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CONCOMITANT INFECTIONS: T. SPIRALIS & T. LEWISI IN RATS

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Ph.D
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IMMUNOLOGICAL ASPECTS OF CONCOMITANT INFECTIONS WITH THE PARASITES *TRICHINELLA SPIRALIS* AND *TRYPANOSOMA LEWISI* IN THE RAT

BY

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ABSTRACT

Immunological aspects of concomitant infections of T. spiralis and T. lewisi have been studied in the rat. Immunopotentiation towards T. lewisi occurs when rats are challenged with trypanosomes after an infection with T. spiralis, such that the development of peak trypanosome parasitemia is inhibited in nematode-infected rats. Splenectomy prior to the challenge infection with T. lewisi abrogates this cross-protection. Immunopotentiation during Trichinellosis is dose dependent and transient.

Primary infections with T. spiralis do not enhance or suppress ablastic or trypanocidal antibody responses to T. lewisi. However, immunization with a soluble antigen extract of T. spiralis muscle larvae enhances trypanosome infections and suppresses acquired humoral immunity to T. lewisi. A comparative study of parasite antigens did not reveal any cross-reactivity.

The granulopectic activity of the RES is enhanced during Trichinellosis; this stimulation is correlated with the time and level of parasitism yielding a maximum immunopotentiation towards T. lewisi. Rats immunized with BCG show a similar potentiation of immunity to T. lewisi, suggesting the importance of a stimulation of non-specific cell-mediated immunity or RES activity in these interspecific interactions. Immunization with only the parenteral, newborn larval stages of T. spiralis also induces immunopotentiation in the rat.

A study of the phagocytic activity of peritoneal and splenic macrophages from nematode-infected rats did not demonstrate enhanced phagocytosis of trypanosomes by these cells in vitro.

Some possible mechanisms of immunopotentiation are discussed in relation to the life cycle of T. spiralis and acquired immunity to T. lewisi.

ABREGE

L'aspect immunologique d'une infection concomittante à partir de T. spiralis et T. lewisi a été étudié chez le rat en laboratoire. Une immunostimulation dirigée contre T. lewisi apparaît lorsque les rats sont infectés avec T. spiralis au préalable. Cette stimulation est remarquable puisqu'elle réussit à inhiber la phase principale de développement des trypanosomes. Par contre, la splénectomie des rats effectuée avant l'infection avec T. lewisi annule cette protection croisée. De plus, cette immunostimulation causée par T. spiralis est dépendante de la dose et transitoire.

Une infection primaire avec T. spiralis n'augmente pas et ne supprime pas l'activité de l'ablastine de même que l'activité des anticorps anti-trypanosome. Toutefois, des rats immunisés avec un extrait antigénique préparé à partir de larves musculaires de Trichinella supprime l'activité humorale du système immunologique contre T. lewisi, augmentant ainsi l'importance de l'infection. Une étude comparative des structures antigéniques des deux parasites ne révèle aucune activité croisée.

L'activité granulopectique de système réticuloendothélial (SRE) est augmentée durant la Trichinellose. Cette stimulation est en corrélation avec la phase de développement de T. lewisi stimulant la réponse immunitaire à son maximum. Des rats immunisés avec BCG montrent une protection similaire contre T. lewisi. Ce résultat met en évidence l'importance de la stimulation non-spécifique de la réponse cellulaire ou bien l'activité du SRE dans de tels systèmes. L'immunisation des rats à partir des larves de T. spiralis nouvellement nés peut induire une immunostimulation comparable.

L'activité phagocytaire étudiée *in vitro*, des macrophages péritonéaux et spléniques prélevés chez des rats infectés avec le nématode n'a pas démontrée une plus grande affinité pour *T. lewisi*.

Des méchanismes possibles pouvant expliquer cette immunostimulation sont proposés en tenant compte du cycle de vie de *Trichinella* et l'immunité acquise contre *T. lewisi*.

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S. J. ACKERMAN

PREFACE

This project was begun in 1973 as a result of the serendipitous discovery by my thesis supervisor, Dr. E. Meerovitch, that rats infected with Trichinella spiralis were sometimes refractory to a challenge infection with Trypanosoma lewisi. A preliminary study of this phenomenon, conducted in the fall of 1973, confirmed this effect and demonstrated that rats infected with T. spiralis for 30 days were partially protected against a challenge infection with T. lewisi. This cross-protection was manifested by a significant inhibition of the development of trypanosome parasitemias in concomitantly infected rats (Meerovitch and Ackerman 1974).

The research presented in this thesis was started in January 1974 and was completed in September 1976 at the Institute of Parasitology, McGill University. The dissertation is organized into 10 chapters. In order to provide the reader with an adequate background, a comprehensive review of the literature, completed in November 1976, is presented in the first four chapters. Chapter I is a general introduction to the subject of concomitant parasitic infections and discusses some of the important factors involved in an explanation of competitive, interspecific, host-parasite interactions that occur during multiple (parasite) species infections. Chapters II and III provide a review of a number of important aspects of the life cycle and immunobiology of Trichinella spiralis and Trypanosoma lewisi that are relevant to this thesis. The final review chapter (IV) presents a discussion of some of the factors involved in a stimulation of non-specific host resistance to parasitic infection and neoplasia.

The next five chapters (V-IX) present details of the experimental work on concomitant infections of T. spiralis and T. lewisi in the inbred rat. Each of these chapters has been organized into a separate introduction, materials and methods, results, and a pertinent discussion. It should be noted that chapters V-IX are in the process of being edited for submission to a number of scientific journals for publication.

Altered states of immunological responsiveness (immunosuppression and immunopotentiation) during experimental Trichinellosis have been investigated for the first time utilizing a model of concomitant infections with the hemoflagellate Trypanosoma lewisi, and the nematode Trichinella spiralis. Chapter V investigates the kinetics and some possible mechanisms of cross-protection between these parasites and demonstrates the dose dependency and transient nature of immunopotentiation in Trichinellosis. Cross-protection is not due to an antigenic cross-reactivity or the presence of non-specific agglutinins or lysins in the serum of infected rats. The granulopectic activity of the reticuloendothelial system (RES) is shown to be enhanced during Trichinellosis in the rat and this stimulation is correlated with the time and level of parasitism yielding maximum immunopotentiation towards T. lewisi. Splenectomy prior to the challenge infection with trypanosomes is shown to abrogate this cross-protection. Chapter VI explores the mechanism of immunopotentiation in this system and evidence is presented that cross-protection is not due to a functional alteration in the acquired humoral immune response of the rat to T. lewisi. Chapter VII investigates for the first time, the role of specific developmental stages of the life cycle of T. spiralis in immunopotentiation, and demonstrates a requirement for living parenteral, newborn larval and encysted

intracellular muscle stages in the induction of this phenomenon. Immunization of rats with a soluble antigen extract of T. spiralis muscle larvae does not stimulate cross-protection, and instead is shown to enhance trypanosome infections and to suppress the development of acquired humoral immunity to T. lewisi. In a comparative study presented in chapter VIII, rats immunized with BCG show a similar potentiation of immunity to T. lewisi, suggesting the importance of a stimulation of non-specific cell-mediated immunity or RES activity in these interspecific interactions. A study of the phagocytic activity of peritoneal and splenic macrophages from nematode-infected rats in chapter IX, however, failed to demonstrate a correlation between enhanced phagocytosis of trypanosomes by these cells *in vitro* and immunopotentiation towards T. lewisi *in vivo*. The concluding chapter (X) presents a summary and discussion of some possible mechanisms of immunopotentiation in relation to the life cycle of T. spiralis and acquired immunity to T. lewisi.

It is hoped that the research presented in this dissertation has done justice to an experimental approach that utilizes concomitant infections to investigate basic immunological phenomena in parasitism.

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The research presented in this thesis was conducted at the Institute of Parasitology of McGill University under the supervision of Dr. E. Meerovitch, to whom I am grateful. I was given total liberty in the conception and realization of my research project, and at any given moment, facilities, research funds, and constructive criticism were readily available.

I am most grateful to Dr. Gaetan Faubert for his enthusiastic interest in my research and progress, for valuable and illuminating scientific discussions and collaborations during the course of my research and degree program, and for providing a most excellent translation of the thesis abstract.

I am also grateful to Dr. C. E. Tanner for his time and interest as my acting supervisor during the sabbatical year of Dr. Meerovitch. I am equally grateful to Dr. J. McLaughlin for many stimulating discussions, constructive criticisms, and instruction in the preparation of parasite antigens, and immunoelectrophoretic and spectrophotometric techniques used in this study.

Special thanks are due to Dr. R. Harpur for many provocative discussions on experimental design and statistical analysis of results, to Mr. G. Bingham for his diligence and expertise in the maintenance of experimental animals, and to Ms. S. Dirienzo for her technical assistance in staining slides and taking spectrophotometer readings during the long gruelling hours required for the counting of trypanosomes and for the carbon clearance assay.

I am also indebted to Dr. N.A. Croll, director of the Institute

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Lastly, I would like to extend my thanks to each and every member of the Institute (staff, student, or friend) without whose continued support and interest, this work might not have progressed so well.

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To my devoted mother and father, my in-laws, Ruth and Claude, and my loving wife and daughter, Karen and Geneviève.

CHAPTER I

CONCOMITANT PARASITIC INFECTIONS: AN INTRODUCTION AND REVIEW

A. INTRODUCTION

The biology of the host-parasite relationship is highly complex. For most, if not all host-parasite relationships, further complexities are introduced by the presence of multiple (parasite) species infections. Concomitant infections are not uncommon, and it is generally recognized that, in its natural environment, the host is usually infected with more than one parasite species. It is only recently, however, that the parasitologist has become interested in the influence of parasitic infection on the ability of the host to resist secondary infection by an unrelated parasite species. Indeed, a detailed knowledge of parasite-mix, and the interspecific interactions that occur in the host, is essential to a comprehensive understanding of the phenomenon of parasitism.

Although many aspects of the immunobiology of single species infections are still not clearly understood, the use of experimental concomitant infections is gaining momentum as a tool for basic research in the immunology of parasites.

B. HOST-PARASITE INTERACTIONS IN CONCOMITANT PARASITIC INFECTIONS

There are numerous reports in the literature that deal with experimental concomitant infections (Table 1.1-1.3).*

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* Many of these reports are not directly relevant to this thesis. Some of the reports on experimental concomitant infections will be discussed in this and later chapters, but a comprehensive review of this field will not be presented.

TABLE 1.1 - 1.3

EXPERIMENTAL STUDIES ON CONCOMITANT PARASITIC INFECTIONS

+ = SYNERGISTIC EFFECTS - enhanced infections of one or both parasites (e.g. - enhanced pathogenicity or virulence, or immunosuppression).

- = ANTAGONISTIC EFFECTS - suppressed infections or growth and development of one or both parasites (e.g. = direct competition, antigenic cross reactions, immunopotentiation).

± = INCONCLUSIVE RESULTS or NO OBSERVABLE EFFECTS on either the host or the parasites.

TABLE 1.1 - EXPERIMENTAL STUDIES ON CONCOMITANT INFECTIONS WITH HELMINTHS

CONCOMITANT INFECTIONS	HOST	EFFECT	REFERENCES
Trichinella spiralis,			
Ancylostoma caninum	mice	–	Cox 1952; Goulson 1958
Ascaris suum	mice	–	Matov and Kamburov 1968
Hymenolepis diminuta	rat	–	Silver *et al.* 1976
Hymenolepis nana	mice	–	Larsh and Campbell 1952
Nippostrongylus brasiliensis	rat	–	Louch 1962; Sinski 1972
Schistosoma mansoni	mice	±	Weinman 1960
"	"	–	Jachowski and Bingham 1961
Strongyloides ratti	rat	–	Kazacos 1976
Trichuris muris	mice	–	Wakelin and Bruce 1974
Nippostrongylus brasiliensis,			
Angiostongylus cantonensis	rat	–	Kocan 1974
Ascaris suum	mice	–	Crandall *et al.* 1967
Nematospiroides dubius	mice	+	Jenkins 1975
Schistosoma mansoni	mice	–	Hunter *et al.* 1967
Strongyloides ratti	rat	–	Kazacos and Thorson 1975
Hymenolepis diminuta,			
Hymenolepis nana	mice	–	Heyneman 1953, 1962
Moniliformis dubius	rat	–	Holmes 1957, 1961, 1962
Nippostrongylus brasiliensis	mice	–	Larsh and Donaldson 1944
"	"	–	Morock and Roberts 1976
Cooperia oncophora,			
Cooperia pectinata	calves	–	Herlich 1965
Haemonchus contortus,			
Ostertagia circumcincta	calves	–	Turner *et al.* 1962
Trichostrongylus axei	calves	–	"
Trichostrongylus axei,			
Ostertagia circumcincta	calves	±	Turner *et al.* 1962

TABLE 1.2 - EXPERIMENTAL STUDIES ON CONCOMITANT INFECTIONS WITH PROTOZOA AND HELMINTHS

CONCOMITANT INFECTIONS	HOST	EFFECT	REFERENCES
Eimeria spp.,			
Cooperia punctata	calves	+	Davis *et al.* 1959
Ostertagia ostertagi	"	±	Davis *et al.* 1960a
Strongyloides papillosus	"	+	Davis *et al.* 1960b
Trichostrongylus colubriformis	"	±	Davis *et al.* 1960c
Entamoeba histolytica,			
Schistosoma mansoni	mice	+	Knight and Warren 1973
Toxocara canis	guinea pig	+	Krupp 1956
Trichuris muris	mice	+	Knight and Chew 1974
Plasmodium berghei,			
Schistosoma mansoni	vole	-	Yoeli 1956
"	mice	±	Lewinsohn 1975
Trichinella spiralis	mice	+	Bruce and Phillips 1974
Trichuris muris	mice	+	Phillips *et al.*1974
Salmonella typhimurium,			
Trichinella spiralis	mice	-	Brewer 1955
Salmonella typhi,			
Trichinella spiralis	rat	-	Weiner and Neely 1964
Toxoplasma gondii,			
Schistosoma mansoni	mice	-	Mahmoud *et al.* 1976
Trypanosoma brucei,			
Nippostrongylus brasiliensis	rat	+	Urquhart *et al.* 1972
Trichinella spiralis	mice	+	Bruce and Phillips 1974
Trichuris muris	mice	+	Phillips *et al.* 1974
Trypanosoma congolense,			
Nippostrongylus brasiliensis	rat	-	Simaren and Bammeke 1970

TABLE 1.2 - CONT'D.

CONCOMITANT INFECTIONS	HOST	EFFECT	REFERENCES
Trypanosoma cruzi,			
Schistosoma mansoni	mice	+,-	Kloetzel *et al.* 1971, 1973
Trichinella spiralis	mice	±	Campbell *et al.* 1976
Trypanosoma equiperdum,			
Schistosoma mansoni	mice	+	Ee-Siriporn and Wagner 1969
Trypanosoma lewisi,			
Hymenolepis diminuta	rat	-	Rigby and Chobotar 1966
"	"	-	Freeman *et al.* 1973
Nippostrongylus brasiliensis	rat	±	Ashley 1962

TABLE 1.3 - EXPERIMENTAL STUDIES ON CONCOMITANT INFECTIONS WITH PROTOZOA

CONCOMITANT INFECTIONS	HOST	EFFECT	REFERENCES
Plasmodium berghei,			
Toxoplasma gondii	mice	+	Strickland *et al.* 1972
Trypanosoma brucei	mice	+	Dallas 1976
Trypanosoma lewisi	rat	+	Hughes and Tatum 1956
"	"	±	Jackson 1959; Shmuel *et al.* 1975
Trypanosoma musculi	mice	+	Bungener 1975; Cox 1975
Toxoplasma gondii,			
Trypanosoma cruzi	mice	+	Kloetzel *et al.* 1975
Eimeria nieschulzi,			
Eimeria separata	rat	+	Duszynski 1972

It is unfortunate that in most of these studies, research has been mostly phenomenological and has provided only limited observations on basic parasitological parameters. Very few detailed studies in this field have been conducted in an attempt to unravel the mechanisms involved in an explanation of the synergistic or antagonistic interactions that occur during intercurrent infections.

The following factors are all probably involved in an explanation of the competitive interactions that occur during concomitant infections:

1) EXPLOITATION OF A COMMON HABITAT IN THE HOST LEADING TO DIRECT COMPETITION FOR AVAILABLE RESOURCES

(e.g. - competitive exclusion as a result of limited space (crowding effects), nutritional factors, etc., or direct antagonistic effects due to parasite-induced alterations in the habitat).

2) THE "REACTIVITY" OF THE IMMUNE RESPONSE

(e.g. - parasite-induced changes in immunological responsiveness, including enhanced non-specific inflammatory reactions or non-specific cell-mediated responses, immunosuppression and immunopotentiation).

3) ANTIGENIC CROSS-REACTION

(e.g. - cross-reactions of the humoral type directly associated with antigenic relationships of parasites and synthesis of homologous antibodies by the host).

Some of the models in which these types of interactions have been studied are briefly discussed below.

EXPLOITATION OF COMMON HABITATS IN THE HOST

This type of interaction has been most widely studied in models that employ intercurrent infections of intestinal helminths in a variety of experimental and natural hosts. For example, Holmes (1957,1961,1962) has extensively studied the interactions between the cestode Hymenolepis diminuta, and the acanthocephalan Moniliformis dubius, in intercurrent infections in rats and hamsters. Concurrent infections demonstrated that the bulk of one species was reduced by about the bulk of the other one present, with differences in worm localization noted as well. Studies of this type have been aimed at an understanding of the influence of competitive interspecific interactions on the evolution and development of helminth communities, site selection, and niche diversification. A comprehensive review of this subject has been presented by Holmes (1973).

"REACTIVITY" OF THE IMMUNE RESPONSE

Numerous investigations have been conducted on the influence of parasitic infections on host immunological responsiveness (Hudson 1973; Capron et al. 1976). The thrust of much of this research has been in the elucidation of the mechanisms by which parasites manage to evade the potentially hostile environment of the host immunological response. It is well documented that parasites are capable of altering the "reactivity" of natural resistance or acquired immunity such that the host may respond more or less efficiently to a primary or secondary infection. Parasitic infections have been shown to suppress or enhance non-specific inflammatory reactions, non-specific cell-mediated immunity, RES activity, antibody responses, and specific cell-mediated responses by a number of possible mechanisms including antigenic competition, induction of immunological tolerance, and induction of suppressor cell function. The effects of parasite-induced immunosup-

pression or immunopotentiation on intercurrent infections has not been adequately investigated. The most detailed studies in this area have been on the cross-resistance between phylogenetically unrelated intracellular parasites (see Chapter IV for review). Many other models are now being used to study these types of interactions and they include concomitant infections of helminths (Table 1.1), protozoa and helminths (Table 1.2), and protozoa (Table 1.3).

ANTIGENIC CROSS-REACTIONS

This type of interaction has been studied in models using concomitant infections with related parasite species (e.g. - same genus) or parasites in which antigenic relationships have been demonstrated. For example, antigenic relationships and interference phenomena between spirochetes and trypanosomes are well documented (Dallas 1976) and Felsenfeld and Wolf (1973) have examined the interactions between borreliae and trypanosomes in a model using Borrelia turicatae, Trypanosoma cruzi, and their antigenic fractions. Whole extracts as well as antigenic fractions of B. turicatae were shown to suppress T. cruzi parasitemia and prolong the life span of the immunized mice.

In parasitic infections where specific immunoglobulin plays an essential role in acquired immunity, this type of interaction may be of primary importance in the host response to concomitant infections with antigenically related species. However, in infections where antibody response is not a factor in acquired immunity, antigenic cross-reactions may be of little consequence, especially where cross-reacting antigens prove to be of a non-functional nature.

The three categories of interactions discussed above have been implicated in many of the previous studies of concomitant infections presented in Tables 1.1-1.3, but to date, no clear picture has emerged for any

single host-parasite system. Further research is required before we will be able to fully understand the multiplicity of host-parasite interactions that occur during multiple (parasite) species infections.

CHAPTER II

TRICHINELLA SPIRALIS: A LITERATURE REVIEW

A. THE NEMATODE

INTRODUCTION AND TAXONOMY

Trichinella spiralis (Owen, 1835) Raillet, 1895 is an aphasmid nematode parasite of vertebrate carnivores. *T. spiralis* was first recognized in a human cadaver by Paget in 1835 (Paget, 1866), but Owen (1835) was the first to publish a definitive description of this nematode. The basic features of its life cycle were described independently by Leuckart and Virchow in 1860 (Schwartz 1960).

According to Chitwood's classification of the Nematoda in 1950 (Chitwood 1950), *T. spiralis* has the following taxonomic position:

Phylum	:	Aschelminthes
Class	:	Nematoda
Sub Class	:	Aphasmidia
Order	:	Enoplida
Sub Order	:	Dorylaimina
Super Family	:	Trichuroidea
Family	:	Trichinellidae
Genus	:	Trichinella
species	:	spiralis

LIFE CYCLE AND COURSE OF INFECTION

Significant contributions towards an understanding of the life cycle of T. spiralis were made independently by Virchow and Leuckart in 1860 (Schwartz, 1960). Detailed aspects of the life cycle including a description of molting, as well as a discussion of the enteral and parenteral phases of infection in the host, have been provided recently by Kozek (1971 a, b).

Infective larvae encysted in the striated skeletal muscles of an infected host are ingested by a vertebrate carnivore. The larvae are freed from their cysts in the stomach of the host and go through four quick molts (L1-Adult) in the small intestine where they become sexually mature adults. Mating and insemination occur within approximately 30 hours after ingestion of infected muscle tissue (Gould et al. 1955; Wu and Kingscote 1957; Kozek 1971a). At approximately four to seven days, depending upon the host and strain of T. spiralis, the inseminated females produce newborn larvae viviparously, and deposit them in either the mucosa or directly into the central lacteals or capillaries of the villi.

The newborn larvae migrate from the intestine to the muscles by a number of different routes, with approximately 70% migrating by way of the blood-lymph circulatory system and approximately 30% by other routes including the peritoneal cavity and the hepatic-portal circulatory system (Harley and Gallicchio 1971). Larviposition and migration of newborn larvae can occur from day 4-14 post ingestion of infective muscle larvae, with a peak at about 7-11 days (Harley and Gallicchio 1971).

Newborn larvae that reach the striated skeletal muscle cells enter into an intracellular stage of development where they encyst and

become infective. The first infective larvae appear approximately 17 days after ingestion of infective muscle larvae and by 28 days nearly all larvae have encysted and become infective (Phillipson and Kershaw 1960, 1961).

The intestinal phase of infection is completed after two weeks in the rat. Worm expulsion begins shortly after the first week and is completed by the end of the second week of infection (Gursch 1949; Fernandez et al. 1969; Gore et al. 1974; Ruitenberg 1974; Love et al. 1976).

The life cycle of this nematode is therefore composed of three distinct parasitic stages which occur during two phases of infection (Fig. 2.1):

ENTERAL PHASE

1) The Intestinal, Adult Stage -------- Day 7

PARENTERAL PHASE

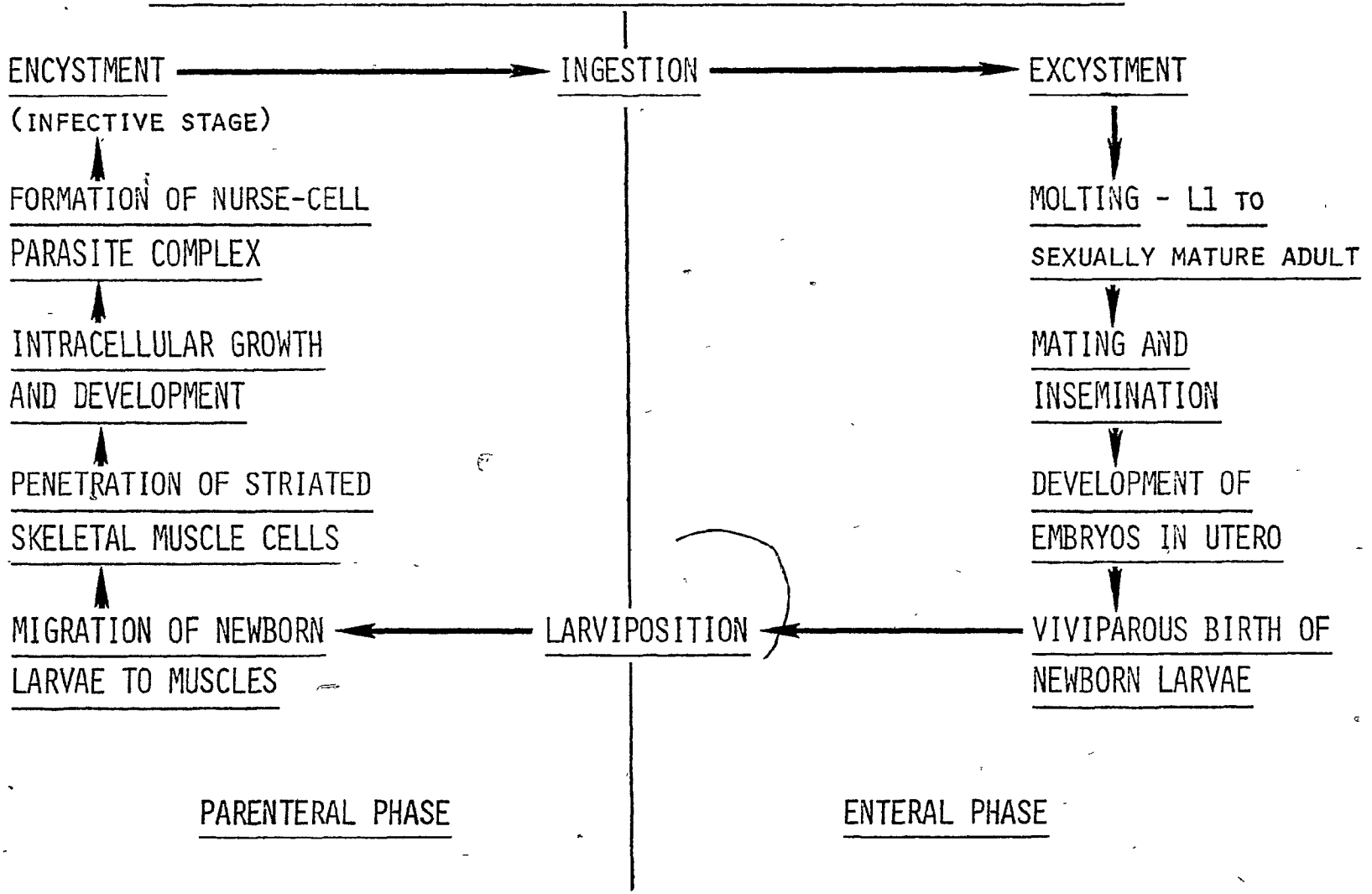
2) The Newborn, Migrating Stage ------- Day 14

3) The Intracellular, Infective Stage - Day 28

Thus, the entire life cycle from adult to infective stages is completed within a single host species.

FIGURE 2.1

LIFE CYCLE OF TRICHINELLA SPIRALIS IN THE VERTEBRATE CARNIVORE



TRICHINELLA SPIRALIS: AN INTRACELLULAR PARASITE

The newborn larva of T. spiralis is capable of penetrating and infecting striated skeletal muscle cells of most mammalian species (Gould 1970). Shortly after the penetration of the larva, the infected muscle cell modulates to become a functionally distinct unit which is now termed the Nurse cell (Purkerson and Despommier 1974). The larva of T. spiralis survives in the Nurse cell as an intracellular parasite (Ribas-Mujal and Rivera-Pomar 1968; Purkerson and Despommier 1974), and this combination has been called the Nurse cell-parasite complex (Purkerson and Despommier 1974; Despommier 1975; Despommier 1976). The intracellular stage of T. spiralis has been called the Nurse cell-parasite complex because the modified host cell (i.e. - striated skeletal muscle cell) functions by obtaining nutrients for the larva from the surrounding extracellular space (Stoner and Hankes 1955; Hankes and Stoner 1958; Steward and Read 1972).

The morphological events that occur during penetration and infection of striated skeletal muscle cells by T. spiralis have been examined in detail. The gross histological changes that occur were described by Gould (1970), and Faaske and Themann (1961), Ribas-Mujal and Rivera-Pomar (1968), Purkerson and Despommier (1974), and Despommier (1975) have described the ultrastructural characteristics of the Nurse cell-parasite complex. This morphological evidence strongly suggests that the larva of T. spiralis acts much like a virus in that it is capable of directing a host cell transformation that provides a suitable niche for the parasite (Ribas-Mujal and Rivera-Pomar 1968). A review of the ecological considerations regarding the Nurse cell-parasite complex has been provided recently by Despommier (1976).

Recent morphological and biochemical studies have demonstrated that the relationship between the intracellular muscle larva and the infected muscle cell is a very complicated, ecologically balanced system resulting in the survival of both the nematode and the host cell (Despommier 1976).

Aspects of the immunobiology of this stage of T. spiralis are only now being uncovered and explored and a precise understanding of the functional interactions of the host muscle cell with the parasite is still lacking.

B. ALTERED IMMUNOLOGICAL RESPONSIVENESS IN TRICHINELLOSIS

INTRODUCTION

The immunology of parasitism offers the immuno-parasitologist numerous opportunities for the elucidation of fundamental immunological mechanisms. Many features of the host-parasite relationship at the immunological level remain poorly understood at this time. The nature and effectiveness of the efferent arc of the immune response have not been well correlated with the various modes of antigenic stimulation by parasites. The origin and nature of functional parasite antigens and their significance in stimulating an immunological response during inter-specific and intraspecific competition between parasites in the host have not been adequately determined.

Parasites can survive in the potentially hostile environment of the host's immunological system and it is reasonable to assume that they should have evolved mechanisms to evade the host response. Some of the mechanisms by which parasites avoid the immunological response of the host have recently been discussed by Hudson (1973) and Capron et al. (1976).

Schistosomes and probably other metazoan and protozoan parasites may reduce the antigenic disparity between parasite and host by the acquisition of host substances on the surface of the parasite or by the endogenous synthesis of host-like antigens (Smithers and Terry 1976). Parasites such as trypanosomes, sporozoans and some piroplasms undergo antigenic variation in which the expression of cell surface antigens may change under the influence of the immune response of the host (Brown 1976).

Other parasites such as T. spiralis or Echinococcus sp., may evade the immune response of the host by the process of encystment, which

serves to isolate the parasite from an attack by the cellular or humoral components of the immunological system. Still other parasites such as Leishmania spp. and Toxoplasma have evolved mechanisms for surviving intracellularly in host tissues.

A fifth and highly effective mechanism for evasion of the immune response resides in a specific alteration in the immunological responsiveness of the host to antigenic stimulation by parasite immunogens. Suppression of immunological responsiveness by microorganisms such as viruses and bacteria is well documented and has recently been reviewed by Salaman (1969, 1970), Floersheim (1969), and Schwab (1975). Hudson (1973) and Capron et al. (1976) have recently attempted to characterize the importance of immunosuppression in the evolution of the host-parasite relationship.

A few of the parasitic diseases in which suppression of humoral or cell-mediated immune responses to homologous or heterologous antigens, or intercurrent parasitic infections occurs include:

1) Trypanosomiasis (Goodwin et al. 1972; Greenwood et al. 1973; Greenwood 1974; Mansfield and Wallace 1974; Murray et al. 1974; Urquhart et al. 1973; Voller 1972)
2) Toxoplasmosis (Huldt et al. 1973; Strickland et al. 1972; Strickland et al. 1973)
3) Leishmaniasis (Cassimos et al. 1969; Chung and Reimann 1930; Clinton et al. 1969)
4) Malaria (Abdel-Wahab et al. 1974; Barker 1971; Barker and Powers 1971; Greenwood et al. 1971; Greenwood 1974; Krettli and Nussenzweig 1974;

Loose et al. 1972; Salaman et al. 1969; Senger et al. 1971)

5) Amoebiasis (Ortiz-Ortiz et al. 1975)

6) Babesiosis (Phillips and Wakelin 1976)

7) Schistosomiasis (Capron et al. 1976)

8) Ascariasis (Crandall et al. 1976)

Research in this field has been mostly phenomenological and we are sorely in need of experimentation that will help to clarify the mechanisms of immunosuppression as well as aid in our understanding of the importance of this phenomenon in the evolution and maintenance of the host-parasite relationship.

A number of studies have begun to take a mechanistic approach. In vitro studies by Criswell et al. (1971) have shown that malaria infections enhance the ability of mouse peritoneal macrophages to take up parasitized erythrocytes, but their ability to function effectively in other ways is diminished. Loose et al. (1971) found that peritoneal macrophages from mice infected with malaria were ineffective in detoxifying endotoxins, and Loose et al. (1972) showed that these cells were unable to function normally in the induction of an immune response. Murray et al. (1974) demonstrated that in Trypanosoma brucei infections, the mononuclear phagocytic system was functionally intact but that there was a defect in the B-cell system. The antibody response to B-cell dependent antigens was completely suppressed while the T-cell response was normal. These authors suggested that immunosuppression in T. brucei infections might be due to the stimulation of a population of suppressor T-cells.

Sufficient evidence is not yet available to clearly indicate the mechanisms by which parasites can modulate host immunological responsiveness, but a number of theories have been suggested. These include the specific induction of tolerance to parasite antigens (Jarrett 1971); antigenic competition (Goodwin 1970; Lubiniecki and Cypess 1975); non-specific activation of immunoglobulin synthesis leading to inhibition of subsequent immune responses (Urquhart et al. 1973); a breakdown in the cooperation between the macrophage, T-cell, and B-cell components of the humoral response (Terry et al. 1973); and the specific induction of suppressor cell function (Murray et al. 1974). Suppression of immunological responsiveness may also occur by the excretion or secretion of immunosuppressive or cytotoxic substances by the parasite (Faubert and Tanner 1975; Faubert 1976; Ackerman and Faubert 1977; Capron et al. 1976).

Further detailed investigation will be required in order to clarify the relevance of these proposed mechanisms in the immunological unresponsiveness generated by many parasitic infections.

IMMUNOSUPPRESSION IN TRICHINELLOSIS

Evidence that infections with T. spiralis can induce a state of immunological unresponsiveness to heterologous antigens, allografts, and viral infections has been supplied by a number of studies. Immunosuppression during Trichinellosis has been characterized by an enhanced retention of skin allografts in mice (Cherniakhovskaia et al. 1971, 1972; Faubert and Tanner 1975; Ljungstrom 1976; Svet-Moldavsky et al. 1970) and heart allografts in mice (Ljungstrom 1976); by a suppression of complement-fixing and neutralizing antibody responses to Japanese B Encephalitis virus (JBE) in mice (Cypess et al. 1973; Lubinieck et al. 1974; Lubiniecki and Cypess 1975), the humoral antibody response of mice to Vaccinia virus (Chimishkian and Ovumian 1975), and the humoral and cell-bound antibody responses of mice to sheep erythrocytes (Barriga 1975; Faubert and Tanner 1971, 1974; Faubert 1976; Lubiniecki et al. 1974; Lubiniecki and Cypess 1975); by pathological changes that occur in the lymph nodes of mice during various stages of the infection (Faubert and Tanner 1974); by the leuco-agglutinating and cytotoxic characteristics of the serum of infected mice and soluble extracts of the muscle larvae of T. spiralis for lymph node cells in vitro (Faubert and Tanner 1974; Faubert 1973); by the failure of spleens cells from infected donor mice to induce Graft vs. Host reactions in hybrid mice (Chimishkian et al. 1974); and by the inability of mouse bone marrow cells from infected animals to reconstitute immunological competence in thymectomized and lethally irradiated mice (Faubert 1973; Faubert and Tanner 1974).

Immunosuppression during experimental Trichinellosis has also been characterized by an increased susceptibility to Japanese B Encephalitis (JBE) and Encephalomyocardial (EMC) viruses in mice and rats re-

spectively (Cypess et al. 1973; Kilham and Olivier 1961; Lubiniecki et al. 1974) and Vaccinia virus in mice and rabbits (Chimishkian and Ovumian 1975).

Nematode infections, such as Trichinellosis, that involve the systemic migration of larvae, have the ability to enhace the virulence of concurrent viral infections. This phenomenon has recently been reviewed and the enhancement of viremia has been attributed to mechanical traumatization of the target tissues of the virus during migration of the nematode larva (Woodruff 1968).

Kilham and Olivier (1961) have reported that rats infected with T. spiralis were more susceptible to disease and death caused by encephalomyocardial virus (EMC) than normal rats. More recently, Cypess et al. (1973), Lubiniecki et al. (1974), and Lubiniecki and Cypess (1975) have extended these observations and demonstrated a synergistic action of infections with T. spiralis on JBE virus in mice. Inoculation of mice with T. spiralis seven days before peripheral challenge with JBE virus, increased the susceptibility of the mice to fatal CNS disease. Mice challenged twenty-one or twenty-eight days after infection with T. spiralis showed a similar susceptibility to the virus as the uninfected controls. The levels of complement fixing and nuetralizing antibodies to JBE virus were significantly reduced at both seven and twenty-eight days after infection with T. spiralis.

Cypess et al. (1973) and Lubiniecki et al. (1974) did not find any direct evidence for mechanical traumatization of the target tissues of the virus by the migration of T. spiralis larvae to the muscles of the host. Although infection with T. spiralis was shown to cause elevated levels of RES activity (assayed by carbon clearance) at fourteen

and twenty-eight days after infection, this activity, as well as the suppression of antibody response, failed to correlate with the peak of enhanced susceptibility to the virus that occured at seven days after infection. Cypess et al. (1973) concluded that "increased corticosteroid levels, suppression of the humoral response, or changes in the fixed macrophage phagocytic activity of the RES, did not appear to constitute a potential mechanism for increased susceptibility of T. spiralis-infected mice to JBE virus".

A number of possible mechanisms may be operative in the immunological unresponsiveness observed during Trichinellosis in mice and rats. Lubiniecki and Cypess (1975) studied the suppression of the antibody response to JBE virus and sheep erythrocytes in detail and suggest that their data are consistent with the hypothesis of sequential antigenic competition as proposed by Pross and Eidinger (1974). These authors suggest that the mechanism of antigenic competition may reside in an alteration of the macrophage cell membrane receptors, thereby interfering with antigen processing and the normal macrophage-T-B cell interactions. Faubert (1976) has also discussed the mechanism of antigenic competition in relation to immunosuppression in Trichinellosis and suggests that the term may be misleading, and advocates the use of the term antigen-induced suppression as proposed by Kerbel and Eidinger (1971).

Other possible explanations and mechanisms have been proposed to account for immunological unresponsiveness in Trichinellosis and these include:

1) The presence of agglutinating and leucotoxic factors in the serum of infected animals and antigen extracts of muscle larvae (Faubert 1973; Faubert and Tanner 1974, 1975).

2) The secretion of immunosuppressive factors by the newborn larvae of T. spiralis (Faubert 1976; Ackerman and Faubert 1977).

3) A reduction or depletion of the thymus derived, antigen reactive T-cell population in infected hosts (Faubert and Tanner 1974; Ljungstrom 1976).

4) The stimulation of suppressor T-cells in the spleens of infected mice (Jones et al. 1975).

Recent research by Faubert (1976) and Ackerman and Faubert (1977) has indicated that immunosuppression in Trichinellosis may be associated with an immunosuppressive factor secreted by a specific developmental stage in the life cycle of T. spiralis. Faubert (1976) has shown that the newborn larvae of T. spiralis can secrete substances in an in vitro culture system that can diffuse through a Millipore membrane (0.45u) and suppress the induction of an in vitro antibody response to sheep erythrocytes. Adult worms or muscle larvae failed to suppress the humoral response in this system.

More recently, Ackerman and Faubert (1977) have shown that in mice inoculated intravenously with either viable or freeze and thawed, non viable newborn larvae, the splenic plaque forming cell response to sheep erythrocytes is reduced by 50% or 80% respectively, as compared to sham-injected controls. This immunosuppression occurred when mice were challenged with sheep erythrocytes only four days after inoculation with the newborn stage of T. spiralis.

Maximum immunosuppression during the natural course of infection with T. spiralis occurs at fourteen days after infection and is correlated with the migration of newborn larvae to the muscles of the host (Faubert 1976). These results suggest that the newborn larvae may be capable of

actively suppressing the immunological response of the host by secreting or excreting toxic or immunosuppressive factors.

The isolation, purification, and identification of these substances will aid in our understanding of the mechanisms of immunosuppression in Trichinellosis.

IMMUNOPOTENTIATION IN TRICHINELLOSIS

In contrast to the immunosuppressive effects of T. spiralis, a number of studies have demonstrated that this nematode can induce a state of non-specific resistance to heterologous bacteria, protozoa and tumors (Table 2.1). In view of the known immunosuppressive effects of this infection on humoral immunity, this stimulation of host immunity probably manifests itself in the form of a non-specific potentiation of cell-mediated immune mechanisms (Cypess et al. 1974; Meerovitch and Ackerman 1974).

Cypess et al. (1974) have shown that mice infected with T. spiralis show enhanced resistance and prolonged survival to intravenous or intraperitoneal injections of Listeria monocytogenes. Mice were challenged with L. monocytogenes at 7, 21 or 49 days after T. spiralis infections and enhanced resistance to bacterial infection was evident at 7 or 21 days, but was abrogated after 49 days. The livers of concomitantly infected mice harbored significantly fewer viable bacteria than control mice. The LD_{50} of helminth infected animals was approximately 8 fold higher than that of controls without T. spiralis at 7 and 21 days after infection but was identical by day 49. Increased survival time of nematode-infected mice was evident at 7 and 21 days after T. spiralis infection but not at 49 days. In addition, the reduced susceptibility to the bacterial infection was independent of the challenging dose. An enhancement of general RES activity (carbon clearance assay) in nematode-infected mice did not correlate with these observations because RES activity was not stimulated until 14 days after T. spiralis infections (Cypess et al. 1974). The lack of correlation between enhanced RES activity (carbon clearance) and immunity to infection by intracellular

TABLE 2.1

IMMUNOSUPPRESSION AND IMMUNOPOTENTIATION IN EXPERIMENTAL TRICHINELLOSIS

---------- IMMUNOSUPPRESSION ----------

1) Increased survival time of skin and heart allografts.
2) Failure of parental spleen cells from infected animals to induce Graft vs. Host reactions in F1 hybrids.
3) Suppression of cell bound and humoral antibody responses to sheep erythrocytes.
4) Suppression of humoral antibody response to Vaccinia virus.
5) Suppression of primary and secondary IgG responses to JBE virus.
6) Enhanced susceptibility of mice and rats to JBE and EMC viruses.
7) Enahnced susceptibility of mice and rabbits to Vaccinia virus.
8) Transient suppression of DTH response to BCG.
9) Immunological incompetence of Bone Marrow cells in the reconstitution of thymectomized, lethally irradiated mice.

---------- IMMUNOPOTENTIATION ----------

1) Decreased susceptibility to Listeria monocytogenes in mice.
2) Suppression of Trypanosoma lewisi parasitemia in rats.
3) Inhibited development of Erysipelothrix insidiosa in rats.
4) Potentiation of cell-mediated (delayed type) hypersensitivity to BCG.
5) Decreased incidence of spontaneous mammary carcinoma in mice.
6) Partial inhibition of the induction and development of Sarcoma-180 Ascites tumors in mice.
7) Anti-neoplastic activity towards B-16 Melanoma and Lewis Lung Carcinoma.
8) Stimulation of RES fixed macrophage phagocytic activity in mice.
9) Cytostatic effect of mouse peritoneal macrophages on R1 Leukemia cells in vitro
10) Enhanced longevity of Swiss mice.

parasites has been reported previously (Lucia and Nussenzweig 1961; Ruskin et al. 1969).

Cypess et al. (1974) failed to detect any serological or antigenic cross reactivity between T. spiralis and L. monocytogenes. These authors have suggested that protection may be due to the activation of macrophages by T. spiralis infections, but make no conclusions as to the stage of infection (enteral or parenteral) responsible for this effect or whether the activation is of a specific or non-specific origin. A similar increase in anti-bacterial resistance (due to non-specifically activated macrophages) and humoral immunosuppression has been shown to occur during Grafts vs. Host reactions in mice (Blanden 1969).

Cypess et al. (1974) and Molinari et al. (1974) have shown that infections with T. spiralis can potentiate a cell-mediated immune response to Bacillus Calmette Guérin (BCG). Cypess et al. (1974) inoculated mice with 200 larvae of T. spiralis 14 days after intravenous administration of a sensitizing dose of BCG. These mice were tested for 24 hour delayed type hypersensitivity (DTH) footpad responses to Old Tuberculin (OT) at 14, 20, 29, 57 or 85 days after infection with T. spiralis. An initial suppression of the classical cell-mediated immune response to BCG was observed at 14 days and cell transfer studies suggested that there was a defect in the adoptively transferred spleen cells, rather than in the physiological state of nematode-infected donors or recipients. There was an enhanced DTH response to OT in BCG-sensitized, parasitized mice at 20, 29, 57 or 85 days after infection as compared to BCG-sensitized controls.

Molinari et al. (1974) sensitized mice with BCG from 14-112 days after oral infection with 200 T. spiralis larvae and found that there

was an enhanced DTH footpad response to OT at all times in T. spiralis - infected mice as compared to non-parasitized controls. Thus Cypess et al. (1974) and Molinari et al. (1974) have shown that a potentiation of CMI can be accomplished by infecting mice with T. spiralis either before or after prolonged periods following administration of the sensitizing dose of BCG. These authors offer a number of possible explanations for these results which include:

1) Increased replication of BCG leading to increased antigenic stimulation.
(Cypess et al. 1974)
2) Non-specific activation of the RES.
(Cypess et al. 1973)
3) Enhancement of T-cell function.
(Miller et al. 1973)

Molinari and Cypess (1975) have examined the possibility that increased bacterial replication might lead to enhanced antigenic stimulation. These authors ruled out this possibility when they found that infections with T. spiralis enhanced the ability of mice to respond to both viable and heat-killed BCG. In the case of heat-killed BCG, the potentiation seemed to be route dependent (i.v. or i.p. inoculations were successful whereas s.c. inoculation was unsuccessful). The fact that T. spiralis potentiated a response against non-viable BCG suggests that this nematode has a marked adjuvant-like effect on cell-mediated immune responses.

Infections with T. spiralis and a number of other metazoan and protozoan parasites have been reported to partially inhibit or completely suppress the development of spontaneous as well as experimental tumors in mice and rats (Lubiniecki and Cypess 1975; Molinari and Ebersole 1976;

Kagan et al. 1968; Weatherly 1970; Keller et al. 1971; and Capron et al. 1972).

Lubiniecki and Cypess (1975) found that T. spiralis infections lengthened the incubation period and survival time of mice injected with Sarcoma-180 Ascites tumor cells. The phenomenon was not observed when mice were challenged with tumor cells 56 days after infection with T. spiralis. This temporal pattern of immunopotentiation is consistent with the immunosuppression, enhanced RES activity and increased resistance to L. monocytogenes that is observed during Trichinellosis in the mouse (none of which occurs at 56 days after infection) (Cypess et al. 1973; Lubiniecki et al. 1974). However, the enhanced response to BCG does not correlate with this temporal relationship (Molinari et al. 1974).

There are a number of possible explanations for the antineoplastic activity of T. spiralis. Infections with this nematode may activate macrophages at the systemic or peritoneal level and these cells may have phagocytosed and killed more tumor cells than would occur in normal mice. A significant cytostatic or cytotoxic effect leading to a reduction in the size of the tumor cell inoculum might account for the increased incubation period (Lubiniecki and Cypess 1975). Old et al. (1960, 1961) have published similar results to those above in Swiss mice given BCG followed by Sarcoma-180 or Ehrlich's Ascites tumors. In view of the known ability of BCG (Old et al. 1961) and T. spiralis (Ackerman and Meerovitch 1975; Meerovitch and Bomford 1977) to activate macrophages, and the similarity of their effects on Sarcoma-180 Ascites production, it is possible that both these agents inhibit neoplasia by a common mechanism of activation of macrophages.

Lubiniecki and Cypess (1975) also suggested the possibility that humoral immunosuppression during Trichinellosis might lead to a decreased

production of "blocking antibody". This is unlikely, however, because immunosuppression is transient in the mouse (Faubert 1976) and is no longer evident at 28 days after oral infection with 200 T. spiralis larvae.

Weatherly (1970) has reported an increased host survival and lowered incidence of spontaneous mammary carcinoma in outbred Swiss mice that were infected with T. spiralis at 8 weeks of age. It is difficult to determine whether the mechanism of this enhanced longevity and suppression of spontaneously occuring neoplasia is similar to that discussed above because a direct comparison of the two systems is not possible. One study employed the use of large numbers of tumor cells while the other was hypothetically employing extremely small number of spontaneously occuring tumor cells (Lubiniecki and Cypess 1975).

Kagan et al. (1968) have similarly shown that the development of spontaneous mammary carcinoma in mice is inhibited when the mice are infected with Trypanosoma cruzi. The proportion of infected mice that developed mammary tumors was significantly less than in non-infected control mice although the difference in mortality, weight gain, and onset of neoplasia were not significantly different in those parasitized or non-parasitized mice that did develop tumors. The authors suggest that these results might be due to a non-specific stimulation of RES activity by T. cruzi infections.

Molinari and Ebersole (1976) have continued to investigate the anti-neoplastic activity of T. spiralis. They have assessed the effects of short term and long term (6 months) infections with 200 T. spiralis larvae, on the induction and progression of the transplantable murine tumors B-16 Melanoma, and Lewis Lung Carcinoma. In experiments using B-16 Melanoma, all control mice developed tumors by day 28 and died within

60 days of tumor challenge, whereas none of the nematode-infected mice showed any signs of neoplasia. Similar results were obtained with Lewis Lung Carcinoma in long term, 6 month, infections with T. spiralis, but 50% of the animals with short term infections of T. spiralis died from progressive tumor growth. These results suggest that a well established, chronic, intracellular muscle infection with T. spiralis is capable of stimulating host anti-neoplastic activity.

Meerovitch and Bomford (1977) have recently examined the mechanism of immunopotentiation in Trichinellosis. Their investigation involved a study of the cytostatic effects of peritoneal macrophages from T. spiralis-infected mice and normal mice on Rl Leukemia cells in vitro. The results suggest that peritoneal macrophages from mice infected with T. spiralis are cytostatic for Rl Leukemia cells in vitro from 6-36 days after infection. This effect may be dependent upon the dose of T. spiralis, as well as the timing of various stages of the life cycle of the parasite in the host.

Thus we have evidence in vitro that T. spiralis is capable of stimulating a cell-mediated immunity in the form of non-specifically activated macrophages. It is highly possible that these activated macrophages are also active in vivo as effector cells in the potentiation of immunity towards T. lewisi, L. monocytogenes, BCG, and experimental or spontaneous neoplasms. Further study is needed, however, to clarify these results.

A number of other helminths have been shown to stimulate humoral and cell-mediated immunity (Schistosoma sp., Trichostrongylus colubriformis, Ascaris suum, and Nippostrongylus brasiliensis)(Larsh 1967; Soulsby 1970, 1972). Under certain conditions, infections with N. brasiliensis have been shown to potentiate the reagin response to previously administered immunogens

such as egg albumin and con albumin (Orr et al. 1971; Orr and Blair 1969; Jarrett 1972; Bloch et al. 1973). This potentiation was shown to be of a short duration and did not involve immunoglobulin classes other than IgE (Bloch et al. 1973). The potentiation of cellular and humoral immunity by other parasites has not been thoroughly investigated.

The clinical significance of these parasite-induced immunological alterations in the host response to infectious agents and neoplasia will only be resolved by further study in this area.

CHAPTER III

TRYPANOSOMA LEWISI: A LITERATURE REVIEW

A. THE TRYPANOSOME

INTRODUCTION AND TAXONOMY

Trypanosoma (Herpetosoma) lewisi (Kent, 1880), Laveran and Mesnil (1901), was one of the first rodent trypanosomes to be discovered and was first noted over 100 years ago (Laveran and Mesnil 1912; Hoare 1972). T. lewisi is the most extensively studied species of the Genus Trypanosoma, due to its worldwide distribution, easy accessibility in the host and ease of maintenance in the laboratory. It is also considered to be the type-species of the non-pathogenic trypanosomes of rodents.

Taxonomically, T. lewisi is classified as follows: (Hoare 1966)

Phylum	:	Protozoa
Sub Phylum	:	Sarcomastigophora
Super Class	:	Mastigophora
Class	:	Zoomastigophorea
Order	:	Kinetoplastida
Sub Order	:	Trypanosomatina
Family	:	Trypanosomatidae
Genus	:	Trypanosoma
species	:	lewisi

LIFE CYCLE AND COURSE OF INFECTION

The life cycle of *T. lewisi* has been described by Minchin and Thomson (1915) and was thoroughly reviewed by Hoare (1972).

The vector and intermediate host for *T. lewisi* is the rat flea, most commonly *Nosopsyllus fasciatus* in temperate zones and *Xenopsylla cheopis* in tropical and subtropical zones.

After the flea ingests infected rat blood, the parasites undergo a cyclical development in the stomach and then the rectum of the flea. Approximately 6 days are required for the development of infective metacyclic trypanosomes, which appear in the rectum and feces of infected fleas (Minchin and Thomson 1915). Infection of the vertebrate host, usually *Rattus rattus* or *Rattus norvegicus*, takes place by ingestion of the infected flea or its feces. The metacyclic trypanosomes presumably penetrate the oral mucous membranes (Hoare 1972) and enter the blood stream and visceral capillaries where they develop and reproduce extracellularly.

Experimental infections of the rat are initiated by intraperitoneal or intravenous injection of infective, dividing epimastigote stages or non-dividing, adult trypomastigote stages from the blood of infected animals.

B. IMMUNOBIOLOGY OF TRYPANOSOMA LEWISI

ACQUIRED HUMORAL IMMUNITY

Differences exist in the intensity and length of parasitemia, the length of the reproductive period, and the immune response to infection depending on the strain of T. lewisi, the strain or species of rat host, host age, sex and diet, and the presence of concomitant infections. (For a comprehensive review of these factors, see D'Alesandro, 1970; Concomitant infections are reviewed in Chapter IV)

Experimental and natural infections with T. lewisi display a number of common features and these have been described by Taliaferro (1926) (see Fig. 3.1). After a period of incubation, which depends upon the size and route of the dose inoculated (Augustine 1941), the trypanosomes appear in the peripheral circulation and visceral capillaries where they show intense reproductive activity. Parasitemia rises sharply to a peak between days 7 and 10 after inoculation of the infective stages. During the logarithmic growth phase, the intensity of reproductive activity is measured by determining either the percentage of dividing trypanosomes (epimastigotes and developmental forms clearly not adult stage trypomastigotes) (Taliaferro et al. 1931) or by a more sensitive technique that measures the coefficient of variation (CV) in the lengths of trypanosomes. The latter technique provides a statistical index of reproductive activity (Taliaferro and Taliaferro 1922). The trypanosomes reproduce by unequal multiple fission (Rabinowitz and Kempner 1899; Laveran and Mesnil 1904; Minchin 1912; Wenyon 1926) of the epimastigote stage and show great variations in length during active multiplication. The CV is a measure of the variability in length during this period. When the CV is high (20-25%), the rate of reproduction is high. When the CV is low (3-5%), reproductive

FIGURE 3.1

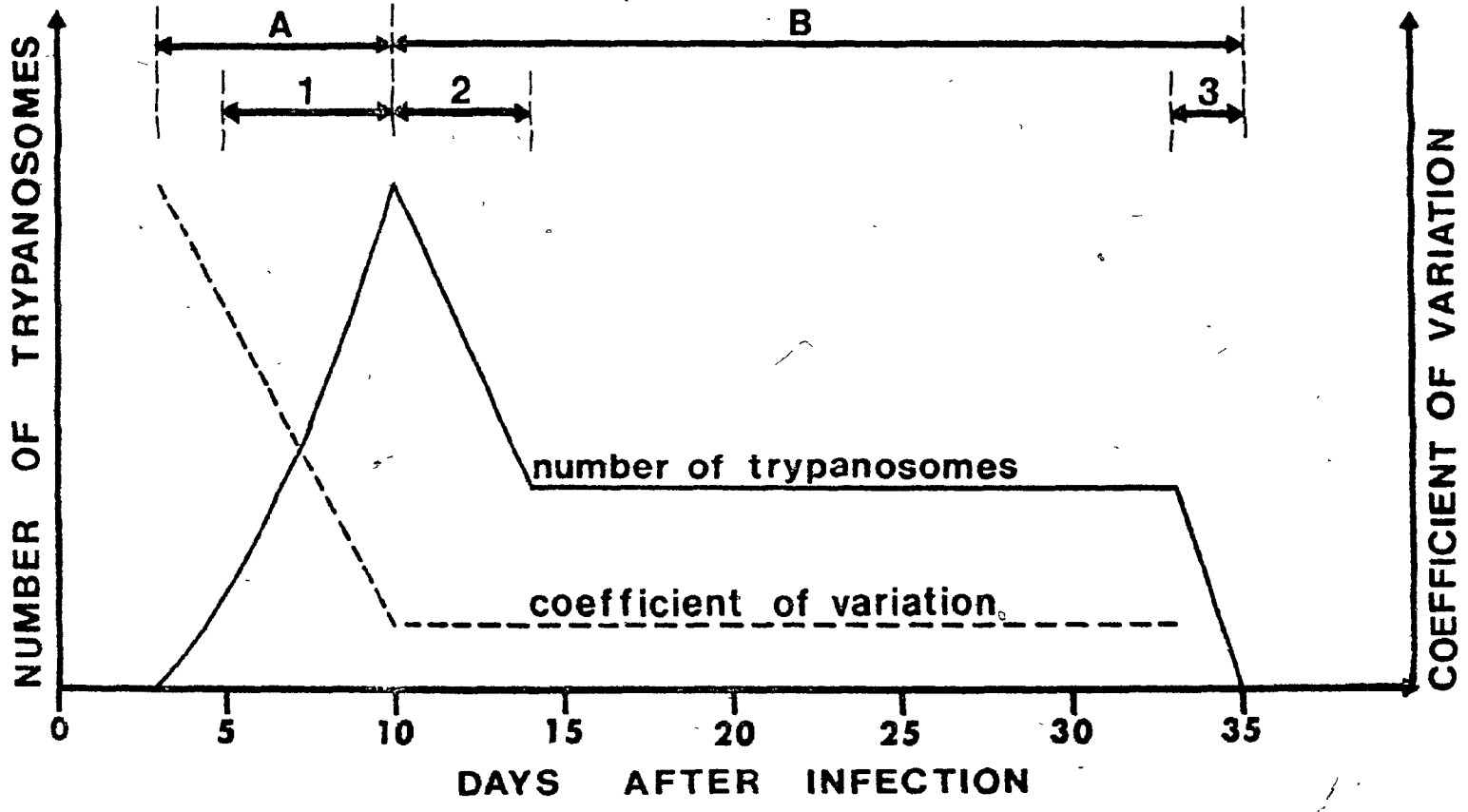
THE COURSE OF T. LEWISI INFECTIONS IN THE RAT

A typical course of infection with T. lewisi in the blood of the rat showing changes in the numbers of trypanosomes and the coefficient of variation (CV). The numerals indicate the three primary manifestations of acquired humoral immunity. The letters indicate the stage of parasite development (Modified and redrawn from Taliaferro 1926).

1) Inhibition of parasite reproduction by ablastin as reflected by the decline in the coefficient of variation
2) The first crisis in which the majority of trypanosomes are destroyed by the 1st trypanocidal antibody which is specific for division stages
3) The terminal crisis caused by the 2nd trypanocidal antibody which is specific for non-dividing adult stages

A) Actively dividing trypanosomes (epimastigotes and developmental forms

B) Non-dividing, inhibited adult stages (trypomastigotes)


A
B
1
2
3
NUMBER OF TRYPANOSOMES
COEFFICIENT OF VARIATION
number of trypanosomes
coefficient of variation
0
5
10
15
20
25
30
35
DAYS AFTER INFECTION

activity ceases and the uniformity in trypanosome size represents a non-dividing "adult" or inhibited population.

As the parasitemia rises, the CV or percentage dividing forms decreases due to the production of a reproduction inhibiting antibody called ablastin (Taliaferro 1924). After approximately 10 days, when reproductive activity has practically ceased due to the presence of ablastin (Coventry 1925), a crisis occurs during which the majority of division-form trypanosomes are killed by a trypanocidal antibody (Coventry 1930). Non-dividing, adult trypanosomes that survive the action of the first trypanocidal antibody, remain in the blood stream of the host for a few weeks to a few months, but are unable to reproduce because of the presence of ablastin.

The infection is terminated either gradually or suddenly by a second trypanocidal antibody (Coventry 1930). After recovery the host possesses a solid, sterile, long lasting immunity (Corradetti 1963; Lee and Lincicome 1972). The immunity to reinfection in rats is considered to be sterile, and this claim has been upheld by the failure to demonstrate the persistence of *T. lewisi* adult or dividing forms in the kidneys or other organs and tissues of rats that have recovered from infection (Wilson *et al.* 1973; Targett and Viens 1975).

The fact that acquired humoral antibodies are responsible for the inhibition of reproduction and the two distinct trypanocidal responses, has been well documented by studies on the passive transfer of immune serum (Rabinowitz and Kempner 1899; Laveran and Mesnil 1900; Coventry 1925, 1930; Taliaferro 1924).

Taliaferro (1924) was the first to demonstrate that a true inhibition of parasite reproduction occurred and that it was due to an acquired

humoral antibody (ablastin) which was distinct from the trypanocidal antibodies of the 1st and 2nd crisis. Coventry (1925) used *in vivo* titration techniques and showed that the titer of ablastin changed during the course of an infection. The titer of ablastin increased during the 5th and 6th day of infection, reached a peak after the 1st crisis, and declined during the remainder of the infection. Studies by Taliaferro and others have continued to confirm and extend these observations (Taliaferro 1932, 1938; Taliaferro *et al*. 1958; D'Alesandro 1959, 1962, 1966, 1972, 1975;Taliaferro and Pizzi 1960).

Acquired humoral immunity to *T. lewisi* in the rat host can therefore be divided into three distinct antibody responses: (see Fig. 3.1)

1) Ablastin - Inhibition of parasite reproduction.
2) 1st Trypanocidal Antibody - causes 1st crisis and is specific for division stages.
3) 2nd Trypanocidal Antibody - Terminates the infection and is specific for non-dividing adult stages.

As far as can be tested, the titers of these three antibodies can vary independently during the infection (Coventry 1925, 1930; Taliaferro 1932, 1941). In addition they all have distinguishing physicochemical and immunological properties and these have been described extensively by D'Alesandro (1959, 1962, 1966, 1970) (see Table 3.1).

TABLE 3.1 - IMMUNOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF ANTIBODIES PRODUCED BY THE RAT TO T. LEWISI INFECTIONS

PROPERTIES	ABLASTIN	1ST TRYP AB	2ND TRYP AB
Passively transferred	+	+	+
Removed from immune serum by adsorption with living trypanosomes	+*	+	+
Trypanosomes sensitized by exposure to antibody	+R	+IR	+IR
In Vitro activity	+	+	+
High degree of specificity for *T. lewisi* antigens			
a) Blood stream forms	+	+	+
b) Culture forms	-	-	-
Opsonizing and Agglutinating activity *in vitro* and *in vivo*	-	+	+
Complement Dependent	-	+	+
Electrophoretic Locus	Between β&γ Globulins	Between β&γ Globulins	Between β&γ Globulins
Sedimentation Constant (Svedbergs)	6S	6S	16S
Immunoglobulin Class	IgG	IgG	IgM

+ = YES

- = NO

R = REVERSIBLE

IR = IRREVERSIBLE

* = D'ALESANDRO 1976, personal communication

(MODIFIED FROM D'ALESANDRO 1959, 1962, 1966, 1970)

CELL-MEDIATED ASPECTS OF IMMUNITY

Until recently, research concerning the immunological response of the rat to T. lewisi has failed to provide positive evidence for the role of cell-mediated immunity in controlling infections with this parasite, other than a subsidiary function for phagocytosis in clearing lysed or agglutinated parasites from the circulation (Taliaferro 1924, 1929, 1932, 1938). Laveran and Mesnil (1901) were the first to consider the possibility that phagocytosis played an active role in T. lewisi infections, after they observed phagocytosis of trypanosomes in the peritoneal cavity of actively and passively immunized rats. Delanoe (1911, 1912) and Roudsky (1911) came to similar conclusions when they observed a phagocytic response in the peritoneal cavity of mice inoculated with T. lewisi. However, these authors were actually studying natural immunity, since the mouse is normally refractory to infection. Delanoe (1912), however, obtained similar results when he used a susceptible strain of mice that developed an acquired immunity. These results have been refuted by other workers (MacNeal 1904; Manteufel 1909; Taliaferro 1924, 1932, 1938) who stressed the importance of lysis and agglutination in parasite destruction.

Brown (1915) observed agglutination and phagocytosis of trypanosomes during the course of infection in rats, and similar observations were made by Augustine (1943) in immune rats reinfected with large numbers of trypanosomes. Augustine (1943) concluded that division stages were destroyed principally by phagocytosis and adult stages by agglutination and mechanical removal from the peripheral circulation.

Regendanz and Kikuth (1927) and Regendanz (1932) supported the view that ablastin was the only acquired humoral antibody produced by the

rat against T. lewisi and that inhibition of parasite reproduction was followed by the non-specific phagocytosis of trypanosomes. The fact that three humoral antibodies are involved in the control of the infection is no longer disputed, however, the importance of phagocytosis and specific or non-specific cell-mediated responses in controlling infections is difficult to determine.

It is quite likely that several trypanocidal mechanisms are operative during T. lewisi infections, especially agglutination, lysis, and phagocytosis. Taliaferro (1932) has suggested that these mechanisms may depend upon the titer of the trypanocidal antibodies. Thus, lysis may occur when trypanocidal antibody titer is high and agglutination and phagocytosis may occur when it is low. There is some evidence to support this view; lysis is rarely observed with immune rat serum but occurs more consistently with hyperimmune rat sera (Taliaferro 1932, 1938).

There is little doubt that humoral antibody plays a primary role in trypanocidal activity, especially in view of the fact that trypanosomes may require sensitization with antibody before a stimulation of macrophage activity or other cellular responses can occur Lange and Lysenko (1960) have reported that immune serum enhanced the phagocytosis of T. lewisi in vitro by peritoneal exudate cells, and that immune serum absorbed with trypanosomes no longer enhanced this activity. Based on these results, Lange and Lysenko (1960) postulated the presence of an opsonin in immune serum.

Taylor and Becker (1948) observed T. lewisi in the Kupffer cells of the liver in pantothenate deficient rats, and more recently Greenblatt (1973) used electron microscopy to demonstrate the presence

of T. lewisi in the spleen cells of infected rats.

Although the trypanocidal mechanism may principally involve lysis and agglutination, it is obvious from studies such as those cited above, that the reticuloendothelial system and macrophages may play an important role in the processing of parasite antigens and in the eventual removal of parasites and parasite debris from the circulation. The relationship between trypanocidal antibody and phagocytosis in the removal of trypanosomes from the peripheral circulation was studied by Taliaferro (1938) who used passively immunized, splenectomized and blockaded rats. In these experiments, trypanosomes were cleared quickly from the blood of passively immunized normal rats, but there was a small but significant loss of trypanocidal activity in splenectomized and blockaded animals. This result could be explained by a depression of phagocytic function, which might impede the removal of opsonized parasites from the circulation or by a decrease in complement levels which could prevent trypanolysis. The former was shown to be untrue as trypanosomes sensitized with trypanocidal antibody before injection were removed from circulation equally well in splenectomized, blockaded rats and normal rats. A definitive answer to this problem has not been provided, but it is possible that severe reticuloendothelial blockade may interfere with antigen-antibody reactions or plasma opsonic activity (Pisano et al. 1968).

Patton (1965, 1972) has investigated the interactions of sera and peritoneal exudate cells on T. lewisi infections both in vitro and in vivo. He has shown that normal rats and rats immunosuppressed with dexamethasone, could be protected against T. lewisi infections only if treated with both hyperimmune serum and normal or hyperimmune peritoneal exudate cells. Trypanosomes given intraperitoneally in this case, were

detained in the peritoneal cavity, agglutinated, lysed and phagocytized. Patton (1972) also observed the *in vitro* phagocytosis of *T. lewisi* in the presence of normal and immune sera plus normal or immune peritoneal exudate cells. A quantitative index of phagocytic activity was not provided in these studies.

Greenblatt *et al.* (1972) have demonstrated the ability of immune spleen cell transplants to transfer immunity to *T. lewisi* in normal syngeneic rats. In their experiments, spleen cells from normal animals were incapable of transferring immunity, but spleen cells from early or late convalescent animals (12-39 days after infection of donors) were capable of transferring immunity. In an earlier study, Greenblatt and Tyroler (1971) showed that *T. lewisi* infections produced a marked increase in the percentage of activated macrophages in the spleens of infected animals, and it is possible that this population of cells may play an important role in either specific or non-specific cellular responses to *T. lewisi*.

The role of activated macrophages and phagocytosis in immunity to other members of the Genus *Trypanosoma* has been investigated recently with respect to infections with *Trypanosoma cruzi* (Hoff 1975; Kierszenbaum *et al.* 1973, 1974; Kress *et al.* 1975; Milder 1973; Milder *et al.* 1973; Tanowitz *et al.* 1975; Ortiz-Ortiz *et al.* 1975) and *Trypanosoma gambiense* (Takayanagi and Nakatake 1974; Takayanagi *et al.* 1974). It is probable that in the near future, the importance of the interaction between humoral and cellular factors in Trypanosomiasis will become evident, and a review of the subject has been presented by Mauel and Behin (1974).

FACTORS AFFECTING THE IMMUNE RESPONSE

1) SPLENECTOMY AND RES BLOCKADE

Splenectomy and blockade of the reticuloendothelial system has been shown to affect the immune response of the host to T. lewisi. The spleen of the rat forms a relatively large part of the RES as well as plays an important role in antibody formation and phagocytosis. Immunosuppressive treatments such as splenectomy and blockade or other stress factors that can suppress the activity of the RES are capable of interfering with the immune response of the host with adverse effects on the course of infections.

Splenectomy was first shown to enhance T. lewisi parasitemia by Regendanz and Kikuth (1927), and was confirmed by Regendanz (1932) who concluded that splenectomy alone could induce severe or fatal infections. Perla and Marmorston-Gottesman (1930) obtained similar results, and found that autotransplants of splenic tissue alleviated the effects of splenectomy and reduced the severity of the infection.

These and previous studies were complicated by the presence of latent, concomitant Bartonella infections. Haleem and Minton (1966) reported similar enhanced parasitemias (without fatalities), but no mention was made of the time of the splenectomy or if the rats were free from concomitant Bartonella. Schwetz (1931) performed splenectomy during the course of infection, and although division forms reappeared in some animals, he concluded that the effect of splenectomy was doubtful.

Early experimental work on the effects of splenectomy were complicated by latent Bartonella and Paratyphoid infections. Taliaferro

et al. (1931) re-examined the effects of splenectomy in Bartonella-free rats and found that in young rats, splenectomy may lengthen the reproductive phase of the parasite if performed on the day of infection. Production of ablastin was not significantly reduced, but parasitemias were higher and infections were of greater duration in splenectomized rats. Taliaferro et al. (1931) concluded that splenectomy of Bartonella-free rats did not significantly alter the ablastic response, but interfered with the production of the trypanocidal antibodies. A combination of RES-blockade and splenectomy (which yielded maximum immunosuppression) effectively interfered with ablastin and trypanocidal antibody production in Bartonella-free rats (Taliaferro 1938).

The pathogenic infections with T. lewisi that have been attributed to the effects of splenectomy (Regendanz and Kikuth 1927; Linton 1929; Vassiliadis 1930; Perla and Marmorston-Gottesman 1930), were most probably the result of the additional stress of virulent Bartonella infections. Taliaferro et al. (1931) showed that fatal infections of T. lewisi in splenectomized rats were always due to concomitant Bartonella or Paratyphoid infections.

2) BACTERIAL ENDOTOXINS

Pre-treatment of rats with small serial doses of bacterial endotoxin has been shown to enhance resistance to T. lewisi. Styles (1965) showed that small serial doses of endotoxin given before infection, depressed peak parasitemias of T. lewisi, whereas parasitemia was elevated when a single large dose of endotoxin was given before, or concurrently with the inoculation of the trypanosomes. Pre-treatment with endotoxin did not affect the prepatent period, but animals made endotoxin-tolerant over a long period of time were more resistant to trypanosome infections.

Singer et al. (1964) found enhanced resistance of mice to Trypanosoma rhodesiense, Trypanosoma congolense, and Trypanosoma duttoni when endotoxin was administered prior to infection. Goble and Boyd (1957) failed to show any effects using a different endotoxin and Trypanosoma congolense infections in mice. Singer et al. (1963) did not find significant alterations in trypanosome infections when endotoxin was administered simultaneously with trypanosomes.

Immunopotentiation and immunosuppression during experimental trypanosome infections after treatment with bacterial endotoxins is not clearly understood. Some investigators have suggested that endotoxin exerts its influence via stimulation of the reticuloendothelial system (Thomas 1975; Howard et al. 1958; Rosen 1961). Goble and Singer (1960) have presented a review of the various agents that can stimulate and depress the RES in Trypanosomiasis and this will be discussed in greater detail in Chapter IV.

Styles (1970) has obtained almost identical results to those of bacterial endotoxins using the toxin holothurin, a steroid saponin

derived from the Bahamian sea cucumber, Actinopyga agassizi. Pretreatment of rats with holothurin increased their resistance to subsequent infections with T. lewisi, whereas holothurin that was administered concurrently with the trypanosomes caused an enhancement of the infection. Holothurin had been shown to inhibit tumor growth in mice (Nigrelli 1975; Sullivan et al. 1955; Sullivan and Nigrelli 1956) and Styles (1970) has suggested that the biphasic effects observed in his study may be due to a stimulation or depression of RES activity by this toxin.

Numerous other investigators have described an increased resistance to experimental infections with a variety of infectious agents after treatment with killed bacteria (Rowley 1955; Landy 1956; Gledhill 1959; Hook and Wagner 1959; Brener and Cardoso 1976; Swartzberg et al. 1975; Nussenzweig 1967; Kierszenbaum 1975).

3) OTHER FACTORS AFFECTING THE IMMUNE RESPONSE TO T. LEWISI

Numerous other factors have been shown to alter the immune response of the rat to T. lewisi, many of which are not relevant to this thesis. These include the effects of host age, sex, and diet, X-irradiation, immunosuppression, thymectomy, adrenalectomy, hypophysectomy, hypoxia, heterologous anti-lymphocyte and antithymocyte sera, etc., on innate and acquired immunity. A comprehensive review of these factors has been presented by D'Alesandro (1970) and more recently by Dusanic (1975).

CHAPTER IV

ASPECTS OF NON-SPECIFIC HOST RESISTANCE TO PARASITIC INFECTIONS AND NEOPLASIA

A. NON-SPECIFIC RESISTANCE TO UNRELATED INTRACELLULAR PARASITES

A number of detailed studies have recently been conducted on the mechanisms of resistance to phylogenetically unrelated organisms that produce intracellular infections. Cross-protection among intracellular parasites has been demonstrated with respect to a number of phylogenetically diverse intracellular infectious agents such as bacteria, protozoa and viruses (see Table 4.1). The demonstration of cross-resistance against organisms that are phylogenetically and antigenically unrelated suggests the possibility that a common mechanism of immunity underlies intracellular infections of all types (Remington and Ruskin 1969; Ruskin et al. 1969).

One of the common features of the immunological response to most intracellular parasites is the non-specific enhancement of antimicrobial activity of host macrophages (Mackaness 1964, 1967; Frenkel 1967). The role of the macrophage in this type of cell-mediated immune response has been investigated in great detail. Interest in macrophage activity has stemmed from the demonstration that there is a cooperation between immune lymphocytes, specifically T-cells, and macrophages, in the effector arm of many immunological reactions. It has been clearly established that acquired resistance to many intracellular parasites requires a two-step process in which immune T-cells, in the presence of specific antigens, can elaborate humoral factors (lymphokines) that activate macrophages (North 1973, 1974). As a consequence of these specific interactions, macrophages may then acquire increased non-specific microbicidal activity (Mackaness 1969).

TABLE 4.1 - CROSS-RESISTANCE AGAINST UNRELATED INTRACELLULAR INFECTIONS IN MICE

PRIMARY INTRACELLULAR INFECTION:	PROTECTION INDUCED AGAINST THE FOLLOWING INFECTIONS:	REFERENCES:
Toxoplasma gondii	*Listeria monocytogenes*	Ruskin and Remington 1968;
	Besnoitia jellisoni	Ruskin *et al.* 1969
	Salmonella typhimurium	"
	Cryptococcus neoformans	Gentry and Remington 1971
	Trypanosoma cruzi	Williams and Remington 1975
	Mengo Virus	Remington and Merigan 1969
Listeria monocytogenes	Mengo Virus	Remington and Merigan 1969
	Toxoplasma gondii	"
	Cryptococcus neoformans	Gentry and Remington 1971
	Candida albicans	Marra and Balish 1974
Besnoitia jellisoni	*Listeria monocytogenes*	Ruskin and Remington 1968;
	Salmonella typhimurium	Ruskin *et al.* 1969
	Brucella melitensis	"
	Toxoplasma gondii	"
	Cryptococcus neoformans	Gentry and Remington 1971
	Mengo Virus	Remington and Merigan 1969
Leishmania donovani	*Mycobacterium tuberculosis*	Konopka *et al.* 1961
	Plasmodium berghei	Adler 1954
Corynebacterium parvum	*Plasmodium berghei*	Nussenzweig 1967
	Trypanosoma cruzi	Brener and Cardoso 1976
	Listeria monocytogenes	Swartzberg *et al.* 1975
	Toxoplasma gondii	"
Bacillus Calmette-Guérin (BCG)	*Plasmodium berghei yoelii*	Clark *et al.* 1976
	Plasmodium vinckei	"
	Babesia microti	"
	Babesia rodhaini	"
	Trypanosoma cruzi	Ortiz-Ortiz *et al.* 1975
Treponema pallidium	*Listeria monocytogenes*	Schell and Musher 1974

Mackaness (1964, 1967) has described this phenomenon as acquired cellular resistance and subsequently has shown that mononuclear phagocytes from immune hosts are more rapidly activated than macrophages from non-immune hosts (Mackaness 1970). Activated macrophages that are generated during the development of acquired cellular resistance are non-specifically activated to kill a wide array of intracellular parasites, which in the normal situation could multiply and survive within non-activated cells (Mackaness 1969). Hosts in which a population of these activated macrophages exists, become capable of suppressing infections by heterologous organisms and the development of spontaneous or experimental neoplasms.

An important factor that is evident in the studies listed in Table 4.1 and in the studies of Blanden et al. (1966), and Collins (1966), is that infectious agents which produce a sustained and chronic tissue phase, confer the most pronounced degree of non-specific immunity to heterologous challenge organisms (Ruskin et al. 1969). Ruskin et al. (1968) and Ruskin and Remington (1969) have suggested that Toxoplasma gondii and Besnoitia jellisoni produce a chronic and sustained tissue infection in mice, and that the persistence of these organisms intracellularly enables them to produce a long-lived stimulation of host lymphoid cells and activated macrophages. The continuous presence of a population of these cells in infected hosts might account for their remarkable resistance to a large variety of intracellular and probably extracellular invaders.

The likelihood that heterologous challenge organisms, with a predilection for an intracellular existence, are eliminated in resistant

animals by a population of activated, highly microbicidal macrophages has been tested *in vitro* (Ruskin and Remington 1968; Ruskin *et al.* 1969; Gentry and Remington 1971). These authors have shown that peritoneal macrophage monolayers derived from animals infected with *Toxoplasma gondii*, *Besnoitia jellisoni*, and *Listeria monocytogenes* were more resistant to necrotization by a challenge with either *Cryptococcus neoformans* or *Listeria monocytogenes* than macrophage monolayers derived from uninfected controls. These studies suggest that chronic intracellular infection with a wide variety of parasites endows the infected host's free peritoneal macrophage system with enhanced ability to destroy phylogenetically unrelated organisms in a reaction that is specifically stimulated but non-specifically active

The fact that acquired cellular immunity is mediated by sensitized lymphoid cells (Mackaness and Blanden 1967) as are other delayed hypersensitivity and cell-mediated immune phenomena, suggests the possibility that comparable populations of lymphoid cells could be appropriately stimulated by organisms or factors other than the intracellular bacteria or protozoa, to produce non-specific potentiation of macrophage activity and heterologous immunity. This possibility has been examined by Blanden (1969) who has shown that mice undergoing a classical Graft vs. Host reaction are unresponsive to sheep erythrocytes but show enhanced resistance to *Listeria monocytogenes* and *Salmonella typhimurium*. Goihman-Yahr *et al.* (1969) however, failed to demonstrate enhanced heterologous immunity in guinea pigs undergoing delayed hypersensitivity to tuberculin, contact dermatitis, or Graft vs. Host reactions.

B. NON-SPECIFIC STIMULATION OF HOST RESISTANCE TO TUMORS BY PARASITIC INFECTIONS

It is highly likely that non-specific resistance that depends upon macrophage activation extends to many systems other than those of bacteria, protozoa, fungi and viruses. For example, a reciprocal resistance has been reported between chronic Besnoitia jellisoni infections and a congenital virus induced leukemia of AKR mice (Lunde and Gelderman 1971). Hibbs et al. (1971) have also reported that chronic infections in mice with either Toxoplasma gondii or the related protozoan Besnoitia jellisoni confer resistance against transplantable and autochthonous tumors. These authors noted significant resistance to the development of spontaneous mammary carcinoma and spontaneous leukemia in C_3H and AKR mice respectively. Similar resistance was also evident following a challenge with Friend Leukemia Virus and Sarcoma-180 Ascites tumor. Youdim (1976) has described a murine experimental model in which non-specific tumor immunity is mediated both in vivo and in vitro by macrophages activated by Listeria monocytogenes. Various other organisms and materials have been shown to non-specifically stimulate host resistance to isogenic and autochthonous tumors and this subject has been thoroughly reviewed by Yasphe (1971).

The importance of the activated macrophage as the effector cell in acquired cellular resistance to intracellular infections and many neoplasms has been clearly demonstrated (Nelson 1974). There is also excellent evidence that a comparable mechanism may also operate in a number of different helminth infections. Infections with Nippostrongylus brasiliensis have been examined in some detail with respect to non-specific potentiation of macrophage activity. Keller et al. (1971) have

shown that the growth of Walker Sarcoma in rats was either entirely suppressed or greatly enhanced during Nippostrongylus brasiliensis infections, depending upon the timing of the tumor cell inoculum in relation to the parasitic infection. In contrast, the growth of syngeneic adenocarcinoma of mice was suppressed during N. brasiliensis infections regardless of whether the infection was initiated before, after, or with the tumor cell inoculum.

Further studies of this phenomenon (Keller and Jones 1971) have shown that rat peritoneal macrophages taken from nematode-infected or peptone-injected rats are "activated" and can inhibit the growth of Walker Carcinosarcoma cells in vivo and in vitro. These authors have demonstrated that a population of radiosensitive cells in recipients of lymph node cells from helminth-infected donors appears to be required for the anti-neoplastic response; a response that can be inhibited by an IgG_2 fraction of antiserum from rats infected with N. brasiliensis. Keller and Jones (1971) were unable to detect any antigenic relationship between Walker Sarcoma cells and N. brasiliensis and suggest that the mechanism of resistance in this system resides in the non-specific cytotoxicity of specifically activated macrophages, as has also been suggested by Mackaness (1969).

As far as this author is aware, the only other reports dealing with inhibition of tumor growth by helminth infections are those of Lubiniecki and Cypess (1975); Molinari and Ebersole (1976), Weatherly (1970), and Capron et al. (1972), which have been reviewed in Chapter II. The antineoplastic activity of helminth infections in general, remains to be more fully investigated.

C. NON-SPECIFIC STIMULATION OF HOST RESISTANCE IN CONCOMITANT PARASITIC INFECTIONS

A cursory search of the literature in immunology and parasitology will reveal numerous references to interference phenomena and cross-immunity between various parasitic organisms, particularly bacteria viruses and protozoa. Reports of cross-resistance or interference between unrelated nematode species, however, are still relatively few in number and it is only recently that this aspect of helminth immunology has been investigated in any detail.

Cox (1952) and Goulson (1958) have reported that infections in mice with Ancylostoma caninum partially protected them against challenge with T. spiralis. Crandall et al. (1967) have demonstrated that increased resistance to Ascaris suum can be induced in mice by infections with N. brasiliensis and Matov and Kamburov (1968) showed increased resistance to Ascaris suum following T. spiralis infections. Kocan (1974) found that rats inoculated with infective N. brasiliensis larvae, but not with transplanted adults, were protected against a challenge infection with Angiostrongylus cantonensis. More recently, Kazacos and Thorson (1975) and Kazacos (1976) have reported that immunization of rats with N. brasiliensis or T. spiralis causes increased resistance to Strongyloides ratti. Many of the other reports dealing with concurrent nematode infections are concerned primarily with the establishment of mixed infections in ruminants (Herlich 1965; Turner et al. 1962; Goldberg 1973)

Of recent, techniques for studying the immunology of the host-parasite relationship have shifted to include the use of concomitant

parasitic infections. Studies on concomitant infections with helminths or with helminths and a variety of unrelated intracellular or extracellular protozoa have indicated that a stimulation of non-specific immune mechanisms may be of importance in the immunological control of parasitic infections in general. Studies of this type have been mostly phenomenological, but the general importance of non-specific, cell-mediated immunity in concomitant parasitic infections is becoming more and more evident. With respect to our knowledge of the immunology of parasitism, it is important that we do not group all forms of acquired resistance to infection under a general heading of immunity, but try to determine whether the immunity is of specific or non-specific origin. Both types of immunity may provide only partial resistance to infection, and while their kinetics and mechanisms may be different, their effects may be additive.

Recent experiments by Mahmoud et al. (1976), in which infections with Toxoplasma gondii induced resistance in mice to Schistosoma mansoni, strongly suggest that non-specific resistance can be effective in schistosomiasis. These authors followed the recovery of schistosomulae, adult worm pairs, or eggs in the livers of mice that had been infected with T. gondii 4 weeks or 1 day before, or 4 weeks after challenge with S. mansoni cercariae. There was a significant reduction in recovery of schistosomulae, adult worms or eggs from the livers of mice that received T. gondii 4 weeks or 1 day before challenge with cercariae. Specific acquired immunity to reinfection with S. mansoni in the mouse takes about 3 months to develop (Sher et al. 1975), whereas the protective effects of T. gondii or other non-specific agents are usually

evident within a few days. This suggests that a stimulation of non-specific cell-mediated immunity may play an effective role in the control of S. mansoni infections.

Kloetzel et al. (1971, 1973) have also examined the effects of concomitant infections with a heterologous intracellular protozoan on S. mansoni infections. These authors studied mixed infections with Trypanosoma cruzi and S. mansoni in experiments that varied in the order of, and intervals between, the two infections. Their results show a pronounced enhancement of T. cruzi parasitemia in all experimental protocols. However, when T. cruzi infection preceded exposure to cercariae by 5-16 days, there was always a significant inhibition of fluke maturation and lower recovery of adult worm pairs. At longer intervals the number of flukes recovered was always higher in concomitant infections. Kloetzel et al.(1973) hypothesized that T. cruzi might develop within S. mansoni, but make no mention of the possible importance of non-specific immunological mechanisms or immunosuppression during these interactions.

Further evidence of the importance of non-specific resistance in helminth infections has been provided by Hunter et al. (1967) in a detailed study of cross-resistance between Nippostrongylus brasiliensis and Schistosoma mansoni in mice. The immunization of mice by infective larvae of N. brasiliensis and a challenge infection with S. mansoni cercariae 7 days later resulted in a significantly lower recovery of flukes than in controls without N. brasiliensis infections. This cross-resistance was not reciprocal, as a primary infection with S. mansoni failed to influence a challenge infection with N. brasiliensis.

Immunoelectrophoresis and indirect hemagglutination using antisera raised in rabbits against S. mansoni cercarial antigen and N. brasiliensis larval antigen showed at least one common antigenic component, but serological cross reactions were not evident in sera from naturally infected mice. The ability of N. brasiliensis to stimulate non-specific tumor immunity (Keller et al. 1971) and the failure of Hunter et al. (1967) to demonstrate humoral cross reactivity in natural infections with these two parasites, supports the idea that non-specific cell-mediated immunity may be an important part of acquired resistance to schistosome infections.

A non-reciprocal cross resistance has also been reported by Jachowski and Bingham (1961) who found that animals infected with T. spiralis for 5 weeks were partially refractory against a challenge with S. mansoni cercariae. The mechanism of this interaction is still obscure however, since a wide variety of S. mansoni antigens can serologically cross-react with antibodies to T. spiralis infections (Anderson 1960; Senterfit 1958; Weinman 1960). In view of the known ability of T. spiralis to potentiate a non-specific, cell-mediated response to a wide variety of heterologous organisms and tumors (see Chapter II), it is entirely possible that both humoral and non-specific cellular mechanisms may operate in the above interaction.

Sinski (1972) has conducted a preliminary study of the influence of Nippostrongylus brasiliensis infections on resistance to Trichinella spiralis in the rat. These experiments demonstrated that rats which were previously immunized with either single or multiple doses of N. brasiliensis infective larvae before challenge with T. spiralis, showed highly significant

reductions in the recovery of T. spiralis muscle larvae, such that the recovery was 2.5% or 23.2% that of control animals respectively. Louch (1962), in a similar study using concomitant infections of N. muris and T. spiralis, also found significant reductions in the recovery of muscle larvae. Sinski (1972) and Louch (1962) suggested that these effects were probably due to an earlier expulsion of adult Trichinella by an enhanced inflammatory and delayed-type hypersensitivity reaction at the intestinal level as was originally suggested by Larsh (1967). The lack of common precipitating antibodies between these two helminths does not preclude the action of a common humoral mechanism in dual infections, nor do these results exclude the possible importance of a non-specific cellular reaction in the inhibition of larviposition, migration or encystment of T. spiralis in host muscle tissues.

The ability of N. brasiliensis to potentiate non-specific host resistance may not be limited to other helminths and tumors. Schmaren and Bammeke (1970) have examined the pathological and biochemical changes that occur in rats concurrently infected with N. brasiliensis and Trypanosoma congolense. They have shown that animals infected with N. brasiliensis have reduced T. congolense parasitemias and enhanced survival times as compared to controls without nematode infections. These authors concluded that protection was probably due to competition for nutrients between the parasites, a conclusion that is not entirely justifiable on the basis of the data they presented. It is quite possible that this interaction may be an immunological one in which non-specific stimulation of RES activity or cell-mediated responses also play an important role.

Yoeli (1956) has examined a similar type of interaction using

concomitant infections of Schistosoma mansoni and Plasmodium berghei in the field vole (Microtus guentheri). In animals receiving plasmodial infections 1-2 weeks before or after exposure to cercariae, a marked increase in chronic P. berghei infections was observed. However, when plasmodial infections were initiated 4-7 weeks after exposure to cercariae, approximately 38% of the animals showed no parasitemia at all and 50% had only mild parasitemia followed by prolonged latency or complete recovery. The latter effects coincided with a period of maximum tissue reaction, cellular proliferation, and infiltration as a result of the concomitant S. mansoni infections, and Yoeli therefore presumed that an alerted RES with marked increase in phagocytic activity may significantly alter the course of a concomitant plasmodial infection. Although Yoeli's conclusions are not justified by an adequate demonstration of RES hyperplasia or enhanced phagocytic capacity, they nevertheless suggest important avenues for further research into the mechanisms of these types of interactions. As far as this author is aware however, no other studies on this phenomenon have been published.

A clarification of the multiple biological or immunological factors involved in an explanation of these competitive effects will only be forthcoming after further study of these types of host-parasite interactions.

D. THE RES AND NON-SPECIFIC RESISTANCE TO TRYPANOSOMES

One of the most widely investigated functions of the RES has been its position among the non-specific mechanisms of host defence against infection. The evidence that the RES plays an active role in non-specific resistance has been based chiefly upon 3 different experimental approaches: 1) The impairment of the immunological defenses of an experimental animal by the blockade of its RES, 2) The demonstration of enhanced ability to clear intraveneously injected particulate matter (such as colloidal carbon) from the blood stream of infected animals, and 3) The use of chemical or biological agents to stimulate RES activity in infected animals in order to alter the course of the infection. In spite of the most ingenious experimental manipulations, these three approaches have not always provided unequivocal answers. A detailed discussion of this subject is beyond the scope of this thesis. However, all three approaches have succeeded in demonstrating both convincing correlations, and lack of correlation between enhanced RES phagocytic activity and enhanced immunity to infection and neoplasia (Bohme 1960; Old et al. 1960, 1961; Freedman 1960; Ruskin et al. 1969).

It is now well recognized that the mononuclear phagocytes of the RES do play an essential role in host resistance against invading pathogens. Biozzi et al. (1975) have reported that enhanced phagocytic function of the RES is one of the primary mechanisms of host resistance against many gram-negative and gram-positive bacteria. Mims (1964) has presented a review of the literature concerning virus-macrophage interactions and reports that macrophages that phagocytize viruses can either support their growth, allow survival until they are transported to other target cells, or destroy them intracellularly.

The principal functions of the RES in relation to the Haemoflagellates and Haemosporidia were originally recognized by Linton (1929) and Taliaferro (1929). They recognized that certain parasitic diseases were primarily infections of the RES, and that one of the chief defense mechanisms of the host in malaria and trypanosomiasis was probably phagocytosis by the macrophages of the RES (Goble and Singer 1960). There are numerous reports in the literature suggesting that stimulation of RES activity by a number of viral agents that attack reticuloendothelial tissue may non-specifically potentiate an immunological response that suppresses malaria infections. Yoeli et al. (1955) observed this phenomenon in concomitant infections with Plasmodium berghei and West Nile Virus in mice. Jacobs (1957) observed a similar suppression of malaria parasitemia with Plasmodium lophurae and Ornithosis Virus in chicks and Trager (1959) observed the same interaction with P. lophurae and a virus that causes splenic necrosis in ducks.

Observations on the stimulation of RES activity and its role in Trypanosomiasis have been made in systems using concomitant infections of trypanosomes with a variety of different agents. As early as 1904, Nissle (1904) reported that the intraperitoneal inoculation of Serratia marcescens (from potato culture) into rats resulted in lower parasitemia during infections with T. brucei. Massaglia (1906) observed that concomitant infections with streptococci and trypanosomes caused the trypanosomes to dissappear from the blood. Schein (1907) reported a similar finding in a dog infected concurrently with Trypanosoma evansi and Bacillus anthracis.

Observations on the interactions of concomitant infections of spirochetes with trypanosomes have been made continually in the past and have been reviewed by Goble and Singer (1960) and more recently by Felsenfeld (1965, 1971) and Dallas (1976). Trautman (1907) made one of the first observations on the effects of relapsing fever spirochetes (Borrelia duttoni) in suppressing T. brucei infections, and since that time numerous examples of this type of interaction have appeared in the literature. The species of Trypanosoma shown to be suppressed in concomitant infections with spirochetes include T. brucei, T. gambiense, T. rhodesiense, T. evansi, T. equinum, T. equiperdum and T. congolense. The spirochetes of the Genus Borrelia that antagonize the development of these trypanosome species (possibly via a non-specific stimulation of RES activity) include B. duttoni, B. merionesi, B. microti, and B. crodidurae.

Tate (1951) showed that Spirillum minus, the causative agent of Sodoku (rat bite fever), produced similar effects in rats with T. equinum and T. lewisi and earlier it was shown that this spirochete inhibited the development of T. brucei (Grillo and Krumeich 1934) and T. gambiense (Ceccaldi and Guilhaumou 1942) in Guinea pigs. Guinea pigs infected with Spirillum minus and challenged with T. brucei or T. gambiense had fewer trypanosomes in the blood and showed enhanced survival times compared to control animals infected with trypanosomes alone. In the work of Tate (1951), rats infected with Spirillum minus were strongly resistant to infection with T. lewisi and T. equinum when inoculated with these trypanosomes shortly after inoculation with the spirochete. Resistance to trypanosomes was most highly developed for a period of 2-4 weeks after infection with spirochetes. In these concomitant infections, rats inoculated with T.

equinim had only transient primary parasitemias and significantly enhanced survival times while in rats challenged with T. lewisi after spirochete infections, only transient subpatent infections were produced.

It is known that spirochete infections can cause considerable RES damage (Haasko 1931; Schlossberger and Grillo 1935) and it might therefore be suggested that the antagonistic effects of spirochetes on trypanosome infections could be due to hyperplasia and a compensatory hyperactivity of the RES in response to this injury. Additionally, Galliard (1931) pointed out that these antagonistic effects could be effectively reduced when splenectomy was performed just before or just after the infections. Recent studies in this field by Felsenfeld and Wolf (1973) have shown that Borrelia turicatae injected simultaneously with Trypanosoma cruzi, prolonged the life span of mice and reduced the number of circulating trypanosomes. These authors demonstrated a marked antigenic cross-reactivity of these two parasites and found that B. turicatae showed a greater capacity to bind antibody from T. cruzi antiserum than vice versa.

The mechanisms of interference in concomitant infections of Borrelia sp. and Trypanosoma sp. are not well understood as yet. Although these genera are antigenically related, it is difficult to accept the theory that these effects are merely due to antigenic relationships and the synthesis of homologous antibodies by the host, because some species of Borrelia do not influence trypanosome infections (Galliard et al. 1958). As early as 1922, Vincent (1927) suggested that macrophage participation may be important in these interactions. The importance of enhanced RES activity or antigenic cross-reactivity in these antagonistic effects

needs to be more fully investigated. It is highly probable, however, that a combination of these immunological factors will be shown to play a role in the potentiation of immunity observed in these host-parasite interactions.

E. BCG AND OTHER IMMUNOSTIMULANTS IN NON-SPECIFIC HOST RESISTANCE AGAINST PARASITIC INFECTIONS AND NEOPLASIA

An important approach to the treatment of infectious diseases and cancer has involved an attempt to non-specifically alter the natural resistance of the host using a variety of chemical and biological agents including bacterial endotoxins, Bacillus Calmette-Guerin (BCG), and Corynebacterium parvum. The immunological factors involved in non-specific host resistance to parasitic diseases and cancer are not yet clearly understood, but a large variety of materials have been found to non-specifically stimulate either specific immune responsiveness or general host resistance (see Table 4.2). The activity of these agents may be expressed as a non-specific enhancement of the capacity to form antibody (adjuvant effects) (Miller et al. 1973); by alterations in the macrophage elements of the RES including heightened phagocytic activity, and increased bactericidal and cytotoxic activity of individual macrophages (Biozzi et al. 1960; Old et al. 1961); or by stimulation of the production of non-antibody, soluble mediators of immunity that may show direct cytotoxic or cytostatic activity against parasitic organisms or neoplastic cells (Clark and Allison 1976).

The mycobacteria and a number of gram-negative and gram-positive bacteria have long been known to induce an increased non-specific resistance in the host against a variety of viral and bacterial infections and early research on this subject was reviewed by Shilo (1959). In relation to the mycobacteria, Dubos and Schaedler (1957) demonstrated that the injection of living attenuated Mycobacterium bovis (BCG) as well as killed BCG could effectively increase resistance to

TABLE 4.2 - MATERIALS WHICH HAVE BEEN FOUND TO NON-SPECIFICALLY STIMULATE THE SPECIFIC IMMUNE RESPONSE OR HOST RESISTANCE*

I. MICROORGANISMS AND SUB-CELLULAR FRACTIONS

A. Mycobacteria

Human and animal strains of mycobacteria including BCG, MER, and Wax D

B. Gram-negative bacilli

Salmonella, Shigella, E. coli, Serratia, H. influenzae, B. pertussis, Brucella, endotoxin moieties

C. Gram-positive cocci

Staphylococci, Streptococci

D. Gram-positive bacilli

Corynebacterium parvum, Listeria monocytogenes

E. Others

Zymosan, lactic dehydrogenase virus

II. NONBACTERIAL MATERIALS

A. Macromolecules

Nucleic acids (DNA, RNA), synthetic polynucleotides, DNA digests, antilymphocytic serum

B. Small molecules

1. Organic

Vitamin A, fatty acids and lipids, epinephrine

2. Inorganic

Alum, bentonite, silica, beryllium

The materials listed here are not all distinct entities. Thus, Wax D is responsible for the classical adjuvant properties of whole mycobacteria, but its role in the induction of resistance by MER and BCG is unknown; and the lipopolysaccharide portion of the bacterial cell wall is at least one of the common active components of many other microorganisms

* This refers to a material whose administration confers on the recipient the ability to respond, or respond more efficiently, to challenge with a non-crossreacting antigen, bacterium, parasite, or tumor, as measured by either a heightened specific immune response or by a general increase in natural resistance. From a review by Yasphe (1971).

staphylococcal infections. Other non-pathogenic mycobacteria have also been shown to increase the host's non-specific resistance to bacterial infections, and enhance the phagocytic activity of the host's RES (Biozzi et al. 1960). In general, however, the degree of enhancement of both these activities was not necessarily parallel (Bohme 1960).

More recently, the Bacillus Calmette-Guērin (BCG) strain of Mycobacterium bovis has become a popular agent for the immunotherapy of cancer (Hersh et al. 1973). The activity of BCG as an immunotherapeutic agent may involve a combination of several mechanisms:

1) A generalized stimulation of immune responsiveness and lymphoreticular activity, following systemic administration (Old et al. 1960, 1961).

2) A local and non-specific destruction of tumor cells at the site of a delayed-type hypersensitivity reaction to BCG ("innocent bystander" effect) (Bartlett et al. 1972).

3) A true adjuvanticity whereby the administration of BCG in temporal and spatial proximity to tumor cells (either mixed in tumor cell inoculum or by infiltration of tumor nodules) results in augmented development of systemic, tumor specific, rejection immunity (Bartlett and Zbar 1972; Hawrylko and Mackaness 1973).

In terms of the treatment of cancer, the most reliable effects with BCG have been in the local destruction of tumor nodules into which the bacteria have been injected (Hersh et al. 1973). Systemic effects suggestive of stimulated tumor rejection immunity are rare, although they are the ultimate goal of immunotherapy.

The use of BCG and other bacteria such as Corynebacterium parvum in research investigating the importance of non-specific immunity in the host-parasite relationship, has become highly fashionable. Indeed the efficacy of using BCG, C. parvum or other non-specific immunostimulants in the immunotherapy of parasitic infections is currently under intensive investigation. Live BCG has been reported to protect against a number of protozoal infections, the success of which varies according to the route of administration. Thus intraperitoneal inoculation of viable BCG was unable to protect mice against Trypanosoma cruzi (Hoff 1975) or Toxoplasma gondii (Frenkel and Caldwell 1975), and viable BCG injected into the retrobular space of rabbits did not protect the eye from T. gondii (Tabbera et al. 1975).

In contrast, pretreatment intravenously with viable BCG reduced the number of circulating T. cruzi and enhanced survival times of mice (Ortiz-Ortiz et al. 1975) and protected rabbit's eyes from T. gondii (Tabbera et al. 1975). In none of these cases, however, was protection as strong as that observed using BCG immunization against Babesia microti, Babesia rodhaini, Plasmodium berghei yoelii and Plasmodium vinckei (Clark et al. 1976). Kuhn et al. (1975) were unable to suppress T. cruzi parasitemia or enhance survival times by pre-treating mice with BCG The differences observed in the effect of BCG on protozoal infections may simply be a question of the viability of the BCG strain, the dose and route of administration, or the timing of the immunization relative to the challenge infection, rather than any fundamental differences in the protection afforded by BCG immunization against different intracellular protozoa.

This may be especially true in view of the recent work by Kierszenbaum (1975) who studied the effect of C. parvum on T. cruzi in mice and Bartlett et al. (1976) who examined the relationship between doses of BCG and tumor rejection phemonenon. Corynebacterium parvum is one of the most powerful RES stimulants known to date (Halpern et al. 1963), and has been shown to increase resistance to transplanted tumors and bacterial infections (Schwab 1975), as well as significantly protect mice against challenge infections with Plasmodium berghei (Nussenzweig 1967). Kierszenbaum (1975) demonstrated the importance of the route of administration fo this bacterium by showing that intravenous, but not intraperitoneal injection of killed C. parvum either before or after intraperitoneal infection with T. cruzi, produced enhanced resistance against this protozoal infection in mice. Recently, however, Brener and Cardoso (1976) were able to demonstrate delayed mortality and suppressed T. cruzi parasitemia using a different strain and dose of C. parvum injected intraperitoneally. In a study on BCG dosage and tumor rejection phenomenon, Bartlett et al. (1976) found that the dosage of BCG required for optimal suppression of local tumor growth was detrimental to the development of a sustained, systemic tumor immunity. It is obvious from these observations and the conflicting results of Kierszenbaum (1975) and Brener and Cardoso (1976) that the variable effects of dosage and route of administration, on non-specific stimulation of host resistance against protozoal infections and tumors, needs further evaluation and research.

Swartzberg et al. (1975) have reported on in vivo and in vitro experiments that examined the effects of C. parvum on resistance

of mice to Listeria monocytogenes and virulent and avirulent strains of T. gondii. Intravenous administration of C. parvum conferred transient protection against Listeria monocytogenes or the avirulent strain of T. gondii but did not protect against a virulent strain of T. gondii. Results in vivo did not correlate well with the enhanced cytotoxicity of macrophages from C. parvum - immunized mice for T. gondii in vitro. Hoff (1975) also failed to correlate an enhanced in vitro killing of T. cruzi by macrophages from BCG - immunized mice with enhanced immunity in vivo. The apparent dichotomy between macrophage activation and enhanced killing of protozoan parasites in vitro and the lack of protection when BCG or C. parvum - immunized hosts are challenged in vivo also needs further clarification.

The most promising and consistent results to date using BCG or C. parvum in the treatment of protozoal infections are those of Clark et al. (1976) and Clark and Allison (1976). These authors have been able to completely suppress the development of Babesia microti, Babesia rodhaini, Plasmodium berghei yoelii, and Plasmodium vinckei in mice using intravenous or intraperitoneal injection of BCG or C. parvum. The mechanism of this protection is still somewhat obscure, but these researchers present preliminary evidence that protection may not be due to either cross-reacting antibodies, enhanced production of parasite specific immunoglobulins, or enhanced macrophage phagocytic activity. Instead, these authors have presented evidence for a hypothesis that BCG or C. parvum enhances the production of non-antibody, soluble mediators of immunity (possibly lymphokines) that may be able to inhibit intracellular growth and development by interacting with the parasite

directly or by altering host cell metabolism and indirectly inhibiting parasite replication (Clark et al. 1975; Clark and Allison 1976). Further research is needed in order to characterize the origin and nature of the non-antibody, soluble serum factor described by these authors as well as to clarify the mechanism by which parasite development is inhibited intracellularly. If this hypothesis is correct, then it is possible that this type of non-specific immunity may also be active against other intracellular parasites such as T. cruzi and T. gondii. It is the opinion of this author, however, that the non-specific activity of BCG and C. parvum as immunotherapeutic agents in general, will probably be shown to be a result of a combination of the mechanisms discussed in this section.

At this time, very little research has been conducted on the effects of BCG, C. parvum or other non-specific immunostimulants of this type on helminth infections. Rau and Tanner (1975) studied the effects of BCG on the asexually proliferating stage of the cestode Echinococcus multilocularis in the cotton rat (Sigmodon hispidus). The growth and metastasis of these parasitic larvae bears an intriguing resemblance to the growth and metastasis of malignant tumors (Rau and Tanner 1976). Pretreatment of the cotton rat with an intraperitoneal inoculation of BCG one week before an intraperitoneal challenge with the larval stage of E. multilocularis, significantly suppressed the growth and metastasis of this parasite. BCG treatment two weeks after initiation of the hydatid infection, failed to suppress the establishment and growth of the parasite cyst mass but did reduce the number of parasitic foci in the infection. The mechanism of these protective interactions is currently under investigation (Reuben and Tanner 1976, personal communication).

Mahmoud et al. (1976) have examined the influence of BCG on Schistosoma mansoni infections in the mouse. In a well planned and well executed preliminary study, these authors demonstrated that BCG induced a high degree of non-specific resistance against S. mansoni infections when administered intravenously. Protection was related to the strain of BCG used, as well as the dose, timing, and route of inoculation of the BCG relative to the challenge infection with S. mansoni.

It is probable that these preliminary studies will stimulate interest and research into the importance of non-specific immunity in the control and immunotherapy of helminth infections in general.

CHAPTER V

DEVELOPMENT OF CROSS-PROTECTION AGAINST TRYPANOSOMA LEWISI IN RATS INFECTED WITH TRICHINELLA SPIRALIS

INTRODUCTION

As noted in Chapter II, altered immunological responsiveness in experimental Trichinellosis is well documented. Numerous investigations have supplied evidence that Trichinella spiralis can induce immunological unresponsiveness to heterologous antigens, allografts and virus infections in mice and rats. Immunosuppression in Trichinellosis is a transient phenomenon and is probably related to a particular stage of the parasite's life cycle in the host (Faubert 1976). It has been characterized by an enhanced retention of skin allografts (Cherniakhovskaia et al. 1971, 1972; Faubert and Tanner 1975; Ljungström 1976; Svet-Moldavsky et al. 1970) and heart allografts in mice (Ljungström 1976); by a suppression of complement-fixing and neutralizing antibody responses to Japanese B Encephalitis (JBE) virus in mice (Cypess et al. 1973; Lubiniecki et al. 1974; Lubiniecki and Cypess 1975), the humoral antibody response to Vaccinia Virus in mice (Chimishkian and Ovumian 1975), and the humoral and cell-bound antibody responses of mice to sheep erythrocytes (Barriga 1975; Faubert and Tanner 1971, 1974; Faubert 1976; Lubiniecki et al. 1974; Lubiniecki and Cypess 1975); by pathological changes that occur in the lymph nodes of mice during various stages of the infection (Faubert and Tanner 1974); by the leuco-agglutinating and cytotoxic characteristics of the serum of infected mice and soluble extracts of the muscle larvae of T. spiralis for lymph node cells in vitro (Faubert and Tanner 1974); by the failure of spleen cells from infected donor mice to induce Graft vs. Host reactions in hybrid mice (Chimishkian et al. 1974); and by the inability of mouse bone marrow cells

from nematode-infected animals to reconstitute immunological competence in thymectomized and lethally irradiated mice (Faubert and Tanner 1974). Immunosuppression in Trichinellosis has also been characterized by an increased susceptibility to JBE virus and Encephalomycardial virus (EMC) in mice and rats respectively (Cypess et al. 1973; Kilham and Olivier 1961; Lubiniecki et al. 1974), and Vaccinia Virus in mice and rabbits (Chimishkian and Ovumian 1975).

In contrast to the immunosuppressive effects of infections with T. spiralis, a number of recent studies have demonstrated that this nematode can also induce a state of non-specific resistance to heterologous bacteria, protozoa, and tumors. This immunopotentiation has been characterized by a decreased susceptibility of mice to Listeria monocytogenes (Cypess et al. 1974); by inhibited development of Erysipelothrix insidiosa infections in rats (Toshkov et al. 1975); by a potentiation of cell-mediated hypersensitivity (delayed-type) to BCG in mice (Cypess et al. 1974; Molinari et al.1974; Molinari and Cypess 1975); by a decreased incidence of spontaneous mammary carcinoma and enhanced longevity in Swiss mice (Weatherly 1970); by the partial inhibition of the induction and development of S-180 Ascites tumors in mice (Lubiniecki and Cypess 1975); by anti-neoplastic activity towards B-16 Melanoma and Lewis Lung Carcinoma in mice (Molinari and Ebersole 1976); by a stimulation of RES fixed macrophage phagocytic activity in mice (Cypess et al. 1974); and by the cytostatic effect of mouse peritoneal macrophages from nematode-infected hosts on R1 Leukemia cells in vitro (Meerovitch and Bomford 1977).

The mechanisms by which this nematode induces states of altered immunological responsiveness are still unclear. It has been suggested that immunosuppression may be the result of sequential antigenic competition

(Lubiniecki and Cypess 1975), the secretion of immunosuppressive factors by the newborn larvae of T. spiralis (Faubert 1976; Ackerman and Faubert 1977), a reduction or depletion of thymus-derived, antigen-reactive T-cell populations in infected hosts (Faubert and Tanner 1974; Ljungström 1976), or the stimulation of suppressor T-cell activity in the spleens of infected mice (Jones et al. 1975). In view of the known immunosuppressive effects of T. spiralis on humoral immunity, it has been suggested that immunopotentiation is probably a reflection of enhanced non-specific RES activity or non-specific cell-mediated immune responses (Cypess et al. 1974; Meerovitch and Ackerman 1974).

The occurence of both immunosuppression and immunopotentiation in Trichinellosis appears to be contradictory. However, these phenomena may not by mutually exclusive and are probably an expression of the timing of the various modes of antigenic stimulation by particular stages of the parasite's life cycle in the host. A similar effect has been demonstrated in mice in which enhanced anti-bacterial resistance (due to non-specifically activated macrophages) is accompanied by humoral immunosuppression during Graft vs. Host reactions (Blanden 1969). The lack of a satisfactory explanation for these contradictory effects, prompted me to investigate these phenomena further in experimental Trichinellosis in the rat model.

In a preliminary study, Meerovitch and Ackerman (1974) reported that rats infected with T. spiralis for 30 days were partially protected against a challenge infection with either Trypanosoma lewisi or Trypanosoma equiperdum. Protection was manifested by a significant inhibition of the development of trypanosome parasitemias in concomitantly infected rats. Since T. lewisi is a non-pathogenic trypanosome of the rat and the infection is mediated by a well characterized humoral response (D'Alesandro

1970), we felt that a model using concomitant infections with these two parasites would be advantageous in examining immunopotentiation in Trichinellosis as it relates to immunosuppression, the nematode life cycle and level of infection, as well as the immunological mechanisms involved in this host-parasite interaction.

MATERIALS AND METHODS

1) THE ANIMALS

Charles River, CDF, inbred albino, female rats (~100 grams) (Charles River Breeding Laboratories, Wilmington Mass.) were used in all experiments. Animals were housed individually in wire bottom cages and received Purina Rat Chow and water ad libitum. Outbred CD, albino female rats (~100 grams) (a Sprague-Dawley line; Canadian Breeding Farms (CBF), St. Constant, Quebec) were used for maintaining infecting stocks of Trichinella spiralis and Trypanosoma lewisi. Female, New Zealand white rabbits (3-5 Kg)(CBF) were used for the production of antisera to T. lewisi and T.spiralis antigen extracts.

2) THE PARASITES

The strain of Trypanosoma lewisi used in this study was a stabilate of the Winches Farm Strain (London School of Tropical Medicine and Hygiene) and was originally isolated from a wild rat and cryopreserved in 1970. It was obtained as a stabilate (LUMP 122) from Dr. Pierre Viens (Dept. of Microbiology, University of Montreal) in September 1973, and has been passaged bi-weekly since that time in CD female, albino rats by the intraperitoneal inoculation of ~1.0×10^5 trypanosomes in rat blood diluted in Hank's Balanced Salt Solution (HBSS).

Trypanosomes for the initiation of experimental infections were obtained from stock infected rats which had been inoculated 12 days previously. Rats were bled aseptically by cardiac puncture into HBSS containing sodium heparin (100 units/ml) and pure suspensions of trypanosomes were prepared in sterile HBSS, supplemented with 1% glucose, according to the technique described by Lincicome and Watkins (1963). Trypanosomes were immobilized (for counting) by making an appropriate dilution of the suspension in 10% (w/v) sodium citrate solution and were counted in a Spencer Bright Line Neubauer Hemocytometer using phase contrast illumination (400x magnification). Experimental infections were initiated by injecting rats with 5.0×10^3 infective trypomastigotes (0.5ml) intraperitoneally without anaesthesia. Trypanosome parasitemia was followed during the course of the T. lewisi infections by daily hemocytometer counts of tail blood diluted 1/200 using 10µl capillary tubes and "Unopettes" (Unopette #5851, Becton-Dickinson and Co., Rutherford, New Jersey) filled with 2.0 ml of formol-saline (0.85% (w/v) sodium chloride, 0.25% (w/v) formalin).

Trichinella spiralis used in this study is a Canadian porcine strain that was originally obtained in 1959 from the Animal Diseases Research Institute, Hull Quebec, where it was isolated from a domestic pig. It has been maintained since that time at the Institute of Parasitology of McGill University exclusively in Sprague-Dawley rats. Infecting stocks for this study were maintained in CD female, albino rats, inoculated with 3,000 infective muscle larvae.

Experimental infections and the recovery of muscle larvae from experimental animals were done according to techniques adopted from Tanner (1968). T. spiralis muscle larvae for all experimental infections were recovered from donor stock rats which had been inoculated 30 days previously. Infected rats were stunned, decapitated, skinned and eviscerated and

the carcasses were homogenized in a 400 ml stainless steel chamber with a Serval "Omni-Mixer" in digestion fluid (tap water containing 0.5% (v/v) HCL and 0.3% (w/v) pepsin powder (Fisher Scientific)). The muscle larvae were recovered by sedimentation after digesting the homogenized carcasses for 4 hours at 37° C. with constant stirring, in 3.0 liters of digestion fluid. The larvae were washed with phosphate buffered saline (PBS, pH 7.2) and counted (using constant stirring of an appropriate dilution) in McMaster Nematode Egg-counting Chambers (Hawksley Ltd., London, England). Inoculating doses were prepared in 0.5 ml of PBS and experimental animals were inoculated orally without anaesthesia using a blunt-end inoculating needle (#16g - 3 inch, Canlab, Montreal). Muscle larvae were recovered and counted at the end of each experiment as described above, but the weight of each carcass was determined before the digestions were initiated.

3) ANTIGENS

The soluble antigen extract of Trichinella spiralis (TSE) used for immunizing rabbits and in immunodiffusion and immunoelectrophoretic analysis was prepared from washed, lyophilized muscle larvae, essentially as described by Tanner (1970). Lyophilized muscle larvae (in a ratio of 1 gram : 25 ml buffer) were disintegrated for 30 minutes with ultrasounds from a Fisher BP-2, 20 kc, ultrasonic probe (200 watt power supply) with continuous cooling (0° C.). The sonicate was centrifuged at 50,000 x g (30 min.) and 100,000 x g (2 hours) at 2° C. and protein concentration was determined according to the technique of Lowry et al. (1951) and adjusted to 10 mg/ml. TSE for immunoelectrophoretic analysis was extracted further in 0.5% Triton X-100 for 12 hours on ice, with occasional mixing prior to centrifugation. Soluble extracts were stored on ice or frozen at -70° C. until needed.

A crude antigen of Trypanosoma lewisi (TLA) for immunizing rabbits was prepared from trypanosomes collected from infected rats 12-14 days after inoculation, just after the first crisis. Pure washed suspensions of trypanosomes were prepared according to the technique of Lanham (1968) and Lanham and Godfrey (1970), using DEAE-Cellulose Anion Exchanger (coarse mesh, 0.90 meq/gram, Sigma Chemical Corp.) and a modified column design (2.5 x 40.0 cm glass chromatography columns, bed volume 150.0 ml) in a closed, gravity flow system. DEAE-Cellulose was prepared according to the technique of Peterson and Sober (1962). Suspensions of trypanosomes (5.0×10^9 organisms/ml) were disrupted by sonication 3 times for 30 secs. with five minute intervals for cooling, using the Fisher ultrasonic probe described above.

A soluble antigen extract of T. lewisi (TLE) for immunodiffusion and immunoelectrophoretic analysis was prepared by extracting the TLA further in 0.5% Triton X-100 for 12 hours on ice with occasional mixing. This preparation was centrifuged for 2 hours at 100,000 x g and the protein concentration of the extract was adjusted to 10 mg/ml using Aquacide (Calbiochem, Richmond, California). TLE was stored on ice or frozen at -70° C. until use.

Sheep red blood cells (SRBC) for immunizations, hemagglutination (HA) and hemolysin (HL) titrations were prepared by centrifuging citrated blood and washing 3 times in PBS (pH 7.2). Suspensions of the required concentration were prepared in PBS immediately before use.

4) ANTISERA

Rabbit antisera to T. lewisi crude antigen (TLA) were prepared according to the procedure described by Yasuda and Dusanic (1971). Three

female New Zealand white rabbits were injected intranuchally (subcutaneous), with 1.0 ml of TLA ($\sim 5.0 \times 10^9$ trypanosomes) emulsified with and equal volume of Freund's Complete Adjuvant (FCA), three times at three week intervals. Two weeks after the last immunization, the rabbits were bled by cardiac puncture. The serum was collected by centrifugation (1500 x g, 30 min.) after the blood had been allowed to clot at 4^0 C. for 12 hours. Sera were heat inactivated (56^0 C., 30 min.) and stored at -20^0 C. until use.

Rabbit antisera to I. spiralis antigen extract (TSE) were prepared according to the following technique: five female New Zealand white rabbits were injected with TSE (10 mg/ml protein) emulsified with equal volumes of FCA intramuscularly in the hind legs (left and right legs alternately) 3 times per week for a period of one month. At approximately one month intervals, for four months, the rabbits were boosted with further injections of TSE as above. Rabbits received a total of 6 ml of TSE or approximately 60 mg total protein during the course of the immunization. One week after the last injection, the blood was obtained by cardiac puncture and the sera collected and stored as described above.

Rats used for the routine maintenance of I. lewisi stock infections were hyperimmunized after they had recovered from a primary infection ($\sim$40 days) by injecting them intraperitoneally with two doses of a purified suspension of living trypanosomes (1.0×10^7 organisms, prepared according to the technique of Lincicome and Watkins (1963)) given at one month intervals. One week after the last injection, the rats were bled by cardiac puncture and the sera collected and stored as described above. Immune rat sera were collected in the same manner just

after the animals had recovered from a primary infection (~40 days).

Hyperimmune rat antisera to T. spiralis were collected from rats that were inoculated with 3,000 infective muscle larvae and challenged with a second dose of 3,000 larvae 30 days later. Two weeks after the challenge infection, the rats were bled by cardiac puncture and the sera collected as described above. Immune rat antisera to T. spiralis were collected in a similar manner from rats infected with 100 or 1500 larvae/rat at 7, 14, 28, or 56 days after infection.

Rat anti-SRBC antisera were obtained from animals that were injected intraperitoneally with 2.0×10^9 SRBC in 0.5ml volumes prepared as described above. Six days after immunization, the rats were bled by cardiac puncture and the blood allowed to clot at 4° C. for 12 hours before centrifugation (1500 x g, 30 min.). The rat sera were heat-inactivated (56° C., 30 min.) and stored individually at -20° C. until HA or HL titrations were performed.

5) ASSAY OF RETICULOENDOTHELIAL SYSTEM (RES) ACTIVITY

The granulopectic activity of the RES during experimental Trichinellosis in the rat was assayed according to methods adopted from Biozzi et al. (1953). A colloidal suspension of carbon particles (20-30 mμ in size) stabilized in fish glue free from shellac (preparation #C11/1431A-Pelikan Special Biological Ink, Gunther-Wagner, Pelikan Werke, Hanover, Germany) was administered via the tail vein to rats previously anaesthetized intraperitoneally with Nembutal. The dose of carbon was calculated to be 8 mg/100 grams body weight. 10μl samples of blood were obtained from the tail vein of rats at 2, 4, 6, 8, 10, and 12 minutes after the injection

of colloidal carbon using pre-calibrated 10µl capillary tubes and "Unopettes" containing 4.0 ml of 0.1 M sodium carbonate as diluent. The concentration of carbon in these samples was read spectrophotometrically at 650 nm on a Pye Unicam SP1800 Ultraviolet Spectrophotometer that was pre-calibrated to read carbon concentration directly in mg/ml. When the log of the carbon concentration is plotted against sampling time, the clearance rates are linear and can be expressed as a slope K, where $K = \frac{\log C_o - \log C}{T}$ or $C = C_o 10^{-kt}$. C is the concentration of carbon in the blood at time T and C_o the blood concentration of carbon just after the injection and before the particles are absorbed by the RES. K is a constant which characterizes the clearance rate of carbon from the blood and is called the Granulopectic Index (Biozzi et al. 1953). Two-variable linear regression analysis was performed on $\log_{10}$ carbon concentration vs. time, using the preprogrammed functions of a Monroe 1860 Calculator and the slope of the regression lines used as the value of "K" for each rat tested.

6) HEMAGGLUTINATION AND HEMOLYSIN TITRATIONS

The direct hemagglutination (HA) test was done essentially as described by Stavitsky (1954). Serial two-fold dilutions (0.05 ml) in PBS (pH 7.2) of inactivated (56° C., 30 min.) rat anti-SRBC antisera were mixed with 0.025 ml of 2.5% SRBC in "Microtiter" plates (Cooke Laboratory Products, Alexandria, Virginia). After mixing, the plates were incubated at room temperature and the agglutination titer was read after 12 hours as the highest dilution showing a 1+ reaction (Stavitsky 1954). Similar volumes of SRBC were used for hemolysin (HL) titrations and 0.05 ml (5 CH50 units) of Guinea Pig Serum (BBL, Cockeysville, Maryland) diluted in Veronal-

Buffered Saline (pH 7.4, McVicker (1962)) was added as a source of complement to each of the serum dilutions. After mixing, the plates were incubated for 12 hours at room temperature and the hemolysin titer read as the highest serum dilution showing 50% hemolysis. In both tests the appropriate positive and negative controls were included.

7) IN VITRO LYSIS AND AGGLUTINATION OF TRYPANOSOMES

The in vitro agglutination of trypanosomes was done essentially as described by D'Alesandro (1976). Serial two-fold dilutions (0.05 ml) in PBGS (PBS pH 7.2, containing 1% glucose (w/v)) of inactivated (56^0 C., 30 min.) rat antisera were mixed with equal volumes of PBGS containing approximately 5.0×10^7 trypanosomes (collected either 6 or 14 days after infection) in "Microtiter" plates. After mixing, the plates were incubated at 37^0 C. for 30 min. and the agglutination titer was read microscopically as the highest dilution to show a 1+ agglutination reaction (D'Alesandro 1976). For trypanolytic titrations, 0.025 ml (5 CH50 units) of Guinea Pig Serum (BBL, Cockeysville, Maryland) diluted in Veronal-Buffered Saline was added as a source of complement to each of the serum dilutions. After mixing, the plates were incubated at 37^0 C. for 30 minutes and the lytic titer read microscopically as the last dilution to show lysed organisms. In both tests the appropriate positive and negative controls were included.

8) IMMUNOCHEMICAL ANALYSES

Ouchterlony double immunodiffusion was performed according to the principles outlined by Ouchterlony (1958) on microscope slides (25 x 75 mm) coated with 1% Agarose as described below for immunoelectrophoresis.

Microslide immunoelectrophoresis was performed following the

principles described by Schiedegger (1955) using microscope slides (25 x 75 mm) coated with 1% Agarose (Bio-Rad, Mississauga, Ontario) in 50 mM Tris - 133 mM Glycine electrophoresis buffer (pH 8.5) containing 0.5% (v/v) Triton X-100 and 0.01% sodium azide. Antigen wells were charged with 10µl of TLE (100µg protein) or TSE (100µg protein) and electrophoresed at a potential of 6 V/cm (actual output, 350 Volts). A prestained serum albumin reference (Amido-Schwartz Stain) was allowed to migrate 5 cm (~1 hour) after which electrophoresis was stopped. Serum troughs were filled with the appropriate antiserum and slides were allowed to develop for 24 hours at room temperature in a humidified atmosphere. Slides were washed extensively for 72 hours in 6 changes of 0.1 M sodium chloride to remove unprecipitated proteins and for 24 hours in 3 changes of distilled water. Gels were dried overnight at 37° C. and subsequently stained with Amido-Schwartz B10 (Uriel and Grabar 1956) for 10-15 minutes and decolorized with 2% acetic acid for 30 minutes. In some cases, the slides were stained with the more sensitive protein stain, Coomassie Brilliant Blue, in order to identify very weak precipitin lines (0.5 grams Gurr's Coomassie Brilliant Blue, #1137, Esbe, Toronto, dissolved in 40.0 ml ethanol, 50.0 ml saturated Mercuric Chloride, and 10.0 ml Glacial Acetic acid). After a 5 minute staining period, the slides were washed in ethanol/water/acetic acid 40:50:10 for 10-15 minutes.

Two dimensional crossed-immunoelectrophoresis is a very sensitive technique that is particularly well suited to the characterization of complex antisera with weak precipitin lines, where it is necessary to identify reactions of identity between heterologous antisera and antigens. The techniques for two dimensional crossed-immunoelectrophoresis (CIEP) and crossed-immunoelectrophoresis with intermediate gels (CIEPIG) have been extensively described by Axelsen et al. (1973). Briefly, glass microscope slides

(50 x 75 mm) were coated with 1% Agarose in 50 mM Tris - 133 mM Glycine electrophoresis buffer (pH 8.5) containing 0.5% (v/v) Triton X-100 and 0.01% Sodium Azide. Antigen wells were charged with 10µl of the appropriate antigen (TSE or TLE, 100µg protein) and were electrophoresed in the first dimension at 6 V/cm (actual output, 350 Volts) until the prestained albumin reference (see above) was 0.5 cm from the edge of the slide (~50-60 min.). Second dimension electrophoresis with intermediate gels was carried out using 400µl or 100µl of antiserum in the reference or intermediate gels respectively and control slides received 100µl of normal rabbit serum in the intermediate gels. Antigens were electrophoresed in the second dimension into the antibody-containing gels at a potential of 2 V/cm (actual output, 110 Volts) for 14 hours in a humidified atmosphere at room temperature. The gels were washed in four changes of 1.0 M sodium chloride for 72 hours to remove soluble proteins and detergent, and after rinsing in distilled water for 24 hours, the gels were dried overnight at 37° C. and subsequently stained with Coomassie Brilliant Blue as described above.

9) SPLENECTOMY

Rats were anaesthetized with intraperitoneal injections of Nembutal, laparotomized, and the spleen exposed. Splenic mesenteries were tied using absorbable sutures (Ethicon Sutures Inc., Peterborough, Ontario) and the spleen was resected. The abdominal cavity was closed using absorbable sutures and surgical clips (Wound Clip - 9 mm, Clay Adams, New Jersey). The entire operation took 10 minutes and the rats recovered quickly for the surgery. Control animals were sham-operated by performing laparotomy and exposing the spleen.

10) EXPERIMENTAL PROTOCOLS

EXPERIMENTS I-IV - CONCOMITANT INFECTIONS OF *T. spiralis* AND *T. lewisi*

The following set of experiments was designed to examine the influence of *T. spiralis* on host immunological responsiveness to concomitant infections with *T. lewisi*. Four factors were investigated relative to the potentiation of acquired immunity to *T. lewisi* during Trichinellosis in the rat host:

1) The inoculating dose of *T. spiralis*
2) The intensity of muscle parasitism
3) The stage of the nematode life cycle in the host during which rats were challenged with a trypanosome infection
4) The influence of splenectomy on immunopotentiation

24 Charles River, inbred albino, female rats, were used in each experiment. Three groups of six rats each were inoculated with 100, 500, or 1500 infective muscle larvae of *T. spiralis* and six animals were kept as uninfected controls (controls received a sham inoculation of PBS). Four separate experiments were conducted in which rats were challenged with *T. lewisi* at 7 (Exp. I), 14 (Exp. II), 28 (Exp. III-A), or 56 (Exp. IV) days after infection with *T. spiralis*. As part of experiment III, an additional group of 30 rats were inoculated as follows (Exp. III-B): Three groups of six rats each were inoculated with *T. spiralis* at three levels of infection as above and twelve rats were kept as uninfected controls. 26 days after infection (2 days before trypanosome challenge) the three infected groups plus half of the control group were splenectomized. The other half of the control group received sham operations.

Trypanosome parasitemia was followed in all experimental animals by daily hemocytometer counts of tail blood as described previously. Thirty days after infection with T. lewisi, the T. spiralis-infected rats in each experiment were killed, and the encysted muscle larvae recovered by acid-pepsin digestion of infected carcasses as described above. The number of larvae per gram of carcass was determined for each infected rat. Experimental animals were separated into larval dosage groups and larval recovery groups based on these results. The daily mean trypanosome parasitemias for each larval recovery group and larval dosage group were plotted against days after infection with trypanosomes, and the course of T. lewisi parasitemia in nematode-infected rats compared to that of the uninfected control groups.

EXPERIMENT V-A - COMPARATIVE IMMUNOCHEMICAL ANALYSIS OF PARASITE ANTIGENS

In any experimental model that deals with concomitant parasitic infections, there exists the possibility that the competitive interactions that occur may be associated with antigenic relationships between parasites and the synthesis of homologous antibodies by the host. In parasitic infections where specific immunoglobulin plays an essential role in acquired immunity (as is the case for T. lewisi), this type of interaction may be of paramount importance in the host response to intercurrent infections with antigenically related organisms. The following immunochemical analyses were conducted in order to test for the possibility of cross-reactivity between T. spiralis and T. lewisi antigens:

1) TSE and TLE were compared using rabbit antisera to each extract by Ouchterlony immunodiffusion, microslide immunoelectrophoresis, and two dimensional crossed-immunoelectrophoresis with intermediate gels.

2) TSE and TLE were also compared by immunodiffusion and microslide immunoelectrophoresis using antisera (immune and hyperimmune) produced in rats during natural infections.

EXPERIMENT V-B - TITRATION OF RAT ANTI-*T. spiralis* IMMUNE SERA FOR LYTIC AND AGGLUTINATING ANTIBODIES TO *T. lewisi*

Rat antisera were collected from animals that had been infected with 100 or 1500 larvae of *T. spiralis* for 7, 14, 28, or 56 days and were tested *in vitro* for the ability to lyse or agglutinate living trypanosomes collected either 6 or 14 days after infection (reproducing forms or inhibited adult forms respectively).

EXPERIMENT VI - ASSAY OF RES ACTIVITY IN EXPERIMENTAL TRICHINELLOSIS

The following experiment was conducted in order to assess the influence of *T. spiralis* infections on RES activity as it relates to both the course of the infection and the parasite burden in the rat host.

60 Charles River CDF, inbred albino, female rats were used in this experiment. Two groups of 20 animals each were inoculated with 100 or 1500 infective muscle larvae of *T. spiralis* and 20 rats were kept as age and weight-matched controls. RES activity was assayed at 7, 14, 28, and 56 days after infection with *T. spiralis* by testing the intravascular clearance of colloidal carbon in five animals from each of the two infected groups and the controls. The Granulopectic Index "K" was calculated for each animal as described above using slopes obtained from linear regression analysis of the carbon clearance data (the correlation coefficients (r) in this analysis were always greater than -0.97 and there were no significant

differences in the values of "r" for any of the treated or control groups during the course of the experiment. The grand mean of "r" for all the animals in this experiment was -0.9912 ± .001). The mean Granulopectic Index "K" for each group was plotted against days after infection with T. spiralis and RES activity for infected groups compared to uninfected controls.

EXPERIMENT VII - ASSAY OF IMMUNOSUPPRESSION DURING TRICHINELLOSIS

Immunosuppression is well characterized in experimental Trichinellosis in the mouse model and maximum suppression of the response to SRBC has been shown to occur at 14 days after infection (Faubert 1976). There has not been an adequate investigation, however, for the purpose of comparison, of the time course of immunosuppression in the rat host as it relates to the stages of the nematode life cycle, level of infection, or immunopotentiation. The following experiment was conducted in order to assess the effect of T. spiralis infections on the humoral antibody response of the rat to the heterologous antigen, SRBC.

60 Charles River CDF, inbred albino, female rats were used in this experiment. Two groups of 20 rats each were inoculated with 100 or 1500 infective muscle larvae of T. spiralis and 20 rats were kept as controls. The humoral response to SRBC was assayed in infected animals by immunizing 5 animals from each group with 2.0×10^9 SRBC given intraperitoneally at 7, 14, 28, or 56 days after infection with T. spiralis. Six days after immunization, the rats were bled and the sera collected as described above. HA and HL titers were determined and expressed as $\log_2$ titer and the difference between the mean experimental and mean control values was plotted against the days after infection when the rats were immunized.

11) STATISTICAL ANALYSES

A statistical analysis of experimental results was conducted using the Analysis of Variance, F-test, Student's t-distribution test (Snedecor and Cochran 1967) and Duncan's new multiple range test (Duncan 1955) where appropriate, and when necessary, extraneous values were rejected using the Q-test (Dean and Dixon 1951).

RESULTS

EXPERIMENTS I-IV - CONCOMITANT INFECTIONS OF T. SPIRALIS AND T. LEWISI

The intensity of muscle parasitism was determined in experiments I-IV in order to assess the dose-response relationship of T. spiralis in the rat host as it relates to the potentiation of immunity to challenge infections with T. lewisi. For the purpose of comparing T. lewisi parasitemias in nematode-infected groups to uninfected controls, animals were separated into groups based upon either the initial dose of T. spiralis or the recovery of muscle larvae at the conclusion of each experiment. The three groups in each category for experiments I-IV are shown in Table 5.1. In general, all rats that were inoculated with a particular dose of infective larvae showed recoveries of encysted muscle larvae that were consistent with that dose. Daily mean trypanosome parasitemias for larval recovery groups and larval dosage groups are therefore essentially identical and the results presented in this chapter represent the mean trypanosome parasitemias for the larval recovery groups.

EXPERIMENT I

In experiment I, rats were challenged with T. lewisi 7 days after inoculation with T. spiralis during the enteral phase of the nematode infection. The daily mean trypanosome parasitemias for larval recovery groups

TABLE 5.1 - LARVAL DOSAGE GROUPS AND LARVAL RECOVERY GROUPS FOR EXPERIMENTS I-IV

EXPERIMENT #	LARVAL DOSAGE GROUPS (LARVAE/RAT)	LARVAL RECOVERY GROUPS (LARVAE/GRAM ± SE)
I	100	139 ± 31
	500	1846 ± 229
	1500	3532 ± 834
II	100	209 ± 22
	500	1110 ± 159
	1500	3382 ± 520
III-A	100	155 ± 23
	500	1062 ± 71
	1500	2302 ± 265
III-B	100	189 ± 57
	500	1031 ± 123
	1500	2792 ± 407
IV	100	151 ± 14
	500	425 ± 66
	1500	1653 ± 297

are presented in Fig. 5.1. On day 8 of the T. lewisi infections (day 15 of the T. spiralis infections), the mean parasitemias for animals that received a dose of either 100 or 500 larvae or had recoveries of 139 ± 31 larvae/gram or 1846 ±229 larvae/gram respectively, were significantly lower (52% and 47% of the control value respectively, $P<.05$) than those of controls without T. spiralis infections. The group of rats that received the higher dose of 1500 larvae or had recoveries of 3,532 ±834 larvae/gram showed no significant differences from the control group throughout the course of the experiment. By 14 days into the trypanosome infections however, the mean parasitemias for all nematode-infected groups were not significantly different from the controls. Therefore, a dose of 100 or 500 larvae/rat of T. spiralis 7 days before a challenge infection with T. lewisi, effectively altered the host response to T. lewisi as reflected by the inhibited development of peak parasitemias in these groups as compared to either the control group without T. spiralis or the nematode-infected group that received a higher dose of 1500 larvae.

EXPERIMENT II

In experiment II, rats were challenged with T. lewisi at 14 days after inoculation with T. spiralis during the peak period of the parenteral migration of newborn larvae to the muscle tissues of the host. The daily mean trypanosome parasitemias for larval recovery groups are illustrated in Fig. 5.2. No significant differences in the levels of parasitemia or the course of trypanosome infections were observed between the nematode-infected groups or the uninfected controls. It would appear that at day 22, the parasitemia in the control group was lower than that of the nematode-infected groups but these differences were not significant ($P>.05$). There-

FIGURE 5.1

Course of trypanosome parasitemia in rats challenged with *Trypanosoma lewisi* 7 days after inoculation with *Trichinella spiralis*. Daily mean trypanosome parasitemias are presented for larval recovery groups as shown (l/gm = larvae/gram).

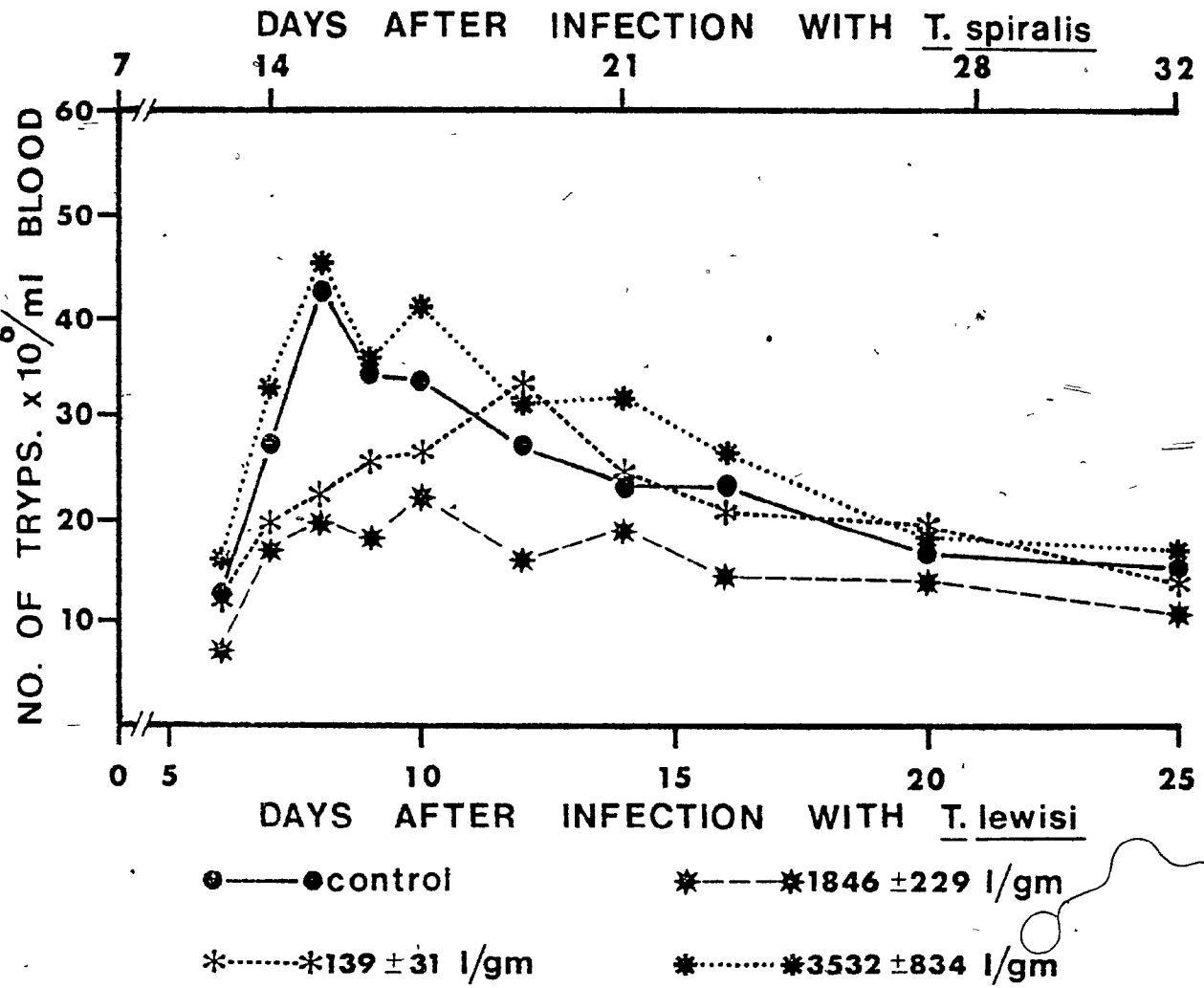
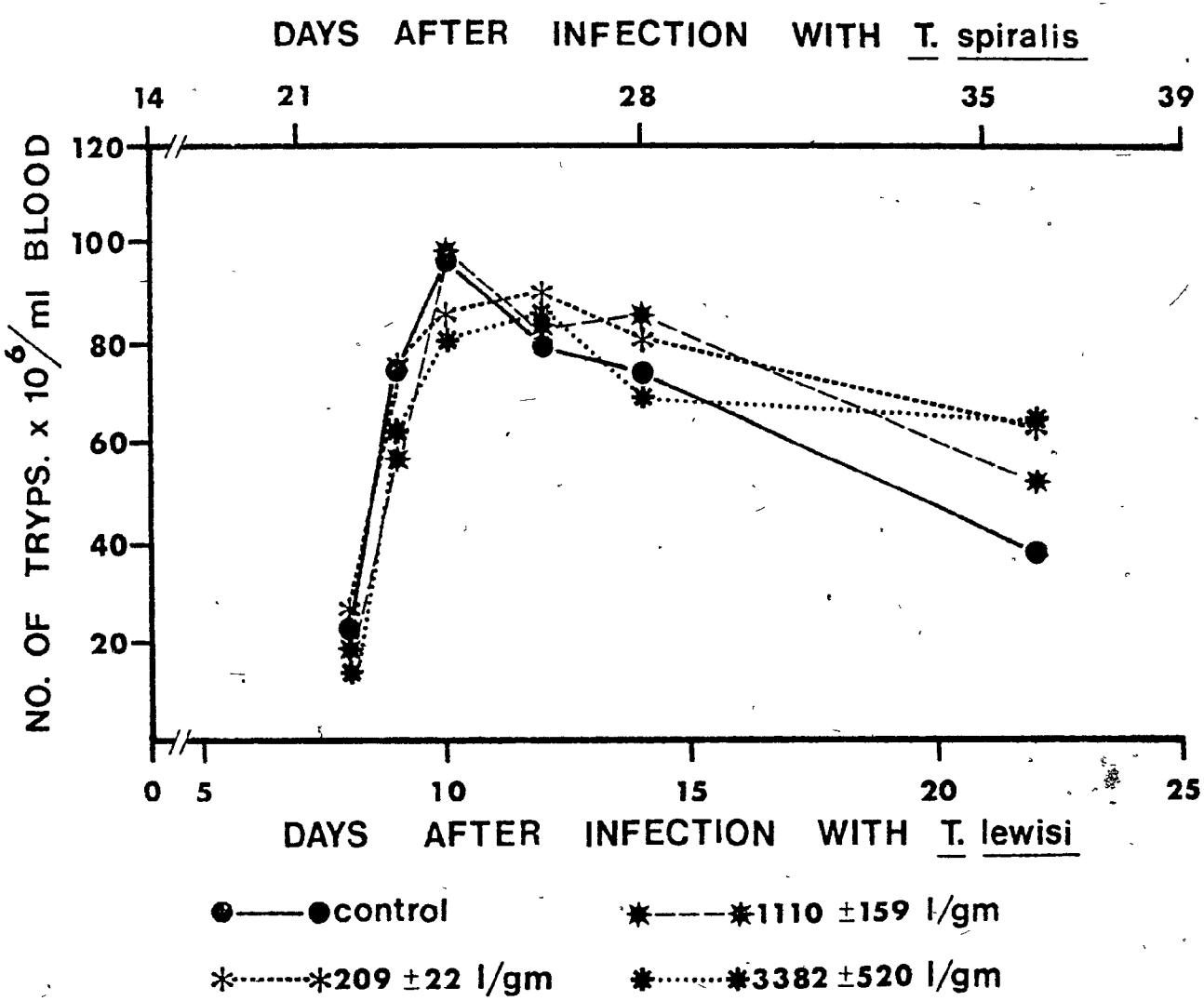

DAYS AFTER INFECTION WITH T. spiralis
7
14
21
28
32
60
50
40
30
20
10
NO. OF TRYPS. x 10⁶/ml BLOOD
0
5
10
15
20
25
DAYS AFTER INFECTION WITH T. lewisi
control
1846 ±229 l/gm
139 ±31 l/gm
3532 ±834 l/gm

FIGURE 5.2

Course of trypanosome parasitemia in rats challenged with *Trypanosoma lewisi* 14 days after inoculation with *Trichinella spiralis*. Daily mean trypanosome parasitemias for larval recovery groups are presented as shown (l/gm = larvae/gram).


DAYS AFTER INFECTION WITH T. spiralis
14
21
28
35
39
NO. OF TRYPS. x 10⁶/ml BLOOD
120
100
80
60
40
20
0
5
10
15
20
25
DAYS AFTER INFECTION WITH T. lewisi
control
1110 ±159 l/gm
209 ±22 l/gm
3382 ±520 l/gm

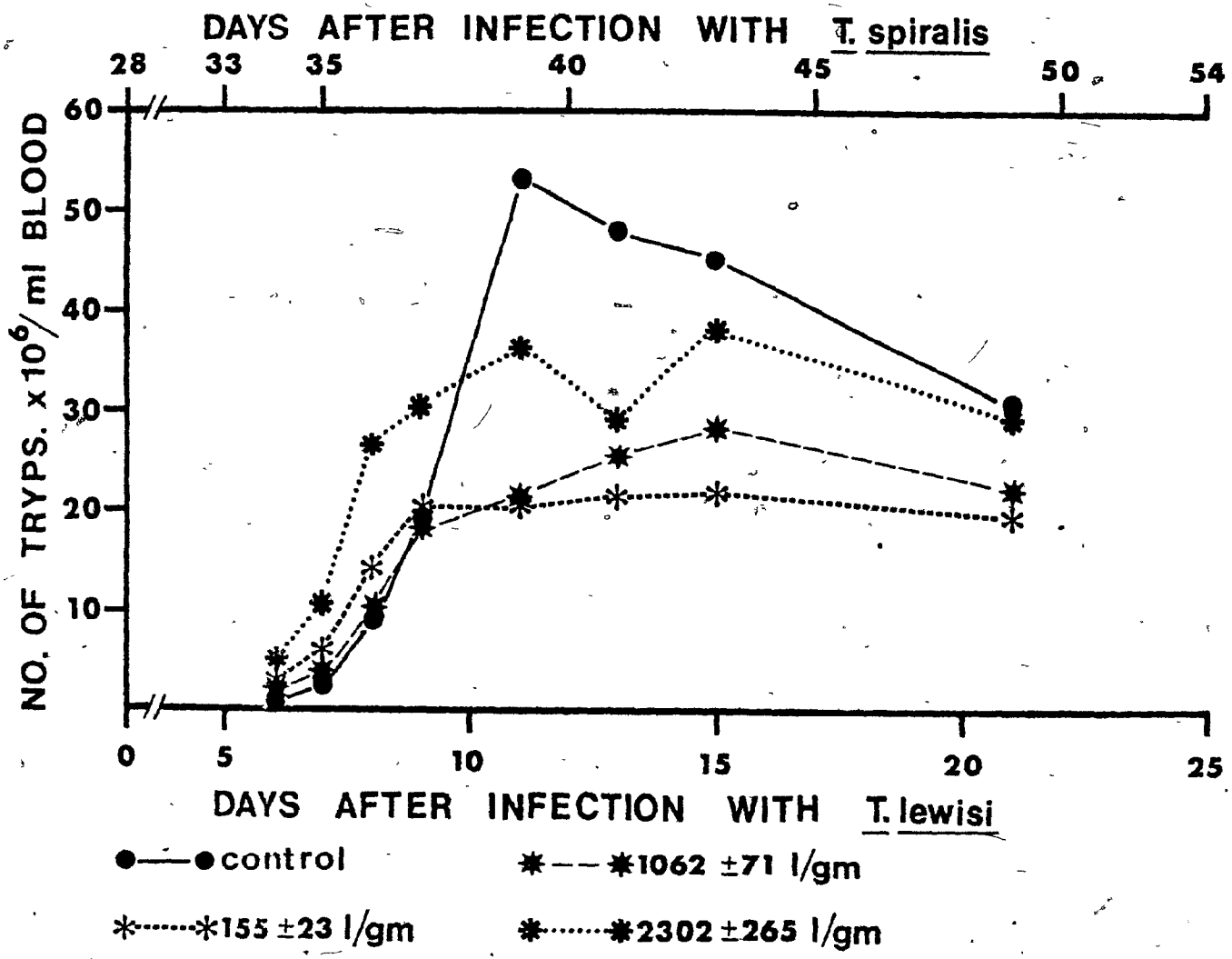
fore, in animals challenged with T. lewisi at 14 days after inoculation with T. spiralis, the course of trypanosome parasitemias was unaffected by the presence of the concomitant nematode infection.

EXPERIMENT III-A

In experiment III-A, rats were challenged with T. lewisi 28 days after inoculation with T. spiralis during the parenteral phase of the infection when T. spiralis is essentially a chronic intracellular parasite in the host's striated muscle cells. The daily mean trypanosome parasitemias for the larval recovery groups are presented in Fig. 5.3. A significant inhibition of T. lewisi parasitemia was observed in all nematode-infected groups in this experiment. On day 11 of the T. lewisi infections (day 39 of the T. spiralis infections), the mean parasitemias for the groups that were inoculated with doses of 100 or 500 larvae/rat or had recoveries of 155 ±23 or 1062 ±71 larvae/gram respectively, were significantly lower (39% of the control value, $P<.05$) than the control group without T. spiralis. These differences were significant until day 15 of the trypanosome infections, after which time the nematode-infected group and control group displayed similar levels of infection. Parasitemia was also inhibited to some degree (69% of the control value) on day 11 in the nematode-infected group that was inoculated with a dose of 1500 larvae/rat or had recoveries of 2302 ±265 larvae/gram, but not to the same extent as in the groups that were inoculated with the lower doses of T. spiralis. It would appear from these results that the inhibition of trypanosome parasitemia or immunopotentiation during these concomitant infections bears an inverse relationship to the level of infection with T. spiralis (dosage or recovery of muscle larvae), such that low level infections

FIGURE 5.3

Course of trypanosome parasitemia in rats challenged with Trypanosoma lewisi 28 days after inoculation with Trichinella spiralis. Daily mean trypanosome parasitemias for larval recovery groups are presented as shown (l/gm = larvae/gram).


DAYS AFTER INFECTION WITH T. spiralis
28
33
35
40
45
50
54
60
50
40
30
20
10
NO. OF TRYPS. x10⁶/ml BLOOD
0
5
10
15
20
25
DAYS AFTER INFECTION WITH T. lewisi
control
1062 ±71 l/gm
155 ±23 l/gm
2302 ±265 l/gm

enable the host to respond more efficiently to a trypanosome challenge than do high level infections.

EXPERIMENT III-B

In experiment III-B, similar groups of nematode-infected and control animals were splenectomized two days before trypanosome challenge, in order to assess the influence of the spleen on immunopotentiation in this model. At 28 days after the inoculation of T. spiralis (two days after splenectomy), all splenectomized and control groups were challenged with T. lewisi as in experiment III-A. The daily mean trypanosome parasitemias for the larval recovery groups are illustrated in Fig. 5.4. No significant differences were observed in trypanosome parasitemia between splenectomized, nematode-infected rats and the splenectomized uninfected controls. In splenectomized animals however, the mean peak parasitemias in all groups were enhanced approximately 5-6 fold over that of sham-operated controls. Therefore, splenectomy seemed to effectively interfere with the immunopotentiation that occurred in experiment III-A (Fig. 5.3), with low level infections of T. spiralis.

EXPERIMENT IV

In experiment IV, rats were challenged with T. lewisi 56 days after inoculation with T. spiralis during the chronic, intracellular muscle stage of the infection. Daily mean trypanosome parasitemias for the larval recovery groups are presented in Fig. 5.5. No significant differences were observed between the nematode-infected groups and the uninfected controls in either the mean levels of parasitemia or the general course of the trypanosome infections. Therefore, the potentiation of the host response

FIGURE 5.4

Course of trypanosome parasitemia in splenectomized rats challenged with Trypanosoma lewisi 28 days after inoculation with Trichinella spiralis (2 days after splenectomy). Daily mean trypanosome parasitemias for larval recovery groups are presented (splen. = splenectomy; l/gm = larvae/gram).

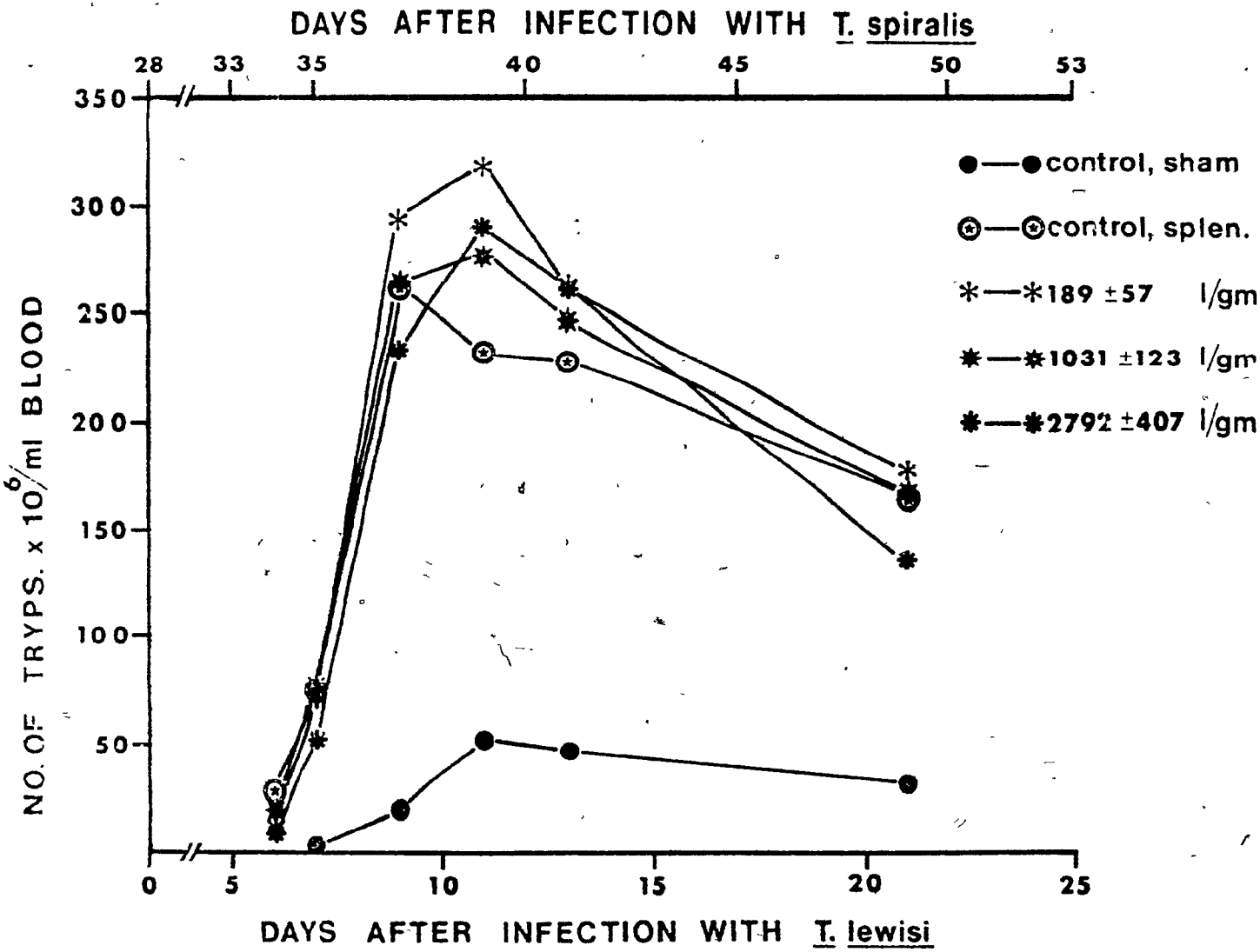
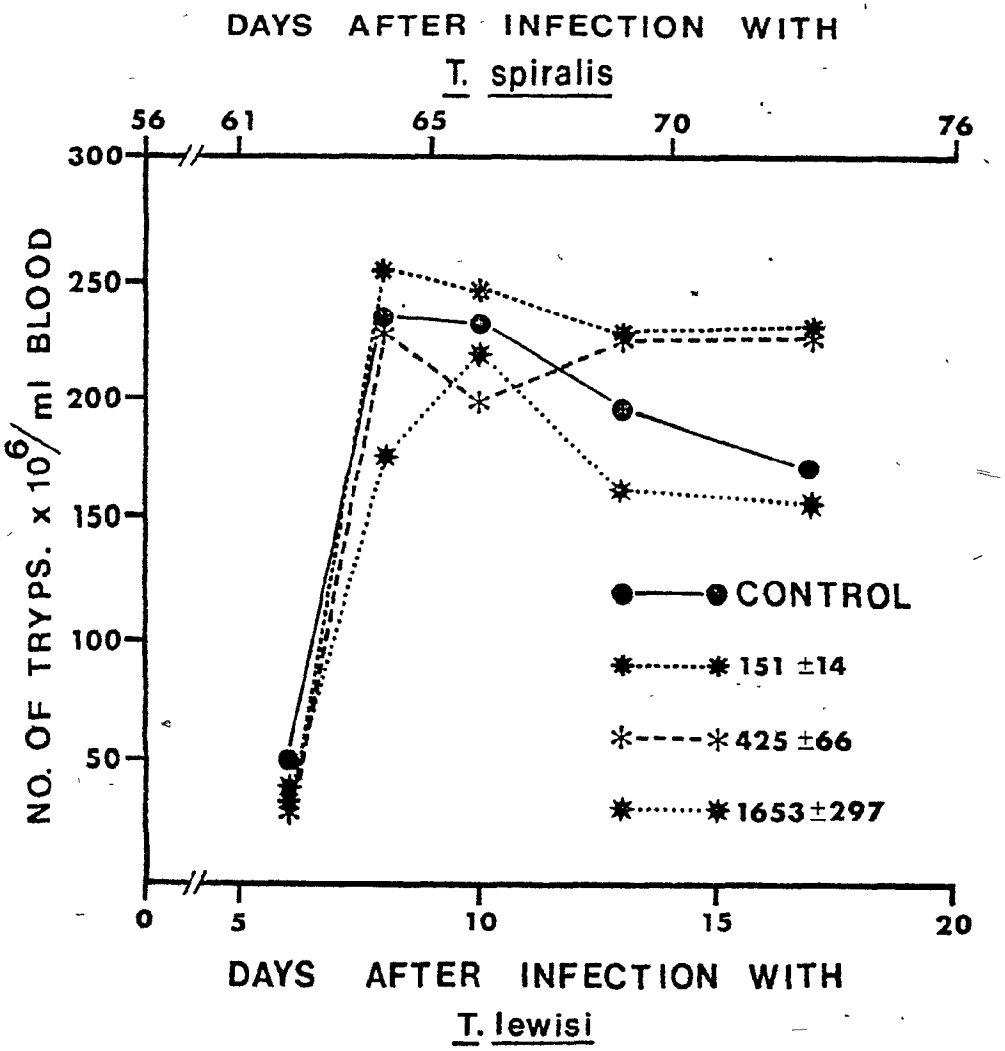

DAYS AFTER INFECTION WITH T. spiralis
28
33
35
40
45
50
53
350
300
250
200
150
100
50
NO. OF TRYPS. x 10⁶/ml BLOOD
control, sham
control, splen.
189 ±57 l/gm
1031 ±123 l/gm
2792 ±407 l/gm
0
5
10
15
20
25
DAYS AFTER INFECTION WITH T. lewisi

FIGURE 5.5

Course of trypanosome parasitemia in rats challenged with Trypanosoma lewisi 56 days after inoculation with Trichinella spiralis. Daily mean trypanosome parasitemias for larval recovery groups are presented as shown (l/gm = larvae/gram).


DAYS AFTER INFECTION WITH
T. spiralis
56
61
65
70
76
300
250
200
150
100
50
NO. OF TRYPS. x 10⁶/ml BLOOD
CONTROL
151 ±14
425 ±66
1653±297
0
5
10
15
20
DAYS AFTER INFECTION WITH
T. lewisi

to T. lewisi that was evident when rats were challenged at 7 or 28 days after infection with T. spiralis (Exp. I and Exp. III-A) was abrogated 56 days after the nematode infection.

EXPERIMENT V-A - COMPARATIVE IMMUNOCHEMICAL ANALYSES OF PARASITE ANTIGENS

A comparative immunochemical analysis of T. lewisi and T. spiralis antigens was conducted in order to rule out the possibility that immunopotentiation during concomitant infections with these two parasites may be the result of cross-reacting antigens and the synthesis of homologous antibodies by the host.

Ouchterlony immunodiffusion, using immune or hyperimmune sera from rats infected with either T. spiralis or T. lewisi as well as antisera from rabbits immunized with antigen extracts of these parasites, did not detect the presence of any cross-reacting antigens (Fig. 5.6). Immunoelectrophoresis using these same antisera and antigens also failed to detect any antigenic cross-reactivity (Fig. 5.7). The highly sensitive technique of CIEPIG using antisera from rabbits immunized with antigen extracts of both parasites did not reveal any common antigenic determinants (Fig. 5.8).

EXPERIMENT V-B - TITRATION OF RAT ANTI-T. SPIRALIS IMMUNE SERA FOR LYTIC AND AGGLUTINATING ANTIBODIES TO T. LEWISI

Rat antisera collected from animals that had been infected with 100 or 1500 larvae of T. spiralis for 7, 14, 28, or 56 days were tested in vitro for their ability to lyse or agglutinate trypanosomes. In vitro lysis or agglutination of trypanosomes was not observed in any serum dilution for any of the antisera tested, using either reproducing forms (first antigenic variant) or inhibited adult forms (second antigenic variant),

FIGURE 5.6

OUCHTERLONY IMMUNODIFFUSION

A - TSE (10 mg/ml)

B - TLE (10 mg/ml)

1 - Pooled sera, rabbit anti-TSE

2 - Pooled sera, rabbit anti-TLE

3 - Pooled sera, rat anti-*T. spiralis*, 100 larvae/rat, Day 28

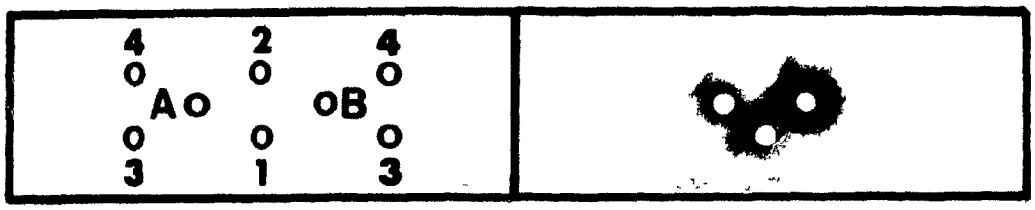
4 - Pooled sera, rat anti-*T. spiralis*, 1500 larvae/rat, Day 28

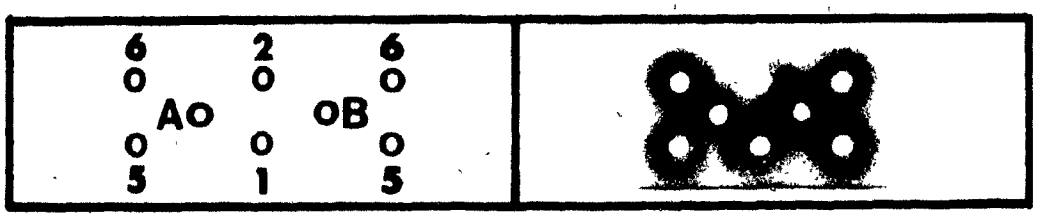
5 - Pooled sera, rat anti-*T. spiralis*, 100 larvae/rat, Day 56

6 - Pooled sera, rat anti-*T. spiralis*, 1500 larvae/rat, Day 56

7 - Pooled sera, hyperimmune, rat anti-*T. spiralis*

8 - Pooled sera, hyperimmune, rat anti-*T. lewisi*


4 2 4
0 0 0
AO OB
0 0 0
3 1 3


6 2 6
0 0 0
AO OB
0 0 0
5 1 5

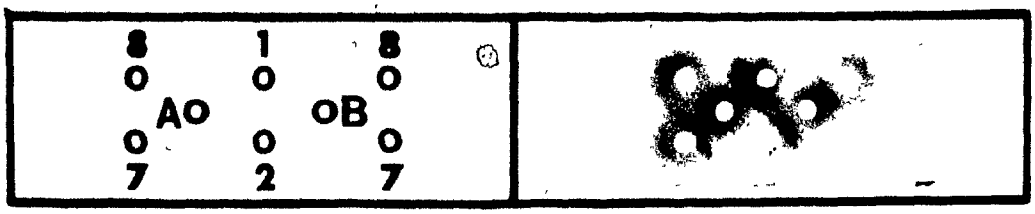

8 1 8
0 0 0
AO OB
0 0 0
7 2 7

FIGURE 5.7

IMMUNOELECTROPHORESIS

A - TLE (10 mg/ml)

B - TSE (10 mg/ml)

1 - Pooled sera, rabbit anti-TSE

2 - Pooled sera, rabbit anti-TLE

3 - Pooled sera, rat anti-*T. spiralis*, 100 larvae/rat, Day 28

4 - Pooled sera, rat anti-*T. spiralis*, 1500 larvae/rat, Day 28

5 - Pooled sera, rat anti-*T. spiralis*, 100 larvae/rat, Day 56

6 - Pooled sera, rat anti *T. spiralis*, 1500 larvae/rat, Day 56

7 - Pooled sera, hyperimmune, rat anti-*T. spiralis*

8 - Pooled sera, hyperimmune, rat anti-*T.lewisi*

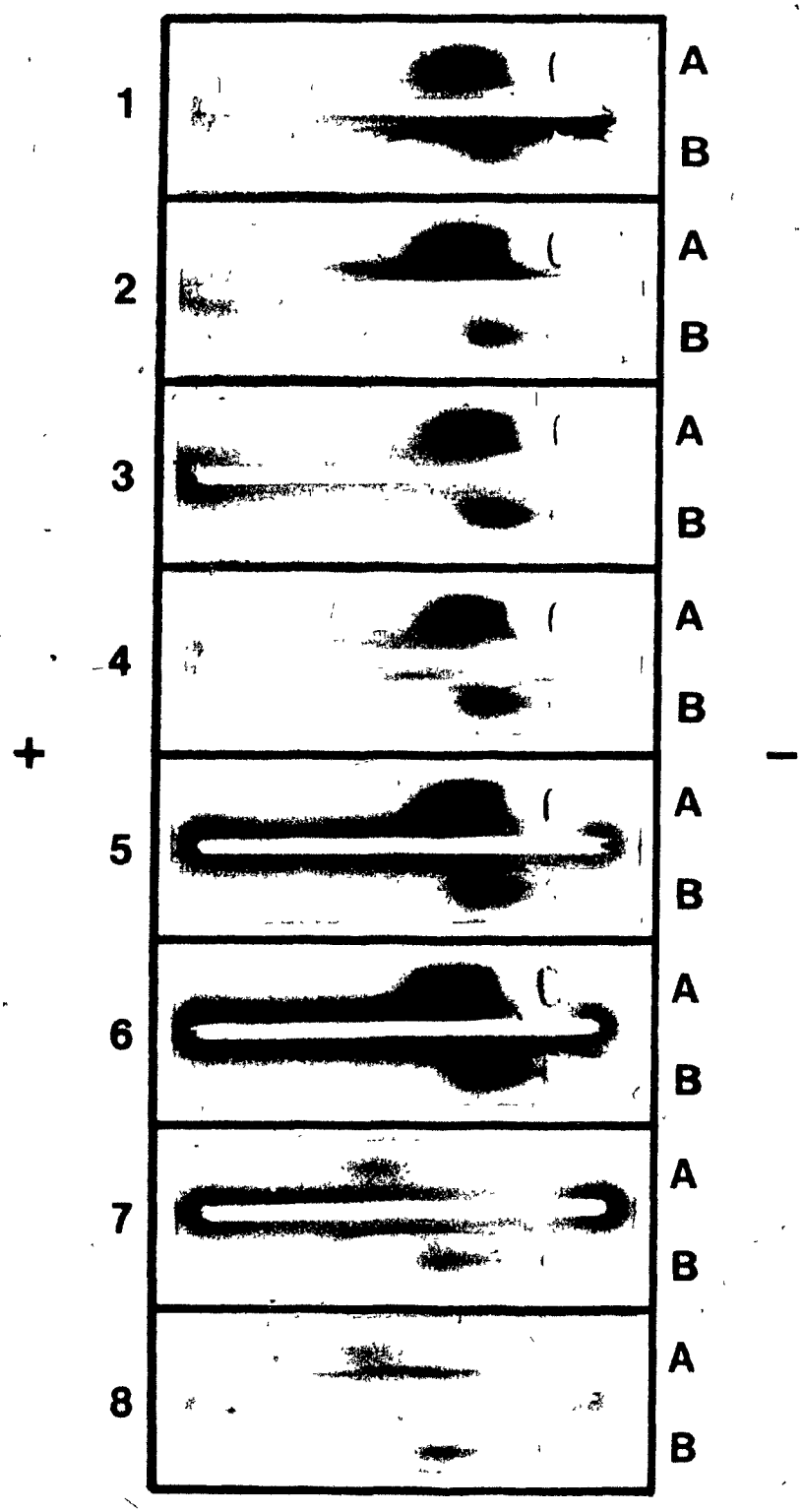

1
2
3
4
5
6
7
8
A
B
+
−

FIGURE 5.8

TWO DIMENSIONAL CROSSED-IMMUNOELECTROPHORESIS

WITH INTERMEDIATE GELS

A - CONTROL SLIDE FOR TSE

1 - Antigen : 10µl TSE (10 mg/ml)

3 - Intermediate Gel : 100µl Normal Rabbit Serum

4 - Reference Gel : 400µl Pooled Rabbit Anti-TSE

B - EXPERIMENTAL SLIDE FOR TSE

1 - Antigen : 10µl TSE (10 mg/ml)

5 - Intermediate Gel : 100µl Pooled Rabbit Anti-TLE

4 - Reference Gel : 400µl Pooled Rabbit Anti-TSE

C - CONTROL SLIDE FOR TLE

2 - Antigen : 10µl TLE (10 mg/ml)

3 - Intermediate Gel : 100µl Normal Rabbit Serum

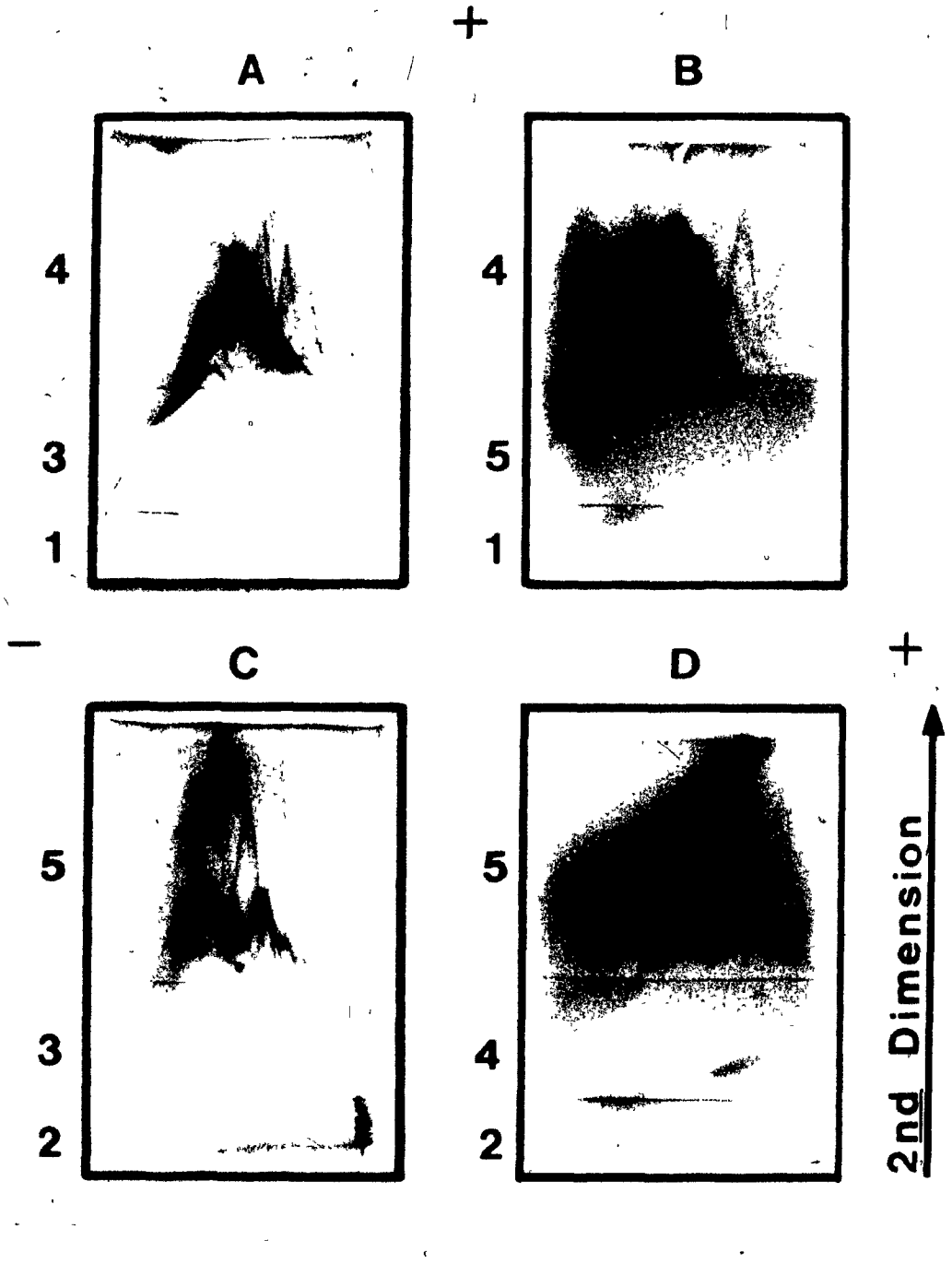
5 - Reference Gel : 400µl Pooled Rabbit Anti-TLE

D - EXPERIMENTAL SLIDE FOR TLE

2 - Antigen : 10µl TLE (10 mg/ml)

4 - Intermediate Gel : 100µl Pooled Rabbit Anti-TSE

5 - Reference Gel : 400µl Pooled Rabbit Anti-TLE


+
A
B
4
3
1
4
5
1
—
C
D
+
5
3
2
5
4
2
2nd Dimension
—
1st Dimension

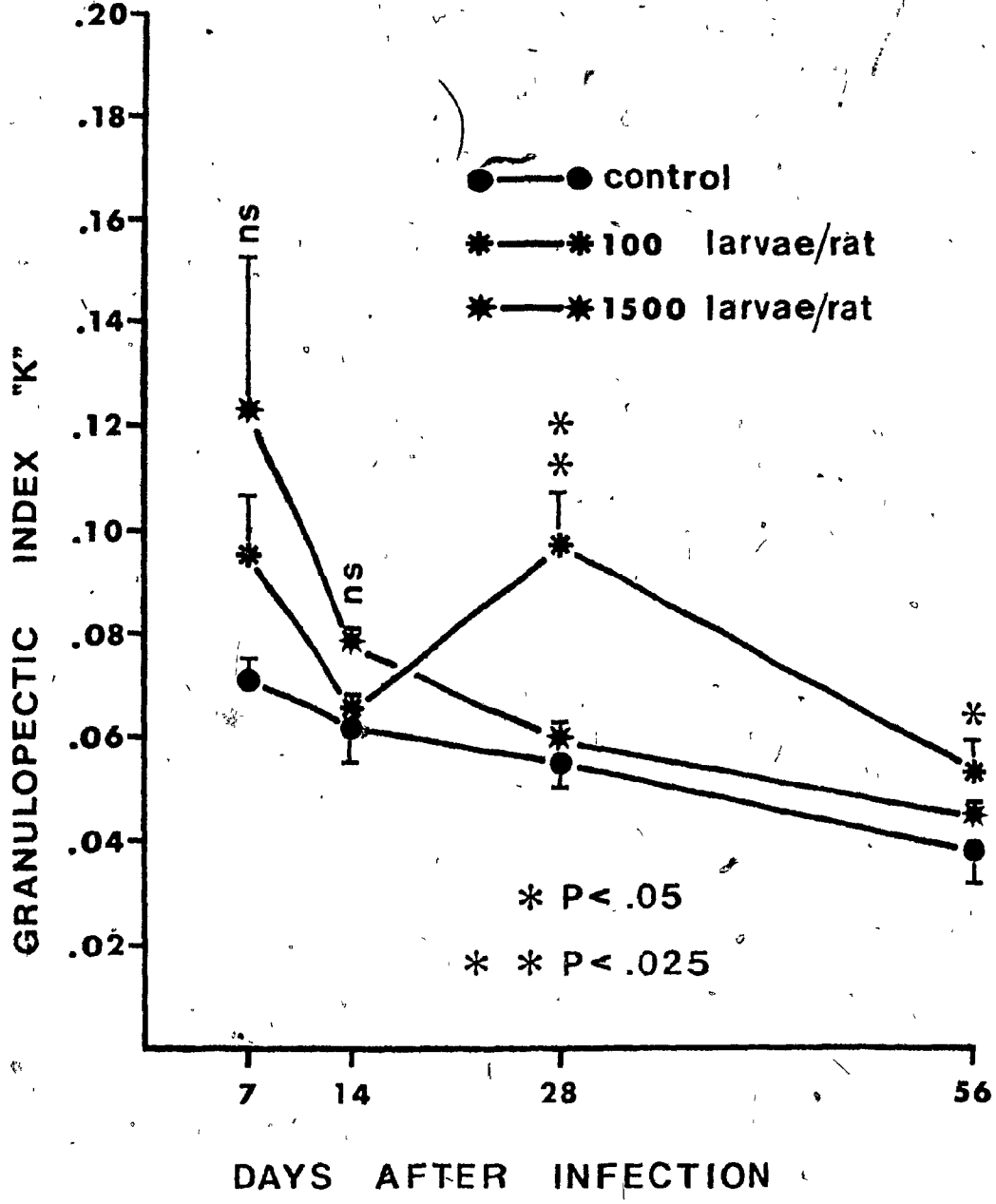
collected 6 and 14 days after infection respectively. Control antisera pooled from rats immune to T. lewisi showed positive titers of 1/16 and 1/32 for lysis and 1/32 and 1/64 for agglutination when tested with inhibited adult forms or reproducing forms respectively. Control antisera pooled from rats hyperimmunized against T. lewisi showed positive titers of 1/32 and 1/64 for lysis and agglutination when tested as above with the first or second antigenic variants respectively.

EXPERIMENT VI - ASSAY OF RES ACTIVITY IN EXPERIMENTAL TRICHINELLOSIS

Immunopotentiation in this model may be related to an expanded fixed-macrophage phagocytic system, resulting in an enhanced removal of trypanosomes from the blood stream during concomitant infections. The functional status of the RES was therefore assessed in order to evaluate the possibility of a correlation between enhanced fixed-macrophage phagocytic activity and the immunopotentiation that occurred at 7 and 28 days after the nematode infection. The phagocytic activity of the RES was examined in rats infected with T. spiralis as it relates to both the level of infection as well as the various stages of the parasite's life cycle in the host. Granulopectic activity, as reflected by the intravascular clearance of colloidal carbon particles, was followed at 7, 14, 28, and 56 days after inoculation with T. spiralis. The mean granulopectic index "K" for each of the infected groups (100 and 1500 larvae/rat) as well as the controls without nematode infections was plotted against days after infection and the results are presented in Fig. 5.9. A significant enhancement of RES activity was observed in rats inoculated with a low dose of 100 larvae at 28 and 56 days after infection, but not at 14 days. At day 7, RES activity in this group appeared to be enhanced, but the difference was not statistically significant ($P<0.1$). In rats that were inoculated with the high dose of 1500 larvae, RES activity was not significantly differ-

FIGURE 5.9

Reticuloendothelial System activity during Trichinellosis in the rat as measured by the rate of the intravascular clearance of colloidal carbon particles. Each point represents the mean granulopectic index "K" for five experimental animals.


.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
GRANULOPECTIC INDEX "K"
control
100 larvae/rat
1500 larvae/rat
ns
ns
* P< .05
* * P< .025
7
14
28
56
DAYS AFTER INFECTION

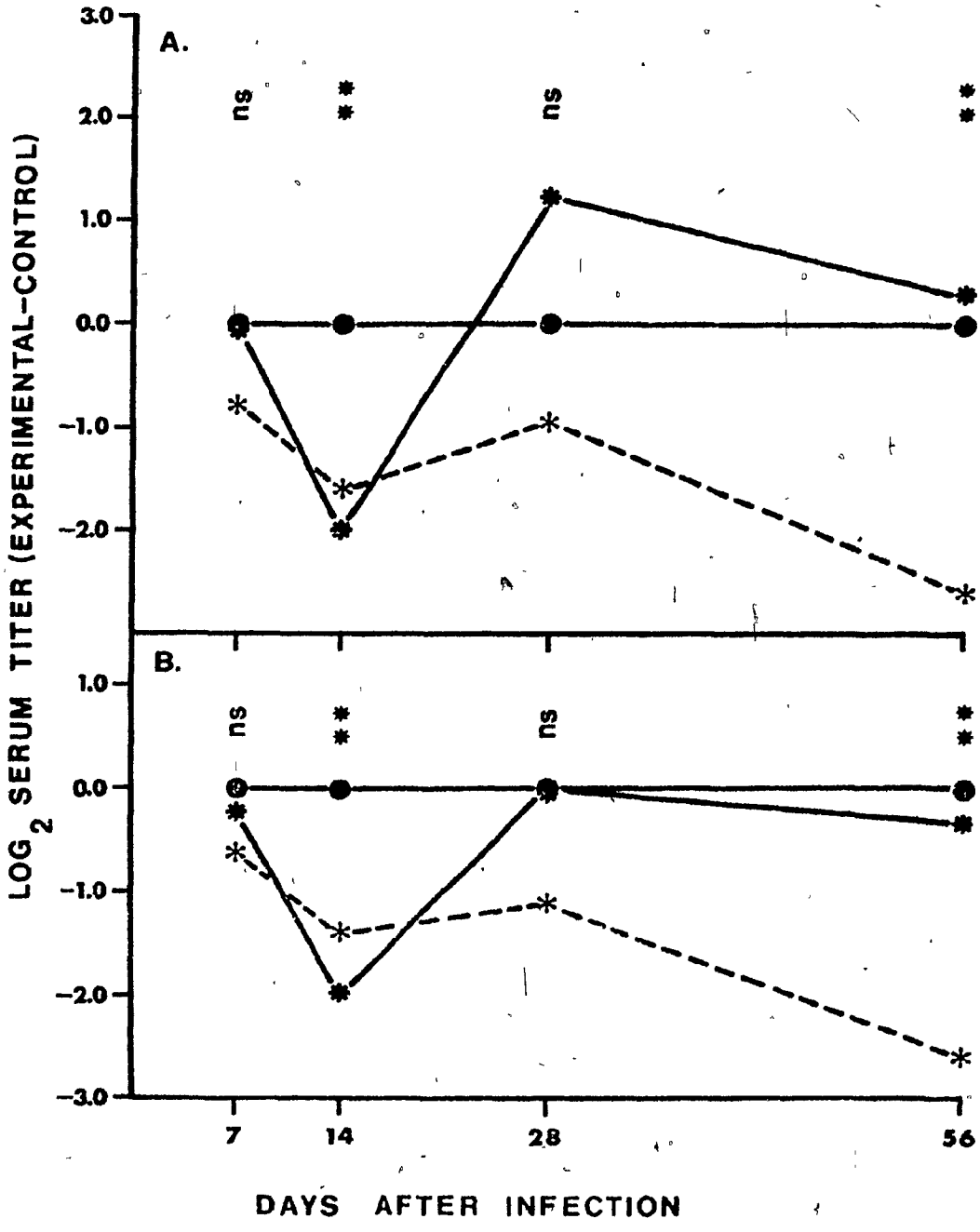
ent from the control group at 14, 28, or 56 days after infection. There appeared to be an enhancement of RES activity in this group at day 7, but this difference was not statistically significant ($P<0.1$) due to the excessive variations in the clearance rates for these animals. A low level infection of 100 larvae/rat was therefore capable of stimulating RES activity at 28 days and possibly 7 days after infection, with gradual return to normal values by day 56. A high level infection with 1500 larvae/rat failed to produce significant stimulation of RES activity during the course of the infection, although a possible stimulation may have occurred at day 7. Both high and low doses of T. spiralis failed to produce a stimulation of RES activity at 14 days after infection.

EXPERIMENT VII - ASSAY OF IMMUNOSUPPRESSION DURING TRICHINELLOSIS

Although immunosuppression is well characterized in the mouse model, no information is available on the influence of T. spiralis on immunological responsiveness to heterologous antigens in the rat host. Whereas maximum immunosuppression in the mouse model occurs at 14 days after infection (Faubert 1976), trypanosome parasitemias were unaffected in nematode-infected rats challenged with T. lewisi at this time (Exp. II). For the purpose of comparing the course of immunosuppression to immunopotentiation during Trichinellosis in the rat model, as it relates to both the level of infection and the nematode life cycle, rats were immunized with the heterologous antigen, SRBC, at 7, 14, 28, and 56 days after infection. Six days after immunization, the serum hemagglutinating (HA) and hemolysin (HL) antibody titers were determined as described above and the results are presented in Fig. 5.10, as the difference between the mean experimental and mean control titers. Humoral responsiveness to SRBC in the group inoculated with 100 larvae/rat was depressed only when animals

FIGURE 5.10

The humoral antibody response to SRBC during Trichinellosis in the rat. Log_2 serum titer (experimental - control) for A) Hemolysin and B) Hemagglutinating antibody titers to SRBC in rats immunized intraperitoneally with 2.0×10^9 SRBC at 7, 14, 28, or 56 days after inoculation with Trichinella spiralis. Each point represents the difference between the means for five animals. ((●) - Control, no T. spiralis; (❋) - 100 larvae/rat; (✳) - 1500 larvae/rat; (✷) - $P<.01$; (ns) - Not significant)


A.
B.
ns
ns
ns
ns
3.0
2.0
1.0
0.0
-1.0
-2.0
1.0
0.0
-1.0
-2.0
-3.0
7
14
28
56
LOG2 SERUM TITER (EXPERIMENTAL-CONTROL)
DAYS AFTER INFECTION

were immunized at 14 days after the nematode infection. The HL and HA titers of infected rats in this group were not significantly different from the controls when rats were immunized at 7, 28, or 56 days after infection. In animals that were inoculated with the high dose of 1500 larvae/rat, the humoral antibody response to SRBC appeared to be depressed throughout the course of the nematode infection, although the differences from the control titers at 7 and 28 days were not necessarily significant ($P<0.1$). At 28 days after infection, however, the difference between the HL titers of the two nematode-infected groups proved to be highly significant ($P<.005$). Therefore, in animals inoculated with the low dose of 100 larvae/rat, immunosuppression was only a transient phenomenon, whereas the higher inoculating dose of 1500 larvae/rat produced a long-lived depression of humoral responsiveness to the heterologous antigen.

DISCUSSION

A summary of the results in this study are presented in Table 5.2. These findings indicate that immunopotentiation occurred with low level inoculating doses of *T. spiralis* when nematode-infected rats were challenged with *T. lewisi* at 7 or 28 days after infection with *T. spiralis*. Immunopotentiation was a transient phenomenon however, and was abrogated when trypanosome challenge was initiated at 14 or 56 days after the nematode infection. The inhibited development of *T. lewisi* parasitemia was related to both the inoculating dose and the level of muscle parasitism. In animals challenged with *T. lewisi* at 7 days after the nematode infection, immunopotentiation was manifested by a significant inhibition of trypanosome parasitemia in those groups that were inoculated with 100 or 500 larvae but not with 1500 larvae.

TABLE 5.2

IMMUNOPOTENTIATION, IMMUNOSUPPRESSION, AND ENHANCED RES ACTIVITY DURING TRICHINELLOSIS IN THE RAT HOST : A SUMMARY OF RESULTS

DAYS AFTER INFECTION -	7			14			28			56		
INOCULATING DOSE -	100	500	1500	100	500	1500	100	500	1500	100	500	1500
IMMUNOPOTENTIATION	+	+	-	-	-	-	+	+	±	-	-	-
IMMUNOSUPPRESSION	-	ND	±	+	ND	+	-	ND	±	-	ND	+
ENHANCED RES ACTIVITY	±	ND	±	-	ND	-	+	ND	-	-	ND	-

+ = YES, - = NO, ± = UNCLEAR (NOT STATISTICALLY SIGNIFICANT), ND = NOT DONE

The development of peak trypanosome parasitemias was also inhibited in all nematode-infected groups challenged with T. lewisi at 28 days after infection but the degree of inhibition appeared to be inversely related to the level of infection (dose or recovery of muscle larvae).

The extensive immunochemical analyses of parasite antigens in experiment V-A did not detect any common antigenic determinants between T. lewisi and T. spiralis, and rat antisera that were collected throughout the course of a primary infection with T. spiralis (experiment V-B), did not lyse or agglutinate trypanosomes in vitro. These results show that immunopotentiation during concomitant infections with these parasites is probably not related to a functional antigenic cross-reactivity and the synthesis of homologous antibodies by the host. Under certain conditions, infections with metazoan parasites such as Nippostrongylus brasiliensis have been shown to potentiate the humoral response (primarily IgE) to previously administered heterologous immunogens (Bloch et al. 1973; Jarrett 1972; Orr and Blair 1969), and the results of experiments V-A and V-B do not rule out the possibility that an infection with T. spiralis may potentiate the normal humoral responsiveness (ablastic or trypanocidal) of the host to T. lewisi. However in view of the known immunosuppressive effects of T. spiralis on the humoral antibody responses of the mouse to heterologous antigens and viruses (see Chapter II), it seemed unlikely that a potentiaiton of humoral responsiveness should occur in the rat model. For purposes of comparison however, the immunosuppressive activity of T. spiralis in the rat was assessed by examining humoral responsiveness to the heterologous antigen, SRBC. The results summarized in table 5.2 show that humoral immunosuppression was related to both the inoculating

dose and the course of the infection. In rats inoculated with the low dose of 100 larvae, immunosuppression was only a transient phenomenon and occurred maximally at 14 days after infection, while the higher inoculating dose of 1500 larvae produced a long-lasting depression of the antibody response to SRBC. Although humoral immunosuppression was evident in both groups at 14 days after infection, T. lewisi parasitemias were never enhanced in nematode-infected rats challenged with trypanosomes at this time. Therefore, T. spiralis did not appear to exhibit any immunosuppressive activity towards acquired humoral immunity to T. lewisi during concomitant infections, although immunopotentiation did not occur when the trypanosome challenge was made during the times after the nematode infection when a depression of humoral responsiveness to SRBC was observed.

The mechanisms by which T. spiralis induces immunopotentiation are still unclear. Cypess et al. (1974) and Meerovitch and Ackerman (1974) have suggested that immunopotentiation is a function of enhanced non-specific cell-mediated immunity (CMI) with the activated macrophage as the effector cell. The fact that T. spiralis infections have been shown to decrease the susceptibility of mice to intracellular parasitism by Listeria monocytogenes (Cypess et al. 1974; Molinari and Cypess 1975) strongly supports this suggestion. More recently, Meerovitch and Bomford (1977) have demonstrated that peritoneal macrophages taken from T. spiralis-infected mice from 6-36 days after infection were cytostatic for R1 luekemia cells in vitro and these results lend further support to the role of the activated macrophage as the effector cell in immunopotentiation during Trichinellosis.

Research on the immunological response of the rat to T. lewisi has not provided definitive evidence for a role of specific or non-specific cell-mediated responses or non-specific RES activity in controlling infections

with this parasite, other than a subsidiary function for phagocytosis in clearing lysed or agglutinated parasites from the blood stream (Taliaferro 1929; D'Alesandro 1970). However, recent research by Greenblatt (1973) demonstrated the presence of T. lewisi in reticular spleen cells (macrophages) in close association with plasma cells in the spleens of infected rats. Although the trypanocidal mechanism may principally involve lysis and agglutination, it is highly probable that splenic macrophages play an important role in both the processing of parasite antigens and in the eventual removal of parasites from the circulation (Greenblatt 1973). The inhibition of trypanosome parasitemia that occurred in nematode-infected hosts (experiments I, III-A) may be due to an expanded fixed-macrophage phagocytic system in the spleen, resulting in accelerated antigen processing or removal of trypanosomes from the blood stream. In order to evaluate this possibility, the functional status of the RES was assessed during Trichinellosis in the rat. The results from experiment VI suggest that a correlation may exist between enhanced RES granulopectic activity and immunopotentiation, both of which occurred primarily with low level inoculating doses of 100 larvae at 7 and 28 days after the nematode-infection, but not with the higher inoculating dose of 1500 larvae (Table 5.2). Other workers have described a lack of correlation between enhanced RES phagocytic activity (as assessed by the rate of intravascular clearance of colloidal carbon particles) and increased resistance to infections with intracellular parasites (Bohme 1960; Lucia and Nussenzweig 1969; Ruskin et al.1969), and Cypess et al. (1974) failed to correlate enhanced RES activity with a decreased susceptibility to L steria monocytogenes in mice infected with T. spiralis. However, T. lewisi is not an intracellular

parasite, and the results of these studies do not preclude the possible involvement of enhanced reticular phagocytic activity in the spleens of concomitantly infected rats. The spleen forms a relatively large part of the RES in the rat and the fact that splenectomy (experiment III-B) interfered with immunopotentiation, while enhancing infections five to six fold over that of sham-operated controls, lends credence to this arguement.

A number of diverse agents that are known to non-specifically stimulate RES activity have also been shown to inhibit T. lewisi parasitemia. Styles (1965, 1970) was able to obtain a similar inhibition of T. lewisi parasitemia by pretreating rats with small serial doses of bacterial endotoxin or the toxin holothurin, a steroid saponin of the Bahamian Sea Cucumber, Actinopyga agassizi. Tate (1951) demonstrated similar results in rats infected previously with the spirochete Spirillum minus, such that rats challenged with T. lewisi after the spirochete infection developed only transient, subpatent parasitemias. Both authors suggested that these agents may act via the non-specific stimulation of RES activity, but neither attempted to correlate enhanced granulopectic activity and increased resistance to infection.

The results of this study suggest that immunosuppression and immunopotentiation are not mutually exclusive in Trichinellosis, nor are they necessarily expressed against all heterologous antigens or organisms. Their expression may be a function of the type of antigen employed and the route of administration (Lubiniecki and Cypess 1975), the intensity of the infection (dose or muscle parasitism), or the timing of the various modes of antigenic stimulation by particular stages of the nematode's life cycle in the host, as has also been suggested by Faubert (1976).

The research presented in the remaining chapters of this thesis examines a number of the possible mechanisms of immunopotentiation in this model of concomitant infections including the potentiation of humoral responsiveness to trypanosome antigens and the role of non-specific CMI, as well as investigates the role of the various stages of the nematode life cycle in the induction of this phenomenon.

CHAPTER VI

THE EFFECT OF *TRICHINELLA SPIRALIS* ON THE DEVELOPMENT OF ACQUIRED HUMORAL IMMUNITY TO *TRYPANOSOMA LEWISI* IN THE RAT

INTRODUCTION

The mechanism of cross-protection during concomitant infections of *Trichinella spiralis* and *Trypanosoma lewisi* in the rat is not clear. The extensive immunochemical analyses of parasite antigens presented in the previous chapter failed to detect any common antigenic determinants between *T. spiralis* and *T. lewisi*, and rat antisera that were collected throughout the course of a primary infection with *T. spiralis* did not agglutinate or lyse trypanosomes *in vitro*. These results suggest that the inhibition of trypanosome parasitemia that occurred during concomitant infections with these parasites is probably not a function of antigenic cross-reactivity and the synthesis of homologous antibodies by the host. However, these results do not rule out the possibility that *T. spiralis* infections may potentiate the normal humoral responsiveness (ablastic or trypanocidal) of the host to *T. lewisi* either by enhancing antibody production (adjuvant effects) or by accelerating the initial processing of parasite antigens. Under certain conditions, infections with a number of metazoan parasites have been shown to potentiate the humoral response of the host to previously administered heterologous immunogens (Bloch *et al.* 1973; Jarrett 1972; Orr and Blair 1969). However, in view of the known immunosuppressive effects of *T. spiralis* on the humoral response of the mouse to heterologous antigens and viruses (Barriga 1975; Chimishkian and Ovumian 1975; Cypess *et al.* 1973; Faubert 1976; Faubert and Tanner 1971, 1974; Lubiniecki *et al.* 1974; Lubiniecki and Cypess 1975), it seemed unlikely that *T. spiralis*

should potentiate the humoral response of the rat to T. lewisi. An assessment of the humoral responsiveness of the rat during Trichinellosis to the heterologous antigen SRBC (Chapter V), showed that the dose (100 larvae) of T. spiralis that gave a maximum inhibition of trypanosome parasitemia, produced only a transient depression of the humoral response to SRBC at 14 days after the nematode infection. Although T. spiralis failed to exhibit any immunosuppressive activity towards challenge infections with T. lewisi, immunpotentiation did not occur when the trypanosome challenge was made at this time.

The experiments presented in this chapter were conducted in order to examine the effect of T. spiralis on acquired humoral immunity to T. lewisi, since an accelerated or enhanced ablastic antibody response or first trypanocidal antibody response might account for the inhibition of trypanosome parasitemia that occurs during concomitant infections with these parasites.

MATERIALS AND METHODS

1) THE ANIMALS

Charles River, CDF, inbred albino, female rats (~100 grams) (Charles River Breeding Laboratories, Wilmington, Massachusettes) were used in this study. The animals were housed individually in wire bottom cages and received Purina Rat Chow and water ad libitum. Stock infections of T. lewisi and T. spiralis were maintained in Outbred, CD, albino female rats (~100 grams)(a Sprague-Dawley line; Canadian Breeding Farms, St. Constant, Quebec).

2) THE PARASITES

The strain of T. lewisi used in this study is described in detail in Chapter V. Trypanosomes for initiating experimental infections were obtained from stock-infected rats 12 days after inoculation. Experimental infections were initiated by injecting rats with 5×10^3 infective trypomastigotes (0.5ml) intraperitoneally without anaesthesia. Trypanosome parasitemias were determined during the course of T. lewisi infections by daily hemocytometer counts of tail blood diluted 1/200 using 10 µl capillary tubes and "Unopettes" as described previously (Chapter V).

The strain of T. spiralis used in this study is described in Chapter V. Infecting stocks were maintained in CD female rats inoculated orally with 3,000 infective muscle larvae, and muscle larvae for initiating experimental infections were obtained from donor stock rats which had been inoculated thirty days previously. Experimental and stock infections and the recovery of muscle larvae from experimental animals at the end of the study were done according to techniques adopted from Tanner (1968) and are described in detail in the previous chapter.

3) EVALUATION OF THE ABLASTIC ANTIBODY RESPONSE TO T. LEWISI

The production of ablastin, the reproduction-inhibiting antibody to T. lewisi, was followed by a sensitive technique that measures reproductive activity by determining the coefficient of variation (CV) in the lengths of trypanosomes in a dividing population (Taliaferro and Taliaferro 1922). This technique provides a statistical index of parasite reproductive activity which is a true reflection of the changes in the titer of ablastin during the course of the infection (Coventry 1925).

The CV was determined essentially as described by Taliaferro and Taliaferro (1922). Thin blood films were prepared during the course of the infections, fixed in absolute methanol, and stained with Giemsa's Blood Stain. The slides were examined using oil immersion optics (1000X magnification) and the midlines (total length from posterior end to the end of the flagellum) of 100 trypanosomes were drawn from random fields with the aid of a camera lucida. The lengths of these midlines were determined using a map measure and the degree of variation in size in the sample population of trypanosomes was obtained by calculating the CV according to the following formula:

$CV = \frac{100 \times SD}{\bar{x}}$ where SD = standard deviation of the mean and $\bar{x} = \Sigma x/n$.

The mean coefficients of variation for animals in the nematode-infected and control groups were plotted against days after infection with T. lewisi in order to compare the ablastic activity of these two groups during the course of the infection.

4) COLLECTION OF SERA

Experimental animals were bled from the tail and the blood was allowed to clot at 4° C. for 12 hours before centrifugation (1500 x g, 30 min.). The rat sera were heat-inactivated (56° C., 30 min.) and stored individually at -20° C. until agglutination titrations were performed.

5) IN VITRO TITRATION OF TRYPANOCIDAL ANTIBODIES

The in vitro agglutination titration of the first and second trypanocidal antibodies to T. lewisi was done essentially as described by D'Alesandro (1976). Serial two-fold dilutions (.05ml) in PBGS (PBS, pH 7.2, containing 1% glucose (w/v) of inactivated (56° C., 30min) in-

fected rat sera were mixed with equal volumes of PBGS containing approximately 5.0 x 10^7 trypanosomes in "Microtiter" plates. After mixing, the plates were incubated at 37° C. for 30 minutes and the agglutination titer was read microscopically, using phase contrast illumination, as the highest serum dilution to show a 1+ reaction (D'Alesandro 1976). The titrations were made using pure suspensions of trypanosomes that were collected at 6 days (1st antigenic variant, reproducing stages) or 14 days (2nd antigenic variant, inhibited adult stages) after infection according to the technique of Lincicome and Watkins (1963). The appropriate positive and negative controls were included in the titrations. Agglutination titers were expressed as $\log_2$ titer and the daily mean titers plotted against days after infection with T. lewisi.

6) EXPERIMENTAL PROTOCOL

This experiment was designed to examine the influence of a low level infection with T. spiralis on acquired humoral immunity to T. lewisi. 20 Charles River CDF, inbred female rats (~100 grams) were used in this experiment. Two groups of 5 rats each were inoculated with 100 infective muscle larvae of T. spiralis (Groups I and III) and two groups of 5 rats each were kept as uninfected controls (Groups II and IV). Control animals recieved a sham inoculation of PBS. 28 days after infection with T. spiralis all groups were challenged with T. lewisi (5.0 x 10^3 trypanosomes given intraperitoneally). The course of T. lewisi parasitemias in groups I and II were followed by daily hemocytometer counts of diluted blood, and thin blood films prepared at the same time were used to follow the coefficient of variation. The rats in groups III and IV were bled from the tail at 10, 12, 15, 20, 25, 30, 35, and 40 days after the challenge infection with T. lewisi

and the sera were collected as described above. The agglutination titers of the 1st and 2nd trypanocidal antibodies were determined during the course of the infection by titrating these sera with dividing form (1st antigenic variant) or inhibited adult form (2nd antigenic variant) trypanosomes respectively. 40 days after infection with T. lewisi, the rats infected with T. spiralis were killed and the number of encysted muscle larvae per gram of carcass determined for each experimental animal.

7) STATISTICAL ANALYSIS

A statistical analysis of the results was performed using the Analysis of Variance, F-test, Student's t-distribution test (Snedecor and Cochran 1967), and Duncan's new multiple range test (Duncan 1955) where appropriate, and when necessary, extraneous values were rejected using the Q-test (Dean and Dixon 1951).

RESULTS

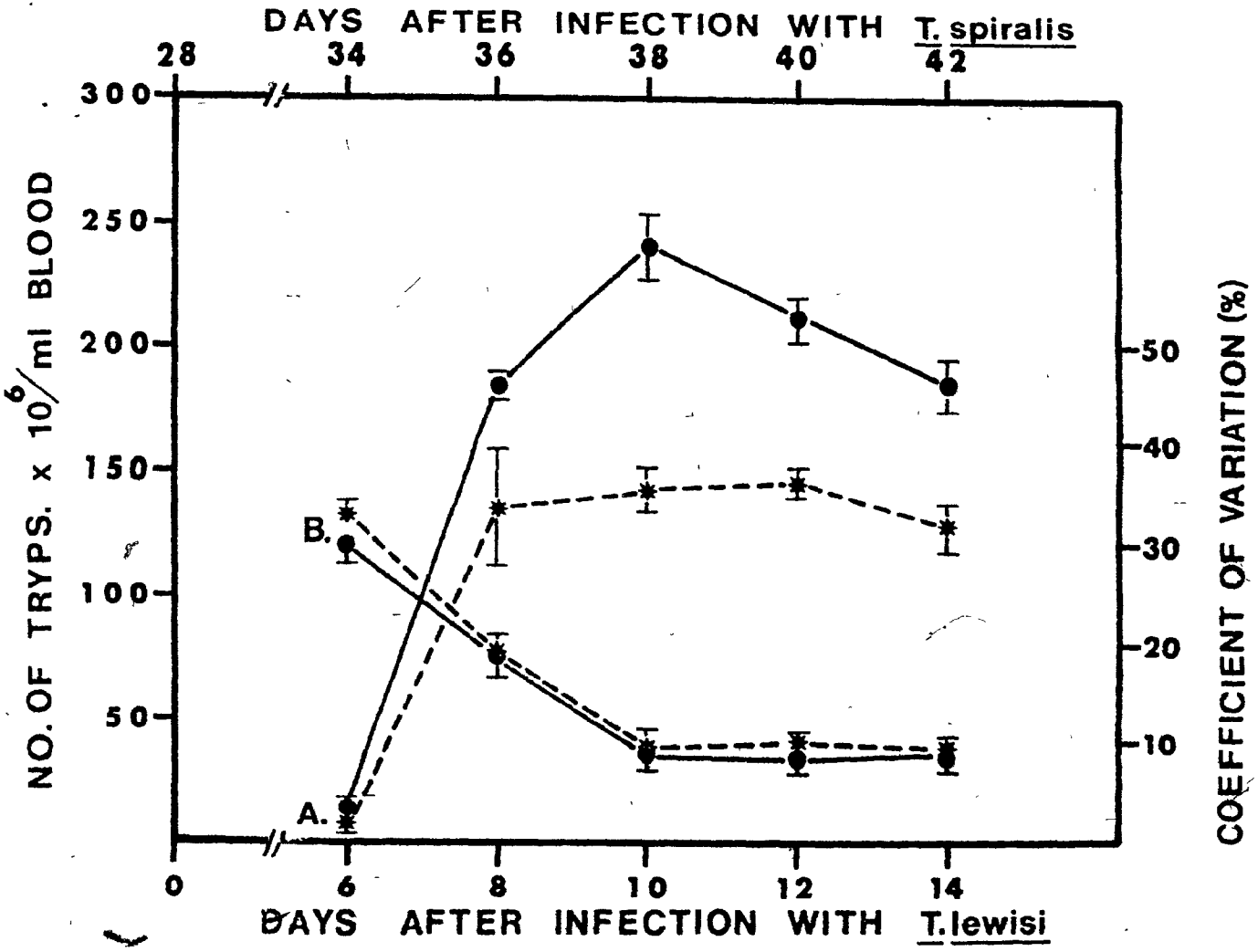
The coefficient of variation in the lengths of trypanosomes was followed in order to assess the influence of T. spiralis on the ablastic antibody response of the rat to T. lewisi. The course of trypanosome parasitemia and the CV for rats challenged with T. lewisi at 28 days after the nematode infection are displayed in Fig. 6.1. The mean CV of the nematode-infected group (I) and control group (II) were not significantly different throughout the course of the trypanosome infection, whereas the normal peak in trypanosome parasitemia that occurred in the control group was inhibited in the nematode-infected group as reported previously (Chapter V). On day 10 of the trypanosome infections, the mean parasitemia in the nematode-infected group was 58% of the mean control value ($P<.01$) and significant

FIGURE 6.1

A) The course of trypanosome parasitemia (mean number of trypanosomes/ml blood, ±SE) and B) The coefficient of variation (comparative measure of the rate of reproduction, mean ±SE) in rats challenged with T. lewisi at 28 days after inoculation with T. spiralis.

(✱) = 100 larvae/rat, recovery of 149 ±17 larvae/gram (Group I)

(●) = Controls, no T. spiralis (Group II)


DAYS AFTER INFECTION WITH T. spiralis
28
34
36
38
40
42
300
250
200
150
100
50
NO. OF TRYPS. x 10⁶/ml BLOOD
B.
A.
50
40
30
20
10
COEFFICIENT OF VARIATION (%)
0
6
8
10
12
14
DAYS AFTER INFECTION WITH T. lewisi

differences between these two groups were still evident at 14 days after infection. Therefore, while trypanosome parasitemia was inhibited in the nematode-infected animals, the ablastic antibody response of the rat was neither enhanced nor suppressed by the previous infection with T. spiralis.

The production of the two trypanocidal antibodies were followed during the course of the T. lewisi infections by titrating the infected rat sera for agglutinating antibody to either the 1st antigenic variant or the 2nd antigenic variant and the results are presented in Fig. 6.2. The 1st trypanocidal antibody was initially detected on day 10 of the trypanosome infection and continued to increase in titer until day 25. There were no significant differences, however, in the 1st trypanocidal antibody titers between the nematode-infected group (III) and the control group (IV). The 2nd trypanocidal antibody was first detected on day 25 of the trypanosome infection and continued to increase in titer until the end of the experiment (day 40). There were also no significant differences in the 2nd trypanocidal antibody response of nematode-infected and control groups. These results show that the previous infection of the rat with T. spiralis had no effect on the production of the two trypanocidal antibodies to T. lewisi.

DISCUSSION

Acquired humoral immunity to T. lewisi is well characterized and can be divided into three separate and distinct antibody responses: 1) the ablastic antibody response which inhibits the reproduction of the parasite, 2) the 1st trypanocidal antibody response which is specific for dividing stages (1st antigenic variant) and causes the first crisis, and 3) the 2nd trypanocidal antibody repsonse which is specific for non-dividing,

FIGURE 6.2

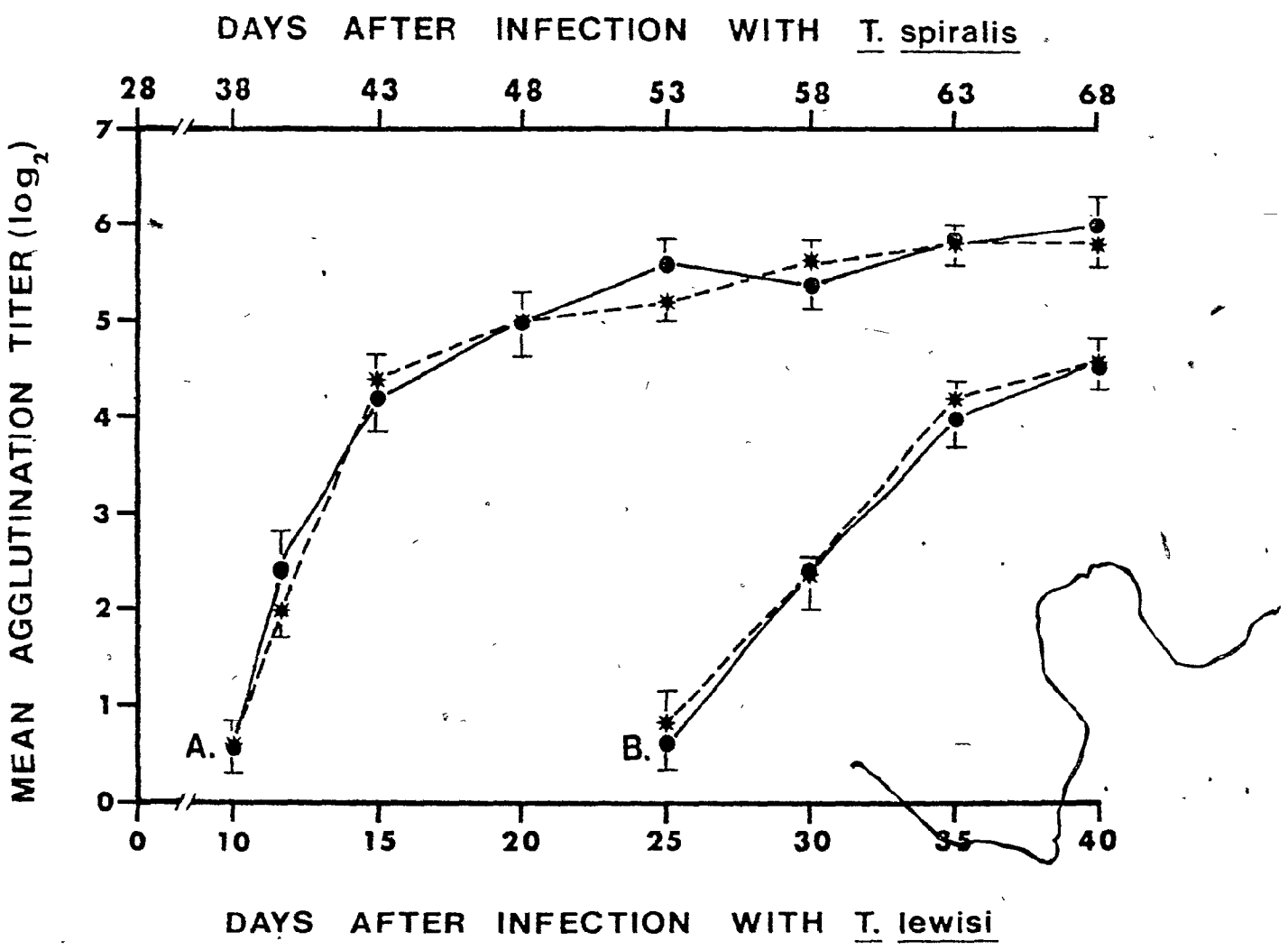
Mean agglutination titers ($\log_2$ ±SE) for rat sera collected during the course of *T. lewisi* infections in animals challenged with trypanosomes at 28 days after inoculation with *T. spiralis*.

A) Agglutination titers of the 1st trypanocidal antibody (titrated with living reproducing stages, 1st antigenic variant).

B) Agglutination titers of the 2nd trypanocidal antibody (titrated with non-dividing, inhibited adult stages, 2nd antigenic variant).

(✱) = 100 larvae/rat, recovery of 129 ±23 larvae/gram (Group III).

(●) = Controls, no *T. spiralis* (Group IV).


DAYS AFTER INFECTION WITH T. spiralis
28
38
43
48
53
58
63
68
MEAN AGGLUTINATION TITER (log_2)
7
6
5
4
3
2
1
0
A.
B.
0
10
15
20
25
30
35
40
DAYS AFTER INFECTION WITH T. lewisi

inhibited adult stages (2nd antigenic variant) and causes the termination of the infection (Taliaferro 1926). A suppression or potentiation of the production of any of these humoral antibodies may effectively alter the normal course of trypanosome parasitemia. The enhanced production of ablastin may cause parasite reproductive activity to be inhibited at an earlier stage in the infection resulting in an overall decrease in peak parasitemia. The enhanced production of the 1st trypanocidal antibody may have a similar effect by causing an accelerated first crisis with an earlier switch to the 2nd antigenic variant and the enhanced or accelerated production of the 2nd trypanocidal antibody would probably result in an earlier termination of the infection.

The immunopotentiation that was observed in concomitantly infected hosts in this study (Fig. 6.1) and in the studies in Chapter V, occurred during the early stages of the trypanosome infection, such that peak trypanosome parasitemias were inhibited in T. spiralis-infected hosts by day 10 after inoculation with T. lewisi. Although immunopotentiation occurred during the period of the infection when ablastin and the 1st trypanocidal antibody were increasing in titer, the results of this study indicate that the titers of these immunoglobulins were essentially identical in nematode-infected and control animals. It would therefore appear that the inhibited development of T. lewisi infections in this model is not related to a potentiation of humoral responsiveness to trypanosome antigens.

Under certain conditions, infections with a number of parasitic helminths have been shown to potentiate humoral or cell-mediated immune responsiveness to heterologous antigens and tumors. Infections with Nippostrongylus brasiliensis, for instance, have been shown to potentiate the reaginic (IgE) antibody response of the rat to previously administered

heterologous immunogens such as egg albumin and con albumin (Orr and Blair 1969; Jarrett 1972). This potentiation was shown to be of short duration however, and did not involve immunoglobulin classes other than IgE (Bloch et al. 1973). Keller et al. (1971) have shown that the growth of Walker Sarcoma cells in rats was either entirely suppressed or greatly enhanced during N. brasiliensis infections, depending upon the timing of the tumor cell inoculum in relation to the parasitic infection. The growth of a syngeneic adenocarcinoma was also suppressed in mice infected with N. brasiliensis regardless of whether the infection was initiated before, after or with the tumor cell inoculum. Keller and Jones (1971) have investigated the mechanism of this host-parasite interaction and suggest that the anti-neoplastic activity of this nematode resides in a stimulation of non-specific cell-mediated immunity in the form of non-specific cytotoxicity of specifically activated macrophages. Simaren and Bammeke (1970) have also demonstrated immunopotentiation during infections with N. brasiliensis such that rats concurrently infected with Trypanosoma congolense show reduced trypanosome parasitemias and enhanced survival times as compared to controls without the nematode infection.

Immunopotentiation is also well characterized in Trichinellosis. The ability of T. spiralis infections to decrease the susceptibility of mice to Listeria monocytogenes (Cypess et al. 1974) and potentiate cell-mediated (delayed type) hypersensitivity to BCG is well documented (Cypess et al. 1974: Molinari et al.1974; Molinari and Cypess 1975). Additionally, T. spiralis-infected mice demonstrate considerable antineoplastic activity against B-16 Melanoma and Lewis Lung Carcinoma (Molinari and Ebersole 1976), spontaneous mammary carcinoma (Weatherly 1970), and S-180 Ascites tumors (Lubiniecki and Cypess 1975). Although T. spiralis has been shown to stimu-

late specific or non-specific cell-mediated immunity, a potentiation of humoral immunity, which might play a protective role either in Trichinellosis or towards heterologous challenge organisms, has not yet been demonstrated. In view of this marked ability of T. spiralis to potentiate cell-mediated immunity and stimulate reticuloendothelial system activity (Chapter V), and the failure of the present study to demonstrate an enhanced humoral response to T. lewisi, it is highly probable that the inhibition of trypanosome parasitemia during concomitant infections with these two parasites is a reflection of enhanced non-specific RES activity or non-specific cell-mediated immunity as was suggested previously (Meerovitch and Ackerman 1974; Ackerman and Meerovitch 1975).

CHAPTER VII

THE EFFECT OF IMMUNIZATION WITH AN ENTERAL OR PARENTERAL INFECTION, OR A SOLUBLE ANTIGEN EXTRACT OF *TRICHINELLA SPIRALIS* ON THE IMMUNOLOGICAL RESPONSE OF THE RAT TO *TRYPANOSOMA LEWISI*

INTRODUCTION

The study presented in Chapter V examined the kinetics of immunopotentiation in Trichinellosis in the rat host during concomitant infections of *T. spiralis* and *T. lewisi* and demonstrated the transient nature of this phenomenon. The inhibitory effect of *T. spiralis* infections on trypanosome parasitemias occurred only when rats were challenged with *T. lewisi* at 7 or 28 days after the nematode infection, and was abrogated if challenge was made at 14 or 56 days after infection. The expression of immunopotentiation towards *T. lewisi*, the depression of humoral responsiveness to sheep red blood cells (SRBC), and the stimulation of reticuloendothelial system (RES) granulopectic activity during Trichinellosis, were all a function of the intensity of the infection (dose or level of muscle parasitism) and more importantly, they appeared to be related to the timing of the various developmental stages (adult, migratory, or encysted intracellular muscle stage) of the nematode life cycle in the host.

The transient nature of immunopotentiation and immunosuppression in Trichinellosis has been reported previously in the mouse model (Cypess *et al.* 1973, 1974; Lubiniecki *et al.* 1974; Lubiniecki and Cypess 1975; Vernes *et al.* 1975). Recent research by Faubert (1976), investigating the kinetics of immunosuppression in Trichinellosis, has indicated that depressed immunological responsiveness to SRBC is well correlated with the peak migration of the newborn larval stage to the muscle tissues of the host around

14 days after infection, and that this phenomenon may be associated with the *in vitro* secretion of an immunosuppressive factor by this developmental stage of *T. spiralis*. More recent work by Ackerman and Faubert (1977) has indicated that a marked suppression of the splenic plaque-forming cell response to SRBC can be obtained by injecting mice intravenously with either living or freeze-thaw-killed newborn larvae four days before immunization.

Earlier studies by Faubert and Tanner (1974, 1975) have shown that antigen extracts of *T. spiralis* muscle larvae or sera from infected animals have leucoagglutinating or cytotoxic activity for homologous lymphoid cells *in vitro*. These extracts and sera were also capable of prolonging skin allografts and suppressing the humoral and cell-bound antibody responses to heterologous antigens such as SRBC in immunized mice. Barriga (1975) has demonstrated a selective depression of T-cell activity in mice immunized with extracts of *T. spiralis* muscle larvae such that the t-dependent response to SRBC was suppressed while a B-cell response to polyvinylpyrrolidone (PVP) was unaffected.

Previous studies on immunopotentiation in Trichinellosis have not implicated nor investigated the role of specific developmental stages of the parasite's life cycle or parasite antigens in the induction of this phenomenon. The purpose of the present study was therefore two-fold: 1) To determine whether or not immunopotentiation in Trichinellosis could be associated with a specific developmental stage of the parasite's life cycle in the host, and 2) To determine if immunopotentiation required the presence of the living parasite or if it could be induced by immunization with an antigen extract of *T. spiralis*. To this purpose, the life cycle of *T. spiralis* was effectively divided into two distinct phases, enteral and parenteral (see figure 2.1, Chapter II). The rat host was exposed to only

the enteral (intestinal adult phase) phase of the infection by inoculating animals orally with infective muscle larvae, and terminating the infection before larviposition occurred using the anti-helminthic drug Methyridine. The parenteral phase of infection was initiated by inoculating rats intravenously with newborn larval stages, which migrate to the striated muscles, encyst intracellularly and become infective, as would normally occur during the course of a natural infection. Rats immunized with either the enteral or parenteral phases of T. spiralis, or with a soluble antigen extract of the muscle stage larva, were challenged with trypanosomes to examine the effect of immunization on the immunological response of the rat to T. lewisi.

MATERIALS AND METHODS

1) THE ANIMALS

Inbred albino, female rats (~100 grams) of the Charles River, CDF strain (Charles River Breeding Laboratories, Wilmington, Massachusettes), were used in these experiments. Outbred CD, albino female rats (~200 grams) (Canadian Breeding Farms, St. Constant, Quebec), were used for the collection of large numbers of intestinal, adult stages of T. spiralis, and stock infections of T. lewisi and T. spiralis were maintained in this strain of rat as described previously (Chapter V). Experimental animals were housed individually in wire bottom cages and fed Purina Rat Chow and water ad libitum.

2) EXPERIMENTAL INFECTIONS

The immunization of experimental animals with only the enteral stages of T. spiralis was accomplished as follows: enteral infections were initiated by inoculating rats orally with 100 infective muscle larvae of T. spiralis without anaesthesia. The intestinal infections were terminated

96 hours after inoculation (before larviposition could occur) by injecting rats subcutaneously (s.c.) with Methyridine ("Promintic", Imperial Chemical Industries Ltd.) in a dose of 50 mg/100 grams body weight (Denham 1965). Control animals for this treatment received an oral inoculation with PBS and the subsequent injection (s.c.) of Methyridine 96 hours later as above. In order to ensure that larviposition had not occurred, animals in the experimental group were killed at the conclusion of the experiment and checked for the presence of encysted muscle larvae using acid-pepsin digestion of the carcasses as described previously.

The experimental infection of rats with only the parenteral (migratory and encysted) stages of T. spiralis was done essentially as described by Despommier (1971). Large numbers of newborn larvae (NBL) were obtained free of adults using the methods described previously by Dennis et al. (1970). Briefly, 20 CD female rats (~200 grams) were inoculated orally with 10,000 infective muscle larvae of T. spiralis and 6 days after inoculation the rats were killed with ether and the entire length of the small intestine was removed. Large numbers of adult worms were collected by slitting the intestines longitudinally and placing them in a circular thermal migration device designed for the rapid collection of large numbers of intestinal helminths Despommier (1973). Adult worms collected over a period of two hours, were washed four times in 0.85% saline at 37° C. and transferred to two 250 ml Falcon Tissue Culture Flasks containing 100 ml each of the following medium: "Medium 199 with sodium bicarbonate (70% by volume), "Dialyzed Calf Serum" (29% by volume), and "Antibiotic-Antimycotic" (Grand Island Biological Co.) (1% by volume), pH 7.3. The adult worms were incubated at 37° C. in a humidified atmosphere containing 5% CO_2 for 30 hours during which time the gravid female worms shed newborn larvae. All operations after this incubation were

done under sterile conditions. The medium containing the adults and newborn larvae was passed through three layers of a stainless steel mesh (25μ pore size, George A. Williams and Son, New York) and the adult-free larvae in the filtrate sedimented by centrifugation (225 x g) for five minutes in 50 ml conical centrifuge tubes at room temperature. The newborn larvae were pooled, washed three times with sterile culture medium at 37° C. and the suspension of larvae counted (using an appropriate dilution) in a Spencer Neubauer Brightline Hemocytometer (100X magnification). Approximately 2.0 x 10^6 NBL were collected in this manner. Quantities of the suspension containing the desired number of NBL were withdrawn for inoculation after the larvae were suspended by a vortex mixer. 98% of the larvae were motile just prior to inoculation. Ether-anaesthetized rats were injected intravenously (i.v.) with 20,000 NBL(0.5ml)in the tail vein with a 1.0 ml tuberculin syringe fitted with a #26 gauge needle. Approximately 50-75% of the NBL injected via this route are infective and can be found encysted in the striated muscle tissues of the rat after ∿21 days (Dennis et al. 1970; Despommier 1971).

Freeze-thaw-killed newborn larvae (FTNBL) were prepared by immersing a 15 ml glass centrifuge tube containing culture medium and NBL in liquid air until the contents of the tube were frozen solid. The tube was defrosted at 37° C. until the contents were completely melted and this freeze-thaw procedure was repeated five times. Anaesthetized rats were injected i.v. in the tail vein with 20,000 FTNBL (0.5 ml) as described above.

3) IMMUNIZATIONS

The soluble antigen extract of T. spiralis (TSE) used for immunizing rats or for sensitizing SRBC for the indirect hemagglutination test was prepared in phosphate-buffered saline (PBS)(pH 7.2) from washed lyophilized

muscle larvae essentially as described by Tanner (1970)(see Chapter V). The TSE was sterilized by filtration through a Millipore filter (0.45μ) and stored at -70° C. in sterile, stoppered serum bottles until use. The protein concentration of the TSE was determined according to the technique of Lowry et al. (1951). Rats were immunized intramuscularly (i.m.) three times per week for three weeks by alternately injecting the thigh muscle of the right or left hind leg with 0.1 ml of TSE (10 mg protein/ml), injected either alone or emulsified with an equal volume of Freund's Incomplete Adjuvant (FIA). Rats were immunized with a total of 9 mg protein over the three week period. Controls were injected in a similar manner with either sterile PBS or PBS emulsified with FIA.

4) INDIRECT HEMAGGLUTINATION

The indirect passive hemagglutination test (IHA) was done essentially as described by Stavitsky (1954). Sheep red blood cells (SRBC) were tanned with 1:40,000 tannic acid (10 min., 37° C.) in PBS (pH 7.2) and sensitized with TSE (5 mg protein/ml) for ten minutes at room temperature in PBS (pH 6.4). All sera and the normal rabbit serum used in the diluent were heat inactivated (56° C., 30 min.) and pre-absorbed (room temperature, 15 min.) with an equal volume of packed SRBC to remove "natural" Forssman antibody. Serial two-fold dilutions (0.05 ml) in 1% normal rabbit serum (pH 7.2) of inactivated rat antisera were mixed with 0.025 ml of 2.5% sensitized SRBC in "Microtiter" plates. After mixing, the plates were incubated at room temperature and the agglutination titer was read after 12 hours as the highest serum dilution to show a 1+ reaction (Stavitsky 1954). The appropriate positive and negative controls were included in all titrations.

5) EVALUATION OF THE ABLASTIC AND TRYPANOCIDAL ANTIBODY RESPONSE

The ablastic antibody response of the rat to T. lewisi was followed by determining the coefficient of variation (CV) in the lengths of trypanosomes during the course of the infection according to the technique of Taliaferro and Taliaferro (1922) as described in the previous chapter. The trypanocidal antibody responses were determined by titrating the rat sera in vitro for agglutinating antibodies to either dividing stages (1st antigenic variant) or the inhibited adult stages (2nd antigenic variant) as described previously in Chapter VI.

6) EXPERIMENTAL PROTOCOLS

EXPERIMENT I - THE EFFECT OF ENTERAL OR PARENTERAL INFECTIONS OF T. SPIRALIS ON THE HOST RESPONSE TO T. LEWISI

The following experiment was designed to examine the role of the enteral or parenteral phases of the life cycle of T. spiralis in the induction of immunopotentiation towards challenge infections with T. lewisi. The 30 Charles River CDF rats used in this experiment were divided into six groups of 5 rats each and the treatment regimen for these groups is displayed in Table 7.1. Immunization with enteral, parenteral or natural infections of T. spiralis were done as described above and control groups (I and V) were injected with culture medium (i.v.) or Methyridine (s.c.) respectively. All experimental and control animals were challenged with T. lewisi at the same time and the immunizations with either enteral or parenteral infections (before trypanosome challenge) were timed to correspond with the period during which these phases of the life cycle normally occur during the course of the natural infection (Group II). The dose of newborn larvae was estimated to produce an equivalent number of encysted

TABLE 7.1

TREATMENT REGIMEN FOR ANIMALS IMMUNIZED WITH ENTERAL, PARENTERAL, OR NATURAL INFECTIONS OF I. SPIRALIS AND CHALLENGED WITH I. LEWISI

GROUP #	I. SPIRALIS (P.O.) [A]	NBL (I.V.) [B]	FTNBL (I.V.) [C]	METHYRIDINE (S.C.) [D]	I. LEWISI (I.P.) [E]	DAYS BEFORE CHALLENGE [F]
I	-	-	-	-	+	
II	+	-	-	-	+	28
III	-	+	-	-	+	21
IV	-	-	+	-	+	21
V	-	-	-	+	+	24
VI	+	-	-	+	+	28, 24 [D]

(A) ORAL INOCULATION OF 100 INFECTIVE MUSCLE LARVAE PER OS (P.O.)
(B) INTRAVENOUS INOCULATION OF 20,000 LIVE NEWBORN LARVAE
(C) INTRAVENOUS INOCULATION OF 20,000 FREEZE-THAW-KILLED NEWBORN LARVAE
(D) SUBCUTANEOUS INOCULATION OF METHYRIDINE TO TERMINATE THE INFECTION
(E) INTRAPERITONEAL INOCULATION OF 5×10^3 TRYPANOSOMES
(F) DAYS BEFORE TRYPANOSOME CHALLENGE WHEN RATS INOCULATED WITH A, B, C, OR D

muscle larvae as would be recovered from an animal inoculated orally with 100 infective muscle larvae (Group II).

Trypanosome parasitemias were followed in all groups by hemocytometer counts of tail blood diluted 1/200 using 10 µl capillary tubes and "Unopettes" as described previously in Chapter V. The number of encysted muscle larvae of T. spiralis in groups II, III, IV, and VI was determined by acid-pepsin digestion of infected carcasses at the end of the experiment.

EXPERIMENT II - THE EFFECT OF IMMUNIZATION WITH A SOLUBLE ANTIGEN EXTRACT OF T. SPIRALIS ON THE IMMUNE RESPONSE OF THE RAT TO T. LEWISI

The following experiment was designed to investigate the influence of immunization with a soluble antigen extract of T. spiralis muscle stage larvae (TSE), on the humoral immune response of the rat to T. lewisi. 20 Charles River CDF rats were divided into 4 groups of 5 animals each. Experimental groups were immunized over a three week period with either TSE (group I) or TSE plus FIA (group III) and controls were injected in the same manner with either PBS (group II) or PBS plus FIA (group IV). One week after the last injection, all rats were challenged intraperitoneally with 5×10^3 trypanosomes. The course of trypanosome parasitemias and the coefficients of variation (C.V.) were followed as described above. 40 days after the challenge infection with T. lewisi, all rats were bled by cardiac puncture for 1.0 ml of blood and the sera collected as described previously. Sera were titrated for anti-TSE antibodies by the IHA test and for trypanocidal antibodies by the in vitro agglutination of living trypanosomes. Three days after collecting the sera, the rats in all groups were challenged orally

with 100 infective muscle larvae of T. spiralis to assess the relative protection of the host due to the previous immunization with TSE. Thirty days after this challenge infection, all rats were killed and the number of encysted muscle larvae determined by acid-pepsin digestion of infected carcasses.

7) STATISTICAL ANALYSIS

Results were analyzed statistically using the Student's t-test and the Analysis of Variance F-test (Snedecor and Cochran 1967) where appropriate, and when necessary, extraneous values were rejected using the Q-test (Dean and Dixon 1951).

RESULTS

EXPERIMENT I

The course of trypanosome parasitemias for groups of rats immunized with enteral, parenteral or natural infections of T. spiralis and challenged with T. lewisi, are displayed in Figure 7.1-7.3. A comparison between animals injected with living NBL (group III), sham-injected controls (group I), or animals that received a natural infection with 100 muscle larvae of T. spiralis is depicted in Figure 7.1. As reported in the two previous chapters, the peak trypanosome parasitemias in rats challenged with T. lewisi at 28 days after the nematode infection (group II) were significantly inhibited when compared to controls without T. spiralis (group I)(50.2% of the control value on day 8, $P<.01$). Peak trypanosome parasitemias for animals that were injected intravenously with 20,000 NBL and challenged 21 days later with T. lewisi were also inhibited to some degree (73.6% of the control value on day 8, $P<.05$) although not to the

FIGURE 7.1

Course of trypanosome parasitemia in rats immunized with parenteral or natural infections of T. spiralis and challenged with T. lewisi. Each point represents the mean ±SE for five animals.

(●) = GROUP I - Controls, no T. spiralis

(✱) = GROUP II - 100 infective muscle larvae per os

(✱) = GROUP III - 20,000 live NBL i.v.

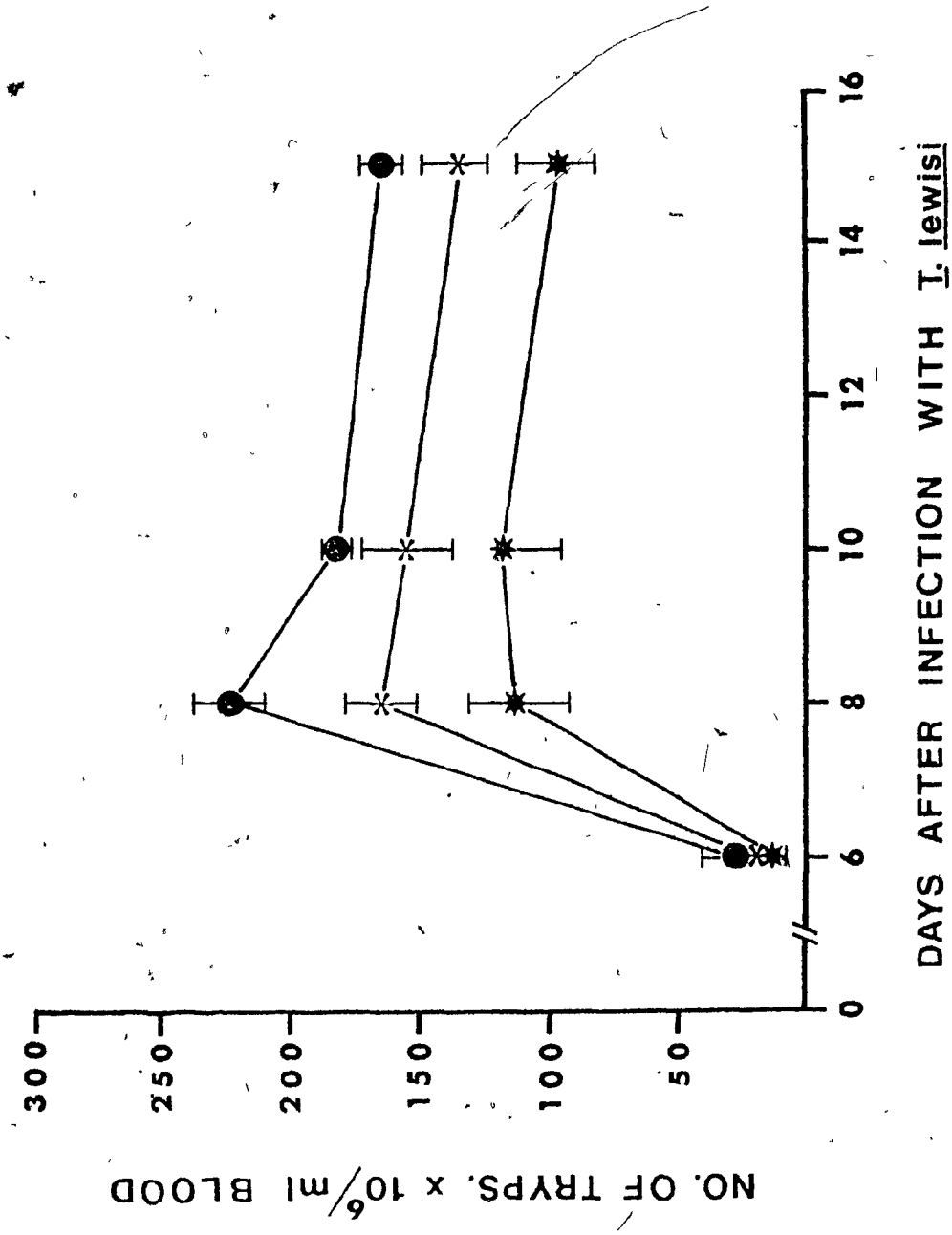

NO. OF TRYPS. x 10⁶/ml BLOOD
300
250
200
150
100
50
0
6
8
10
12
14
16
DAYS AFTER INFECTION WITH T. lewisi

FIGURE 7.2

Course of trypanosome parasitemias in rats immunized with freeze-thaw-killed newborn larvae (FTNBL) or natural infections of T. spiralis and challenged with T. lewisi. Each point represents the mean ±SE for five animals.

(●) = GROUP I - Controls, no T. Spiralis

(✳) = GROUP II - 100 infective muscle larvae per os

(✱) = GROUP IV - 20,000 FTNBL i.v.

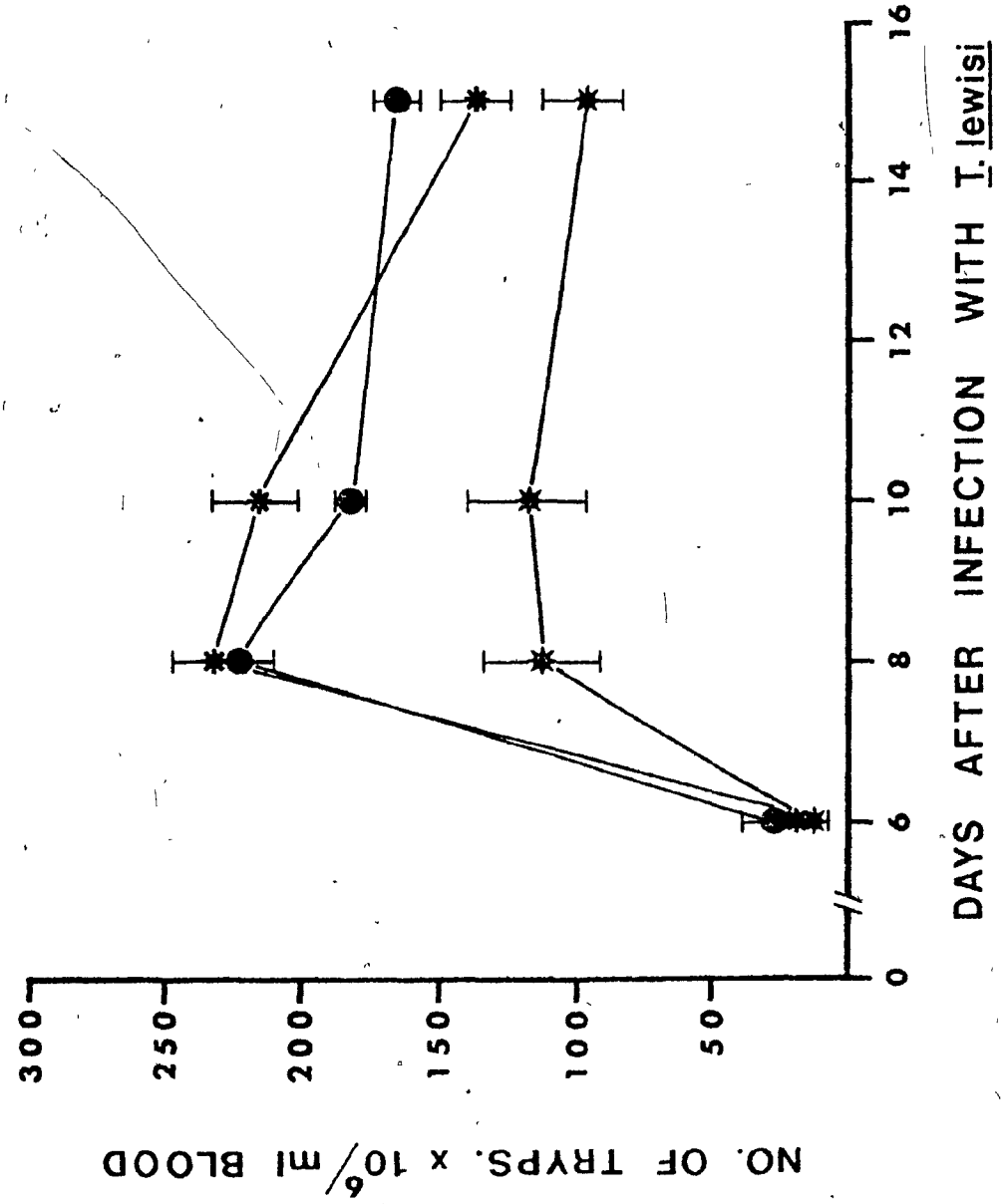

NO. OF TRYPS. x 10^6/ml BLOOD
300
250
200
150
100
50
0
6
8
10
12
14
16
DAYS AFTER INFECTION WITH T. lewisi

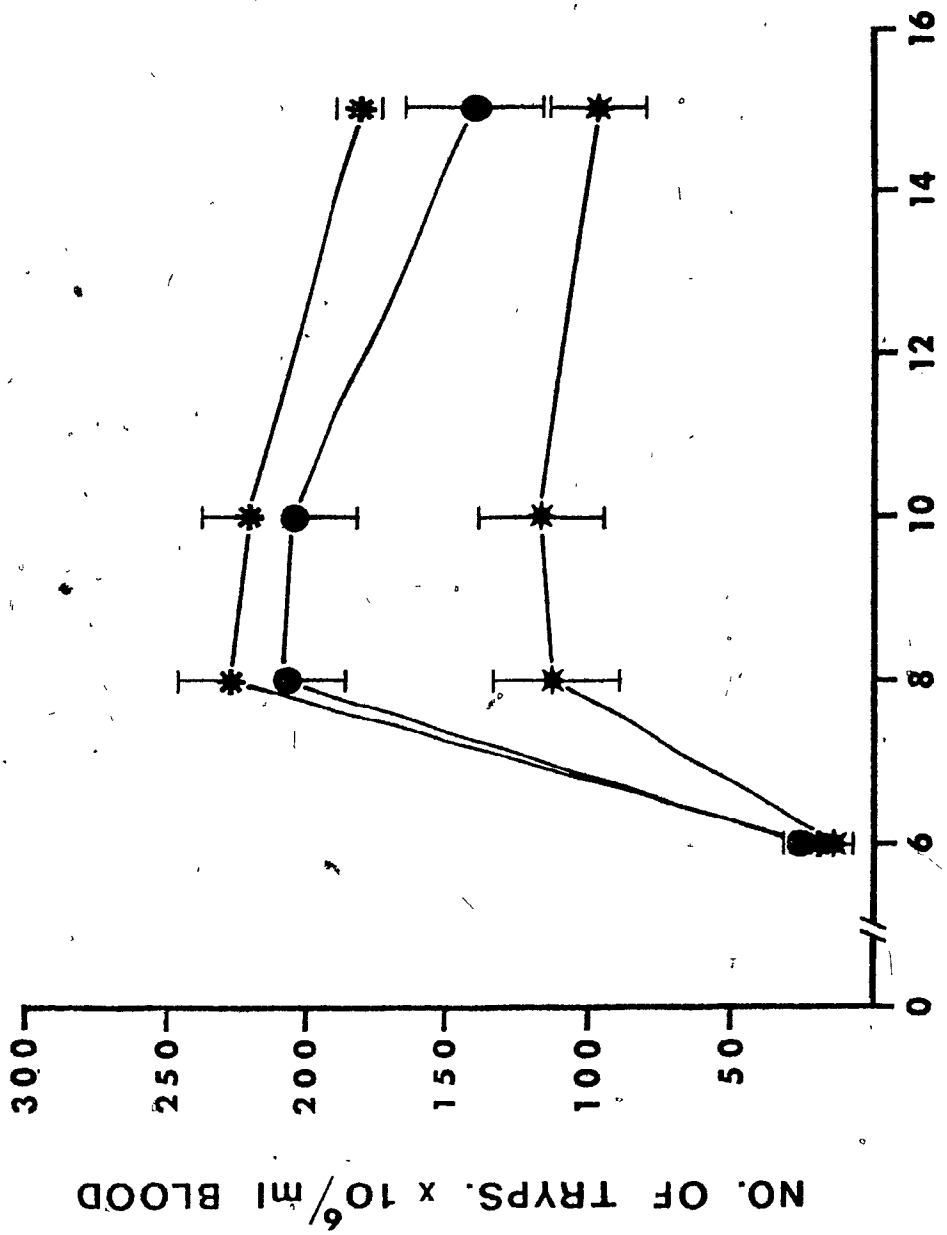
FIGURE 7.3

Course of trypanosome parasitemia in rats immunized with drug-abbreviated enteral infections or natural infections of T. spiralis and challenged with T. lewisi. Each point represents the mean ±SE for five animals.

(✱) = GROUP II - 100 infective muscle larvae per os

(●) = GROUP V - Controls, no T. spiralis, Methyridine s.c.

(✱) = GROUP VI - 100 infective muscle larvae per os, Methyridine s.c. (drug-abbreviated enteral infection)


NO. OF TRYPS. x 10⁶/ml BLOOD
300
250
200
150
100
50
0
6
8
10
12
14
16
DAYS AFTER INFECTION WITH T. lewisi

same extent as in group II. Therefore, immunopotentiation was induced in rats by immunization with only the parenteral phase (migratory and encysted intracellular stages) of the T. spiralis infection.

An inhibition of trypanosome parasitemia did not occur in rats injected with an equivalent dose of freeze-thaw-killed newborn larvae (Figure 7.2) indicating the importance of immunization with living migratory or encysted muscle stages in the induction of this phenomenon. Rats that were immunized with a drug-abbreviated enteral infection (group VI) did not show any significant differences in parasitemia from the controls (group V, Figure 7.3) and these results suggest that a 96 hour exposure to only an intestinal infection with T. spiralis is not sufficient to induce immunopotentiation in this model.

The number of encysted muscle larvae in animals inoculated either orally with 100 infective larvae (group II) or intravenously with 20,000 NBL (group III) was determined at the end of the experiment in order to compare the level of muscle parasitism in these groups. The results presented in Table 7.2 show that an average of 52% of the inoculating dose of 20,000 NBL was recovered as encysted muscle larvae. The total number of larvae recovered from animals in these groups was not significantly different ($P>.05$) although the difference in the number of larvae recovered per gram of carcass was statistically significant ($P<.005$). No encysted muscle larvae were recovered from animals given either drug-abbreviated infections (group VI) or intravenous injections with FTNBL (group IV).

EXPERIMENT II

The course of trypanosome parasitemias for rats immunized intramuscularly with TSE (group I) or TSE emulsified with FIA (group III) are

TABLE 7.2

THE RECOVERY OF MUSCLE LARVAE FROM RATS INOCULATED ORALLY WITH INFECTIVE MUSCLE LARVAE OR INTRAVENOUSLY WITH LIVE NEWBORN LARVAE OF *T. SPIRALIS*

TREATMENT	CARCASS WT. (GRAMS)	TOTAL LARVAE RECOVERED	LARVAE/GRAM CARCASS	% RECOVERY*
GROUP III	118.5	10,000	84.4	50%
20,000 NBL	82.5	7,200	87.3	36%
(I.V.)	118.5	13,533	114.2	68%
	76.8	5,800	75.5	29%
	111.5	15,400	138.1	77%
	MEAN	10,387	99.9	52%
	± SE	±1,821	±11.5	±9%
GROUP II	75.6	12,266	162.2	
100 MUSCLE	84.2	15,533	184.5	
LARVAE (P.O.)	86.3	18,066	209.3	
	79.3	11,733	148.0	
	81.3	14,200	174.7	
	MEAN	14,360	175.7	
	± SE	±1,149	±10.3	

* % RECOVERY OF NEWBORN LARVAE INJECTED I.V. = (TOTAL LARVAE RECOVERED/20,000) x 100

presented in Figure 7.4. The immunization of rats with TSE alone (group I) enhanced parasitemias (as compared to the controls, group II), as well as delayed the onset of the 1st crisis in parasite numbers, which occurred in the controls on day 8 but was delayed until day 10 in the immunized group. The enhanced trypanosome parasitemias in group I were also reflected by a significantly higher mean coefficient of variation ($P<.05$) on day 8 of the infection (Figure 7.5), indicating that the ablastic antibody response was either delayed or depressed in this group as compared to the non-immunized controls.

In rats immunized with TSE plus FIA (group III), trypanosome parasitemias were significantly enhanced over that of animals immunized with TSE (group I) or non-immunized controls (group II)(Figure 7.4). However, this result was difficult to interpret in view of the fact that the control group injected with PBS plus FIA (group IV) also showed significantly enhanced parasitemias as compared to those of groups I and II. Enhanced parasitemias in groups III and IV were also reflected by higher mean coefficients of variation on day 8 of the trypanosome infections (as compared to control group II, Figure 7.5), indicating that parasite reproductive activity continued in the presence of a delayed or depressed ablastic antibody response in both these groups. The 1st crisis in the controls injected with PBS plus FIA (group IV) occurred on day 8 of the infection, as was the case for controls in group II, but the onset of the 1st crisis was delayed until day 10 in rats immunized with TSE plus FIA (group III).

The sera of animals in groups I-IV were collected 40 days after infection and titrated for the 1st and 2nd trypanocidal antibodies to *T. lewisi* as well as for specific anti-TSE immunoglobulin. The mean agglutinating titers for the trypanocidal antibodies are displayed in Table

FIGURE 7.4

Course of trypanosome parasitemias in rats immunized with TSE or TSE plus FIA and challenged with T. lewisi. Each point represents the mean ±SE for five animals.

(✱) = GROUP I - TSE i.m.

(●) = GROUP II - PBS i.m.

(✱) = GROUP III - TSE plus FIA i.m.

(◉) = GROUP IV - PBS plus FIA i.m.

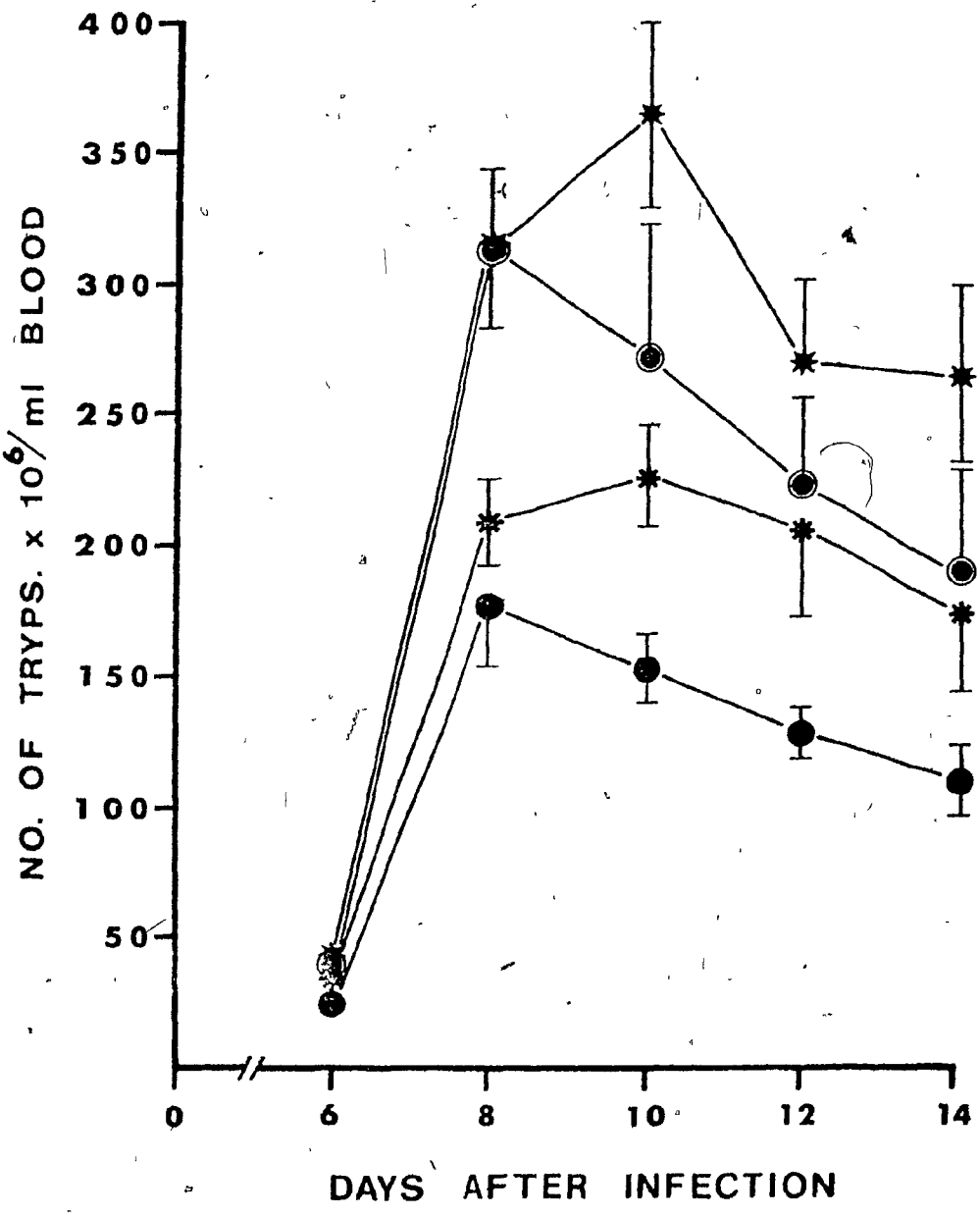
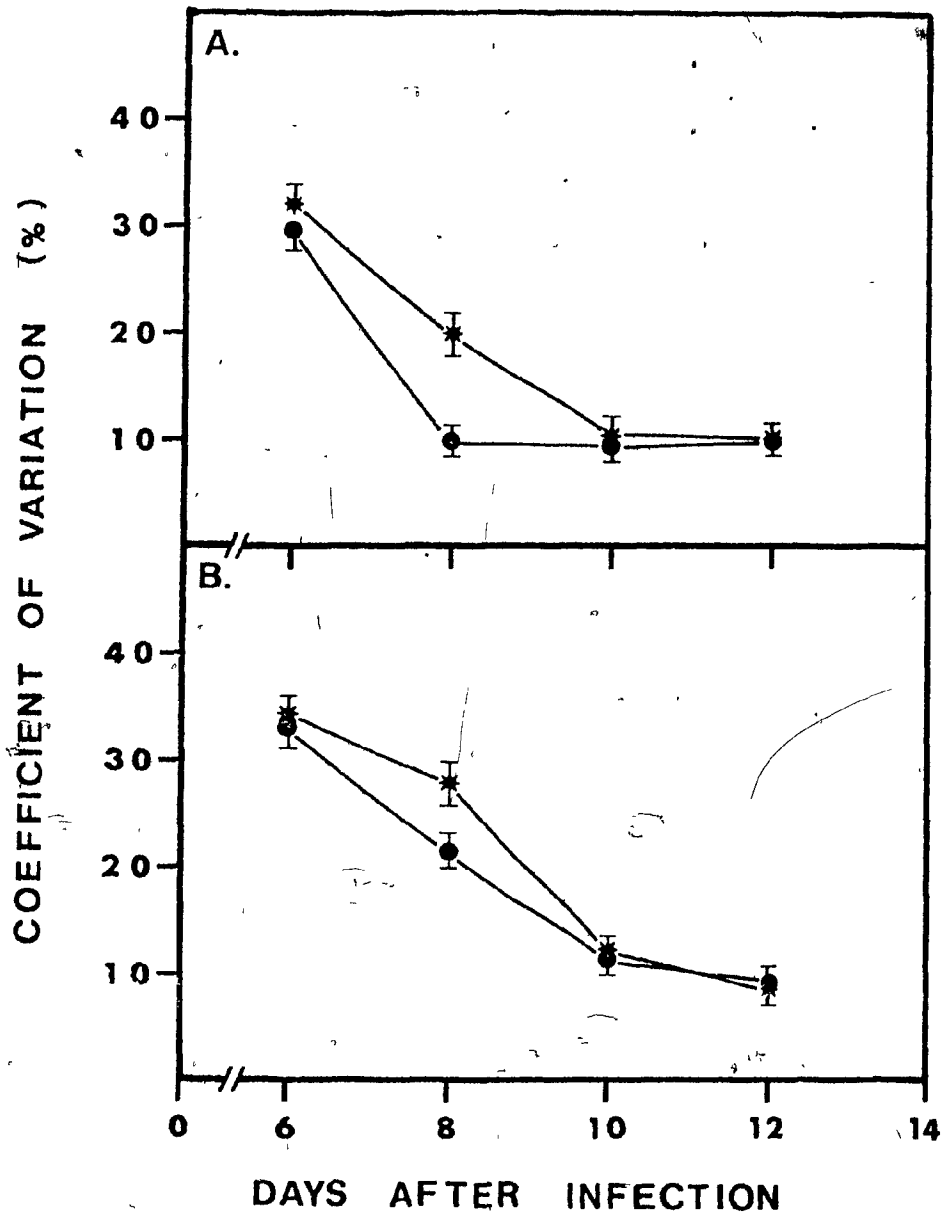

400
350
300
250
200
150
100
50
NO. OF TRYPS. x 10⁶/ml BLOOD
0
6
8
10
12
14
DAYS AFTER INFECTION

FIGURE 7.5

The coefficient of variation (CV) in the lengths of trypanosomes (index of reproductive activity) during the course of T. lewisi infections in rats immunized with TSE or TSE plus FIA. Each point represents the mean ±SE for five animals.

A) CV for rats immunized with TSE (group I)(✱) and for controls injected with PBS (group II)(●).

B) CV for rats immunized with TSE plus FIA (group III)(✱) and for controls injected with PBS plus FIA (group IV)(●).


A.
B.
40
30
20
10
0
6
8
10
12
14
COEFFICIENT OF VARIATION (%)
DAYS AFTER INFECTION

7.3. Although the mean titers for the 1st and 2nd trypanocidal antibodies for rats immunized with TSE (group I) or TSE plus FIA (group III) appeared to be slightly lower than those of the control groups, these differences were not statistically significant. Therefore, although the production of the 1st trypanocidal antibody or the onset of the first crisis appeared to be delayed until day 10 in both of the immunized groups (I and III), the antibody titers of these groups were not significantly different from the controls at 40 days after the trypanosome infection.

The sera of rats immunized intramuscularly with TSE (group I) or TSE plus FIA (group III) were also titrated for antibodies to TSE in order to assess the relative effectiveness of these immunization procedures in stimulating the production of specific immunoglobulin. The mean IHA titers for these groups are presented in Table 7.4. Those rats immunized with TSE alone had relatively low IHA titers as compared to animals immunized with antigen plus adjuvant, and these differences were highly significant ($P<.001$). The effectiveness of the immunizations with TSE in protecting animals against challenge infections of T. spiralis was assessed by inoculating the rats in groups I-IV orally with 100 infective muscle larvae and determining the total number of encysted muscle larvae 30 days later. The results displayed in Table 7.5 show that both methods of immunization were equally effective in protecting rats against a challenge infection with T. spiralis. Although the anti-TSE IHA antibody titers of rats in group III were significantly higher than those of group I (Table 7.4), the degree of protection in these two groups was not significantly different.

TABLE 7.3

THE MEAN AGGLUTINATING (TRYPANOCIDAL) ANTIBODY TITERS (LOG_2) FOR RATS IMMUNIZED WITH TSE AND CHALLENGED WITH TRYPANOSOMA LEWISI

TREATMENT	1ST TRYP. AB.(A) (MEAN ± SE)	P-VALUE*	2ND TRYP. AB.(B) (MEAN ± SE)	P-VALUE*
GROUP I TSE (I.M.)	5.0 ±.32		4.0 ±.28	
		P>.05		P>.05
GROUP II PBS (I.M.)	5.6 ±.25		4.4 ±.40	
GROUP III TSE + FIA (I.M.)	4.8 ±.37		3.8 ±.37	
		P>.05		P>.05
GROUP IV PBS + FIA	5.4 ±.25		4.2 ±.20	

(A) TITRATED WITH LIVING REPRODUCING STAGES (1ST ANTIGENIC VARIANT)

(B) TITRATED WITH INHIBITED ADULT STAGES (2ND ANTIGENIC VARIANT)

* STUDENT'S T-TEST

TABLE 7.4

LOG_2 ANTI-TSE IHA* ANTIBODY TITERS FOR RATS IMMUNIZED WITH TSE

TREATMENT	LOG_2 IHA TITER (MEAN ± SE)	P-VALUE**
GROUP I		
TSE (I.M.)	2.4 ±.24	
GROUP III		P<.001
TSE + FIA (I.M.)	10.6 ±.20	

* TITRATED WITH TSE-SENSITIZED SRBC

** STUDENT'S T-TEST

TABLE 7.5

THE RECOVERY OF MUSCLE LARVAE FROM RATS IMMUNIZED WITH TSE AND CHALLENGED ORALLY WITH 100 INFECTIVE MUSCLE LARVAE OF *I. SPIRALIS*

TREATMENT	TOTAL LARVAE RECOVERED	% PROTECTION**	P-VALUE***
GROUP I			
TSE (I.M.)	8,400	53.6%	
	8,867	51.0%	
	14,133	21.9%	
	8,400	53.6%	
	11,933	34.0%	
	MEAN 10,347	42.8%	P<.025
	± SE ±1,154	±6.4%	(I vs II)
GROUP II			
PBS (I.M.)	MEAN 18,106		
	± SE ±2,498		
GROUP III*			
TSE + FIA	5,400	73.9%	
(I.M.)	3,467	83.2%	
	10,000	51.7%	
	MEAN 6,289	69.6%	P<.005
	± SE ±1,938	±9.3%	(III vs IV)
GROUP IV			
PBS + FIA	MEAN 20,720		
(I.M.)	± SE ±1,844		

* TWO RATS DIED BEFORE CHALLENGE

** PERCENT DIFFERENCE BETWEEN IMMUNIZED AND CONTROL GROUPS

*** STUDENT'S T-TEST

DISCUSSION

The object of the present study was to determine whether the immunopotentiation observed in Trichinellosis towards challenge infections with *T. lewisi* is associated with a specific developmental stage of the parasite's life cycle, and to determine if it required the presence of the living parasite or could be induced by immunization with an antigen extract. The results of experiment I indicate that immunopotentiation could be stimulated by the intravenous inoculation of living newborn larvae, which produces only the parenteral phase of infection in the host. However, the intravenous injection of an equivalent dose of freeze-thaw-killed newborn larvae did not significantly alter trypanosome parasitemias, indicating a requirement for either living migratory or encysted, intracellular muscle stages in the induction of this phenomenon. The immunization with a drug-abbreviated enteral infection, exposing the host to only the molting muscle larvae and sexually mature adults, did not significantly effect the trypanosome infections, suggesting that a four day exposure to the enteral phase of the nematode infection was insufficient for inducing immunopotentiation in the rat model.

The temporal patterns of immunopotentiation, enhanced RES activity, and humoral immunosuppression during Trichinellosis (Fig. 5.2, Chapter V) are consistent with the above findings. These altered states of immunological responsiveness were a function of the intensity of infection, but more importantly, appeared to be related to the mode and timing of antigenic stimulation by the various developmental stages of the parasite's life cycle in the host. Low level infections of *T. spiralis* in the rat (100 larvae), produced a depression of humoral responsiveness to SRBC, that was restricted to 14 days after infection (during the peak period in which the newborn larvae migrate to the muscle tissues of the host), a result which is consistent with

the temporal pattern of immunosuppression in the mouse model (Faubert 1976). Immunopotentiation on the other hand, did not occur at 14 days, but was evident when rats were challenged with T. lewisi at 7 days (during the peak period of larviposition), or at 28 days (during the chronic intracellular muscle stage) after the nematode infection. This temporal pattern of immunopotentiation in the rat is consistent with several others previously demonstrated in T. spiralis-infected mice: Cypess et al.(1974) have reported an increased resistance to i.p. or i.v. challenge infections with Listeria monocytogenes, which occurred at 7 or 21 days after infection but was abrogated after 49 days, and Lubiniecki and Cypess (1975) have demonstrated small but significant increases in the incubation period and survival time of nematode infected mice injected with Sarcoma-180 Ascites tumors at 28 days but not at 56 days following the helminthic infection. Other studies, however, have indicated that immunopotentiation may occur in both short-term and long-term infections of T. spiralis: Cypess et al. (1974) and Molinari et al. (1974) have observed enhanced cell-mediated (delayed type) hypersensitivity to BCG in mice infected with T. spiralis either before or after prolonged periods following the administration of a sensitizing dose of BCG, and Molinari and Ebersole (1976) have demonstrated complete resistance to the transplantable murine tumors B-16 Melanoma and Lewis Lung Carcinoma in mice injected with tumor cells after long-term (6 month) infections of T. spiralis.

Recent research by Faubert (1976), investigating the temporal relationship between depressed humoral responsiveness to SRBC and the developmental stages of the life cycle of T. spiralis in the mouse model, has indicated that immunosuppression is well correlated with the peak of the migration of newborn larvae to the muscle tissues of the host. In this

study, the newborn larval stage of T. spiralis was shown to secrete substances in an in vitro culture system that could diffuse through a Millipore membrane and suppress the induction of an in vitro antibody response to SRBC. Adult worms or muscle stage larvae failed to suppress the antibody response in this system. More recently, Ackerman and Faubert (1977) have shown that in mice inoculated intravenously with either viable or freeze-thaw-killed newborn larvae, the splenic plaque-forming cell response to SRBC was reduced 50% and 80% respectively when mice were challenged with SRBC only four days after the inoculation of the parasite larvae.

The ability of the newborn larval stage of T. spiralis to induce both immunosuppression in the mouse and immunopotentiation in the rat appears somewhat paradoxical. However, an important difference between the present study and that of Ackerman and Faubert (1977) resides in the time period between the inoculation of the newborn larvae and the challenge with the heterologous antigen or parasite. Ackerman and Faubert (1977) challenged mice with SRBC only 4 days after the injection of newborn larvae, whereas in the present study, 21 days were allowed for the newborn larvae to complete their migration and to encyst intracellularly in the striated muscle cells before the trypanosome challenge. In terms of the natural course of the nematode infection, the former case corresponds to approximately 14 days after infection (period of maximum immunosuppression), whereas the latter case corresponds to approximately 28 days after the helminthic infection (period of maximum immunopotentiation). Therefore, if the newborn larvae of T. spiralis are capable of secreting immunosuppressive factors during the migration to the muscles (Faubert 1976), then it would appear from the results of the present study, that these factors are either not produced, or no longer secreted once larval development has progressed to

to the intracellular, encysted stage of the infection.

In spite of the apparent requirement for the parenteral phase of the T. spiralis infection in stimulating the host response to T. lewisi in experiment I, an attempt was made to induce immunopotentiation by immunizing rats with a soluble antigen extract of the muscle stage larvae (experiment II). Rats immunized intramuscularly with TSE, either alone or with Freund's Incomplete Adjuvant, had significantly enhanced trypanosome parasitemias as compared to non-immunized controls. Enhanced parasitemias were a result of a suppressed or delayed ablastic antibody response as well as a delayed 1st crisis in immunized animals. Although the delayed 1st crisis might have resulted from a suppression of the 1st trypanocidal antibody response, the agglutination titers of this antibody were not significantly different from non-immunized controls at 40 days after infection.

The ability of Freund's Incomplete Adjuvant to enhance parasitemias in the controls (group IV) is difficult to explain. This effect may possibly be related to an excessive amount of stress induced in this group with a consequent cortisone release (Christian and Davis 1964) due to the three week regimen of intramuscular injections of adjuvant in the hind leg muscles of the rat. Among its several reported functions, cortisone is known to inhibit antibody production (McMaster and Franzl 1961; Nelson 1969). An inhibition of the ablastic and 1st trypanocidal antibody responses of the rat to T. lewisi, due to the immunosuppressive effects of cortisone, would result in enhanced parasitemias and a delayed 1st crisis in this group. The ability of cortisone treatment to produce virulent T. lewisi infections in rats by interfering with the ablastic and trypanocidal antibody responses has been reported previously (Sherman and Ruble 1967).

The intramuscular immunization regimen used in this experiment

was highly effective in stimulating the production of specific anti-TSE immunoglobulin in the rat, and although the host's humoral responsiveness to a heterologous challenge organism (T. lewisi) was depressed by TSE, immunized rats were significantly protected against a homologous challenge with T. spiralis. These results confirm those of earlier studies on the immunosuppressive activity of soluble extracts of T. spiralis muscle larvae (Barriga 1975; Faubert and Tanner 1974, 1975).

The isolation, purification, and identification of the potent immunosuppressive factors secreted by the newborn larvae and present in soluble extracts of T. spiralis muscle stage larvae should aid in our understanding of why infections with this nematode are capable of both a suppression of specific cellular and humoral immune responsiveness and a potentiation of non-specific cell-mediated immunity to heterologous antigens and organisms.

CHAPTER VIII

THE EFFECT OF NON-SPECIFIC IMMUNIZATION WITH BCG ON THE IMMUNOLOGICAL RESPONSE OF THE RAT TO T. LEWISI

INTRODUCTION

The mechanism by which T. spiralis induces a potentiation of immunological responsiveness to heterologous organisms is still unclear. Cypess et al. (1974) and Meerovitch and Ackerman (1974) have suggested that immunopotentiation is a function of enhanced non-specific cell-mediated immunity in which the activated macrophage is the effector cell. The fact that T. spiralis infections decrease the susceptibility of mice to intracellular parasitism by Listeria monocytogenes (Cypess et al. 1974) and potentiate cell-mediated (delayed type) hypersensitivity to BCG in mice (Cypess et al. 1974; Molinari et al. 1974; Molinari and Cypess 1975), strongly supports this suggestion. More recently, Meerovitch and Bomford (1977) have demonstrated that peritoneal macrophages taken from T. spiralis-infected mice are cytostatic for R1 luekemia cells in vitro; this result lends further support to the role of the activated macrophage as the effector cell in immunopotentiation during experimental Trichinellosis.

The mechanism by which T. spiralis infections can inhibit the development of a concomitant infection with T. lewisi remains unclear. The results presented in Chapter VI indicate that T. spiralis infections do not potentiate the humoral responsiveness of the rat to T. lewisi and the extensive immunochemical analyses in Chapter V, failed to reveal any cross-reacting antigens between these parasites. The results of Chapter V, however, do demonstrate that the ability of T. spiralis infections to enhance non-specific RES activity is correlated with the inhibited development of

trypanosome parasitemia that occurs when rats are challenged with T. lewisi at 7 or 28 days after the nematode infection. Although previous workers have described an apparent lack of correlation between enhanced RES phagocytic activity and increased resistance to intracellular parasitism (Bohme 1960; Lucia and Nussenzweig 1969; Ruskin et al. 1969), their results do not preclude the possible involvement of enhanced reticulocyte phagocytic activity in the spleens of rats concomitantly infected with T. spiralis and T. lewisi.

Research on the immunological response of the rat to T. lewisi has failed to provide definitive evidence for the role of specific or non-specific cell-mediated immunity or non-specific RES activity in controlling infections with this parasite, other than a subsidiary function for phagocytosis in clearing lysed or agglutinated trypanosomes from the blood stream (Taliaferro 1929; D'Alesandro 1970). Recent research by Greenblatt (1973), however, has demonstrated the presence of T. lewisi in reticular spleen cells (macrophages) that are in a close association with plasma cells in the spleens of infected rats. Therefore, although the normal trypanocidal mechanism may principally involve lysis and agglutination, it is highly likely that splenic macrophages play an important role in both antigen processing and in the eventual removal of trypanosomes from the circulation (Greenblatt 1973).

A number of diverse agents that are known to enhance RES activity non-specifically, have also been shown to inhibit the development of T. lewisi infections. Styles (1965, 1970) was able to obtain an inhibition of T. lewisi parasitemia (similar to that presented in this dissertation), by pre-treating rats with small serial doses of E. coli endotoxin or the toxin holothurin, a steroid saponin of the Bahamian Sea Cucumber Actinopyga

agassizi. Similarly, Singer et al. (1963, 1964) found that the resistance of mice was enhanced when bacterial endotoxin was administered prior to infections with T. rhodesiense, T. congolense, and T. duttoni. Tate (1951) has demonstrated inhibited trypanosome parasitemias in rats previously infected with the spirochete Spirillum minus, such that rats challenged with T. lewisi after the spirochete infections developed only transient, subpatent parasitemias. While these authors suggest that endotoxin moieties or spirochete infection may act via the non-specific stimulation of RES activity, none of the above mentioned studies attempted to correlate enhanced reticulocyte phagocytic activity with increased resistance to infection.

The immunological factors involved in non-specific resistance to parasitic diseases in general, are not yet clearly understood, but a large variety of materials have been found to stimulate non-specifically either specific immune responsiveness or general host resistance (for a review see Yasphe 1971; Shilo 1959). Bacillus Calmette-Guérin (BCG), an attenuated strain of Mycobacterium bovis, provides non-specific resistance against a wide variety of diseases, including parasitic, infectious, and neoplastic (for a review see Chapter IV). In view of the known ability of systemically administered BCG to produce marked alterations in the macrophage elements of the RES, including heightened phagocytic activity and increased bactericidal and cytotoxic activities of individual macrophages (Biozzi et al. 1960; Old et al. 1961), the following study was conducted for the purpose of comparing the effects of a BCG-stimulated non-specific cell-mediated immunity with the effects of T. spiralis infections on the host response to T. lewisi. To this purpose, rats were immunized either intraperitoneally or intravenously with graded doses of BCG and subsequently challenged with Trypanosoma lewisi.

MATERIALS AND METHODS

1) EXPERIMENTAL INFECTIONS

Inbred albino, female rats (~100 grams) of the Charles River, GDF strain (Charles River Breeding Laboratories, Willmington, Mass.) were used in this experiment. Stock infections of Trypanosoma lewisi were maintained in outbred CD, albino female rats (~100 grams)(Canadian Breeding Farms, St. Constant, Quebec) as described previously in Chapter V. Experimental animals were housed individually in wire-bottom cages and fed Purina Rat Chow and water ad libitum.

The strain of T. lewisi used in this study is described in detail in Chapter V. Trypanosomes for initiating experimental infections were obtained from stock-infected rats 12 days after inoculation. Purified suspensions of trypanosomes were prepared according to the technique of Lincicome and Watkins (1963)(see Chapter V) and experimental animals were inoculated with 5.0×10^3 infective trypanosomes (0.5 ml) intraperitoneally without anaesthesia. Trypanosome parasitemias were followed during the course of the infections by daily hemocytometer counts of tail blood diluted 1/200 using 10µl capillary tubes and "Unopettes" as described previously in Chapter V. Thin blood films prepared during the course of the infections were stained with Giemsa's Blood Stain and used for a determination of the Coefficient of Variation (CV).

2) IMMUNIZATIONS WITH BCG

Rats were immunized either intraperitoneally or intravenously in the tail vein with 5.0×10^5 or 5.0×10^6 colony forming units (CFU) of lyophilized BCG (Lot #1707-6; Institute de Microbiologie et d'Hygiéne de Montréal, Quebec) in 0.5 ml (i.p.) or 0.25 ml (i.v.) sterile distilled

water, injected using a 1.0 ml syringe and a #26 gauge needle. In a preliminary experiment, rats were immunized with 5.0×10^7 CFU injected i.p. in 0.5 ml distilled water.

3) ASSAY FOR DELAYED TYPE HYPERSENSITIVITY

Experimental animals were tested for delayed-type hypersensitivity responses (DTH) 44 days after immunization with BCG in order to assess the level of specific, cell-mediated immunity stimulated by the different doses and routes of inoculation. Footpad swelling was used as a measure of DTH (Gray and Jennings 1955). The level of immunity was assessed after challenge with 0.1 ml (5.0 mg) of old tuberculin (OT; Connaught Medical Research Laboratories, University of Toronto, Canada) injected intradermally with a #30 gauge needle in the dorsal aspect of one hind footpad, whereas the contralateral hind pad (control) was injected with an equal volume of sterile physiological saline. The degree of swelling was measured after 24 hours according to the technique of Axelrad (1968). This method depends upon the principle that an object immersed in a fluid will displace its own volume. If the object (of volume V) has a lower specific gravity (S_o g/ml) than that of the fluid (S_f g/ml), then the pressure (P_g) needed to achieve total immersion in the fluid is given by the formula: $P = (S_f - S_o)V$.

A Mettler (Model P1200) top loading, single pan balance with an optical scale calibrated in 10 mg divisions was used. The balance was tared for a 250 ml beaker filled with mercury and the deflection produced by immersing the rat's paw (to the level of a line drawn in the groove immediately distal to the lateral malleolus) was noted. Rats were ether-anaesthetized and the hand immersing the paw rested on a firm bridge over the surface of the mercury. This method can clearly detect a paw volume difference of 0.02ml

(Axelrad 1968).

Results were calculated as the percentage difference in volume between the experimental and control footpads, and a reaction was considered positive if the swelling in the OT-challenged footpad was at least 20% greater than that measured for the saline control. Statistical analyses were performed on the absolute and percentage differences in footpad swelling using the Student's t-test, and the results were essentially identical for both analyses.

4) EVALUATION OF THE HUMORAL RESPONSE TO T. LEWISI

The ablastic antibody response of the rat to T. lewisi was followed by determining the coefficient of variation (CV) in the lengths of trypanosomes at 6, 8, and 10 days after infection according to the technique of Taliaferro and Taliaferro (1922)(see Chapter VI). The 1st and 2nd trypanocidal antibody responses to T. lewisi were examined by titrating rat sera (collected 40 days after infection) in vitro for agglutinating antibodies to either dividing stages (1st antigenic variant) or inhibited adult stages (2nd antigenic variant) according to the technique of D'Alesandro (1976) as described previously in Chapter VI.

5) EXPERIMENTAL PROTOCOL

EXPERIMENT I

A small preliminary experiment was conducted in order to examine the effect of BCG on the development of T. lewisi parasitemia. Ten Charles River CDF rats were divided into two groups of five rats each. Five rats were inoculated with 5.0×10^7 CFU (0.5 ml) of BCG intraperitoneally and five rats were injected i.p. with an equal volume of physiological saline.

Seven days after immunization with BCG, all rats were challenged with 5.0×10^3 trypanosomes given i.p..

EXPERIMENT II

Experiment II was designed to investigate the effect of different doses and routes of inoculation of BCG on the host's immunological response to T. lewisi. The 25 Charles River CDF rats used in this experiment were divided into five groups of five rats each. The treatment regimen for these experimental groups is displayed in Table 8.1. Four groups of rats were injected either i.v. or i.p. with two different doses of BCG. Controls were injected in a similar manner with sterile physiological saline. 14 days after immunization with BCG, all groups were challenged i.p. with 5.0×10^3 trypanosomes. 30 days after infection with T. lewisi, the animals in all groups were tested for a DTH response to OT as described above. 40 days after the trypanosome infections, all rats were bled by cardiac puncture and the sera collected and titrated for agglutinating (trypanocidal) antibodies to T. lewisi.

6) STATISTICAL ANALYSIS

Results were analyzed for statistical significance using the Analysis of Variance F-test and the Student's t-test where appropriate (Snedecor and Cochran 1967), and when necessary, extraneous values were rejected using the Q-test (Dean and Dixon 1951).

RESULTS

EXPERIMENT I

The development of parasitemias in rats immunized i.p. with 5.0×10^7 CFU of BCG or controls injected with saline is displayed in

TABLE 8.1

TREATMENT REGIMEN FOR GROUPS OF RATS IMMUNIZED WITH BCG AND CHALLENGED WITH I. LEWISI

GROUP	BCG DOSE (C.F.U.)*	ROUTE	TIME INTERVAL (DAYS)	CHALLENGE INFECTION**	TIME INTERVAL (DAYS)	DTH FOOTPAD TEST***
I	5×10^5	I.V.	14	I. LEWISI	30	+
II	5×10^5	I.P.	14	I. LEWISI	30	+
III	5×10^6	I.V.	14	I. LEWISI	30	+
IV	5×10^6	I.P.	14	I. LEWISI	30	+
V	CONTROL	SHAM	14	I. LEWISI	30	+

* COLONY FORMING UNITS

** 5×10^3 TRYPANOSOMES I.P.

*** DELAYED TYPE HYPERSENSITIVITY TESTED WITH OLD TUBERCULIN

Figure 8.1. Parasitemias in the two groups paralleled each other until day 9 of the infection after which time the rats pre-treated with BCG developed and sustained considerably enhanced parasitemias over those of the controls. Rats pre-treated with BCG sustained enhanced infections throughout the period in which the parasitemias were followed. Pre-treatment with BCG also induced considerable variation in parasitemias as evidenced by the very large standard errors in this group as compared to the non-immunized controls.

EXPERIMENT II

The development of trypanosome parasitemias in rats immunized with BCG according to the treatment regimen shown in Table 8.1 are displayed in Figures 8.2 and 8.3. Rats immunized intraperitoneally with BCG (groups II and IV) developed lower peak parasitemias on day 8 of the infections than the non-immunized controls in group V (Figure 8.2). However, the lower parasitemia values recorded for the BCG-treated rats in these groups were not significantly different from the control values at any time during the infection ($P>.05$).

In contrast, rats immunized intravenously with BCG (groups I and III) developed significantly lower peak parasitemias on day 8 of the infection than non-immunized controls in group V (Figure 8.3, $P<.05$). Although these BCG-treated groups (I and III) sustained lower mean parasitemias than the controls throughout the course of the infection, these differences were only statistically significant on day 8. The parasitemias in the two groups treated intravenously with 5×10^5 or 5×10^6 CFU of BCG (groups I and III respectively) were not significantly different from each other during the course of the infections.

The level of specific, cell-mediated immunity induced in rats by the various doses and routes of inoculation of BCG was assessed 44 days

FIGURE 8.1

The course of trypanosome parasitemias in rats immunized with 5.0×10^7 CFU of BCG intraperitoneally and challenged 7 days later with Trypanosoma lewisi. Each point represents the daily mean trypanosome parasitemia ±SE for five animals.

(●) = Controls, saline i.p.; (✱) = 5.0×10^7 CFU of BCG i.p.

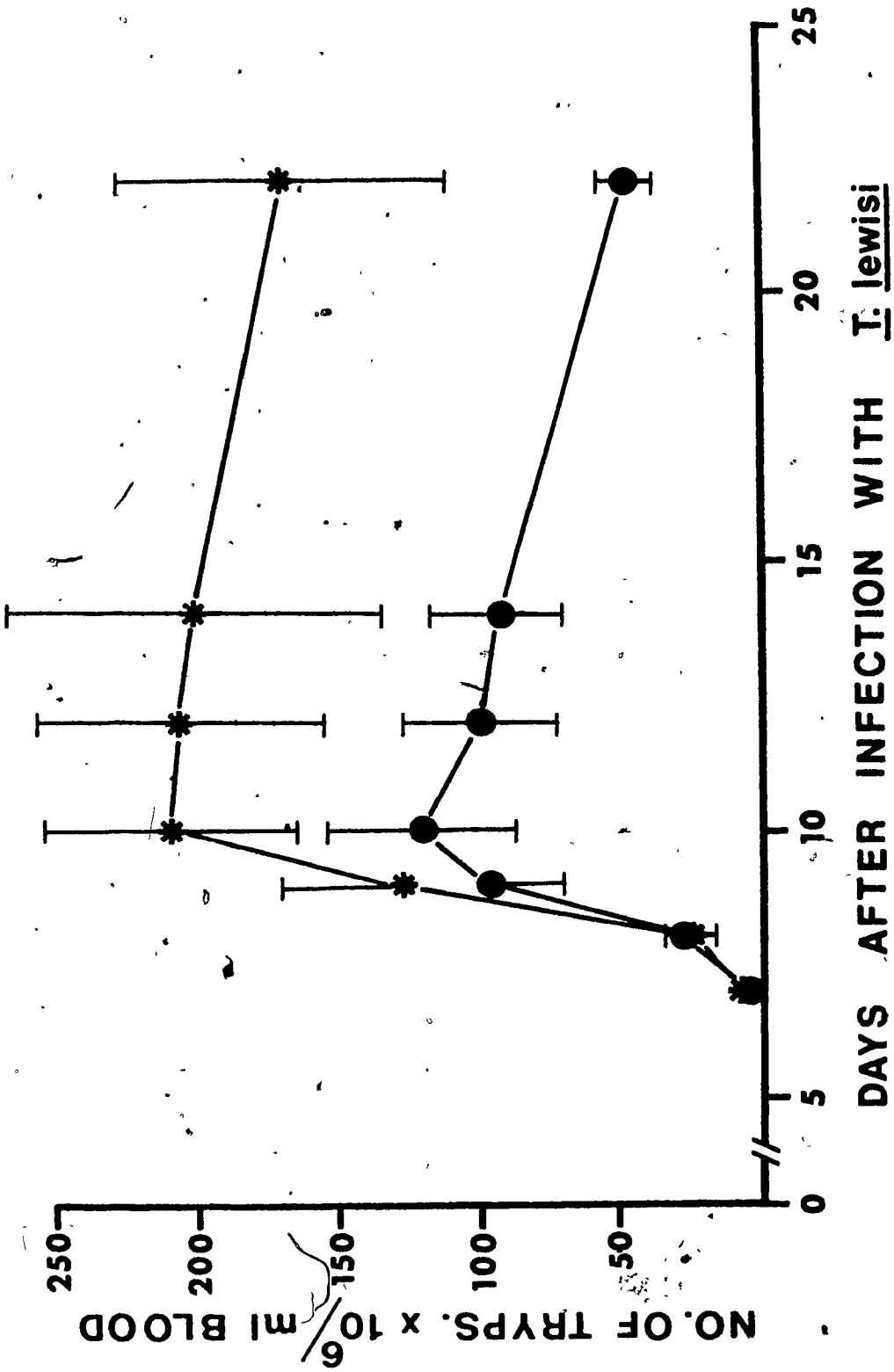

NO. OF TRYPS. x 10^6/ml BLOOD
250
200
150
100
50
0
5
10
15
20
25
DAYS AFTER INFECTION WITH T. lewisi

FIGURE 8.2

The course of trypanosome parasitemias in rats immunized intraperitoneally (i.p.) with BCG and challenged 14 days later with Trypanosoma lewisi. Each point represents the daily mean trypanosome parasitemia ±SE for five animals.

(●) = GROUP V - Controls, no BCG

(✱) = GROUP II - 5.0×10^5 CFU of BCG i.p.

(✸) = GROUP IV - 5.0×10^6 CFU of BCG i.p.

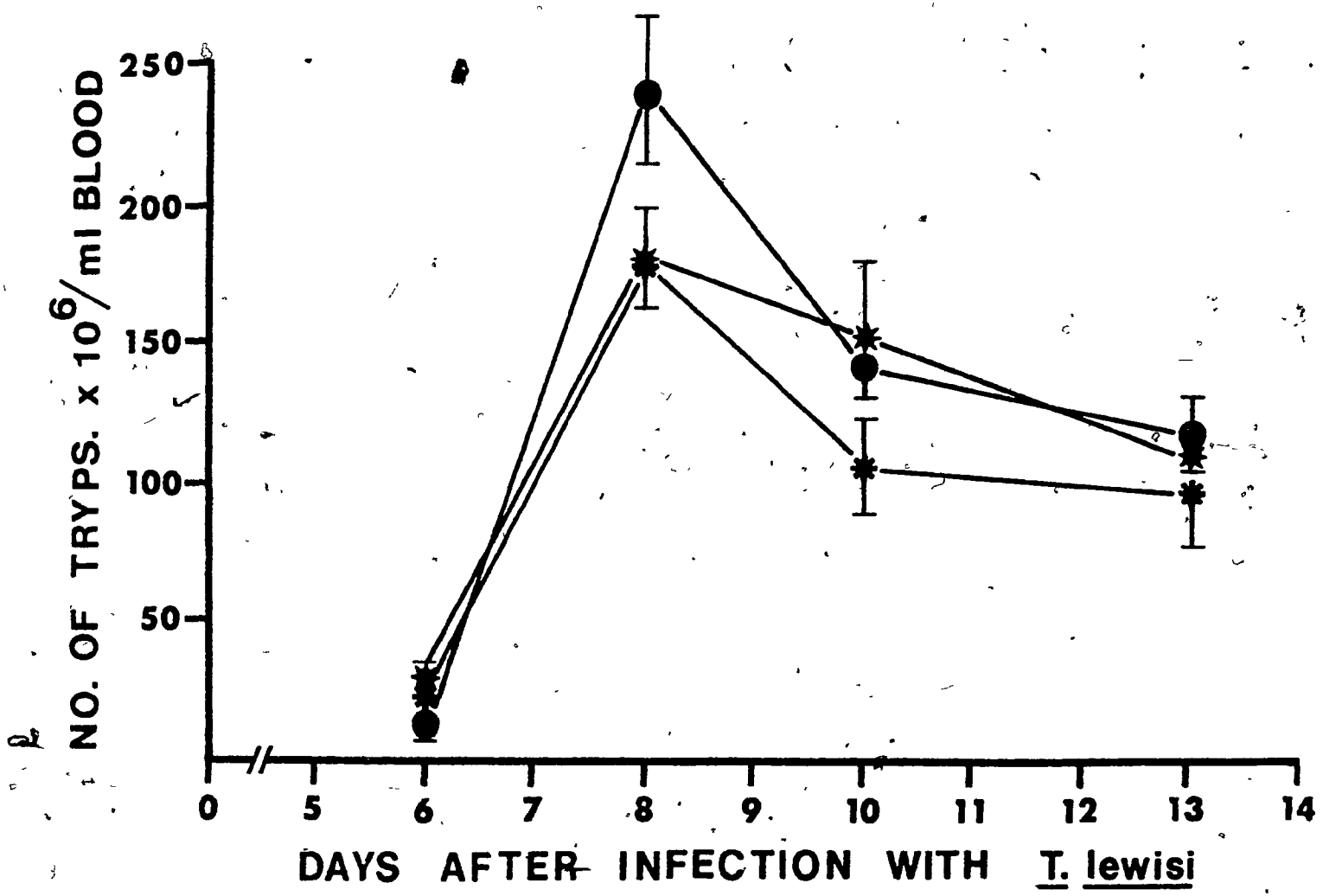

NO. OF TRYPS. x 10^6/ml BLOOD
250
200
150
100
50
0
5
6
7
8
9
10
11
12
13
14
DAYS AFTER INFECTION WITH T. lewisi

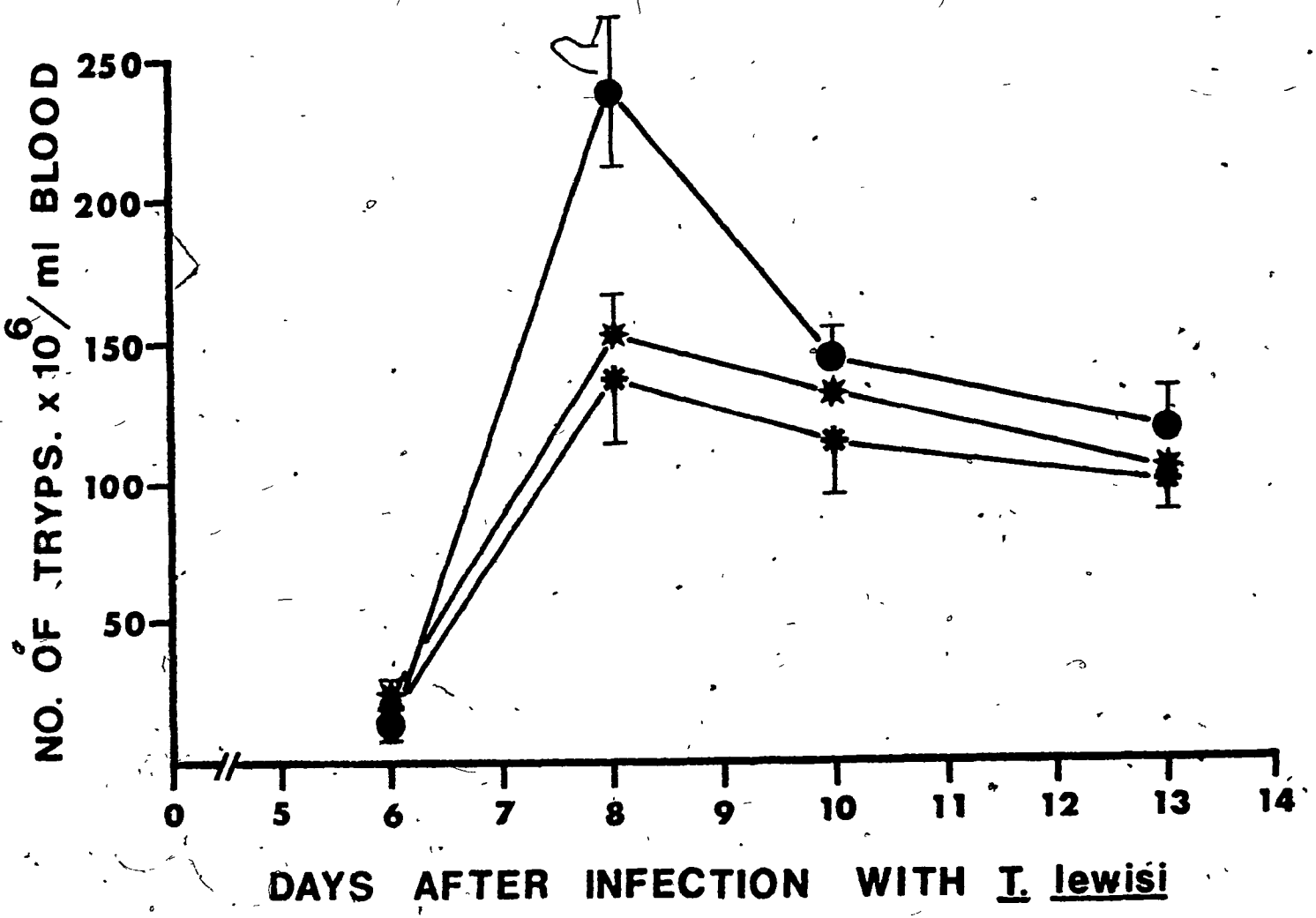
FIGURE 8.3

The course of trypanosome parasitemias in rats immunized intravenously (i.v.) with BCG and challenged 14 days later with Trypanosoma lewisi. Each point represents the daily mean trypanosome parasitemia ±SE for five animals.

(●) = GROUP V - Controls, no BCG

(✱) = GROUP I - 5.0×10^5 CFU of BCG i.v.

(✱) = GROUP III - 5.0×10^6 CFU of BCG i.v.


NO. OF TRYPS. x 10⁶/ml BLOOD
250
200
150
100
50
0
5
6
7
8
9
10
11
12
13
14
DAYS AFTER INFECTION WITH T. lewisi

after immunization by determining the extent of a 24 hour DTH footpad swelling reaction after the injection of old tuberculin. The results presented in Table 8.2 indicate that all rats immunized intravenously with BCG showed positive DTH reactions to OT (groups I and III), although the two doses of BCG did not produce significantly different levels of DTH. Rats immunized intraperitoneally with BCG (groups II and IV) did not all exhibit positive DTH responses, although the average percent swelling in these groups was significantly different from that of the controls in group V. The BCG dose of 5.0×10^6 CFU i.p. in group IV was considerably better in stimulating a cell-mediated DTH response than was the lower dose of 5.0×10^5 CFU i.p. in group II.

The effect of BCG treatment on the development of acquired humoral immunity to T. lewisi in the rat was followed by examining both the ablastic and the trypanocidal antibody responses in immunized animals. The ablastic antibody response was followed by determining the coefficient of variation (CV) in the lengths of trypanosomes on days 6, 8, and 10 of the infections. The CV is a statistical index of parasite reproductive activity (Taliaferro and Taliaferro 1922) and is a direct reflection of the changes in the titer of ablastin, the reproduction-inhibiting antibody to T. lewisi (Coventry 1925). The results presented in Table 8.3 show that the pre-treatment of rats with BCG, either i.v. or i.p. (groups I-IV), had no effect on the CV as compared to non-immunized controls (group V) on days 6, 8, or 10 after infection. The inhibited development of peak parasitemias in groups I and III (Figure 8.3) on day 8 of the infection was not reflected by lower coefficients of variation (enhanced production of ablastin) in these groups. Therefore, BCG-treatment had no effect on the ablastic antibody response to T. lewisi.

The trypanocidal antibody responses were examined by titrating

TABLE 8.2

DELAYED HYPERSENSITIVE FOOTPAD SWELLING REACTIONS

24 HOURS AFTER CHALLENGE WITH OLD TUBERCULIN*

GROUP	BCG DOSE (CFU)	ROUTE OF INOCULATION	# ANIMALS(A) POS./GROUP	AVERAGE(B) (%) ±SE	P-VALUE(C)
I	5×10^5	I.V.	5/5	33.8 ±2.6	<.001
II	5×10^5	I.P.	2/5	16.2 ±4.4	<.05
III	5×10^6	I.V.	5/5	37.3 ±3.3	<.001
IV	5×10^6	I.P.	3/5	23.3 ±8.1	<.001
V	CONTROL	SHAM	0/5	10.8 ±2.6	

I vs II: P<.001; III vs IV: P<.001; I vs III: P>.05; II vs IV: P<.05

* TESTED 30 DAYS AFTER IMMUNIZATION WITH BCG

(A) REACTION CONSIDERED POSITIVE IF CHALLENGED FOOTPAD EXHIBITED 20% GREATER SWELLING THAN CONTROL FOOTPAD

(B) AVERAGE % SWELLING = (EXPERIMENTAL-CONTROL)/CONTROL X 100

(C) STUDENT'S T-TEST: P-VALUES WERE SIMILAR USING ABSOLUTE VOLUME INCREASES

TABLE 8.3

THE MEAN COEFFICIENTS OF VARIATION (CV) FOR RATS IMMUNIZED WITH BCG AND CHALLENGED WITH I. LEWISI

GROUP	BCG DOSE (CFU)	ROUTE OF INOCULATION	CV (MEAN ±SE)* DAYS AFTER INFECTION 6	8	10
I	5×10^5	I.V.	27.8 ±2.7	9.0 ±1.5	9.8 ±1.5
II	5×10^5	I.P.	30.0 ±2.3	8.6 ±1.4	8.4 ±1.3
III	5×10^6	I.V.	29.0 ±2.4	8.4 ±1.2	8.2 ±1.0
IV	5×10^6	I.P.	29.4 ±2.4	12.2 ±1.4	10.0 ±1.2
V	CONTROL	SHAM	27.4 ±2.1	10.4 ±1.7	8.8 ±1.4

* THE MEAN VALUES FOR GROUPS I-IV ARE NOT SIGNIFICANTLY DIFFERENT FROM THE CONTROL GROUP ON DAYS 6, 8, OR 10 AFTER THE INFECTION ($P > .05$).

rat sera collected 40 days after infection for agglutinating antibodies to either living reproducing stages (1st antigenic variant) or inhibited, non-dividing adult stages (2nd antigenic variant)(collected 6 or 14 days after infection respectively). The results presented in Table 8.4 indicate that the mean agglutinating (trypanocidal) antibody titers for rats immunized with BCG (groups I-IV) were not significantly different from those of non-immunized controls in group V at 40 days after infection. Therefore, pre-treatment with BCG did not appear to effect the development of the trypanocidal responses of the rat to T. lewisi.

DISCUSSION

Bacillus Calmette-Guérin (BCG) provides enhanced non-specific resistance in a wide variety of infectious (Nakamura and Cross 1972; Larson et al. 1972), neoplastic (Old et al. 1959; Bluming et al. 1972; Hersh et al. 1973; Gutterman et al. 1973), and parasitic (Ortiz-Ortiz et al. 1975; Tabbera et al. 1975; Rau and Tanner 1975; Clark et al. 1976; Mahmoud et al. 1976) diseases. Although the specific modes of action of BCG in stimulating non-specific resistance in this broad spectrum of diseases are still somewhat controversial, it is generally recognized that immunization with live BCG can enhance antibody production (Miller et al. 1973), and produce marked alterations in the macrophage elements of the RES, including heightened phagocytic activity and increased microbicidal and cytotoxic activities of individual macrophages (Biozzi et al. 1960; Old et al. 1961). It has been suggested that the activity of BCG as an anti-cancer agent may involve a combination of several mechanisms (Bartlett et al. 1976) including: 1) a generalized stimulation of immune responsiveness and lymphoreticular activity following systemic administration (Old et al.

TABLE 8.4

THE MEAN AGGLUTINATING (TRYPANOCIDAL) ANTIBODY TITERS (LOG_2) FOR RATS IMMUNIZED WITH BCG AND CHALLENGED WITH *TRYPANOSOMA LEWISI*

GROUP NO.	1ST TRYP. AB.(A) (MEAN ± SE)	P-VALUE*	2ND TRYP. AB.(B) (MEAN ± SE)	P-VALUE*
I	4.8 ±.49	NS	4.0 ±.32	NS
II	4.4 ±.50	NS	4.2 ±.58	NS
III	4.6 ±.51	NS	4.6 ±.24	NS
IV	5.0 ±.31	NS	4.4 ±.60	NS
V	4.8 ±.73		4.6 ±.25	

(A) TITRATED WITH LIVING REPRODUCING STAGES (1ST ANTIGENIC VARIANT)

(B) TITRATED WITH INHIBITED ADULT STAGES (2ND ANTIGENIC VARIANT)

* STUDENT'S T-TEST, NS = NOT SIGNIFICANT ($P>.05$)

1960, 1961), 2) a local and non-specific destruction of tumor cells at the site of a delayed-type hypersensitivity reaction to BCG ("innocent bystander" effect)(Bartlett et al. 1972), and 3) a true adjuvanticity whereby the administration of BCG in temporal and spacial proximity to tumor cells (either mixed in the tumor cell inoculum or by infiltration of the tumor nodules) results in augmented development of systemic, tumor-specific, rejection immunity (Bartlett and Zbar 1972; Hawrylko and Mackaness 1973).

The use of BCG in investigating the role of non-specific immunity in the host-parasite relationship has now become highly fashionable. Indeed, the efficacy of using BCG or other non-specific immunostimulants such as Corynebacterium parvum in the immunotherapy of parasitic diseases is now under extensive investigation (Brener and Cardoso 1976; Kierszenbaum 1975; Swartzberg et al. 1975). Live BCG has been reported to protect against a number of experimental protozoal infections, the success of which has varied considerably with the dose, strain, and route of administration. Thus, intraperitoneal inoculations of viable BCG did not protect mice against T. cruzi (Hoff 1975) or Toxoplasma gondii (Frenkel and Caldwell 1975), and BCG injected into the retrobulbar space of rabbits did not protect the eyes from T. gondii (Tabbera et al. 1975). In contrast, pre-treatment intravenously with viable BCG reduced the number of circulating T. cruzi and enhanced survival times of mice (Ortiz-Ortiz et al. 1975) and protected rabbits' eyes from T. gondii (Tabbera et al. 1975). However, Kuhn et al. (1975) were unable to suppress T. cruzi parasitemia or enhance survival times by pretreating mice intravenously with BCG. The most promising and consistent results to date using BCG are those of Clark et al. (1976) and Clark and Allison (1976). These authors have been able to completely suppress the development of Babesia microti, Babesia rodhaini, Plasmodium berghei, and Plasmodium vinckei in mice using either intravenous or intraperitoneal in-

jections of BCG.

The extensive differences observed in the effects of BCG on protozoal infections may simply be a question of the BCG strain, the dose and route of inoculation, or the timing of the immunization relative to the challenge infection, rather than any fundamental differences in the protection afforded by BCG immunization against different protozoan parasites (Clark et al. 1976). This may be especially true in view of the results of the present study.

In a preliminary experiment (experiment I), a dose of 5.0×10^7 CFU of BCG given i.p. caused considerable enhancement of T. lewisi parasitemias when immunization preceded the trypanosome challenge by seven days. However, immunization with lower doses of BCG (5.0×10^5 or 5.0×10^6 CFU) also given i.p. (experiment II) did not enhance trypanosome parasitemias and may have actually inhibited the development of peak parasitemia to some degree, when immunization preceded the trypanosome challenge by 14 days. In contrast, the same doses of BCG given intravenously 14 days before trypanosome challenge (experiment II), significantly inhibited the development of peak T. lewisi parasitemias on day 8 of the infections.

The results of experiments I and II do not confirm those of a recent study by Tizard and Ringleberg (1975) on the effects of bacterial adjuvants on T. lewisi infections in rats. These authors failed to demonstrate either enhanced or suppressed T. lewisi infections after the intramuscular administration of BCG. These conflicting results confirm the observations of other workers that BCG-induced, non-specific resistance to protozoal infections and neoplasia is a function of both the dosage and the route of inoculation. For example, in a recent study of BCG dosage and tumor rejection phenomenon, Bartlett et al. (1976) found that the dose of

BCG required for optimal suppression of local tumor growth, was detrimental to the development of a sustained and systemic tumor immunity. It is obvious from observations such as these, and the conflicting reports of Kuhn et al. (1975), Ortiz-Ortiz et al. (1975), and Hoff (1975) that the variable effects of dosage and route of administration of BCG on non-specific stimulation of host resistance needs further evaluation and research.

The present study was conducted for the purpose of comparing the effects of non-specific, cell-mediated immunity stimulated by BCG, with the effects of T. spiralis infections on the immunological response of the rat to T. lewisi. Although there is considerable evidence to suggest that immunopotentiation in Trichinellosis is a function of enhanced non-specific, cell-mediated immunity (Cypess et al. 1974; Molinari et al. 1974; Molinari and Cypess 1975), the mechanism of immunopotentiation during concomitant infections of T. spiralis and T. lewisi is unclear. It was hoped that this comparative study would aid in an understanding of how a stimulation of cell-mediated immunity might affect the host response to T. lewisi. The results of experiment II indicate that the systemic administration of BCG (i.v.) was capable of causing an inhibited development of peak trypanosome parasitemia, while the normal humoral response (ablastic and trypanocidal) of the rat appeared unaffected. These results mirror those obtained for rats infected with T. spiralis, in which peak parasitemia was inhibited, while the ablastic and trypanocidal responses were unchanged (Chapter VI).

A host undergoing a high level of cell-mediated (delayed-type) hypersensitivity to BCG, may upon secondary stimulation, release soluble factors (lymphokines) which activate macrophages and enable them to destroy unrelated organisms more effectively (Mackaness 1969). In this situation,

the non-specific stimulator must be antigenic, and the host must be undergoing the corresponding immune response. Although the effector cell (macrophage) is specifically activated, it is non-specifically active and can destroy a wide variety of phylogenetically and antigenically unrelated organisms (Ruskin et al. 1969). It is therefore not difficult to see a similarity between the effects that stimulation with BCG (experiment II), T. spiralis (Chapters V and VI), or bacterial endotoxin (Styles 1965) might have on the host response to infection with T. lewisi.

Definitive evidence for a role of specific or non-specific cell-mediated immunity or RES phagocytic activity in controlling infections with T. lewisi has not been presented in the past (Taliaferro 1929; D'Alesandro 1970). In spite of the fact that T. lewisi infections are principally controlled by three well defined humoral antibody responses (Taliaferro 1926), the results of the present study and those of Styles (1965, 1970) lead me to presume that an appropriate stimulation of non-specific cell-mediated immunity or RES phagocytic activity by agents such as BCG, T. spiralis, or bacterial endotoxin, can result in enhanced resistance to T. lewisi.

CHAPTER IX

THE PHAGOCYTIC ACTIVITY OF PERITONEAL AND SPLENIC MACROPHAGES FROM TRICHINELLA SPIRALIS-INFECTED AND BCG-IMMUNIZED RATS ON TRYPANOSOMA LEWISI IN VITRO

INTRODUCTION

Research in the past concerning the immunological responsiveness of the rat to T. lewisi has not provided affirmative evidence for a role of specific or non-specific cell-mediated immunity or RES activity in controlling infections with this trypanosome (Taliaferro 1929; D'Alesandro 1970). Recent studies by a number of people, however, have clearly demonstrated the capacity of splenic or peritoneal macrophages from normal or infected hosts to phagocytize T. lewisi in vitro (Lange and Lysenko 1960; Patton 1972; Greenblatt and Tyroler 1971) as well as the capability of whole spleen cell or peritoneal cell transplants from immune or hyperimmune rats to confer protection against T. lewisi in non-immune recipients or immunosuppressed hosts (Greenblatt et al. 1972; Patton 1965, 1972).

The results presented in Chapter VIII of this thesis suggest that an appropriate stimulation of non-specific, cell-mediated immunity or RES activity can effectively inhibit the full development of T. lewisi infections. The similarities between the effects of infection with T. spiralis (Chapters V and VI), BCG immunization (Chapter VIII), or pre-treatment with E. coli bacterial endotoxin (Styles 1965) or the toxin holothurin (Styles 1970) on T. lewisi infections, suggests the possibility that a common mechanism of immunopotentiation may underlie the activity of all of these different agents.

Cypess et al. (1974) and Meerovitch and Ackerman (1974) have

suggested that immunopotentiation during Trichinellosis probably manifests itself in the form of non-specific, cell-mediated immunity in which the activated macrophage is the effector cell. In this situation, macrophages specifically activated by infection with T. spiralis would be non-specifically active against a wide variety of heterologous organisms or neoplastic cells (Lubiniecki and Cypess 1975; Ruskin et al. 1969). Meerovitch and Bomford (1977) have tested this hypothesis in vitro and have shown that peritoneal macrophages taken from mice infected with T. spiralis for 6 to 36 days are activated and highly cytostatic for R1 Leukemia cells as compared to macrophages from normal animals.

In view of the fact that activated peritoneal or splenic macrophages have been continually alluded to as the effector cells in immunopotentiation during experimental Trichinellosis, it was of interest to explore the potential of this cell type for phagocytic activity towards T. lewisi. Therefore, the purpose of the experiments reported in this chapter was to investigate the ability of splenic or peritoneal macrophages from rats infected with T. spiralis, or immunized with BCG, to phagocytize trypanosomes in vitro. To this purpose, macrophages from the spleens or peritoneal cavities of normal animals, rats immunized with BCG, or rats infected with T. spiralis or T. lewisi were cultured and compared in vitro for the ability to phagocytize T. lewisi in the presence of homologous or heterologous immune sera.

MATERIALS AND METHODS

1) THE PARASITES, EXPERIMENTAL INFECTIONS, AND IMMUNIZATIONS

TRYPANOSOMA LEWISI

The strain of T. lewisi used in this study was maintained by serial transfers at two week intervals in outbred CD, albino female rats

(∿100 grams)(Canadian Breeding Farms, St. Constant, Quebec) and is described in detail in Chapter V. Charles River CDF, inbred albino, female rats (∿200 grams) were experimentally infected i.p. with ∿1.0 x 10^5 trypanosomes from a previously infected stock rat. Twelve days after inoculation, the rats were bled by cardiac puncture and trypanosomes free of host blood cell components were obtained by means of DEAE-Cellulose Anion Exchange Columns (Lanham 1968; Lanham and Godfrey 1969; see Chapter V). The trypanosomes were washed three times in phosphate-buffered saline (pH 7.2) containing 1% glucose, by centrifugation at 4^0 C. (1000 x g, 10 min.), resuspended in sterile tissue culture medium (see below) at a concentration providing suitable numbers for the phagocytic test, and stored on ice until use.

TRICHINELLA SPIRALIS

The strain of T. spiralis used in this study is described in detail in Chapter V. Infecting stocks were maintained in outbred CD rats inoculated orally with 3,000 infective muscle larvae, and muscle larvae for initiating experimental infections were obtained from donor stock rats which had been inoculated thirty days previously. Experimental and stock infections, and the recovery of muscle larvae were done according to techniques adopted from Tanner (1968) and are described in detail in Chapter V. For the purpose of this study, Charles River CDF, inbred rats (∿100 grams), were inoculated per os with 100 infective muscle larvae, 28 days before the collection of the peritoneal or spleen cells that were used in the phagocytic test.

BACILLUS CALMETTE-GUERIN (BCG)

Charles River CDF, inbred albino, female rats (∿100 grams) were immunized intravenously in the tail vein with 5.0 x 10^6 colony forming units (CFU) of lyophilized BCG (Lot # 1707-6; Institute de Microbiologie

et d'Hygiéne de Montréal, Quebec) in 0.25 ml of sterile distilled water, injected using a 1.0 ml syringe and a #26 gauge needle. Immunizations were done 14 days before peritoneal exudate or spleen cells were collected for the in vitro phagocytic test.

2) COLLECTION OF IMMUNE SERA

The various immune and normal rat sera (see below) used in the in vitro phagocytic test were collected from Charles River CDF, inbred albino, female rats previously infected with T. lewisi or T. spiralis, or hyperimmunized with T. lewisi. Rats were bled by cardiac puncture and the blood was allowed to clot for 12 hours at 4° C.. The sera were collected by centrifugation (1500 x g, 30 min.) and heat inactivated (56° C., 30 min.). Sera were pooled and sterilized by filtration through a "Millipore" filter (0.45μ) and stored in sterile, stoppered serum bottles at -20° C. until use.

1) Normal Rat Sera (NRS) - Normal rat sera were collected from naive animals.

2) Immune Rat Sera - T. lewisi (IRSTL) - Immune sera from rats infected with T. lewisi were obtained from animals which were inoculated with ∿1.0 x 10^5 trypanosomes and bled approximately 40 days later, when an examination of the blood no longer revealed circulating trypanosomes.

3) Immune Rat Sera - T. spiralis (IRSTS) - Immune sera from rats infected with T. spiralis were obtained from animals which had been inoculated 28 days previously with 100 infective muscle larvae per os.

4) Hyperimmune Rat Sera - T. lewisi (HRSTL) - Hyperimmune sera were obtained from rats which had recovered from a primary

infection with T. lewisi (~40 days) and were subsequently challenged i.p. with two doses of a purified suspension of living trypanosomes (~1.0 x 10^7 organisms, prepared according to the technique of Lincicome and Watkins (1963)) given at two week intervals. Sera were collected one week after the last injection of the trypanosomes.

3) COLLECTION OF PERITONEAL EXUDATE AND SPLEEN CELLS

PERITONEAL EXUDATE CELLS (PEC)

Peritoneal exudate cells were collected according to the technique of Takayanagi et al. (1974). Rats were killed rapidly with ether and the peritoneal cavity injected with 10.0 ml of Hank's Balanced Salt Solution (HBSS) pH 7.3, containing 10 I.U./ml sodium heparin. The peritoneum was gently massaged and the fluid containing the PEC was withdrawn aseptically using a sterile syringe, and transferred to a 12 ml centrifuge tube. The cells were sedimented and washed twice by centrifugation at 4° C. in a clinical centrifuge at approximately 1,500 rpm. The cells were gently resuspended in 5.0 ml of tissue culture medium (TCM) consisting of TCM 199, 20% fetal calf serum, 50 I.U./ml penicillin, and 50 µg/ml streptomycin, pH 7.3, and stored on ice until use. Viable cells were counted with 0.2% Trypan Blue in a Spencer Brightline Hemocytometer and the concentration adjusted to 2.0 x 10^6 viable cells/ml. The entire procedure was done with the cells either refrigerated (4° C.) or kept on ice.

SPLEEN CELLS (SC)

Spleen cell suspensions were prepared according to the technique of Greenblatt and Tyroler (1971). The spleen of the rat was removed aseptically and a cell suspension was prepared by gently sieving the spleen through

a #80 mesh stainless steel screen into 10 ml of ice cold TCM. The suspension was transferred to a sterile 12 ml centrifuge tube and the spleen cells were gently sedimented and washed with TCM three times by centrifugation at approximately 1,500 rpm at 4^{o} C. in a refrigerated clinical centrifuge. The cells were gently resuspended in 10.0 ml of TCM and stored on ice until use. Viable cells were counted as described above and the concentration adjusted to 1.0×10^{7} viable cells/ml. The entire procedure was done with the cells either refrigerated (4^{o} C.) or kept on ice.

4) PREPARATION AND CULTURE OF MACROPHAGE CELL MONOLAYERS

The macrophage monolayers (adherent cells) used for the phagocytic test were prepared according to the technique of Greenblatt and Tyroler (1971). Spleen cell suspensions (1.0×10^{7} cells/ml) or peritoneal exudate cell suspensions (2.0×10^{6} cells/ml), prepared as described above, were dispensed in volumes of 2.0 ml or 1.0 ml respectively into Leighton tubes containing 10 x 40 mm coverslips. Tubes were incubated at 37^{o} C. in a humidified, CO_2-gas flow incubator (5% CO_2 and air). After two hours of incubation, each culture tube was vigorously agitated and the culture medium withdrawn. The cell layer was next washed twice with 2.0 ml of TCM by pipetting the culture medium across the surface of the coverslip and gently agitating the tube. The wash fluid was removed, the cells overlaid with 1.5 ml of TCM, and the tubes were incubated as above for a few hours until used for the phagocytic test. The agitation and washing procedure removed more than 95% of the non-adherent lymphocytes and in some preparations it was difficult to find any cells other than macrophages on the coverslips.

5) INCUBATIONS OF MACROPHAGE MONOLAYERS WITH TRYPANOSOMES AND SERA

The phagocytic activity of peritoneal or splenic macrophages was assessed by incubating the macrophage cell monolayers with trypanosomes and various immune or normal sera. Before adding the trypanosomes and sera, the culture medium was first discarded. Each Leighton tube culture then received 0.25 ml of a suspension of trypanosomes containing 1.0×10^8 organisms/ml. After approximately one minute, 0.25 ml of immune or normal serum and 0.5 ml of TCM was added to each tube. After mixing thoroughly, the culture tubes were incubated at 37^0 C. for two hours as described above. At the end of this incubation period, the coverslips in the Leighton tubes were washed twice with TCM to remove free trypanosomes. Coverslips were air dried, fixed in absolute methanol for five minutes, and stained in Giemsa's Blood Stain.

6) EVALUATION OF THE PHAGOCYTIC INDEX (PI)

The stained coverslips were mounted on microscope slides and examined using oil immersion optics (1000X magnification). Random fields were examined and all macrophages encountered were examined for trypanosomes either adhered to their surface or phagocytized. The phagocytic index, PI(%), was calculated for 100 macrophages per slide according to the following formula:

$$PI(\%) = \frac{\text{\# of macrophages with adherent or phagocytized trypanosomes} \times 100}{\text{total \# of macrophages observed}}$$

Since adherence is considered to be the first step to phagocytosis, the phenomenon was included in a calculation of the PI (Jones et al. 1972; Takayanagi et al. 1974).

7) EXPERIMENTAL PROTOCOL

A total of 24 Charles River CDF, inbred albino rats were used in these experiments. Peritoneal exudate cells or spleen cells were collected from groups of three rats, each group of which received one of the following treatments:

GROUP I - Normal rats not previously exposed to BCG, T. lewisi, or T. spiralis.

GROUP II - Rats infected 7 days previously with T. lewisi (5.0×10^3 trypanosomes i.p.).

GROUP III - Rats infected 28 days previously with 100 infective muscle larvae of T. spiralis per os.

GROUP IV - Rats immunized 14 days previously with 5.0×10^6 CFU of BCG given i.v..

Adherent peritoneal (experiment I) or adherent spleen (experiment II) cells from three rats in each of these four groups were tested in vitro for their ability to phagocytize T. lewisi in the presence of NRS, IRSTL, HRSTL, or IRSTS according to the culture system displayed in Table 9.1. The spleen cells or peritoneal cells from each rat constituted a separate series of four macrophage monolayer cultures, each of which was treated with one of the four different sera described above.

Results are presented as the mean phagocytic index (PI) ±SE for the cell monolayers from 3 rats in each group receiving the same treatment.

RESULTS

The phagocytic activity of peritoneal or splenic macrophages from rats in groups I-IV was assessed in vitro in the presence of normal rat serum (NRS), rat anti-T. lewisi immune serum (IRSTL), rat anti-T. lewisi

TABLE 9.1

IN VITRO CULTURE SYSTEM FOR PERITONEAL AND SPLENIC MACROPHAGE CELL MONOLAYERS

SOURCE OF CELLS*	TUBE #**	VOLUME ADDED PER TUBE (ML)					
		ICM	NRS	IRSTL	HRSTL	IRSTS	TRYPANOSOMES $1x10^8$ TRYPS/ML
GROUP I	1	0.5	0.25				0.25
	2	0.5		0.25			0.25
	3	0.5			0.25		0.25
	4	0.5				0.25	0.25
GROUP II	1	0.5	0.25				0.25
	2	0.5		0.25			0.25
	3	0.5			0.25		0.25
	4	0.5				0.25	0.25
GROUP III	1	0.5	0.25				0.25
	2	0.5		0.25			0.25
	3	0.5			0.25		0.25
	4	0.5				0.25	0.25
GROUP IV	1	0.5	0.25				0.25
	2	0.5		0.25			0.25
	3	0.5			0.25		0.25
	4	0.5				0.25	0.25

* EXP. I - PERITONEAL CELLS; EXP. II - SPLEEN CELLS (SEE TEXT - 7) EXPERIMENTAL PROTOCOL)

** FOUR MACROPHAGE CULTURES WERE PREPARED FOR EACH OF THE THREE RATS IN GROUPS I-IV, EACH OF WHICH WAS TREATED WITH ONE OF FOUR DIFFERENT SERA.

hyperimmune serum (HRSTL), and rat anti- T. spiralis immune serum (IRSTS). The phagocytic index, % PI, was calculated for cell monolayers prepared from 3 rats in each group and are presented in Tables 9.2 and 9.3 as the mean PI ±SE. Since adherence of the parasite to the macrophage cell surface is considered to be the first step to engulfment, this phenomenon was included in an evaluation of the PI.

EXPERIMENT I - PHAGOCYTIC ACTIVITY OF PERITONEAL MACROPHAGES

Attachment and ingestion of trypanosomes by peritoneal macrophages were greatly enhanced by the addition of immune or hyperimmune homologous antisera against the parasites (IRSTL and HRSTL respectively) with cells from all sources (Groups I-IV). The addition of normal rat serum (NRS) or the heterologous rat anti-T. spiralis immune serum (IRSTS) produced similar levels of phagocytosis with cells in any one particular group. Phagocytosis did not occur appreciably with cells from uninfected control animals (group I) or cells from animals infected with T. spiralis (group III) in the presence of NRS or IRSTS although phagocytosis was enhanced in these groups by the addition of immune or hyperimmune (IRSTL or HRSTL) homologous antiserum against the parasites. Peritoneal macrophages from rats immunized with BCG (group IV) showed considerable phagocytic activity with all four sera as compared to cells from the controls (group I), or from T. spiralis-infected rats (group III), and the phagocytic indices in group IV compared favorably with the homologous situation in which cells derived from rats infected with T. lewisi (group II) were incubated in rat anti-T. lewisi immune or hyperimmune serum.

The results of this experiment suggest that immunization intravenously with BCG produced a significant activation of peritoneal macrophages which was reflected by an enhanced ability to phagocytize trypano-

TABLE 9.2

THE PHAGOCYTIC ACTIVITY* OF PERITONEAL MACROPHAGES FROM RATS INFECTED WITH I. SPIRALIS, I. LEWISI, OR BCG ON I. LEWISI IN VITRO

SOURCE OF SERUM***	SOURCE OF PERITONEAL MACROPHAGES**			
	GROUP I CONTROL	GROUP II I. LEWISI	GROUP III I. SPIRALIS	GROUP IV BCG
NRS	2.3 ±1.5	16.7 ±3.2	4.3 ±1.5	10.3 ±1.5
IRSTL	9.6 ±2.0	28.3 ±1.8	13.3 ±1.5	23.6 ±2.3
HRSTL	14.0 ±2.1	32.3 ±3.8	16.7 ±2.4	31.7 ±4.1
IRSTS	2.0 ±1.2	16.0 ±2.9	4.7 ±1.5	9.0 ±2.5

* MEAN PHAGOCYTIC INDEX (PI) FOR CELLS FROM 3 RATS, ±SE

** SEE TEXT - 7) EXPERIMENTAL PROTOCOL

*** SEE TEXT - 2) COLLECTION OF IMMUNE SERA

NRS = NORMAL RAT SERUM

IRSTL = IMMUNE RAT SERUM - I. LEWISI

HRSTL = HYPERIMMUNE RAT SERUM - I. LEWISI

IRSTS = IMMUNE RAT SERUM - I. SPIRALIS

somes *in vitro*. This macrophage activity was equivalent to that exhibited in the homologous reaction with cells derived from rats previously infected with *T. lewisi*. Although the phagocytic indices for peritoneal macrophages from *T. spiralis*-infected rats were slightly higher than those for normal cells in all cases, these differences would not appear to be significant. Therefore, infection with *T. spiralis* 28 days previously, did not appear to stimulate macrophage activity towards *T. lewisi* as assessed by this *in vitro* test of the phagocytosis of trypanosomes.

EXPERIMENT II - PHAGOCYTIC ACTIVITY OF SPLENIC MACROPHAGES

The process of attachment and ingestion of trypanosomes by splenic macrophages occurred with cells from all sources (groups I-IV)(Table 9.3) and was greatly enhanced by the addition of immune or hyperimmune antisera against the parasites (IRSTL and HRSTL respectively). As was the case for peritoneal macrophages, the addition of normal rat serum (NRS) or the heterologous rat anti-*T. spiralis* immune serum (IRSTS) produced similar levels of phagocytosis for the cells in any one particular group. Phagocytic activity was very low, however, (<5%) for cells from uninfected control animals (group I) or cells from rats previously infected with *T. spiralis* (group III) in the presence of NRS or IRSTS. Phagocytosis was enhanced to some extent in these two groups by the addition of the homologous antiserum against the trypanosomes. Splenic macrophages from rats immunized intravenously with BCG (group IV) were highly activated and the PI for these cells was considerably higher with all four sera tested when compared with cells from controls (group I) or from rats infected with *T. spiralis* (group III). The phagocytic activity of cells in group IV also compared quite favorably with the homologous combination of cells derived from rats infected with *T. lewisi* (group II) and rat anti-*T. lewisi* immune

TABLE 9.3

THE PHAGOCYTIC ACTIVITY* OF SPLENIC MACROPHAGES FROM RATS INFECTED WITH I. SPIRALIS, I. LEWISI OR BCG ON I. LEWISI IN VITRO

SOURCE OF SERUM***	SOURCE OF SPLENIC MACROPHAGES**			
	GROUP I CONTROL	GROUP II I. LEWISI	GROUP III I. SPIRALIS	GROUP IV BCG
NRS	1.7 ±1.2	20.3 ±2.9	3.3 ±1.5	19.3 ±3.5
IRSTL	7.7 ±1.8	32.7 ±2.9	10.3 ±1.5	35.0 ±3.2
HRSTL	12.7 ±2.3	37.3 ±3.5	15.3 ±2.3	36.0 ±3.2
IRSTS	2.7 ±1.2	18.3 ±2.3	4.7 ±2.3	17.3 ±3.9

* MEAN PHAGOCYTIC INDEX (PI) FOR CELLS FROM 3 RATS, ±SE

** SEE TEXT - 7) EXPERIMENTAL PROTOCOL

*** SEE TEXT - 2) COLLECTION OF IMMUNE SERA

NRS = NORMAL RAT SERUM

IRSTL = IMMUNE RAT SERUM - I. LEWISI

HRSTL = HYPERIMMUNE RAT SERUM - I. LEWISI

IRSTS = IMMUNE RAT SERUM - I. SPIRALIS

or hyperimmune sera.

These results suggest that immunization with BCG by the intravenous route produced considerable activation of splenic macrophages which was reflected *in vitro* by an enhanced capacity to phagocytize trypanosomes in the presence of normal, or homologous or heterologous immune rat sera. This macrophage activity was comparative with that exhibited *in vitro* by spleen cells from rats previously infected with *T. lewisi* for 7 days. Splenic macrophages from rats infected with *T. spiralis*, however, did not exhibit enhanced *in vitro* phagocytic activity towards *T. lewisi*, although the phagocytic indices for cells from this source were slightly higher than control values with all four sera tested. Therefore, previous infection with *T. spiralis* did not appear to potentiate the *in vitro* phagocytic activity of splenic macrophages in this system.

DISCUSSION

Until recently, research concerning the immunological responsiveness of the rat to *T. lewisi* has failed to provide positive evidence for a role of cell-mediated immunity in controlling infections with this parasite, other than a subsidiary function for phagocytosis in clearing lysed or agglutinated parasites from the circulation (Taliaferro 1924, 1929, 1932, 1938). Laveran and Mesnil (1901) were the first to consider the possibility that phagocytosis played an active role in *T. lewisi* infections after they observed phagocytosis in the peritoneal cavity of actively and passively immunized rats. Delanoe (1911, 1912) and Roudsky (1911) came to similar conclusions when they observed a phagocytic response in the peritoneal cavities of mice inoculated with *T. lewisi*. However, these authors were probably studying natural immunity, since the mouse is normally refractory

to this trypanosome infection. Delanoe (1912) obtained similar results, however, using a susceptible strain of mice that developed an acquired immunity. Brown (1915) considered phagocytosis an essential mechansim in relieving the host of infection with this parasite after observing agglutination and phagocytosis during the course of infection in rats, and Augustine (1943) made similar observations in immune rats reinfected with large numbers of trypanosomes. These results were refuted by other workers (MacNeal 1904; Manteufel 1909; Taliaferro 1924, 1932, 1938) who stressed the importance of humoral antibodies in parasite destruction.

There is little doubt today that specific humoral antibodies play a primary role in trypanocidal activity, especially in view of the fact that trypanosomes may require sensitization (opsonization) with specific immunoglobulin before a stimulation of macrophage activity or other cell-mediated response can occur. The results of the present study would tend to confirm this view, since phagocytosis in vitro did not occur appreciably when trypanosomes and peritoneal or splenic macrophages were incubated in the presence of normal rat serum or heterologous immune serum from rats rats infected with T. spiralis. Lange and Lysenko (1960) reported that immune serum enhanced the phagocytosis of T. lewisi in vitro by peritoneal exudate cells, and that immune serum adsorbed with trypanosomes no longer enhanced this activity. The ability of immune serum to enhance phagocytosis in vitro was confirmed by Patton (1972) and is consistent with the results of the present study.

Patton (1965, 1972) has investigated the interactions between sera and peritoneal exudate cells on T. lewisi in vivo and found that rats immunosuppressed with dexamethasone could only be protected against T. lewisi infections if treated with both hyperimmune serum and normal or hyperimmune

peritoneal exudate cells. Trypanosomes given i.p. in this case were detained in the peritoneal cavity, agglutinated, lysed and phagocytized. Patton (1972) also observed phagocytosis of T. lewisi in vitro in the presence of normal or immune sera plus normal or immune peritoneal exudate cells, but a quantitative index of phagocytic activity was not provided in these studies.

Greenblatt and Tyroler (1971), Greenblatt et al. (1972), and Greenblatt (1973) have extensively studied the role of the spleen and splenic macrophages in immunity to T. lewisi. The spleen of the rat occupies a central role in the development of acquired immunity to T. lewisi and it is expected that individual reticular macrophages should express this activity (Perla and Marmorston-Gottesman 1930; Taliaferro 1929). Greenblatt and Tyroler (1971) have shown that T. lewisi infections produce a marked increase in the percentage of activated macrophages in the spleens of infected animals which peaks at approximately seven days after infection. These cells were also highly active in vitro in phagocytizing trypanosomes, a result which is confirmed by the present study. Greenblatt et al. (1972) showed that immune spleen cells (taken from infected rats 12-39 days after infection) were capable of transferring immunity, and electron microscope studies have demonstrated the presence of T. lewisi in reticular cells (macrophages) in close association with plasma cells in the spleens of infected rats (Greenblatt 1973). Although the trypanocidal mechanism may principally involve lysis and agglutination, it is obvious from studies such as those cited above, that splenic macrophages may play an important role in both the processing of parasite antigens, and in the eventual removal of parasites from the circulation.

In view of the fact that non-specifically activated peritoneal

or splenic macrophages have been continually alluded to as effector cells in immunopotentiation during Trichinellosis (Cypess et al. 1974; Lubiniecki and Cypess 1975; Meerovitch and Ackerman 1974; Meerovitch and Bomford 1977), it was of interest to explore the potential of this cell type for phagocytic activity towards T. lewisi. The results presented in Chapter VIII of this thesis had suggested that an appropriate stimulation of non-specific cell-mediated immunity or RES activity by BCG could effectively inhibit the development of T. lewisi parasitemias in immunized rats. The apparent similarities between the effects of T. spiralis infections (Chapter V and VI), BCG immunizations (Chapter VIII), or pre-treatment with E. coli bacterial endotoxin (Styles 1965) or the toxin holothurin (Styles 1970) on T. lewisi infections in the rat, suggests the possibility that a common mechanism of resistance may underlie the activity of these different agents. Therefore, the purpose of the present study was to compare the in vitro phagocytic activity of splenic or peritoneal macrophages from rats infected with T. spiralis or immunized with BCG, towards T. lewisi, with that of macrophages from normal rats or rats infected with T. lewisi.

The results of this study indicate that splenic or peritoneal macrophages from rats immunized with BCG are highly activated and can phagocytize trypanosomes in vitro in the presence of normal, or homologous or heterologous immune serum. The activity of these cells was equivalent to that exhibited by splenic or peritoneal macrophages from rats infected 7 days previously with T. lewisi, and was considerably enhanced by the addition of immune or hyperimmune serum specific for the parasites. In contrast, the phagocytic activity of macrophages from T. spiralis-infected rats (cells collected 28 days after the nematode infection during the period of maximum immunopotentiation towards T. lewisi and enhanced RES activity

(see Chapter V)) was not enhanced over that of cells from the uninfected control animals. These results suggest that immunopotentiation and macrophage activation during Trichinellosis (Meerovitch and Ackerman 1974; Meerovitch and Bomford 1977; Lubiniecki and Cypess 1975) was not reflected in this *in vitro* system by a potentiation of splenic or peritoneal macrophage phagocytic activity towards *T. lewisi*. Therefore, while the immunopotentiation by BCG *in vivo* (Chapter VIII) correlates well with the enhanced ability of BCG-activated macrophages to phagocytize *T. lewisi in vitro*, the potentiation of the host response to *T. lewisi* during Trichinellosis (Chapter V), was not reflected *in vitro* by enhanced phagocytic activity of splenic or peritoneal macrophages.

These differences are difficult to reconcile, but previous workers have also demonstrated a dichotomy between the *in vitro* and *in vivo* functioning of non-specifically activated macrophages (Hoff 1975; Kress *et al.* 1975; Swartzberg *et al.* 1975). Although *T. spiralis* has been shown to potentiate cell-mediated immunity to BCG (Cypess *et al.* 1974), it is also quite possible that *T. spiralis* and BCG may stimulate different elements of the RES, such that phagocytosis of trypanosomes in *T. spiralis*-infected rats may occur more readily in the fixed cells lining the sinusoids of the liver, or other macrophage elements of the reticuloendothelial system not examined in this study. Indeed, Taylor and Becker (1948) have found that phagocytosis of *T. lewisi* can also occur in the Küpffer cells of the liver during infections in pantothenate-deficient rats. The correlation between enhanced RES granulopectic activity and immunopotentiation towards *T. lewisi* during Trichinellosis reported in Chapter V, lends credence to this argument, since the liver is generally responsible for the greater part of the granulopectic activity of the RES (Biozzi *et al.* 1953).

The only other *in vitro* study to date, dealing with this aspect of immunopotentiation in Trichinellosis, is that of Meerovitch and Bomford (1977). These authors have reported that peritoneal macrophages from mice infected with *T. spiralis* are highly activated and can inhibit DNA synthesis of syngeneic mouse R1 Leukemia cells *in vitro*. These cytostatic effects were comparable to those obtained in the same experimental system using macrophages from mice injected with *Corynebacterium parvum* (Olivotto and Bomford 1974). The apparent differences in the *in vitro* activity of rat peritoneal macrophages in the present study and mouse peritoneal macrophages in the study of Meerovitch and Bomford, may be due to host differences and level of infection (rats in the present study were inoculated with a dose of 100 muscle larvae whereas Meerovitch and Bomford used doses of approximately 500 and 1000 larvae per mouse), or basic functional differences between the *in vitro* phagocytosis of opsonized cells (trypanosomes) and the inhibition of cell growth by non-specifically activated macrophages.

CHAPTER X

GENERAL DISCUSSION AND SUMMARY

The immunology of parasitism offers numerous opportunities for the elucidation of fundamental immunological mechanisms. However, new concepts in immunology have emerged less rapidly in relation to the immunology of parasitism because of the difficulties involved in introducing sound experimental manipulations into complex host-parasite systems. The paucity of good experimental models in parasitology has also weakened the conclusiveness of many existing experimental findings. Many characteristics of host-parasite relationships at an immunological level remain poorly understood. The nature and effectiveness of the efferent arc of the immune response have not been precisely correlated with the specific modes of antigenic stimulation in parasitic infections. An understanding of the origin and nature of functional parasite antigens is lacking; their significance in inducing altered states of immunological responsiveness during interspecific or intraspecific competition between parasites has not been adequately determined.

The literature in experimental parasitology is replete with examples of competitive, interspecific host-parasite interactions during concomitant infections (reviewed in chapter I). The immunobiology of the host-parasite relationship is highly complex, and for most if not all host-parasite interactions, further complexities are introduced by the presence of multiple (parasite) species infections. It is generally recognized as the rule, rather than the exception, that in its natural environment, the host supports infections by more than one parasite species. It is only recently, however, that immunoparasitologists have become inter-

ested in the influence of parasitism on host immunological responsiveness towards secondary infections by antigenically or phylogenetically unrelated organisms. Indeed, a detailed knowledge of parasite-mix, and the effect of parasitic infection on the immune status of the host, is essential to a comprehensive understanding of the phenomenon of parasitism and evolutionary concepts of adaptation and counteradaptation by parasites and hosts.

Many of the aspects of the immunobiology of single species infections are still not clearly delineated, but the use of experimental concomitant infections has gained acceptance as a tool for basic research in the immunology of parasitism. Studies on concomitant infections with helminths or helminths and a variety of phylogenetically unrelated intracellular or extracellular protozoa have indicated that a stimulation of non-specific immune mechanisms may be of importance in the immunological control of parasitic infections in general. Studies of this type have been mostly phenomenological to date, but the value of non-specific cell-mediated immunity in concomitant parasitic infections is becoming more and more evident. With respect to our knowledge of the immunology of parasitism, it is of primary importance that we do not indiscriminately group all forms of acquired resistance to infection under a general heading of immunity, but try to determine whether immunity is of specific or non-specific origin. Both types of immunity may provide only partial resistance to infection, and while their kinetics and mechanisms may be different, their effects may be additive.

In the present study, an attempt was made to gain some insight into the basic immunological relationships and specificities of altered immunological responsiveness during experimental Trichinellosis in the rat

model. In 1974, this author reported on a preliminary experiment that demonstrated that rats infected with T. spiralis for thirty days were partially protected against a challenge infection with T. lewisi. Cross-protection was manifested by a significant inhibition of the development of trypanosome parasitemia in concomitantly infected rats (Meerovitch and Ackerman 1974). Since T. lewisi is a non-pathogenic trypanosome of the rat, and the infection is mediated by a well-characterized humoral antibody response (reviewed in chapter II), it was felt that a model system using concomitant infections with these two parasites would be advantageous in investigating the altered states of immunological responsiveness (immunosuppression and immunopotentiation) in Trichinellosis as they relate to the nematode life cycle and course of infection, and the mechanisms involved in these host-parasite interactions.

As was noted earlier in chapters II and V, altered immunological responsiveness in experimental Trichinellosis is well documented. Numerous investigators have supplied evidence that T. spiralis can produce immunological unresponsiveness to heterologous antigens, allografts, and virus infections in mice and rats. In contrast to these immunosuppressive effects, T. spiralis can also induce a state of enhanced resistance to heterologous bacteria, protozoa, and tumors (see Table 2.1). The mechanisms by which this nematode generates immunosuppression or immunopotentiation are not entirely clear. Lubiniecki and Cypess (1975) have provided evidence that immunosuppression is the result of sequential antigenic competition as proposed by Pross and Eidinger (1974). Others have suggested that immunosuppression may be due to the presence of agglutinating and leucotoxic factors in the serum of infected animals (Faubert and Tanner 1974, 1975); the secretion of immunosuppressive substances by the newborn larval stages of T.

spiralis (Faubert 1976; Ackerman and Faubert 1977); the reduction or depletion of thymus-derived, antigen-reactive, T-cell populations in infected hosts (Faubert and Tanner 1974;Ljungström 1976); or by the stimulation of suppressor T-cell activity in the spleens of infected animals (Jones et al. 1975). In view of the considerable immunosuppressive activity of T. spiralis infections on humoral responses, Cypess et al. (1974) and Meerovitch and Ackerman (1974) suggested that immunopotentiation is most probably a reflection of enhanced non-specific RES activity, or non-specific cell-mediated immunity.

The occurrence of both immunosuppression and immunopotentiation in Trichinellosis appears contradictory. However, the results presented in this thesis indicate that these phenomena are not mutually exclusive nor are they necessarily expressed against all heterologous antigens or organisms. A suppression of acquired humoral immunity to T. lewisi was never found to occur during T. spiralis infections in any of the experiments reported in chapters V-VII. The expression of immuno- potentiation or suppression in Trichinellosis is a function of the type of antigen employed and the route of administration (Chapter V; Barriga 1975; Lubiniecki and Cypess 1975), the intensity of infection (dose or level of muscle parasitism) (Chapter V), and most importantly, a function of the timing of the various modes of antigenic stimulation by parenteral, newborn larval stages and encysted intracellular muscle stages of the parasite's life cycle in the host (Chapter VII; Faubert 1976; Ackerman and Faubert 1977).

In any experimental model that deals with concomitant parasitic infections, there exists the possibility that competitive interspecific interactions may be associated with antigenic relationships between parasites and the synthesis of homologous, cross-reacting antibodies by the

host. In parasitic diseases where specific immunoglobulin plays an essential role in acquired resistance to infection (as is the case for T. lewisi), this type of interaction may be of paramount importance in the host response to intercurrent infections with antigenically related organisms. However, in infections were specific antibodies are not a factor in acquired immunity, antigenic cross-reactions may be of little consequence, especially where cross-reacting antigens prove to be of a non-functional nature.

In order to rule out this possibility in the present study, a comparative immunochemical analysis of parasite antigens was performed using both natural antisera produced during the course of T. lewisi and T. spiralis infections in the rat, and heterologous antisera produced in rabbits against antigen extracts of either parasite. Immunoelectrophoretic analyses presented in chapter V failed to reveal the presence of antigenic cross-reactivity, and it was concluded that immunopotentiation was not related to the presence of common antigenic determinants in these parasites. However, these results did not rule out the possibility that T. spiralis might stimulate the normal humoral responsiveness (ablastic or trypanocidal) of the host to T. lewisi either by enhancing antibody production (adjuvant effects) or by accelerating the initial processing of parasite immunogens. The study presented in chapter VI of this dissertation ruled out this possibility by demonstrating that infections with T. spiralis do not enhance or suppress the ablastic or trypanocidal antibody responses of the rat to T. lewisi at any time during the course of the trypanosome infection. Therfore, the inhibited development of trypanosome parasitemias in this experimental model is not related to either antigenic cross-reactivity, or a potentiation of humoral responsiveness to trypanosome antigens.

Faubert (1973) and Faubert and Tanner (1975) have reported the presence of leucoagglutinating and leucotoxic factors in the serum of infected mice, rats and rabbits infected with T. spiralis, which can agglutinate and lyse homologous lymph node cells in vitro. These factors appeared in the sera of infected mice on the 7th day following the inoculation of the parasite and titers rose to a maximum by the 30th day of the infection. The possible presence of heterophile agglutinins or lysins (capable of agglutinating or lysing trypanosomes) in the serum of infected rats was investigated in chapter V. Sera from rats infected with 100 or 1500 larvae of T. spiralis for 7, 14, 28, or 56 days were titrated in vitro for lytic or agglutinating activity towards reproducing stage (1st antigenic variant) or non-dividing, inhibited adult stage (2nd antigenic variant) trypanosomes. Agglutinins or lysins were not observed in the sera of infected rats, thereby ruling out lysis or agglutination as a possible mechanism of immunopotentiation during concomitant infections of T. spiralis and T. lewisi.

The study of the kinetics of immunopotentiation and immunosuppression in chapter V demonstrates the transient nature of these phenomena in the rat host, and indicates that they are a function of the nematode dose or level of muscle parasitism, a relationship that has not been previously reported in the literature. In addition, these altered states of immunological responsiveness appear to be related to the timing of antigenic stimulation by particular developmental stages of the parasite's life cycle in the host. The transient nature of immunopotentiation and immunosuppression in Trichinellosis has been reported previously in the mouse model (Cypess et al. 1973, 1974; Lubiniecki et al. 1974; Lubiniecki and Cypess 1975; Vernes et al. 1975; reviewed in chapter II) and Faubert (1976) has indicated that depressed immunological responsiveness to SRBC is correlated

with the peak migration of the newborn larval stage of T. spiralis to the muscle tissues of the host.

Previous studies on immunopotentiation in Trichinellosis have not investigated nor implicated a role for specific developmental stages of the parasite's life cycle or parasite antigens in the induction of this phenomenon. The experimental results presented in chapter VII of this thesis indicate for the first time, that immunopotentiation can be induced by the intravenous inoculation of living newborn larvae, which produces only a parenteral infection in the host. The intravenous injection of non-viable newborn larvae does not significantly alter trypanosome parasitemias, indicating a requirement of either living migratory or encysted intracellular muscle stages for immunopotentiation to occur. Drug-abbreviated enteral infections, exposing the host to only the molting muscle larvae and sexually mature adults, are not sufficient to induce immunopotentiation; a result which suggests that larviposition may also be required for a stimulation of the host response to T. lewisi. This may be especially true in view of the fact that immunopotentiation was shown to occur in rats challenged with T. lewisi at 7 days after the nematode infection, during the peak period of larviposition in the host (chapter V).

Recent research by Faubert (1976) and Ackerman and Faubert (1977) has indicated that the newborn larval stage of T. spiralis can function in vitro and in vivo to suppress the induction of a humoral response to the heterologous antigen, SRBC, and that this may occur by the active secretion of an immunosuppressive factor by the parasite. The ability of an intravenous injection of living newborn larvae to produce both immunosuppression in the mouse and immunopotentiation in the rat appears somewhat paradoxical. However, an important difference between the study presented in

chapter VII and the study of Ackerman and Faubert (1977), resides in the time period between the inoculation of the newborn larvae and the challenge with heterologous antigen or parasite. Ackerman and Faubert challenged mice with SRBC only four days after injection of the parasite, whereas in the present study, 21 days were allowed for the newborn larvae to complete their migration and to encyst intracellularly in the muscle cells, before rats were challenged with trypanosomes. In terms of the natural course of the nematode infection, the former case corresponds to approximately 14 days after infection (period of maximum immunosuppression (Faubert 1976; experiment VII, chapter V)), whereas the latter case corresponds to approximately 28 days after the helminthic infection (a period of maximum immunopotentiation towards T. lewisi (chapter V-VII)). Therefore, if the newborn larvae of T. spiralis are capable of secreting immunosuppressive factors during migration to muscle tissues (Faubert 1976), then it would appear from the results of the present study, that these factors cease to be produced, or are no longer secreted once larval development progresses to the intracellular stage of the infection. These results, however, will require further clarification.

The isolation, purification, and identification of the potent immunosuppressive factors secreted by the newborn larvae and present in soluble extracts of T. spiralis encysted muscle stage larvae (Barriga 1975; Faubert and Tanner 1974, 1975; Experiment II, chapter VII) should aid in our understanding of why infections with this nematode are capable of both a suppression of specific cellular and humoral immune responsiveness, as well as a potentiation of non-specific cell-mediated immunity to heterologous antigens, organisms, and neoplastic cells.

An important but unresolved question in this dissertation regards

the mechanism of immunopotentiation towards T. lewisi. It was suggested in chapter V that immunopotentiation in this model might be due to an expanded fixed-macrophage phagocytic system in the RES, resulting in enhanced removal of trypanosomes from the blood stream of the rat. The functional status of the RES was assessed during Trichinellosis in order to evaluate this possibility, and a convincing correlation was shown to exist between enhanced RES granulopectic activity (as reflected by the intravascular clearance of colloidal carbon) and immunopotentiation, both of which occurred primarily with a low inoculating dose of 100 larvae at 7 and 28 days after the nematode infection, but not with a higher inoculating dose of 1500 larvae (Table 5.2, chapter V). Other workers have described a lack of correlation between enhanced RES activity and increased resistance to intracellular parasitism, and definitive evidence for a role for the RES in controlling infections with this parasite has not been presented in the past (see chapter II for review). The results presented in this thesis, however, lead this author to believe that an appropriate stimulation of RES activity and/or non-specific cell-mediated immunity may result in enhanced resistance to T. lewisi. This may be especially true in view of the fact that intravenous immunization with BCG (chapter VIII) and a number of other diverse agents known to non-specifically stimulate RES activity and cell-mediated immunity have also been shown to inhibit the development of T. lewisi parasitemias, while the acquired humoral response of the rat remained unaffected (Styles 1965, 1970; Tate 1951).

The similarities between the effects of T. spiralis infections (chapters V-VII) and BCG immunization (chapter VIII) in the present study and that of bacterial endotoxin or the toxin holothurin (Styles 1965, 1970) on the response of the rat to T. lewisi, suggests that a common mechanism

of resistance underlies immunostimulation by all these different biological agents.

A common feature of the immunological response to most intracellular parasites is the non-specific enhancement of anti-microbial activity of host macrophages (Mackaness 1964, 1976; Frenkel 1967). The role of the activated macrophage in this type of non-specific cell-mediated immunity has been investigated in great detail and it has been clearly established that acquired cellular resistance requires a two-step process in which immune T-cells, in the presence of specific antigens, can elaborate humoral factors (lymphokines) that activate macrophages. A consequence of these specific interactions is that specifically activated macrophages are non-specifically active against a wide array of intracellular and probably extracellular parasites and neoplastic cells (Mackaness 1969). Hosts in which a population of these activated macrophages exists become capable of suppressing infections by heterologous organisms and the development of spontaneous or experimental neoplasms.

An important factor that is evident in the studies reviewed in chapter IV, is that infectious agents that produce sustained and chronic tissue phases of infection can confer the most pronounced degree of non-specific immunity to heterologous challenge organisms (Ruskin et al. 1969). Trichinella spiralis may fulfil this requirement since it has been shown to produce a sustained and chronic intracellular infection in host muscle cells (see chapter II). The newborn larvae of T. spiralis are capable of penetrating and infecting striated skeletal muscle cells of most species (Gould 1970). After penetration by the larvae, the infected muscle cells modulate to become functionally distinct units which are termed Nurse cells (Purkerson and Despommier 1974) and the larvae of T. spiralis survive

in the Nurse cells as chronic intracellular parasites (Ribas-Mujal and Rivera-Pomar 1968; Despommier 1976). The intracellular persistence of T. spiralis may produce a long-lived antigenic stimulation of host lymphoid cells and activated macrophages. The continuous presence of a population of these activated cells during Trichinellosis might account for the remarkable non-specific resistance of nematode-infected hosts to a wide variety of intracellular and extracellular invaders (such as Listeria monocytogenes and T. lewisi respectively) and to the development of spontaneous or experimental neoplasms.

Although this dissertation has not provided direct evidence for the role of the activated macrophage as the effector cell in immunopotentiation towards T. lewisi, it is the considered opinion of this author that further research will serve to clarify the importance of this cell type in immunopotentiation during Trichinellosis.

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