using C.I.E. Houba et al., (1976) detected soluble antigen and relevant specific antibodies to Schistosoma mansoni in the sera of baboons, while Phillips and Draper (1975) detected circulating immune complexes in the sera of humans infected with Schistosoma mansoni.

The kinetics of T. spiralis infection in adult NIH strain mice have been studied by many authors (Wakelin and Lloyd, 1976a; Kennedy, 1980a; Alizadeh and Wakelin, 1982). Encapsulation of migrating first stage larvae begins about day 19 post-infection, and by day 35 the majority of the larvae are encapsulated. In the present study, soluble antigen was first detected on day 12 post-primary infection, and also on day 19 (Plate 4.4, A & B). Newborn larvae initially released on day 5 post-infection (Denham, 1965) remain for a period of 48 hours in the general circulation before becoming intracellular muscle parasites (Berntzen, 1965). Thus the majority of newborn larvae would have entered striated muscle cells by about day 12. At this stage of infection metabolic products of migrating newborn larvae could cause a situation of circulating antigen excess, as no circulating antibodies were detected by precipitation. By day 26 post-primary infection no antigen precipitin bands could be seen, but on day 40 soluble antigen precipitin bands were again noted.

From day 47 post-primary infection onwards no further antigen precipitin bands were observed.

Antibody precipitin bands were not detected until 26 days post-primary infection, but remain present until the termination of the experiment (day 82 post-infection). Perrudet-Badoux, Binaghi and Biozzi (1975) detected low titres of IgM antibody to T.spiralis antigen 45 days post-infection using the passive haemagglutination test and low titres of IgG antibody at 70 days post-infection using immunodiffusion in mice genetically selected to produce high antibody levels. Perhaps this particular study highlights the bias inherent in selection studies and the merit which should be accorded to different tests. Also Mota, Sadun, Bradshaw and Gore (1969) could not detect different antibodies including IgG_1 against T. spiralis in mice by passive cutaneous anaphylaxis (PCA) until 5 weeks after infection.

On secondary infection (day 35 after primary infection) circulating soluble antigen was detected on day 5 post-infection, but had disappeared by day 12. Whether the presence of circulating soluble antigen precipitin bands on day 5 post-secondary infection is due to the reappearance of circulating antigen on day 40 following a primary infection is not clear from the present investigation. Antibody precipitin bands were present throughout the reinfection period until the termination of the experiment (day 82 post-secondary infection). Houba et al.(1976) suggested that the simultaneous presence of both antigen and antibody components in the same serum sample was due to the splitting of antigen and low affinity antibody complexes by electrophoresis. They also suggested that as the infection progressed, complexes are found with a

high-affinity type antibody, this type of complex not being disassociated by electrophoresis. In the present study, soluble antigen was the only component detected on day 12 and 19 post-primary infection, indicative of circulating non-complexed soluble antigen, whilst its disappearance is indicative of either the formation of antigen and high-affinity type antibody complexes, or its absence from the general circulation.

On reinfection, the simultaneous existence of antigen and antibody (day 5 post-secondary infection, Plate 4.5,B) is indicative of antigen and low-affinity type antibody complexes which may be suppressed by high-affinity type antibodies by day 12. Since in secondary infections in NIH mice, intestinal worms persist for only 6-8 days with little in the way of production of newborn larvae into the circulation it would be surprising if circulating antigens were to persist for any length of time. The functional role of the circulating antigens during parasitic infections is as yet unknown. It is interesting to note that Wakelin and Lloyd (1976b) demonstrated that a serum pool taken from mice 21 days following a primary infection with T. spiralis was the most effective in transferring immunity to recipient mice. The present study shows that the soluble antigen was detected on day 19 post-primary infection, and it might well be that soluble antigens or antigen and low affinity-type antibody complexes which are normally transferred in 'immune serum' given to recipient mice induce active immunity as well as passively transferring immunity in these recipient mice.

Immunological transfer between mothers and children have been demonstrated or suggested in many diseases found in humans. For example,

delayed hypersensitivity to Schistosoma mansoni adult antigens was observed in uninfected children born to infected mothers (Camus, Carlier, Bina, Borojevic, Prata and Capron, 1976), and one of the hypotheses provided by these authors to explain the transfer of this sensitization from mothers to their children was the transplacental and/or mammary gland transfer of parasitic circulating antigens or immune complexes. In further studies Santoro, Borojevic, Bout, Tachon, Bina and Capron (1977), demonstrated the presence of two circulating S. mansoni antigens (parasite "M" antigen and antigen "4") in milk from mothers suffering from Schistosomiasis. To explain the presence of these antigens in the milk they suggested that antigens might have been derived from the blood and concentrated in the mammary gland, or complexed in the blood circulation with specific antibodies as immune complexes before appearing in the mammary gland. In this regard, circulating immune complexes were demonstrated in human Schistosomiasis (Phillips and Draper, 1975; Bout, Santoro, Carlier, Bina and Capron, 1977) and may be involved in the induction of delayed hypersensitivity. Uhr, Salvin and Pappenheimer (1957) had shown with diphtheria toxins that immune complexes were associated with delayed hypersensitivity. Prior to the demonstration of parasite antigen in human milk (Santoro et al., 1977) which might be important in the immunological relationship between mother and child during suckling, Duckett et al., (1972) had suggested that T. spiralis antigen which circulates in mothers might actively immunize the suckling infants. In the experiments which have been dealt with in Section 1, there was no evidence that this was the case. Worm antigens which circulate in sera early in the infection as shown from the present study, might appear in maternal milk and reach the suckling

infants. However, neither long term infection and/or immunization of the mothers, or in short term infection in mothers had any affect on the course of T. spiralis infection in infant mice. In fact no increased protection in terms of establishment of T. spiralis worms was observed in infants which were suckling short term infected mothers in comparison to infants suckling long term infected mothers, which suggests that this possible source of stimulation for the infants had no effect. Further it was shown that an increased rate of expulsion of primary infection in infants did not occur, indicating that orally administered antigen, even if it was not denatured in the infant gut, was not involved significantly in sensitization of the infants.

4.6 Summary:

1. The use of the enzyme linked immunosorbent assay (ELISA) showed that, in sera of adult mice infected with T. spiralis, IgG antibody was first detectable on day 15 post-primary infection, and the titre rose steadily to the day 35-40 period. IgM antibody was just positive as early as day 5 post-infection with variable high titre responses from day 15. IgA antibody was not detected. Both antibodies were also positive in sera from adult and lactating mice following immunization with T. spiralis antigen and adjuvant.

2. Both IgG and IgM antibodies were observed in lower titres in sera from infected lactating mice. A primary infection of 16-17 days in mothers yields a positive antibody response. IgA antibody was not detected.

3. IgG, IgM and IgA antibodies against T. spiralis larval antigen, were all demonstrated in maternal milk, and milk taken from infant stomachs. The levels of antibodies in milk, were always lower than that observed in sera. IgG antibody was detected at the moderately high level of 1:120, while IgM and also IgA could be detected at low titres.

4. IgG antibody only was observed in sera of naive infant mice which were born of and suckled immune mothers by 8 days of age. The titre was maximal by day 10 but falls substantially by day 19. This indicates the uptake of maternal IgG by the suckling infants. IgM and IgA antibodies were not detected in the sera of these infant mice.

5. Infant mice infected at 1 week of age were shown to be capable of producing antibodies of IgM and IgG classes which were detectable in the sera after 14 days of a primary infection with T. spiralis infective larvae. IgM antibody titre was much higher than that of IgG.

6. The total amounts of both immunoglobulin A and G in mid-lactation maternal milk from infected mice showed a marked increase, while total IgM levels were low in both naive and infected milk. To some degree the levels in milk reflect increased total IgG and IgA in post-infection sera, in which notable increasesof IgA, and IgG were observed in comparison to naive sera. No significant increase in the amount of IgM was detected in infected sera. Significant amounts of IgA and IgG immunoglobulins were detected in milk recovered from the stomachs of infant mice. Very small amounts of IgM were detected.

7. Soluble T. spiralis antigen was detected in sera of adult mice during a primary infection, but there is no evidence for the occurrence of worm antigen in milk.

Table 4.1. Microelisa. T. spiralis larval antigen. NIH mice = SERA. IgG antibody levels in primary and secondary infection sera using different concentrations of larval antigen.

Antigen concentration		Optical Density (O.D.) value at 492 nm											
		0.7 µg/ml			1 µg/ml			3.5 µg/ml			7 µg/ml		
Pooled sera (6 mice)		secondy. infect.	primary infect.	naive control	secondy. infect.	primary infect.	naive control	secondy. infect.	primary infect.	naive control	secondy. infect.	primary infect.	naive control
dilution	1:100	0.906	0.235	0.051	1.020	0.312	0.069	1.133	0.458	0.131	1.142	0.612	0.199
	1:200	0.725	0.221	0.042	0.872	0.274	0.075	1.060	0.365	0.081	1.101	0.478	0.112
	1:400	0.477	0.185	0.061	0.619	0.205	0.065	0.823	0.263	0.066	0.926	0.366	0.11
	1:800	0.291	0.116	0.038	0.382	0.126	0.042	0.573	0.182	0.063	0.700	0.246	0.085
	1:1600	0.159	0.066	0.038	0.219	0.069	0.035	0.388	0.106	0.053	0.517	0.145	0.077
	1:3200	0.108	0.065	0.036	0.135	0.075	0.035	0.297	0.082	0.047	0.388	0.123	0.083
	1:6400	0.064	0.036	0.023	0.099	0.031	0.010	0.189	0.048	0.034	0.266	0.077	0.056
Mean ±s.d			0.0412 ±0.012			0.0472 ±0.023			0.0678 ±0.0315			0.103 ±0.046	

Primary infection: 26 days post-infection
Secondary infection: 47 days post-secondary infection.
Positive reaction outlined in heavy columns; positivity - any reading ≥ twice the mean of the naive control.

PLATE 4.1

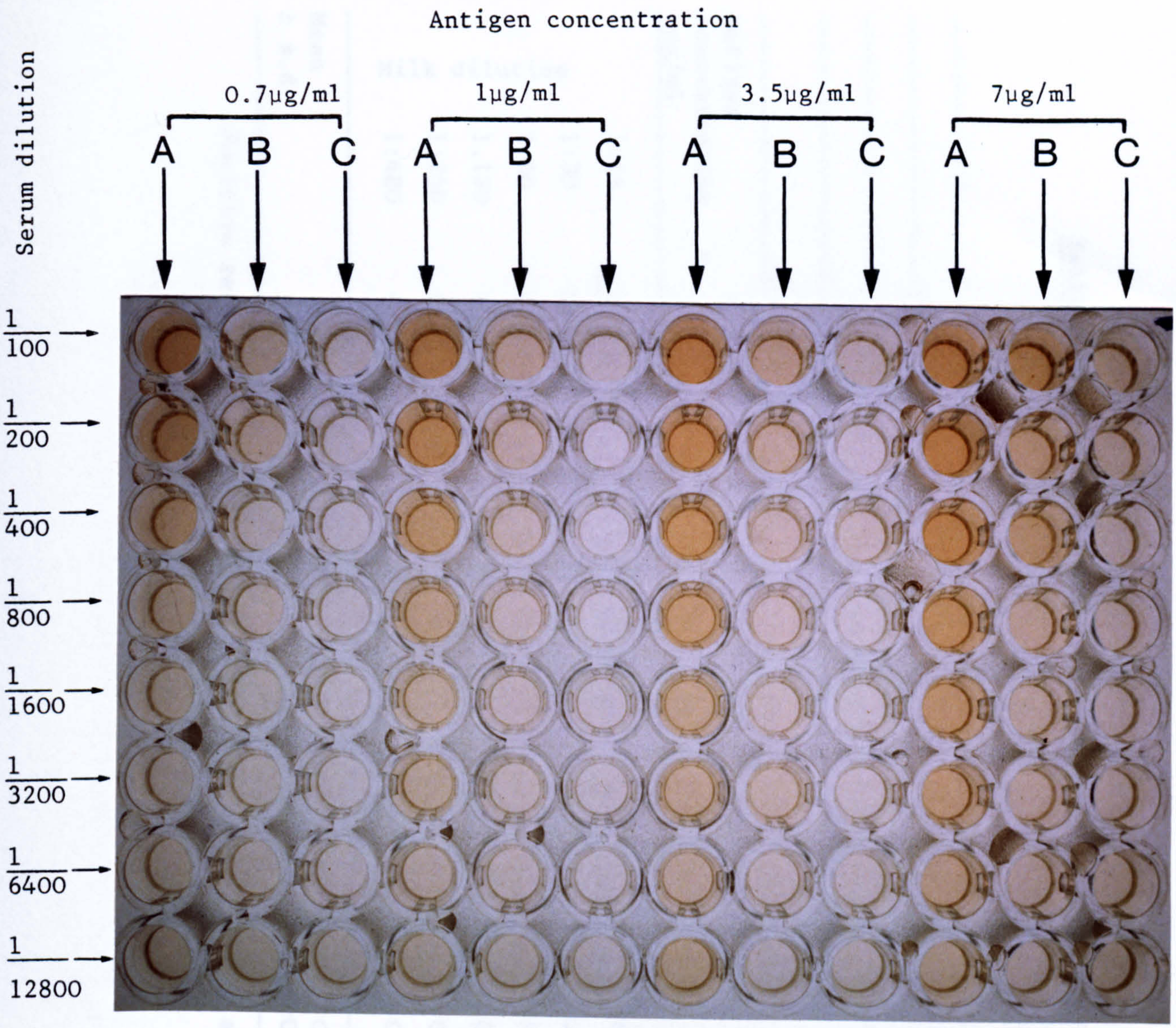


Plate 4.1

Microelisa. T. spiralis larval antigen, NIH mice - SERA. IgG antibody levels in primary and secondary infection sera using different concentration of larval antigen.

A: Serum from infected mice (47 days after secondary infection)

B: Serum from infected mice (26 days after primary infection)

C: Serum from uninfected (naive) mice.

Table 4.2. Microelisa. T. spiralis larval antigen. NIH mice - MILK. IgG antibody levels in infected mice - early and mid-lactation, using different concentrations of larval antigen.

Optical Density (O.D.) value at 492 nm													
Stage of Lactation													
		Early lactation						Mid lactation					
		pooled immune milk (6 mice)			pooled naive milk (6 mice)			pooled immune milk (6 mice)			pooled naive milk (6 mice)		
Antigen concentration µg/ml →		2	5	10	2	5	10	2	5	10	2	5	10
Milk dilution	1:15	0.311	0.389	0.393	0.113	0.145	0.136	0.367	0.570	0.500	0.102	0.137	0.136
	1:30	0.193	0.262	0.234	0.145	0.136	0.136	0.223	0.266	0.277	0.114	0.140	0.132
	1:60	0.159	0.201	0.207	0.115	0.139	0.149	0.181	0.219	0.225	0.121	0.136	0.155
	1.120	0.128	0.181	0.174	0.122	0.130	0.143	0.158	0.183	0.206	0.107	0.143	0.145
	1:240	0.126	0.167	0.152	0.161	0.163	0.178	0.150	0.178	0.181	0.138	0.132	0.143
	1:480	0.119	0.155	0.152	0.188	0.152	0.178	0.113	0.157	0.180	0.128	0.174	0.179
Mean ± s.d.					0.140 ±0.029	0.144 ±0.011	0.153 ±0.019				0.118 ±0.013	0.143 ±0.015	0.148 ±0.016

Positive reactions outlined in heavy columns; positivity - any reading ≥ twice the mean of the naive control.

FIG.4.1

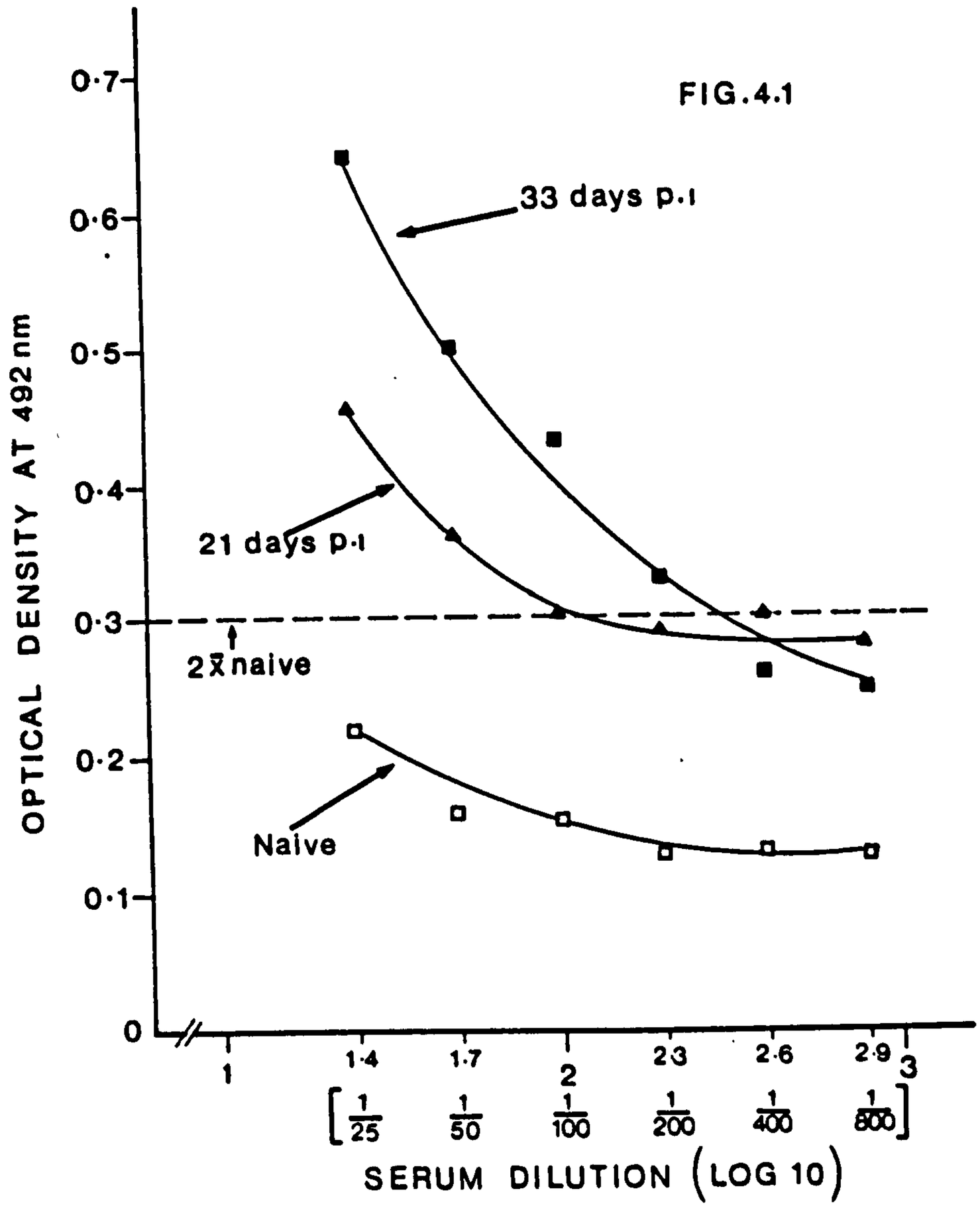


Figure 4.1.

Microelisa. T. spiralis larval antigen.

NIH mice - SERA.

IgG antibody levels in primary infections.

Sera pooled from 6 mice.

FIG 4.2

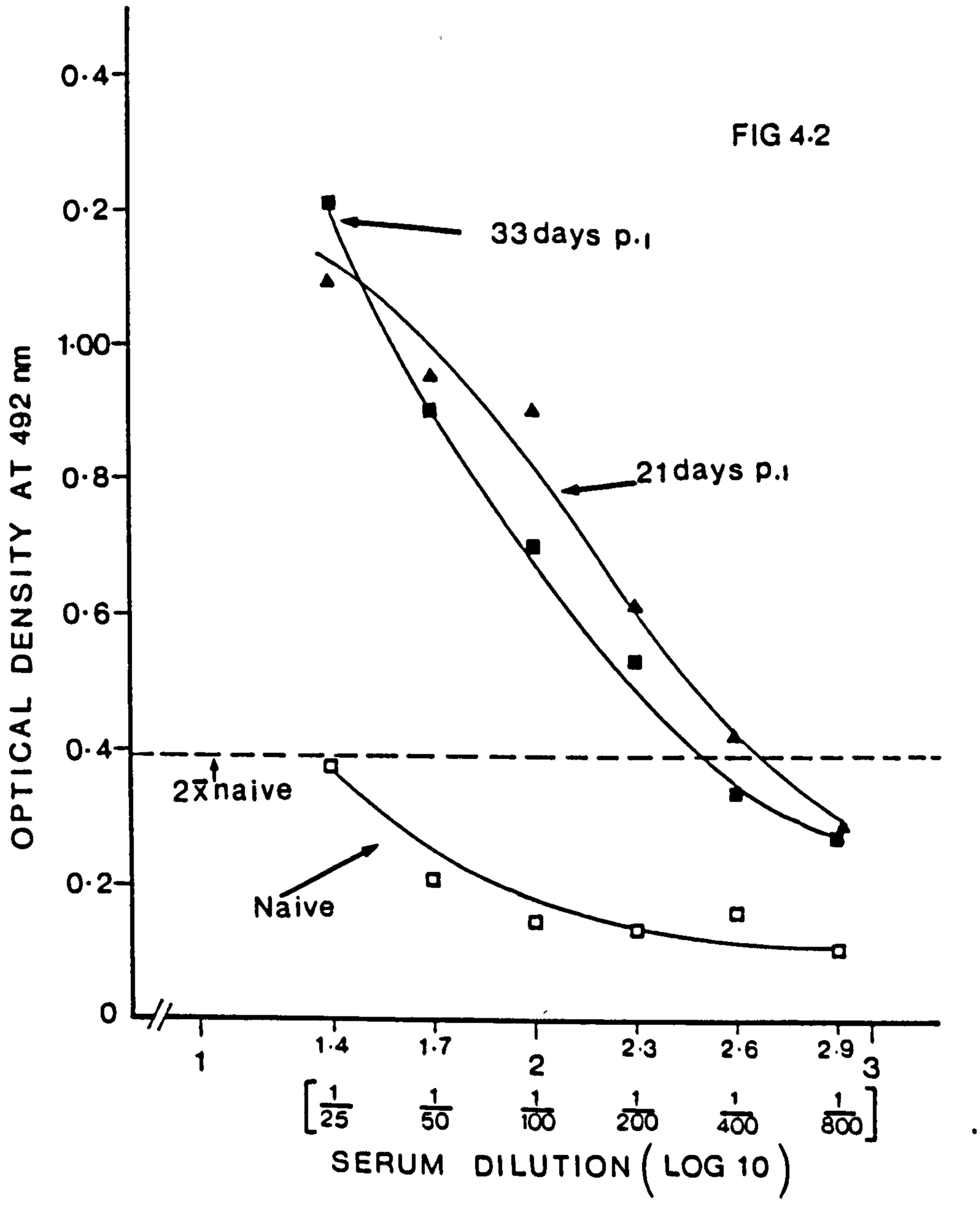


Figure 4.2.

Microelisa. T. spiralis larval antigen,

NIH mice - SERA

IgM antibody levels in primary infections.

Sera pooled from 6 mice.

Table 4.3. Microelisa. T. spiralis larval antigen, NIH mice - SERA.
IgG antibody levels in primary and secondary infections.

	Optical Density (O.D.) value at 492 nm												
pooled sera (6 mice)	Days post-infection											Control Sera	
dilution	5	10	15	18	21	24	27	30	33	36	40	47 p.i sec.	naive
1:25	0.220	0.336	0.364	0.398	0.458	0.490	0.666	0.572	0.643	0.841	0.800	1.296	0.221
1:50	0.171	0.316	0.344	0.382	0.360	0.341	0.437	0.457	0.502	0.744	0.829	0.917	0.158
1:100	0.196	0.207	0.204	0.267	0.302	0.366	0.381	0.486	0.439	0.499	0.508	0.645	0.156
A 1:200	0.178	0.221	0.177	0.293	0.296	0.301	0.455	0.438	0.332	0.437	0.429	0.430	0.127
1:400	0.218	0.178	0.154	0.285	0.300	0.323	0.334	0.314	0.268	0.346	0.429	0.306	0.126
1:800	0.136	0.154	0.170	0.223	0.282	0.239	0.231	0.238	0.255	0.356	0.408	0.262	0.122
Mean ±s.d													0.151 ±0.037
1:100	0.067	0.128	0.161	0.178	0.213	0.262	0.193	0.254	0.283	0.440	0.369	0.740	0.077
1:200	0.056	0.071	0.115	0.137	0.174	0.180	0.152	0.189	0.202	0.280	0.277	0.618	0.082
B 1:400	0.047	0.067	0.105	0.110	0.157	0.166	0.129	0.154	0.145	0.239	0.202	0.425	0.062
1:800	0.044	0.049	0.091	0.113	0.153	0.138	0.109	0.131	0.082	0.142	0.157	0.320	0.068
1:1600	0.052	0.050	0.087	0.104	0.120	0.121	0.081	0.103	0.097	0.004	0.119	0.235	0.066
1:3200	0.049	0.046	0.069	0.102	0.113	0.101	0.097	0.094	0.093	0.074	0.094	0.184	0.103
Mean ±s.d													0.076 ±0.015

Positive reactions outlined in heavy columns.

Table 4.4. Microelisa. T. spiralis larval antigen, NIH mice - SERA.
IgM antibody levels in primary and secondary infections.

		Optical Density (O.D.) value at 492 nm												
pooled sera (6 mice)		Days post-infection											control sera	
	dilution	5	10	15	18	21	24	27	30	33	36	40	47 p.i.sec.	Naive
A	1:25	0.630	0.703	1.082	1.298	1.099	1.101	1.080	1.157	1.211	1.155	1.244	0.730	0.393
	1:50	0.385	0.306	1.044	0.857	0.952	0.856	0.936	0.899	0.892	0.935	1.066	0.395	0.210
	1:100	0.304	0.239	0.851	0.835	0.900	0.663	0.810	0.838	0.698	0.795	0.853	0.254	0.152
	1:200	0.354	0.134	0.666	0.678	0.613	0.476	0.658	0.620	0.531	0.542	0.638	0.211	0.143
	1:400	0.119	0.101	0.390	0.422	0.424	0.354	0.451	0.441	0.346	0.332	0.386	0.166	0.166
	1:800	0.203	0.145	0.263	0.244	0.282	0.219	0.275	0.320	0.287	0.192	0.284	0.157	0.110
	Mean ±s.d													0.195 ±0.102
B	1:100	0.283	0.336	0.842	0.903	0.697	0.692	0.654	0.771	NOT DONE			0.487	0.221
	1:200	0.155	0.179	0.537	0.725	0.673	0.543	0.436	0.517				0.205	0.109
	1:400	0.173	0.145	0.408	0.558	0.598	0.450	0.317	0.354				0.228	0.159
	1:800	0.164	0.193	0.240	0.311	0.328	0.257	0.216	0.285				0.190	0.116
	1:1600	0.141	0.170	0.222	0.276	0.270	0.266	0.243	0.267				0.192	0.116
	1:3200	0.184	0.156	0.214	0.248	0.215	0.263	0.275	0.207				0.258	0.117
	Mean ±s.d.													0.139 ±0.043

Positive reactions outlined in heavy columns.

Table 4.5. Microelisa. T. spiralis larval antigen. NIH mice - SERA. IgA antibody levels in primary and secondary infections.

Optical Density (O.D.) value at 492 nm

Adult sera

	Infected						Naive
	Days post-infection						
	dilution	18	21	30	33	40	46	
A	1:10	0.345	0.343	0.345	0.355	0.333	0.379	0.323
	1:20	0.336	0.330	0.337	0.350	0.352	0.370	0.290
	1:40	0.326	0.316	0.323	0.335	0.336	0.363	0.291
	1:80	0.310	0.292	0.310	0.305	0.312	0.315	0.277
	1.160	0.293	0.278	0.279	0.256	0.310	0.283	0.227
	1:320	0.256	0.243	0.244	0.234	0.279	0.246	0.209
	Mean ±s.d.							0.269 ±0.043

	Infected				Naive
	Days post-infection				
	dilution	10	21	28	26 p.i.sec.	
B	1:10	0.421	0.521	0.521	0.506	0.367
	1:20	0.416	0.518	0.493	0.502	0.323
	1:40	0.384	0.483	0.469	0.469	0.309
	1:80	0.349	0.446	0.458	0.442	0.288
	1:160	0.308	0.418	0.415	0.393	0.287
	1:320	0.271	0.368	0.373	0.347	0.295
	Mean ±s.d.					0.312 ±0.03

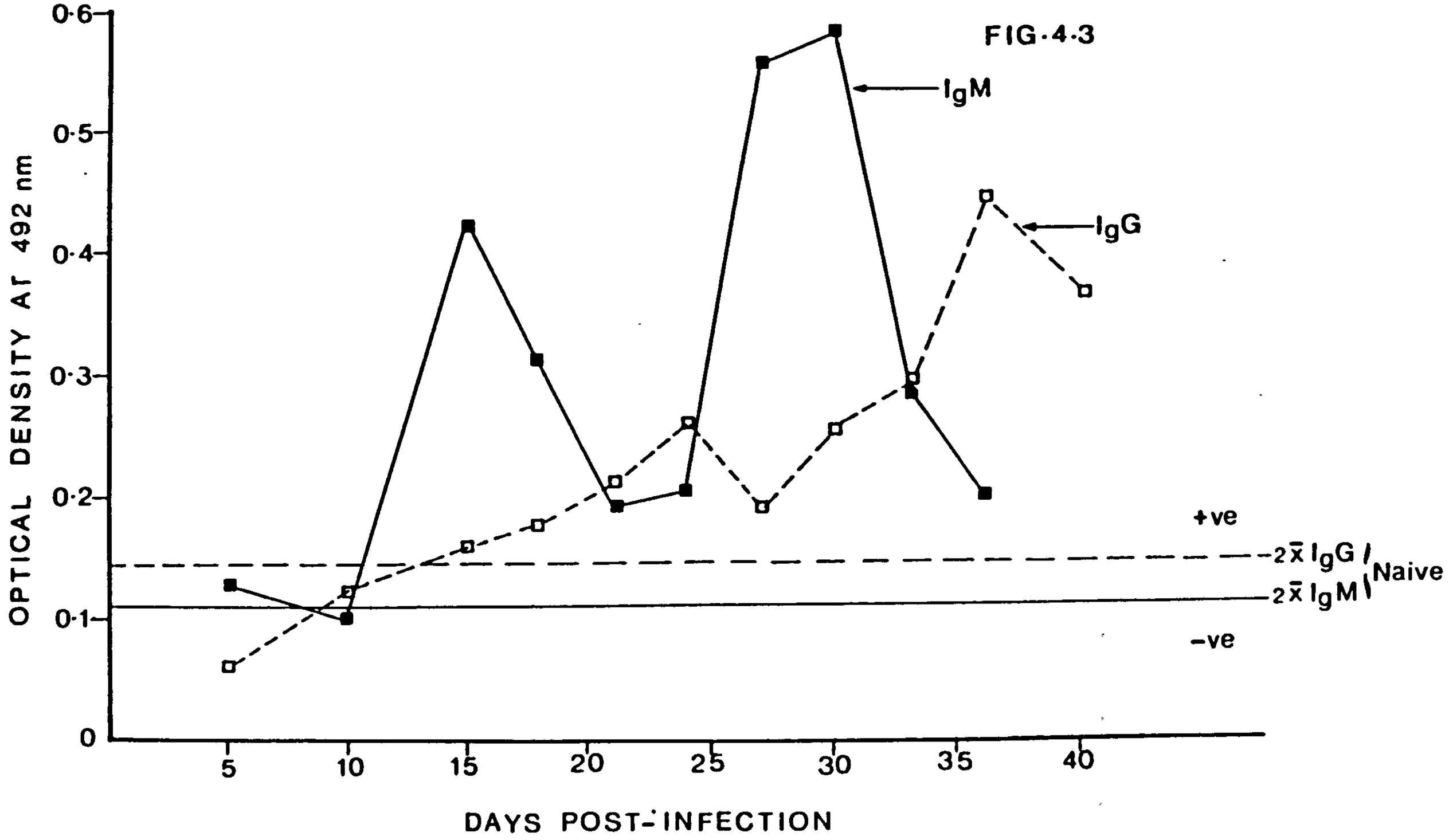


Figure 4.3. Microelisa. T. spiralis larval antigen, NIH mice - SERA primary infections, IgG and IgM antibodies. OD readings of 1/100 dilution samples.

FIG. 4.4

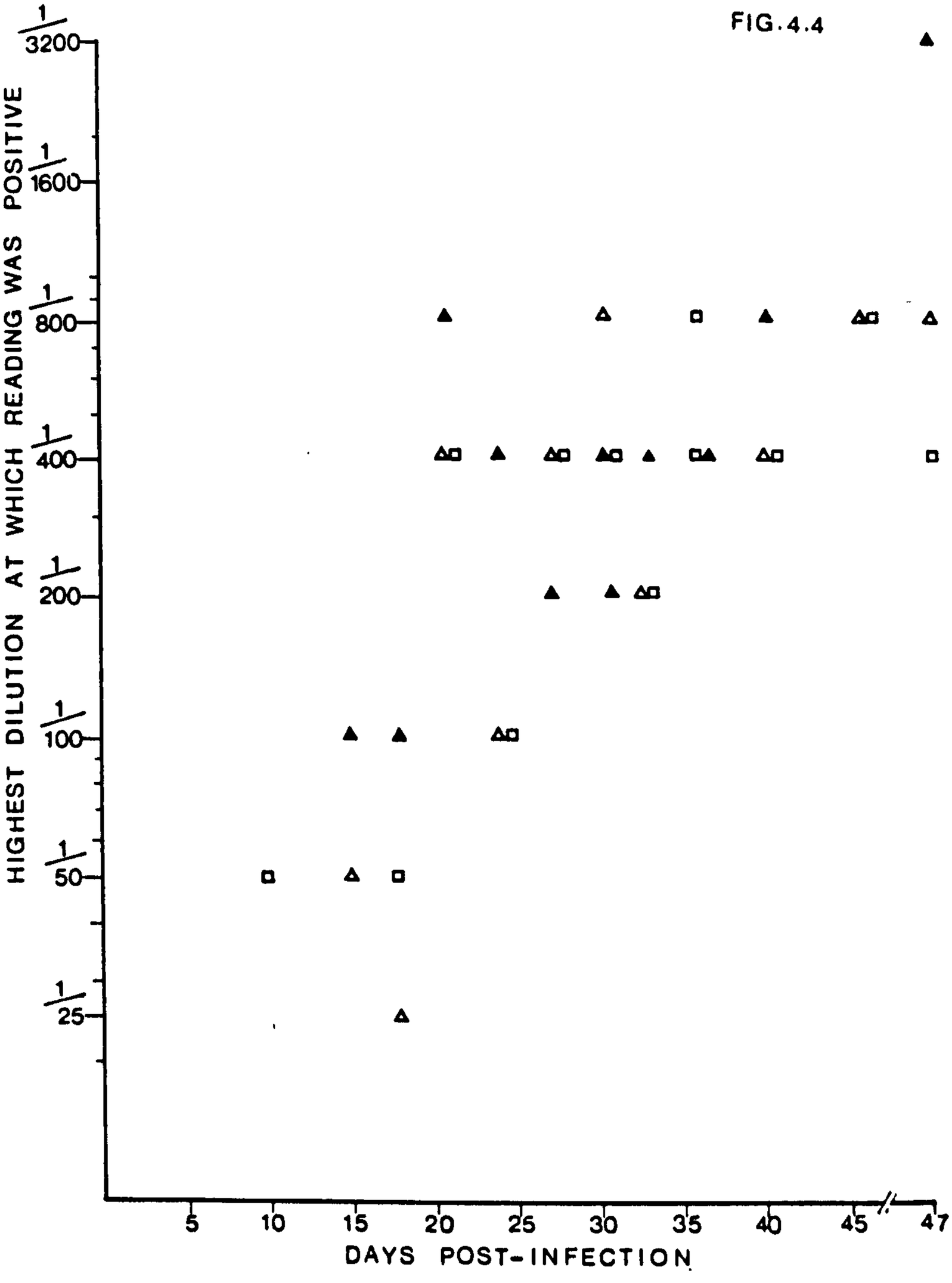
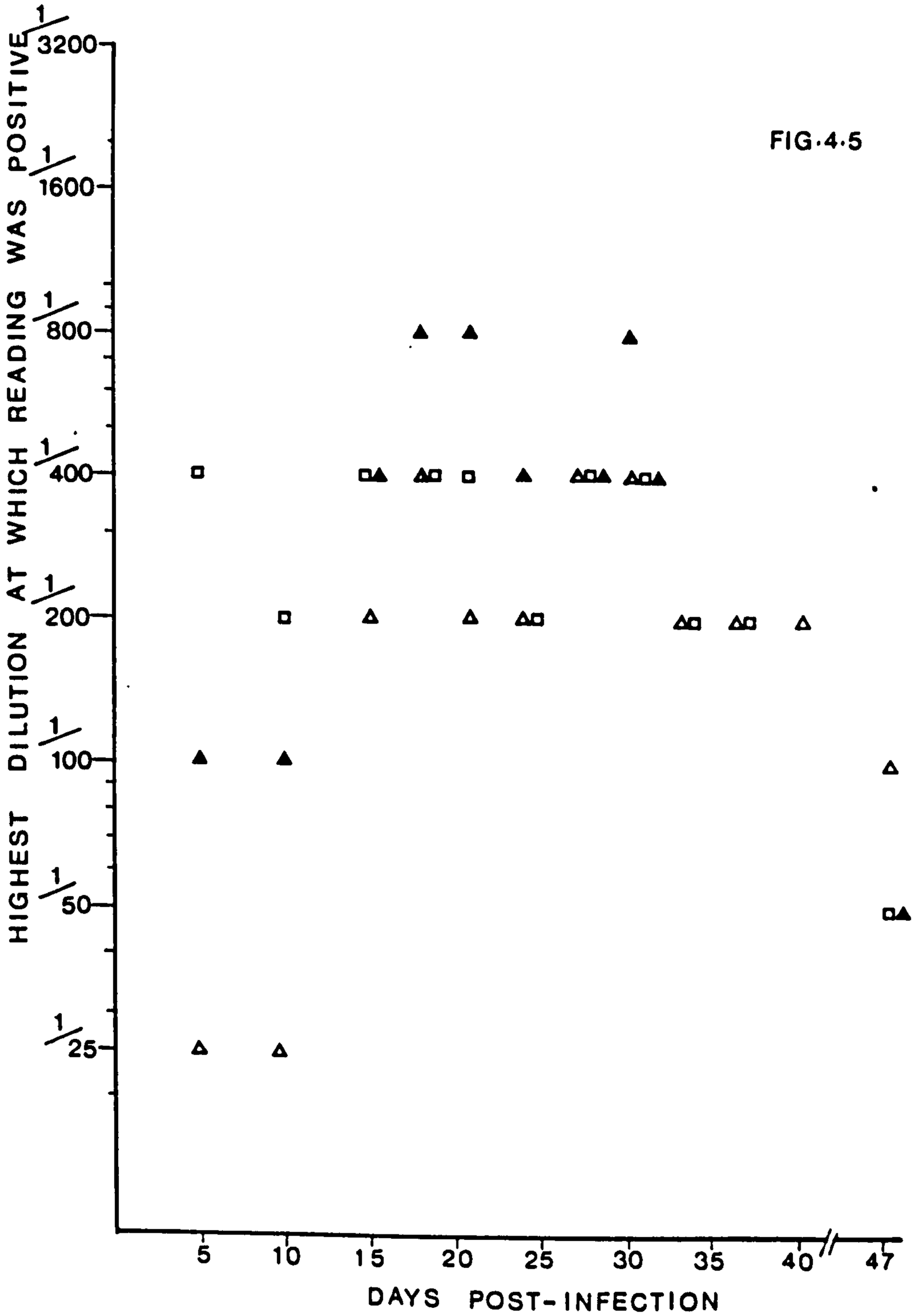


Figure 4.4.
Microelisa. T. spiralis larval antigen. NIH mice - SERA. IgG antibody levels in three runs of serum samples, from primary and secondary infections. Two readings only for day 46 primary P.I. Secondary infection at day 47 P.I after challenge infection.



FIG·4·5

Figure 4.5

Microelisa. T.spiralis larval antigen, NIH mice - SERA - IgM antibody levels in three runs of serum samples from primary and secondary infections. Two readings only for day 33 & 36 primary P.I, and one reading for day 40 primary P.I. Secondary infection at day 47 P.I after challenge infection.

Table 4.6: Microelisa. T. spiralis larval antigen. NIH mice - SERA. IgG, IgM and IgA antibody levels in infected lactating mice.

	Optical Density (O.D.) value at 492 nm					
	Stage of lactation "mid-lactation"					
	pooled sera (6 mice)	dilution	35-40 days post secondary infections	Naive	dilution	Primary infections of 16-17 days	Naive
IgG		1:25	0.324	0.098	1:25	0.340	0.119
		1:50	0.244	0.090	1:50	0.222	0.119
		1:100	0.196	0.097	1:100	0.210	0.118
		1:200	0.207	0.090	1:200	0.188	0.107
		1:400	0.165	0.076	1:400	0.169	0.114
		1:800	0.142	0.085	1:800	0.164	0.114
	Mean ±s.d.			0.089 ±0.008			0.115 ±0.004
IgM		1:25	0.280	0.176	1:25	0.309	0.166
		1:50	0.211	0.128	1:50	0.264	0.145
		1:100	0.177	0.113	1:100	0.254	0.140
		1:200	0.161	0.093	1:200	0.227	0.118
		1:400	0.131	0.094	1:400	0.192	0.118
		1:800	0.114	0.103	1:800	0.172	0.117
	Mean ±s.d.			0.117 ±0.031			0.125 ±0.013
		Stage of lactation					
IgA		dilution	Primary infection of 11 days	Naive	dilution	Primary infection of 40-45 days	Naive
		1:10	0.383	0.338	1:10	0.398	0.289
		1:20	0.368	0.309	1:20	0.354	0.230
		1:40	0.350	0.292	1:40	0.349	0.230
		1:80	0.345	0.275	1:80	0.344	0.212
		1.160	0.308	0.227	1:160	0.301	0.171
		1:320	0.277	0.207	1:320	0.288	0.174
	Mean ±s.d.			0.274 ±0.049			0.218 ±0.042

positive reactions outlined in heavy columns.

Table 4.7. Microelisa. T. spiralis larval antigen. NIH mice - SERA. IgG and IgM antibody levels after primary and secondary immunization with T. spiralis larval antigen + Freund's complete adjuvant.

	Sera dilution	Optical Density (O.D.) Value at 492 nm — Days Post-immunization: Primary 5	Primary 10	Primary 20	Primary 27	Secondary 19 Late pregnancy	Secondary 26 Early lactation	Control Sera 47 p.i. sec.	Naive
IgG	1:25	0.180	0.455	0.736	0.898	1.350	1.168	1.097	0.218
	1:50	0.139	0.210	0.672	0.725	1.278	1.022	0.934	0.148
	1:100	0.149	0.215	0.511	0.509	1.124	0.802	0.644	0.102
	1:200	0.114	0.197	0.426	0.477	1.013	0.665	0.447	0.203
	1:400	0.099	0.134	0.275	0.329	0.928	0.607	0.348	0.164
	1:800	0.129	0.114	0.251	0.210	0.744	0.447	0.226	0.149
	Mean ±s.d.								0.164 ±0.041
IgM	1:25	0.770	1.004	0.768	0.783	0.959	0.964	0.643	0.422
	1:50	0.442	0.753	0.535	0.483	0.796	0.773	0.444	0.333
	1:100	0.280	0.560	0.303	0.304	0.567	0.448	0.299	0.159
	1:200	0.230	0.373	0.217	0.186	0.423	0.337	0.262	0.113
	1:400	0.153	0.219	0.151	0.143	0.302	0.235	0.197	0.109
	1:800	0.185	0.158	0.150	0.121	0.250	0.167	0.176	0.142
	Mean ±s.d.								0.213 ±0.131

Positive reactions outlined in heavy columns.

Table 4.8 Microelisa. T. spiralis larval antigen, NIH mice - MILK. IgG, IgM and IgA antibodies at different lactation periods. Milk pooled from 6 mice.

	Optical Density (O.D.) value at 492 nm								
	dilu-tion	Early Lactation		dilu-tion	Mid Lactation		dilu-tion	Late Lactation	
		immune	Naive		immune	Naive		immune	Naive
IgG	1:15	0.552	0.119		0.687	0.146		0.877	0.145
	1:30	0.293	0.109		0.341	0.132		0.443	0.132
	1:60	0.223	0.127		0.227	0.128		0.336	0.144
	1:120	0.171	0.120		0.224	0.164		0.249	0.130
	1:240	0.194	0.118		0.117	0.153		0.204	0.178
	1:480	0.128	0.160		0.151	0.132		0.153	0.131
	Mean ±s.d.		0.125 ±0.017			0.142 ±0.014			0.143 ±0.018
IgM	1:15	0.217	0.161		0.310	0.154		0.348	0.223
	1:30	0.189	0.121		0.217	0.159		0.199	0.172
	1:60	0.177	0.111		0.190	0.179		0.182	0.147
	1:120	0.134	0.120		0.164	0.196		0.300	0.123
	1:240	0.170	0.136		0.249	0.145		0.171	0.167
	1:480	0.128	0.128		0.204	0.170		0.141	0.132
	Mean ±s.d.		0.129 ±0.017			0.167 ±0.018			0.160 ±0.036
IgA	1:10	0.294	0.187	1:15	0.290	0.179	1:10	0.306	0.250
	1:20	0.275	0.187	1:30	0.210	0.155	1:20	0.271	0.254
	1:40	0.287	0.168	1:60	0.163	0.144	1:40	0.245	0.241
	1:80	0.286	0.181	1:120	0.153	0.106	1:800	0.249	0.250
	1:160	0.254	0.169	1:240	0.157	0.111	1.160	0.242	0.233
	1:320	0.226	0.162	1.480	0.139	0.105	1:320	0.216	0.181
	Mean ±s.d.		0.175 ±0.010			0.133 ±0.03			0.234 ±0.027

Positive reactions outlined in heavy columns.

Table 4.9.

Microelisa. T. spiralis larval antigen. NIH mice MILK. Determination of IgG, IgM and IgA antibodies in T. spiralis infected mice.

Anti-body	*Milk batch	Stage of lactation: Colostrum	Early - Lactation	Mid - Lactation	Late- Lactation
IgG	1	$\frac{1}{15}$	$\frac{1}{15}$ - $\frac{1}{50}$	$\frac{1}{15}$ - $\frac{1}{50}$	$\frac{1}{15}$
	2	N.D.	$\frac{1}{30}$	$\frac{1}{30}$	$\frac{1}{60}$
	3	N.D.	$\frac{1}{64}$ - $\frac{1}{120}$	$\frac{1}{64}$ - $\frac{1}{120}$	$\frac{1}{64}$ - $\frac{1}{120}$
IgM	1	- ($\frac{1}{15}$)	- ($\frac{1}{15}$)	- ($\frac{1}{15}$)	- ($\frac{1}{15}$)
	2	N.D.	- ($\frac{1}{15}$)	-($\frac{1}{15}$)	+ $\frac{1}{15}$
	3	N.D.	- ($\frac{1}{15}$)	$\frac{1}{15}$	- ($\frac{1}{15}$)
IgA	1	N.D.	- ($\frac{1}{10}$)	$\frac{1}{15}$	- ($\frac{1}{10}$)
	2	N.D.	- ($\frac{1}{10}$)	- ($\frac{1}{10}$)	- ($\frac{1}{10}$)

- ($\frac{1}{x}$) not detectable at lowest starting dilution.

*Each batch of milk consisted of a pool taken from 6 different lactating mice.

N.D. = not done.

Table 4.10: Microelisa. T. spiralis larval antigen. NIH mice MILK IN INFANTS. IgG, IgM and IgA antibodies. Batches of milk collected from the stomachs of 5-6 infants at different lactation periods. Mothers were given a primary infection of 400 T. spiralis larvae, followed after 4 weeks by another infection of 200 larvae. The last infection was given one week prior to mating.

Positive reactions outlined in heavy columns

	Optical density (O.D.) value at 492 nm					
	Stage of lactation					
	Early-lactation			Mid-lactation		
	dilution	immune	naive	dilution	immune	naive
IgG	1:5	0.576	0.157	1:15	0.473	0.100
	1:10	0.316	0.122	1:30	0.217	0.106
	1:20	0.286	0.121	1:60	0.134	0.121
	1:40	0.226	0.108	1:120	0.136	0.102
	1:80	0.196	0.116	1:240	0.117	0.104
	1:160	0.148	0.127	1:480	0.153	0.113
Mean ±s.d.			0.125 ±0.016			0.107 ±0.007
IgM	1:5	0.391	0.137	1:15	0.246	0.140
	1:10	0.217	0.214	1:30	0.193	0.106
	1:20	0.219	0.138	1:60	0.217	0.113
	1:40	0.228	0.122	1:120	0.167	0.124
	1:80	0.306	0.159	1:240	0.184	0.130
	1:160	0.279	0.165	1:480	0.149	0.095
Mean ±s.d.			0.155 ±0.032			0.118 ±0.016
	Mid-lactation			Late-lactation		
IgA	1:5	0.313	0.109	1:5	0.211	0.210
	1:10	0.229	0.103	1:10	0.210	0.216
	1:20	0.217	0.105	1:20	0.205	0.229
	1:40	0.178	0.095	1:40	0.204	0.218
	1:80	0.137	0.102	1:80	0.198	0.219
	1:160	0.096	0.145	1:160	0.182	0.215
Mean ±s.d.			0.109 ±0.017			0.217 ±0.006

Average volume of milk recovered from the stomachs of two week old infants was 0.2 ml.

Table 4.11: Microelisa. T. spiralis larval antigen - NIH mice. Milk in infants. Determination of IgG, IgM and IgA antibodies in the milk recovered from the stomachs of groups of 5-6 infants at different lactation periods.

Antibody	Milk from different groups of infants	Stage of lactation		
		Early - lactation	Mid - lactation	Late - lactation
IgG	1	$-(\frac{1}{15})$	$\frac{1}{30}$	$-(\frac{1}{15})$
	2	$\frac{1}{20}$	N.D.	$\frac{1}{30}$
	3	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$
IgM	1	$-(\frac{1}{15})$	$-(\frac{1}{15})$	$-(\frac{1}{15})$
	2	$\frac{1}{5}$	$\frac{1}{15}$	N.D.
	3	$\frac{1}{5}$	N.D.	$\frac{1}{30}$
IgA	1	N.D.	$\frac{1}{10}$	$-(\frac{1}{5})$
	2	N.D.	$-(\frac{1}{10})$	$-(\frac{1}{10})$

$-(\frac{1}{x})$ not detectable at lowest starting dilution.

N.D. = not done.

FIG.4.6

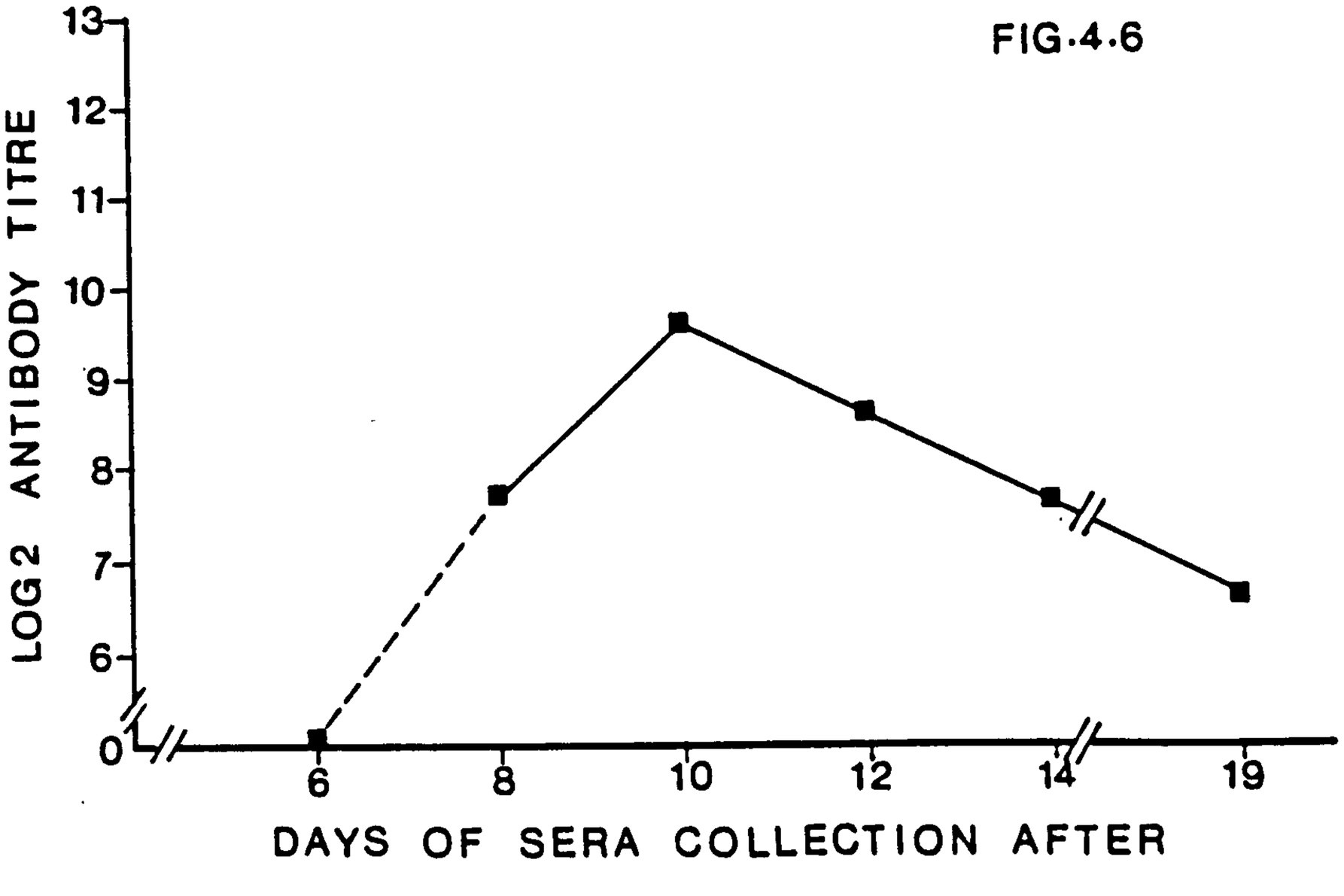


Figure 4.6

Microelisa. T. spiralis larval antigen.

NIH Mice - SERA of NAIVE INFANTS suckling infected/immune mothers.

IgG antibody.

Negative control - 6 day old or 12 day old naive infants.

Infected/immune mothers: a primary infection of 400 T. spiralis larvae followed after 4 weeks by a secondary infection of 200 larvae. The last infection was given a week prior to mating.

FIG.4.7

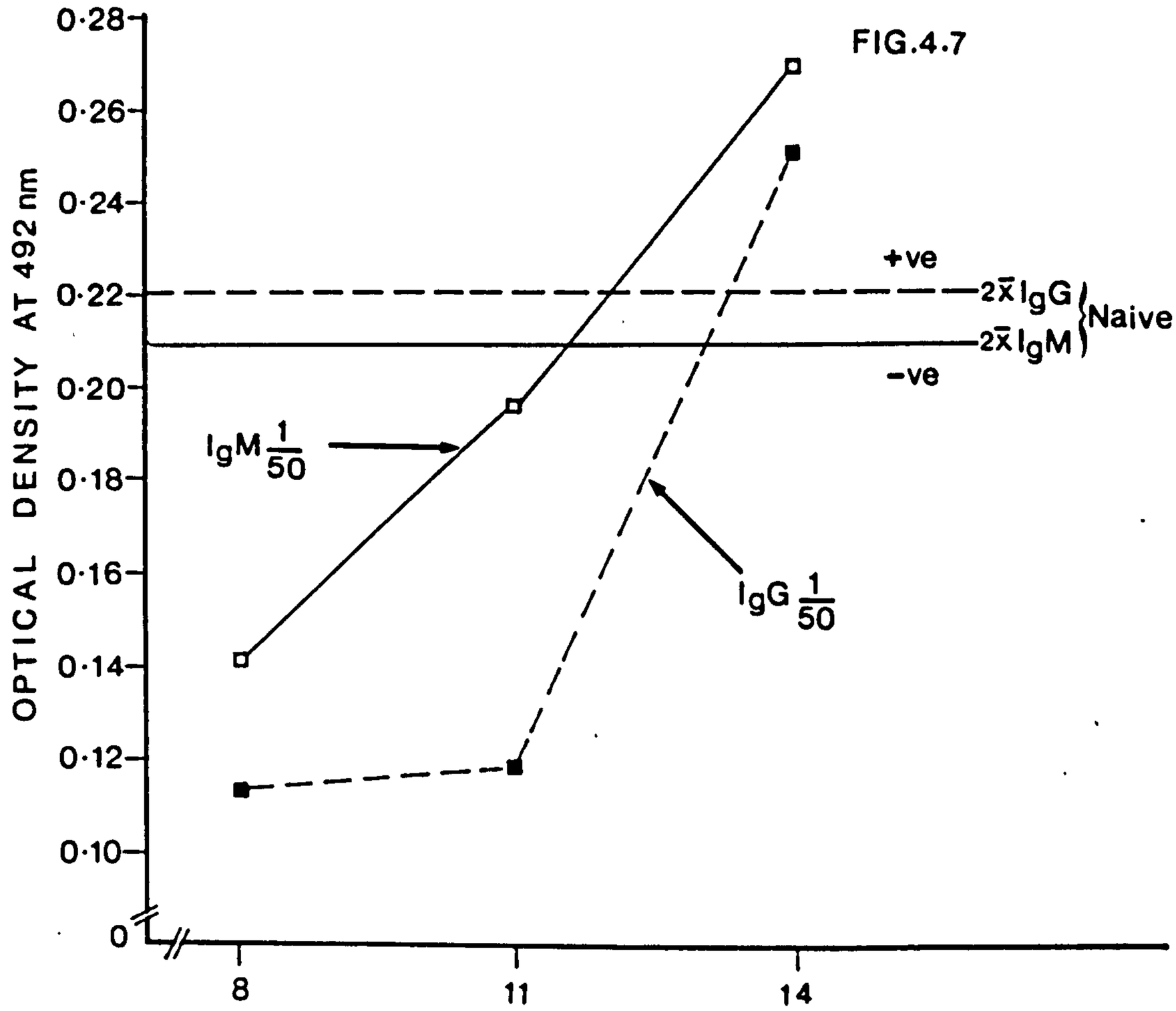


Figure 4.7

Microelisa T.spiralis larval antigen NIH mice. SERA of INFECTED INFANTS. IgG and IgM antibodies. O.D. readings of 1/50 dilution samples. Infants infected at 1 week of age with 100 T.spiralis larvae. Sera recovered from groups of 5-6 infants.

Table 4.12: IgG, IgM and IgA antibodies in adult and infant mice. Highest titres at which positivity was observed.

Sera/Milk		Antibody		
		IgG	IgM	IgA
SERA from adult mice with infection	Primary	1:800	1:800	-(1:10)
	Secondary	1:3200	1:100	
SERA from infected lactating mice	Primary	1:25	1:100	-(1:10)
	Secondary	1:200	1:25	-(1:10)
MILK from infected lactating mice	Primary (17 days)	1:30	1:15	-(1:15)
	Secondary	1:120	1:15	1:15
MILK from stomachs of infant mice suckling infected/ immune mothers		1:100	1:30	1:10
SERA from 10 day old infant mice suckling infected/immune mothers		1:800	-(1:25)	-(1:10)
SERA from infant mice infected at one week of age, and suckling naive mothers. Sera collected on day 14 post-infection		1:50	1:200	-(1:10)

- (1:x); not detectable at lowest starting dilution

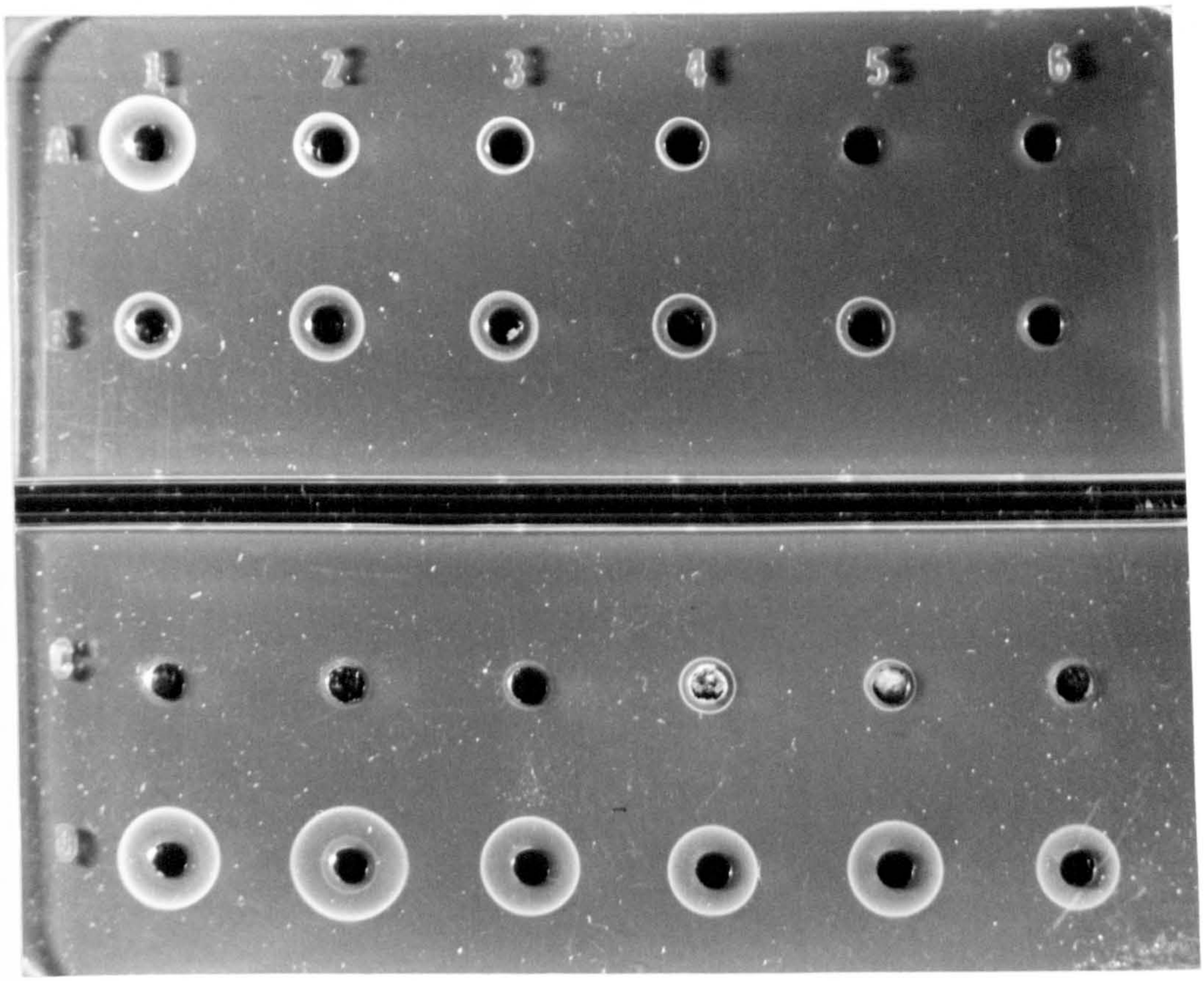


Plate 4.2: Radial immunodiffusion plate (R.I.D.) for total IgA immunoglobulins in milk and sera.

FIG 4·8

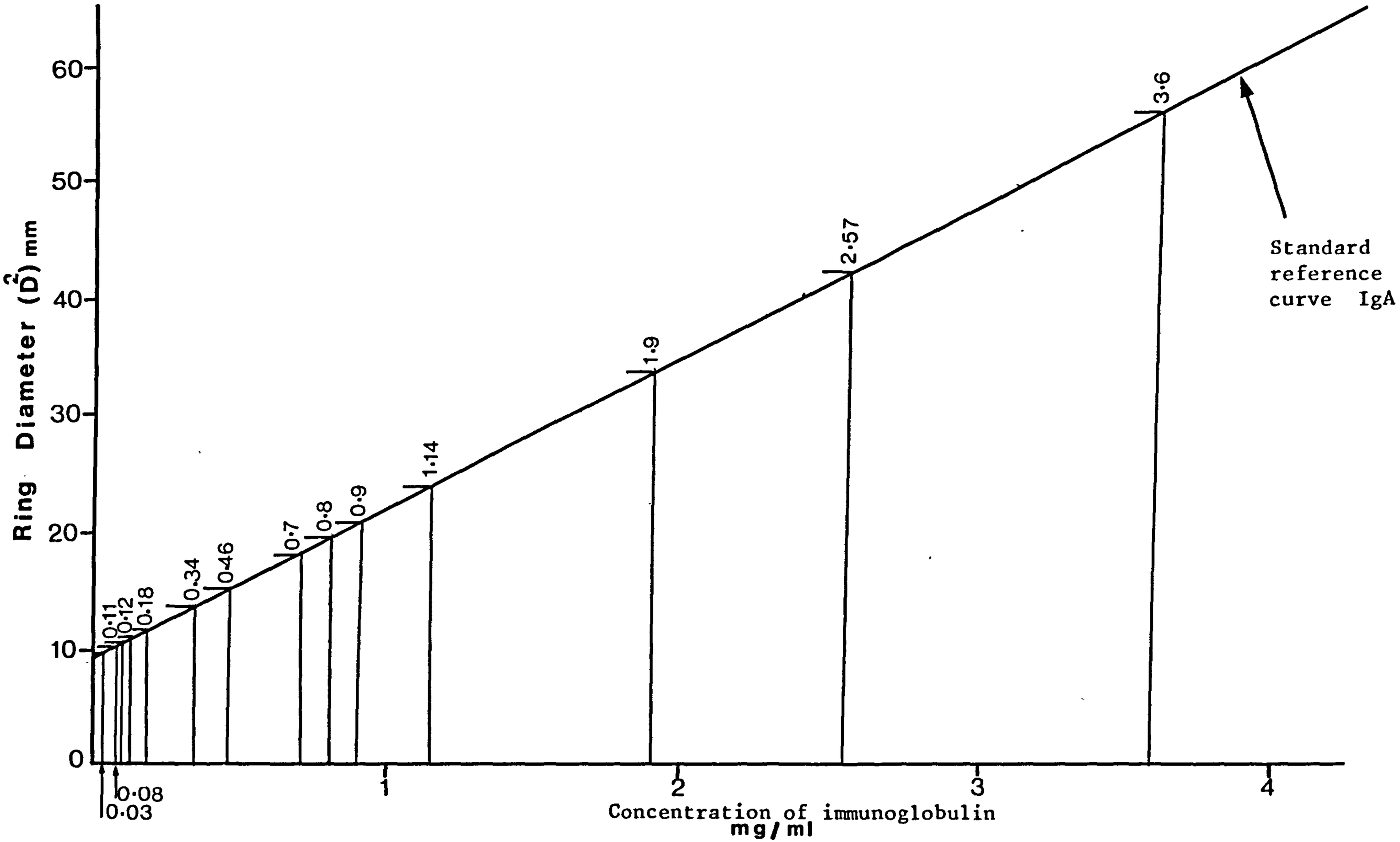


Figure 4.8: Radial immunodiffusion (R.I.D.) IgA immunoglobulin concentrations in milk and sera.

FIG 4.9

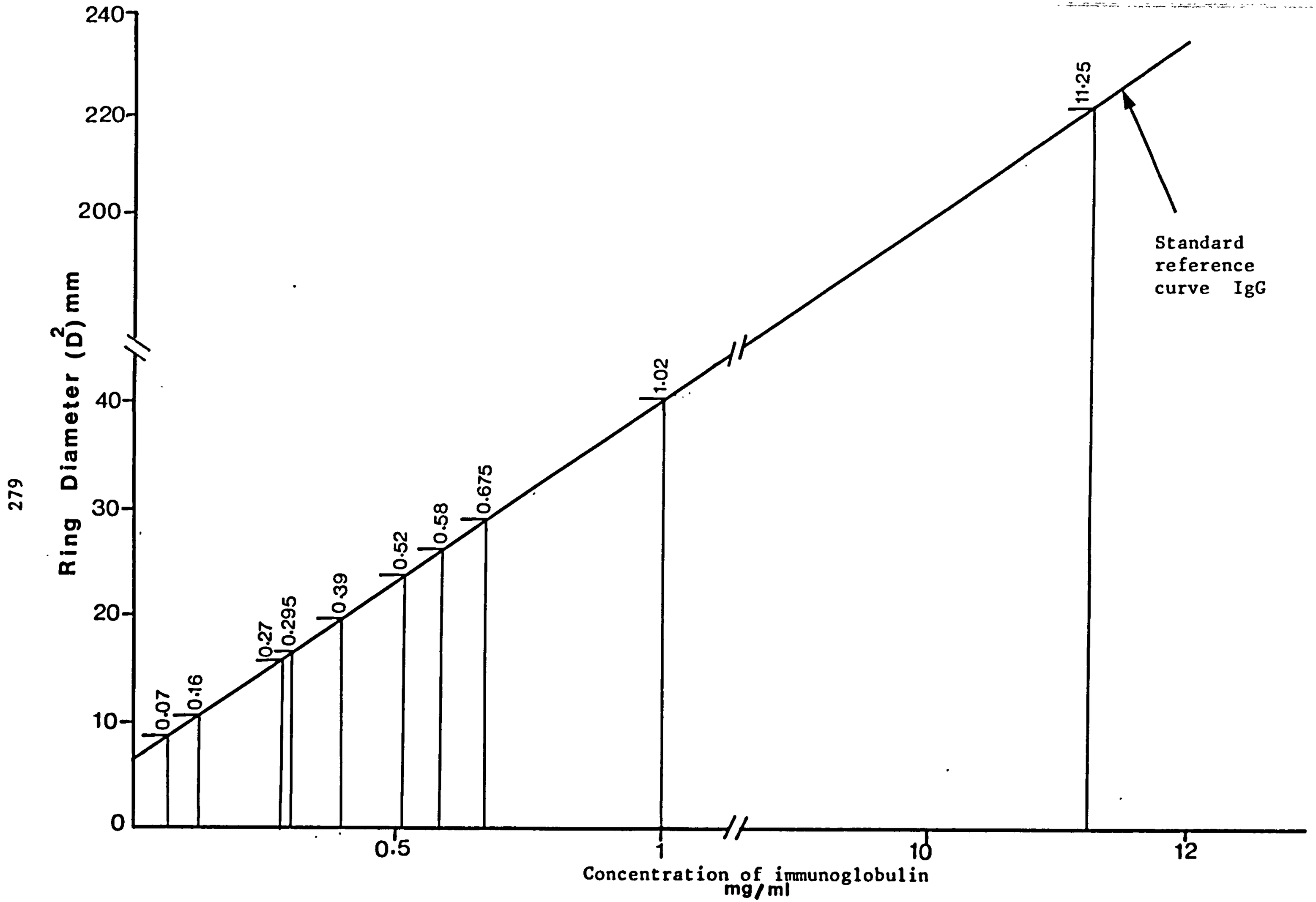


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Figure 4.9: Radial immunodiffusion (R.I.D.) IgG immunoglobulin concentrations in milk and sera.

FIG 4.10

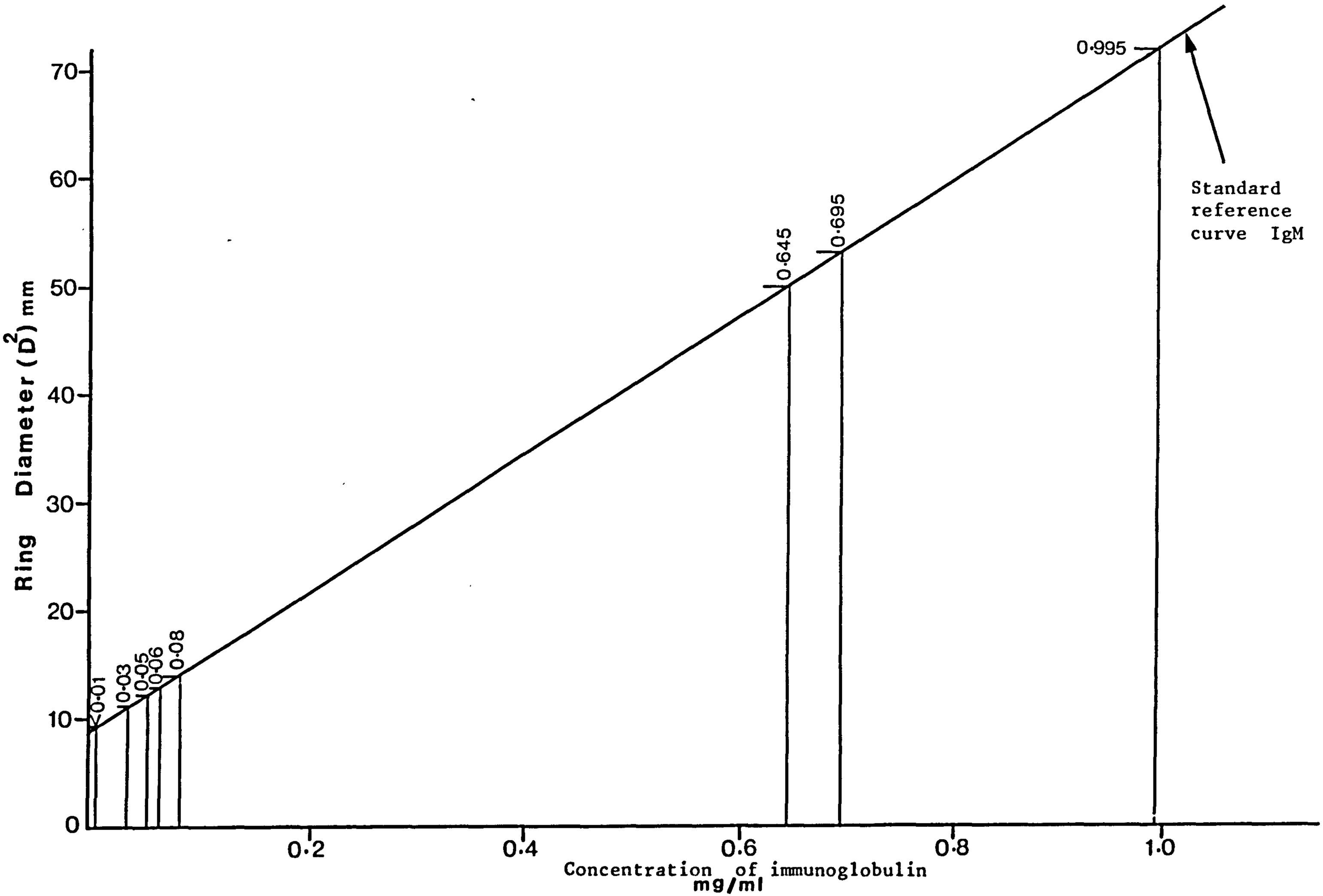


Figure 4.10: Radial immunodiffusion (R.I.D) IgM immunoglobulin concentrations in milk and sera.

Table 4.13: Radial immunodiffusion (R.I.D.). Concentration of IgA, IgG and IgM in maternal milk, in milk collected from infant stomachs during mid-lactation period, and in sera from naive and 21 day infected NIH mice.

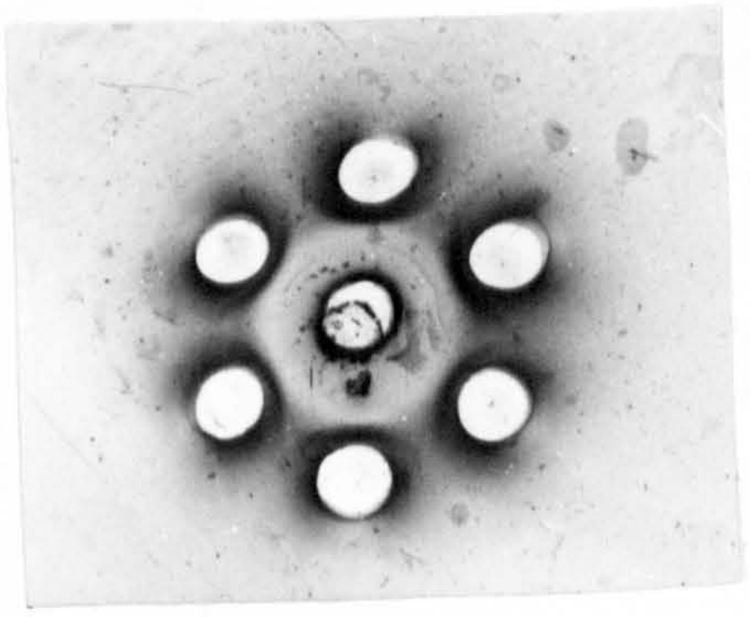
For milk - mean ± s.d.

Milk/sera	Mice	Immunoglobulin concentration mg/ml		
		IgA	IgG	IgM
Maternal Milk	Naive	0.29 ±0.14 (n=5)	0.32 ±0.24 (n=4)	0.02 ±0.014 (n=2)
	Infected/ immune	0.88 ±0.23 (n=5)	0.629 ±0.304 (n=4)	0.067 ±0.015 (n=4)
Milk from infant stomachs	Naive	0.02 ±0.049 (n=5)	0.09 ±0.15 (n=3)	0 (n=2)
	Infected/ immune	0.11 ±0.04 (n=7)	0.336 ±0.218 (n=3)	0.07 ±0.017 (n=3)
Sera	Naive	0.9 - 2.57	0.39	0.695
	Infected for 21 days primary	1.9 - 3.6	11.25	0.645 - 0.995

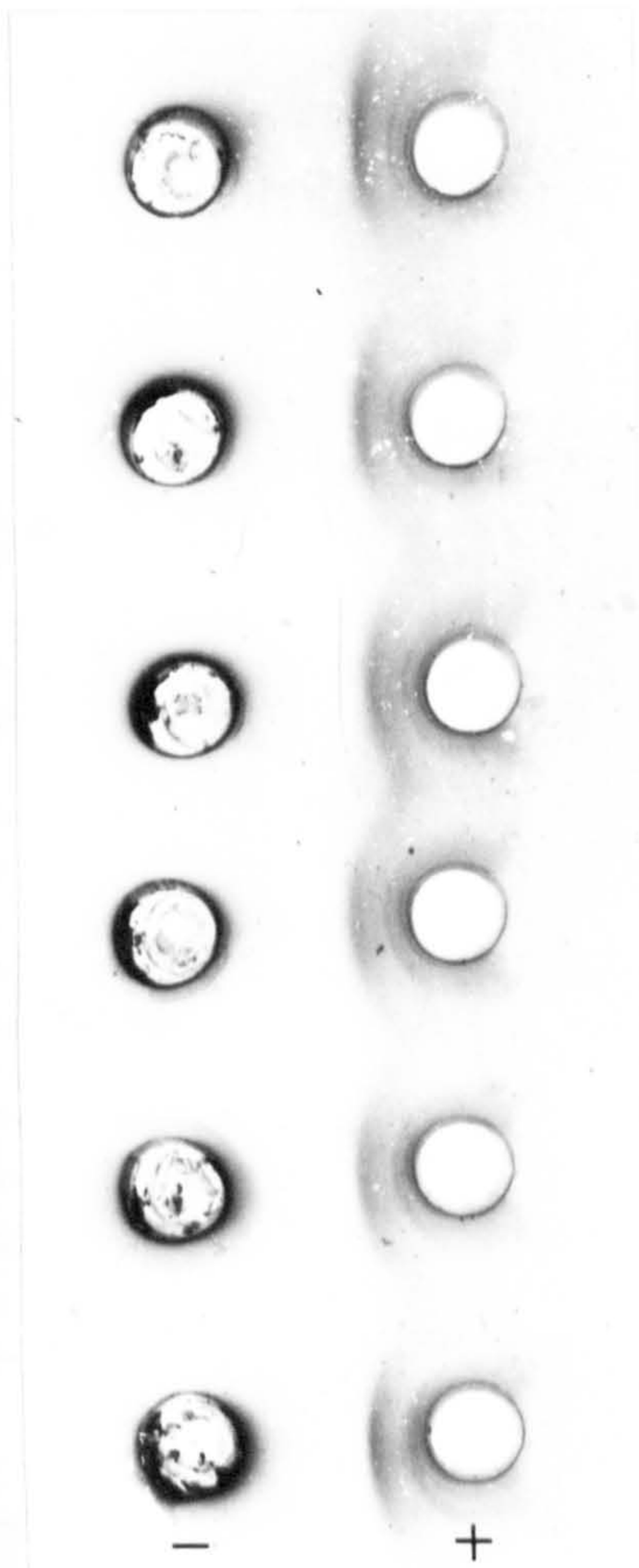
n = number of batches (pooled samples from 5-6 animals)

sera - one or two individual sera only.

Ouchterlony immunodifussion



C.1.E.



Immunoelectrophoresis

Plate 4.3: The detection of antibody activity in anti T.spiralis larval serum (ALaS) using T. spiralis larval antigen (Lag) by means of Ouchterlony immunodiffusion, C.1.E. and immunoelectrophoresis.

Precipitin bands or arcs were produced in all three tests by the reaction between (Lag) and (ALaS).

FIG 4·11

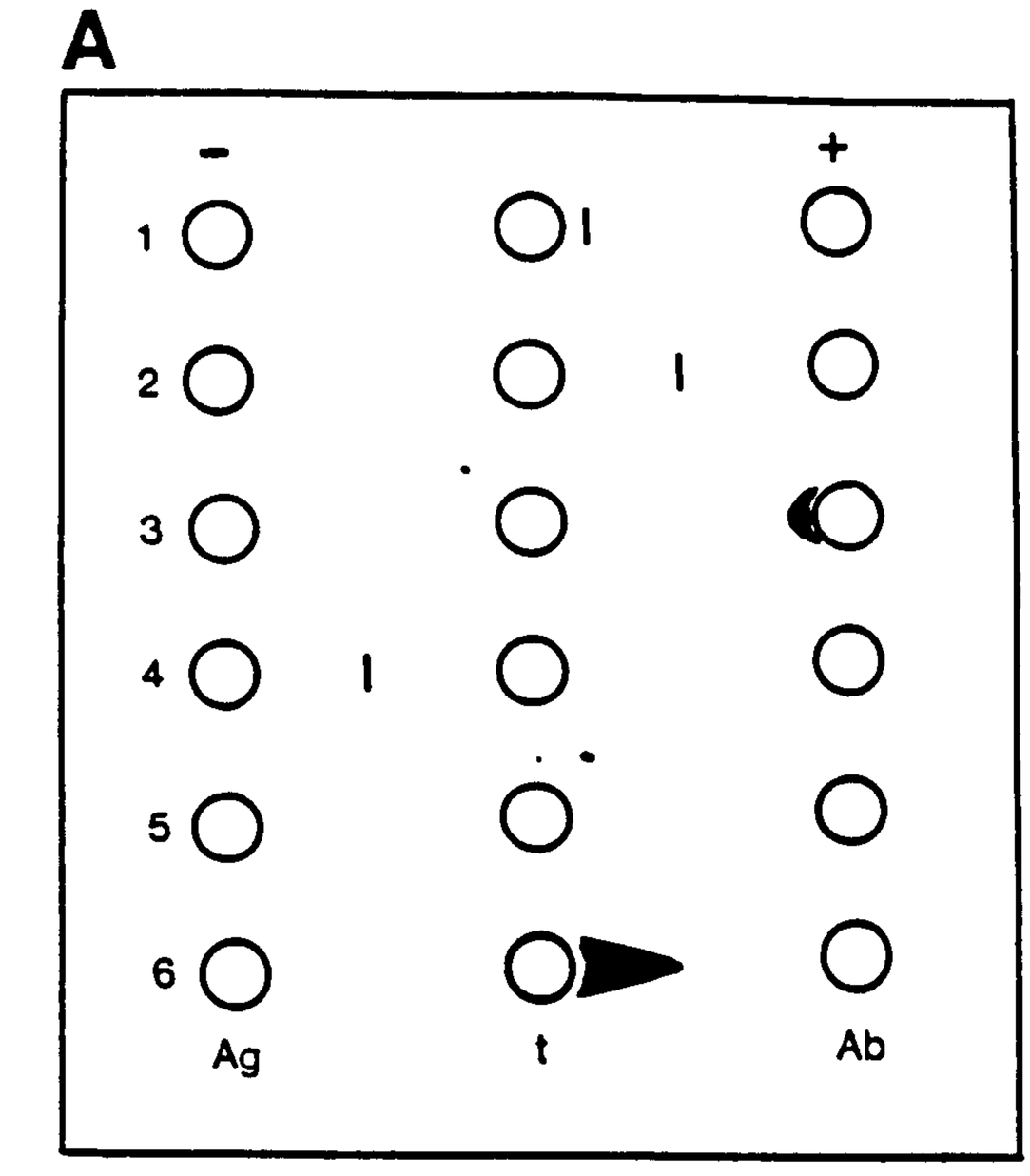


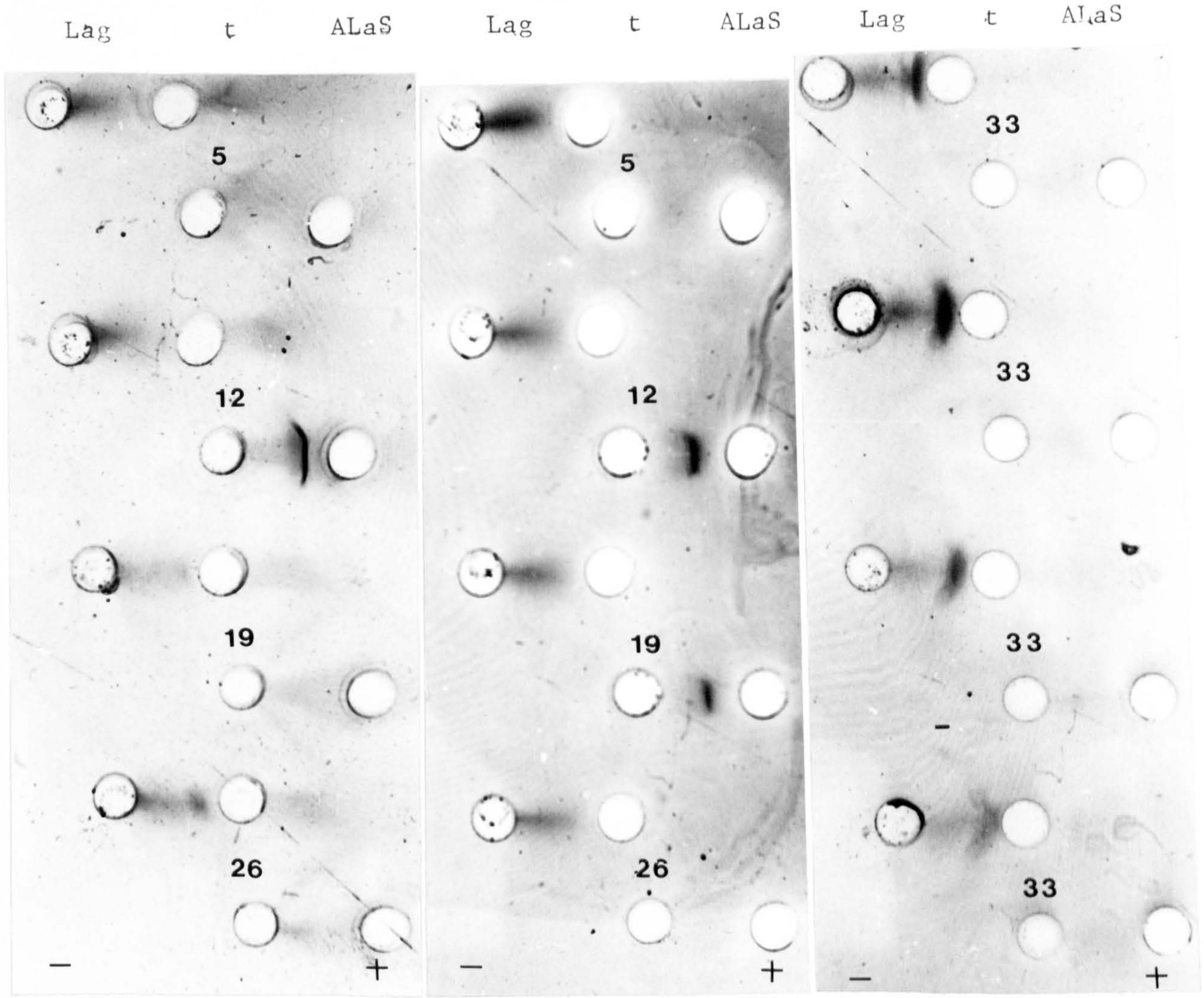
Figure 4.11: Diagramatic representation for simultaneous detection of antigen and antibody using straight line (A) or offset (B) counter current immunoelectrophoresis.

1. antigen weak positive
2. antigen positive
3. antigen strong positive
4. antibody positive
5. negative
6. false positive reaction due to lipaemic serum.

Ab = known antibody Ag = known antigen
t = test sera

Pattern (A) and results: Reference = Thompson, R.A.(1977).

PLATE 4·4



(A) single sera

(B) pooled sera (6 mice)

(C) single sera

Plate 4.4 D.C.I.E, NIH mice -SERA. Primary infection.

Double counter current immunoelectrophoresis of ALaS and Lag in outer wells, and mice test sera (t) in inner wells - sera collected between 5 and 33 days post-infection.

4.4.A: Single precipitin band in day 12 single serum - indicating reaction between antigen from test serum and antibody in ALaS.

Diffuse faint band in day 26 single serum (arrow) indicating antibody in test serum reacting with Lag.

4.4.B: Single bands in days 12 and 19 pooled test sera - indicating antigen in test sera reacting with ALaS.

4.4.C: Diffuse bands present in 4 different single serum - indicating antibody in test serum reacting with Lag.

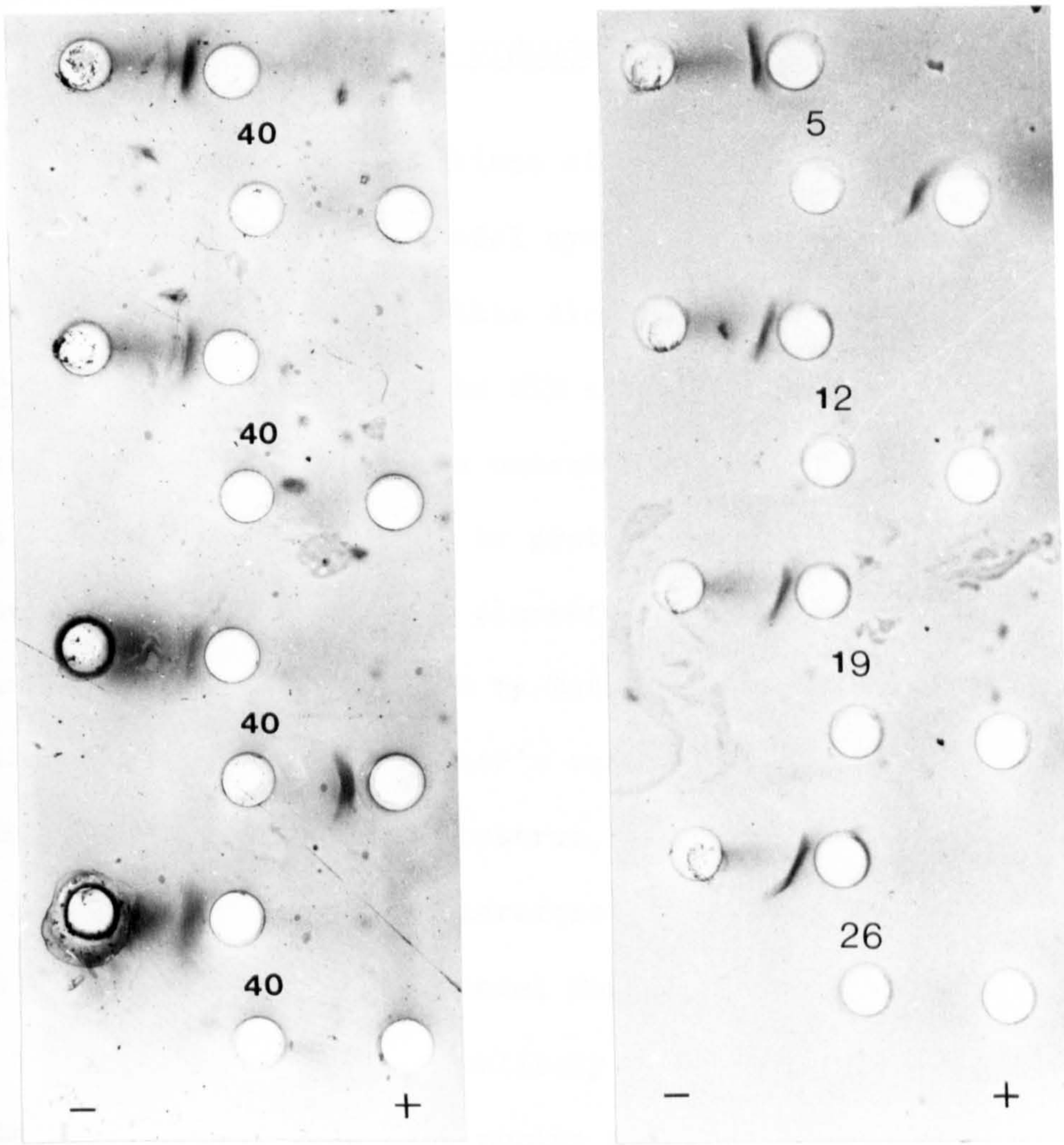
Table 4.14: Detection of soluble antigen and antibodies in sera of adult NIH mice during primary and secondary infections of T. spiralis by double counter current immunoelectrophoresis (D.C.I.E.).

Days after infection	Test sera from mice: soluble antigen		antibodies	
	primary infection	secondary infection	primary infection	secondary infection
5	-	+	-	+
12	+	-	-	+
19	+	-	-	+
26	-	-	+	+
33	-	-	+	+
40	+	-	+	+
47	-	-	+	+
54	-	-	+	+
61	-	-	+	+
68	-	-	+	+
75	-	-	+	+
82	-	-	+	+

In primary infection mice were infected with 450 T. spiralis larvae. Sera were collected from these mice 5 days post-infection and weekly thereafter.

In secondary infection, mice were infected twice, first with 450 T. spiralis larvae, followed after 5 weeks by a second infection of 350 larvae - Sera were collected from these mice 5 days post-secondary infection and weekly thereafter.

PLATE 4·5



(A) Single sera

- primary infection -

(B) Pooled sera.

- secondary infection -

Plate 4.5: D.C.I.E, NIH mice - SERA. Primary and secondary infection.

Double counter current immunoelectrophoresis of ALaS and Lag in outer wells, and mice test sera (t) in inner wells. Single sera were collected on day 40 post-infection, and pooled sera were collected on day 5-26 secondary post-infection.

4.5.A: Diffuse precipitin bands in day 40 of one single sera indicating reaction between antigen from test sera and antibody in ALaS.

One or two bands present in four different single sera on day 40 post-infection indicating antibody in test sera reacting with Lag.

4.5.B: Single precipitin band in day 5 post-secondary infection indicating reaction between antigen from test sera and ALaS.

Precipitin bands produced by the reaction of Lag and test sera from day 5-26 secondary post-infection, indicating the presence of antibody in the test sera.

GENERAL DISCUSSION

In most experimental investigations of parasite immunity, practical considerations require the use of model systems utilising laboratory animals. In the work presented in this dissertation the model-host parasite system is Trichinella spiralis in the NIH strain mouse.

It must be appreciated from the outset that the mouse, in terms of the methods of transfer of immunity or protection from the mother to the infant, conveys immunity across the placenta and via the milk, i.e. mixed transfer, and was thus characterised by Butler (1973) as belonging to Group 2 along with dogs and rats. In Butler's scheme, the ungulates are Group 3 - transfer almost entirely via the colostrum, and humans and rabbits are classified in Group 1. There is therefore no pretence that T. spiralis in NIH mice is a particularly good model for human maternal infant transfer and in any case, human infants are unlikely to experience infection with the parasite much earlier than nine months of age. With respect to the pig, in which such a large proportion of the maternal endowment is conveyed via the milk, and in which infection of the infants with T. spiralis is a distinct possibility from the age of four weeks or so, T. spiralis in the mouse can be compared more directly if so desired. From a perusal of the literature at the commencement of this investigation it was concluded that the knowledge of the mechanisms of transfer of immunity with respect to parasitic infections was at such an embryonic state that any objective study would be beneficial. Nonetheless for technical as well as comparability reasons, rabbits would have been the preferred animals. However, the numbers of animals which were required, together with the lack of extensive housing facilities for this species precluded their use.

In addition to differences between species there are considerable

differences in the host-parasiterelationship between strains of one species of host, including mice infected with T. spiralis, as was shown by Manson-Smith et al. (1979b).

Infant mice have seldom been used for parasitic infections and it is not known whether they are good host animals in general. T. spiralis infection in very young mice has been reported on only a few occasions. Bass and Olsen (1965) studied the T. spiralis burdens of very young Fairfield-Webster white mice and noted that mice infected within the first week after birth were 5-10 times less susceptible to infection than adults of that strain. They also noted that the course of infection was of approximately the same duration as that in adult mice indicating a very considerable degree of immunocompetence.

One of the preliminary aspects of this study confirmed these findings for T. spiralis in infant NIH mice, although the earliest at which mice were infected was 1 week of age. Infections in mice of this age were highly variable and they were clearly not suitable for quantitative experimentation. With two week old mice sufficiently consistent infections were achieved and such mice formed the basis for much of the study of maternal-infant transfer of immunity. It should be noted that the distributiqn/localisation of intestinal T. spiralis in young NIH mice differs from that in adult mice in that more of the worms are located in the posterior half of the small intestine.

The immunocompetence of NIH mice, two weeks old at the time of infection, in expelling a burden of intestinal T. spiralis is not profoundly different from that of adult mice; they are a little slower in clearing the gut of adult worms. Apart from being less susceptible to intestinal infection than adult mice, they would otherwise appear to be adequate host animals in that

the fecundity of the worms is unimpaired, leading to equivalent, if not slightly higher, yields of infective larvae in the muscles.

Given the information that young mice are deficient in lymphocyte populations in the gut until approaching three weeks of age (Ferguson and Parrott, 1972) and their capacity of endogenous antibody production is undeveloped (Brambell, 1970) such suggested immunocompetence in young mice is at first sight a little puzzling. However it should be borne in mind that cell traffic studies and comprehensive histological studies of the intestines of young mice undergoing major assault and tissue injury as in a T. spiralis infection have not yet been undertaken. It should also be appreciated that the development of immunocompetence during the third week of life may be a very rapid process and given the possibility of a lower than average general antigenic assault, together with maternal protection to the commoner antigens, during this period, a population of T. spiralis may be subjected to a highly focussed immunological response. Clearly all of these considerations present an area for fruitful research.

The major issue of the recruitment of maternal cells - in particular lymphocytes, into the tissues of the suckling infant remains unresolved. The principal technique chosen to investigate this issue, namely the quantification of radiolabelled maternal cells internalized to the tissues of the infant from the ingesta proved unsatisfactory in that very significant amounts of ^{125}I-UdR were liberated from the cells which were orally intubated to the infants, were taken up very rapidly by the infant gut and therefore completely masked the presence, if any, of cells which migrated into the infant gut tissue. Chromium radiolabelling of cells although not suffering from this problem yielded such small amounts of radiolabel in the infant tissues as to be unquantifiable consistently. Autoradiography, which was

to be the second string of this part of the investigation was not undertaken.

Immunofluorescence studies did show the presence of a few Ig-containing cells in the lamina propria of the infant small intestine. Both IgA and IgM-containing cells were detectable with very faint fluorescence from the sixth day of age, with traces of IgM present in the enterocytes. Such cells were present in naive infants suckling naive and infected mothers, but the occurrence of the cells was so patchy and infrequent that meaningful quantification could not be produced. However this observation would appear to be the earliest at which Ig-containing cells have been observed in the intestine of infant mice, Crabbe et al. (1970) having recorded IgA cells only from day 10 followed by IgM and IgG cells from day 13 in C_3H mice. A considerable proportion of the Ig-containing cells observed in this study were identified as lymphoid cells. All of the cells observed were present in the lamina propria or submucosal regions with no such cells observed in the intraepithelial positions which would have permitted a firmer conclusion of cells being internalized. Nonetheless it must remain a possibility that these Ig-containing cells were of maternal origin and as such offered the infant potential protection.

IgG was quite readily vizualised in the infant gut tissues at a very young age. Very few cells were observed, with a very large proportion of fluorescence associated with ducts in the lamina propria and submucosa. Very little IgG could be vizualised in enterocytes and as with IgA and IgM none could be detected in/on the brush border. However when the results of the study of T. spiralis antibodies with ELISA are taken into account, namely, that a titre of 1:200 IgG antibody can be detected in the sera of eight day old naive infants suckling infected mothers there can be little doubt that the IgG vizualised in the infant gut tissues by immunofluorescence

is of maternal origin. Any consideration to the contrary would involve a major reassessment of the well established work of Halliday (1955 a & b) and the considerations of Brambell (1970) and Morris and Morris (1978). The degree and methods by which such an endowment may afford protection to the infants will be discussed later.

A further assessment of the immunocompetence of young mice vis-à-vis intestinal T. spiralis was the study of the production of antibody in young mice infected at one week of age and suckling naive mothers. Such mice were shown to be able to produce detectable amounts of IgM and IgG antibodies in their sera within 14 days of infection, i.e. at the age of three weeks. The probability of significant tissue levels of these antibodies, and possibly also of IgA antibodies, prior to their apperance in sera, remains to be investigated. The early part of this dissertation documents comprehensively that maternal protection to T. spiralis infection in the infant is entirely an endowment conveyed through the milk. As such this work confirms the study of Duckett et al. (1972). However in this study there is clarification that protection against T. spiralis in the infant is wholly protection against the establishment of infective and developing larvae in the gut and is not protection involving the transfer or induction of the varied components of the immunological response which give rise to early expulsion of an established infection. Thus irrespective of the possible uptake of immunocompetent maternal cells by the neonate together with the uptake of at least the IgG class of antibodies, together with the continued presence in the milk of IgA and IgM class antibodies (at least to the infective larvae), T. spiralis once it has circumvented the initial 'barrier' is apparently untroubled by the presence of these components in the lumen of the gut, in the enterocytes or in the subepithelial tissues

of the gut.

It has been known for some time and in a number of host/parasite systems that lactating mice and rats are less competent than non-lactating animals in expelling a burden of nematodes from the gut, e.g. N. brasiliensis in rats (Connan 1970), T. spiralis in mice (Ngwenya 1977). Provided that lactating mice can elicit and/or transfer a protective response to their infants, such incompetence within the gut of lactating mice is not of material significance to the T. spiralis/NIH mouse model. In passing it should be acknowledged that lactation incompetence is of enormous significance in the majority of gastro-intestinal helminth infections in which the continued presence of adult worms in the gut, often with enhanced fecundity, ensures a subsequent large population of infective larvae on pasture which are ingested by young animals. Trichostrongyle infection in lambs is one of the earlier well documented examples of the significance of lactation suppression (Connan, 1968).

In adult NIH mice infected with T. spiralis, the studies using radio-labelled mesenteric lymphoblasts confirmed the prior report of Rose et al. (1978), to the effect that there is a significant accumulation of mesenteric derived lymphoblasts in the mammary gland of lactating mice. Analysis of the data in this study does suggest however that a considerable degree of diversion of lymphoblasts from gut localisation does occur which may explain in part the relative incompetence of lactating mice in expelling an intestinal burden of adult worms.

In lactating NIH mice there are in addition considerable populations of immunoglobulin-containing cells, most of which were identified as lymphoid cells in the tissues of the mammary gland, and immunoglobulin-containing cells are also to be observed in the secreted milk in the ducts. Thus the findings

of Weisz-Carrington et al. (1977) are extended and the reports of Seelig et al. (1979) and Head and Beer (1978) confirmed. By a considerable factor IgA-containing cells were the most frequent immunoglobulin-containing cells in the mammary gland. IgG and IgA-containing cells were observed more frequently in the milk than IgM-containing cells. In T. spiralis infected lactating mice, there was a statistically significantly greater number of immunoglobulin-containing cells, of all three classes examined, in the mammary gland during the mid-lactation period although there was not a marked increase in the total number of leucocytes in milk recovered from infected mice. Although the numbers of immunoglobulin-containing cells in collected milk could not be determined satisfactorily, the numbers of lymphocytes delivered to the infants was of the order of 1.1×10^5 lymphocytes per ml of milk and the number of macrophages as 3.0×10^5 cells per ml. These figures although lower than those recorded by Head and Beer (1978), nevertheless represent a considerable cellular endowment from the mother.

In adult non-lactating mice the occurrence of detectable amounts of antibodies in the serum was observed by day 5 post-infection for IgM and consistently by day 15 for IgG antibodies. IgA antibodies were not detected. IgG antibodies persisted for a considerable time through a primary infection and were sustained at a titre of in excess 1:400 in secondary infections. IgM antibodies which were produced at high titres early in a primary infection were not maintained at such high levels during a secondary infection. In infected lactating mice the serum antibody titres of both IgG and IgM were lower-max. titre for IgG antibody 1:200 in a secondary infection and 1:25 early in a primary infection and 1:100 for IgM antibody early in a primary infection.

In milk from infected mice IgG antibody levels were 1:120 in a secondary infection and 1:30 in a primary infection of 17 days duration. IgM antibodies were just detectable at a titre of 1:15 in both primary and secondary infections and IgA antibodies, undetectable in sera of adult mice were just detectable in the milk of secondary infection mice.

When total immunoglobulin levels were assessed by R.I.D. milk from infected mice was shown to contain approximately two to three times the amounts of IgG and IgA present in milk from uninfected mice, with only minute amounts of IgM. IgA levels in the milk of infected mice were 0.88 mg/ml and IgG levels 0.63 mg/ml with less than 0.1 mg/ml of IgM. It is therefore the case that of the three measurements of components of the milk endowment, namely total Ig, antibodies and cells, mice undergoing a secondary infection of T.spiralis are shown to be highly immunocompetent.

The majority of the studies of protection in infant mice were conducted with mothers which were experiencing a secondary infection and which were in mid-lactation. It is possible to summarise the milk endowment of these mothers as follows. Each ml of milk contained approximately 0.63 mg of IgG with antibody at a titre of 1:120, 0.88 mg IgA with antibody at a titre of 1:15 and less than 0.1 mg of IgM with antibody at a titre of 1:15 and 1.1×10^5 lymphocytes and 3.0×10^5 macrophages. An undetermined proportion of both the types of cells were observed to contain immunoglobulins. No such comparable studies in parasite infected animals have been encountered in the literature.

In studies of cell populations in human milk Ho and Lawton (1978) observed phagocytic activity in macrophages and Parmely et al. (1976) and Ogra and Ogra (1978) noted reactivity of milk lymphocytes. It may therefore be possible to attribute a potential immunological competence to

these milk cells other than the synthesis or transport of maternally induced or produced immunoglobulins.

In the most interesting experiments of the transfer of protection to infants it was shown that suckling milk as described above for as short a period as 6 hours conferred a statistically significant degree of protection to infants. Direct measurements of the amount of milk suckled in 6 hours after fostering were not undertaken. However by inference from the experiments utilizing ^{51}Cr-labelled cells (Table 2.8) it would appear that approximately 75% of ingested milk had passed through the stomach within 3 h and 95% of the original ingesta by 6 h. Thus about 1½-2 fills of the stomach had passed into the intestine within 6 hours. Given the very slight delay in the commencement of suckling following the transfer of infants to the infected mother it would still seem reasonable to assume that at least one fill of the stomach of milk from infected foster mothers had passed through into the small intestine within the period of six hours. Since the average volume of milk contained in the stomachs of two week old infants was recorded as 0.2 ml (Table 4.10) approximately one-fifth of the maternal endowment listed earlier can be presumed to be sufficient to confer protection to the infants. Remarkably similar figures are derived from the experiment in which 0.3 ml of milk from infected mothers was given to infants by oral intubation in two aliquots over a period of 24 h and which yielded good protection to the infants. If the figures from this latter experiment, which involves less in the way of conjecture, are determined it would appear that an endowment of 0.19 mg of IgG with antibody of a titre of 1:120, 0.26 mg of IgA with antibody of a titre of 1:15, 0.02 mg of IgM with antibody of a titre of 1:15 and 0.33×10^5 lymphocytes and 0.9×10^5 macrophages confers protection to two week old infant mice. However in both of the experiments

under consideration the milk and the components would be diluted by additional milk of non-infected origin.

It was desirable to demonstrate that maternal immunoglobulins and antibodies were present in and internalized to some degree by the infants. These issues have been dealt with in the earlier part of this discussion, but for additional proof, the milk in the stomach was tested for the presence of antibodies and total immunoglobulins. All of the components listed in the maternal milk were detected in the milk in the stomachs of infants. The presence of viable maternal cells was also assessed. Although attempts to quantify the numbers of cells present in the infant stomach did not yield satisfactory results, apparently viable cells were observed in histological sections of the infant stomach. Maternal cells were not visualized in the intestine of infant mice but Bruce (unpublished observation) observed a considerable number of orally intubated maternal cells in the small intestine of infant mice. It would seem therefore that the potential for transport and/or secretion of immunoglobulins in the intestine of infants does exist in NIH mice together with the potential for internalization of maternal cells as was postulated by Head (1977) and Ogra et al (1977).

The most significant missing piece of evidence from the studies undertaken in this project is the lack of the determination of the presence of detectable amounts of maternal immunoglobulins in the brush border and within the enterocytes of the small intestine of the infants. The process of brush border attachment and internalisation of immunoglobulins has been well documented by several authors from the early studies of Halliday (1955 a & b) through Brambell (1966) to Mackenzie, Morris and Morris (1983) amongst others. The shortfall of the present investigation may perhaps

be attributed to an insufficiently sensitive immunofluorescent technique to detect the small amounts of maternal immunoglobulins dispensed over the relatively very large surface area of the small intestine. Even if that supposition can be verified with refined techniquesit will remain a salutary indication that the amount of one or more immunological component which is necessary for the profound degree of protection conferred to infants may be remarkably small.

Such a consideration is given weight when the results of the experiment concerned with the required duration of infection of the lactating mice are assessed. In this experiment it was shown that maternal infection of as short a period as 9 days is sufficient to confer protection via the milk. At this point of infection in fully expressive non-lactating adult NIH mice, significant levels of IgG antibodies are not detectable in the sera. It is the period however of maximal rate of expulsion of adult worms from the gut. The nearest comparable evidence for the transfer of protection early in infection is that of Miller (1980) who was able to demonstrate transferrable protection (early expulsion) to N. brasiliensis in the sera of rats with a 6 or 8 day primary infection.

To determine the potential function of the antibodies detectable in serum and in milk of infected animals in addition to their occurrence, the reactivity and specificity of the antibodies to worm components was examined by immunofluorescence studies in sections of infective larvae. Obviously the external surface of the parasite and the internal surface of the worms alimentary canal together with its glands, in this case the stichocytes associated with the osophagus, are the structures/components of the worm which are the first to come into contact with the host components. In addition

the cuticle and the stichosome have been shown by various authors to be associated with 'metabolic' secretions or excretions some of which may be enzymes as well as being antigens. Antibody reactivity to the surface of the cuticle, to the contents of the worms intestine, which is the only part of the alimentary canal large enough to be easily assessed, and presumed secretory material within some stichocytes was demonstrated. Weak, moderate titre, reactivity to all these components was observed with IgM antibody, with weak fluorescence at moderate to high titre with IgA antibody to the surface of the cuticle and externally adherent material and to the gut contents but not to the stichocytes, and moderate to strong fluorescence at high titre with IgG antibody to all three components. There is therefore a clear distinctiveness of the antigenecity of the material contained within some stichocytes without any assumption of common identity for the antigens of the cuticle and gut. It may be significant that the stichocyte material elicits the earliest detectable response in the sera, although the point has been made earlier that the occurrence of small amounts of antibodies within certain tissues is likely to be of considerably greater significance than any amount of antibody detectable in the sera.

This antibody reactivity to these components of the worm in addition to being part of a recognition system, which in adult mice might be an essential feature of a cascade of reactions leading to expulsion of an established infection as early expulsion in a sensitized animal, might have a simple and direct effect of blocking or neutralising the functions of such antigens on a worm surface or externally to the worm such that disorientation or impaired ability to secure nutrients or inability to penetrate the brush border and epithelial barrier would result. The consequence of any of these effects would be non-establishment of the worm.

The knowledge of the declining levels of maternal IgG antibody located in naive infant sera by the end of the third week of life, with a presumed half-life of 4.1 days (Fahey and Robinson 1963) indicates that the degree of protection which persists for at least one week after the termination of suckling may be achieved by small amounts of the maternal endowment. In addition the evidence of Bruce (unpublished observation) that the life of enterocytes in NIH mice is not longer than 4 days would also add to the known rate of loss of maternal components and would encourage the view that effective if small quantities of maternal components would be present on the brush border of the new enterocytes or be present in the new enterocytes only if there is charging of the new epithelia from the lamina propria or via an intestinal-liver circulation as demonstrated in adult rats by Lemaitre-Coelho, Jackson and Vaerman,(1978).

The formulation of roles for maternal leucocytes in the infants, other than (a) a proportion of them being internalized and possibly conferring a capacity for earlier immune responsiveness than would otherwise occur, or (b) acting as vehicles synthesising or transporting immunoglobulins, cannot be achieved at the present time. Before direct anti-worm activities could be postulated experiments determining the duration of their existence in the small intestine of the infants, their adherence and/or toxicity to the parasite or their involvement in a sequence of cellular or physiological events of remarkably rapid development (6 hours) would have to be accomplished. Given also the evidence of the size of the cell population, a mere 110,000 lymphoblasts and 300,000 lipid laden macrophages in the stomach and small intestine of a two week old mouse, it would seem improbable that a significant direct role for these leucocytes is likely to

exist. If parasite sensitized lymphocytes are internalized it is clear that they do not have a role in short or long term memory conferring enhanced expulsion of the parasite at a subsequent primary infection.

The final points of record are that, the conferment to infants of antibodies and cells does not induce tolerance or immunological incompetence vidé Jacobsen et al. (1978) and Santoro et al. (1977); the possible conferment of parasite antigens (detected only in the sera of adults) has no apparent effect, and exposure to infections at a very early age also does not induce tolerance, indeed infection at an early age induces a high degree of immunity in infants suckling naive or infected mothers.

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A P P E N D I X

Table APX 1.1: Number of T. spiralis recovered after primary infection in adult NIH (14-16 weeks old) after 4 days of infection.

Experiment No.	Group	No. of T. spiralis larvae (infective dose *mean ± s.d.)	Localisation of worms in the small intestine (mean ±s.d.) of 6 mice			% of establishment
			Anterior	Posterior	Total	
1	A	57± 1	17.8± 5.64	6 ± 2.37	23.8± 6.55	41.7
	B	160± 5	48.2±15.47	11.8 ± 5.84	60 ±13.89	37.5
	C	448±10	168 ±28.86	48.7 ± 25.59	216.7±21.86	48.4
2	A	66± 5	11.8± 4.26	12 ± 4.86	23.8± 5.56	36.1
	B	148± 3	58.8± 4.62	13.7 ± 4.23	72.5± 4.5	49
	C	434±28	145 ±54.96	52.5 ± 49.17	197.5±16.03	45.5
3	A	52± 3	15.7± 4.63	7.1 ± 3.12	22.8± 6.14	43.8
	B	163±10	49.7±15.2	20.5 ± 13.25	70.2± 3.54	43.1
	C	469± 9	160.2±29.75	76 ± 36.85	236.2±30.33	50.4
4	A	51± 1	16.5± 5.97	8.7 ± 3.93	25.2± 3.97	49.4
	B	148± 3	51.7± 8.94	16.8 ± 4.79	68.5± 8.45	46.3
	C	435± 4	167.2±19.63	46.8 ± 4.53	214 ±18.21	49.2
5	A	55± 3	14.5± 4.41	12.7 ± 2.34	27.2± 4.44	49.4
	B	161± 6	67.5± 5.54	17.3 ± 6.5	84.8± 5.64	52.7
	C	465±17	159.2±19.99	76 ± 31.23	235.2±13.85	50.6
6	A	59± 2	20.7± 3.5	5 ± 3.29	25.7± 5.57	43.5
	B	165± 3	49 ± 7.4	21.8 ± 6.94	70.8± 5.91	42.9
	C	465+ 6	176.3±54.09	68.5 ± 23.4	244.8±34.18	52.6

Dose volume = 0.3 ml

s.d. = standard deviation

* Mean of five estimates

Table APX 1.2 Expulsion of primary infection of Trichinella spiralis from adult NIH mice (8-11 weeks old) infected with 50 larvae

Exp. No.	No. of T.spiralis larvae (infective dose) mean ± s.d. of 5 estimates		Number of T.spiralis recovered from the small intestine (Mean ± s.d) of 5 mice						Statistical significance P.value	
			Days post-infection						day	days
			4 p.i	6 p.i	8 p.i	10 p.i	12 p.i.	14 p.i.	6 vs 8	8 vs 10
1	51 ± 2.06	A	13.8±3.42	12.6±4.72	2.8±3.03	1.8±1.3	0.6±0.89			
		P	5.6±3.21	7.4±3.78	2.2±1.48	1.4±2.07	1 ±1.73	N.D.		
		T	19.4±2.07	20 ±1.22	5 ±3.81	3.2±3.34	1.6±2.5		P<0.001	N.S.
	% of establishment		38%							
2	50 ± 3.7	A	14.2±2.17	14.8±2.23	6.8±2.16	4.2±3.34	0.2±0.44			
		P	5.4±2.88	2.8±3.34	5.8±2.48	2.6±2.5	0.8±1.3	N.D.		
		T	19.6±1.82	17.6±2.19	12.6±1.81	6.8±4.76	1 ±1.4		P<0.01	P<0.05
	% of establishment		39.2%							
3	52.6 ± 6.8	A	17.83±5.8	16.33±6.21	5.6±2.4	1.2±1.78				
		P	3.16±1.94	2.33±1.36	5 ±2.91	0.8±0.83	0	0		
		T	21 ±4.64	18.66±5.92	10.6±2.5	2 ±2.34			P<0.05	P<0.01
	% of establishment		39.9%							
4	50.6 ± 3.4	A	14.2 ±1.09	8.2 ±2.86	5.8±2.28	2 ±2.91	0.4±0.54			
		P	4.2 ±2.28	10.2 ±5.97	5.6±3.36	1.6±1.14	0.2±0.45	N.D.		
		T	18.4 ±1.94	18.4 ±4.04	11.4±4.84	3.6±3.26	0.6±0.89		P<0.05	
	% of establishment		36.4%							
5	51.5 ± 6	A	23 ±5.66	18.8 ±1.3	8.6±2.97	0.4±0.55	0			
		P	3.8 ±1.3	8 ±5.38	7.2±3.96	0.6±0.55	0	N.D.	P<0.01	
		T	26.8 ±4.97	26.8 ±5.02	15.8±3.63	1 ±0.71	0			
	% of establishment		52.5%							

A: anterior part of the small intestine

P: posterior part of the small intestine

T: total worms recovered

N.D: not done

Table APX 1.3 The course of infection of T. spiralis in infant mice
Expulsion of a primary infection of T. spiralis from 1 week old mice

Exp. No.	Estimated no. of larvae (inoculum)	mice		Worm burden recovered (Mean± s.d) of 5 mice						Statistical significance (P.value)	
				Days of post-infection						Days	
				4	6	8	10	12	14	6 vs 8	8 vs 10
1	100	infant	A	0	0	0	0	0			
			P	8.8±8.52	4 ± 4	2.4±3.2	3.6±2.07	2.6±1.34	N.D.		
			T	8.8±8.52	4 ± 4	2.4±3.2	3.6±2.07	2.6±1.34		N.S.	N.S.
		Adult (control)	A	20.2±2.77							
			P	5.6±2.96			N.D.				
			T	25.8±4.65							
2	150	infant	A								
			P	N.D.	16.2±8.34	12.6±9.86	6.4±5.59	3 ±1.87	1.6±1.14		
			T		16.2±8.34	12.6±9.86	6.4±5.59	3 ±1.87	1.6±1.14	N.S.	N.S.
		Adult (control)	A		27.4±8.44	15.2±7.49	2.6±3.57	0.4 ±0.89			
			P	N.D.	10.8±2.58	11.4±5.59	2.2±3	1.8 ±3	0	N.S.	P<0.01
			T		38.2±10.4	26.6±8.2	4.8±6.57	2.2 ±3.89			

A: anterior part of the small intestine
P: posterior part of the small intestine
T: total worms recovered
s.d.: standard deviation

Table APX 1.4 — T. spiralis infection in young mice. Effect of immunity in mothers on the establishment and the rate of expulsion in 2 weeks old mice.

infants infected with 200 larvae at 2 weeks of age	Worm burden recovered (mean ± s.d) of 5-9 mice														
	Days post-infection														
	2 p.i			4 p.i.			6 p.i.			8 p.i.			10 p.i.		
	A	P	T	A	P	T	A	P	T	A	P	T	A	P	T
Born of naive	6	36	42	13	41	54	8	36	44	0	19	19	0	23	23
Suckling naive	1	37	38	0	32	32	11	41	52	1	13	14			
	3	39	42	11	43	54	1	34	35	5	25	30	1	11	12
	9	32	41	6	35	41	5	32	37	1	16	17	4	13	17
	7	42	49	8	32	40	12	40	52	0	19	19			
	5	28	33	15	40	55	9	32	41	4	24	28	5	16	21
							5	38	43	3	38	41	2	10	12
							12	40	52	6	35	41			
							3	33	36	2	11	13	0	8	8
Mean	5.16	35.66	40.83	8.83	37.16	46	7.33	36.22	43.55	2.44	22.22	24.66	2	13.5	15.5
± s.d	±2.85	±5	±5.27	±5.41	±4.79	±9.65	±4.03	±3.63	±7.02	±2.18	±9.31	±10.87	±2.09	±5.39	±5.82
Born of immune	4	18	22	1	22	23	1	17	18	0	13	13	1	18	19
Suckling immine	1	26	27	5	23	28	0	12	12	1	13	14			
	9	20	29	3	19	22	4	18	22	0	2	2	0	2	2
	1	19	20	8	22	30	1	21	22	1	5	6	1	1	2
	11	16	27	0	29	29	5	15	20	2	6	8			
							4	7	11	0	19	19	3	3	6
							8	15	23	0	8	8	0	5	5
							2	9	11	1	2	3			
							0	17	17	3	18	21	0	0	0
Mean	5.2	19.8	25	3.4	23	26.4	2.77	14.55	17.33	0.88	9.55	10.44	0.83	4.83	5.66
± s.d.	±4.6	±3.76	±3.8	±3.2	±3.67	±3.64	±2.68	±4.47	±4.89	±1.05	±6.46	±6.72	±1.16	±6.67	±6.88

* On days 2, 4 & 10,6 mice were killed (three from two litters) on days 6 & 8, 9 mice were killed (three from three litters).

Table APX 1.5 T.spiralis infection in young mice. Duration of protective immunity gained from mothers

Infants	Age of infants	*Mean worm burden ± s.d. established in anterior (A) and posterior (P) of the small intestine and total recovery (T)								
		Days post-infection								
		6 P.i			8 P.i			10 P.i		
		A	P	T	A	P	T	A	P	T
Born of naive Suckling naive	1 wk	2.4 ±5.36	22.4 ± 7.12	24.8 ±8.44	0.17 ±0.41	13.83 ±8.73	14 ±8.94	N.D.	N.D.	N.D.
	10 days	1 ±1	45.4 ± 3.05	46.4 ±2.3	0.2 ±0.45	31.6 ±22.12	31.8 ±22.39	0.6 ±0.89	27.2 ±14.34	27.8 ±13.88
	2 wks	12.7 ±4.27	32 ± 4.34	44.7 ±5.24	7.2 ±3.76	19.3 ±3.07	26.5 ±5.47	2 ±2.45	12.25 ±5.19	14.25 ±6.7
	3 wks	25 ±11.47	17.22 ± 9.92	42.22 ±12.41	10.39 ± 8.46	10.55 ±6.62	20.94 ±12.62	6 ±5.72	5.16 ±5.39	11.16 ±9.47
	4 wks	31.16 ±10.13	21.5 ± 6.65	52.66 ±9.74	14.44 ±8.18	11.94 ±7.21	26.38 ±13.45	7.5 ±5	5.33 ±6.09	12.83 ±9.45
	6 wks	36.06 ±12.37	15.47 ± 6.99	51.53 ±8.52	18.2 ±7.43	12.53 ±9.63	30.73 ±9.36	10.3 ±4.8	7.5 ±4.24	17.8 ±7.84
	9 wks	42.8 ±11.97	12.6 ± 5.01	55.4 ±9.78	19.93 ±10.44	9.73 ±5.72	29.66 ±12.59	6.8 ±8.29	4.3 ±3.71	11.1 ±11.39
Born of immune Suckling immune	1 wk	0.8 ±1.09	7.4 ± 4.92	8.2 ±5.06	0	3.8 ±4.14	3.8 ±4.14	N.D.	N.D.	N.D.
	10 days	0	12.8 ± 9.88	12.8 ±9.88	0.2 ±0.45	8.6 ±12.74	8.8 ±13.18	0	7.4 ±5.18	7.4 ±5.18
	2 wks	4.8 ±2.32	12 ± 3.63	16.8 ±3.97	2.66 ±2.8	10.16 ±5.71	12.83 ±6.31	1.5 ±1.91	4.75 ±2.06	6.25 ±2.99
	3 wks	9.61 ±5.03	6.27 ± 3.39	15.88 ±5.79	5.44 ±6.03	6.33 ±4.77	11.77 ±8.43	3.08 ±4.27	2.58 ±3.47	5.66 ±7.46
	4 wks	20.22 ±7.52	10.11 ± 5.66	30.33 ±10.39	4.77 ±7.57	3.61 ±4.43	8.38 ±11.12	1.17 ±1.64	1.16 ±1.85	2.33 ±2.8
	6 wks	36.46 ±8.02	11 ± 4.69	47.46 ±5.93	27 ±5.33	9.8 ±5.89	36.8 ±6.26	15.3 ±6.75	6.6 ±4.71	21.9 ±6.87
	9 wks	43.4 ±8.62	14.33 ± 7.5	57.73 ±8.04	23.67 ±12.69	9.13 ±5.19	32.8 ±12.11	4.6 ±5.96	5.7 ±4.16	10.3 ±8.73

N.D. = not done

Dost of infection = 200 larvae

*Each value represent a mean of
4 mice (2 wks day 10 p.i)
5 mice (1 wk & 10 days old, days 6, 8, 10 p.i)
6 mice (2 wks, days 6&8)
18 mice (3 wks, & 4 wks, days 6, 8 P.i)
12 mice (3 wks, & 4 wks, day 10 P.i)
15 mice (6 wks & 9 wks, days 6, 8 P.i)
10 mice (6 wks & 9 wks, day 10 P.i)

Table APX 1.6 T. spiralis infection in young mice (1 week old)
Duration of protective immunity gained from the mothers.

	Number of worms established in the small intestine								
	Group 1 Born of naive Suckling naive								
	Days post-infection								
	4 P.i (n=5)			6 P.i (n=5)			8 P.i (n=8)		
	A	P	T	A	P	T	A	P	T
	5	16	21	0	29	29	1	23	24
	1	20	21	0	13	13	0	11	11
	6	16	22	0	30	30	0	22	22
	0	21	21	12	21	33	0	19	19
	0	33	33	0	19	19	0	3	3
							0	5	5
Mean	2.4	21.2	23.6	2.4	22.4	24.8	0.17	13.83	14
±s.d	±2.89	±6.98	±5.27	±5.36	±7.12	±8.44	±0.41	±8.73	±8.94

	Group 2 Born of immune Suckling immune								
	Days post-infection								
	4 P.i (n=5)			6 P.i (n=5)			8 P.i (n=5)		
	A	P	T	A	P	T	A	P	T
	0	4	4	0	8	8	0	4	4
	0	3	3	0	1	1	0	0	0
	1	8	9	2	11	13	0	10	10
	1	9	10	0	13	13	0	5	5
	0	1	1	2	4	6	0	0	0
Mean	0.4	5	5.4	0.8	7.4	8.2	0	3.8	3.8
±s.d	±0.55	±3.39	±3.91	±1.09	±4.92	±5.06		±4.14	±4.14

Dose of infection 200 T.spiralis larvae

Statistical analysis (total recovery)
Group 1 day 4 vs 6 = N.S.
6 vs 8 = N.S.
Group 2 day 4 vs 6 = N.S.
6 vs 8 = N.S.
Group 1 day 4 vs group 2 day 4 = P<0.001
Group 1 day 6 vs group 2 day 6 = P<0.01
Group 1 day 8 vs group 2 day 8 = P<0.05

A: anterior part of the small intestine
P: posterior part of the small intestine
T: total worms recovered
n: number of mice used

Adult establishment (n=5)
day 6 post-infection

	A	P	T
Mean	47.4	12.2	59.6
± s.d.	±9.34	±3.63	±7.23

Table APX 1.7 T. spiralis infection in young mice (10 days old). Duration of protective immunity gained from the mothers.

		Number of worms established in the small intestine																	
		Group 1 Born of naive Suckling naive									Group 2 Born of immune Suckling immune								
		Days post-infection									Days post-infection								
		6 (n=5)			8(n=5)			10 (n=5)			6(n=5)			8(n=5)			10(n=5)		
		A	P	T	A	P	T	A	P	T	A	P	T	A	P	T	A	P	T
		0	49	49	0	17	17	0	24	24	0	4	4	1	31	32	0	10	10
		1	43	44	1	55	56	0	49	49	0	15	15	0	7	7	0	11	11
		2	45	47	0	20	20	0	31	31	0	13	13	0	2	2	0	4	4
		0	48	48	0	56	56	2	22	24	0	4	4	0	2	2	0	0	0
		2	42	44	0	10	10	1	10	11	0	28	28	0	1	1	0	12	12
Mean	1		45.4	46.4	0.2	31.6	31.8	0.6	27.2	27.8	0	12.8	12.8	0.2	8.6	8.8	0	7.4	7.4
± s.d.	1		±3.05	±2.3	±0.45	±22.12	±22.39	±0.89	±14.34	±13.88		±9.88	±9.88	±0.45	±12.74	±13.18		±5.18	±5.18

Dose of infection 200 T.spiralis larvae

Statistical analysis (total recovery)

Group 1 day 6 vs 8 = N.S.
8 vs 10 = N.S.
6 vs 10 = P< 0.08

Group 2 day 6 vs 8 = N.S.
8 vs 10 = N.S.
6 vs 10 = N.S.

Group 1 day 6 vs group 2 day 6 = P<0.001
" 1 " 8 vs " 2 " 8 = N.S.
" 1 " 10 vs " 2 " 10 = P<0.05

A : anterior part of the small intestine
P : posterior part of the small intestine
T : total worms recovered
n : number of mice used.

Adult establishment (n=5)

	6 days post infection A	P	T
Mean	50.4	13.6	64
±s.d.	±7.54	±5.86	±10.1

Table APX 1.8 T. spiralis infection in young mice (2 weeks old).
Duration of protective immunity gained from the mothers.

	Number of worms established in the small intestine																	
	Group 1 Born naive, suckling naive									Group 2 Born of immune, suckling immune								
	Days post-infection									Days post-infection								
	6 p.i(n=6)			8 p.i(n=6)			10 p.i.(n=4)			6 p.i.(n=6)			8 p.i.(n=6)			10 p.i. (n=4)		
	A	P	T	A	P	T	A	P	T	A	P	T	A	P	T	A	P	T
	17	28	45	3	22	25	0	16	16	5	16	21	0	14	14	4	6	10
	10	33	43	4	16	20	3	12	15	7	10	17	2	8	10	0	3	3
	18	29	47	8	17	25	5	16	21	2	13	15	1	4	5	2	3	5
	14	38	52	11	18	29	0	5	5	8	12	20	2	19	21	0	7	7
	9	36	45	5	19	24				31	15	18	8	11	19			
	8	28	36	12	24	36				4	6	10	3	5	8			
Mean	12.7	32	44.7	7.2	19.3	26.5	2	12.25	14.25	4.8	12	16.8	2.66	10.16	12.83	1.5	4.75	6.25
±s.d.	±4.27	±4.34	±5.24	±3.76	±3.07	±5.47	±2.45	±5.19	±6.7	±2.32	±3.63	±3.97	±2.8	±5.71	±6.31	±1.91	±2.06	±2.99

Dose of infection: 200 T.spiralis larvae

Statistical analysis (total recovery)

Group 1 day 6 vs 8 = P<0.001
8 vs 10 = P<0.02
6 vs 10 = P<0.001

Group 2 day 6 vs 8 = N.S.
8 vs 10 = N.S.
6 vs 10 = P < 0.01

Group 1 day 6 vs group 2 day 6 = P<0.001
Group 1 day 8 vs group 2 day 8 = P<0.01
Group 1 day 10 vs group 2 day 10 = N.S.

A: Anterior part of the small intestine
P: Posterior part of the small intestine
T: Total worms recovered
n: number of mice used

Adult establishment (n=5)
6 days post-infection

	A	P	T
Mean	45	12.4	57.4
± s.d.	±5.61	±3.29	±5.68

Table APX.1.9 T.spiralis infection in young mice (3 weeks old). Duration of protective immunity gained from the mothers.

Number of worms established in the small intestine																		
Group 1. Born of naive, suckling naive									Group 2 Born of immune, suckling immune									
Days post-infection									Days post-infection									
6 (n=18)			8 (n=18)			10 (n=12)			6 (n=18)			8 (n=18)			10 (n=12)			
A	P	T	A	P	T	A	P	T	A	P	T	A	P	T	A	P	T
35	6	41	20	9	29	22	13	35	10	5	15	3	0	3	0	0	0
39	9	48	14	12	26	7	10	17	5	5	10	2	10	12	0	0	0
29	16	45	28	16	44	3	4	7	4	2	6	4	6	10	1	3	4
21	15	36	12	10	22	5	0	5	11	6	17	6	12	18	10	7	17
20	41	61	1	7	8	4	0	4	9	3	12	3	11	14	0	0	0
8	34	42	2	8	10	3	15	18	0	8	8	15	3	18	0	0	0
38	30	68	2	22	24	7	10	17	18	8	26	3	4	7	12	7	19
15	9	24	24	17	41	11	2	13	13	7	20	18	10	28	4	1	5
40	11	51	3	15	18	3	0	3	7	15	22	8	16	24	2	1	3
35	21	56	13	24	37	1	3	4	8	6	14	0	0	0	7	10	17
40	12	52	18	12	30	4	4	8	7	7	14	20	7	27	0	0	0
8	24	32	13	6	19	2	1	3	13	10	23	1	10	11	1	2	3
16	9	25	5	7	12				18	4	22	4	0	4			
17	12	29	5	10	15				6	3	9	5	3	8			
36	8	44	4	0	4				15	3	18	2	11	13			
15	12	27	17	12	29				6	12	18	3	6	9			
24	16	40	5	3	8				7	4	11	1	3	4			
14	26	39	1	0	1				16	5	21	0	2	2			
Mean	25	17.22	42.22	10.39	10.55	20.94	6	5.16	11.16	9.61	6.27	15.88	5.44	6.33	11.77	3.08	2.58	5.66
± s.d	±11.47	±9.92	±12.41	±8.46	±6.62	±12.62	±5.72	±5.39	±9.47	±5.03	±3.39	±5.79	±6.03	±4.77	±8.43	±4.27	±3.47	±7.46

Dose of infection 200 T.spiralis larvae

Statistical analysis (total recovery)

Group 1, day 6 vs day 8 = P<0.001
8 vs day 10 = P<0.05
6 vs day 10 = P<0.001
Group 2, day 6 vs day 8 = N.S.
8 vs day 10 = N.S.
6 vs day 10 = P<0.001

A : anterior part of the small intestine
P : posterior " " " " "
T : total worms recovered
n : number of mice used

Adult establishment (n = 5)
6 days post-infection

	A	P	T
Mean	49.4	12.6	62
± s.d.	± 3.04	± 2.97	± 4.85

Group 1 day 6 vs group 2 day 6 = P<0.001
Group 1 day 8 vs group 2 day 8 = P<0.02
Group 1 day 10 vs group 2 day 10 = N.S.

Table APX 1.10 T.spiralis infection in young mice (4 weeks old).
Duration of protective immunity gained from the mothers.

Number of worms established in the small intestine																		
Group 1 Born of naive, suckling naice									Group 2 Born of immune, suckling immune									
Days post-infection									Days post-infection									
6 (n=18)			8 (n=18)			10 (n=12)			6 (n=18)			8 (n=18)			10(n=12)			
A	P	T	A	P	T	A	P	T	A	P	T	A	P	T	A	P	T	
30	13	34	21	7	28	10	9	19	24	7	31	2	0	2	0	0	0	
39	23	62	11	21	32	10	21	31	2	4	6	1	0	1	0	0	0	
33	14	47	24	19	43	4	5	9	28	7	35	3	2	5	0	1	1	
33	23	56	29	27	56	9	2	11	12	9	21	0	1	1	0	0	0	
27	13	40	4	10	14	3	0	3	19	6	25	3	0	3	2	0	2	
35	21	56	7	15	22	12	3	15	24	21	45	4	2	6	0	0	0	
53	21	74	14	12	26	4	0	4	25	13	38	0	0	0	4	3	7	
25	28	53	15	5	17	14	2	16	25	11	36	4	2	6	0	2	2	
27	25	52	23	13	36	16	23	28	21	16	37	0	7	7	0	0	0	
36	19	55	14	12	26	4	5	9	18	18	36	5	7	12	1	6	7	
33	27	60	11	9	20	0	1	1	20	18	38	8	14	22	4	2	6	
37	20	57	24	13	37	4	4	8	9	12	21	31	14	45	3	0	3	
21	38	59	10	20	30				23	9	32	16	5	21				
6	25	31	16	12	28				34	15	49	4	3	7				
25	21	46	14	0	14				20	3	23	1	4	5				
29	27	56	1	4	5				15	6	21	0	0	0				
24	19	43	23	16	39				28	3	31	2	4	6				
48	10	58	2	0	2				17	4	21	2	0	2				
Mean 31.16	21.5	52.66	14.44	11.94	26.38	7.5	5.33	12.83	20.22	10.11	30.33	4.77	3.61	8.38	1.17	1.16	2.33	
s.d±10.13	±6.65	±9.74	±8.18	±7.21	±13.45	±5.0	±6.09	±9.45	±7.52	±5.66	±10.39	±7.57	±4.43	±11.12	±1.64	±1.85	±2.8	

Dose of infection 200 T.spiralis larvae

Statistical analysis (total recovery)

Group 1 day 6 vs 8 = P<0.001
8 vs 10 = P<0.01
6 vs 10 = P<0.001
Group 2 day 6 vs 8 = P<0.001
8 vs 10 = P<0.05
6 vs 10 = P<0.001

Group 1 day 6 vs group 2 day 6 = P<0.001
Group 1 day 8 vs group 2 day 8 = P<0.001
Group 1 day 10 vs group 2 day 10 = P<0.001

Adult establishment (n=5)
6 day post-infection

	A	P	T
Mean	50.5	13.25	60.75
± s.d.	±6.56	±2.22	±5.19

A : anterior part of the small intestine
P : posterior part of the small intestine
T : total worms recovered
n : number of mice used

Table APX.1.11 T. spiralis infection in mice (6 weeks old).
Duration of protective immunity gained from the mothers.

Number of worms established in the small intestine																				
Group 1 Born of naive, suckling naive									Group 2 Born of immune, suckling immune											
Days post-infection									Days post-infection											
6 (n=15)			8 (n=15)			10 (n=10)			6 (n=15)			8(n=15)			10 (n=10)					
A	P	T	A	P	T	A	P	T	A	P	T	A	P	T	A	P	T			
20	28	48	21	13	34	7	7	14	39	6	45	30	9	39	20	6	26			
35	16	51	20	19	39	17	9	26	28	12	40	26	15	41	18	3	21			
25	25	50	21	24	45	10	4	14	38	8	46	22	20	42	5	10	15			
36	21	57	8	32	40	7	6	13	29	13	42	22	5	27	24	2	26			
48	10	58	21	24	45	19	13	32	42	11	53	23	15	38	16	1	17			
42	16	58	6	21	27	13	15	28	49	6	55	35	17	52	22	16	38			
39	5	44	24	3	27	6	3	9	38	5	43	25	9	34	6	11	17			
32	18	50	18	3	21	12	2	14	22	15	37	22	13	35	8	8	16			
52	8	60	11	5	16	6	6	12	36	18	54	30	6	36	15	6	21			
61	8	69	19	13	32	6	10	16	40	10	50	26	6	32	19	3	22			
36	12	48	13	15	28				48	3	51	36	2	38						
46	11	57	36	2	38				46	10	56	24	3	27						
19	15	34	20	6	26				28	15	43	22	17	39						
30	13	43	23	4	27				35	17	52	37	3	40						
20	26	46	12	4	16				29	16	45	25	7	32						
Mean 30.06	15.47	51.53	18.2		12.53	30.73	10.3	7.5	17.8	36.46	11	47.46	27	9.8	36.8	15.3	6.6	21.9		
s.d ±12.37	±6.99	±8.52	±7.43		±9.63	±9.36	±4.8	±4.24	±7.84	±8.02	±4.69	±5.93	±5.33	±5.89	±6.26	±6.75	±4.71	±6.87		

Dose of infection 200 T. spiralis larvae

Statistical analysis (Total recovery)

Group 1 day 6 vs 8 = P<0.001
day 8 vs 10 = P<0.001
day 6 vs 10 = P<0.001

Group 2 day 6 vs 8 = P<0.001
8 vs 10 = P<0.001
6 vs 10 = P<0.001

Group 1 day 6 vs group 2 day 6 = N.S.
Group 1 day 8 vs group 2 day 8 = P<0.05
Group 1 day 10 vs group 2 day 10 = N.S.

A : anterior part of the small intestine
P : posterior part of the small intestine
T : total worms recovered
n : number of mice used.

Adult establishment (n = 5)
6 days post-infection

	A	P	T
Mean	47.6	12	59.6
± s.d.	±6.69	±3.39	±8.96

Table APX 1.12 T. spiralis infection in mice (9 weeks old).
Duration of protective immunity gained from mothers

Number of worms established in the small intestine																		
Group 1 Born of naive, suckling naive									Group 2 Born of immune, suckling immune									
Days post-infection									Days post-infection									
6 (n=15)			8(n=15)			10(n=10)			6(n=15)			8(n=15)			10(n=10)			
A	P	T	A	P	T	A	P	T	A	P	T	A	P	T	A	P	T	
49	16	65	32	19	51	21	12	33	55	9	64	27	9	36	1	10	11	
49	7	56	38	5	43	4	6	10	40	29	69	21	13	34	3	0	3	
38	10	48	27	12	39	0	5	5	39	12	51	41	2	43	14	9	23	
49	8	57	20	8	28	16	7	23	47	15	62	16	14	30	2	2	4	
36	19	55	14	5	19	0	0	0	47	8	55	14	23	37	2	5	7	
41	12	53	19	9	28	0	1	1	62	9	71	7	5	12	5	5	10	
61	12	73	14	18	32	0	0	0	39	28	67	45	7	52	17	10	27	
50	6	56	6	10	16	12	6	18	32	22	54	18	5	23	2	11	13	
22	12	34	20	13	33	15	3	18	49	13	62	52	9	61	0	0	0	
28	18	46	3	0	3	0	3	3	41	6	47	26	4	*30	0	5	5	
33	19	52	10	16	26				39	11	50	18	13	31				
40	14	54	16	13	29				32	18	50	13	11	24				
48	8	56	28	3	31				35	20	55	18	8	26				
66	7	73	36	12	48				41	6	47	20	8	28				
32	21	53	16	3	19				53	9	62	19	6	25				
Mean 42.8	12.6	55.4	19.93	9.73	29.66	6.8	4.3	11.1	43.4	14.33	57.73	23.67	9.13	32.8	4.6	5.7	10.3	
s.d±11.97	±5.01	±9.78	±10.44	±5.72	±12.59	±8.29	±3.71	±11.39	±8.62	±7.5	±8.04	±12.69	±5.19	±12.11	±5.96	±4.16	±8.73	

Dose of infection 200 T.spiralis larvae

Statistical analysis (total recovery)

Group 1 day 6 vs 8 = P<0.001
8 vs 10 = P<0.001
6 vs 10 = P<0.001
Group 2 day 6 vs 8 = P<0.001
8 vs 10 = P<0.001
6 vs 10 = P<0.001

Group 1 day 6 vs group 2 day 6 = N.S.
Group 1 day 8 vs group 2 day 8 = N.S.
Group 1 day 10 vs group 2 day 10 = N.S.

A : anterior part of the small intestine
P : posterior part of the small intestine
T : total worms recovered
n : number of mice used.

Table APX 1.13 T. spiralis infection in young mice. Route of protection from immune mothers. Nature of immunization:- Mothers infected with 200 larvae 16 and 10 weeks before parturition.

Groups	infants infected with 200 larvae at 2 weeks of age		*Mean intestinal worm count ± s.d. Days post-infection 6 p.i	8 p.i	10 p.i
1	Born of immune	A	4.22±1.86	3 ± 2.22	1.25±1.42
	Suckling immune	P	10.11±4.56	8.61 ± 5.31	3.08±5.19
		T	14.33±4.89	11.61 ± 7.01	4.33±6.06
2	Born of immune	A	11.55±4.13	5.77 ± 4.24	4.41±3.82
	Suckling naive	P	33.38±6.58	17.77 ± 7.39	11.25±6.96
		T	44.94±7.12	23.55 ± 8.24	15.66±9.84
3	Born of naive	A	4.39±2.14	3.27 ± 2.93	2.25±2.56
	Suckling immune	P	11.77±5.15	9.55 ± 6.45	5.58±5.07
		T	16.16±6.64	12.83 ± 8.49	7.83±6.79
4	Born of naive	A	12.11±3.49	4.83 ± 3.52	1.41±1.93
	Suckling naive	P	31.11±7.57	20.55 ± 8.71	10.91±4.68
		T	43.22±8.93	25.38 ± 9.4	12.33±6.27

*Each value represent a mean of 18 or 12 mice.
A : anterior part of the small intestine
P : posterior part of the small intestine
T : total worms recovered.
s.d : standard deviation

Table APX 1.14 T.spiralis infection in young mice. Route of protection from immune mothers. Nature of immunization: Mothers immunized with saline soluble antigen + complete Freund's adjuvant 8 & 4 weeks before parturition.

Groups	Infants infected with 200 larvae at 2 weeks of age	Mean intestinal worm count * mean ± s.d. Days Post-Infection								
		Day 6 P.i (n=12			Day 8 P.i (n=12)			Day 10 P.i (n=8)		
		A	P	T	A	P	T	A	P	T
1	Born of immune Suckling immune	3.91 ±3.47	19.5 ±6.47	23.41 ±7.3	1.83 ±1.52	15.42 ±6	17.25 ±6.18	0.37 ±0.51	7 ±7.44	7.37 ±7.26
2	Born of immune Suckling naive	10.59 ±6.69	36.41 ±9.68	47.0 ±8.44	5.58 ±5.61	27.83 ±11.93	33.41 ±13.94	0.87 ±1.24	19 ±10.69	19.87 ±11.61
3	Born of naive Suckling immune	2.92 ±2.77	19.41 ±6.02	22.33 ±7.49	1.33 ±1.92	18 ±7.08	19.33 ±7.03	0.37 ±0.74	9.75 ± 8.17	10.12 ± 8.35
4	Born of naive Suckling naive	9.83 ±3.51	35.75 ±11.66	45.58 ±8.77	4.41 ±4.69	25.44 ±8.62	29.85 ±11.03	1.12 ±1.55	15.75 ±7.32	16.87 ±7.91

A: anterior part of the small intestine
P: posterior part of the small intestine
T: total worms recovered

Group E: Adult mice (10-12 wks old) infected with 200 larvae as control for establishment.

* each mean represents either 8 or 12 mice.
n: number of mice used

Group E (n = 5)
Worms recovered at day 6 P.i mean ± s.d.

	A	P	T
1.	50	14	64
2.	37	20	57
3.	55	16	71
4.	53	11	64
5.	41	13	54
Mean	47.2	14.8	62
± s.d.	±7.82	±3.42	±6.67

Table APX 1.15 Trichinella spiralis infection in young mice.
The duration of intake of immune milk and protection in 2 weeks old mice.
Time of fostering before infection 6 hours (Group 1 ref. Table 1.18)

		Worms recovered on day 5 post-infection											
	No.	TREATMENT A			TREATMENT B			TREATMENT C			TREATMENT D		
		A	P	T	A	P	T	A	P	T	A	P	T
first litter (no. of infants = 6)	1	6	13	19	4	16	20				10	35	45
	2	13	16	29	0	14	14				11	37	48
	3	1	19	20	1	34	35				11	32	43
	4	5	12	17	4	17	21	NOT DONE			6	40	46
	5	5	9	14	7	23	30				6	30	36
	6	0	32	32	9	8	17				7	17	24
	Mean	5	16.83	21.83	4.17	18.66	22.83				8.5	31.83	40.33
	±s.d.	±4.6	±8.18	±7.08	±3.43	±8.93	±8.03				±2.42	±8.08	±9.0
second litter no.of infants = 6)	1	0	28	28	2	23	25						
	2	2	18	20	4	7	11						
	3	1	25	26	3	19	22						
	4	7	19	26	0	16	16	NOT DONE			NOT DONE		
	5	1	28	29	4	16	20						
	6	4	15	19	3	9	12						
	Mean	2.5	22.16	24.66	2.66	15.0	17.66						
	± s.d.	±2.59	±5.56	±4.17	±1.5	±6.03	±5.61						
Mean ± s.d. of both litters		3.75 ±3.79	19.5 ±7.22	23.25 ±5.73	3.42 ±2.64	16.83 ±7.51	20.25 ±7.14				8.5 ±2.42	31.83 ±8.08	40.33 ±9.0

n = number of mice used. s.d. = standard deviation
A = anterior part of the small intestine
P = posterior part of the small intestine
T = total worms recovered from both parts
2 week infants fostered to immune and naive mothers.

Treatments:

A : 2 weeks infants born to naive mothers and suckling immune mothers for 6 hours only before infection of the infants with 200 larvae.

B : 2 weeks infants born to immune mothers and suckling naive mothers for 6 hours only before infection of of infants with 200 larvae.

C : 2 weeks infants born to and suckling their immune mothers before infection of the infants with 200 larvae.

D : 2 weeks infants born to and suckling their naive mothers before infection with 200 larvae.

E : Adult mice infected with 200 larvae as control for establishment.

TREATMENT E

Worms recovered on day 6 post-infection

	A	P	T
1	43	12	55
2	43	4	47
3	45	11	56
4	51	4	55
5	39	6	45
Mean	44.2	7.4	51.6
± s.d.	±4.38	±3.85	±5.18

Table APX 1.16 *Trichinella spiralis* infection in young mice.
The duration of intake of immune milk and protection in 2 weeks old mice given 200 larvae.
Time of fostering before infection 6-8 hours (Group 1 ref. Table 1.19)

	No.	Worms recovered on day 6 post-infection											
		TREATMENT A (n=5)			TREATMENT B (n=5)			TREATMENT C (n=7)			TREATMENT D (n=7)		
		A	P	T	A	P	T	A	P	T	A	P	T
first litter	1	1	19	20	7	25	32	2	25	27	8	25	33
	2	3	44	47	13	30	43	0	23	23	7	55	62
	3	3	30	33	2	25	27	6	24	30	4	18	22
	4	7	25	32	8	25	33	1	24	25	15	26	41
	5	9	35	44	7	25	32	3	23	26	6	55	61
	6							1	30	31	12	40	52
	7							1	38	39	19	59	78
	Mean ± s.d.	4.6 ±3.28	30.6 ±9.55	35.2 ±10.75	7.4 ±3.91	26.0 ±2.23	33.4 ±5.85	2.0 ±2.0	26.71 ±5.52	28.71 ±5.31	10.14 ±5.39	39.71 ±16.9	49.85 ±19.17
		(n=7)			(n=4)			(n=6)			(n=6)		
second litter	1	1	35	36	5	27	32	0	30	30	11	37	48
	2	4	36	40	7	14	21	2	26	28	4	54	58
	3	5	18	23	3	43	46	6	19	25	13	40	53
	4	5	45	50	4	28	32	0	16	16	10	41	51
	5	2	23	25				2	32	34	0	60	60
	6	4	41	45				4	19	23	2	34	36
	7	2	20	22									
	Mean	3.28	31.14	34.42	4.75	28.0	32.75	2.33	23.67	26.00	6.67	44.33	51.00
	±s.d.	±1.6	±10.7	±11:27	±1.7	±11.86	±10.24	±2.34	±6.59	±6.22	±5.35	±10.28	±8.57
	Mean ± s.d. of both litters	3.83 ±2.4	30.92 ±9.8	34.75 ±10.55	6.22 ±3.27	26.89 ±7.51	33.11 ±7.52	2.15 ±2.07	25.31 ±5.99	27.46 ±5.68	8.54 ±5.46	41.84 ±13.88	50.38 ±14.66

n = number of mice used. s.d. = standard deviation
A = anterior part of the small intestine
P = posterior part of the small intestine
T = total works recovered from both parts.
2 weeks infants fostered to immune and naive mothers.

Treatments:

A : 2 weeks infants born to naive mothers and suckling immune mothers for 6-8 hours only before infection of the infants with 200 larvae.

B : 2 weeks infants born to immune mothers and suckling naive mothers for 6-8 hours only before infection of the infants with 200 larvae.

C : 2 weeks infants born to and suckling their immune mothers before infection of the infants with 200 larvae.

D : 2 weeks infants born to and suckling their naive mothers before infection with 200 larvae.

E : Adult mice infected with 200 larvae as control for establishment.

TREATMENT E
Worms recovered on day 6 post-infection

	A	P	T
1	52	32	84
2	57	14	71
3	54	29	83
4	69	16	85
Mean	58	22.75	80.75
±s.d.	±7.61	±9.07	±6.55

Table APX 1.17. Trichinella spiralis infection in young mice.
The duration of intake of immune milk and protection in 2 weeks old mice given 200 larvae.
Time of fostering before infection, 12 hours (Group 2 ref. Table 1.18)

	No.	Worms recovered on day 6 post-infection											
		TREATMENT A			TREATMENT B			TREATMENT C			TREATMENT D		
		A	P	T	A	P	T	A	P	T	A	P	T
first litter (no.of infants = 6)	1	4	22	26	8	27	35	7	17	24	13	36	49
	2	4	13	17	8	26	34	4	13	17	6	29	35
	3	7	24	31	4	24	28	2	20	22	9	36	45
	4	11	22	33	11	34	45	5	11	16	7	16	23
	5	7	9	16	4	22	26	9	14	23	8	28	36
	6	9	12	21	5	6	11	5	12	17	8	30	38
	Mean	7.0	17.0	24.0	6.67	23.16	29.83	5.33	14.5	19.83	8.5	29.16	37.66
	±s.d.	±2.76	±6.39	±7.16	±2.8	±9.34	±11.37	±2.42	±3.39	±3.54	±2.42	±7.33	±9.02
second litter (no.of infants = 6)	1	7	16	23	4	17	21				10	32	42
	2	6	24	30	9	19	28				17	19	36
	3	1	11	12	12	18	30				7	27	34
	4	4	18	22	6	6	12	NOT DONE			1	38	39
	5	2	23	25	7	28	35				*-	-	-
	6	5	22	27	5	4	9				-	-	-
	Mean	4.16	19.0	23.16	7.17	15.33	22.5				8.75	29.0	37.75
	±s.d.	±2.31	±4.97	±6.17	±2.93	±8.93	±10.36				±6.65	±8.04	±3.5
Mean ± s.d. of both litters		5.58 ±2.84	18.0 ±5.55	23.58 ±6.39	6.91 ±2.74	19.25 ±9.63	26.16 ±11.06	5.33 ±2.42	14.5 ±3.39	19.83 ±3.54	8.6 ±4.24	29.1 ±7.17	37.7 ±7.02

*only 4 infants available in this litter. s.d. standard deviation
A = anterior part of the small intestine T = total worms recovered from both parts.
P = posterior part of the small intestine
2 weeks infants fostered to immune and naive mothers.

Treatments:

A : 2 weeks infants born to naive mothers and suckling immune mothers for 12 hours only before infection of the infants with 200 larvae.

B : 2 weeks infants born to immune mothers and suckling naive mothers for 12 hours only before infection of the infants with 200 larvae.

C : 2 weeks infants born to and suckling their immune mothers before infection of the infants with 200 larvae.

D : 2 weeks infants born to and suckling their naive mothers before infection with 200 larvae.

E : Adult mice infected with 200 larvae as control for establishment.

TREATMENT E

Worms recovered on day 6 post infection

	A	P	T
1	38	5	43
2	62	2	64
3	40	21	61
4	41	8	49
5	43	11	54
Mean	44.8	9.4	54.2
±s.d.	±9.78	±7.3	±8.58

Table APX 1.18 Trichinella spiralis infection in young mice. The duration of intake of immune milk and protection in 2 weeks old mice given 200 larvae. Time of fostering before infection, 1 day (Group 3 ref. Table 1.18)

	No.	Worms recovered on day 6 post-infection											
		TREATMENT A			TREATMENT B			TREATMENT C			TREATMENT D		
		A	P	T	A	P	T	A	P	T	A	P	T
first litter (no. of infants = 6)	1	6	19	25	3	31	34	3	12	15	6	46	52
	2	2	20	22	5	20	25	2	17	19	6	24	30
	3	3	25	28	3	36	39	0	20	20	4	28	32
	4	4	21	25	10	22	32	3	9	12	4	36	40
	5	4	28	32	4	38	42	1	21	22	3	33	36
	6	4	14	18	9	31	40	4	13	17	4	38	42
	Mean (6)	3.83	21.17	25.0	5.67	29.66	35.33	2.17	15.33	17.5	4.5	34.16	38.66
	±s.d. (6)	±1.33	±4.87	±4.81	±3.08	±7.28	±6.31	±1.47	±4.76	±3.61	±1.22	±7.75	±7.96
second litter (no. of infants = 6)	1	1	7	8	2	26	28	0	22	22	3	42	45
	2	0	15	15	0	19	19	1	24	25	2	38	40
	3	2	12	14	0	22	22	4	9	13	2	39	41
	4	2	15	17	4	16	20	1	12	13	3	38	41
	5	1	15	16	4	34	38	2	21	23	*-	-	-
	6	3	20	23	6	26	32	0	17	17	-	-	-
	Mean	1.5	14.0	15.5	2.67	23.83	26.5	1.33	17.5	18.83	2.5	39.25	41.75
	±s.d.	±1.04	±4.28	±4.84	±2.42	±6.33	±7.52	±1.5	±5.95	±5.23	±0.57	±1.89	±2.21
	Mean	2.67	17.58	20.25	4.16	26.75	30.91	1.75	16.41	18.16	3.7	36.2	39.9
	±s.d. of both litters	±1.67	±5.75	±6.77	±3.06	±7.18	±8.07	±1.48	±5.26	±4.34	±1.41	±6.44	±6.27

*only 4 infants available in this litter. s.d. = standard deviation
A = anterior part of the small intestine
P = posterior part of the small intestine
T = total worms recovered from both parts.
2 weeks infants fostered to immune and naive mothers.
Treatments:

A : 2 weeks infants born to naive mothers and suckling immune mothers for 1 day only before infection of the infants with 200 larvae.

B : 2 weeks infants born to immune mothers, and suckling naive mothers for 1 day only before infection of the infants with 200 larvae.

C : 2 weeks infants born to and suckling their immune mothers before infection of the infants with 200 larvae.

D : 2 weeks infants born to and suckling their naive mothers before infection of the infants with 200 larvae

E : Adult mice infected with 200 larvae as control for establishment.

TREATMENT E
worms recovered on day 6 post-infection

	A	P	T
1	31	19	50
2	34	13	47
3	36	20	56
4	27	17	44
5	38	18	56
Mean	33.2	17.4	50.6
+s.d	±4.32	±2.7	±5.36

Table APX 1.19 Trichinella spiralis infection in young mice. The duration of intake of immune milk and protection in 2 weeks old mice given 200 larvae. Time of fostering before infection, 3 days (Group 4 ref. Table 1.18)

	No.	Worms recovered on day 6 post-infection											
		TREATMENT A			TREATMENT B			TREATMENT C			TREATMENT D		
		A	P	T	A	P	T	A	P	T	A	P	T
first litter (no. of infants =6)	1	2	17	19	4	16	20	0	11	11	3	24	27
	2	3	7	10	1	18	19	0	18	18	5	26	31
	3	1	14	15	3	20	23	2	18	20	6	31	37
	4	2	8	10	1	23	24	0	13	13	7	28	35
	5	1	5	6	1	26	27	0	9	9	2	33	35
	6	1	8	9	3	27	30	1	11	12	6	36	42
	Mean ±s.d.	1.67 ±0.82	9.83 ±4.62	11.5 ±4.67	2.16 ±1.32	21.67 ±4.41	23.83 ±4.16	0.5 ±0.83	13.33 ±3.82	13.83 ±4.26	4.83 ±1.94	29.67 ±4.5	34.5 ±5.12
second litter (no. of infants =6)	1	0	10	10	3	29	32	0	5	5	2	29	31
	2	4	11	15	14	17	31	2	19	21	4	28	32
	3	1	11	12	1	21	22	2	3	5	0	33	33
	4	2	5	7	7	9	16	0	3	3	6	31	37
	5	3	13	16	9	16	25	1	9	10	5	31	36
	6	1	19	20	3	18	21	0	9	9	11	26	37
	Mean ±s.d.	1.83 ±1.47	11.5 ±4.54	13.33 ±4.63	6.17 ±4.83	18.33 ±6.56	24.5 ±6.15	0.83 ±0.98	8.0 ±6.03	8.83 ±6.52	4.66 ±3.78	29.67 ±2.5	34.33 ±2.66
Mean ± s.d. of both litters (n=12)		1.75 ±1.13	10.66 ±4.45	12.41 ±4.54	4.16 ±3.97	20.0 ±5.6	24.16 ±5.02	0.67 ±0.89	10.66 ±5.56	11.33 ±5.87	4.75 ±2.86	29.66 ±3.47	34.41 ±3.89

s.d. = standard deviation n = number of mice used
A = anterior part of the small intestine
P = posterior part of the small intestine
T = Total worms recovered from both parts.
2 weeks infants fostered to immune and naive mothers
Treatments:

A : 2 weeks infants born to naive mothers and suckling immune mothers for 3 days only before infection of the infants with 200 larvae.

B : 2 weeks infants born to immune mothers and suckling naive mothers for 3 days only before infection of the infants with 200 larvae.

C : 2 weeks infants born to and suckling their immune mothers before infection of the infants with 200 larvae.

D : 2 weeks infants born to and suckling their naive mothers before infection of the infants with 200 larvae.

E : Adult mice infected with 200 larvae as control for establishment.

TREATMENT E
Worms recovered on day 6 post-infection

	A	P	T
1	30	12	42
2	46	8	54
3	38	4	42
4	28	18	46
5	38	12	50
Mean	36.0	10.8	46.8
±s.d.	±7.21	±5.21	±5.21

Table APX 1.20 *Trichinella spiralis* infection in young mice.
The duration of intake of immune milk and protection in 2 weeks old mice given 200 larvae.
Time of fostering before infection, 5 days (Group 5 ref. Table 1.18)

		Worms recovered on day 6 post-infection											
	No.	TREATMENT A			TREATMENT B			TREATMENT C			TREATMENT D		
		A	P	T	A	P	T	A	P	T	A	P	T
first litter (No. of infants =6)	1	0	9	9	5	20	25	5	18	23	1	31	32
	2	0	17	17	9	25	34	2	22	24	4	29	33
	3	0	13	13	4	29	33	5	15	20	3	33	36
	4	1	14	15	2	20	22	0	5	5	0	36	36
	5	2	18	20	8	13	21	1	11	12	7	29	36
	6	1	6	7	7	16	23	2	16	18	6	34	40
	Mean ±s.d.	0.67 ±0.82	12.83 ±4.62	13.5 ±4.89	5.83 ±2.63	20.5 ±5.82	26.33 ±5.71	2.5 ±2.07	14.5 ±5.89	17.0 ±7.26	3.5 ±2.73	32.0 ±2.82	35.5 ±2.81
second litter (no. of infants =6)	1	0	1	1	0	18	18	4	13	17	11	19	30
	2	1	19	20	2	33	35	2	20	22	2	25	27
	3	3	8	11	3	26	29	3	14	17	5	22	27
	4	1	22	23	3	30	33	7	11	18	3	19	22
	5	2	15	17	4	18	22	6	16	22	5	26	31
	6	7	14	21	0	16	16	1	12	13	6	31	37
	Mean ±s.d.	2.33 ±2.5	13.17 ±7.63	15.5 ±8.24	2.0 ±1.67	23.5 ±7.14	25.5 ±7.96	3.83 ±2.31	14.33 ±3.26	18.16 ±3.73	5.33 ±3.14	23.67 ±4.63	29.0 ±5.02
	Mean ±s.d. of both litters (n=12)	1.5 ±1.97	12.0 ±6.01	14.5 ±6.54	3.91 ±2.9	22.0 ±6.41	25.91 ±6.62	3.17 ±2.21	14.41 ±4.54	17.58 ±5.45	4.42 ±2.97	27.83 ±5.68	32.25 ±5.15

n = number of mice used s.d. = standard deviation
A = anterior part of the small intestine
P = posterior part of the small intestine
T = total worms recovered from both parts
2 weeks infants fostered to immune and naive mothers

Treatments:

A : 2 weeks infants born to naive mothers and suckling immune mothers for 5 days only before infection of the infants with 200 larvae.

B : 2 weeks infants born to immune mothers and suckling naive mothers for 5 days only before infection of the infants with 200 larvae

C : 2 weeks infants born to and suckling their immune mothers before infection of the infants with 200 larvae.

D : 2 weeks infants born to and suckling their naive mothers before infection of the infants with 200 larvae.

E : Adult mice infected with 200 larvae as control for establishment

TREATMENT E

Worms recovered on day 6 post-infection

	A	P	T
1	35	18	53
2	30	17	47
3	35	24	59
4	28	20	48
5	36	16	52
Mean	32.8	19.0	51.8
±s.d.	±3.56	±3.16	±4.76