497

# Effects of *Trichinella spiralis* on the immune response

A thesis submitted for the degree of Doctor of Philosophy

in the

University of London

Faculty of Medicine

by

Nagwa Abdel Naki ABOUL ATTA

M.B., Ch.B. (Egypt), M.Sc. (London)

From the Department of Medical Helminthology

London School of Hygiene and Tropical Medicine

Keppel Street, London WC1E 7HT.

1979

To my family

ACKNOWLEDGEMENTS

I owe a great debt of gratitude to Professor C. S. Nelson for allowing me to undertake my studies in his department and to Dr. D. A. Denham for his supervision, criticism and advice throughout the whole period of my study. I am also grateful to Dr. M. Steward for introducing me to some of the immunological techniques used and for his kindness, continuous encouragement and helpful discussions.

I am grateful to Mr. H. Furse for facilitating availability of all the equipment and research materials.

I would like to express my sincere gratitude to my husband for his understanding and for giving me the chance to complete my studies, my parents for their kindness in looking carefully after my daughter during the period of my study and to my dear daughter, Hanan, for her understanding and patience.

I am also grateful to all my colleagues in the department for their encouragement and to everyone who contributed to the completion of this work.

Thanks are due to all the staff of the animal house for their care of my experimental animals.

This thesis was typed by Mrs. Manson to her I am thankful.

Finally, I wish to thank the Egyptian government for the study leave and financial support.

ABSTRACT

Effects of Trichinella spiralis on the immune response

By Nagwa Aboul Atta

The immune responses to heterologous antigens of mice infected with T. spiralis has been studied. The humoral responses in infected animals were depressed. The three stages of infection (adults, newborn larvae, and encysted larvae altered the immune response against sheep erythrocytes (SRBC). Assaying humoral responses by haemolytic plaque formation against sheep erythrocytes, it was shown that IgM responses are markedly depressed, but IgG responses were not affected either after primary or secondary immunization.

The antibody responses, measured by the haemagglutination and haemolysin tests, against the T-dependent antigen SRBC were depressed by the three phases of infection. However, the antibody responses against the T-independent antigen, lipopolysaccharide, was only depressed in animals infected for 30 days.

Macrophages have an important role in the immune response and activation of the phagocytic properties of macrophages was demonstrated in animals infected with adult phase of T. spiralis infection. Later phases of infection showed activated macrophage function only when large numbers of larvae were inoculated.

Animals infected with T. spiralis cleared the passively transferred macroglobulins faster than did the uninfected controls showing that their rate of catabolism was increased.

It has also shown that T. spiralis infection increased the affinity of antibody for human serum albumin antigen. The same effect was demonstrated with the three stages of infection.

These findings are discussed in the light of the present knowledge of host parasite relationship. It is suggested that many factors are responsible for the immunodepression induced by T. spiralis.

TABLE OF CONTENTS

Page no.

List of tables 9

List of figures 14

Chapter 1 : Introduction 19

A review of the literature on immunodepression by infectious organisms. 24

Chapter 2 : General materials and methods 56

I. Laboratory animals. 56

II. Parasite 57

1. Strain 57

2. Preparation of infective material 57

3. Determination of rates of infection 61

4. Treatment of infected animals 62

5. Statistical analysis 62

Chapter 3 : Effect of different stages of T. spiralis infection on the number of plaque forming cells. 63

Introduction 63

Determination of antibody forming cells by plaque assay 63

a) Immunization of animals 64

b) Preparation of the medium 65

c) Preparation of the complement 66

d) Preparation of Cunningham chambers 66

e) Preparation of the suspension of lymphoid cells 68

f) Viability test 68

g) Preparation of the RBC for the assay 69

h) Procedure of Cunningham modification of the Jerne plaque assay 69

Page no.

Preliminary experiments 72

Experiments and results 72

Effect of T. spiralis infection on the number of plaque forming cells in mice immunized with SRBC. 79

Effect of T. spiralis on the number of PFC after secondary immunization with SRBC. 113

Discussion 114

Chapter 4 : Effect of T. spiralis infection on antibody levels in the sera of mice. 122

Introduction 122

Materials and methods 123

Preparation of the sera 123

Measurement of anti-SRBC haemagglutinins 123

Measurement of SRBC haemolysins 124

Estimation of minimum haemolytic dose of the complement. 125

Haemolysin assay. 125

Measurement of anti-lipopolysaccaride by complement mediated haemolysis. 126

Coating of SRBC with LPS. 126

The assay. 127

Preliminary experiment 127

Statistical analysis 128

Experiments and results:

I. Effect of T. spiralis infection on haemagglutinin and haemolysin antibodies against SRBC. 128

1. Effect of full infection 128

2. Effect of intestinal phase 131

Page no.

II. Effect of *T. spiralis* on antibody titres against the T-independent antigen LPS. 149

1. Effect of full infection 149

2. Effect of developing and intestinal phases. 154

Discussion 159

Chapter 5 : Effect of *T. spiralis* infection on macrophage activity. 164

a) Effect of *T. spiralis* infection on the clearance of $^{125}$I PVP from the blood as an indication of macrophage activity. 165

b) Effect of *T. spiralis* infection on the clearance of $^{51}$Cr-labelled SRBC. 180

Labelling of SRBC with $^{51}$Cr. 180

Experiments and results 180

Discussion 196

Chapter 6 : Effect of *T. spiralis* on antibody catabolism 198

Introduction 198

Prepration of macroglobulins 198

Experiment and result 202

Discussion 206

Chapter 7 : Effect of *T. spiralis* infection on the affinity of antibody for human serum albumin 209

Introduction 209

Measurement of antibody affinity 209

Experiments and results 211

Discussion 213

General discussion and conclusion 221

References 232

LIST OF TABLES

Table no. Page no.

1. The number of IgM (direct) PFC in spleens of immunized and unimmunized animals at different times after immunization. 71

2. Numbers of PFC of immunized and unimmunized animals by direct and indirect assays using different dilutions of rabbit antimouse IgG. 75

3. The number of IgG PFC in spleens of immunized and unimmunized animals at different times after immunization. 80

4. Effect of 30 day infection of T. spiralis on the number of IgM and IgG PFC produced in the spleen in response to inoculation of SRBC. 82

5. Effect of a 30 day old T. spiralis infection on IgM PFC 7 day response to inoculation of SRBC post infection and assayed. 85

6. Effect of a light 30 day old infection on T. spiralis on spleen IgM PFC assayed 4 days after immunization with SRBC. 88

7. Effect of the intestinal stage of T. spiralis on the PFC response to SRBC when assayed 4 and 9 days post immunization. 91

8. The effect of the intestinal phase of T. spiralis infection on the number of PFC 7 days after immunization with SRBC. 94

| Table no. | | Page no. |
|---|---|---|
| 9. | Effect of infection with irradiated (i.e. sexually sterile) *T. spiralis* on the development of spleen PFC in response to the inoculation of SRBC 11 days after infection. | 97 |
| 10. | Effect of adult stage of *T. spiralis* infection on the number of PFC 7 days after immunization with SRBC. | 100 |
| 11. | Effect of a low level irradiated *T. spiralis* infection on spleen PFC in response to SRBC inoculation. | 102 |
| 12. | Effect of *T. spiralis* on the number of IgM PFC after primary immunization with SRBC using SRBC and HRBC *in vitro*. | 105 |
| 13. | Effect of different ages of *T. spiralis* infection on the primary immune response. | 108 |
| 14. | Effect of different stages of infection of *T. spiralis* on spleen PFC (4 and 9) days after SRBC infection. | 111 |
| 15. | Effect of *T. spiralis* on spleen PFC after secondary immunization. | 115 |
| 16. | A summary of immunodepression induced by *T. spiralis* judged by PFC assay during primary response. | 117 |
| 17. | Titres of total and DTT resistant (i.e. IgG) haemolysins in the serum at different times after SRBC inoculation. | 129 |
| 18. | Effect of a 30 day old *T. spiralis* infection on antibody production upon immunization with SRBC. | 132 |

Table no. Page no.

19. Effect of a 30 day old light *T. spiralis* infection on haemagglutinins after SRBC injection. 134

20. Effect of the intestinal phase of *T. spiralis* infection on antibodies upon SRBC injection. 137

21. Effect of irradiated *T. spiralis* larvae (i.e. intestinal phase only) on the production of antibodies against SRBC. 140

22. Effect of a light irradiated (i.e. intestinal phase) infection on *T. spiralis* on haemagglutinins against SRBC. 142

23. Effect of a light methyridine terminated infection of *T. spiralis* on haemagglutinins against SRBC. 145

24. Effect of methyridine on antibody titres 4 days after SRBC injections. 147

25. Effect of full infection of *T. spiralis* on the haemolysin titres against LPS. 150

26. Effect of full infection of *T. spiralis* on haemolysin antibodies against LPS. 152

27. Effect of a 14 day old *T. spiralis* infection on anti-LPS haemolysins. 155

28. Effect of early phases of *T. spiralis* infection on antibody titres against LPS. 157

29. Effect of *T. spiralis* at different ages of infection on the antibody response against LPS. 158

30. Summary of the effect of *T. spiralis* infection on antibody levels. 160

31. Effect of number of blood samples (n) used to calculate K $^{125}$I PVP $h^{-1}$. 167

| Table no. | | Page no. |
|---|---|---|
| 32. | Effect of sex on $^{125}I$ PVP clearance. | 170 |
| 33. | Effect of *T. spiralis* infection on $^{125}I$ PVP clearance 1 week after infection. | 171 |
| 34. | Effect of *T. spiralis* infection on $^{125}I$ PVP clearance 2 weeks after infection. | 172 |
| 35. | Effect of *T. spiralis* on $^{125}I$ PVP clearance 3 weeks after infection. | 174 |
| 36. | Effect of *T. spiralis* on $^{125}I$ PVP clearance 4 weeks after infection. | 175 |
| 37. | Effect of *T. spiralis* on $^{125}I$ PVP clearance 6 weeks after infection. | 177 |
| 38. | Effect of *T. spiralis* on $^{125}I$ PVP clearance 8 weeks after infection. | 178 |
| 39. | Effect of *T. spiralis* on $^{125}I$ PVP clearance 1 week after infection. | 181 |
| 40. | Effect of *T. spiralis* on $^{125}I$ PVP clearance 2 weeks after infection. | 182 |
| 41. | Effect of full infection and intestinal phase of *T. spiralis* infection on $^{51}Cr$-SRBC clearance. | 185 |
| 42. | Effect of a 14 day old *T. spiralis* infection on $^{51}Cr$-SRBC clearance. | 189 |
| 43. | Effect of different ages of *T. spiralis* infection on $^{51}Cr$-SRBC clearance. | 191 |
| 44. | Catabolism of $^{125}I$ macroglobulins in blood of normal mice infected with *T. spiralis*. | 207 |
| 45. | Effect of a 7 day old *T. spiralis* infection on affinity of antibody for HSA antigen. | 214 |

| Table no. | | Page no. |
|---|---|---|
| 46. | Effect of a 14 day old *T. spiralis* infection on affinity of antibody for HSA antigen. | 216 |
| 47. | Effect of different ages of *T. spiralis* infection on affinity of antibody for HSA antigen. | 218 |

## LIST OF FIGURES


| Fig. no. | | Page no. |
|---|---|---|
| 1. | Life cycle of Trichinella spiralis in the laboratory | 22 |
| 2. | Diagram of the MacMaster counting chamber used for counting excysted muscle larvae | 59 |
| 3. | Cunningham slide chambers: | 67 |
| | a) Making the chambers | 67 |
| | b) Sealing the chamber in molten wax | 67 |
| 4. | Photograph of a plaque showing antibody forming cell at the centre of an area of haemolysis | 71 |
| 5. | The number of IgM (direct) PFC in spleens of immunized and unimmunized animals at different times after immunization | 74 |
| 6. | Plot of KI titration, using spleen cells from mice immunized 4 days before | 77 |
| 7. | Plot of KD titration, using spleen cells 9 days after immunization | 78 |
| 8. | The number of IgG PFC in spleens of immunized and unimmunized animals at different times after immunization | 81 |
| 9. | Effect of 30 days infection of T. spiralis on the number of IgM and IgG PFC produced in the spleens in response to inoculation of SRBC | 83 |
| 10. | Effect of 30 day T. spiralis infection on IgM PFC 7 day response to inoculation of SRBC post infection | 86 |
| 11. | Effect of light 30 day old infection of T. spiralis on spleen IgM PFC assayed 4 days after immunization with SRBC | 89 |

| Fig. no. | | Page no. |
|---|---|---|
| 12a. | Effect of intestinal stage of *T. spiralis* on the IgM PFC response to SRBC when assayed 4 days after immunization | 92 |
| 12b. | Effect of intestinal stage of *T. spiralis* on PFC response to SRBC when assayed 9 days post immunization | 93 |
| 13. | Effect of the intestinal phase of *T. spiralis* infection on the number of IgM PFC 7 days after immunization with SRBC | 95 |
| 14. | Effect of infection with irradiated (i.e. sexually sterile) *T. spiralis* on the development of spleen PFC in response to inoculation of SRBC 11 days after infection | 98 |
| 15. | Effect of adult stage of *T. spiralis* infection on the number of PFC 7 days after immunization with SRBC | 101 |
| 16. | Effect of a low level irradiated *T. spiralis* infection on spleen PFC in response to SRBC inoculation | 103 |
| 17. | Effect of *T. spiralis* on the number of IgM PFC after primary immunization with SRBC using SRBC and HRBC *in vitro* | 106 |
| 18. | Effect of different ages of *T. spiralis* infection on the primary immune response to SRBC | 109 |
| 19. | Effect of different stages of infection of *T. spiralis* on spleen PFC (4 and 9) days after SRBC injection | 112 |
| 20. | Effect of *T. spiralis* on spleen PFC after secondary immunization | 116 |

Fig. no. Page no.

21. Titres of total and DTT resistant (i.e. IgG) haemolysins in the serum of different times after SRBC inoculation 130

22. Effect of a 30 day old T. spiralis infection on antibody production upon immunization with SRBC 133

23. Effect of a 30 day light T. spiralis infection on haemagglutinins after SRBC injection 135

24. Effect of intestinal phase of T. spiralis infection on antibodies upon SRBC injection 138

25. Effect of irradiated T. spiralis larvae (i.e. intestinal stage only) on the production of antibodies against SRBC 141

26. Effect of light irradiated (i.e. intestinal phase) infection of T. spiralis on haemagglutinins against SRBC 143

27. Effect of light methyridine terminated infection of T. spiralis on haemagglutinins against SRBC 146

28. Effect of methyridine on antibody titres 4 days after SRBC injection 148

29. Effect of a 30 day T. spiralis infection on the haemolysins against LPS 151

30. Effect of a 30 day old T. spiralis infection on haemolysins against LPS 153

31. Blood radioactivity at different times after $^{125}$I PVP injection 168

32. Effect of sex on $^{125}$I PVP clearance 168

33. Effect of 1 week T. spiralis on $^{125}$I PVP clearance 173

34. Effect of 2 weeks T. spiralis on $^{125}$I PVP clearance 173

Fig. no. page no.

35. Effect of 3 weeks T. spiralis on $^{125}I$ PVP clearance 176
36. Effect of 4 weeks T. spiralis on $^{125}I$ PVP clearance 176
37. Effect of 6 weeks T. spiralis on $^{125}I$ PVP clearance 179
38. Effect of 8 weeks T. spiralis on $^{125}I$ PVP clearance 179
39. Effect of 1 week T. spiralis on $^{125}I$ PVP clearance 183
40. Effect of 2 weeks T. spiralis on $^{125}I$ PVP clearance 183
41. Effect of 7 day T. spiralis infection on $^{51}Cr$-SRBC clearance 186
42. Effect of 30 day old T. spiralis infection on $^{51}Cr$-SRBC clearance 187
43. Effect of 14 day old T. spiralis infection on $^{51}Cr$-SRBC clearance 190
44. Effect of 7 day old T.spiralis infection on $^{51}Cr$-SRBC clearance 192
45. Effect of 11 day old T. spiralis infection on $^{51}Cr$-SRBC clearance 193
46. Effect of 14 day old T. spiralis infection on $^{51}Cr$-SRBC clearance 194
47. Effect of 30 day old T. spiralis infection on $^{51}Cr$-SRBC clearance 195
48. Elution profile of serum proteins from a column of G.200 Sephadex 199
49. Effect of a 7 day old T. spiralis infection on $^{125}I$ macroglobulin clearance 203
50. Effect of a 14 day old T. spiralis infection on $^{125}I$ macroglobulin clearance 204
51. Effect of a 30 day old T. spiralis infection on $^{125}I$ macroglobulin clearance 205

| Fig. no. | | Page no. |
|---|---|---|
| 52. | Langmuir plot of ideal antibody-antigen binding | 212 |
| 53. | Effect of 7 day old T. spiralis infection on the affinity of antibody for HSA | 215 |
| 54. | Effect of 14 day old T. spiralis infection on the affinity of antibody for HSA | 217 |
| 55. | Effect of different ages of T. spiralis infection on affinity of antibody for HSA | 219 |

Chapter 1

# INTRODUCTION

Many parasitic organisms give rise to chronic infections in their primary hosts. Furthermore, acquired immunity to parasites is often slow to develop and is frequently incomplete (Ogilvie and Wilson, 1976).

It would be advantageous to vaccinate against the various pathogens as successful vaccination should ensure long lasting protection of the host despite continuous exposure to the infective stages of the pathogens. One of the greatest challenges in medicine is the development of vaccines effective against the major parasitic diseases of man. Whilst a great deal of time and effort is currently being devoted to research into vaccines against parasites, the only successful preparation so far in general use against parasitic infection is Dictol, the vaccine prepared from irradiated larvae of *Dictyocaulus viviparus* given as double dose to calves (Jarrett *et al.*, 1959).

It has recently become increasingly apparent that some parasites facilitate their own survival by evading the immune response of the host. Examples of the mechanisms responsible for this are antigenic variation in the trypanosomes (Vickerman, 1978); incorporation of host blood group antigens into the tegument of schistosomes (Smithers and Terry, 1976) and inhibition of complement, depletion of C3 levels and generation of anaphylatoxin activity in normal serum *in vitro* and depression of rat serum complement *in vivo*, by *Taenia taeniaeformis* (Hammerberg *et al.*, 1976). There is, in addition, evidence that some parasites cause immunodepression in their hosts (see next section). Parasite induced

# Chapter 1

## INTRODUCTION

Many parasitic organisms give rise to chronic infections in their primary hosts. Furthermore, acquired immunity to parasites is often slow to develop and is frequently incomplete (Ogilvie and Wilson, 1976).

It would be advantageous to vaccinate against the various pathogens as successful vaccination should ensure long lasting protection of the host despite continuous exposure to the infective stages of the pathogens. One of the greatest challenges in medicine is the development of vaccines effective against the major parasitic diseases of man. Whilst a great deal of time and effort is currently being devoted to research into vaccines against parasites, the only successful preparation so far in general use against parasitic infection is Dictol, the vaccine prepared from irradiated larvae of *Dictyocaulus viviparus* given as double dose to calves (Jarrett *et al.*, 1959).

It has recently become increasingly apparent that some parasites facilitate their own survival by evading the immune response of the host. Examples of the mechanisms responsible for this are antigenic variation in the trypanosomes (Vickerman, 1978); incorporation of host blood group antigens into the tegument of schistosomes (Smithers and Terry, 1976) and inhibition of complement, depletion of C3 levels and generation of anaphylatoxin activity in normal serum *in vitro* and depression of rat serum complement *in vivo*, by *Taenia taeniaeformis* (Hammerberg *et al.*, 1976). There is, in addition, evidence that some parasites cause immunodepression in their hosts (see next section). Parasite induced

immunodepression can affect the host in various ways. Parasites may aggravate host's susceptibility to other micro-organisms such as bacteria, viruses, fungi, protozoa and helminths. It may render the parasitised host more liable to develop spontaneous tumours. Furthermore, parasitic infections may depress the effectiveness of vaccination against other infectious agents and may lead to the host making less effective immune responses against the homologous parasite. Hosts harbouring parasites may be less likely to develop autoimmune diseases.

Since parasitic infections are prevalent throughout the world, an understanding of how they induce altered states of immunologic responsiveness may be important in the ultimate control of many parasitic infections or may provide the means of restoring immune responses. Moreover, in tropical and subtropical countries, most vertebrate hosts are parasitized by more than one parasite at the same time. The importance of this in respect to immunity and immunodepression deserves attention.

In this thesis the effect of Trichinella spiralis on some aspects of the immune response to heterologous antigens will be investigated.

T. spiralis is a gut dwelling nematode which has received considerable attention and study since it was discovered by Paget in the early nineteenth century. This parasite serves as an excellent model for a variety of experimental investigations. All the stages of the life cycle occur within one host, it is easily maintained in the laboratory in various experimental animals such as mice, rats, hamsters and guinea pigs, it is easily handled and counted and presents very little danger to the laboratory worker.

Life cycle of T. spiralis.

In many areas of the world, the pig has been the chief source of Trichinella infection of man. In nature, infection occurs when man or another flesh eating mammal ingests raw or inadequately cooked meat containing viable encysted Trichinella larvae. The action of the digestive juices in the stomach frees the larvae within a few hours. The freed larvae moult 4 times and become adults in the small intestine. The adults burrow into the intestinal mucosa. Gould (1945) has observed that larvae had penetrated the intestinal mucosa as early as 1 hour after experimental infection in rats. In experimental trichinosis, there are approximately twice as many female intestinal adults as males. Copulation and ovulation occurs in the intestine 25-40 hours after infection. It is thought that the difference in time of insemination among various female trichinellae might be influenced by such factors as the sex or the type of the host and, perhaps by different amounts of by-products by the parasite.

The female of this parasitic nematode is ovoviviparous (Christenson, 1950), since the eggs hatch in utero and motile first stage larvae are born in the intestinal mucosa of the host. The number of newborn larvae (NBL) deposited by each female ranges from 1500 to 2300 (Campbell and Yakstis 1969).

Larvae are born from 5 days after infection (Denham and Martinez, 1970). The motile larvae measuring about 100 μm x 6 μm enter the lymphatics of the intestine, and pass to the thoracic duct and finally the blood stream and are distributed throughout the body of the host (Harley and Callicchio, 1971).

It is believed that T. spiralis has a predilection for skeletal muscles, due to a special organotropism. The larvae which reach the

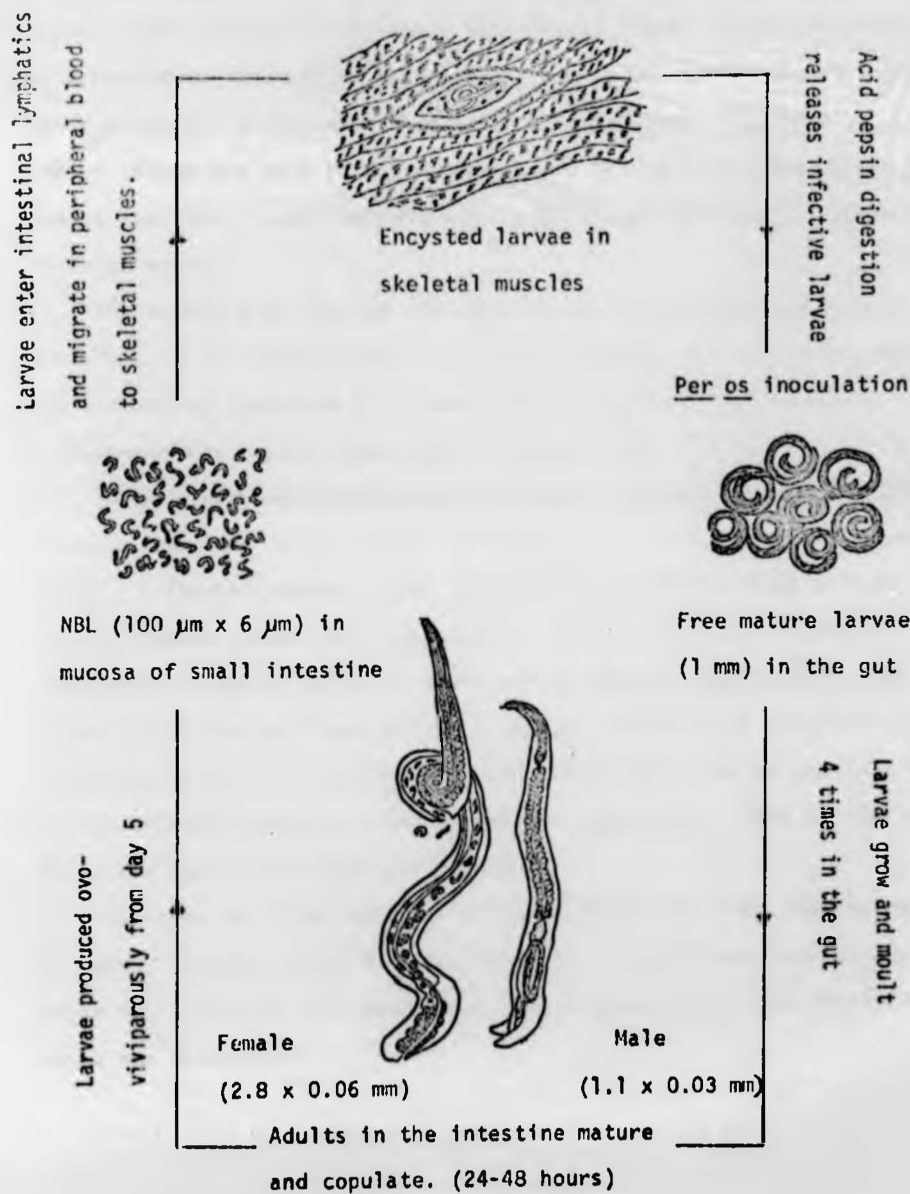



Figure 1 : LIFE CYCLE OF TRICHINELLA SPIRALIS IN THE LABORATORY.

skeletal muscles are the only ones which survive and continue the life cycle. After penetrating the skeletal muscle fibres, the larvae undergo a period of development (attaining a length of about 1 mm, 15 days after infection of the muscle cell), coil and become encapsulated. The mature larvae are able to resist the action of the acid-pepsin digesting juices and hence become infective to another host that might ingest the infected muscle.

The normal life span of the adult worms in the intestine varies according to the species and strain of the animal, but generally worms are eliminated from mice by 15 days after infection. Females are eliminated more rapidly than males (Denham, 1968).

In mice most adult worms parasitize the anterior half of the small intestine for the first 11 days (Larsh et al., 1952 quoted from Larsh, 1970). A tissue response, mild inflammation, is noted about 4 days post infection (Larsh and Race, 1954). About 8 days post infection, inflammation reaches an acute phase accompanied by massive infiltration of polymorphonuclear leucocytes. At about 10 days after infection, the inflammatory reaction becomes subacute (or chronic) and accompanied by infiltrates of mononuclear cells, mainly lymphocytes. Most of the adult worms are expelled in the next few days.

In rats, about one half of the inoculated larvae are lost within 24 hours. Between 9 and 12 days, there is a significant loss of adult worms (Gursch, 1949), and between 15 and 18 days, all of the remaining worms are eliminated.

A REVIEW OF THE LITERATURE ON

IMMUNODEPRESSION BY INFECTIOUS ORGANISMS

The study of the immune response to an unrelated antigen of animals which have been primed previously with one antigen is one of the most important problems in the field of immunology and the factors which regulate the immune responses in infected animals have received particular attention during the last few years.

It is not only infections that cause a lowered immune response. Waterston (1970) showed that immunization of mice with pig erythrocytes (PRBC) caused impairment of antibody responses to subsequent immunization with sheep erythrocytes (SRBC). The same effect was observed in many species of animals with a wide variety of antigens (Adler, 1964).

Alteration of the immune response of mice to SRBC caused by previous inoculation of polyacrylic acid (PAA), Escherichia coli lipopolysaccharide (LPS) and dextrane sulphate (DS), compounds mitogenic for B lymphocytes in vitro, have been reported by Diamantstein et al. (1976). The injection of these mitogens 2 - 4 days before immunization depressed the immune response whereas the same compounds enhanced the immune response when injected half an hour before immunization of animals with suboptimal doses of antigen. Injection of incomplete Freund's adjuvant into mice 2 - 3 days before SRBC can also suppress antibody forming cells (Finger et al., 1973).

Bacteria can modulate the immune mechanism to unrelated antigens in several ways, including enhancing and depressing effects (Munoz, 1964 and Zabriskie, 1967). The earliest reports of immunodepression by a bacterial product was the report of Bradley and Watson (1964), that the endotoxin of E. coli diminished the production of actinophage

neutralizing antibody by BALB/c mice.

Numerous bacterial species or bacterial products are reported to have immunodepressive activity on humoral or cellular levels (summarized by Schwab, 1975). The dose of agent, timing of injections relative to antigen, nature and dose of antigen are important factors which affect the immune response. Malakian and Schwab (1968 and 1971) demonstrated an immunodepressant in partially purified extracts of mechanically disrupted group A streptococci. A single injection of this extract into mice one or seven days before SRBC antigen depressed both IgM and IgG plaque forming cells in the spleens of mice but injection of the extract 1 or 2 days after the injection of antigen had no effect.

Several reports have shown that Corynebacterium parvum is a good adjuvant when injected before an antigen (Howard et al., 1973 and Neveu et al., 1964). However, lymphocytes from mice injected with C. parvum show a depressed responsiveness to phytohaemagglutinin (PHA), as well as a reduced mixed lymphocyte culture response and graft versus host reactivity (Scott, 1972). Johnson et al. (1967), Franzl and McMaster (1968) and McMaster and Franzl (1968) demonstrated that Salmonella typhi endotoxin could either enhance or depress antibody formation in mice immunized with SRBC depending on the time of its administration. If an antigen was given with endotoxin or shortly thereafter, an increased antibody response was obtained whereas, in contrast, injection of endotoxin one or two days before antigen, caused depression and often prevented antibody formation.

Chisari et al. (1974) showed that cholera endotoxin is an adjuvant when 0.05 μg is injected with antigen and a depressant when injected 12 hours before or after antigen.

The importance of the dose of an antigen in regulating the immune response has been shown by Finger et al. (1972a&b). With a suboptimal dose

of $2 \times 10^7$ SRBC both 19 S and 7 S antibody-forming cells were temporarily depressed but when Bordetella pertussis was injected after $4 \times 10^8$ antigen, there was an enhanced response. The secondary response to SRBC could be depressed if the organisms were given 2 days before a second injection of $4 \times 10^8$ SRBC (Finger et al., 1972a). Howard et al. (1973) observed that B. pertussis vaccine injected into mice with 5 μg of pneumococcal type III capsular polysaccharide (S III) reduced spleen plaque forming cells (PFC) against S III 6 days later. The vaccine had little effect when given 4 days before S III but there was an increased response to SRBC when this antigen was injected with B. pertussis vaccine.

Wilkie et al. (1976) reported that Pasteurella haemolytica type I in combination with Freund's complete adjuvant (FCA) can induce depression of the primary immune response to SRBC in mice, while failing to elicit detectable antibacterial serum agglutinins. It is most interesting that they showed that depression could be adoptively transferred to syngeneic mice with viable spleen cells treated with antithymocyte serum (ATS) or anti-mouse globulin (AMG) plus complement but is abrogated by treatment of transferred cells with some batches of normal rabbit serum. They suggested that depression was mediated by a viable cell other than T or B lymphocytes.

Spleen cells from mice previously primed with virulent Listeria monocytogenes organisms have been tested in culture for their ability to develop a humoral immune response to SRBC (Kongshavn et al., 1977). Spleen cells from primed mice did not develop an anti-SRBC PFC response to SRBC in cultures. In addition, when L. monocytogenes primed spleen cells were cultured with normal spleen cells and SRBC, the anti-SRBC response of normal cells was depressed. The immunodepressive effect

developed two days after L. monocytogenes inoculation and peaked by day 6. Effective immunodepression was also demonstrated when L. monocytogenes primed spleen cells from T-cell depleted donors were used. Low doses of L. monocytogenes produced some enhancement rather than the depressive effect.

The production of antibodies or cell mediated immune (CMI) responses can be increased, decreased or qualitatively changed with bacterial antigens, however, changes in the humoral response are not related to the CMI response so that depression or delay of the antibody response can occur with or without depression or stimulation of the CMI response.

Immunodepression of the CMI response caused by bacteria have been reported by many workers. Evidence for the capacity of C. parvum to depress the CMI response has been presented by Collins and Scott (1974). They showed that mice injected with 700 µg of killed organisms did not develop characteristic delayed hypersensitivity against Salmonella enteritidis, although the mice were more resistant to infection with this organism. Depression of the CMI response by endotoxin has also been reported (Floersheim and Szeszak, 1972). In this report, however, only a depression of the effector mechanisms of delayed hypersensitivity was demonstrated. The in vivo depression of CMI response described by Henney et al. (1973), is also primarily on the efferent side. C57 BL/6 mice were immunized with BDA/2 mastocytoma cells intraperitoneally and cytolytic activity of spleen cells obtained 11 days later was measured, in vitro, by $^{51}Cr$ release. One microgram of cholera endotoxin (CE) injected on the day of immunization had no effect, whereas injection of CE 4 days after immunization gave 70% inhibition of cytolytic effect and injection on days 7, 8, 9 or 10 gave 100% inhibition. In contrast no prolongation of skin graft across this same allogeneic barrier was

obtained. Using granuloma formation in response to Schistosoma mansoni eggs as a model of delayed hypersensitivity, Warren et al. (1974) concluded that CE produced a more impressive depression of the CMI response than antilymphocyte serum or other immunodepressive measures tested.

Apart from the experimental demonstration of depression with bacterial agents, Turk and Bryceson (1971) found that patients with lepromatous leprosy had delayed rejection of skin grafts.

Recently, impairment of the immune response induced by viruses has been studied. Bro-Jørgensen et al. ( 1975) reported that mice infected with lymphocytic choriomeningitis virus (LCM) showed a 92-96% reduction of the thymus-dependent, anti-SRBC PFC responses 2-4 weeks after infection. However, the responses to the thymus independent antigens, LPS, S III and polyvinyl pyrrolidone (PVP), were close to normal at all stages of the infection. On the other hand, there was a significant impairment of the allograft response of infected mice. Dunmire et al. (1975) demonstrated that the addition of both measles and purified protein derivatives (PPD) to lymphocyte cultures can have a variable effect on lymphocyte responsiveness to PPD alone in vitro. The effect varies from marked inhibition to enhancement beyond a summation effect. The variation was related to lymphocyte donor, dose and concentration of either antigens. Earlier, it had been demonstrated that live, attenuated measles vaccine also caused diminution of skin test responsiveness to tuberculin (Bech et al., 1962; Mellman and Wetton, 1963; Starr and Berkovick, 1964; Friday et al., 1968; Brody and McAlister, 1964; Zweiman et al., 1971) to PPD, Candida, vaccinia and diphtheria toxoid (Fireman et al., 1969; Friday et al., 1968).

When spleen cells from mice infected with Rowson-Parr virus (RPV) were cultivated with SRBC, they showed markedly lower PFC responses than those in spleen cells from normal mice. Addition of as few as $10^3$ spleen

cells from RPV infected mice to cultures of normal splenocytes also markedly depressed the expected response (Bendinelli et al., 1975). They also showed that normal peritoneal exudate cells, but not thymus, bone marrow or unfractionated spleen cells, restored immunocompetence to cultures of spleen cells from RPV infected mice but did not affect the depressive properties of the infected cells on normal splenocytes.

In humans, the effect of attenuated rubella virus infection upon cell-mediated immunity has been studied (Ganguly et al., 1976). The vaccine was given to volunteers either by nose drops or by subcutaneous injection. The rubella vaccine induced transient depression of the CMI response which lasted until week 4 after challenge in most volunteers but the response returned to normal in all volunteers by week 6. The three parameters studied were delayed hypersensitivity to Candida, mumps and PPD, lymphocyte stimulation by PHA and spontaneous production of macrophage migration inhibition factor (MIF). Kauffman et al. (1974) have confirmed the depressive effect of rubella virus on CMI. Dermal hypersensitivity was markedly impaired in volunteers during the height of illness after experimentally induced rubella infection. However, the response to PHA and the number of T lymphocytes in peripheral blood were not changed.

A number of other viruses are also known to depress thymus-dependent immune functions in animals (reviewed by Notkins et al., 1970). Examples are smallpox (Hughes et al., 1968), mumps (Kupers et al., 1970), poliomyelitis (Berkovich and Starr, 1966), influenza (Reed et al., 1972), RNA leukaemogenic viruses (Dent, 1972), Friend leukemia virus (Mortensen et al., 1974), Moloney sarcoma virus (Kirchner et al., 1974), and murine sarcoma virus (Gorczynski, 1974).

Infection with protozoan parasites can also alter the immune response.

Immunodepression induced by malaria was reported by McGregor and Barr (1962) who noticed that children in malarious areas, in the Gambia, produced poor antibody responses to vaccination with tetanus toxoid. Other clinical evidence for immunodepressive effect of malaria had been reported earlier as Salmonella spp. infections are recognized complications of malaria therapy for neurosyphilis (Hayasaka, 1933 quoted by Bennett and Hook, 1959) and bacterial infections are commonly seen in children in the tropics with acute Plasmodium falciparum malaria. Greenwood et al. (1972) and Greenwood (1974b) reported that children with acute malaria showed a diminished antibody response to tetanus and S. typhi vaccination although their CMI responses were normal. Mice experimentally infected with malaria also produce lower than normal levels of antibodies upon immunization with SRBC (Salaman et al., 1969) and depression was most marked at the peak of parasitaemia. Much more work has confirmed immunodepression by malaria infections using a wide variety of heterologous erythrocytes as antigens, (Greenwood et al., 1971 ; Barker, 1971; Sengers et al., 1971; Weidanz and Rank, 1975a), human gamma globulin (Greenwood et al., 1971 ), Salmonella typhimurium (Kaye et al., 1965), murine sarcoma virus and urethane leukemia virus (Salaman et al., 1969), T. spiralis (Bruce and Philips, 1974) and tetanus toxoid (Voller et al., 1972, and Greenwood et al., 1972). Recently, Cox et al. (1974) have shown that Rowson-Parr virus causes depression of the splenic response of mice infected with Plasmodium vinckei chaubaudi and with Plasmodium berghei yoelii. However a normal immune response to keyhole limpet haemocyanin (KLH), normal contact sensitivity reactions and PHA transformation of lymphocytes have been demonstrated in malaria infected mice (Greenwood et al. 1971 ).

Immunodepression induced by malaria was reported by McGregor and Barr (1962) who noticed that children in malarious areas, in the Gambia, produced poor antibody responses to vaccination with tetanus toxoid. Other clinical evidence for immunodepressive effect of malaria had been reported earlier as Salmonella spp. infections are recognized complications of malaria therapy for neurosyphilis (Hayasaka, 1933 quoted by Bennett and Hook, 1959) and bacterial infections are commonly seen in children in the tropics with acute Plasmodium falciparum malaria. Greenwood et al. (1972) and Greenwood (1974b) reported that children with acute malaria showed a diminished antibody response to tetanus and S. typhi vaccination although their CMI responses were normal. Mice experimentally infected with malaria also produce lower than normal levels of antibodies upon immunization with SRBC (Salaman et al., 1969) and depression was most marked at the peak of parasitaemia. Much more work has confirmed immunodepression by malaria infections using a wide variety of heterologous erythrocytes as antigens, (Greenwood et al., 1971 ; Barker, 1971; Sengers et al., 1971; Weidanz and Rank, 1975a), human gamma globulin (Greenwood et al., 1971 ), Salmonella typhimurium (Kaye et al., 1965), murine sarcoma virus and urethane leukemia virus (Salaman et al., 1969), T. spiralis (Bruce and Philips, 1974) and tetanus toxoid (Voller et al., 1972, and Greenwood et al., 1972). Recently, Cox et al. (1974) have shown that Rowson-Parr virus causes depression of the splenic response of mice infected with Plasmodium vinckei chaubaudi and with Plasmodium berghei yoelii. However a normal immune response to keyhole limpet haemocyanin (KLH), normal contact sensitivity reactions and PHA transformation of lymphocytes have been demonstrated in malaria infected mice (Greenwood et al. 1971 ).

Malaria-induced depression has been demonstrated experimentally using T-dependent antigens. However it seems difficult to confirm its T-cell dependency, since Weidanz and Rank (1975a) reported that the PFC response to E. coli LPS (a T-independent antigen) was suppressed in P. gallinaceum infected chickens compared to normal birds; the same degree of depression was observed in the response to SRBC. Steward and Voller (1973) made the interesting observation that although the total antibody response to transferin was not reduced by malaria infection, the affinity of the antibody formed was significantly reduced.

More recently, depression of the immune response by P. berghei has been shown in BALB/c mice. A profound impairment in splenic direct PFC formation occurred in infected mice which had been immunized with SRBC 2 or 4 days after P. berghei NYu-2 strain infection. Serum haemagglutinins were depressed in mice which received SRBC 4 days but not 2 days after infection. Coincident with the depressed immune response, there was a profound increase in the vascular clearance of $^{51}$Cr-SRBC with an enhanced hepatic uptake of $^{51}$Cr-SRBC and depressed splenic uptake of the $^{51}$Cr-SRBC (Loose and Di Luzio, 1976). Suppressed response of PFC in mice infected with P. berghei NK 65 was also reported. The response of infected mice showed 2 distinct phases: in the initial period of infection (days 0 - 3), the PFC response to SRBC was depressed whereas the PFC response to PVP was normal. Also the PFC response to PVP was not depressed in infected athymic nude (nu/nu) mice. When the infection proceeded further (days 5 - 7), the PFC response to SRBC was greatly depressed and the response of spleen cells to PVP was also depressed (Tanabe et al., 1977). Furthermore, acute P. berghei infection in BALB/c mice caused permanent and complete depression of

the mice's immune response to SRBC measured by PFC assay. In the period of severe depression, tolerance to SRBC was induced. Responsiveness was restored when infected mice were cured with chloroquine. Considerable elevation of antibodies predominently IgG were produced during immunodepression suggesting a possible relationship between immunodepression and hyperimmunoglobulinaemia (Poels et al., 1977 , and Greenwood, 1974a).

McBride and Micklem (1977 ) have studied the effect of malaria on the immune response against bovine serum albumin. The primary response to alum-adsorbed bovine serum albumin was depressed in CBA mice infected with P. yoelii yoelii. Responses initiated within approximately 3 weeks of infection were reduced in quantity, but not in avidity. Depressed responses were also seen in infected compared to control splenectomized mice, this was accompanied by impaired priming for a secondary response.

The effect of malaria on the immune response was also studied against type III pneumococcal polysaccharide (S III). Acute P. y. yoelii and chronic P. berghei infections of CBA mice were accompanied by a reduced capacity to give an antibody response to S III antigen measured by PFC and haemagglutinins. A substance which cross reacted serologically with S III was found in blood of infected mice (McBride et al., 1977 , Wedderburn and Dracott, 1977).

It has been shown that malaria induced regional immunodepression. P. berghei yoelii infection in mice severely depressed the splenic antibody response to SRBC measured by PFC but little effect was demonstrated on antibody formation in lymph nodes (Weidanz and Rank, 1975b). P. b. yoelii showed also depression in haemagglutinin of pertussis bacteria particularly in mice infected 13 days before vaccination. Malaria infection also significantly reduced the protective effect of

the vaccine when the mice were challenged by intracerebral inoculation of the virulent bacteria (Tarzaali et al., 1975).

The effect of experimental malaria on cellular immunity has been controversial. Greenwood et al. (1971 ) reported that neither allograft rejection nor contact hypersensitivity was impaired in mice with P. berghei yoelii. Similar findings have been reported by Weidanz and RanK (1975a) who showed that skin grafts from histoincompatible donors were rejected at approximately the same time by P. gallinaceum infected and uninfected chickens. However, Wedderburn (1974) has observed a significant increase in graft survival time in P. b. yoelii infected mice. P. b. berghei infected mice showed a delayed allograft rejection (Sengers et al., 1971) and depressed contact sensitivity to oxazolone (Jayawardena et al., 1975).

In vitro studies on malaria infected mice showed also depressed immune response. Adherent spleen cells from mice infected with P. b. yoelii were defective in their ability to allow non-adherent spleen cells of both normal and infected mice to respond to HRBC. On the other hand, adherent spleen cells did not depress the PFC response of unfractionated spleen cells from normal mice to HRBC and they contained macrophages which were unable to take up HRBC (Warren and Weidanz, 1976) Peritoneal exudate and spleen cells taken from mice infected with P. berghei did not migrate when incubated with sonicate of erythrocytes of infected mice although cells from normal mice migrated well (Coleman et al., 1976). P. berghei infections also caused depressed responses to non specific T-cell stimulators such as PHA (Jayawardena et al., 1975 and Golenser et al., 1975).

In P. falciparum infection, Taylor and Siddiqui (1978) have reported that Colombian monkeys infected with the Uganda-Palo Alto strain of

P. falciparum decreased the PHA, Con A and Pokeweed mitogen (PWM) responses in cultures of peripheral blood lymphocytes when the parasitaemia was more than 50%. Spleen cells from all acutely infected monkeys were depressed to PHA and Con A but not to PWM stimulation.

Malarial infection decreases resistance to infection by assorted microbial agents such as Moloney leukaemia virus (Wedderburn, 1970), toxoplasma (Strickland et al., 1972), trypanosomes (Cox, 1975) and S. typhimurium (Kaye et al., 1965; Jerusalem, 1968). Wedderburn (1970) and Bomford and Wedderburn (1973) have shown that malaria infection potentiated the induction of lymphomas by the moloney leukaemia virus (MLV) and reduced the levels of circulating neutralising antibodies to MLV particularly IgG antibodies.

It has been suggested that low incidence of auto-immune diseases in some parts of the tropics might be related to the occurrence of parasitic infections (Greenwood, 1968) and it was later shown that early infection with P. yoelii suppressed the spontaneous auto-immune disease which normally occurs in NZB and NZB x NZW mice (Greenwood and Voller, 1970a and b).

Trypanosomiasis causes a more extensive immune defect than malaria, involving both humoral and cellular immunity. Goodwin (1970) and Goodwin et al. (1972) observed much lower SRBC agglutinin levels in mice and rabbits infected with Trypanosoma brucei brucei than in normal animals and this effect became more marked as infection progressed. Later, it was shown that T. brucei infections caused mice to make poor PFC responses to SRBC (Longstaff et al., 1973; Murray et al., 1974) and to LPS antigens (Murray et al., 1974). Mackenzie et al. (1975) found that sheep infected with T. congolense reduced the haemagglutination titres against SRBC when compared to normal uninfected animals. Moulton and

Coleman (1977) have confirmed the immunodepressing effect of Trypanosoma infection to SRBC responses. Deer mice infected with T. equiperdum showed depression in the number of PFC in response to SRBC injection. The effect was due to the presence of the live parasite since radio-attenuated trypanosomes had normal to enhanced immunological responses to SRBC.

Infection with T. congolense diminished the antitoxin responses to the various components (tetanus, septicum and oedematiens) of clostridial vaccine when the vaccine is injected into cattle 3 weeks after T. congolense infection (Holmes et al., 1974). Greenwood (1974b) demonstrated impaired antibody response to S. typhi vaccine in 38 patients with Trypanosoma gambiense infection.

Attempts to investigate depression of CMI responses by trypanosomes have been conflicting. Responses to contact sensitising agents were largely unimpaired until the terminal stages of T. brucei infection (Freeman et al., 1973; Murray et al., 1974). However a significant reduction in the GVH reactivity of spleen cells from T. brucei infected mice has been reported (Freeman, 1975). In addition there was an impairment of cell-mediated immunity using skin testing with purified protein derivatives (PPD) and Candida antigens in sleeping sickness patients compared to controls (Greenwood, 1974b). Experimental confirmation of this result has been reported by Mansfield and Kreier (1972b) and Mansfield and Wallace (1974) who found that skin test responses to PPD were less marked in infected, immunized rabbits than in uninfected, immunized rabbits.

Recently, Reed et al. (1977) demonstrated that mice infected with Tulahuin strain of T. cruzi showed a depressed immune response when skin-tested with either BCG protoplasm or oxazolone to detect

hypersensitivity. Depression was observed during marked parasitaemia. They also showed that mice which responded to oxazolone before infection lost their ability to respond as the infection progressed.

A group of human patients infected with T. gambiense had depressed hypersensitivity to PPD, Candida and streptococcal antigens (Greenwood et al., 1973).

Allt et al. (1971) have shown that infection of rabbits with T. brucei protects them from developing allergic neuritis, an autoimmune disease in which cell-mediated immunity is thought to play an important part. However autoantibodies to normal tissue antigens have been demonstrated in experimental Trypanosoma congolense infections of rabbits (Mansfield and Kreier, 1972a; Muschel et al., 1961; Seed and Gam, 1967). Henderson-Begg (1946) and Houba et al. (1969) showed a rise in heterophile agglutinins and rheumatoid factor-like antibodies.

It has been reported that the protective immune response of mice to the intestinal nematode Trichuris muris was abolished when the mice were concurrently infected with T. brucei (Phillips et al., 1974), and Urquhart et al. (1972 and 1973) have reported a diminished immune response to Nippostrongylus brasiliensis infection in mice and rats with trypanosomiasis.

Human patients with sleeping sickness have an increased susceptibility to secondary bacterial infection (Greenwood et al., 1973), a feature which was recognized in some of the earliest studies of this disease (Low and Castellani, 1903 quoted from Greenwood, 1973). In sheep infected with T. vivax, a predisposition to bacterial infections was also noted (Hull, 1971; Mackenzie et al., 1975). Furthermore, it has been shown that T. b. gambiense infected voles increased the susceptibility to Ehrlich's tumour growth. When Ehrlich's ascites tumour

cells were injected i.p. or subcutaneously into uninfected and animals infected with *T. b. gambiense*, over 78% of infected animals developed tumours while the uninfected controls were totally resistant after i.p. challenge. When solid tumours were implanted subcutaneously, the tumour mass decreased in size in the controls while it expanded and viable solid tumours were recovered from over 70% of the infected animals (Ackerman and Seed, 1976).

*In vitro* culture of peripheral blood lymphocytes (PBL), from animals infected with *T. gambiense*, with PPD and PHA produced a lower response compared to PBL from uninfected animals and there was no detectable MIF production by infected rabbits (Mansfield and Wallace, 1974). Further *in vitro* studies showed that *T. equiperdum* depressed the T-lymphocyte response to PHA and Con A and the B-cell response to LPS and pokeweed mitogens (Moulton and Coleman, 1977).

The *in vitro* immunodepressing effect was observed with other trypanosomes. Spleen cell cultures prepared from mice between days 4 and 24 after infection with *T. musculae* failed to respond to SRBC or to the mitogens PHA, Con A or LPS. Serum prepared from the blood of infected mice markedly inhibited the ability of spleen cell cultures of normal mice to respond to SRBC. Similar effects were observed when saline extracts of frozen-thawed trypanosomes inhibited the humoral antibody responses of normal spleen cell cultures (Albright *et al.*, 1977). More *in vitro* studies have confirmed the altered immuno response due to trypanosome infections. Lack of responsiveness of T-cells to PHA was demonstrated in splenic lymphoid populations of mice infected with *T. brucei* strain 42. The ability to proliferate in response to LPS was severely depressed by day 7 and almost totally absent by day 12. Moreover, macrophages obtained from infected mice profoundly depressed the

ability of normal spleen cells to proliferate and secrete immunoglobulin in LPS cultures, (Corsini et al., 1977). It was suggested that the mechanism of depression in trypanosomes could be attributed to clonal exhaustion of B-cell potential due to undefined blastogenic stimulus from the parasites which may operate at least in part by the generation of suppressive T cells and macrophages. This theory of clonal exhaustion of B-cell has been suggested in trypanosomiasis, in vitro (Urquhart et al., 1973), and in vivo (Hudson et al., 1976 ) when an increased number of background PFC in T. b. brucei infected animals was demonstrated. Mice showed depressed immune response to SRBC starting from 5 days after T. b. brucei infection and persisted for the remainder of the infection. The work of Jayawardena and Waksman (1977) is in agreement with Albright et al. (1977), however they suggested that macrophages are not the primary source of suppressor activity. Immunodepression was demonstrated when they added T-cells and splenic macrophages from mice infected with T. brucei strain 42 to normal spleen cell cultures, measured by the ability of spleen cells to produce DNA synthetic responses to the mitogens Con A, PHA and LPS and by PFC responses to SRBC and DNP-Ficoll. Further studies presented evidence suggesting that suppressor cells are involved in the immunological hyporesponsiveness in trypanosome infections. While spleen cells from infected BALB/c mice caused significant depression of PHA and LPS mitogen responses, spleen cells from infected nude mice at the same stage of infection failed to suppress the mitogen induced DNA synthetic responses of normal cells indicating that the suppressors in the spleens of infected mice were either T-cell or were dependent on T-cell influence for its generation.

Eardley and Jayawardena (1977) and Jayawardena and Waksman (1977) confirmed earlier reports that

thymus-dependent antibody responses are depressed in mice soon after infection with T. brucei. They showed that suppressor cells appeared at the same time as the immunodepression of the primary in vitro antibody responses and that depression can be mediated at least in part by T1 cells based on insensitivity to ALS. Both plastic adherent (most likely macrophages) and non-adherent cells were depressive.

Altered reactivity to immunologically unrelated antigen has been shown with other protozoan infections. Depression of humoral responses in Leishmania was indicated as early as 1930 when Chung and Reimann showed that kala-azar patients who had been inoculated with typhoid vaccine had lower titres of specific agglutinins than normal controls. This was confirmed by Cassimos et al. (1966). Later, Clinton et al. (1969) showed that anti-ovalbumin antibodies in hamsters infected with Leishmania donovani were significantly lower than controls. Bryceson et al. (1974) demonstrated that selective depression of cell-mediated immunity occurs during the course of cutaneous leishmaniasis in the guinea-pig. They concluded that the immunodepressive effects of heavy infecting inocula were due to desensitization of rapidly acquired cell-mediated immunity rather than to the induction of immunological tolerance.

Strickland et al. (1972), who used an acute strain of Toxoplasma gondii in mice which were subsequently infected with P. b. yoelii, found that the malarial parasitaemia was more severe and prolonged in mice infected with both protozoa than when malaria was given alone. There was also a greater anaemia in these animals. Antibody production to both T. gondii and P. b. yoelii was depressed and the authors suggest that this immunodepression was due to antigenic competition.

Later, Mahmoud et al. (1977) showed that animals infected with T. gondii one day before S. mansoni had a higher mortality rate than

animals infected with either parasite alone. They also showed that *T. gondii* had profound and prolonged depressive effect on cell-mediated granulomatous hypersensitivity *in vivo* although this effect resulted in considerable alleviation of hepatic schistosomiasis under one set of circumstances. Mice with combined infections had markedly smaller hepatic granulomas and lower mean portal pressures and less oesophageal varicosity than those with *S. mansoni* alone. Strickland *et al.* (1973) showed that mice infected with *T. gondii* had depressed levels of haemagglutinins, haemolysins and PFC to SRBC. Immunodepression occurred in mice within a week of infection and lasted for 1 month. Immunodepression was mainly in IgM antibodies and corresponded with the period of maximum increase in spleen size and *T. gondii* antibodies. Huldt *et al.* (1973) also showed reduced antibody responses to SRBC and to killed poliomyelitis vaccine in *T. gondii* infected animals. Later, Strickland and Sayles (1977) studied the effect of *T. gondii* on the immune response after primary and secondary immunization with SRBC. They found that IgM haemagglutinins and haemolysins, IgG and IgG-secreting PFC were depressed after primary immunization while there was a delay but not actual depression after secondary immunization. Furthermore, it has been shown that injecting *T. gondii* antiserum, a small dose of *T. gondii* ($5 \times 10^4$) or a combination of both, decreased the number of rosette forming cells (RFC) to intravenous injection of $10^6$ toxoplasms one hour after the first injection (Masihi and Werner, 1977). *T. gondii* also showed a reduced blastogenic response to T and B cell mitogens in *T. gondii* infected animals (Strickland *et al.*, 1975).

The two piroplasms , *Babesia microti* and *Babesia hylomysci*, have been shown to temporarily depress the immune response of mice to the nematode *Trichuris muris* and delayed the expulsion of the worm from the intestine (Philips and Wakelin, 1976). Depression was most marked when

the patent phase of the Babesia infections coincided with the pre-expulsion period of the nematode infection. Acute Babesia infections also depressed the primary agglutinating antibody response of mice to SRBC. Cox (1976, 1977 ) also working with B. microti, found that super-imposed T. musculi infections were prolonged and enhanced, and that antibodies to the trypanosomes were decreased. Purvis (1977) showed that mice infected with B. microti depressed the ability of mice to mount an immune response to SRBC. The immune response recovered gradually with the gradual disappearance of the parasites from the blood. Her work showed also that the phagocytic activity as measured by carbon clearance tests was increased in B. microti infected animals and that increased phagocytic activity was correlated with the para-sitaemia. Cell mediated responses as determined by contact sensitivity to oxazolone and allograft survival were not affected.

It has also been shown that the intestinal flagellate Hexamita muris depressed the T-helper cell function and the B-cell function in mice. Animals infected with H. muris showed lower haemagglutination titres against tetanus toxoid and type 3 pneumococcal polysaccharide (S III). However, the CMI response, judged by skin allograft survival, was not affected. The infected mice were resistant to Listeria monocytogens when the challenge was 2, 4 and 6 days after H. muris infection. The authors suggested that all these phenomena might be explained by increased macrophage activity (Ruitenberg and Kruyt, 1975).

Duszynski et al. (1978) have found that the protozoa Eimeria nieschulzi depressed the immune response of the host. Rats immunized with T. spiralis larvae then inoculated with sporulated oocysts of E. nieschulzi, had 4 times more T. spiralis larvae than the control immune rats when the T. spiralis larvae were given 2 and 10 days after

the protozoa infection.

Until recently, little work has been done on the depression of the immune response by metazoan parasites. Of the trematodes, it has been shown that Schistosoma infections depress the immune response in the hosts. Yoeli (1956) reported that in mixed P. berghei and Schistosoma mansoni infections, a marked increase in the persistence of plasmodial infections was observed. The effect was conspicuous in animals infected with P. berghei 1-2 weeks before or 1-2 weeks after exposure to cercariae. This primary invasive period of schistosomiasis is marked by its general toxic reaction. However animals receiving their plasmodial infection 4-7 weeks after exposure to cercariae showed a mild course of parasitaemia and no deaths were recorded. He suggested that the reticulo-endothelial system was altered with a marked increase in its phagocytic power due to chronic bilharzial infections and that this markedly affects the course of a concomitant plasmodial infection. Kloetzel et al. (1973) showed that S. mansoni increased the levels of peak parasitaemia of Trypanosoma cruzi and parasitaemia also persisted longer.

It has been reported that, in Egypt, bacteriuria is often present in hospital patients with S. haematobium infections (Abdallah, 1946; Shokeir et al., 1972; Lehman et al., 1973) and chronic pyelonephritis has been associated with S. haematobium infection (Abo Gabal et al., 1970; Higazi et al., 1972; Smith et al., 1974). Neves and Da Luz Lobo Martins (1967) found that S. mansoni infection was common in cases having a peculiar long duration of septicaemic salmonellosis in Brazil and this may be due to the immunodepression induced by S. mansoni infection.

Brito et al. (1976) studied the role of schistosomiasis as a depressor of the immune response to an antigenic stimulus. Their results show that infection with S. mansoni depresses the antibody response of

mice to tetanus toxoid. The depression was significant when the tetanus toxoid was given 9 weeks after cercarial infection and was maximal when the toxoid was given 15 weeks after infection. Mota Santos et al. (1976) demonstrated that low, moderate and severe S. mansoni infection in mice had different effects on the immune response upon immunization with SRBC. Moderate infection (25 worm/mouse) showed a transient immunodepression from the fourth week of infection which lasted about 10 days. The group of heavily infected animals (50 worms/mouse) did not recover from the immunodepression during the period of observation (50 days). However the spleens of the lightly infected group (15 worms/mouse) developed almost as many PFC and RFC as the control mice.

Ramalho-Pinto et al. (1976) have studied the role of thymus-derived lymphocytes in immunodepression induced by S. mansoni by using schistosomules as carriers for a hapten and measuring the anti-hapten response in normal and nude, infected and vaccinated mice. The anti-TNP response was very much less in nude mice than in controls, suggesting that the carrier effect was largely T cell mediated. A massive anti-TNP response was produced in mice infected with 40 cercariae when TNP schistosomules were given 10 days after infection and thereafter declined to the level of normal response about week 12 after infection. Infected mice showed a normal response to TNP-HRBC, showing that the altered T cell response was immunologically specific. A smaller helper T cell response was seen with mice vaccinated with 40 formalin-fixed schistosomules which lasted at least 13 weeks after vaccination. This suggests that both the early increase and subsequent decline may be partly due to the living infection. The authors also observed that the background PFC in S. mansoni infected mice began to rise from 4 weeks after infection, an indication of non-specific B-cell activation.

The *in vitro* responses of lymph node and spleen cells to non-specific mitogens (Con A and PHA) at various time periods after *S. mansoni* infection in mice has been studied by Pelley *et al.* (1976). Cells from mesenteric lymph nodes of animals infected with *S. mansoni* for 7 weeks and maintained *in vitro* in the absence of exogenous stimuli, synthesized eight times as much DNA ("baseline" DNA) as did those from uninfected controls. This elevation reached a maximum 8 weeks after infection and subsequently declined. In spleen cells the elevation was not as pronounced as in mesenteric lymph node cells. The addition of the T cell mitogens PHA and Con A to cultures of mesenteric lymph node and spleen cells from *S. mansoni* infected and control mice showed that cells of mice 7 weeks after infection had depressed responses to PHA and Con A. The depression was progressive from week 7 onwards. 12 weeks after infection, all the parameters studied (DNA synthesis as well as mitogen stimulation) were profoundly suppressed. The authors showed a relationship between the onset of egg laying and the degree of depression detected and they suggested that suppressor T cell activity is the most likely explanation for this depression of mitogen reactivity that develops during chronic schistosomiasis.

Dessaint *et al.* (1977) have confirmed the immunodepressing effect of *S. mansoni* *in vitro*. Cell-free supernatants of *S. mansoni* cultures and incubation products of the parasite decreased tritiated thymidine and leucine uptake in normal spleen cells from CBA mice, rats and peripheral human blood lymphocytes stimulated either by PHA, Con A, or LPS. The depressing factors were heat-resistant, dialysable and of molecular weight 500-1000. Serum and its dialysable fractions obtained from rats infected with *S. mansoni* for 4 weeks demonstrated the same depressing activity.

CMI response is also depressed in S. mansoni infected mice. Araujo et al. (1977) showed that rejection of skin grafts is altered in mice infected with S. mansoni. Depression was evident in mice infected for 60 days whereas it was not demonstrated in mice infected 30 days prior to grafting. The authors related this depression of the CMI response either to substances secreted by adult worms or to soluble antigens secreted by eggs. Mota-Santos et al. (1977) have shown that S. mansoni infections in mice induced marked diminution in the cellular and humoral response to SRBC and LPS, judged by foot pad swelling and PFC respectively. They demonstrated also that adult worms or products released by them but not by egg extract caused this profound alteration in the immune system. Mice retained their normal immune responses 4 weeks after treatment with the anthelmintic Oxamniquine.

Little work has been done regarding the effect of Fasciola infections on the immune response. Aitken et al. (1976) found that cattle orally infected with 1000 metacercariae of Fasciola hepatica had increased susceptibility to Salmonella dublin given intravenously 13 weeks later. Sewell (1963) showed that antigens prepared from Fasciola show an anti-complementary activity when added to guinea pig serum. The antigenic component concerned was demonstrated in preparations from adult and immature flukes of both Fasciola hepatica and F. gigantica and from the metabolic products of F. gigantica. Goose (1977) also showed that F. hepatica infections reduced responses to SRBC in the experimental system.

Depression of the immune response to unrelated antigens can be a feature of cestode infections. Good and Miller (1976) showed that intraperitoneal infection of mice with the larval form of the cestode Taenia crassiceps depresses both primary and secondary humoral responses to SRBC in vivo. Secondary PFC responses were consistently depressed in

both spleen and mesenteric lymph node preparations from infected mice, the primary *in vitro* responses to SRBC were consistently depressed in mesenteric lymph nodes but not always in spleen cell preparations. Haemagglutination titres of infected mice were depressed compared to controls. Depression of the secondary response was more pronounced than depression of the primary response. Neither depression of the primary response nor depression of secondary response was accompanied by a shift in kinetics. The authors suggested that antigenic competition, mediated by soluble products released from living larvae was a possible mechanism of immunodepression by *T. crassiceps*.

Studying the larval cestode, *Mesocestoides corti*, i.p. injection of SRBC or dinitrophenylated Ficoll (DNP-Ficoll), resulted in, at least, 20 times fewer PFC in the spleens of infected mice and fewer haemagglutinins than uninfected animals. By contrast intravenous injection of SRBC leads to normal PFC responses. However CMI responses judged by delayed hypersensitivity responses to FGG were normal. Infected mice showed also poor absorption of $^{125}$I-human gamma globulin or $^{125}$I mouse erythrocytes from the peritoneal cavity. Mitchell and Handman (1977) suggested that sequestration of antigen, and its subsequent local destruction, accounts for the markedly suppressed systemic immune responses induced by i.p. injected antigens in *M. corti* infected mice. In addition, it was found that anti-DNP-antibody responses were defective in mice infected with *M. corti* larvae when DNP-*M.corti* larvae and DNP-human gamma globulin were injected after infection (Mitchell *et al.*, 1977).

There has been a growing interest in the effect of nematodes on the immune response. Woodruff (1968) had noticed that infection with parasitic nematodes is often associated clinically with increased susceptibility of the host to bacterial and viral diseases, both in man

and animals. One possible contributing mechanism for such an association is depression of the host's immune system as a result of the helminth infection.

The occurrence of depressed antibody production to heterologous antigen infections was investigated in hamsters (Mesocricetus auratus) and Mongolian jirds (Meriones unguiculatus) infected with Dipetalonema viteae (Dalesandro and Klei, 1976). Comparisons of the mean passive haemagglutinating antibody titres between infected and uninfected animals, made one week following BSA injections, revealed a significant decrease in antibody levels in both infected hamsters and jirds. It was not possible to relate the level of immunodepression to either mean, peak or duration of microfilaraemia. Using SRBC as an antigen, the mean number of PFC in spleens of infected animals 10 weeks after D. viteae inoculation was significantly less (40%) than that in spleens of uninfected animals. However, no significant difference was seen when the response of infected and uninfected animals were compared 5 weeks after infection. Dalesandro and Klei (1976) suggested a possible association between microfilaraemia and immunodepression since immunodepression was detected at 10 weeks and not at 5 weeks post infection. A defective immune response was also found in Litomosoides carinii infected albino rats (Sharma and Ramachandran, 1976). Infected animals developed significantly lower agglutinin titres when compared to controls. In man, haemagglutinating antibodies to tetanus toxoid and precipitating antibodies to Salmonella typhi H antigen were impaired in patients with bancroftian filariasis. Delayed hypersensitivity skin reactions to Candida albicans, mumps skin test antigens and streptokinase-streptodornase were also depressed (Grove and Forbes, in press). Antigen specific cellular unresponsiveness was demonstrated in patients

chronically infected with Wuchereria bancrofti (Ottesen et al., 1977).
Blood lymphocytes from infected patients showed poor cellular responsiveness to filaria antigens, Brugia and Dirofilaria, in an in vitro lymphocyte transformation assay. However normal responses were detected to tuberculin (PPD) and streptococcal (SK-SD) antigens. Portaro et al. (1976) have demonstrated that PHA and Con A reactivity of splenocytes from jirds, Meriones unguiculatus, with Brugia pahangi infections was found to be depressed in comparison to uninfected controls. However serum from infected animals did not show the same effect.

The nematode Heligmosomoides polygyrus (= Nematospiroides dubius) caused a lower serum haemagglutinin titre in mice following a series of oral inoculations of SRBC when compared to similarly inoculated uninfected mice (Shimp et al., 1975). There was also a low splenic PFC response in infected animals compared to controls. Oral and intraperitoneal routes of administration of SRBC into infected mice produced a similar reduction in antibody titres. However, immunodepression following i.p. infection was not consistently observed. Earlier, Cypess et al. (1974c) have found that mice infected with N. dubius showed a significant depression of PFC response to Escherichia coli when the mice were challenged parenterally, but not orally, with E. coli.

It has been reported that IgG levels were persistently elevated in H. polygyrus infected animals (Crandall et al., 1974) which indicates sustained antigenic stimulation. Shimp et al. (1975) suggested that malnutrition in H. polygyrus infected mice is a contributing factor in the immune depression. Recently, Brown et al. (1976) demonstrated accelerated clearance of passively transferred mouse IgG in H. polygyrus infected mice. To assess the influence of H. polygyrus infection [illegible] catabolic rate of IgG, the biologic half life (T ½) were determined using $^{125}$I IgG. The results showed a decrease in $IgG_1$ [illegible] [illegible]ly H. polygyrus infected mice, the rate of loss of IgG [illegible]

twice that of controls. The catabolic rates of all IgG classes are accelerated by increased serum levels of any IgG class (Fahey and Sell, 1965). Brown et al. (1976) suggested that the elevated IgG levels of H. polygyrus infected mice are a major cause of increased $IgG_1$ catabolism in infected mice.

Crandall and Crandall (1976) showed that infection with A. suum 4, 11 or 21 days before immunization with SRBC reduced splenic IgM and [illegible] responses and HA antibody titres to SRBC. The mean PFC responses ranged from 18 to [illegible]% of the control values. However infection on the day of immunization did not produce any statistical difference in antibody responses. The results did not demonstrate a significant reduction in either the primary or secondary IgG responses although the mean numbers of PFC were consistently lower being 40 to 90% of the control values in the primary response. A reduced primary antibody response to ovalbumin was also demonstrated in A. suum infected mice. Delayed hypersensitivity responses, footpad swelling and contact hypersensitivity, mediated by peripheral lymphoid tissues were essentially normal during infection, but footpad swelling response following i.v. sensitization with SRBC was inhibited.

Three possibilities have been suggested to explain immunodepression induced by A. suum infection.

1) Reduction of the quantity of antigen reaching the lymphoid tissues in an immunogenic form;
2) Alteration in T cell function or that macrophage activity which facilitates antibody response;
3) Antigenic competition in which products induced during the immune response to the parasite restrict development of a subsequent immune response.

Kasuya et al. (1977) showed that purified eosinophils from peritoneal exudate cells of immunized guinea pigs depressed the in vitro DNA synthesis of lymph node cells sensitised with A. suum and then activated by the antigen or by PHA. Addition of eosinophils did not affect the viability of lymph nodes. However, blastformation was remarkably suppressed when 5-10 x $10^{15}$ eosinophils were added to $10^{6}$ lymph node cells within 48 hours after stimulation by A. suum.

Much work has been done over the last few years to study the effect of T. spiralis infection on the immune response to unrelated antigens and there is considerable evidence for depression of both humoral and cellular immune response to foreign antigen.

The first published work was with allogeneic skin grafts. Svet-Moldavsky et al. (1970) showed that allogeneic skin grafts survived considerably longer in mice infected with T. spiralis than in normal, uninfected controls. They suggested that T. spiralis produces substances which depress the host's immunity and postulated that these substances were produced particularly during larval migration. Chernyakhovskaya et al. (1971) also showed that T. spiralis depresses the immune response to allogeneic skin grafts and that the more T. spiralis larvae in the muscles, the more pronounced the depression of the transplantation reaction. Ljungstrom and Huldt (1977) have shown that T. spiralis depresses tissue rejection. The cellular immune response judged by split heart allograft rejection was subject to severe and long lasting depression specially when the graft was made 1 week after infection with T. spiralis. Barriga (1978a) showed that mice infected for 7 days or mice which received T. spiralis extract, rejected primary skin allografts later (18-23 days) than the controls (12-18 days) and rejected secondary skin allografts at 12-16 days while the controls rejected

them immediately. He suggested that in the depression of graft rejection only T lymphocytes were affected by the infection or the inoculation of T. spiralis extract, either by a direct action on the lymphocytes or indirectly by affecting macrophage activity. He also showed that inoculation of increasing doses of spleen cells from mice pre-treated with T. spiralis extract proportionally reduced the capacity of the inoculated spleen cells to induce a graft versus host reaction while proportionally stronger reactions were produced after inoculation of increasing doses of spleen cells from normal mice or mice pre-treated with saline or BSA.

The cell mediated response to BCG after infection with T. spiralis has been studied in mice by Cypess et al. (1974b). Results indicate that infection with T. spiralis produces an initial depression followed by a prolonged potentiation of the delayed foot pad responses to BCG. The immunodepression was detected 14 days after infection but no longer evident after 20 days. Adoptive transfer experiments suggest that the initial failure to develop a positive foot pad reaction to old tuberculin at 14 days was due to a defect in the adoptively transferred splenic cells. These authors suggested that increased bacterial replication leading to increased antigenic stimulation, a nonspecific activation of the reticuloendothelial system, and enhancement of T cell function were contributed to the potentiation in cell-mediated response to BCG in T. spiralis.

Faubert and Tanner (1974a) have shown that T. spiralis infected mice or mice treated with serum from T. spiralis infected animals or parasite extract contained fewer spleen Rosette-forming cells to SRBC. They showed also that bone-marrow cells from infected mice were considerably less efficient than normal cells in reconstituting thymectomized,

irradiated animals.

The results of Faubert and Tanner (1971), using the SRBC system in mice, indicate clearly that immunodepression to heterologous antigens is a feature of T. spiralis infection. They also claimed that the serum of infected animals induces this effect in normal mice. Depression of the production of SRBC agglutinins was caused also by rabbit antilymphocyte serum as well as infected rabbit serum. However, it must be conclud[illegible] that this phenomenon is not significant since normal rabbit serum, used as a control produced the same effect.

In addition, mice produced lower antibody titres to vaccinia virus when infected with T. spiralis (Chimishkyan and Ovumyan (1975). Barriga (1975) suggested that immunodepression is T cell dependent, since infection in his mice reduced the capacity of mice to form antibodies to the T cell dependent antigen, SRBC, but not to the T cell independent antigen, PVP. Later, Faubert (1976) suggested that the depression of the immune response to SRBC in mice infected with T. spiralis is transitory and is related to the migrating phase of the parasite. He showed that newborn larvae produce substances which can diffuse through a Millipore filter and killed spleen cells. Furthermore, Faubert and Tanner (1975) showed that lymphoid cells were agglutinated and killed in vitro by a factor present in the sera of mice infected with T. spiralis. The agglutinating factor appeared in the serum 7 days post infection, rose to a maximum on the 30th day of infection and then decreased but the intensity of infection had no relation to the leucocyte-agglutinating titres. Mice injected with an extract of T. spiralis larvae showed the same leucocyte agglutinating and leucocyte toxic activity.

Jones et al. (1976) have shown that a 20 day old T. spiralis infection induces a depression of the primary antibody response to SRBC,

measured by the number of PFC or the haemagglutinin titres. This depression was also demonstrated in cultures of splenocytes immunized *in vitro*. This depression is T lymphocyte dependent since the depressive activity of the splenocytes is abolished by lysis with anti-thy 1 serum, which kills T cells, but is enhanced by treatment with anti-immunoglobulin serum, which kills B cells. *In vitro* stimulation of splenocytes from normal mice using the T cell mitogens, con A and PHA, and the B lymphocyte mitogen, LPS, showed normal LPS response of cells from infected mice and depressed response to the T cell mitogens. They showed also that the addition of supernatant fluids of cultures of cells from mice which had been infected for 20 days significantly depressed the response of normal splenocytes.

Ljungstrom and Huldt (1977) confirmed the ability of *T. spiralis* to depress the immune response to unrelated antigens at humoral and cellular levels. The humoral immune response to SRBC showed transient depression. There were significantly fewer IgM PFC in mice infected with *T. spiralis* 3 and 6, but not 1, week after infection. IgG PFC were only depressed in mice immunized 6 weeks after *T. spiralis* infection.

The effect of *T. spiralis* on haemagglutinin production and PFC to sheep erythrocytes has also been studied by Chimishkyan *et al*. (1974). Depression of haemagglutinin production was observed on day 25 after infection but not earlier. Depression of PFC was greatest 20 days after infection.

Cypess *et al*. (1973) and Lubiniecki and Cypess (1975a) showed that *T. spiralis* infection caused a depression of the neutralising and complement fixing antibody responses to JBE virus. In contrast infection with gamma irradiated larvae of the parasite had no effect on antibody production. Lubiniecki and Cypess (1975a) have found fewer antibody

forming cells to SRBC in the spleens of mice parasitized with T. spiralis.

Relative unresponsiveness to passive cutaneous anaphylaxis (PCA) induced with hen albumin and its corresponding antibodies, has been reported in T. spiralis infected mice (Munoz and Cole, 1977). The unresponsiveness was to PCA produced either with $IgG_1$ or IgE antibodies but more with the latter. Sera of infected mice inhibited mainly PCA induced by IgE 35 days but not 10 months after infection. These authors believed that the relative unresponsiveness of infected mice is due to an increase in production of IgE which competitively blocks the mast cell sites for other IgE molecules.

In a study on the effect of T. spiralis on the thymus, spleen and lymph nodes, Tanner and Lim (1974) found that light infections enhance antibody response to sheep erythrocytes. There was an overall cell depletion in the thymus 14 days after T. spiralis infection but an increase in the population of the spleen and lymph node cells. The enhancement of response to SRBC in low infections and the depression of this response in heavy infections was ascribed to changes in the activity of helper T cells and depressor T cells.

T. spiralis can also increase the susceptibility of the hosts to secondary infections. Kilham and Oliver (1961) reported that T. spiralis infection increased the susceptibility of rats to viral infection.

Cypess et al. (1973) and Lubiniecki et al. (1974a) showed that infection of mice with 200 T. spiralis larvae 7 days prior to challenge with Japanese B encephalitis (JBE) virus, greatly increased susceptibility to fatal, viral, central nervous system disease but gamma irradiated larvae had no effect on subsequent viral infection. In their work enhanced intracerebral replication of the virus and higher mortality of mice peripherally inoculated with JBE virus was observed after

infection with T. spiralis. The effect was maximal when the virus was inoculated 7 days after T. spiralis infection and was absent by 21 days post T. spiralis infection. It was suggested that T. spiralis abrogates the host defence mechanism which normally aborts JBE virus replication in the brain. Chimishkyan and Ovumyan (1975) demonstrated that T. spiralis increases the susceptibility of mice and rabbits to vaccinia virus.

The interactions between parasites and particularly T. spiralis and the host which lead to immunodepression are clearly of great complexity. There is at present no completely satisfactory explanation for the immunodepression induced by T. spiralis.

The objective of the present study was to:

1) Study the effect of different stages of infection and its effect on the immune response to a T-dependent antigen (SRBC) and a T-independent antigen (LPS).
2) Show the effect of T. spiralis infection on antibody catabolism.
3) Detect the effect of T. spiralis on macrophage activity.
4) Study the effect of T. spiralis infection on the affinity of antibody for human serum albumin (HSA) antigen.

# CHAPTER 2

# GENERAL MATERIALS AND METHODS

## 1) Laboratory animals:

### Mice:

1. Outbred:

Male and female T.O. albino mice aged 8 weeks were used in many of the experiments. Their body weight at the start of the experiments was approximately 25 g. Animals were obtained from A. J. Tuck and Son Ltd., Essex, England.

2. Inbred mice:

Male and female mice of the Simpson strain, 8 weeks old, kindly supplied by Dr. M. Steward (London School of Hygiene and Tropical Medicine) were used in some experiments.

### Rats:

Adult Wistar albino rats obtained from A. J. Tuck and Son Ltd., Essex, England were used.

Mice and rats were marked and kept in plastic cages in groups of five animals per cage and were fed with pellets and water ad libitum. The pellets were either RGP 86 diet obtained from Peter Fox Scientific Animal Services, Oxoid diet obtained from Lillico and Son Ltd., or mouse diet (expanded) R&M No. 1 obtained from B.P. Nutrition (UK) Ltd. Oxoid or R&M No. 1 diet were used for the later experiments, since diet 86 was found to contain the anthelmintic Parbendazole on two occasions and this prevented the

mice from becoming infected.

Guinea pigs:

Guinea pigs were used as a source of complement. The animals were obtained from A. J. Tuck and Son Ltd. and were provided with guinea pig diet obtained from Peter Fox Scientific Animal Services, Herts., England.

II) Parasite:

1. Strain

T. spiralis of the "London strain" (Nelson et al, 1966) was used throughout these studies. The parasite has been maintained in L.S.H.T.M. by continuous passage through young mice. It was originally isolated by Dr. R. T. Leiper in 1939 from a domestic cat obtained from Penrith.

2. Preparation of infective material:

a) Preparation of normal muscle larvae:

Mice used for preparation of infective muscle larvae had been infected for at least five weeks. One or more infected mice were killed by cervical dislocation, skinned and evicerated. The ears, tail, paws, muzzles and upper and lower teeth were removed. The rest of the animal was minced twice through a kitchen mincer and put into a plastic bucket containing 1-2 litres of digesting fluid prepared as follows:

| | | |
|---|---|---|
| Pepsin | 15 g (BDH Chemicals) | |
| Hcl | 10 ml | 31% W/W (BDH Chemicals) |

Warm tap water up to 1 litre

The mixture was kept in the incubator at 37$^{o}$C under continuous agitation for 2-3 hours. The coarse particles were removed by passing the digested mixture through a 50 mesh/inch sieve. The excysted larvae then collected on a 200 mesh/inch sieve, washed twice with tap water, then suspended in 150 ml of saline. The larvae were counted using a McMaster counting chamber (Fig. 2) and the number of larvae was calculated as follows:

Total number of larvae in 1 ml =

$$\frac{\text{Mean number of larvae in both sides of the chamber}}{0.3}$$

to get the infective dose needed, either dilution or concentration of the mixture was performed. Adjustments were usually made to prepare the number of larvae required for infection in 0.25 ml.

b) Preparation of irradiated muscle larvae:

Cobalt 60 was adopted as the irradiation source, as high energy gamma radiation is less susceptible to the various physical variables than is an x-ray or U-V source (Evans, 1970). Infective larvae were prepared as described previously and were irradiated with 8,500 rads. This dose level sterilises adult T. spiralis while otherwise restricting the development of these worms as little as possible (James, 1974).

c) Infection with muscle larvae:

Mice were infected with the required number of larvae by inserting a 1 mm wide, blunt-tipped, needle attached to a 1 ml syringe into the oesophagus. The syringe was filled from a suspension of larvae which was gently stirred with a magnetic stirrer to ensure good distribution of larvae.

Figure 2 :

Diagram of the Mac Master counting chamber used for counting excysted muscle larvae. Actual size (Each ruled area is 10 x 10 mm and holds a volume of 0.15 ml).

d) Preparation of new born larvae (NBL):

Ten rats were usually used for each experiment. They were infected with 1-2 x $10^4$ larvae *per os* *T. spiralis* muscle larvae, prepared as described above. Six days later the rats were killed by cervical dislocation after being anaesthetized with Nembutal. The small intestine was removed as quickly as possible, split open and rinsed in warm tap water to remove the detritus. The intestines were then soaked in 0.85% saline at 37°C for an hour. The intestines were removed and discarded and the adult worms collected on a 200 mesh per inch sieve and then transferred to NCTC 135 medium after 3 washings in 0.85% saline. The adult worms were transferred to sterile bottles and incubated for 24 hours at 37°C in culture medium NCTC 135 (see below). After incubation the medium containing adults and NBL was passed through a 50 mesh/inch sieve. NBL passed through the sieve into a sterile container while adults were held on the sieve. The NBL were then washed by centrifugation three times with warm saline, and finally suspended in saline. The NBL in 0.05 ml were counted using a slide scratched to form a grid and the volume adjusted by concentration or dilution to prepare the needed number of NBL in 0.25 ml.

The culture medium was composed of the following:

| | |
|---|---|
| NCTC-135 medium with L-Glutamine | 100 ml |
| Mycostatin suspension | 0.5 ml |
| Penbritin solution | 1 ml |
| Calf serum | 10 ml |

Preparation of Mycostatin suspension:

The contents of 1 vial of Mystatin 500,000 units (E. R. Squibb and Sons, Inc., New York) were added to 20 ml of basic culture medium,

and kept at 4°C until needed.

Preparation of Penbritin solution:

The contents of 2 vials Penbritin (500 mg Penbritin ampicillin sodium, Beecham Research Laboratories, Brentford, England) were added to 20 ml of the basic culture medium and stored at 4°C until used.

e) Infection with NBL:

Mice in cages were left in the incubator at 37°C for 10 minutes to ensure that the tail vein was engorged. A mouse was then held inside a small plastic container with the tail protruding through a hole at the end. The required number of NBL prepared was injected into the tail vein.

3. Determination of rates of infection:

a) Determination of the number of larvae in the muscles:

Mice were digested as described in 2a) above and the muscle larvae collected and washed on a 200 mesh per inch screen. The larvae were suspended in tap water and stirred with a magnetic stirrer. Aliquots were counted in a McMaster chamber and the number of larvae in the mouse calculated as in 2a) (above).

b) Determination of the number of adult worms in the intestine of mice:

The mouse was killed by cervical dislocation, the abdominal wall opened and the small intestine was removed from the body and slit open longitudinally. This was done immediately after death. The

intestine was washed quickly in warm tap water to remove the detritus, then put in a small container containing 50 ml of 0.85% saline which was incubated at 37°C for 1 hour. The intestine was shaken well in the solution, rinsed again in a similar amount of saline and finally removed and discarded. The worms were allowed to sink to the bottom of the container and the larger part of the saline decanted. The remainder of the saline containing worms was poured into a petri dish with parallel lines etched in its base and counted under a dissecting microscope.

4) Treatment of infected animals:

Termination of the intestinal stage in infected animals was needed in some experiments. A 5% solution of the anthelmintic drug, Methyridine, was used. A dose of 500 mg per kg body weight, i.e. 0.1 ml of the 5% solution per 10 g was given unless otherwise mentioned. The drug was inoculated subcutaneously in the back of each mouse at 3 hourly intervals.

Statistical evaluation:

The Student's 't' test was used to evaluate the significance of data. P values $\leq$ 0.05 were considered significant.

CHAPTER 3

# Effect of different stages of T. spiralis infection on the number of plaque forming cells

## Introduction

Like many other parasitic agents T. spiralis causes an altered state of cellular and humoral immune responsiveness to heterologous antigens.

Chimishkyan et al. (1974) and Jones et al. (1976) demonstrated that depression of SRBC-PFC in T. spiralis infected animals was greatest when the infection was 20 days old. It has been reported that SRBC-PFC were depressed when mice were infected with T. spiralis larvae 14 days (Lubiniecki and Cypess, 1975a) but not 7 days (Lubiniecki et al., 1974b) before immunization with SRBC. Faubert suggested that the depression of the immune response induced by T. spiralis is a transitory phenomenon related to the migrating phase of the parasite. The depression of PFC in his animals was detected 14 but not 7 days after infection. Ljungstrom and Huldt (1977) confirmed the ability of 3 and 6 weeks but not 1 week old infections to depress PFC responses to SRBC.

In this study, it was decided to adopt different approaches to determine which stage of T. spiralis induced immunodepression in mice.

## Determination of antibody forming cells by plaque assay:

In 1963 Jerne and Nordin modified the virologist's plaque technique

to detect those cells in fresh lymphoid tissue which were producing antibody. They showed that a plaque, or a clear area of haemolysis, formed around antibody-producing cells suspended in target cells in the presence of complement. In this way a population of lymphoid cells can be screened for Plaque Forming Cells (PFC) (antibody producers). The technique can be used to detect not only those cells producing IgM antibodies but also those producing IgG and IgA (Dresser and Wortis, 1965) by adding anti-IgG or anti-IgA respectively to the system.

The plaque technique has been modified for use on a microscale by Cunningham and Szernberg (1968). In principle, a suspension of fresh lymphoid cells from a mouse, immunized with sheep red blood cells, is mixed with SRBC and complement and pipetted into chambers (formed by sealing together two microscope slides), thus forming a monolayer of cells. Complement dependent lysis occurs around those lymphoid cells which have produced specific haemolysing antibody. With suitable illumination those plaques can be counted under the dissecting microscope.

a) Immunization of animals:

Sheep red blood cells (SRBC) were obtained from Oxoid Ltd., England, as 25 ml of SRBC in Alsever's solution. The cells were always checked for signs of haemolysis (crenation of cells and pink supernatant), if either feature was present the cells were discarded. Cells were not used if older than 4 weeks. To prepare cells for use they were centrifuged at 1000 g for 5 minutes with an equal quantity of phosphate buffered saline (PBS), pH 7.2. The supernatant was removed and the cells resuspended in PBS and washed 3 times. After the last wash, the

packed cells were resuspended in PBS to form 10% suspension. Fresh preparation of the 10% suspension was made for each experiment. The cells were counted in an improved Neubauer haemocytometer and a preparation was made to get $10^9$ cells in 0.25 ml then injected intraperitoneally into each mouse.

b) Preparation of the medium:

Gey's solution (Dresser and Wortis, 1967) was used throughout the assays. The composition of the medium used was as follows:

Solution A:

| | |
|---|---|
| NaCl | 70 g |
| KCl | 3.7 g |
| $NaHPO_4 12H_2O$ | 3.01 g |
| $KH_2PO_4$ | 0.237 g |
| Glucose | 10 g |
| Phenol red | 0.10 g |
| Distilled $H_2O$ | to 1 litre |

Solution B:

| | |
|---|---|
| $MgCl.6H_2O$ | 0.42 g |
| $MgSO_4.7H_2O$ | 0.14 g |
| $CaCl_2$ | 0.34 g |
| Distilled $H_2O$ | to 100 ml |

Solution C:

| | |
|---|---|
| $NaHCO_3$ | 2.25 g |
| Distilled $H_2O$ | to 100 ml |

Solutions A, B and C were sterilized by autoclaving for 10 minutes at 10 lb per square inch pressure and stored at 4°C. The final solution was prepared fresh on the day of the experiment by mixing 10 ml of solution A, 5 ml of solution B, 5 ml of solution C and 80 ml of distilled water to give a final volume of 100 ml Gey's solution. The pH was adjusted to 7.2 with $CO_2$ gas.

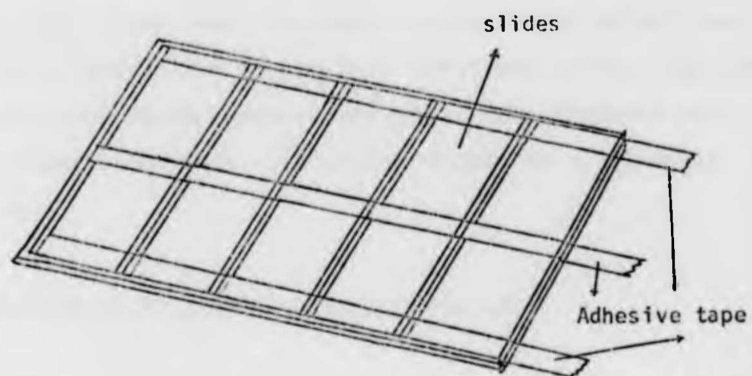
c) Preparation of the complement (C'):

Albino guinea-pigs (the coloured variety are not such a good source of C') were bled by heart puncture and the blood left at room temperature for 30 minutes, then at 4°C overnight. Next day, the blood clot was detached from the sides of the tubes. The samples were centrifuged twice, in a refrigerated centrifuge for 20 minutes. The collected sera were pooled and absorbed with 1 ml of packed, washed SRBC to every 20 ml guinea pig serum at 4°C for 20 minutes. After centrifugation at 1300 g for 5 minutes the supernatant was removed and either used immediately or stored in aliquots of 1 ml at -70°C. Immediately before use, the required volume of serum was thawed and diluted 1:1 with cold Gey's solution.

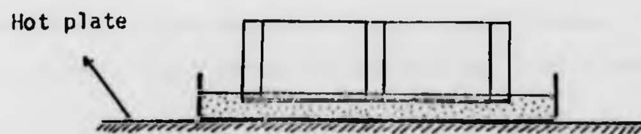
d) Preparation of Cunningham chambers:

A row of scrupulously clean microscope slides was laid side by side on a flat surface against a straight edge and strips of 6 mm wide, pressure sensitive, double sided, self adhesive Scotch tape applied in 3 parallel stripes, to divide the slides into two areas (Fig. 3a). The backing of the tape was peeled off and another layer of slides

Figure 3 : Cunningham slide chambers.




a) Making the chambers.




b) Sealing the chamber in molten wax.

placed exactly on top of the first row, then the two layers of slides were pressed together with a roller. The tape was trimmed off at the ends of the row of the double slides. Each chamber was marked into squares with a diamond or a Pentel pen and were kept dust free. Before use, slide chambers were separated by breaking them apart from their neighbours. Each slide chamber had a volume of 0.18-0.2 ml.

e) Preparation of the suspension of lymphoid cell:

A mouse which had been previously sensitized by the intraperitoneal injection of $10^9$ washed SRBC was killed by cervical dislocation. The abdominal cavity was opened and the spleen dissected free from fat and removed into a plastic tea-strainer lying in a pool of ice-cold Gey's solution in a small petri dish. The spleen was gently macerated with a syringe plunger and the spleen suspension transferred to a centrifuge tube placed on ice. The strainer and dish were washed and the suspension was made up to 8 ml with cold Gey's solution, then it was centrifuged at 550 g for 5 minutes. The supernatant was discarded and the spleen cells suspended incold Gey's solution and centrifuged again and then resuspended in a final volume of 4 ml for each spleen. Dilutions were made as 1/100 on day 4 assay and 1/50 on day 7 and 9 assays.

f) Viability test (trypan-blue exclusion test):

To determine how many spleen cells were alive and how many were dead during the preparation of the spleen cell suspension a dye exclusion test with 0.2% trypan blue was used. Immediately before use, trypan blue was diluted with 4.25% NaCl (4:1) to render it isotonic. Spleen

cell suspensions were diluted 1:20 in the prepared trypan blue solution and well mixed. The cells were counted in a Neubauer haemocytometer and the coloured and natural cells recorded (dead cells will take up the stain, enlarge and then burst, while living cells retained their size, shape and colour). This was done for each spleen cell suspension, and the test was only considered valid when the viability was more than 75%.

g) Preparation of RBC for the assay:

SRBC were obtained and washed 3 times in PBS as mentioned before in (a). After the last wash, the packed cells were resuspended 1:6.6 in fresh Gey's solution.

Horse red blood cells (HRBC) were obtained from Oxoid Ltd. as defibrinated blood. The cells were washed in the same way as SRBC. However, HRBC, having no preservative, were more sensitive and were not used if older than two weeks. After the last wash the cells were suspended up to the required volume.

h) Procedure of Cunningham modification of the Jerne plaque assay: (Cunningham and Szenberg, 1968)

The chambers were prepared as described before (d). The following suspension mixture was prepared in duplicate for each spleen in plastic disposable haemagglutination plates (Biocult, Linbro). The mixture was mixed well and then was pipetted into each chamber using an Oxford dispenser:

cell suspensions were diluted 1:20 in the prepared trypan blue solution and well mixed. The cells were counted in a Neubauer haemocytometer and the coloured and natural cells recorded (dead cells will take up the stain, enlarge and then burst, while living cells retained their size, shape and colour). This was done for each spleen cell suspension, and the test was only considered valid when the viability was more than 75%.

g) Preparation of RBC for the assay:

SRBC were obtained and washed 3 times in PBS as mentioned before in (a). After the last wash, the packed cells were resuspended 1:6.6 in fresh Gey's solution.

Horse red blood cells (HRBC) were obtained from Oxoid Ltd. as defibrinated blood. The cells were washed in the same way as SRBC. However, HRBC, having no preservative, were more sensitive and were not used if older than two weeks. After the last wash the cells were suspended up to the required volume.

h) Procedure of Cunningham modification of the Jerne plaque assay:
(Cunningham and Szenberg, 1968)

The chambers were prepared as described before (d). The following suspension mixture was prepared in duplicate for each spleen in plastic disposable haemagglutination plates (Biocult, Linbro). The mixture was mixed well and then was pipetted into each chamber using an Oxford dispenser:

| | |
|---|---|
| Gey's solution (or antiserum for indirect plaques) | 0.04 ml |
| SRBC (1:6.6 ml in Gey's solution) | 0.02 ml |
| Complement (1:1 in Gey's solution) | 0.02 ml |
| Spleen cell suspension (1/50 or 1/100) | 0.10 ml |

Any residual space was filled with Gey's solution. For each spleen cell suspension, duplicate chambers were set up, unless otherwise mentioned. Chambers were sealed on both edges with molten wax (equal parts of paraffin wax, m.p 56°C and petroleum jelly) (Fig. 3b). Chambers were incubated at 37°C, horizontally in a moist box, for 60-75 minutes to allow cells to form a monolayer.

Plaques (Fig. 4) were counted under a dissecting microscope. The substage mirror was adjusted to give semidark ground illumination. Each plaque was counted (if in doubt, they were examined under the binocular microscope x 40 , to be sure that a clear area had a lymphoid cell in the centre). The number of plaque forming cells (PFC) per spleen were calculated as follows:

Number of PFC per spleen = N x 10 x d where:

N = Mean number of PFC per chamber (in 0.1 ml spleen).

d = dilution used.

Figure 4 :

Photograph of a plaque showing antibody forming cell at the centre of an area of haemolysis (x 40).

## Plaque Forming Cell Assay: Preliminary Experiments

### Experiment 3.1

PFC assay relies on antibody-mediated complement-dependent lysis. Where a single IgM molecule on an erythrocyte can fix complement and cause lysis, several thousands of IgG antibody molecules are required for the same effect. The addition of an optimum dilution of anti IgG helps the formation of γ globulin complexes which fix complement and cause lysis.

This experiment was designed to determine the optimum day for assaying IgM and PFC and to determine the optimum dilution of rabbit antimouse IgG to be used in the indirect assay for development of IgG PFC.

60 mice were injected i.p. with 0.25 ml of a 10% suspension of SRBC ($10^9$ cells). 5 immunized and 5 unimmunized controls were killed and a direct assay performed daily from a pool of the spleens of each group. Indirect PFC, using different dilutions of rabbit antimouse IgG (developing serum obtained from Miles-Yeda Ltd.) in 0.85% NaCl (1/10, 1/25, 1/50, 1/100, 1/250, 1/500, 1/1000), were determined on days 4, 8, 9 and 10. Two chambers were made for each dilution of antiserum.

The results of the assay for direct PFC (Table 1 and Fig. 5) show a sharp peak at day 4 and it was decided that in future experiments the assay for IgM PFC would be performed normally on day 4. Table 2 shows the number of indirect PFC found when different dilutions of developing serum were used on days 4, 8, 9 and 10. The maximum numbers of indirect PFC were detected at 1/500 or 1/250 dilutions, but plaques were clearer

Table 1.

The number of IgM (direct) PFC in spleens of immunized and unimmunized animals at different times after immunization.

| Days after immunization | Mean no. of PFC/spleen x $10^3$ | |
|---|---|---|
| | immunized | unimmunized |
| 2 | 86 | 2 |
| 3 | 60 | 24 |
| 4 | 780 | 12 |
| 5 | 630 | 24 |
| 6 | 614 | 16 |
| 7 | 286 | 16 |
| 8 | 306 | 26 |
| 9 | 116 | 14 |
| 10 | 106 | 28 |
| 11 | 136 | 10 |
| 12 | 176 | 6 |

Figure 5 :

The number of Ig M ( direct ) PFC in spleens of immunized and un immunized animals at different times after immunization

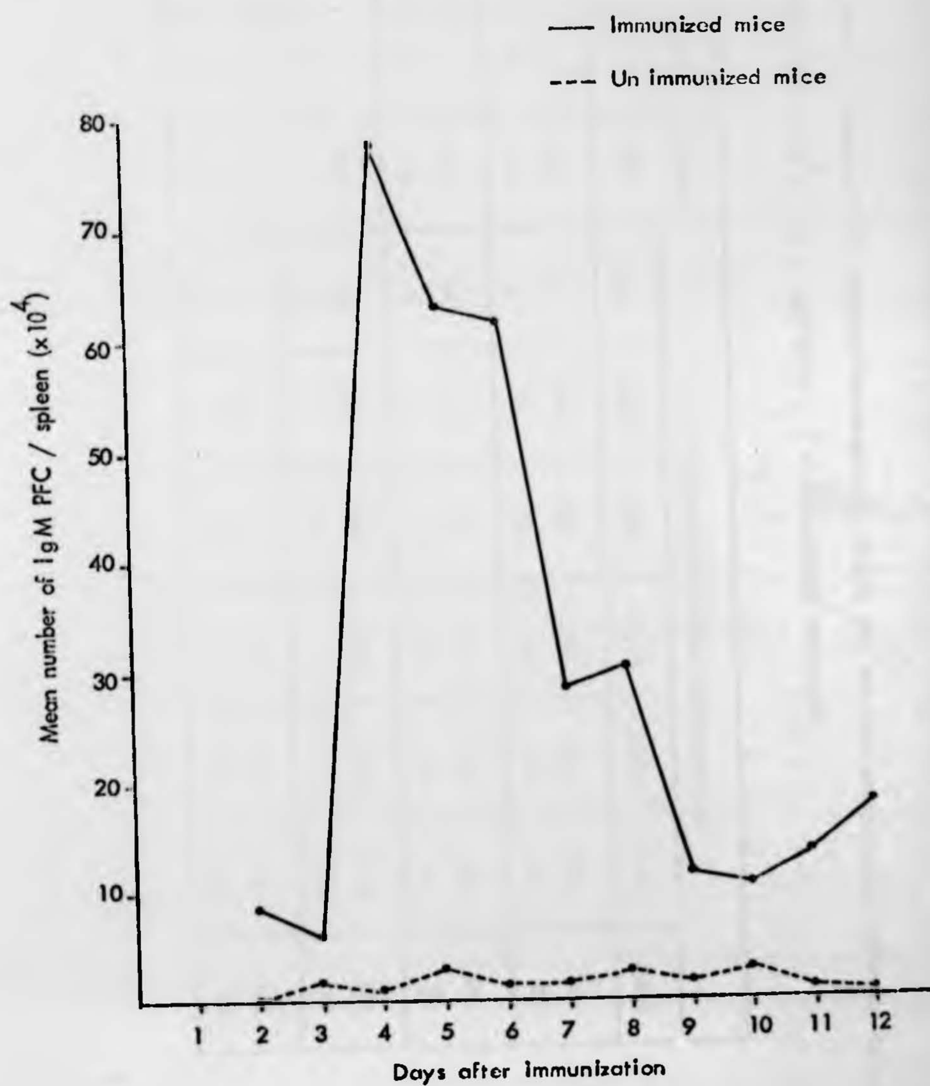


Table 2

Numbers of PFC found in immunized and unimmunized animals by direct and indirect assays using different dilutions of rabbit antimouse IgG (x $10^3$). (I = Immunized ; C = Unimmunized controls)

| Days after immunization | Group | Direct PFC | Dilutions of rabbit antimouse IgG | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1/10 | 1/25 | 1/50 | 1/100 | 1/250 | 1/500 | 1/1000 |
| 4 | I | 780 | 61 | 558 | 558 | 598 | 750 | 758 | 638 |
| | C | 12 | 4 | 4 | 12 | 8 | 12 | 44 | 28 |
| 8 | I | 306 | 14 | 8 | 80 | 512 | 554 | 546 | 188 |
| | C | 26 | 0 | 8 | 4 | 14 | 14 | 20 | 22 |
| 9 | I | 116 | 32 | 196 | 346 | 328 | 344 | 370 | 302 |
| | C | 14 | 2 | 2 | 4 | 6 | 8 | 14 | 14 |
| 10 | I | 106 | 4 | 116 | 102 | 166 | 134 | 160 | 126 |
| | C | 28 | 2 | 2 | 28 | 26 | 32 | 40 | 20 |

and larger at 1/500. Inhibition of IgM PFC was detected at high concentrations of antiserum. The inhibitory effect of different dilutions of antisera on mouse IgM plaques 4 days after immunization are plotted in Fig. 6 and the inhibition constant (KI) of the dilution of anti IgG, it was decided to use (1/500), was calculated as follows:

$$\text{KI } 1/500 = \frac{\text{Mean PFC with antiserum per spleen}}{\text{Mean PFC without antiserum per spleen}} = \frac{758}{780}$$

$$= 0.97$$

When the experiment was repeated using spleen cells immunized 9 days before, the developing effect of the different dilutions of antiserum was measured (Fig. 7). The developing constant (KD) of the chosen dilution was as follows:

$$\text{KD } 1/500 = \frac{\text{Maximum PFC} - (\gamma\text{M} \times \text{KI at that concentration})}{\text{PFC at } 1/500 - (\gamma\text{M} \times \text{KI at } 1/500)}$$

$$= \frac{370 - (116 \times 0.97)}{370 - (116 \times 0.97} = 1.0$$

Ideally the KI and KD of a developing serum should be 1.0 if the serum does not react with IgM and if used at optimum concentration (Dresser and Wortis, (1967). The basis for calculation of IgM (direct) and IgG (indirect) PFC/spleen for each animal was as follows:

Developed PFC = /Total PFC in treated chamber - KI (PFC in untreated chamber)/ KD

Since the KI in this experiment was 0.97 for easier calculation it was decided to use the following:

IgG PFC = Total PFC in treated chamber - PFC in untreated chamber.

Figure 6 :

Plot of K I titration, using spleen cells from mice immunized 4 days before. The dashed line represents the mean number of direct PFC and the arrow indicates the dilution of anti-serum it was decided to use 1/500

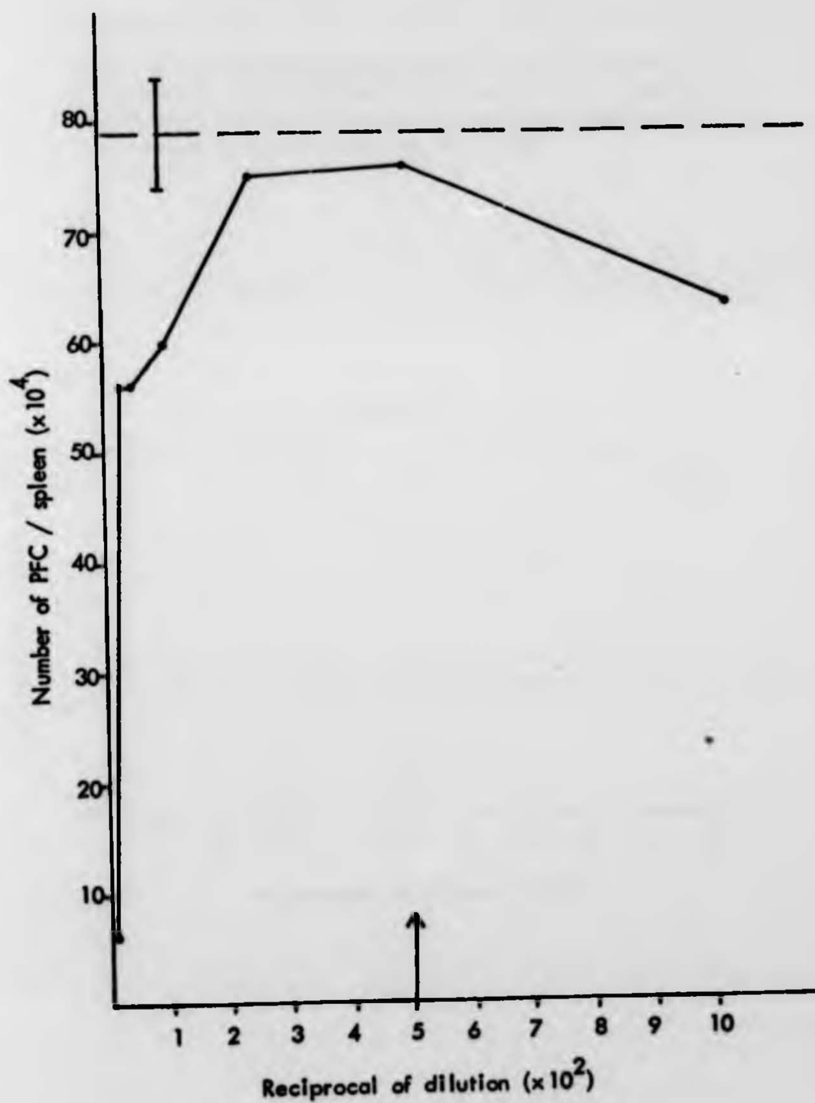
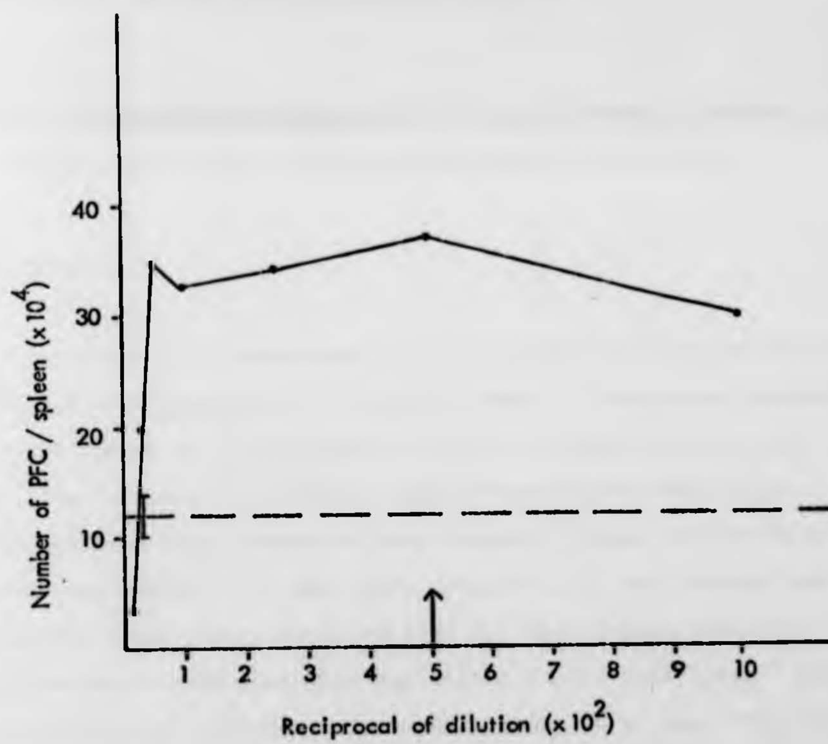


Figure 7 :

Plot of KD titration, using spleen cells 9 days after immunization. The dashed line represents the mean number of direct PFC and the arrow indicates the dilution of anti serum it was decided to use 1/500



Experiment 3.2

The aim of this experiment was essentially to determine the optimum day for assaying IgG PFC.

A daily assay for indirect PFC using 1/500 dilution of antimouse IgG was carried out on mice which had been given $10^9$ SRBC i.p. The results are shown in Table 3 and Fig. 8. From these results it can be seen that the greatest number of PFC were found on day 9 and this was chosen as the day of the indirect assay.

## Effect of Trichinella spiralis infection on the number of plaque forming cells in mice immunized with sheep red blood cells

Experiment 3.3

The aim of this experiment was to study the immunodepressive effect of a 30 day old *T. spiralis* infections in mice. 15 mice were infected with 400 larvae of *T. spiralis* and 10 kept as uninfected controls. 3 days after infection, 5 infected animals were killed and adults recovered from their intestines were counted. A mean of 167 (SE 69) adults were counted. 30 days after infection, all the infected and uninfected animals were immunized with $10^9$ SRBC intraperitoneally. 5 infected and 5 uninfected mice were killed 4 and 9 days later. Spleens were removed and assayed for the number of IgM PFC on day 4 and for the number of IgM and IgG PFC on day 9.

Results are shown in Table 4 and Fig. 9. Significantly fewer IgM PFC were detected in spleens of infected mice 4 days after immunization

Table 3

The number of IgG PFC in the spleens of immunized and unimmunized animals at different times after immunization.

| Days after immunization | Mean no. of PFC/spleen x $10^3$ | |
|---|---|---|
| | immunized | unimmunized |
| 2 | 6 | 8 |
| 3 | 72 | -4 |
| 4 | -22 | 32 |
| 5 | 108 | 18 |
| 6 | 202 | -2 |
| 8 | 240 | -6 |
| 9 | 254 | 0 |
| 10 | 54 | 12 |
| 11 | -44 | -4 |
| 12 | 18 | 6 |

Figure 8 :

The number of IgG PFC in spleens of immunized and unimmunized animals at different times after immunization

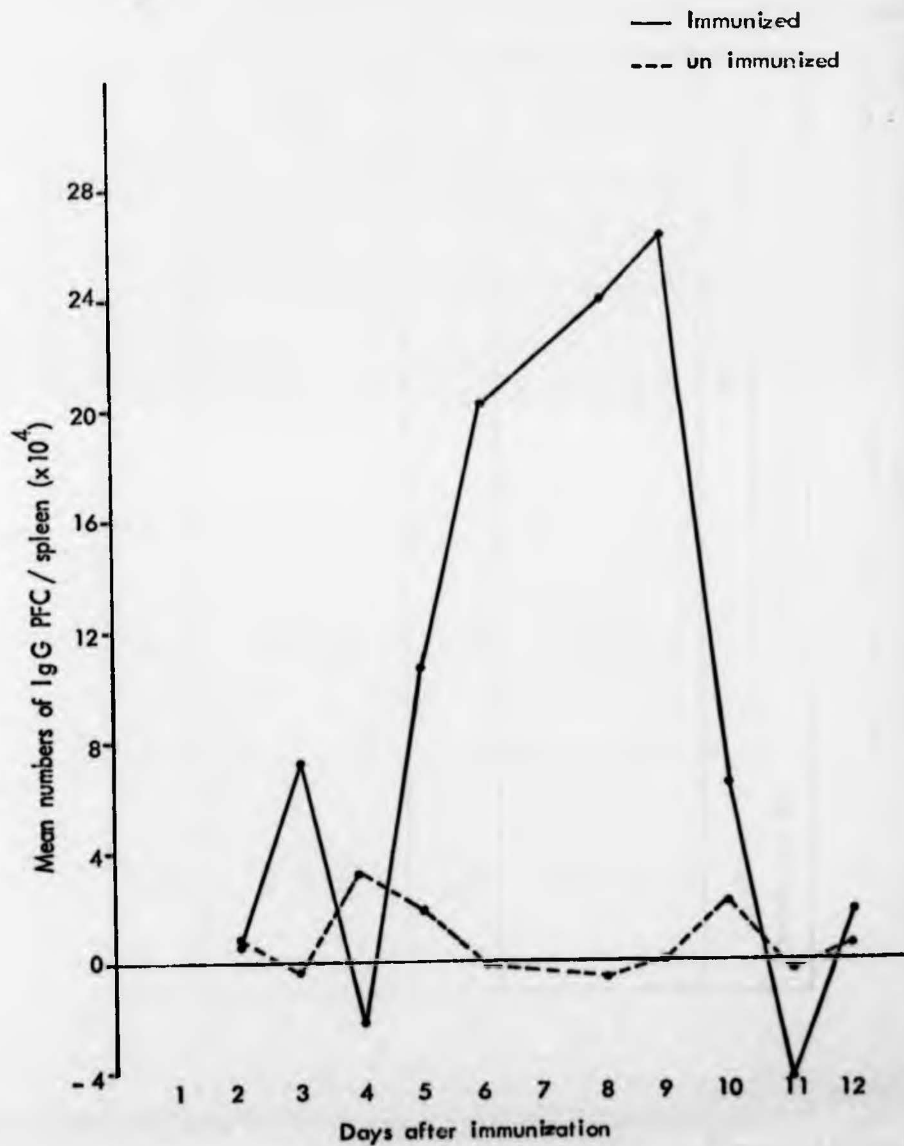


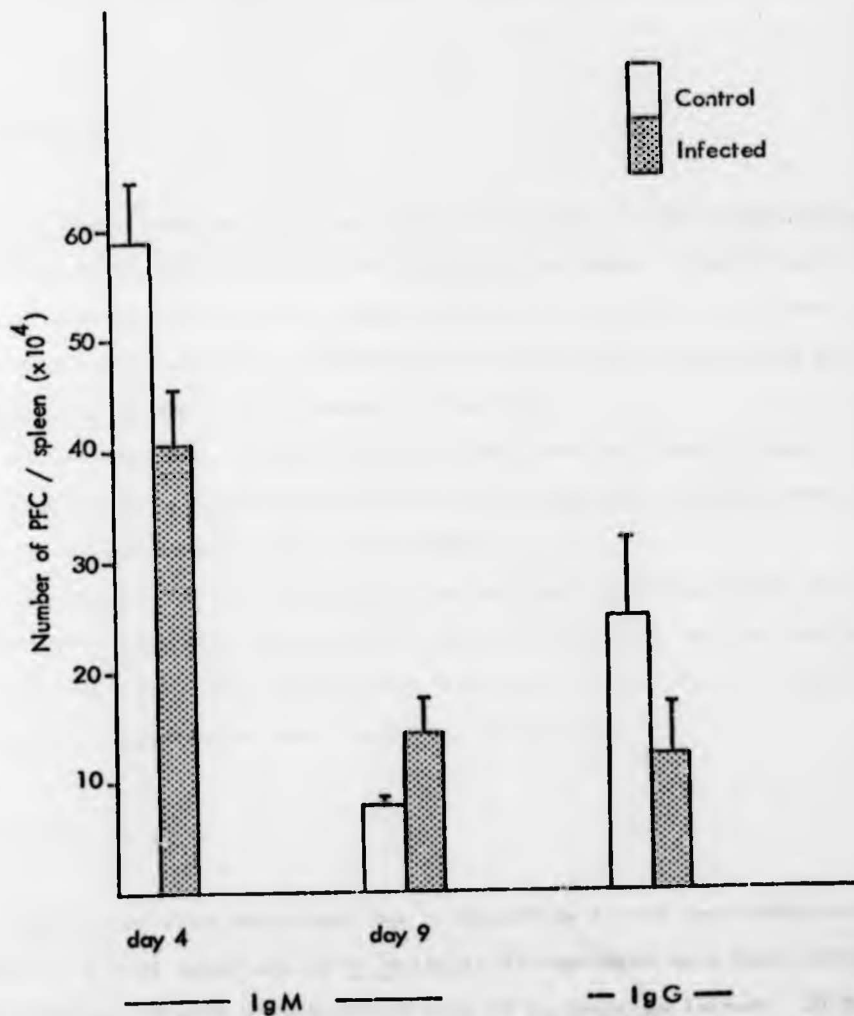
Table 4. Effect of a 30 day old *T. spiralis* infection on the number of IgM and IgG PFC produced in the spleen in response to inoculation of SRBC.

| Mouse no. | | IgM PFC/spleen ($\times 10^3$) | | IgG PFC/spleen ($\times 10^3$) |
|---|---|---|---|---|
| | | day 4 | day 9 | |
| Infected | 1 | 312 | 82 | 1 |
| | 2 | 320 | 96 | 96 |
| | 3 | 340 | 190 | 173 |
| | 4 | 496 | 210 | 223 |
| | 5 | 560 | | |
| Mean ± SE | | * 406 ± 51 | * 145 ± 32 | 123 ± 48 |
| Control | 1 | 440 | 54 | 103 |
| | 2 | 540 | 80 | 145 |
| | 3 | 552 | 82 | 348 |
| | 4 | 638 | 96 | 394 |
| | 5 | 770 | | |
| Mean ± SE | | 588 ± 55 | 78 ± 9 | 247 ± 72 |

* statistically significant

Figure 9 :

Effect of 30 days infection of T.spiralis on the number of IgM and IgG PFC produced in the spleens in response to inoculation of SRBC.



when compared to controls ($p < 0.025$) while there was a significant increase in IgM PFC on day 9. There was no significant difference in the number of IgG PFC in spleens of infected and control animals on day 9.

The results of this experiment indicate that T. spiralis infection depresses the immune response to SRBC. Depression was only of IgM PFC on day 4.

Experiment 3.4

This experiment was also carried out to study the immunodepression caused by a 30 day old infection of T. spiralis in mice. 22 mice were used in this experiment on the same basis as the previous experiment but animals were killed 7 days after sheep erythrocyte inoculation and spleens were assayed for the number of IgM PFC.

Table 5 and Fig. 10 show the individual data and means. There were significantly fewer PFC in the spleens of infected animals than in those of the control group ($p < 0.0005$).

The results of this experiment confirm that immunodepression is a feature of T. spiralis infection in mice and when compared with results of experiment 3.3 would suggest that depression of IgM PFC is a temporary phenomenon (depressed at day 4 and 7 but not day 9).

Experiment 3.5

The aim of this experiment was to determine if the immunodepressive effect of a full infection of T. spiralis is dependent on a high level of infection. 5 mice were infected with 50 T. spiralis larvae. 30 days

Table 5

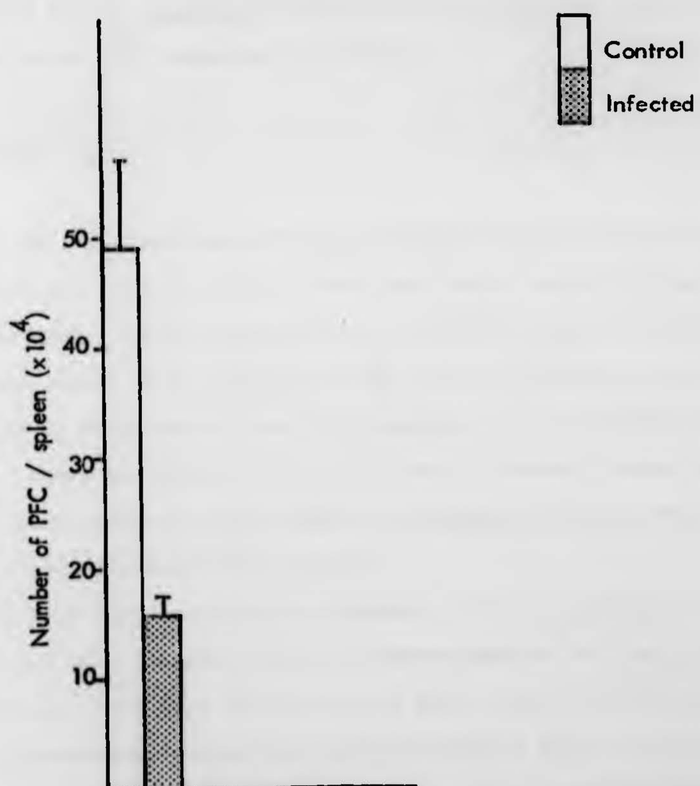
Effect of a 30 day old T. spiralis infection on IgM PFC 7 day response to inoculation of SRBC.

| Mouse no. | No. of PFC/spleen x $10^3$ | |
|---|---|---|
| | Infected | Control |
| 1 | 21 | 149 |
| 2 | 87 | 193 |
| 3 | 142 | 283 |
| 4 | 143 | 305 |
| 5 | 148 | 421 |
| 6 | 153 | 511 |
| 7 | 154 | 602 |
| 8 | 176 | 735 |
| 9 | 187 | 839 |
| 10 | 188 | 856 |
| 11 | 228 | |
| 12 | 250 | |
| Mean ± SE | * 156 ± 17 | 489 ± 83 |

* statistically significant

Figure 10 :

Effect of 30 day T. sprialis infection on IgM PFC 7 day response to inoculation of SRBC post infection



later, the infected animals and a group of uninfected mice were given $10^9$ sheep erythrocytes each. 4 days later all these mice were killed and their spleens removed and assayed for the number of IgM PFC. The numbers of PFC from spleens of infected animals were significantly less than those of the control group ($p < 0.005$) (Table 6 and Fig. 11). The infected mice were digested and yielded a mean of 3186 (SE 539) muscle larvae.

The results of experiments 3.3, 3.4 and 3.5 confirm the previous reports that a full T. spiralis infection can decrease the number of PFC produced after SRBC have been inoculated.

Experiment 3.6

One of the complications with T. spiralis infections is that from 5 days onwards the host is infected with both adult worms and their parenteral progeny. This experiment was designed to test the effect of the intestinal phase of T. spiralis on the production of PFC against SRBC by removing adult worms from the intestine by use of anthelmintic methyridine before any newborn larvae had been produced. Methyridine is a potent anthelmintic against adult T. spiralis (Denham, 1965).

40 mice were divided into 4 groups:

Group 1 : 10 mice received an infection of 400 T. spiralis larvae per os each and were treated twice with methyridine at 500 mg/kg 5 days after infection. They were immunized with SRBC a day after treatment. This group is hereafter called the infected-treated group. Group 2 : 10 mice were not infected but received the same dose of methyridine and SRBC, to determine the effect of methyridine, if any, on the immune response. This group is hereafter called the uninfected-treated group. Group 3 : 10 mice were neither infected nor treated but were immunized

later, the infected animals and a group of uninfected mice were given $10^9$ sheep erythrocytes each. 4 days later all these mice were killed and their spleens removed and assayed for the number of IgM PFC. The numbers of PFC from spleens of infected animals were significantly less than those of the control group ($p < 0.005$) (Table 6 and Fig. 11). The infected mice were digested and yielded a mean of 3186 (SE 539) muscle larvae.

The results of experiments 3.3, 3.4 and 3.5 confirm the previous reports that a full T. spiralis infection can decrease the number of PFC produced after SRBC have been inoculated.

Experiment 3.6

One of the complications with T. spiralis infections is that from 5 days onwards the host is infected with both adult worms and their parenteral progeny. This experiment was designed to test the effect of the intestinal phase of T. spiralis on the production of PFC against SRBC by removing adult worms from the intestine by use of anthelmintic methyridine before any newborn larvae had been produced. Methyridine is a potent anthelmintic against adult T. spiralis (Denham, 1965).

40 mice were divided into 4 groups:

Group 1 : 10 mice received an infection of 400 T. spiralis larvae per os each and were treated twice with methyridine at 500 mg/kg 5 days after infection. They were immunized with SRBC a day after treatment. This group is hereafter called the infected-treated group. Group 2 : 10 mice were not infected but received the same dose of methyridine and SRBC, to determine the effect of methyridine, if any, on the immune response. This group is hereafter called the uninfected-treated group. Group 3 : 10 mice were neither infected nor treated but were immunized

Table 6

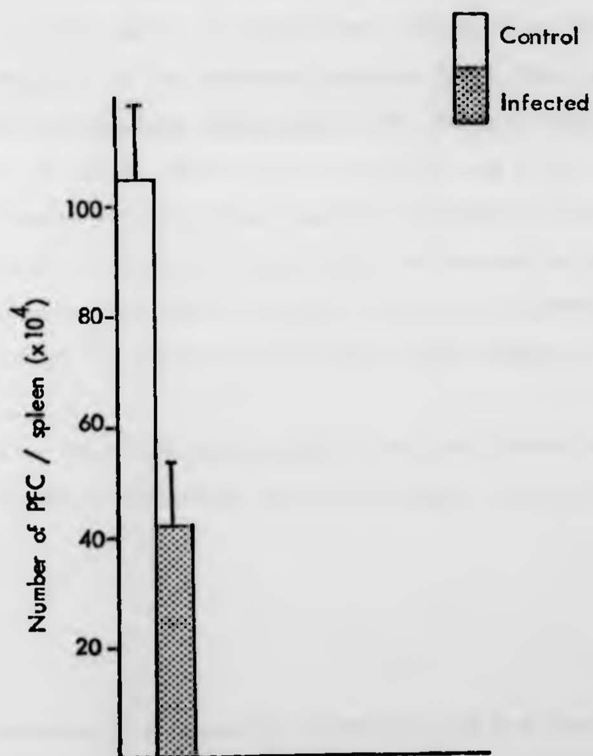
Effect of a light 30 day old infection of T. spiralis on spleen IgM PFC assayed 4 days after immunization with SRBC.

| Mouse no. | No. of PFC/spleen (x $10^3$) | |
|---|---|---|
| | Infected | Control |
| 1 | 132 | 716 |
| 2 | 300 | 802 |
| 3 | 416 | 1010 |
| 4 | 420 | 1490 |
| 5 | 834 | 1220 |
| Mean ± SE | * 420 ± 116 | 1048 ± 141 |

* statistically significant

Figure 11 :

Effect of light 30 day old infection of T. spiralis on spleen IgM PFC assayed 4 days after immunization with SRBC



with SRBC. 5 mice of each group were killed 4 and 9 days after immunization. Spleens were assayed for the number of IgM and IgG PFC. Group 4 : 10 mice were infected with 400 T. spiralis larvae. A mean of 251 (SE 50) adult worms were recovered from 5 animals killed 3 days post infection. The rest of the animals were treated with methyridine 5 days post infection and killed 2 days later to check for the therapeutic effect of methyridine. No worms were found in these mice.

The results of PFC assay are shown in Table 7 and means are represented in Fig. 12a and b. A significant reduction in the number of IgM PFC was detected in the infected group on day 9 when compared to the methyridine and control groups ($p < 0.01$ and $p < 0.025$) respectively but not on day 4. The number of IgG PFC was significantly higher in the infected-treated group than the uninfected-treated one ($p < 0.05$). However there was no significant difference between the infected and uninfected untreated control. The drug treatment had no significant effect on the number of IgM and IgG PFC either on day 4 or day 9.

The results of this experiment suggest that the intestinal stage of T. spiralis infection depresses the IgM response to SRBC but not to IgG response.

Experiment 3.7

This was essentially a repeat of experiment 3.6 but the mice were killed 7 days after immunization with SRBC and their spleens assayed for the number of IgM PFC. There were 4 mice in each group. Results of PFC assays are shown in Table 8 and Fig. 13. Methyridine treatment

Table 7

Effect of the intestinal stage of T. spiralis on the PFC response to SRBC when assayed 4 and 9 days post immunization.

| Group | Mouse no. | No. of PFC/spleen x $10^3$ | | |
|---|---|---|---|---|
| | | IgM PFC | | IgG PFC |
| | | day 4 | day 9 | |
| Infected/ methyridine treated | 1 | 168 | 8 | 32 |
| | 2 | 168 | 10 | 52 |
| | 3 | 192 | 12 | 72 |
| | 4 | 548 | 16 | 74 |
| | 5 | 554 | 18 | 78 |
| Mean ± SE | | 326 ± 92 | * 13 ± 2 | * 62 ± 9 |
| Uninfected methyridine treated | 1 | 272 | 16 | -6 |
| | 2 | 280 | 24 | 18 |
| | 3 | 432 | 32 | 40 |
| | 4 | 436 | 34 | 46 |
| | 5 | 460 | 46 | 64 |
| Mean ± SE | | 376 ± 41 | 30 ± 5 | 32 ± 12 |
| Uninfected/ untreated control | 1 | 140 | 12 | 12 |
| | 2 | 298 | 28 | 16 |
| | 3 | 320 | 30 | 74 |
| | 4 | 410 | 66 | 98 |
| | 5 | 656 | 70 | 124 |
| Mean ± SE | | 365 ± 85 | 41 ± 11 | 65 ± 22 |

* statistically significant

Figure 12a :

Effect of intestinal stage of T. spiralis on the IgM PFC response to SRBC when assayed 4 days after immunization

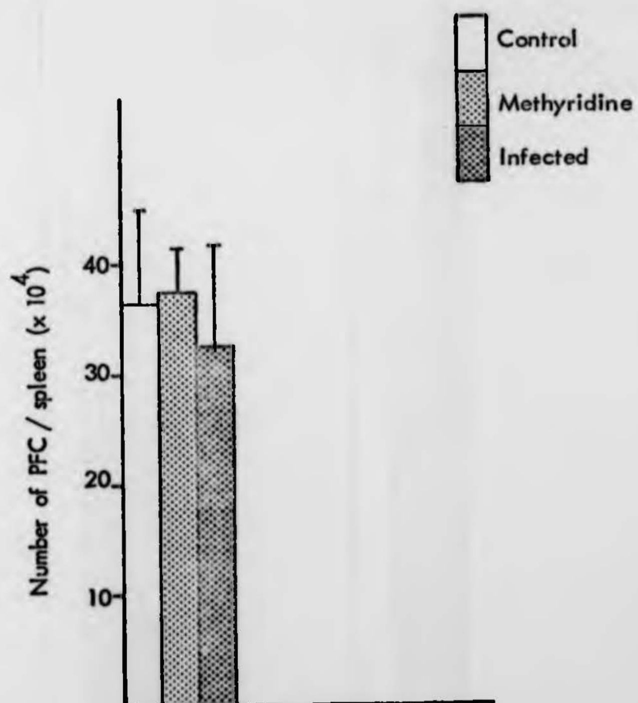


Figure 12b

Effect of the intestinal stage of _T. spiralis_ on the PFC response to SRBC when assayed 9 days post immunization

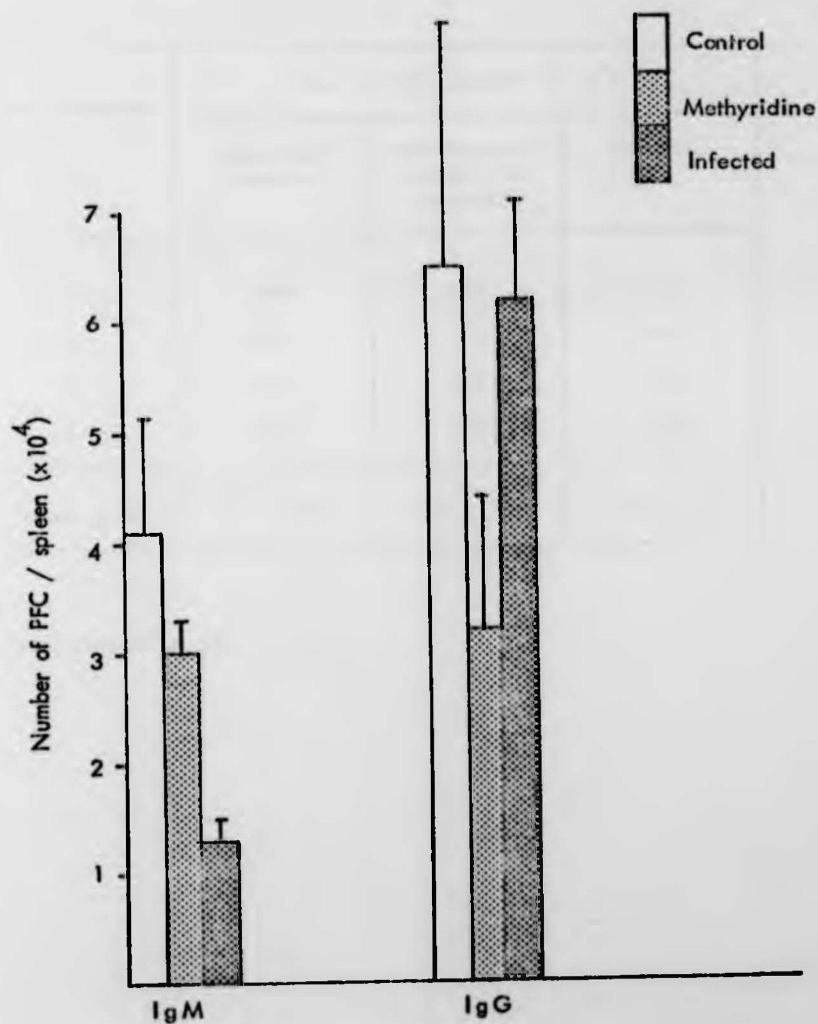


Table 8

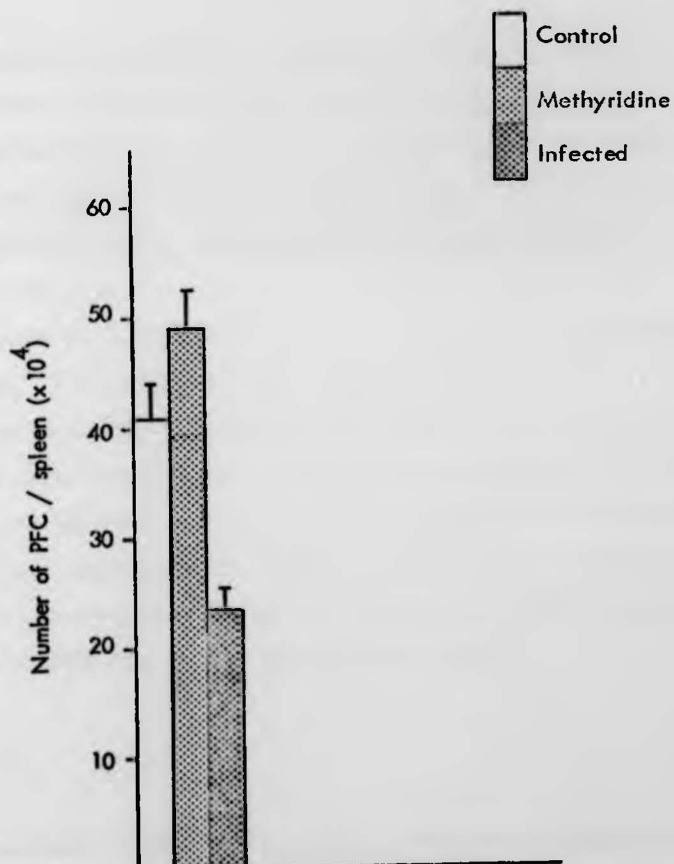
The effect of the intestinal phase of *T. spiralis* infection on the number of PFC 7 days after immunization with SRBC

| No. of mice | No. of PFC/spleen (x $10^3$) | | |
|---|---|---|---|
| | Infected/ treated | Uninfected/ methyridine treated | Control |
| 1 | 186 | 416 | 348 |
| 2 | 221 | 467 | 407 |
| 3 | 261 | 491 | 424 |
| 4 | 272 | 589 | 459 |
| Mean ± SE | * 235 ± 20 | 491 ± 36 | 404 ± 23 |

* statistically significant

Figure 13 :

Effect of the intestinal phase of *T. spiralis* infection on the number of IgM PFC 7 days after immunization with SRBC



had no effect on the number of PFC compared to the control group. A statistically highly significant reduction in the number of PFC occurred in infected animals as compared to the uninfected-treated or control groups ($p < 0.0005$ and $p < 0.0025$) respectively.

Experiment 3.8

This experiment was designed to examine further the immuno-depressive effect of the intestinal stage of T. spiralis infection. On this occasion irradiated larvae were used for infection as these are sexually sterile but develop into adult worms which live for a reasonable time in the gut. A group of 15 mice received 2000 T. spiralis irradiated larvae each. These mice were given large numbers of larvae as irradiation reduces the longevity and viability of T. spiralis. 5 mice were killed 3 days later and a mean of 453 (SE 75) adults recovered. 10 animals were used as uninfected controls. 11 days after infection,each animal received an injection of $10^9$ SRBC. 4 days later all mice were killed, spleens were pooled and 6 chambers were filled from suspensions of spleens of each group. Table 10 and Fig. 15 show that the number of IgM PFC in infected animals was significantly less than that in the controls ($p < 0.01$).

Experiment 3.9

This experiment aimed to study a lower level of irradiated larvae on the number of PFC to SRBC.

10 mice were infected with 400 irradiated T. spiralis larvae and 10 mice were left uninfected and used as controls. 11 days post infection

Table 9

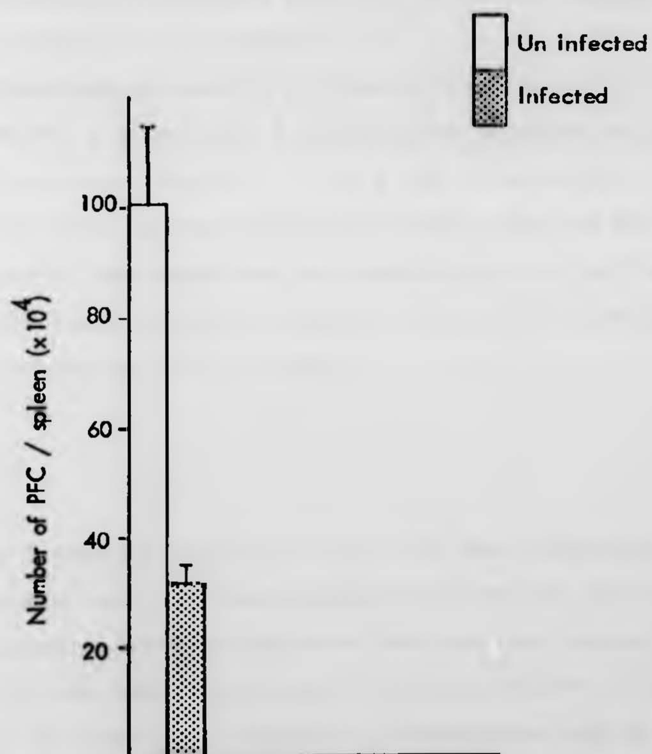
Effect of infection with irradiated (i.e. sexually sterile) *T. spiralis* on the development of spleen PFC in response to the inoculation of SRBC 11 days after infection.

| Mouse no. | Mean no. of PFC/spleen (x $10^3$) | |
|---|---|---|
| | Infected | Uninfected |
| 1 | 188 | 390 |
| 2 | 228 | 393 |
| 3 | 248 | 492 |
| 4 | 275 | 924 |
| 5 | 288 | 1071 |
| 6 | 291 | 1083 |
| 7 | 294 | 1142 |
| 8 | 354 | 1426 |
| 9 | 408 | 1427 |
| 10 | 519 | 1688 |
| Mean $\pm$ SE | * 309 $\pm$ 30 | 1004 $\pm$ 144 |

* statistically significant

Figure 14 :

Effect of infection with irradiated (i.e. sexually sterile) T. spiralis on the development of spleen PFC in response to inoculation of SRBC 11 days after infection



all the animals were immunized with SRBC. Mice were killed 7 days later, spleens were assayed for IgM PFC. The results (Table 9 and Fig. 14) show highly significant reductions in the number of PFC in infected mice compared to controls ($p < 0.0005$).

Experiment 3.10

This experiment was conducted to determine the effect of a low level of infection with irradiated larvae on the immune response to SRBC. It is comparable with experiment 3.5.

13 mice were infected with 50 irradiated larvae each (mean adult recovery 22 SE 3). 8 animals and 9 controls were immunized with $10^9$ SRBC each 11 days after infection. 4 and 9 days later spleens of infected and uninfected groups were assayed for IgM and IgG PFC.

The results of this experiment are shown in Table 11 and Fig. 16 and show no significant difference between infected and uninfected groups either on IgM and IgG PFC numbers.

Experiment 3.11

Since the previous experiments proved that there was depression of the immune response when mice were immunized against SRBC after full *T. spiralis* infection (30 days) and after the intestinal stage, the next step was to see whether the results could be obtained at intermediate stages (14 days) after infection. Observations made at this time after infection show the effect of the migrating stage and the intestinal stage. 55 mice divided into three groups were used in this experiment.

Table 10

Effect of adult stage of *T. spiralis* infection on the number of PFC 7 days after immunization with SRBC. The infection was of irradiated larvae.

| No. of chamber | No. of PFC/spleen (x $10^3$) | |
|---|---|---|
| | Infected | Control |
| 1 | 384 | 552 |
| 2 | 496 | 572 |
| 3 | 532 | 632 |
| 4 | 572 | 712 |
| 5 | 572 | 716 |
| 6 | 580 | 828 |
| Mean ± SE | * 523 ± 31 | 669 ± 42 |

* statistically significant

Figure 15 :

Effect of adult stage of *T. spiralis* infection on the number of PFC 7 days after immunization with SRBC

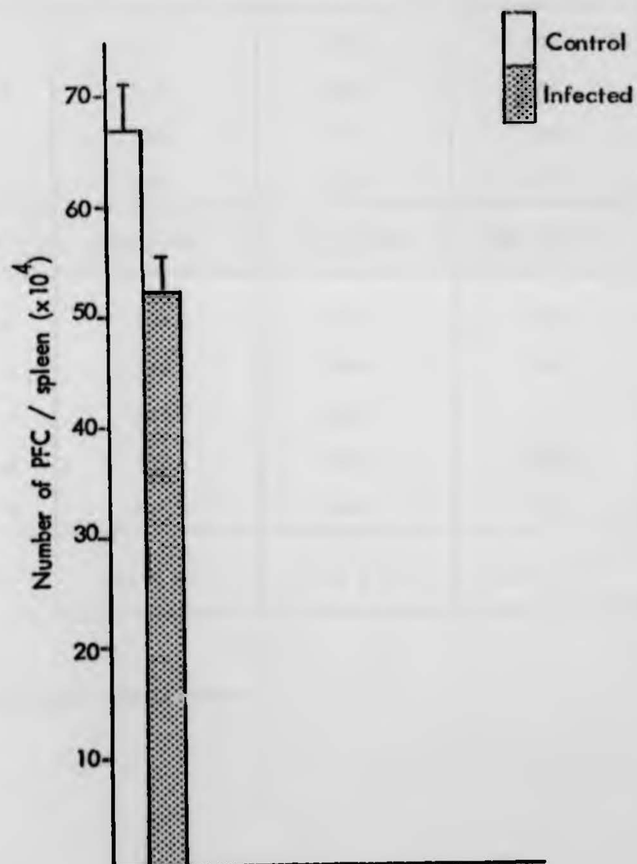


Table 11

Effect of a low level irradiated *T. spiralis* infection on spleen PFC in response to SRBC inoculation

| Group | Mouse no. | No. of PFC/spleen (x $10^3$) | | |
|---|---|---|---|---|
| | | IgM on day 4 | IgM on day 9 | IgG |
| Infected | 1 | 72 | 54 | -52 |
| | 2 | 256 | 106 | -26 |
| | 3 | 324 | 152 | 34 |
| | 4 | 264 | 212 | 201 |
| Mean ± SE | | 229 ± 54 | 131 ± 34 | 39 ± 57 |
| Control | 1 | 184 | 56 | -53 |
| | 2 | 244 | 106 | -27 |
| | 3 | 329 | 120 | -6 |
| | 4 | 376 | 124 | 52 |
| | 5 | n.d. | 164 | 124 |
| Mean ± SE | | 283 ± 42 | 114 ± 17 | 18 ± 32 |

* statistically significant

Table 11

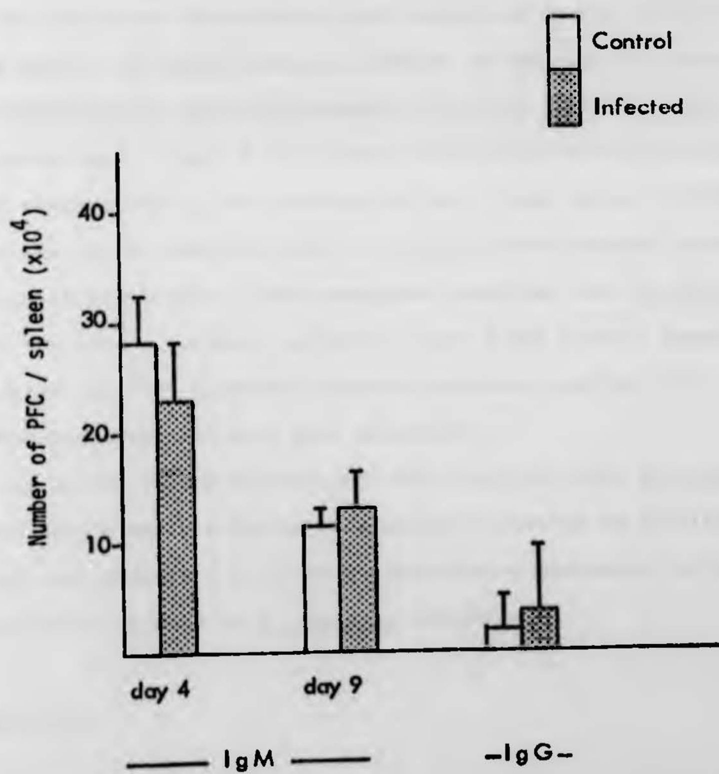
Effect of a low level irradiated T. spiralis infection on spleen PFC in response to SRBC inoculation

| Group | Mouse no. | No. of PFC/spleen (x $10^3$) | | |
|---|---|---|---|---|
| | | IgM on day 4 | IgM on day 9 | IgG |
| Infected | 1 | 72 | 54 | -52 |
| | 2 | 256 | 106 | -26 |
| | 3 | 324 | 152 | 34 |
| | 4 | 264 | 212 | 201 |
| Mean ± SE | | 229 ± 54 | 131 ± 34 | 39 ± 57 |
| Control | 1 | 184 | 56 | -53 |
| | 2 | 244 | 106 | -27 |
| | 3 | 329 | 120 | -6 |
| | 4 | 376 | 124 | 52 |
| | 5 | n.d. | 164 | 124 |
| Mean ± SE | | 283 ± 42 | 114 ± 17 | 18 ± 32 |

* statistically significant

Figure 16 :

Effect of a low level irradiated T. spiralis infection on spleen PFC in response to SRBC inoculation



Group 1 : 20 mice were infected with 400 T. spiralis larvae for 30 days. 5 of them were killed at the end of the experiment and muscle larvae, a mean of 33,500 (SE 5,910) found. Group 2 : 20 mice infected for 14 days with the same number of a different batch of T. spiralis larvae. 204 (SE 60) were found in the intestines of 5 mice. Group 3 : 15 mice were left uninfected to serve as controls.

The experiment was designed for all the mice to be inoculated with $10^9$ SRBC on the same day. 4, 7 and 9 days later 5 mice of each group were killed, their spleens assayed for the number of IgM PFC. To test for polyclonal stimulation, HRBC were used in the plaque assay on some slides. The results obtained (Table 12 and Fig. 17) demonstrated significantly decreased numbers of IgM PFC in 14 day and 30 day old infections at days 7 and 9 after SRBC inoculation ($p < 0.005$ and $< 0.025$ respectively). In the assay at day 4 both groups produced lower numbers of PFC than the control group but the decrease was not significant statistically. This experiment confirms that T. spiralis depresses the immune response to SRBC at days 7 and 9 after inoculation and indicates that the migrating stage of infection and the adult stage can immunodepress as much as a full infection.

Nearly no PFC were developed when HRBC replaced SRBC in vitro indicating that even in infected mice plaque formation is specific and that polyclonal stimulation is not a contributing mechanism in the immunodepression induced by T. spiralis infection.

Experiment 3.12

This study aimed to examine immunodepression at earlier stages of T. spiralis infection and to undertake a different approach to study

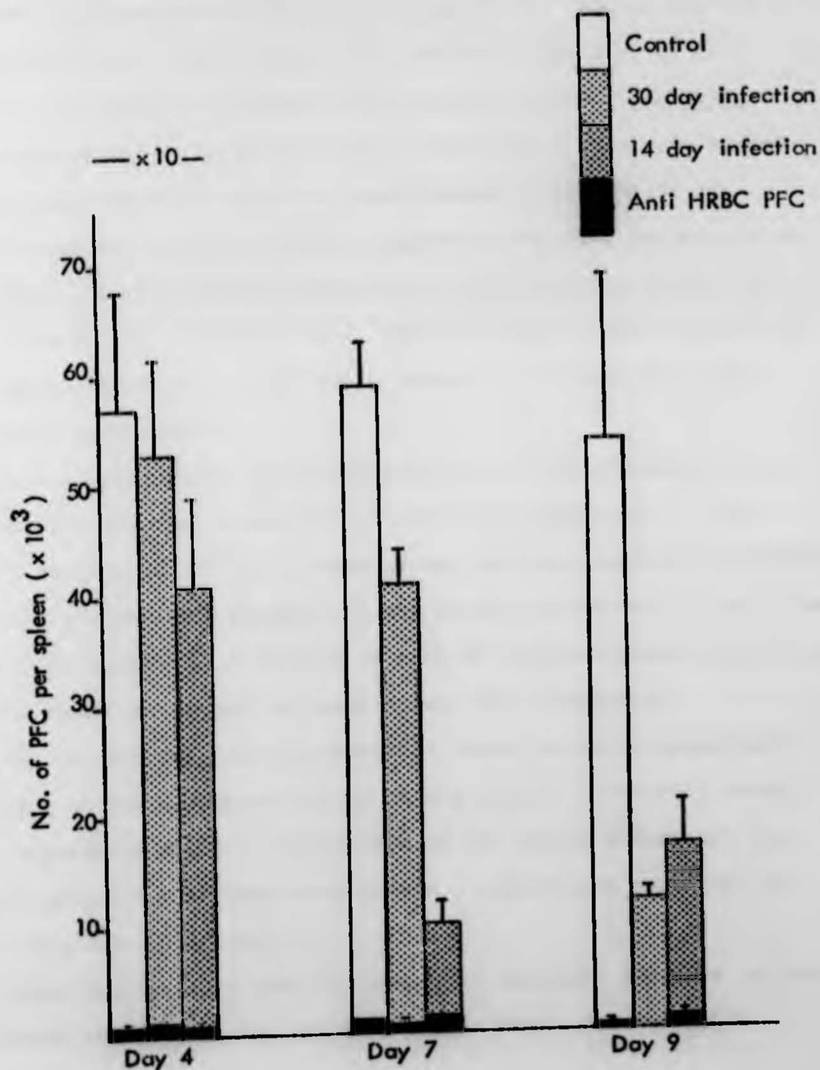
Table 12. Effect of *T.spiralis* on the number of IgM PFC after primary immunization with SRBC using SRBC and HRBC *in vitro*.

| Group | Mice no. | Mean no. of PFC/spleen x $10^3$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | Day 4 | | Day 7 | | Day 9 | |
| | | SRBC | HRBC | SRBC | HRBC | SRBC | HRBC |
| Control | 1 | 274 | 0 | 48 | 2 | 36 | 0 |
| | 2 | 422 | 1 | 52 | 0 | 38 | 0 |
| | 3 | 536 | 1 | 62 | 1 | 39 | 2 |
| | 4 | 792 | 2 | 62 | 2 | 45 | 0 |
| | 5 | 814 | 0 | 72 | 1 | 113 | 0 |
| Mean ± SE | | 568 ± 107 | 0.8 ± 0.3 | 59 ± 4 | 1.2 ± 0.3 | 54 ± 15 | 0.4 ± 0.3 |
| 30 day old infection | 1 | 212 | 0 | 34 | 1 | 9 | 0 |
| | 2 | 412 | 2 | 36 | 0 | 9 | 0 |
| | 3 | 520 | 1 | 38 | 2 | 13 | 0 |
| | 4 | 666 | 2 | 48 | 1 | 14 | 0 |
| | 5 | 834 | 1 | 48 | 0 | 16 | 0 |
| Mean ± SE | | 529 ± 108 | 1.2 ± 0.3 | * 41 ± 3 | 0.8 ± 0.3 | * 12 ± 1 | 0 ± 0 |
| 14 day old infection | 1 | 264 | 2 | 6 | 1 | 7 | 2 |
| | 2 | 278 | 0 | 8 | 3 | 11 | 0 |
| | 3 | 332 | 0 | 8 | 2 | 16 | 0 |
| | 4 | 516 | 0 | 14 | 1 | 25 | 2 |
| | 5 | 660 | 2 | 16 | 0 | 27 | 2 |
| Mean ± SE | | 410 ± 78 | 0.8 ± 0.4 | * 10 ± 2 | 1.4 ± 0.5 | * 17 ± 4 | 1.2 ± 0.4 |

* statistically significant

Figure 17.

Effect of T. spiralis on the number of IgM PFC after primary immunization with SRBC using SRBC and HRBC in vitro



the effect of the migrating stage of infection on the PFC response.

This experiment involved 5 groups of mice.

Group 1 : 20 mice were infected for 7 days with 400 T. spiralis larvae. The mean number of adults in the intestines of 5 animals 3 days post infection was 187 (SE 17). Group 2 : 20 mice were infected for 10 days with 400 T. spiralis larvae. Adults counted in the intestine of 5 mice 3 days after infection were 269 (SE 69). Group 3 : 20 mice were infected for 14 days with 400 T. spiralis muscle larvae each. Three days after infection, 5 mice were killed and the mean number of adults in their intestine was 88 (SE 7). Group 4 : 10 mice received 40,000 newborn larvae (NBL) by intravenous injection. 32,100 (SE 3000) muscle larvae were counted at the end of the experiment in 5 of these mice. NBL were left in the host for 25 days before immunization with SRBC. Group 5 : 15 mice were left uninfected as controls.

At the appropriate time after infections, the infected and the control mice received a dose of $10^9$ SRBC on the same day. 4 days later 5 animals of the first three groups and the controls were killed and their spleens were assayed for the number of IgM PFC. 7 and 9 days after SRBC inoculation 5 animals of each of the five groups were killed and the number of IgM PFC of their spleen were determined.

The results (Fig. 18 and Table 13) showed a highly significant reduction in the number of IgM PFC in all groups of infected animals when compared to controls at all days of the assays except for the group infected for 14 days which showed a significant reduction on day 4 and 7 but not on day 9.

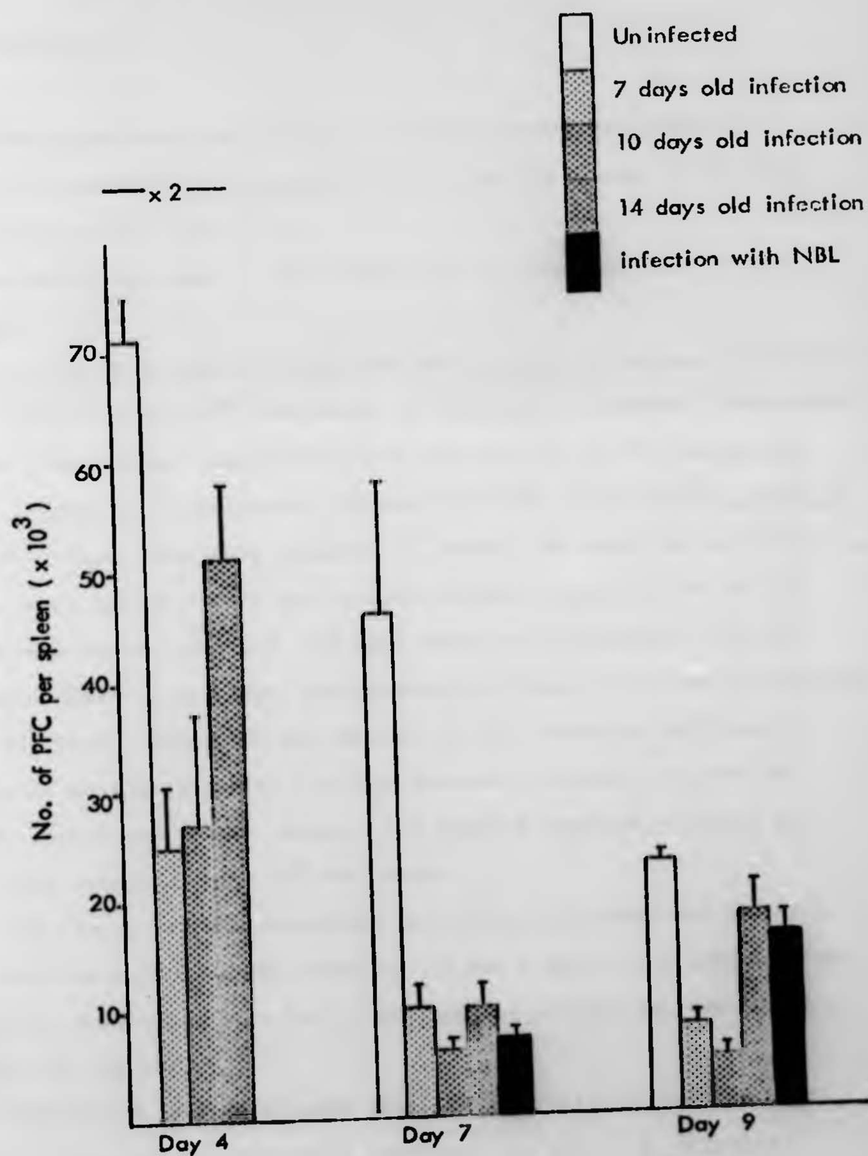
These results show that all stages of infection are able to alter the immune response to the second antigen, SRBC. The fact that

Table 13. Effect of different ages of T. spiralis infection on the primary immune response to SRBC.

| Group | Mean PFC/spleen x $10^3$ ± SE and p value | | | |
|---|---|---|---|---|
| | No. of mice | Day 4 | Day 7 | Day 9 |
| 7 day old infection | 5 | 50<br>± 11<br>< 0.0005 | 10<br>± 2<br>< 0.01 | 8<br>± 1<br>< 0.0005 |
| 10 day old infection | 5 | 54<br>± 20<br>< 0.0025 | 6<br>± 1<br>< 0.005 | 5<br>± 1<br>< 0.0005 |
| 14 day old infection | 5 | 102<br>± 14<br>< 0.025 | 10<br>± 2<br>< 0.01 | 18<br>± 3<br>n.s. |
| Infection with NBL | 5 | n.d. | 7<br>± 1<br>< 0.005 | 16<br>± 2<br>< 0.0125 |
| Uninfected controls | 5 | 142<br>± 8 | 46<br>± 12 | 23<br>± 1 |

Figure 18.

Effect of different ages of T. spiralis infection on the primary immune response to SRBC



immunodepression was not consistent on each of the days after SRBC inoculation when plaques were assayed, may indicate that this immune unresponsiveness is a temporary phenomenon.

Experiment 3.13

This experiment was designed to study further the effect of different stages of T. spiralis infection on the number of PFC after immunization with SRBC.

40 mice were used in this experiment divided into the following groups.

Group 1 : 15 mice were infected with 400 T. spiralis larvae and 30 days later they received $10^9$ SRBC each. To check the infection 5 mice were killed 3 days after infection when a mean of 159 (SE 73) adults was found. Group 2: 15 mice were infected with 800 irradiated T. spiralis larvae, 5 days later they received $10^9$ SRBC. To check the infection 5 mice were killed 3 days post infection when a mean of 167 (SE 29) adults was found. Group 3 : 10 mice received an infection dose of 40,000 newborn larvae (NBL) intravenously 25 days before being immunized with $10^9$ SRBC. Infection was checked in this group by counting the number of muscle larvae in 5 of the animals. A mean of 24,880 (SE 4,630) larvae was found. Group 4 : 5 animals were not infected but were only immunized with $10^9$ SRBC each.

The timing of this experiment was arranged so that all the mice received their SRBC on the same day. 4 and 9 days after immunization 5 mice of each group were killed and spleens assayed for the number of IgM and IgG PFC.

Results of this experiment (Fig. 19 and Table 14) confirm that a full infection, an adult only infection and NBL of T. spiralis

Table 14

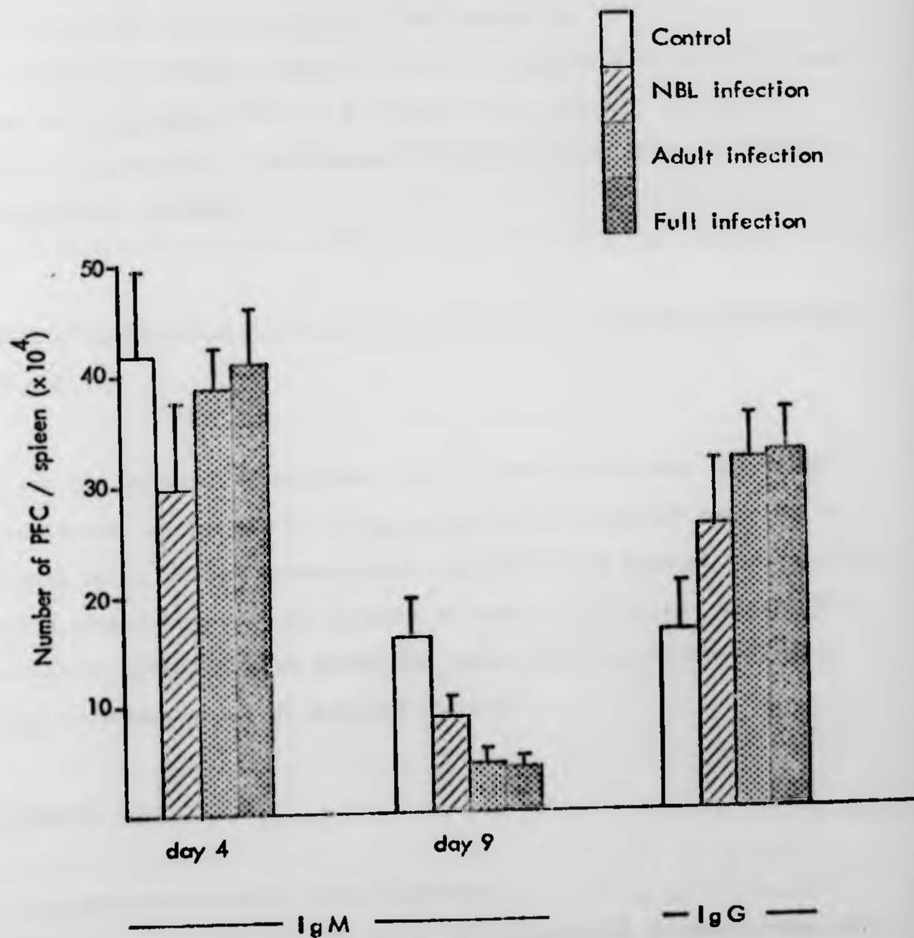
Effect of different stages of infection of *T. spiralis* on spleen PFC (4 and 9) days after SRBC injection.

| Group | Mouse no. | Mean no. of PFC/spleen (x $10^3$) | | |
|---|---|---|---|---|
| | | IgM on day 4 | IgM on day 9 | IgG |
| Full infection | 1 | 232 | 18 | 206 |
| | 2 | 372 | 20 | 277 |
| | 3 | 428 | 42 | 321 |
| | 4 | 484 | 54 | 411 |
| | 5 | 528 | 60 | 414 |
| | Mean | 409 | * 39 | * 326 |
| | ± SE | 51 | 9 | 40 |
| Adult infection | 1 | 280 | 12 | 202 |
| | 2 | 356 | 26 | 253 |
| | 3 | 372 | 34 | 374 |
| | 4 | 424 | 54 | 384 |
| | 5 | 496 | 88 | 389 |
| | Mean | 386 | * 43 | * 320 |
| | ± SE | 36 | 13 | 39 |
| NBL infection | 1 | 108 | 38 | 135 |
| | 2 | 128 | 86 | 192 |
| | 3 | 316 | 88 | 338 |
| | 4 | 464 | 102 | 376 |
| | 5 | 472 | | |
| | Mean | 298 | * 79 | 260 |
| | ± SE | 78 | 14 | 58 |
| Control | 1 | 256 | 62 | 55 |
| | 2 | 320 | 118 | 104 |
| | 3 | 352 | 138 | 167 |
| | 4 | 464 | 236 | 175 |
| | 5 | 692 | 244 | 319 |
| | Mean | 417 | 160 | 164 |
| | ± SE | 77 | 35 | 44 |

* statistically significant

Figure 19:

Effect of different stages of infection of T. spiralis on spleen PFC ( 4 and 9 ) days after SRBC injection



depress the immune response upon immunization with SRBC. Depression was shown by the lower number of IgM PFC of infected animals compared to controls on day 9 ($p < 0.01$, $p < 0.01$, $p < 0.05$ respectively). Numbers of IgM PFC on day 4 were lower than the control but the depression was not statistically significant. Surprisingly the number of IgG PFC of infected animals was more than the controls. The increase was significant in full infection ($p < 0.025$) and adult infection ($p < 0.025$) but not significant in NBL infection.

This would confirm results of previous experiments that the three stages of T. spiralis infection (adults, NBL, and full infection) depress the primary IgM PFC immune response to SRBC either separately or conjointly in mice.

Effect of T. spiralis on the number of PFC after a second immunization with SRBC

In the previous experiments there was no significant depression in the number of IgG PFC in T. spiralis infected animals compared to controls after primary immunization with SRBC. As most of the antibody after a second injection of antigen is usually IgG it was decided that it would be interesting to study the number of IgG and IgM PFC after secondary immunization in infected animals.

Experiment 3.14

24 mice were divided into 3 groups.

Group 1 : 9 mice were infected with 500 T. spiralis larvae, 4 mice were immunized 15 and 30 days later with $10^9$ SRBC, the rest of the mice were

used to check the infection by counting larvae in the digested muscles; with a mean recovery of 61,900 (SE 7,964). Group 2 : 10 mice were immunized with $10^9$ sheep erythrocytes 10 days before being infected with 500 T. spiralis larvae. 5 mice received a secondary dose of SRBC 5 days after infection. The rest of the animals of this group were digested 30 days after infection when a mean of 54,000 (SE 1,802) muscle larvae were found. Group 3 : 5 mice were not infected but received 2 doses of SRBC 15 days apart.

The experiment was arranged so that all the animals received their injections of SRBC on the same days. 7 days after the second immunization all the mice were killed and their spleens were assayed for direct and indirect PFC. Details of results are shown in Table 15 and means represented in Fig. 20. IgM but not IgG PFC of intestinal stage group was less than the control ($p < 0.05$). However, there was no difference between full infection group and controls in the number of IgM or IgG PFC.

## Discussion

The result of all the experiments reported in this chapter are summarized in Table 16. It can be seen that in nearly every experiment a 30 day old infection caused a reduction in the numbers of IgM PFC. There were two exceptions to this; in experiment 3.3 (Table 4) there were more IgM PFC nine days after inoculation of SRBC and in experiment 3.13 (Table 14) there was no difference between the infected and uninfected mice 4 days after inoculation of SRBC. No explanation is available so far for these results, but they may indicate that day 7 is an optimum day to detect immunodepression in T. spiralis infections.

Table 15

Effect of *T. spiralis* on spleen PFC after secondary immunization

| Group | Mouse no. | No. of PFC/spleen (x $10^3$) | |
|---|---|---|---|
| | | IgM | IgG |
| Full infection | 1 | 92 | 50 |
| | 2 | 118 | 68 |
| | 3 | 150 | 102 |
| | 4 | 210 | 113 |
| Mean ± SE | | 143 ± 25 | 83 ± 15 |
| Intestinal stage | 1 | 68 | 52 |
| | 2 | 86 | 68 |
| | 3 | 90 | 102 |
| | 4 | 114 | 110 |
| | 5 | 138 | 208 |
| Mean ± SE | | * 99 ± 12 | 108 ± 27 |
| Control | 1 | 110 | 10 |
| | 2 | 118 | 66 |
| | 3 | 134 | 70 |
| | 4 | 134 | 88 |
| | 5 | 150 | 144 |
| Mean ± SE | | 129 ± 7 | 76 ± 21 |

* statistically significant

Table 15

Effect of *T. spiralis* on spleen PFC after secondary immunization

| Group | Mouse no. | No. of PFC/spleen (x $10^3$) | |
|---|---|---|---|
| | | IgM | IgG |
| Full infection | 1 | 92 | 50 |
| | 2 | 118 | 68 |
| | 3 | 150 | 102 |
| | 4 | 210 | 113 |
| Mean $\pm$ SE | | 143 $\pm$ 25 | 83 $\pm$ 15 |
| Intestinal stage | 1 | 68 | 52 |
| | 2 | 86 | 68 |
| | 3 | 90 | 102 |
| | 4 | 114 | 110 |
| | 5 | 138 | 208 |
| Mean $\pm$ SE | | * 99 $\pm$ 12 | 108 $\pm$ 27 |
| Control | 1 | 110 | 10 |
| | 2 | 118 | 66 |
| | 3 | 134 | 70 |
| | 4 | 134 | 88 |
| | 5 | 150 | 144 |
| Mean $\pm$ SE | | 129 $\pm$ 7 | 76 $\pm$ 21 |

* statistically significant

Figure 20 :

Effect of T. spiralis on spleen PFC after secondary immunization

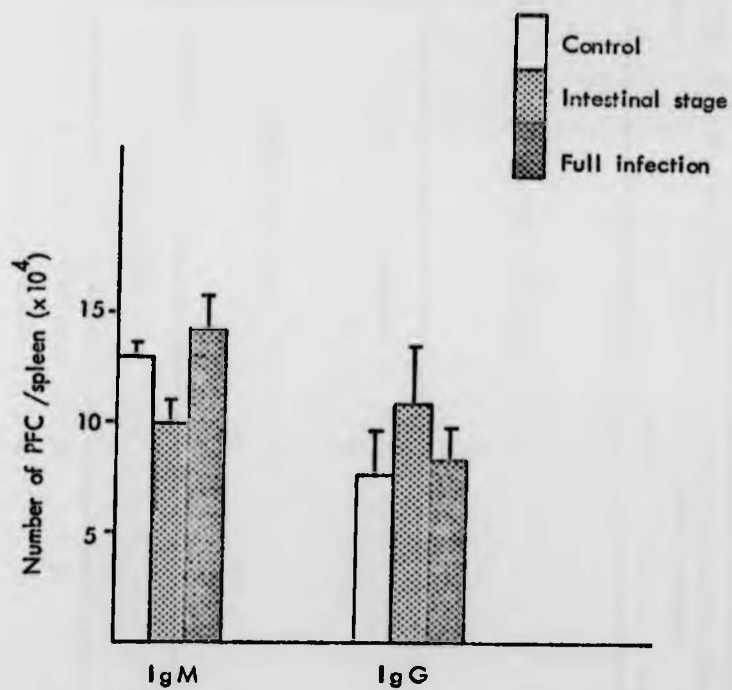


Table 16. A summary of immunodepression induced by *T. spiralis* judged by PFC assay during primary response (D = significant depression, S = significant stimulation, n = no effect).

| Experiment No. | Table No. | No. of *T. spiralis* larvae inoculated | Type of infection | Effect on IgM PFC after SRBC injection | | | Effect on IgG PFC |
|---|---|---|---|---|---|---|---|
| | | | | Day 4 | Day 7 | Day 9 | |
| 3.3 | 4 | 400 | | D | - | S | n |
| 3.4 | 5 | 400 | | - | D | - | - |
| 3.11 | 12 | 400 | 30 day old infection | n | D | D | - |
| 3.13 | 14 | 400 | | n | - | D | S |
| 3.5 | 6 | 50 | | D | - | - | - |
| 3.6 | 7 | 400 | Methyridine treated | n | - | D | S |
| 3.7 | 8 | 400 | | - | D | - | - |
| 3.8 | 10 | 2000 | | D | - | - | - |
| 3.13 | 14 | 800 | Irradiated larvae | n | - | D | - |
| 3.9 | 9 | 400 | | - | D | - | - |
| 3.10 | 11 | 50 | | n | - | n | n |
| 3.13 | 14 | 40,000 | New born larvae | n | - | D | - |
| 3.12 | 13 | 40,000 | | - | D | D | - |
| 3.12 | 13 | 400 | 7 day old infection | D | D | D | - |
| 3.12 | 13 | 400 | 10 day old infection | D | D | D | - |
| 3.11 | 12 | 400 | 14 " " " | n | D | D | - |
| 3.12 | 13 | 400 | 14 " " " | D | D | n | - |

The remaining experiments attempted to determine which part of the life cycle is responsible for this immune depression.

The effect of the intestinal phase of the infection was studied in two ways. In experiments 3.6 and 3.7 the infections were terminated by treatment with methyridine before any larvae had been born. No depression was seen on day 4 but on days 7 and 9 there was significant depression. This suggests that the intestinal phase may depress the immune response by reducing the duration of the IgM response. In experiments 3.8, 3.13, 3.9 and 3.10, irradiated, sexually sterile infections were used. When 2000 larvae were used depression was seen on day 4 but when lower levels of infection were used depression was only seen 7 or 9 days after inoculation of SRBC. Very light infection did not cause depression (experiment 3.10) whereas a similar level of normal larvae left for 30 days did (experiment 3.5). Dose dependency has been reported to be important in induction and maintenance of immunodepression. Usually the larger the dose of the antigen, the more complete is the unresponsive state and the longer the duration (Smith and Bridges, 1958; Kitzman and Smith, 1959).

In addition the minimal dose of antigen required to induce unresponsiveness can vary from one strain of host to another (Golub and Weigle, 1969). This point may explain the controversy about the effects of the intestinal phase of infection. Further research may be worth doing to detect the optimum dose responsible for immunodepression by adult T. spiralis infection.

When the new born larvae (NBL) were injected intravenously (experiments 3.12 and 3.13), thus producing a muscle infection but no intestinal phase, depression was seen on days 7 and 9 but not on day 4.

In experiments 3.12 and 3.13 the effects of different ages of

infection were compared. Strong depression was evident on days 4, 7 and 9.

It appears, then, that a mixture of intestinal adults and migrating and developing larvae is more effective in depressing the immune response than is either stage on its own. It is conceivable that this is because the stimulus from these "natural" infections continues for longer than it does in the artificial infections.

These results are in agreement with those of Faubert (1976), Chimishkyan et al. (1974), Jones et al. (1976), Lubiniecki and Cypess (1975a) and Ljungstrom and Huldt (1977) concerning the ability of infections which are 14 or more days old to reduce the number of PFC but neither Lubiniecki et al. (1974b) nor Ljungstrom and Huldt (1977) found depression before this. In experiment 3.12, 7 and 10 day old infection caused a diminution of the PFC response. The results of this part of the study are supported by the work of Faubert and Tanner (1975) who found that sera collected from animals infected with T. spiralis for 7 days agglutinated and killed homologous lymphoid cells in vitro. Cypess et al. (1973) and Lubiniecki et al. (1974a) found that increased susceptibility to JBE virus was maximal 7 days post infection with T. spiralis. However they found that mice infected with irradiated T. spiralis, had the same low susceptibility to JBE virus as the controls (Cypes. et al., 1973). This may be because of the high level of irradiation used in their work would markedly reduce the longevity of adult worms (James, 1974). Ljungstrom and Huldt (1977) and Barriga (1978a) have demonstrated that allograft rejection was delayed in mice infected with T. spiralis especially 7 days after infection. It is, therefore, clear that the early part of infection is capable of depressing the immune response although this is the

first report of depressed SRBC-PFC immune response induced by intestinal stage of T. spiralis infection in mice.

It can be seen (Table 16) that only IgM PFC were depressed during T. spiralis infection. IgG PFC were not depressed either after primary or secondary immunization. Ljungstrom and Huldt (1977) found that IgG PFC were not depressed until 42 days post infection. This suggests that IgG PFC are affected by encysted muscle larvae but not by the intestinal or developing muscle stages. A differential unresponsive state has also been reported between different immunoglobulins (Weigle, 1973). An explanation may be the existence of two populations of B lymphocytes, committed to production of antibody of various classes or subclasses which respond differently to contact with antigen and may contain receptors of different avidity (Playfair and Purves, 1971). Alternatively, the receptors may be present on the cell surface in different amounts. Also it has been shown that both 19 S and 7 S synthesis is amenable to suppression (Möller and Wigzell, 1965) but there is general agreement that 7 S production is more resistant to depression.

In experiment 3.11 an attempt was made to demonstrate polyclonal B cell stimulation in T. spiralis infected animals.

Under the conditions made in the experiment 3.11, it was demonstrated that animals immunized with SRBC and either infected with T. spiralis or uninfected, produced the same number of PFC when HRBC were used in the assay. This excludes non specific B cell stimulation as a partial cause of specific PFC depression. Non specific polyclonal B cell activation leading to the exhaustion of B cell potential has been suggested as a mechanism for immunodepression in trypanosomiasis (Urquhart et al., 1973 and Hudson et al., 1976 ) and in S. mansoni

infections (Ramalho-pinto et al., 1976). The experiment reported above does not support the possibility of this happening in T. spiralis infection. Different approaches to study non specific B cell activation in T. spiralis infection may be useful in detecting this phenomenon.

# CHAPTER 4

## Effect of T. spiralis infection on antibody levels in the sera of mice

### Introduction

In view of the observations that T. spiralis affects the number of antibody forming cells studies were now directed towards determining the levels of antibodies in the sera of animals infected with T. spiralis larvae.

It has been shown that anti-SRBC but not anti-PVP agglutinins were depressed in animals infected with T. spiralis larvae for 7 days (Barriga, 1975). In contrast, Lubiniecki et al. (1974b) have demonstrated that a 7 day old T. spiralis infection in mice had no significant effect on antibody titres against SRBC. Lubiniecki and Cypess (1975a) reported that anti-SRBC haemagglutinins were depressed in mice 14 days after T. spiralis infection but Jones et al. (1976) found that depression of haemagglutinins occurred only 20 days post infection. Ljungstrom and Huldt (1977) showed that anti-SRBC agglutinins were depressed 3 and 6 weeks but not 1 week after infection.

Agglutinating and haemolysin levels in sera of animals sensitized previously with an antigen may be compared semi-quantitatively by determining the end point of their titration curve. The sera are diluted until they no longer give a visible reaction with antigen by the agglutination or haemolysin tests.

In this part of the study, it was decided to study the effect of different phases of T. spiralis infection on the immune response to

T- dependent and T- independent antigens SRBC and LPS using agglutination and haemolysin tests.

## Materials and Methods

### Preparation of the sera:

Mice were killed with nembutal and then bled individually by heart puncture. Blood was left at room temperature for 1 hour and then overnight at 4°C to allow shrinkage of the blood clot to get the maximal amount of serum. Next day centrifugation was carried out at 1000 g for 5 minutes. The collected sera were kept in aliquots at -70°C until assayed to avoid denaturation of proteins by repeated thawing and refreezing.

### Measurement of anti-SRBC haemagglutinins:

Anti-SRBC agglutinins were measured in the sera of different groups using the simplest form of haemagglutination test. 0.025 ml of each serum (measured by a standard dropping pipette) was diluted with an equal volume of pH 7.2 phosphate buffered saline (PBS), in the V-shaped wells of Microhaemagglutination trays (Flow Laboratories Ltd., Irvine, Scotland). Serial dilutions in PBS were made using standard diluting loops. For each mouse serum, two rows of dilutions of antiserum in PBS were prepared. Between each serum the diluters were washed in distilled water then heated in a Bunsen flame until they were red hot. 0.025 ml of 2% SRBC which had been washed three times in PBS and then suspended in PBS was added to each serum dilution and to a well containing 0.025 ml of PBS, as a control for

spontaneous agglutination. A positive and a negative control were used to check the validity of the test. The trays were covered by a sealer strip, the contents of the wells mixed by shaking and incubated at 37°C for 30 minutes and left at 4°C overnight. The results were read on a white surface or using a mirror. Agglutination titres were read as the last well which shows complete agglutination (even deposits). For negative results the red cells fell into the bottom of the well forming a red button.

Measurement of SRBC haemolysins:

The buffer used for this assay was Barbitone buffered saline (BBS) pH 7.6, prepared as follows:

Solution A : 85.0 g Sodium chloride
3.75 Sodium diethylbarbiturate
Made up to 1400 ml with distilled water

Solution B : 5.75 Diethylbarbituric acid in 500 ml hot distilled water

Solution C : 20.3 g $MgCl_2 6H_2O$ (2.0 M) dissolved in 50 ml distilled water + 30 ml 1.0 M Calcium chloride solution. Adjust to 100 ml with distilled water. (Final concentration $MgCl_2$ 1.0 M , $CaCl_2$ 0.3 M).

Solutions A and B were mixed and left to cool at room temperature. 5 ml of solution C was added and the final volume adjusted to 2 litres with distilled water and stored at 4°C. The buffer was diluted 5 times just before use.

Estimation of minimum haemolytic dose of the complement (MHD):

Sensitization of SRBC with antibody:

SRBCs were washed three times in BBS and 6% of SRBC in the buffer were prepared. 15 ml of BBS were mixed with 0.1 ml of rabbit haemolytic serum (obtained from Wellcome Reagent Ltd., UK) then 15 ml of 6% SRBC were added. The cell suspension was incubated at 37°C for 15 minutes. The sensitized cells were used within 24 hours of preparation.

The complement was prepared from a pool of guinea pig serum as described in Chapter 3. Dilutions of the complement were set up as follows:

| | Tube number | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Barbitone buffered saline (ml) | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 |
| Guinea-pig serum (ml) initial dilution 1:10 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Final complement dilution | 1:20 | 1:30 | 1:40 | 1:50 | 1:60 | 1:70 | 1:80 |

0.1 ml of each complement dilution was added to 0.2 ml of BBS + 0.1 ml of sensitized erythrocytes then incubated for 30 minutes at 37°C and left overnight at 4°C. The dilution of the tube next to the first one which showed a button of erythrocytes was taken as the MHD. In the assay 4 MHD were used (1:20).

Haemolysing assay:

Anti-SRBC haemolysins were measured in the sera of individual animals. Two rows of serial dilutions of sera were prepared as

described in haemagglutination test but using BBS instead of PBS. Dilutions were made in the U-shaped wells of microtitration trays. 0.025 ml of 2% SRBC (washed and prepared in BBS) was added to each serum dilution and 0.025 ml of the diluted complement was added. A positive and a negative control were used to check the validity of the assay. The trays were then covered with a sealer strip, shaken well, incubated at 37°C for 30 minutes, then left at 4°C overnight. The end points were taken as the last well which showed complete haemolysis.

Measurement of anti-Lipopolysaccharide (LPS) by complement-mediated haemolysis:

The buffer used in this assay was balanced salt solution (BSS) prepared as follows:

| | |
|---|---|
| Calcium chloride | 0.14 g |
| Sodium chloride | 8.00 g |
| Potassium chloride | 0.40 g |
| Magnesium sulphate, Mg $SO_4$ $7H_2O$ (0.8 mM) | 0.20 g |
| Magnesium chloride, $MgCl_2$ $6H_2O$ (1.0 mM) | 0.20 g |
| Potassium dihydrogen phosphate (0.4 mM) | 0.06 g |
| Di-sodium hydrogen phosphate, $Na_2HPO_42H_2O$ (1.4 mM) | 0.24 g |

All components were dissolved in 1000 ml and kept at 4°C until used.

Coating of SRBC with LPS:

3 mg of LPS of Escherichia coli serotype No. 055:B5 (obtained from Sigma Chemical Company) were dissolved in 3 ml BSS in a Bijou bottle which was kept in a boiling water bath for 2 hours; the pH was adjusted to 7-8 during boiling by adding 0.1 M NaOH. The LPS

solution was left to cool at room temperature. SRBC were washed 3 times in BSS and 1 ml of packed cells were added to the LPS solution and then incubated at 37°C for 45 minutes. The coated cells were then washed three times in BSS then diluted to 2%. The coated cells were used within two days of preparation.

The assay:

The anti-LPS haemolysin assay was done as described in anti-SRBC haemolysin assay, except that LPS coated SRBC were used instead of normal SRBC and BSS were used as a buffer instead of BBS.

Preliminary experiment:

This experiment was designed to study the levels of IgM and IgG antibodies against SRBC in the mouse sera, at different times after SRBC injection, using the method described by Olson et al. (1976) for inactivation of antibodies using Dithiothreitol (DTT). 25 mice were used in this experiment. 20 animals received a dose of $10^9$ SRBC and 5 left unimmunized. 5 immunized mice were killed 4, 6, 9 and 12 days later and were bled individually from the heart and sera were separated. The sera of unimmunized mice were also prepared individually. Equal volumes (0.025 ml) of serum + DTT (Sigma) or BBS were incubated at 37°C for 30 minutes. Doubling dilutions were then made for each serum sample. The total and DTT resistant antibodies were measured using the assay for haemolysin antibodies as described before. The titre of DTT resistant antibodies is equivalent to that due to IgG in the serum and the titre obtained without DTT was due to IgM plus IgG.

The results obtained (Table 17, Fig. 21) showed that the titre of antibodies detected at day 4 was due to DTT sensitive antibodies, while DTT resistant antibodies were of low titre (1:5). This result showed that anitbody levels in the serum at day 4 represents mainly IgM antibodies. DTT resistant antibodies (IgG) started to appear in the serum in a reasonable level at day 6, increased gradually to constitute the major part of the total antibody levels at day 9 and 12 while DTT sensitive antibodies (IgM) were of very low titres. This result indicates that the antibody levels in the serum at day 9 and 12 represents mainly IgG. From the results of this experiment, it was decided that day 4 and day 9 were suitable days for measuring IgM and IgG antibodies, respectively, in the serum.

Statistical analysis:

The geometric mean (95% confidence interval) was more suitable for the results of this chapter since the data tended to be skewed.

## I. EFFECT OF *T. SPIRALIS* INFECTIONS ON HAEMAGGLUTININ AND HAEMOLYSIN ANTIBODIES AGAINST SRBC.

### 1. Effect of full infection:

Experiment 4.1

14 mice were infected with 400 *T. spiralis* larvae and 9 kept as controls. 30 days after infection 9 mice of each group were immunized with $10^9$ SRBC and exsanguinated 4 and 9 days later and haemagglutinin and haemolysin titres were estimated in the sera. Infection was

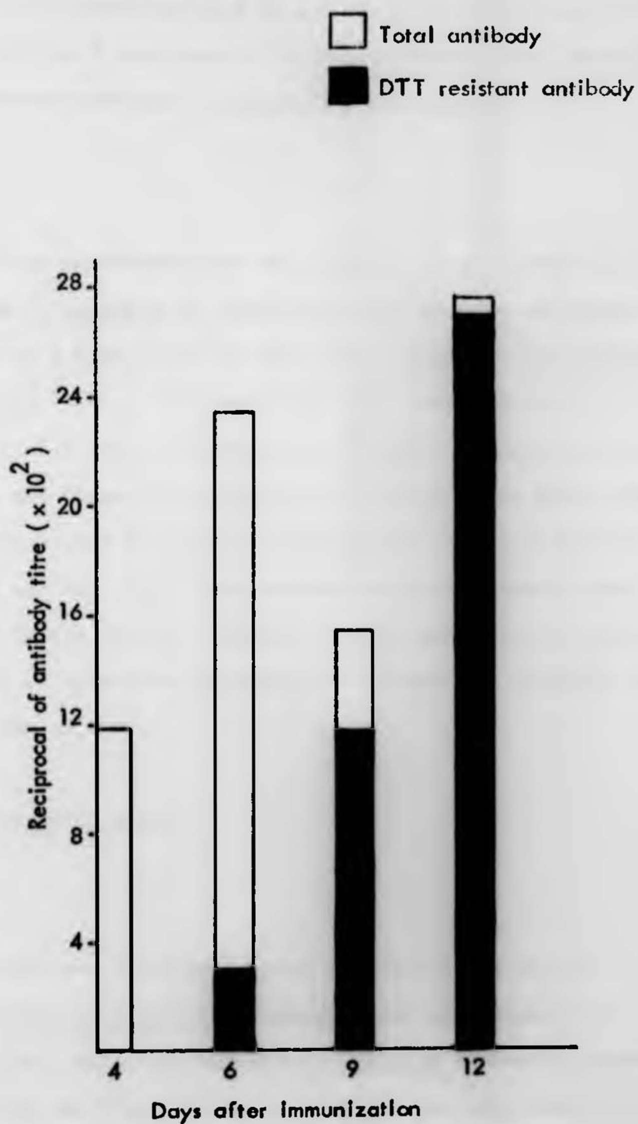
Table 17 Titres of total and DTT resistant (i.e. IgG) haemolysins in the serum at different times after SRBC inoculation.

| Group | Days after immunization | Number of mice | Geometric mean of reciprocal titre of total antibodies (95% confidence interval) | * Geometric mean of reciprocal titre of DTT resistant antibodies |
|---|---|---|---|---|
| Immunized (SRBC) | 4 | 5 | 1184 (631 - 2221) | 5 (3 - 8) |
| | 6 | 5 | 2347 (1354 - 4098) | 294 (124 - 695) |
| | 9 | 5 | 1552 (902 - 2669) | 1156 (620 - 2158) |
| | 12 | 5 | 2767 (1831 - 4178) | 2702 (1882 - 3878) |
| Unimmunized | - | 5 | 000 | 000 |

* represents IgG antibodies

Figure 21.

Titres of total and DTT resistant (i.e. IgG) haemolysins in the serum at different times after SRBC inoculation



checked by counting the number of adults in the intestines of 5 infected mice; a mean worm recovery of 167 (SE 69) was found.

Results are shown in Table 18 and Fig. 22 and show that on day 4 haemagglutinins and haemolysins were significantly less in infected animals than in uninfected controls ($p < 0.01$, $p < 0.0005$) respectively. Antibody titres on day 9 were less in infected animals than controls but these differences were not statistically significant.

Experiment 4.2

The aim of this experiment was to determine if the effect of a full infection of *T. spiralis* on antibody titres seen in experiment 4.1 is dependent on a high level of infection. 5 mice were infected with 50 *T. spiralis* larvae. 30 days later, the infected mice and a group of 5 normal mice were injected with $10^9$ SRBC. 4 days later all mice were killed and haemagglutinin titres of their serum determined. There were no significant differences between the titres of the two groups (Table 19 and Fig. 23). The infected mice contained a mean of 3186 (SE 539) muscle larvae. Results of this experiment indicate that a high level of infection is needed to depress the antibody level in the sera of mice to SRBC.

2. Effect of intestinal phase:

Experiment 4.3

This experiment was designed to test for the effect of the intestinal stage of *T. spiralis* on haemagglutinin and haemolysin titres. 15 mice were infected with 400 *T. spiralis* larvae. 3 days later a mean of 251 (SE 50) adults were found in the intestine of 5

Table 18

Effect of a 30 day old *T. spiralis* infection on antibody production upon immunization with SRBC

| Group | Mouse no. | Reciprocal of titres | | | |
|---|---|---|---|---|---|
| | | Haemagglutinins | | Haemolysins | |
| | | d 4 | d 9 | d 4 | d 9 |
| Infected | 1 | 128 | 384 | 384 | 128 |
| | 2 | 192 | 384 | 384 | 256 |
| | 3 | 192 | 384 | 512 | 512 |
| | 4 | 256 | 768 | 512 | 1024 |
| | 5 | 256 | - | 768 | - |
| Geometric mean (95% confidence interval) | | *199 (152-262) | 457 (304-686) | *495 (378-648) | 362 (126-1037) |
| Control | 1 | 256 | 384 | 1536 | 384 |
| | 2 | 384 | 512 | 1536 | 4096 |
| | 3 | 384 | 1536 | 3072 | 4096 |
| | 4 | 512 | 4096 | 2048 | - |
| | 5 | 1024 | - | 2048 | - |
| Geometric mean (95% confidence interval) | | 456 (280-745) | 1054 (294-3773) | 1993 (1511-2592) | 1861 (189-18620) |

* statistically significant

Figure 22.

Effect of a 30 day old *T. spiralis* infection on antibody production upon immunization with SRBC

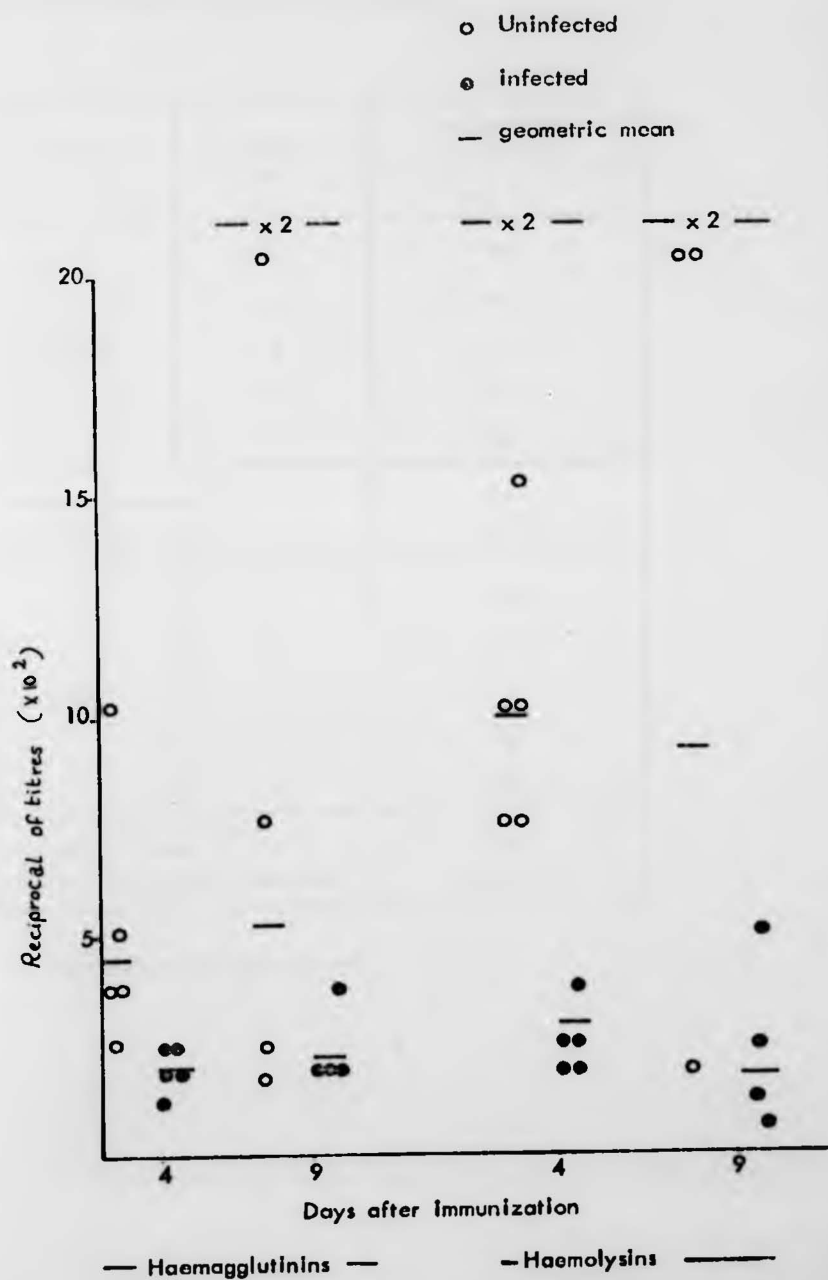


Table 19

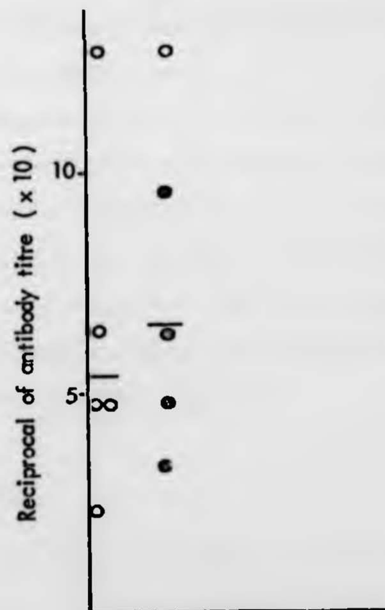
Effect of a 30 day old light *T. spiralis* infection on haemagglutinins after SRBC injection.

| Group | Mouse no. | Reciprocal of titres |
|---|---|---|
| | | Day 4 |
| Infected | 1 | 32 |
| | 2 | 48 |
| | 3 | 64 |
| | 4 | 96 |
| | 5 | 128 |
| Geometric mean (95% confidence interval) | | 66 (39-110) |
| Control | 1 | 24 |
| | 2 | 48 |
| | 3 | 48 |
| | 4 | 64 |
| | 5 | 128 |
| Geometric mean (95% confidence interval) | | 54 (30-96) |

* statistically significant

Figure 23.

Effect of a 30 light T. spiralis infection on heamagglutinins after SRBC injection



mice. Infection was terminated by a dose of Methyridine subcutaneously 5 days post infection. Another group of 10 mice received Methyridine without infection and 10 mice were kept as normal controls.

The mice were immunized with $10^9$ sheep erythrocytes one day after Methyridine treatment. 4 and 9 days later 5 mice of each group were exsanguinated and the serum collected. Haemagglutinins and haemolysins were assayed on individual sera. Results are shown in Table 20 and Fig. 24. Haemagglutinin titres were significantly less on day 4 in infected animals when compared to Methyridine or control groups ($p < 0.0005$ and $p < 0.025$) respectively. Haemolysin titres were also significantly less on day 4 in the infected group compared to Methyridine or control groups ($p < 0.025$ and $p < 0.0125$) respectively.

No significant difference was detected on day 9 either in haemagglutinin or haemolysin titres. Methyridine treatment had no effect on antibody titres on day 9. On day 4 haemagglutinin titres in the Methyridine treated mice were significantly more than the control group ($p < 0.025$). Haemolysin titres in Methyridine treated group were significantly less than the control group ($p < 0.05$). The results of this experiment indicate that the intestinal stage of T. spiralis infection can depress haemagglutinin and haemolysin antibodies in serum of infected animals on day 4.

Experiment 4.4

This experiment was designed to confirm the effect of adult T. spiralis on the immune response to SRBC. 15 mice were infected with 2000 irradiated T. spiralis larvae. 5 mice were killed to check the level of infection and gave a mean of adult recovery of 453 (SE 75). 5 days after infection, 10 infected and 10 uninfected mice were

Table 20

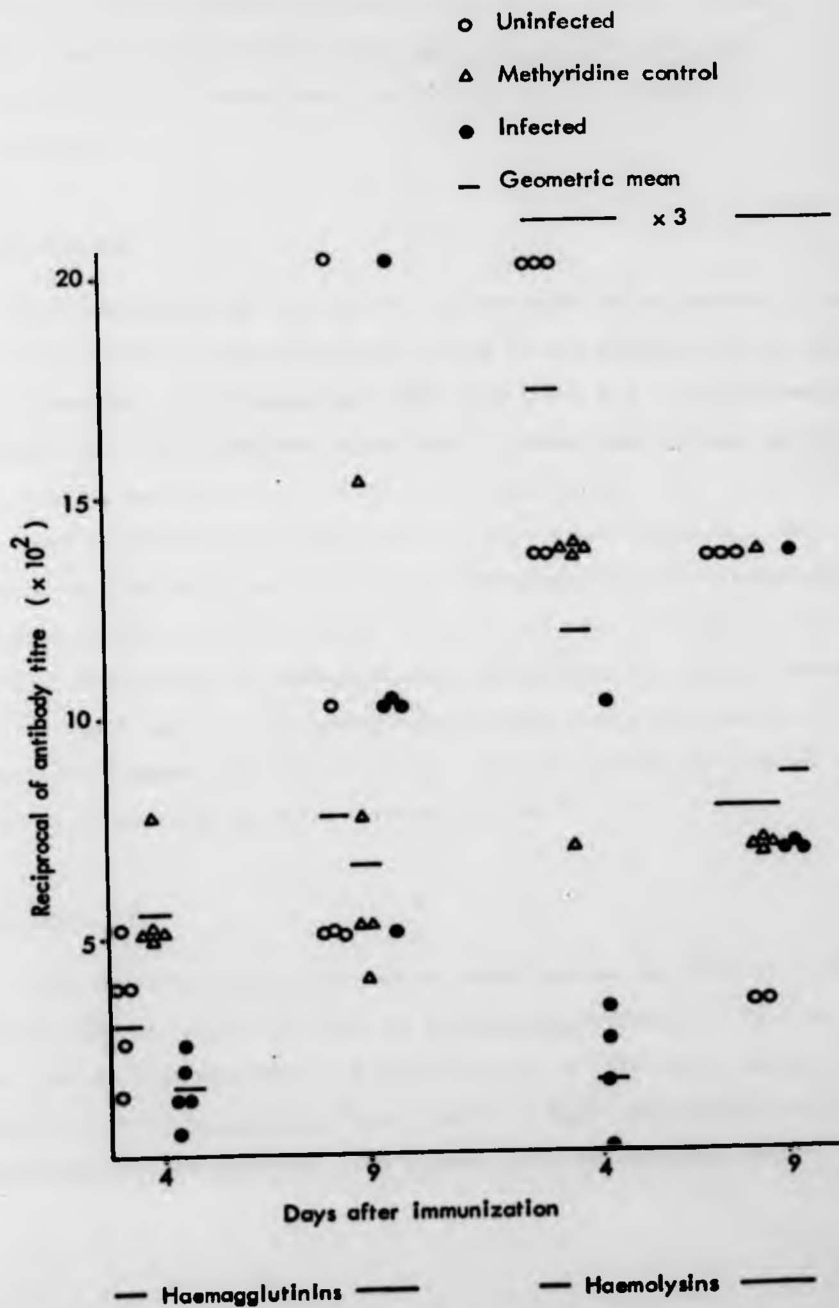
Effect of intestinal phase of T. spiralis infection on antibodies upon SRBC injection

| Group | Mouse no. | Reciprocal of titres | | | |
|---|---|---|---|---|---|
| | | Haemagglutinins | | Haemolysins | |
| | | d 4 | d 9 | d 4 | d 9 |
| Infected then Methyridine treated | 1 | 64 | 512 | 24 | 2048 |
| | 2 | 128 | 1024 | 512 | 2048 |
| | 3 | 128 | 1024 | 768 | 2048 |
| | 4 | 192 | 1024 | 1024 | 4096 |
| | 5 | 256 | 2048 | 3072 | 4096 |
| Geometric mean (95% confidence interval) | | *139 (84-228) | 1024 (642-1632) | *495 (87-2798) | 2702 (1882-3878) |
| Methyridine treated | 1 | 512 | 384 | 2048 | 2048 |
| | 2 | 512 | 512 | 4096 | 2048 |
| | 3 | 512 | 512 | 4096 | 2048 |
| | 4 | 512 | 768 | 4096 | 2048 |
| | 5 | 768 | 1536 | 4096 | 4096 |
| Geometric mean (95% confidence interval) | | *555 (468-658) | 653 (391-1089) | *3566 (2655-4788) | 2353 (2474-3159) |
| Uninfected, untreated controls | 1 | 128 | 512 | 4096 | 1024 |
| | 2 | 256 | 512 | 4096 | 1024 |
| | 3 | 384 | 512 | 6144 | 4096 |
| | 4 | 384 | 1024 | 6144 | 4096 |
| | 5 | 512 | 2048 | 6144 | 4096 |
| Geometric mean (95% confidence interval) | | 301 (180-502) | 776 (465-1401) | 5244 (4233-6443) | 2353 (1141-4850) |

* statistically significant

Figure 24.

Effect of intestinal phase of T. spiralis infection on antibodies upon SRBC injection



injected i.p. with $10^9$ SRBC. 4 days later, the animals were killed and haemagglutinins and haemolysins were measured in the sera. Results of antibody titres are shown in Table 21 and Fig. 25 and confirm that intestinal phase of T. spiralis could depress the haemagglutinins and haemolysins ($p < 0.0025$ and $p < 0.0005$) respectively.

Experiment 4.5

This experiment was carried out to determine the effect of a low level of infection using irradiated larvae on the antibody titres upon SRBC infection. It is comparable with experiment 4.2. 15 mice were infected with 100 irradiated larvae each. 5 mice were killed and gave a mean adult recovery of 22 (SE 3). The infected mice and 10 uninfected mice were injected with $10^9$ SRBC each 11 days after infection. All animals were killed 4 and 9 days later and haemagglutinin titres were measured in their sera. Results, Table 22 and Fig. 26, show no significant differences in haemagglutinins of infected and control groups both on day 4 and 9. This result demonstrates that a high level of infection is needed for the intestinal stage to depress the immune response as measured by serum antibody levels

Experiment 4.6

This experiment was conducted to study further the necessity for a high level of intestinal phase of T. spiralis infection. 24 mice were used in this experiment, 8 mice each group. The first group was infected with 50 T. spiralis larvae each. 5 days later infection was terminated with Methyridine. The second group was injected with

Table 21

Effect of irradiated T. spiralis larvae (i.e. intestinal phase only) on the production of antibodies against SRBC.

| Group | Mouse no. | Reciprocal of titres | |
|---|---|---|---|
| | | Haemagglutinins | Haemolysins |
| Intestinal phase | 1 | 256 | 192 |
| | 2 | 384 | 384 |
| | 3 | 768 | 512 |
| | 4 | 768 | 512 |
| | 5 | 1024 | 768 |
| | 6 | 1024 | 768 |
| | 7 | 1536 | 768 |
| | 8 | 2048 | 1024 |
| | 9 | 2048 | 1536 |
| | 10 | 4096 | - |
| Geometric mean (95% confidence interval) | | *1048 (651-1689) | *621 (428-901) |
| Control | 1 | 1536 | 768 |
| | 2 | 1536 | 1536 |
| | 3 | 2048 | 1536 |
| | 4 | 2048 | 1536 |
| | 5 | 2048 | 1536 |
| | 6 | 2048 | 3072 |
| | 7 | 3072 | 3072 |
| | 8 | 4096 | 4096 |
| | 9 | 4096 | 4096 |
| | 10 | 4096 | - |
| Geometric mean (95% confidence interval) | | 2478 (1972-3116) | 2063 (1451-2932) |

* statistically significant

Figure 25.

Effect of irradiated T. spiralis larvae (i.e. intestinal stage only) on the production of antibodies against SRBC.

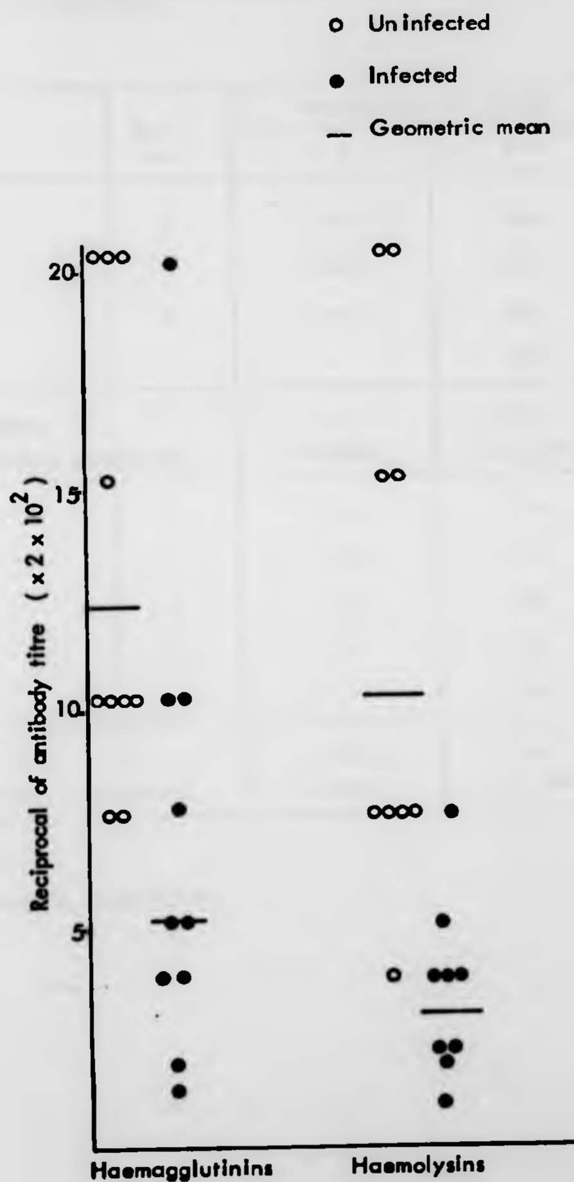


Table 22

Effect of a light irradiated (i.e. intestinal phase) infection of T. spiralis on haemagglutinins against SRBC.

| Group | Mouse no. | Reciprocal of titres | |
|---|---|---|---|
| | | d 4 | d 9 |
| Infected | 1 | 128 | 256 |
| | 2 | 256 | 256 |
| | 3 | 256 | 256 |
| | 4 | - | 384 |
| Geometric mean (95% confidence interval) | | 203 (104-398) | 283 (223-359) |
| Control | 1 | 64 | 64 |
| | 2 | 128 | 128 |
| | 3 | 128 | 256 |
| | 4 | 512 | 256 |
| | 5 | - | 256 |
| Geometric mean (95% confidence interval) | | 153 (54-424) | 169 (93-305) |

* statistically significant

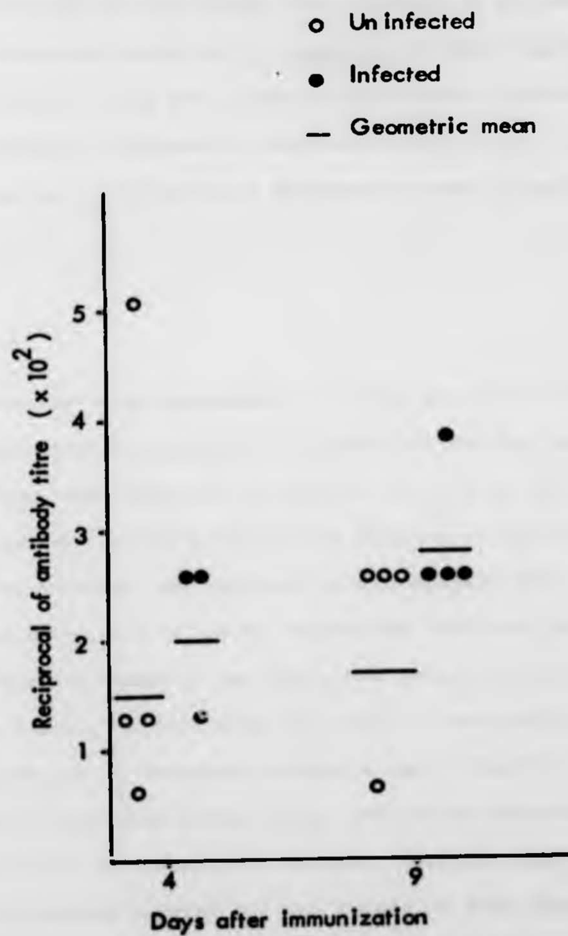
Figure 26.

Effect of light irradiated (i.e. intestinal phase) infection of T.spiralis on haemagglutinins against SRBC

o Un infected

● Infected

— Geometric mean



Methyridine without infection, the third group was used as control. One day after Methyridine injection, all mice received $10^9$ SRBC. Sera of animals were collected 4 and 9 days later and haemagglutinins were measured. Results, Table 23 and Fig. 27, show no significant differences in antibody titres between infected and both Methyridine and control groups on day 4 and 9, confirming the results of the previous experiment and indicating that depression of the immune response by intestinal stage of T. spiralis is dose dependent. The Methyridine treated group had slightly significant increase in haemagglutinins when compared to controls on day 9 ($p < 0.05$). However, there was no significant difference when compared to controls on day 4.

Experiment 4.7

It was noticed from experiment 4.3 that treatment with Methyridine appeared to increase haemagglutinin antibodies and depressed haemolysin antibodies when compared to control animals on day 4. Therefore this experiment was conducted to either confirm or exclude this effect of Methyridine treatment on antibody levels against SRBC. A heavy dose of Methyridine, 1.2 ml of 5% suspension (6000 mg per kg body weight), on divided doses on two days, was injected subcutaneously into each of 8 mice. A day later the group of Methyridine treated mice and a group of 10 untreated controls were injected i.p. with $10^9$ SRBC. All mice were bled 4 days later and serum separated to measure the haemagglutinin and haemolysin titres. Results, Table 24 and Fig. 28 show no difference between the two groups in both types of antibodies, indicating that Methyridine has no effect on antibody titres.

Table 23

Effect of a light Methyridine terminated infection of

*T. spiralis* on haemagglutinins against SRBC

| Group | Mouse no. | Reciprocal of titres | |
|---|---|---|---|
| | | d 4 | d 9 |
| Infected, treated | 1 | 128 | 768 |
| | 2 | 512 | 768 |
| | 3 | 512 | 1024 |
| | 4 | 1024 | 1024 |
| Geometric mean (95% confidence interval) | | 431 (154-1200) | 886 (731-1076) |
| Methyridine treated | 1 | 512 | 1024 |
| | 2 | 512 | 1024 |
| | 3 | 1024 | 2048 |
| | 4 | 1024 | - |
| Geometric mean (95% confidence interval) | | 724 (452-1159) | * 1290 (658-2530) |
| Control | 1 | 192 | 256 |
| | 2 | 768 | 512 |
| | 3 | 2048 | 768 |
| | 4 | 2048 | 1024 |
| Geometric mean (95% confidence interval) | | 886 (237-3311) | 567 (280-1149) |

* statistically significant

Figure 27.

Effect of light Methyridine terminated infection of *T. spiralis* on haemagglutinins against SRBC

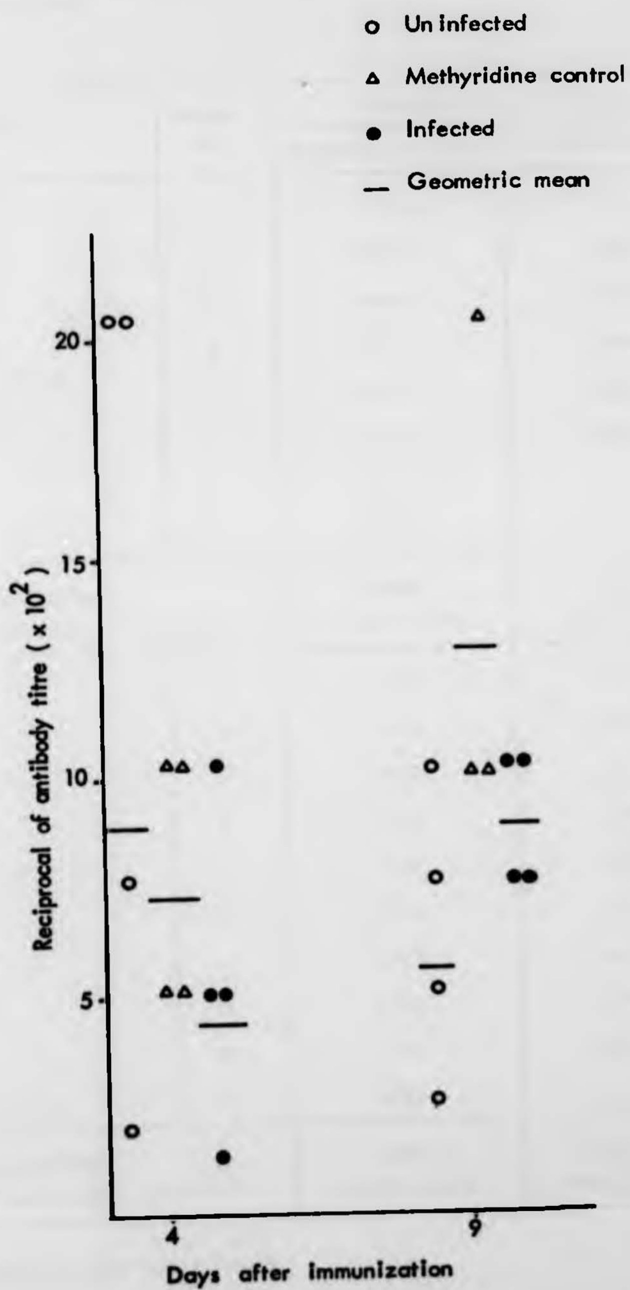


Table 24

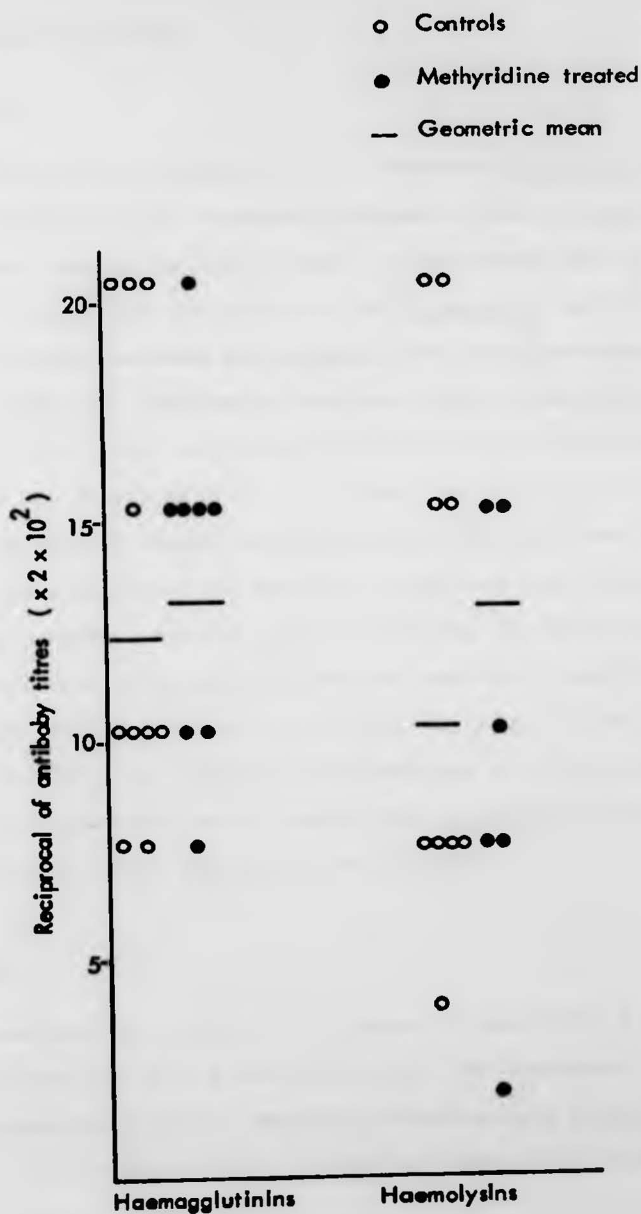
Effect of Methyridine on antibody titres 4 days after SRBC injections

| Group | Mouse no. | Reciprocal of titres | |
|---|---|---|---|
| | | Haemagglutinins | Haemolysins |
| Methyridine treated | 1 | 1536 | 384 |
| | 2 | 2048 | 1536 |
| | 3 | 2048 | 1536 |
| | 4 | 3072 | 2048 |
| | 5 | 3072 | 3072 |
| | 6 | 3072 | 3072 |
| | 7 | 3072 | - |
| | 8 | 4096 | - |
| Geometric mean (95% confidence interval) | | 2638 (2133-3263) | 1612 (857-3027) |
| Control | 1 | 1536 | 768 |
| | 2 | 1536 | 1536 |
| | 3 | 2048 | 1536 |
| | 4 | 2048 | 1536 |
| | 5 | 2048 | 1536 |
| | 6 | 2048 | 3072 |
| | 7 | 3072 | 3072 |
| | 8 | 4096 | 4096 |
| | 9 | 4096 | 4096 |
| | 10 | 4096 | - |
| Geometric mean (95% confidence interval) | | 2478 (1972-3116) | 2063 (1451-2932) |

* statistically significant

Figure 28.

Effect of Methyridine on antibody titres 4 days after SRBC injection



## II. EFFECT OF T. SPIRALIS ON ANTIBODY TITRES AGAINST THE T-INDEPDENT ANTIGEN, LIPOPOLYSACCHARIDE (LPS)

### 1. Effect of full infection:

#### Experiment 4.8

This experiment was conducted to test whether T. spiralis immunodepression is purely T-cell dependent phenomenon. The T-independent antigen LPS was used as the test antigen. 45 mice were used in this experiment. 25 mice were infected with 200 T. spiralis larvae. The mean number of adults counted in the intestines of 5 mice 3 days after infection was 105 $\pm$ 6. The rest of the mice were left as uninfected controls. 30 days later, each mouse received an intravenous injection of $10^{-2}$ µg of LPS dissolved in 0.1 ml of BSS. 4, 6, 9 and 12 days later 5 infected and 5 control mice were killed and bled from the heart. Sera were separated and anti-LPS haemolysins were measured on individual samples. Results (Table 25 and Fig. 29) showed low titres of antibodies in T. spiralis infected mice when compared to the uninfected controls at day 4 ($p < 0.05$), day 6 ($p < 0.0005$) and day 12 ($p < 0.0025$). No antibodies were detected in infected animals at day 9. This experiment shows clearly that T. spiralis infection depresses the host immune response to LPS antigen.

#### Experiment 4.9

This experiment was essentially a repeat of experiment 4.8 except that animals were killed 4, 6 and 9 days after LPS injection. Table 26 and Fig. 30 show the results. Anti-LPS antibodies were suppressed in T. spiralis infected animals when compared to controls at all days

Table 25 Effect of full infection of <u>T. spiralis</u> on the haemolysin titres against LPS

| Mice no. | Group | Reciprocal of titres | | | |
|---|---|---|---|---|---|
| | | Day 4 | Day 6 | Day 9 | Day 12 |
| 1 | *T. spiralis* + LPS | 8 | 256 | 0 | 32 |
| 2 | | 16 | 256 | 0 | 32 |
| 3 | | 16 | 256 | 0 | 32 |
| 4 | | 16 | 512 | 0 | 64 |
| 5 | | 32 | 512 | 0 | 64 |
| Geometric mean | | * 16 | * 338 | * 0 | * 42 |
| (95% confidence interval) | | (10-25) | (235-485) | (0) | (29-60) |
| 1 | LPS | 16 | 1024 | 128 | 64 |
| 2 | | 32 | 1024 | 128 | 128 |
| 3 | | 32 | 2048 | 256 | 128 |
| 4 | | 32 | 2048 | 256 | 128 |
| 5 | | 64 | 4096 | 512 | 256 |
| Geometric mean | | 32 | 1782 | 223 | 128 |
| (95% confidence interval) | | (20-51) | (1026-3096) | (128-387) | (48-342) |

* statistically significant

Figure 29.

Effect of a 30 day *T. spiralis* infection on the haemolysins against Lps.

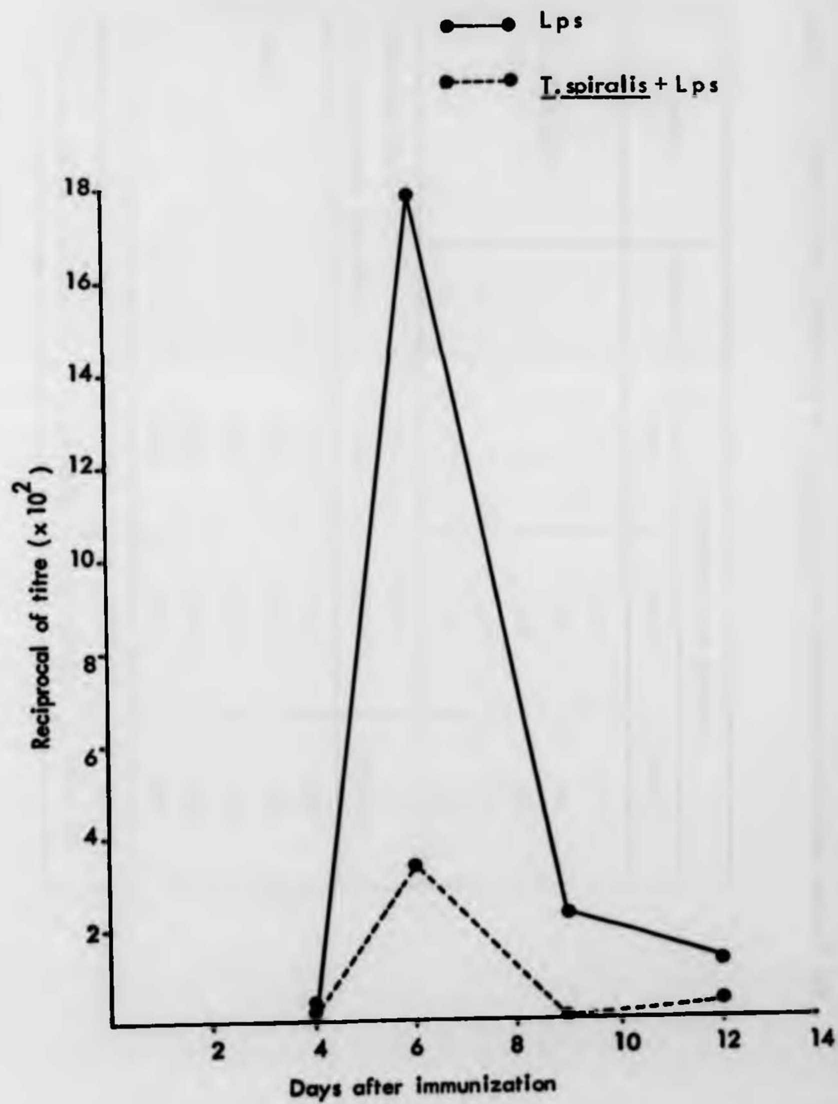


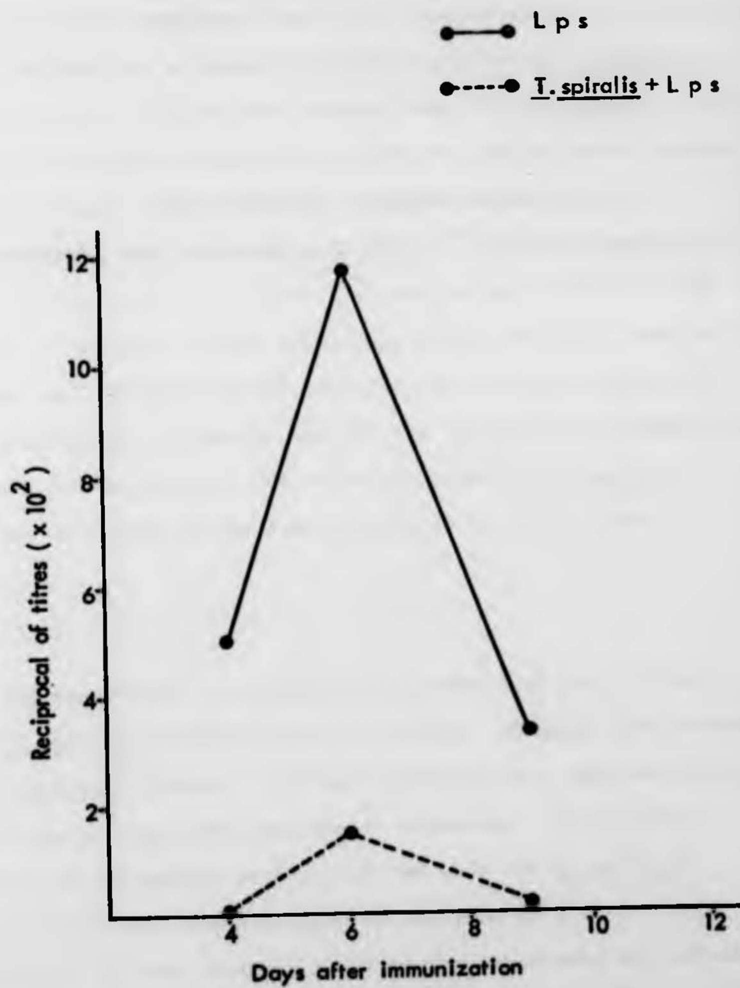
Table 26 Effect of full infection of T. spiralis on haemolysin antibodies against LPS

| Group | Mice no. | Reciprocal of titres | | |
|---|---|---|---|---|
| | | Day 4 | Day 6 | Day 9 |
| T. spiralis + LPS | 1 | 0 | 128 | 8 |
| | 2 | 2 | 128 | 16 |
| | 3 | 2 | 128 | 16 |
| | 4 | 2 | 128 | 16 |
| | 5 | 2 | 256 | 32 |
| Geometric mean (95% confidence interval) | | * 1.7 (1-2) | * 145 (110-197) | * 16 (10-25) |
| LPS | 1 | 128 | 512 | 128 |
| | 2 | 256 | 1024 | 256 |
| | 3 | 512 | 1024 | 256 |
| | 4 | 1024 | 2048 | 512 |
| | 5 | 2048 | 2048 | 1024 |
| Geometric mean (95% confidence interval) | | 501 (180-1455) | 1177 (677-2043) | 333 (160-717) |

* statistically significant

Figure 30.

Effect of a 30 day old T. spiralis infection on haemolysins against Lps.



($p < 0.0005$) confirming that T. spiralis infection depresses the immune response to LPS antigen.

2. Effect of developing and intestinal phases:

Experiment 4.10

The aim of this experiment was to see whether immune unresponsiveness to LPS antigen was a feature of earlier phases of T. spiralis infections in mice. 25 mice were infected with 400 T. spiralis larvae. 3 days later 5 mice were killed and 133 (SE 42) adults found in their intestines. 14 days after infection, infected animals and 10 uninfected controls were injected i.v. with $10^{-2}$ µg LPS dissolved in 0.1 ml BSS. A day later 10 mice of the infected group died. 4 and 6 days later, 5 animals of each group were killed and their sera collected. The anti-LPS haemolysins measured in their sera (Table 27) showed no significant difference between the two groups on either day of the assays indicating that the migrating phase of T. spiralis infection has no effect on the immune response to LPS antigen.

Experiment 4.11

This was essentially a repeat of experiment 4.10 but a third 7 day old T. spiralis infected group was added. 29 mice were infected with 400 T. spiralis larvae. 5 animals were killed 3 days later and 170 (SE 26) adults recovered from their intestines. 7 days later, another group of 29 animals were inoculated with 400 T. spiralis larvae and 115 (SE 18) adults counted in the guts of 5 mice 3 days post infection. 14 days from the start of the experiment all infected animals and 20 uninfected mice were injected i.v. with $10^{-2}$ µg of LPS

Table 27 Effect of a 14 day old <u>T. spiralis</u> infection on anti-LPS haemolysins

| Group | Days after LPS injection | No. of mice | Geometric mean of reciprocal of titres (95% confidence interval) | p value |
|---|---|---|---|---|
| Uninfected controls | 4 | 5 | 1176 (227-6092) | - |
| | 6 | 5 | 1351 (637-2867) | - |
| 14 day old infection | 4 | 5 | 568 (62-5225) | n.s. |
| | 6 | 5 | 524 (60-4613) | n.s. |

n.s. = not significant

dissolved in 0.1 ml of BSS. A day after LPS injection 15 animals of the 14 day old infection group and 3 of the 7 day old infection group died. 4 and 6 days after LPS injection 5 animals of the control and 7 day old infection groups were killed and sera collected. 9 and 12 days after LPS injection a group of animals from each of the 3 groups were killed and sera collected. Anti-LPS haemolysins were measured in all sera collected and the results are presented in Table 28. It was noticed that there was a great variability in individual data. No significant difference was detected between the 7 day old infected group and the controls on either day of the assays. However the 14 day old infected group showed significant reduction in antibody titres on day 12 ($p < 0.05$) but not on day 9.

Experiment 4.12

This experiment was carried out to either confirm or exclude the immunodepressing effect of early phases of *T. spiralis* to LPS antigen. 45 mice were used for each phase of infection and 40 mice used as controls. Adults recovered 3 days after each infection were 103 (SE 34) and 133 (SE 42) for the 14 day old and 7 day old infections respectively. LPS antigen was injected into all animals in the same way as in the last experiment. Again a day after LPS injection 4 animals of the 7 day old group and 28 animals of the group infected for 14 days had died. The rest of the animals and the controls were divided into 4 sub-groups each and killed 4, 6, 9 and 12 days after LPS injections. Sera were collected and anti-LPS haemolysins were measured on individual sera. The results, Table 29, showed no significant difference between the 7 day old infection group and the control group. The 14 day old infection group depressed anti-LPS haemolysins on day 6 ($p < 0.005$) but not on days 4 and 9 after LPS injection.

Table 28 Effect of early phases of *T. spiralis* infection on antibody titres against LPS

| Group | Days after LPS injection | No. of mice | Geometric mean of reciprocal of titres (95% confidence interval) | p value |
|---|---|---|---|---|
| Uninfected controls | 4 | 5 | 50 (5-520) | - |
| | 6 | 5 | 1276 (318-5102) | - |
| | 9 | 5 | 692 (141-3397) | - |
| | 12 | 5 | 1176 (571-2425) | - |
| 7 day old infection | 4 | 5 | 163 (7-3799) | n.s. |
| | 6 | 5 | 1276 (581-2800) | n.s. |
| | 9 | 6 | 164 (30-910) | n.s. |
| | 12 | 5 | 638 (95-4293) | n.s. |
| 14 day old infection | 4 | n.d. | n.d. | n.d. |
| | 6 | n.d. | n.d. | n.d. |
| | 9 | 4 | 400 (115-1393) | n.s. |
| | 12 | 5 | * 42 (1-1250) | $<0.05$ |

* statistically significant

n.s. = not significant

n.d. = not done

Table 29 Effect of T. spiralis at different ages of infection on the antibody response against LPS

| Group | Days after LPS injection | No. of mice | Geometric mean of reciprocal of titres (95% confidence interval) | p value |
|---|---|---|---|---|
| Uninfected controls | 4 | 10 | 562 (323-973) | - |
| | 6 | 10 | 1142 (517-2525) | - |
| | 9 | 10 | 409 (95-1766) | - |
| | 12 | 10 | 772 (363-1641) | - |
| 7 day old infection | 4 | 10 | 664 (266-2547) | n.s. |
| | 6 | 8 | 437 (105-1799) | n.s. |
| | 9 | 9 | 445 (157-1256) | n.s. |
| | 12 | 9 | 342 (152-770) | n.s. |
| 14 day old infection | 4 | 4 | 100 (2-4062) | n.s. |
| | 6 | 4 | * 21 (2-864) | $<0.005$ |
| | 9 | 4 | 576 (96-3351) | n.s. |
| | 12 | n.d. | n.d. | n.d. |

* statistically significant

n.s. = not significant

n.d. = not done

Discussion

Several points can be made from experiments of this chapter which are summarized in Table 30. T. spiralis infection depressed the production of both haemagglutinins and haemolysins against the T-dependent antigen, SRBC. Depression was demonstrated when the three phases of infection were present in the host (experiment 4.1 and 4.2), and also when the infection was limited to the intestinal phase (experiments 4.3 and 4.4). These results agree with results obtained in the previous chapter with PFC and confirm the depression of the immune response to the T-dependent antigen, SRBC in T. spiralis infected mice. Thus not only were the number of lymphocytes depressed by T. spiralis infection, but the total amount of IgM antibody secreted was lower.

Chimishkyan and Ovumyan (1975) have shown that T. spiralis exerts an immunodepressive effect on the production of anti vaccinia virus agglutinin. Results of this study are comparable with their results. The results also confirm the depressed antibody levels against SRBC to T. spiralis infection shown by Faubert and Tanner (1971) and Jones et al. (1976) and against JBE (Cypess et al., 1973). Lubiniecki et al. (1974b) have reported that T. spiralis infection had no effect on anti-SRBC haemagglutinins although later Lubiniecki and Cypess (1975a) demonstrated a depressed antibody response to JBE virus and SRBC in T. spiralis infected animals. They showed that the immunodepression was more on IgG antibodies. Irrespective of the contradictions between their two papers, their data does not agree with that presented here. However the strain of mice and helminth, dose of larvae and route of SRBC inoculation employed here were different than those used in their studies.

Table 30 Summary of the effect of T. spiralis infection on antibody levels (D = significant depression, n = no effect)

| Experiment no. | Table no. | Number of T. spiralis larvae inoculated | Type of infection | Type of antigen | Type of antibody | Effect on antibody titres after antigen injection | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Day 4 | Day 6 | Day 9 | Day 12 |
| 4.1 | 18 | 400 | | | Haemagglutinins | D | | n | |
| 4.2 | 19 | 50 | 30 day old infection | SRBC | " | n | | - | |
| 4.1 | 18 | 400 | | | Haemolysins | D | | n | |
| 4.4 | 19 | 2000 | Irradiated larvae | | Haemagglutinins | D | | - | |
| 4.5 | 22 | 100 | " " | | " | n | | n | |
| 4.3 | 20 | 400 | Methyridine treated | | " | D | | n | |
| 4.6 | 23 | 50 | " " | SRBC | " | n | | n | |
| 4.4 | 21 | 2000 | Irradiated larvae | | Haemolysins | D | | - | |
| 4.3 | 20 | 400 | Methyridine treated | | " | D | | n | |
| 4.8 | 25 | 200 | 30 day old infection | | | D | D | D | D |
| 4.9 | 26 | 200 | " " " " | | | D | D | D | - |
| 4.10 | 27 | 400 | 14 day old infection | | | n | n | - | - |
| 4.11 | 28 | 400 | " " " " | LPS | Haemolysins | - | - | n | D |
| 4.12 | 29 | 400 | " " " " | | | n | D | n | n |
| 4.11 | 28 | 400 | 7 day old infection | | | n | n | n | n |
| 4.12 | 29 | 400 | " " " " | | | n | n | n | n |

Antibody levels against SRBC, induced by both the intestinal phase and full infection, was dependent on high levels of T. spiralis infection (Experiments 4.2, 4.5 and 4.6) confirming the dose dependency of the immune unresponsiveness due to adult phase shown in the previous chapter. However the number of PFC in mice infected for 30 days was not dependent on high level of infection. It seems probable that light infection for 30 days had only a partial effect on the immune response against SRBC (depressed PFC but not antibody levels).

It was observed from Experiments 4.1 - 4.6 that depressed levels of antibodies due to T. spiralis infection were only on day 4 when IgM antibodies predominate (Table 17). This finding is in agreement with the depressed levels of IgM PFC demonstrated in the previous chapter.

Studying the effect of T. spiralis on the immune response to the T-independent antigen LPS showed different results with different phases of infection. Full infection (experiments 4.8 and 4.9) demonstrated long term depression of anti-LPS haemolysins while 7 day old infections (representing the intestinal phase) (experiments 4.11 and 4.12) had no effect on anti-LPS antibody levels when compared to controls. However the 14 day old infection group (experiments 4.10, 4.11 and 4.12) seldom showed a significant difference when compared to controls. The two exceptions were on days 6 and 13 in experiments 4.12 and 4.11 respectively. By the day of the assay the developing larvae are in the muscle cells and, therefore, the depression detected on those days may be an early expression of the effect of the muscle phase rather than of the intestinal or migrating phase. Death of animals with 14 day old infections prevented the use of the same number of animals comparable to the controls and was the reason for cutting short the days of some assays. This made studying the consistency of results at similar days after LPS injection difficult.

However death of animals after LPS challenge may be another indication of depressed immune status during infection with the developing phase. Perrudet-Badoux et al. (1976 and 1977 ) showed that infection with T. spiralis causes potentiation of IgE response which occurs at the time of migration of larvae from the intestine to blood vessels. Their results may be another explanation of the death of large numbers of animals when challenging with LPS was carried out during migrating phase of infection. Further experiments are needed to explain this finding.

LPS antigen selectively stimulates the secretion of 19 S (Andersson et al., 1972). In contrast Zaunderer and Askona, 1976) have shown that precursors to IgG, IgA and IgM secreting cells can be activated by LPS stimulus. This contradiction makes the explanation of the results of this study difficult regarding the effect of T. spiralis on different classes of antibody against LPS antigen. LPS antigen does not require T-lymphocytes to activate B cells (Moller and Michael, 1971; Andersson et al., 1972; and Zaunderer and Askona, 1976) so that these results may indicate that T. spiralis infection depresses the host immune response independently of the T cell helper function.

Barriga (1975) has showed that animals infected with T. spiralis for 7 days depressed the antibody response to the T-dependent SRBC but not to the T-independent antigen PVP. Results presented in experiments 4.3, 4.4, 4.11 and 4.12 are in agreement with his data. In addition, Ljungström and Huldt (1977) found that when CBA mice are infected with T. spiralis they show depressed IgM agglutinins to SRBC but not to PVP when injected 21 days after T. spiralis infection. Later, Barriga (1978b) found that an extract prepared from T. spiralis muscle larvae depressed the blastogenic response to LPS antigen. His

finding supports the results of experiment 4.8 and 4.9.

In conclusion, experiments presented in this part of the study show that early stages of T. spiralis infection depresses the immune response to T-dependent but not to T-independent antigens while late stages of infection depress the immune response to both T-dependent and T-independent antigens SRBC and LPS.

# CHAPTER 5

## Effect of *T. spiralis* infection on macrophage activity

### Introduction

Over the past few years the macrophages have been recognized as playing an increasingly important role in both induction and expression of the immune response. Metchnikoff (1905) maintained that the macrophage is the body's chief defence against particulate foreign matter and many chronic infections. Later workers have demonstrated that the macrophages respond to certain infections with an adaptive increase in its defensive capacities (Suter and Ramseier, 1964). Recently, Mackaness (1964 and 1969) has shown that the enhancement of macrophage function during infection has an immunological basis and involves the lymphocyte. Antibody responses to the majority of multideterminant antigens appear to depend on interactions between at least three functionally distinct cell types, T cells, B cells and macrophages (Unanue, 1972). Unlike B or T lymphocytes, the macrophage does not by itself possess immunological specificity. It is accepted that one way in which macrophages operate in the immune response is by binding and processing certain antigens and mitogens and subsequently presenting them to lymphocytes in a molecular form appropriate for lymphocyte activation. Their function in antigen uptake, catabolism and presentation to T and B cells in the initiation of immune responses have been reviewed by Rosenstreich and Oppenheim (1976). The cells of the reticulo-endothelial system (RES) are all capable of ingesting foreign material and degrading it by means of intracellular enzymes in phago-lysosomes.

Radioactively labelled antigen injected into an animal is taken up by macrophages, and most of the antigen undergoes rapid degradation.

*T. spiralis* induces depression of the immune response to the T-dependent and T-independent antigens SRBC and LPS. It was of interest to find out if changes in the activity of macrophages have any contributing role in *T. spiralis* induced immunodepression. Macrophage activity was studied by the clearance of $^{125}$I PVP and $^{51}$Cr-SRBC in animals infected with *T. spiralis* and controls.

Inbred Simpson mice between 8-12 weeks of age, weighing 20-30 g, were used in this part of the study.

a) Effect of *T. spiralis* infection on the clearance of $^{125}$I poly vinyl pyrolidone from the blood as an indication of macrophage activity

The rate constant (K PVP) of the exponential decay in blood radioactivity between 18 and 48 hours after i.v. injection of 30-80 μg of $^{125}$I labelled polyvinyl pyrolidone (PVP) in mice is a good indicator of the phagocytic activity of macrophages (Morgan and Soothill, 1975b). The test has some advantages over conventional tests of *in vivo* phagocytosis such as the clearance of colloidal carbon or aggregated proteins. It is simple and accurate, the dose to be used is not critical and is well below that which would lead to interference with macrophage activity. Blood radioactivity following the injection of 75 μg $^{125}$I-labelled PVP into the tail vein falls rapidly in the first few hours after injection, but after about 8 hours there is a slower, exponential fall due to the phagocytic activity of macrophages. The rapid phase of loss of radioactivity probably results both from

diffusion of PVP into the extravascular fluid and also from loss of PVP into the urine (Morgan and Soothill, 1975b).

$^{125}$I-labelled PVP (Radiochemical Centre, Amersham, Bucks., specific activity 41 µCi per mg) with a stated average molecular weight of 30-40,000 was used. It was diluted with normal saline to obtain the required dose of 25-50 µg for injection. Blood samples were collected from the retro-orbital venous plexus and whole blood radioactivity counted in 1280 ultrogamma counter.

A preliminary experiment was conducted to study the effect of number of blood samples on the exponential phase of $^{125}$I PVP clearance. 5 mice were injected in the tail vein with 1 µCi of $^{125}$I PVP (25-50 µg) each. 50 µl blood samples were collected from the retro-orbital sinus 18, 25, 40 and 48 hours after injection and radioactivity was measured. Decay constant (K PVP $h^{-1}$) was calculated as follows:

$$\frac{\text{Natural log of sample (A) - Natural log of sample (B)}}{\text{Hours after } ^{125}\text{I PVP injection (B-A)}}$$

Fig. 31 shows that the fall of blood radioactivity was consistently exponential as judged by a straight regression line graph. There is, therefore, no obvious advantage in using more than 2 points (Table 31) and subsequent studies are therefore based on 2 blood samples taken 18 and 48 hours after $^{125}$I PVP injection. This is in agreement with the results of Morgan and Soothill (1975b).

A second, preliminary, experiment was designed to study the effect of sex differences in Simpson mice on $^{125}$I PVP clearance since it was not easy to provide the same sex for each experiment.

9 male and 9 female Simpson mice of the same age were used in this experiment. Each mouse was injected with 1 µci (25-50 µgm) of $^{125}$I PVP into the tail vein. 18 and 48 hours after injection 50 µl of blood was collected from the retro-orbital sinus and the amount of radioactivity

Table 31

Effect of number of blood samples (n) used to calculate K $^{125}$I PVP $h^{-1}$

| Mouse no. | Mean K PVP $h^{-1}$ ± SE (n = 4) | K PVP $h^{-1}$ (n = 2 ) |
|---|---|---|
| 1 | 0.0238 ± 0.0050 | 0.0294 |
| 2 | 0.0309 ± 0.0051 | 0.0347 |
| 3 | 0.0343 ± 0.0062 | 0.0337 |
| 4 | 0.0408 ± 0.0040 | 0.0406 |
| 5 | 0.0268 ± 0.0072 | 0.0292 |
| Mean ± SE | 0.0313 ± 0.0000 | 0.0335 ± 0.0000 |

* statistically significant

Fig. 31 : Blood radioactivity at different times after $^{125}$I PVP injection

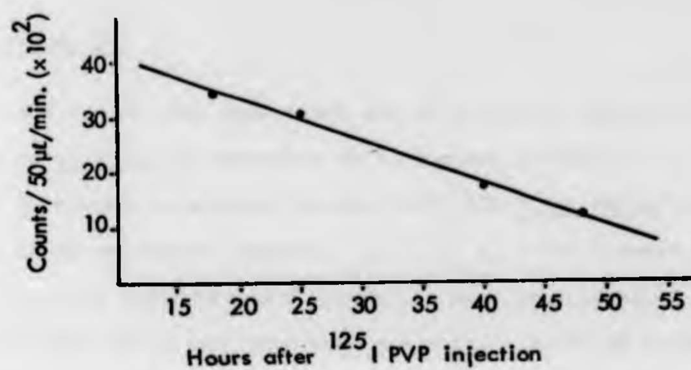
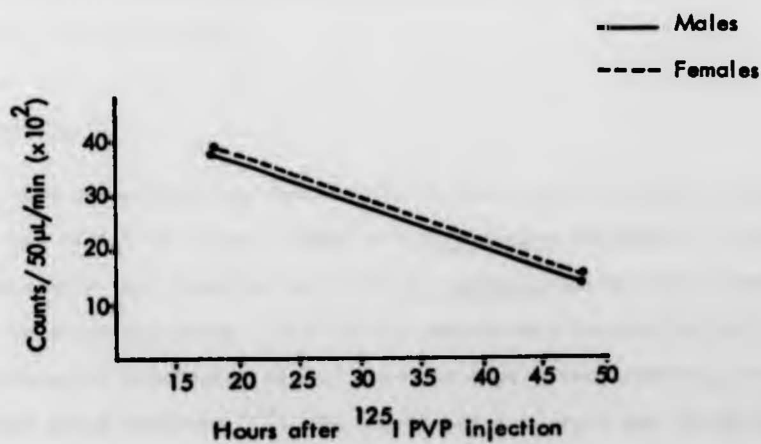



Fig. 32 :Effect of sex on $^{125}$I PVP clearance



per sample per minute was measured in each. Results are shown in Table 32 and Fig. 32. There were no significant differences in K PVP $h^{-1}$ between the two groups indicating that both sexes clear $^{125}$I PVP at the same speed.

Experiment 5.1

The aim of this experiment was to study the effect of different ages of T. spiralis infection on macrophage activity.

35 Simpson mice were infected with 400 T. spiralis larvae, and 35 kept as an uninfected control. 1, 2, 3, 4, 6 and 8 weeks after infection, 6 infected and 6 uninfected mice were injected with 1 μci of $^{125}$I PVP (25-50 μg) into the tail vein. 18 and 48 hours later mice were bled (50 μℓ) and radioactivity per minute was measured. 118,200 (SE 34,200) larvae were counted in the muscles of 5 infected animals. Results show that mice with a one week old T. spiralis infection (i.e. intestinal phase) clear $^{125}$I PVP significantly faster than controls ($p < 0.005$). There were no significant differences in PVP clearance between the other stages of infection and the control groups (Tables 33-38 and Figs. 33-38).

Experiment 5.2

This experiment was carried out on the same basis as 5.1 to confirm the effect of 1 and 2 weeks old T. spiralis infection. 21 male Simpson mice were infected with 400 T. spiralis larvae and 16 were used as a control group. 191 (SE 5) adults were counted in the intestines of 5 infected mice. 7 and 14 days after infection 8 mice of each group received $^{125}$I PVP (25-50 μg) i.v. each and 50 μℓ of blood was collected from the retro-orbital sinus 18 and 48 hours later.

Table 32

Effect of sex on $^{125}$I PVP clearance

| Group | Mouse no. | Count/50 $\mu\ell$/min after $^{125}$I PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Males | 1 | 3819 | 1916 | 0.0230 |
| | 2 | 2932 | 1400 | 0.0246 |
| | 3 | 3983 | 1617 | 0.0300 |
| | 4 | 2927 | 1155 | 0.0310 |
| | 5 | 4884 | 1722 | 0.0348 |
| | 6 | 3814 | 1312 | 0.0356 |
| | 7 | 4042 | 1358 | 0.0364 |
| | 8 | 2501 | 754 | 0.0400 |
| | 9 | 5000 | 1216 | 0.0471 |
| Mean | | 3767 | 1383 | 0.0336 |
| ± SE | | 286 | 114 | 0.0025 |
| Females | 1 | 4028 | 2203 | 0.0201 |
| | 2 | 3885 | 1804 | 0.0220 |
| | 3 | 3227 | 1328 | 0.0296 |
| | 4 | 5428 | 2214 | 0.0300 |
| | 5 | 4210 | 1580 | 0.0327 |
| | 6 | 3984 | 1425 | 0.0343 |
| | 7 | 3049 | 1028 | 0.0362 |
| | 8 | 3611 | 1091 | 0.0399 |
| | 9 | 3689 | 882 | 0.0477 |
| Mean | | 3901 | 1506 | 0.0325 |
| ± SE | | 228 | 163 | 0.0028 |

** K PVP $h^{-1}$ = rate constant of exponential fall of $^{125}$I PVP/hour.

* statistically significant

Table 33

Effect of T. spiralis infection on $^{125}$I PVP clearance

a) 1 week after infection

| Group | Mouse no. | Count/50 $\mu\ell$/min after $^{125}$I PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Control | 1 | 4028 | 2203 | 0.0201 |
| | 2 | 3885 | 1804 | 0.0220 |
| | 3 | 3819 | 1916 | 0.0230 |
| | 4 | 2932 | 1400 | 0.0246 |
| | 5 | 3049 | 1028 | 0.0362 |
| | 6 | 2501 | 754 | 0.0400 |
| Mean ± SE | | 3369<br>255 | 1518<br>227 | 0.0277<br>0.0000 |
| Infected | 1 | 5556 | 1816 | 0.0373 |
| | 2 | 3321 | 1004 | 0.0399 |
| | 3 | 4154 | 1243 | 0.0402 |
| | 4 | 2796 | 515 | 0.0410 |
| | 5 | 5113 | 1438 | 0.0423 |
| | 6 | 4241 | 1104 | 0.0449 |
| Mean ± SE | | 4197<br>425 | 1187<br>178 | * 0.0409<br>0.0010 |

** K PVP $h^{-1}$ = rate constant of exponential fall of $^{125}$I PVP/hour.

* statistically significant

Table 34

Effect of T. spiralis on $^{125}$I PVP clearance

b) 2 weeks after infection

| Group | Mouse no. | Count/50 $\mu\ell$/min after $^{125}$I PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Control | 1 | 3227 | 1328 | 0.0296 |
| | 2 | 3983 | 1617 | 0.0300 |
| | 3 | 2927 | 1155 | 0.0310 |
| | 4 | 4210 | 1580 | 0.0327 |
| | 5 | 3984 | 1425 | 0.0343 |
| | 6 | 3814 | 1312 | 0.0356 |
| Mean | | 3691 | 1403 | 0.0322 |
| ± SE | | 204 | 72 | 0.0091 |
| Infected | 1 | 3695 | 1514 | 0.0297 |
| | 2 | 3692 | 1423 | 0.0318 |
| | 3 | 4017 | 1284 | 0.0380 |
| | 4 | 3423 | 1051 | 0.0394 |
| | 5 | 3043 | 716 | 0.0482 |
| | 6 | 2289 | 917 | 0.0304 |
| Mean | | 3360 | 1151 | 0.0379 |
| ± SE | | 252 | 127 | 0.0000 |

** K PVP $h^{-1}$ = rate constant of exponential fall of PVP/hour.

* statistically significant

Fig. 33: Effect of 1 week T. spiralis on $^{125}$I PVP clearance

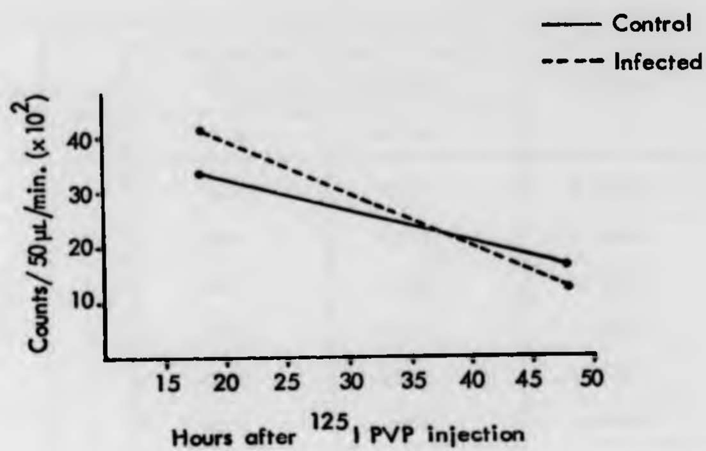



Fig. 34: Effect of 2 weeks T. spiralis on $^{125}$I PVP clearance

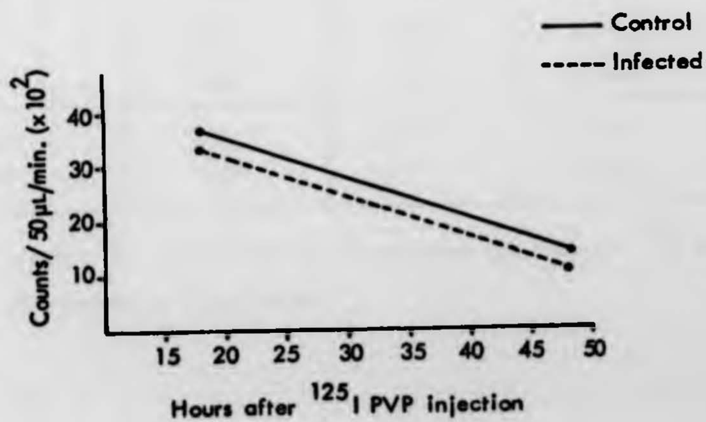


Table 35

Effect of <u>T. spiralis</u> on $^{125}$I PVP clearance

c) 3 weeks after infection

| Group | Mouse no. | Counts/50 μℓ/min after $^{125}$I PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Control | 1 | 5428 | 2214 | 0.0300 |
| | 2 | 4884 | 1722 | 0.0348 |
| | 3 | 4042 | 1358 | 0.0364 |
| | 4 | 3611 | 1091 | 0.0399 |
| | 5 | 5000 | 1216 | 0.0471 |
| | 6 | 3689 | 882 | 0.0477 |
| Mean | | 4442 | 1414 | 0.0393 |
| ± SE | | 311 | 197 | 0.0029 |
| Infected | 1 | 5427 | 2096 | 0.0317 |
| | 2 | 5014 | 1785 | 0.0344 |
| | 3 | 5289 | 1871 | 0.0346 |
| | 4 | 3969 | 1190 | 0.0401 |
| | 5 | 4334 | 1282 | 0.0406 |
| | 6 | 2088 | 621 | 0.0409 |
| Mean | | 4354 | 1474 | 0.0371 |
| ± SE | | 508 | 223 | 0.0016 |

** K PVP $h^{-1}$ = rate constant of exponential fall of $^{125}$I PVP/hour.

* statistically significant

Table 36

Effect of T. spiralis on $^{125}I$ PVP clearance

d) 4 weeks after infection

| Group | Mouse no. | Counts/50 µℓ/min after $^{125}I$ PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Control | 1 | 6496 | 3354 | 0.0220 |
| | 2 | 4025 | 1864 | 0.0257 |
| | 3 | 4688 | 1907 | 0.0300 |
| | 4 | 3928 | 1435 | 0.0336 |
| | 5 | 4063 | 1395 | 0.0356 |
| | 6 | 4088 | 1377 | 0.0363 |
| Mean | | 4548 | 1889 | 0.0305 |
| ± SE | | 405 | 309 | 0.0000 |
| Infected | 1 | 4051 | 1814 | 0.0268 |
| | 2 | 3482 | 1425 | 0.0298 |
| | 3 | 4635 | 1844 | 0.0307 |
| | 4 | 3239 | 1262 | 0.0314 |
| | 5 | 3583 | 1306 | 0.0336 |
| | 6 | 3719 | 1030 | 0.0428 |
| Mean | | 3785 | 1447 | 0.0325 |
| ± SE | | 202 | 132 | 0.0022 |

** K PVP $h^{-1}$ = rate constant of exponential fall of $^{125}I$ PVP/hour.

* statistically significant

Fig.35 : Effect of 3 weeks T. spiralis on $^{125}$I PVP clearance

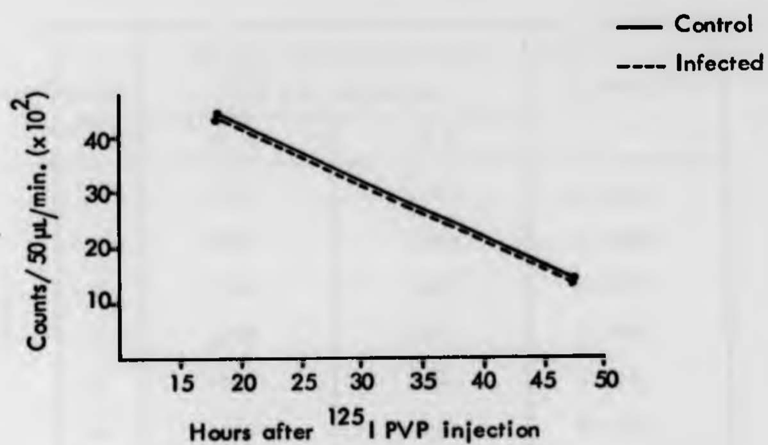



Fig.36 : Effect of 4 weeks T. spiralis on $^{125}$I PVP clearance

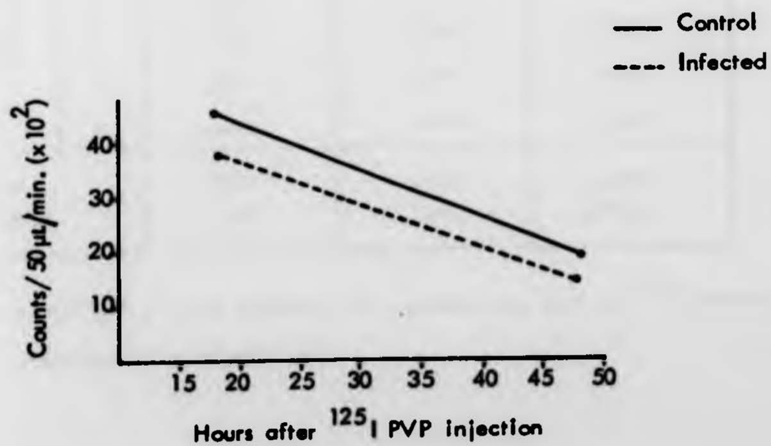


Table 37

Effect of *T. spiralis* on $^{125}$I PVP clearance

e) 6 weeks after infection

| Group | Mouse no. | Counts/ 50 $\mu\ell$/min after $^{125}$I PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Control | 1 | 3790 | 1747 | 0.0258 |
| | 2 | 2935 | 1266 | 0.0280 |
| | 3 | 3766 | 1614 | 0.0282 |
| | 4 | 3998 | 1551 | 0.0316 |
| | 5 | 3856 | 1391 | 0.0340 |
| | 6 | 4255 | 1154 | 0.0435 |
| Mean | | 3767 | 1454 | 0.0319 |
| ± SE | | 182 | 91 | 0.0026 |
| Infected | 1 | 2723 | 2719 | 0.0001 |
| | 2 | 1088 | 1397 | -0.0083 |
| | 3 | 2960 | 1220 | 0.0295 |
| | 4 | 3518 | 998 | 0.0420 |
| | 5 | 3771 | 1007 | 0.0440 |
| | 6 | 3260 | 862 | 0.0443 |
| Mean | | 2887 | 1368 | 0.0400 |
| ± SE | | 391 | 281 | 0.0261 |

** K PVP $h^{-1}$ = rate constant of exponential fall of $^{125}$I PVP/hour.

* statistically significant

Table 38

Effect of T. spiralis on $^{125}$I PVP clearance

f) 8 weeks after infection

| Group | Mouse no. | Counts/50 μℓ/min after $^{125}$I PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Control | 1 | 3014 | 1387 | 0.0259 |
| | 2 | 4052 | 1796 | 0.0271 |
| | 3 | 4581 | 1941 | 0.0286 |
| | 4 | 4052 | 1628 | 0.0304 |
| | 5 | 3291 | 1315 | 0.0306 |
| | 6 | - | - | - |
| Mean ± SE | | 3798<br>284 | 1613<br>119 | 0.0285<br>0.0008 |
| Infected | 1 | 1771 | 822 | 0.0256 |
| | 2 | 2718 | 1143 | 0.0289 |
| | 3 | 2717 | 1062 | 0.0313 |
| | 4 | 2751 | 855 | 0.0390 |
| | 5 | 2999 | 813 | 0.0435 |
| | 6 | - | - | - |
| Mean ± SE | | 2591<br>212 | 939<br>68 | 0.0337<br>0.0033 |

** K PVP $h^{-1}$ = rate constant of exponential fall of $^{125}$I PVP/hour.

* statistically significant

Fig. 37: Effect of 6 weeks T. spiralis on $^{125}$I PVP clearance

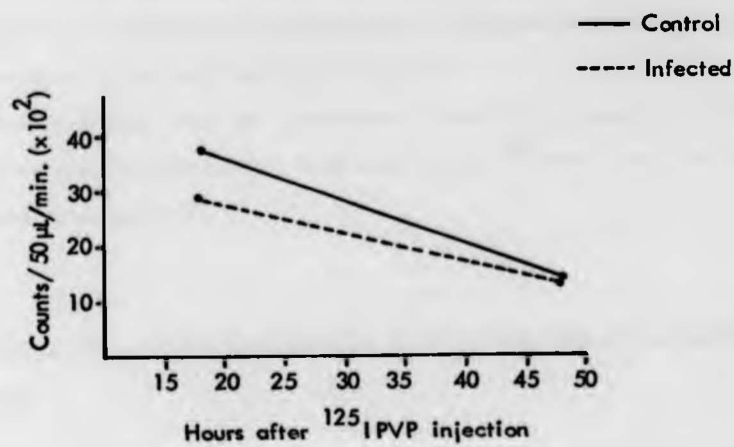
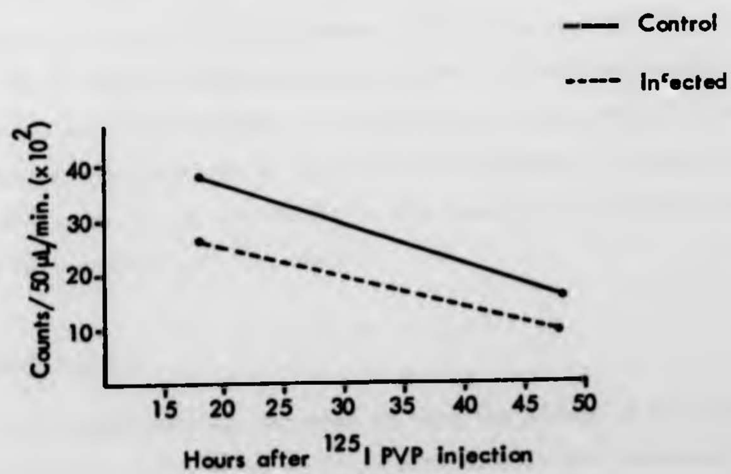



Fig. 38: Effect of 8 weeks T. spiralis on $^{125}$I PVP clearance



Radioactivity/min. are recorded in Tables 39 and 40 and Fig. 39 and 40. $^{125}I$ PVP clearance in 7 day old infection group was significantly faster than the control group ($p < 0.0005$) measured by ($K$ PVP $h^{-1}$). There were no significant differences between 14 day old infection group and the control group.

This confirms that the intestinal stage of T. spiralis infection activates the macrophages so that they clear $^{125}I$ PVP from the circulation more rapidly.

b) Effect of T. spiralis infection on the clearance of $^{51}Cr$ labelled SRBC

Study at this stage was directed towards determining the effect of T. spiralis infection on $^{51}Cr$-SRBC clearance from the blood.

Labelling of SRBC with $^{51}Cr$:

SRBC in Alsever's solution were washed three times in PBS (pH 7.2). After the last wash a 10% suspension of SRBC was made in PBS. 1 ml of $^{51}Cr$ (Na Chromate in aqueous solution obtained from Radiochemical Centre, Amersham, specific activity 1.04 mCi/8.4 µg/ml) was added to the SRBC suspension and incubated at 37°C for 45-90 minutes. The SRBC were then washed 4 times in PBS and finally a 10% suspension in PBS was made (0.25 ml contained $10^9$ $^{51}Cr$-SRBC).

Experiment 5.3

This experiment was designed to test the effect of full infection and intestinal phase of T. spiralis infection on the clearance of $^{51}Cr$

Table 39

Effect of T. spiralis on $^{125}$I PVP clearance

1 week after infection

| Group | Mouse no. | Counts/50 $\mu\ell$/min after $^{125}$I PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Control | 1 | 1991 | 875 | 0.0274 |
| | 2 | 3057 | 1219 | 0.0306 |
| | 3 | 2590 | 997 | 0.0318 |
| | 4 | 2205 | 840 | 0.0322 |
| | 5 | 2610 | 977 | 0.0328 |
| | 6 | 2206 | 1000 | 0.0332 |
| | 7 | 2616 | 959 | 0.0335 |
| | 8 | 2911 | 974 | 0.0365 |
| Mean | | 2523 | 980 | 0.0323 |
| ± SE | | 130 | 40 | 0.0009 |
| Infected | 1 | 2675 | 999 | 0.0328 |
| | 2 | 2343 | 725 | 0.0391 |
| | 3 | 2753 | 850 | 0.0392 |
| | 4 | 3290 | 948 | 0.0415 |
| | 5 | 2696 | 611 | 0.0495 |
| | 6 | 4167 | 923 | 0.0502 |
| | 7 | 2342 | 520 | 0.0502 |
| | 8 | 2661 | 447 | 0.0594 |
| Mean | | 2866 | 753 | * 0.0453 |
| ± SE | | 213 | 74 | 0.0030 |

** K PVP $h^{-1}$ - rate constant of exponential fall of $^{125}$I PVP/hour.

* statistically significant

Table 40

Effect of T. spiralis on $^{125}I$ PVP clearance

2 weeks after infection

| Group | Mouse no. | Counts/50 μl/min after $^{125}I$ PVP injection | | ** K PVP $h^{-1}$ |
|---|---|---|---|---|
| | | 18 h | 48 h | |
| Control | 1 | 2820 | 1511 | 0.0208 |
| | 2 | 3005 | 1328 | 0.0272 |
| | 3 | 3960 | 1675 | 0.0287 |
| | 4 | 2640 | 1064 | 0.0303 |
| | 5 | 2777 | 1112 | 0.0305 |
| | 6 | 4474 | 1775 | 0.0308 |
| | 7 | 3657 | 1325 | 0.0338 |
| | 8 | 3470 | 1025 | 0.0407 |
| Mean | | 3350 | 1352 | 0.0304 |
| ± SE | | 231 | 100 | 0.0020 |
| Infected | 1 | 3253 | 1704 | 0.0216 |
| | 2 | 5327 | 2444 | 0.0260 |
| | 3 | 2422 | 862 | 0.0344 |
| | 4 | 3652 | 1153 | 0.0384 |
| | 5 | 3729 | 1127 | 0.0399 |
| | 6 | 3572 | 1027 | 0.0416 |
| | 7 | 2880 | 752 | 0.0447 |
| Mean | | 3548 | 1296 | 0.0352 |
| ± SE | | 345 | 223 | 0.003 |

** K PVP $h^{-1}$ = rate constant of exponential fall of $^{125}I$ PVP/hour.

* statistically significant

Fig.39 : Effect of 1 week T.spiralis on $^{125}$I PVP clearance

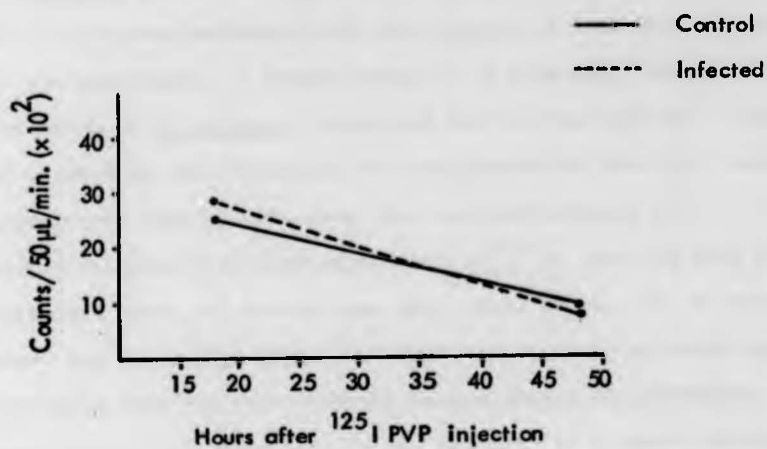
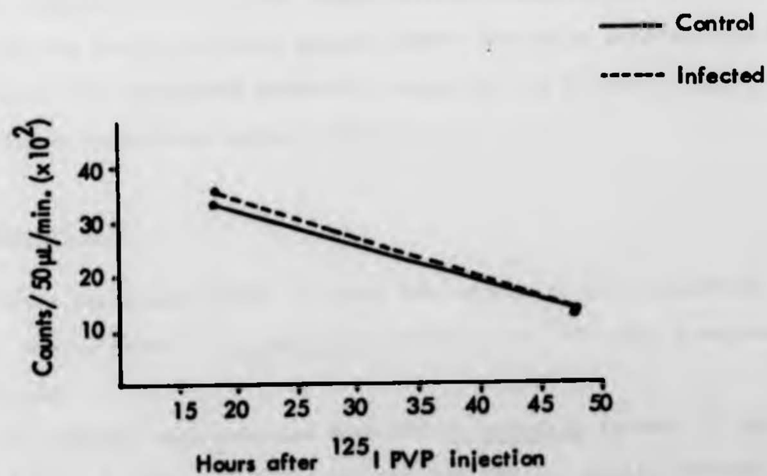



Fig. 40 : Effect of 2 weeks T.spiralis on $^{125}$I PVP clearance



labelled SRBC.

26 mice were used in this experiment. 7 mice were infected with 400 T. spiralis larvae and left in the host for 30 days. 33,500 (SE 12,900) larvae were recovered from the muscles of 5 of them at the end of the experiment. A second group of 12 mice was infected with similar batch of T. spiralis larvae and left in the host for 7 days. Adults counted in the intestines of 5 mice were 396 (SE 136). After the appropriate time of infection, the infected animals and 7 uninfected controls were challenged with $10^9$ $^{51}Cr$ labelled SRBC into the tail vein each, all on the same day. One, 18, 21, 40, 48 hours and every day for 6 days after $^{51}Cr$-SRBC injection 50 $\mu l$ blood samples were obtained from the retro-orbital venous plexus of each mouse. The radioactivity of the blood samples was measured in a gamma counter. Mean radioactivity in blood samples/min. are presented in Table 41 and Fig. 41 and 42 and show that the group infected for 7 days had higher counts 1 hour after $^{51}Cr$-SRBC injection followed by a significant reduction in the counts from 18 hours onwards after $^{51}Cr$-SRBC inoculation compared to controls. There were no significant differences between the counts of blood samples taken from mice infected for 30 days and from uninfected controls, except at day 5 where radioactivity was higher in infected animals ($p < 0.01$).

Experiment 5.4

This experiment aimed to study the effect of late intestinal and early muscle phase of T. spiralis infection on $^{51}Cr$-SRBC clearance from the blood.

12 animals were infected with 400 T. spiralis larvae. 5 animals were killed 3 days post infection and 141 (SE 14) adults counted in

Table 41 Effect of full infection and intestinal phase of <u>T. spiralis</u> infection on $^{51}$Cr-SRBC clearance

| Group | No. of animals | Mean radioactivity/50 $\mu\ell$/min. after $^{51}$Cr-SRBC injection ± SE, p value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 hour | 18 hour | 21 hour | 40 hour | 48 hour | 3 days | 4 days | 5 days | 6 days |
| 30 day old infection | 7 | 960 | 271 | 245 | 137 | 112 | 74 | 58 | 51 | 44 |
| | | ± 159 | ± 28 | ± 20 | ± 9 | ± 10 | ± 4 | ± 3 | ± 2 | ± 2 |
| | | ns | ns | ns | ns | ns | ns | ns | $<0.01$ | ns |
| 7 day old infection | 7 | 7972 | 72 | 63 | 50 | 47 | 40 | 33 | 34 | 33 |
| | | ± 1672 | ± 4 | ± 3 | ± 1 | ± 1 | ± 2 | ± 1 | ± 1 | ± 1 |
| | | $<0.0005$ | $<0.0005$ | $<0.0005$ | $<0.0005$ | $<0.0005$ | $<0.0005$ | $<0.0005$ | $<0.0005$ | $<0.0005$ |
| Uninfected controls | 7 | 1432 | 285 | 246 | 129 | 110 | 72 | 52 | 42 | 42 |
| | | ± 319 | ± 24 | ± 16 | ± 8 | ± 7 | ± 3 | ± 2 | ± 2 | ± 2 |

ns = not significant ($p > 0.05$)

Figure 41.
Effect of 7 day old T. spiralis infection on $^{51}Cr$ - SRBC clearance

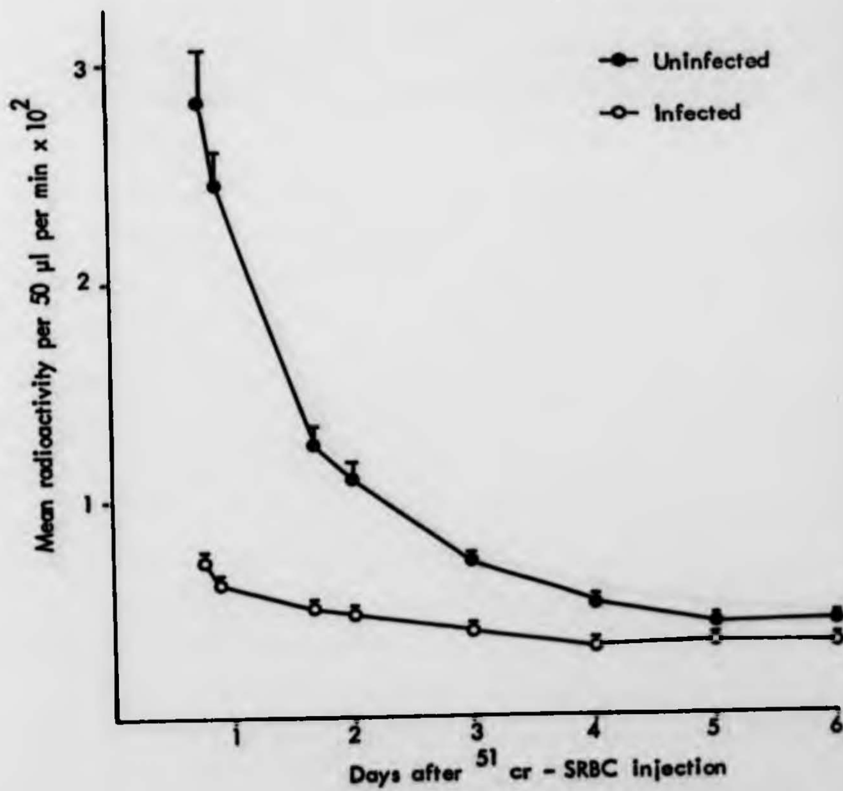
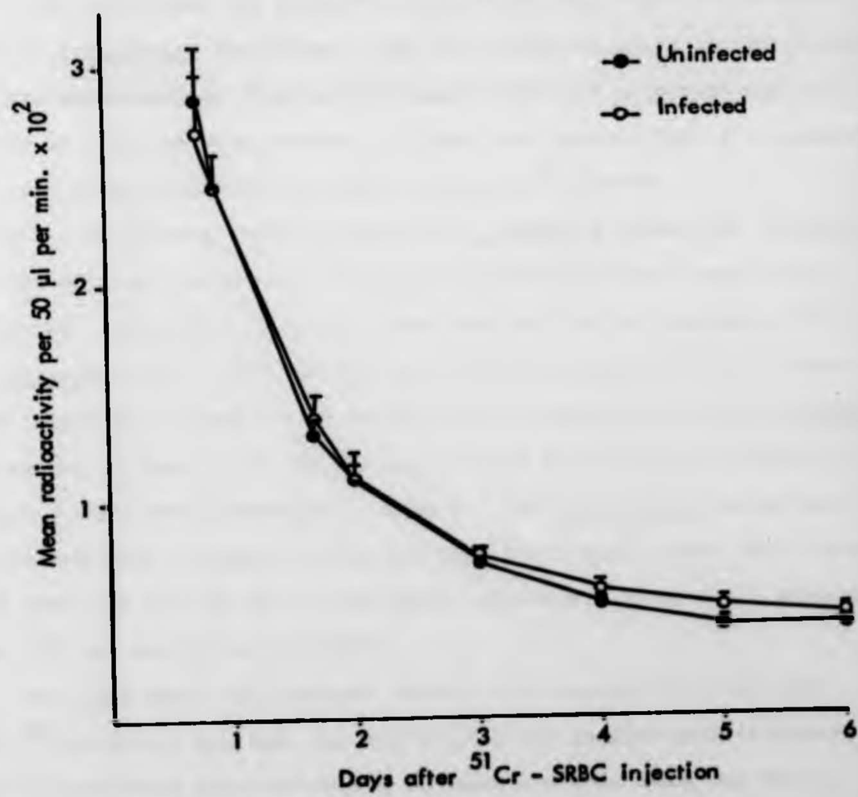


Figure 42.
Effect of 30 day old T. spiralis infection on $^{51}Cr$ - SRBC clearance



their intestines. 14 days later, each of the remaining infected animals and 6 uninfected animals were injected with $10^9$ $^{51}$Cr-SRBC into the tail vein. 50 $\mu\ell$ of blood were collected from the retro-orbital sinus and radioactivity measured 18, 21, 40 and 48 hours and then every day for 6 days after $^{51}$Cr-SRBC injection. Results of blood radioactivity counts (Table 42 and Fig. 43) showed no significant difference between the infected and the control group.

Experiment 5.5

This experiment was carried out to study the effect of different ages of *T. spiralis* infection. Also the infection level was increased in this experiment to find out if higher levels of infection may be needed to activate macrophages. 45 mice were divided into five groups and were treated as follows before injecting $^{51}$Cr-SRBC.
Group 1 : 10 animals infected with 600 *T. spiralis* larvae for 30 days. 598 (SE 60) adult worms were found in 5 animals killed 3 days after infection. Group 2 : 10 animals were infected for 14 days with 600 *T. spiralis* larvae. 247 (SE 23) adults were counted in 5 mice 3 days post infection. Group 3 : 10 animals were infected with 600 *T. spiralis* larvae for 11 days. 207 (SE 28) adults were found in the intestines in 5 mice 3 days after infection. Group 4 : 600 *T. spiralis* larvae were inoculated into 10 mice, left in the host for 7 days. Mean adult count in 5 mice was 269 (SE 69) 3 days after infection. Group 5 : 5 animals were left as uninfected controls.

The experiment was arranged for all the animals to be injected with $^{51}$Cr-SRBC on the same day and 50 $\mu\ell$ blood samples were collected and radioactivity measured 18 and 24 hours and then every day for 6 days post $^{51}$Cr-SRBC injection. Blood counts (Table 43 and Fig. 44-47)

Table 42 Effect of a 14 day old <u>T. spiralis</u> infection on $^{51}$Cr-SRBC clearance

| Group | No. of mice | Mean radioactivity/50 μℓ/min. after $^{51}$Cr-SRBC injection ± SE, p value | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 18 hour | 21 hour | 40 hour | 48 hour | 3 days | 4 days | 5 days | 6 days |
| 14 day old infection | 7 | 305<br>± 62<br>ns | 274<br>± 54<br>ns | 142<br>± 24<br>ns | 128<br>± 19<br>ns | 93<br>± 9<br>ns | 73<br>± 4<br>ns | 56<br>± 2<br>ns | 49<br>± 3<br>ns |
| Uninfected controls | 6 | 345<br>± 38 | 330<br>± 30 | 188<br>± 30 | 165<br>± 25 | 120<br>± 13 | 74<br>± 3 | 59<br>± 4 | 48<br>± 1 |

ns = not significant ($p > 0.05$)

Figure 43.

Effect of 14 day old T. spiralis infection on $^{51}Cr$ - SRBC clearance

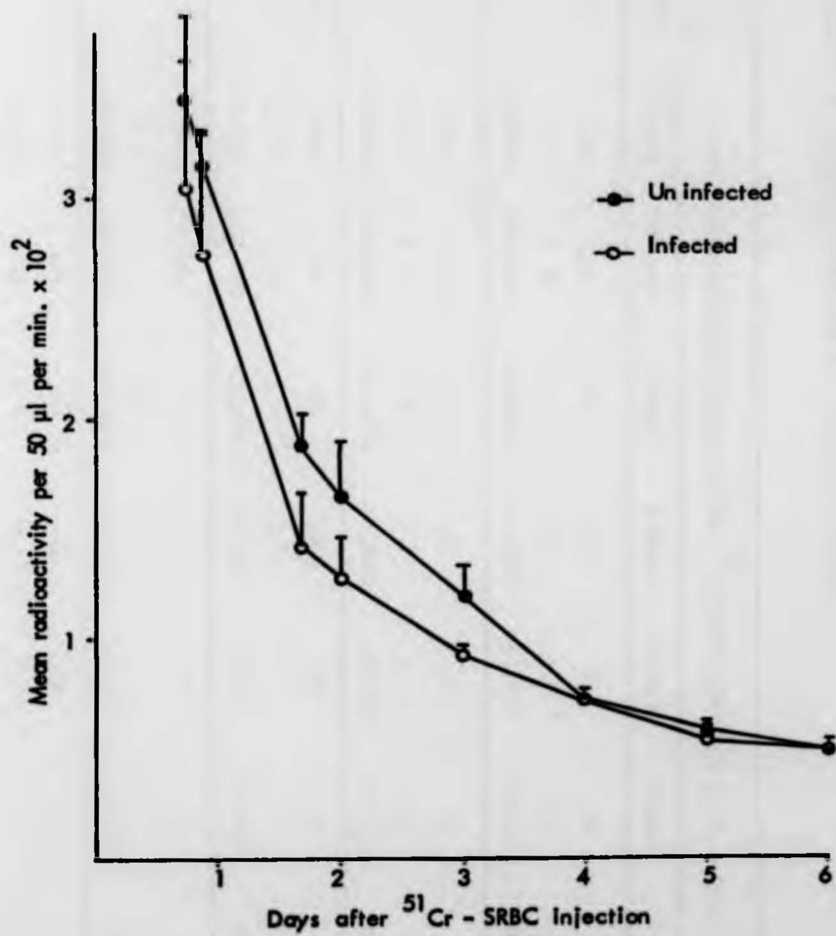


Table 43 Effect of different ages of <u>T. spiralis</u> infection on $^{51}$Cr-SRBC clearance

| Group | No. of mice | Mean radioactivity/50 μℓ/min. after $^{51}$Cr-SRBC injection ± SE, p value | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 18 hour | 24 hour | 2 days | 3 days | 4 days | 5 days | 6 days |
| 30 day old infection | 5 | 154 ± 11 <0.0005 | 119 ± 9 <0.0005 | 67 ± 5 <0.0025 | 41 ± 2 <0.0025 | 39 ± 1 <0.005 | 36 ± 1 <0.0125 | 34 ± 1 <0.05 |
| 14 day old infection | 5 | 150 ± 10 <0.0005 | 104 ± 10 <0.0005 | 65 ± 5 <0.0025 | 42 ± 3 <0.0025 | 37 ± 2 <0.0025 | 35 ± 2 <0.0125 | 35 ± 2 <0.05 |
| 11 day old infection | 5 | 165 ± 25 <0.005 | 106 ± 6 <0.0005 | 65 ± 5 <0.0025 | 44 ± 3 <0.0025 | 37 ± 3 <0.01 | 34 ± 3 <0.025 | 33 ± 2 <0.025 |
| 7 day old infection | 5 | 185 ± 6 <0.0025 | 144 ± 3 <0.0025 | 72 ± 4 <0.0025 | 48 ± 3 <0.005 | 43 ± 1 <0.025 | 37 ± 1 <0.025 | 31 ± 2 <0.025 |
| Uninfected controls | 5 | 282 ± 21 | 221 ± 18 | 95 ± 5 | 62 ± 3 | 50 ± 3 | 42 ± 2 | 43 ± 4 |

Figure 44.

Effect of 7 day old T. spiralis infection on $^{51}Cr$ - SRBC clearance

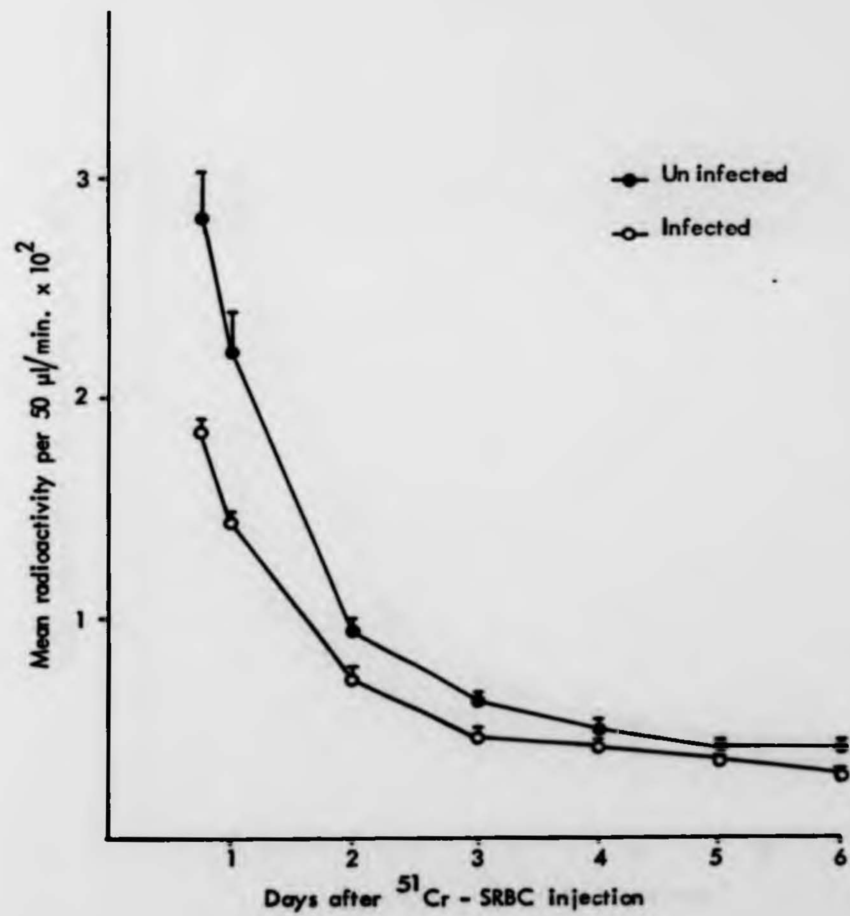


Figure 45.

Effect of 11 day old T. spiralis infection on $^{51}Cr$ - SRBC clearance

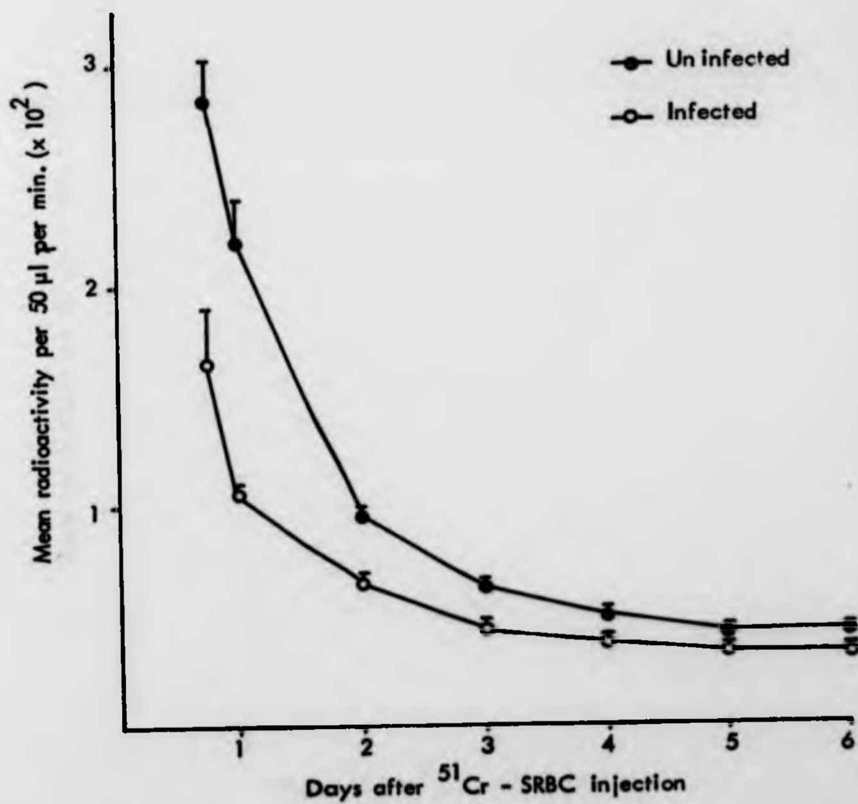


Figure 46.
Effect of 14 day old T. spiralis infection on $^{51}Cr$ - SRBC clearance

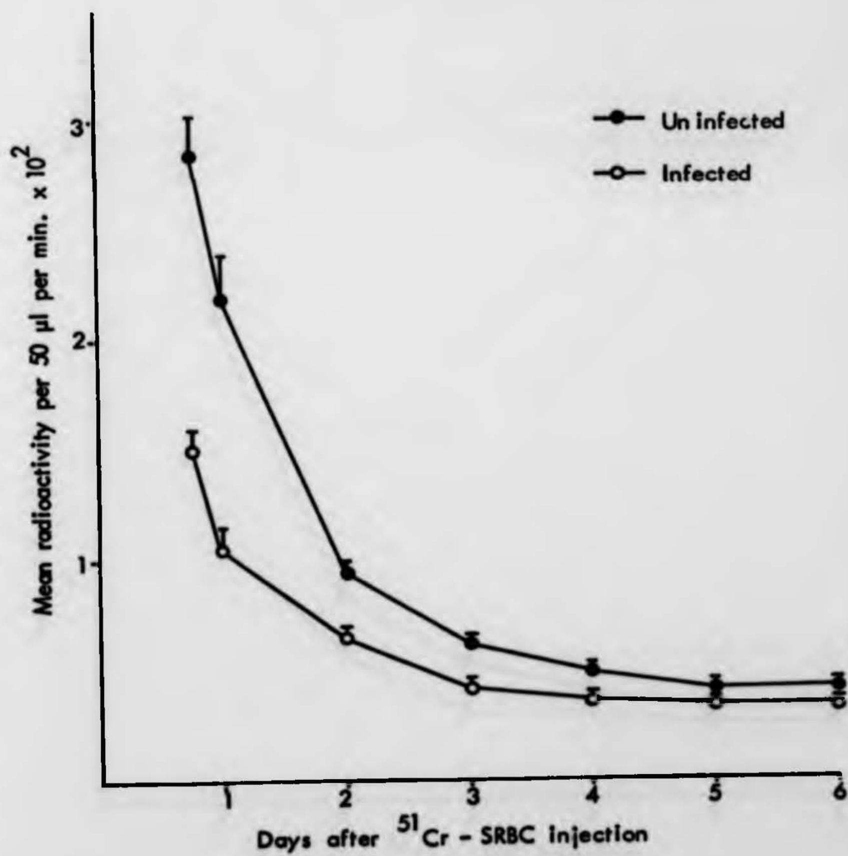
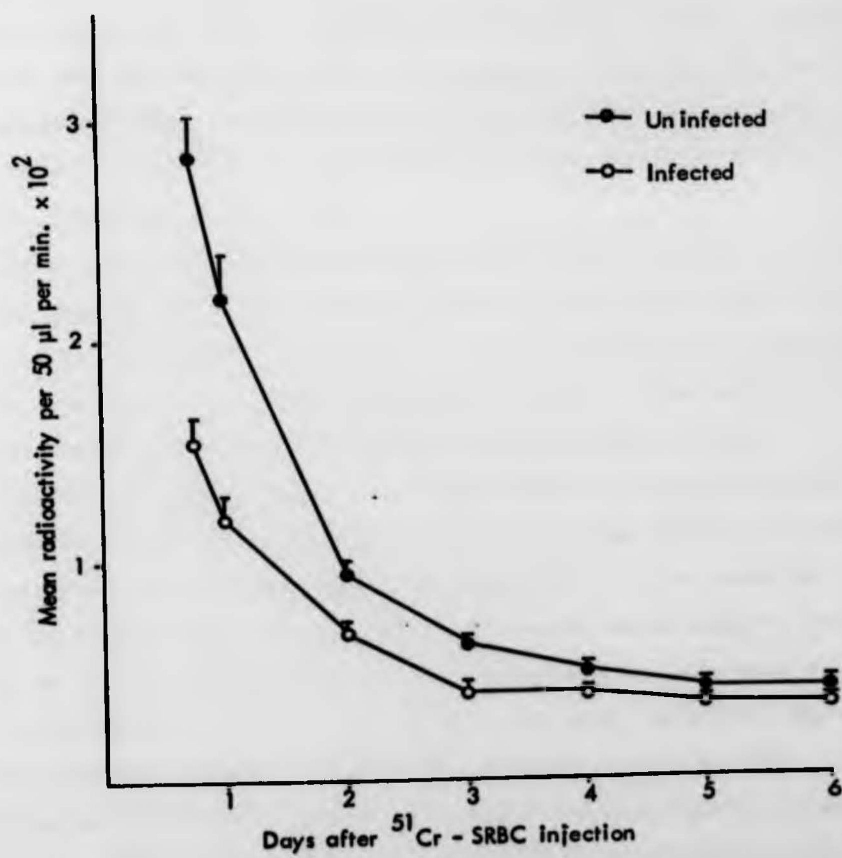


Figure 47.

Effect of 30 day old T. spiralis infection on $^{51}$Cr - SRBC clearance



show significant reduction in all infected animals at all times when blood samples were taken and compared to similar controls.

## Discussion

By inspection of results of the experiments 5.1 and 5.2 it was found that the rate of $^{125}I$ PVP clearance was significantly increased in animals infected for one week (when the intestinal phase of infection is predominating) compared to the uninfected controls. This suggests that the intestinal phase of *T. spiralis* infection stimulates macrophage activity. This activation of macrophages was not detected in animals which had been infected with *T. spiralis* for 2, 3, 4, 6 or 8 weeks (experiment 5.1 and 5.2).

Results of $^{51}Cr$-SRBC clearance test demonstrated that mice infected with *T. spiralis* for 7 days cleared $^{51}Cr$-SRBC significantly faster than the controls (experiment 5.3 and 5.5). Animals infected with *T. spiralis* for 14 days and 30 days showed inconsistent results. While there was no significant difference in $^{51}Cr$-SRBC clearance between animals infected with *T. spiralis* for 14 or 30 days and the uninfected controls (experiment 5.4 and 5.3 respectively), the same groups showed significant fast clearance at all blood samples in experiment 5.5. The level of infection in experiment 5.5 was higher (600 larvae) which suggests that 14 and 30 day old infection can activate macrophages but only when the level of infection is high. This part of the study, therefore, shows clearly that the intestinal phase of *T. spiralis* infection in mice activates the macrophage function. Activation was demonstrated by fast clearance of $^{125}I$ PVP and $^{51}Cr$-SRBC. Later stages of infection showed

activation of macrophages only when the infection was high (experiment 5.5).

Tanner (1968) has reported a direct relationship between the number of T. spiralis muscle larvae and the level of anti T. spiralis antibodies in the serum. Therefore, it seems likely that high levels of infection lead to increased production of antibodies which may form antigen-antibody complexes which facilitate macrophage activation. This suggestion may be supported by the work of Perrudet-Badoux and Binaghi (1977 ) who reported that peritoneal cells from animals infected with T. spiralis adhere firmly to T. spiralis larvae only in presence of serum containing anti-trichinella antibodies.

Macrophage activation in T. spiralis infected animals has been suggested. Meerovitch and Bomford (1977) showed that macrophages from mice infected with T. spiralis were strongly cytostatic to leuckaemia cell DNA synthesis as early as 6 days following infection.

Also T. spiralis infection inhibits a superimposed infection with Trypanosoma equiperdum and T. lewisi in rats (Meerovitch and Ackerman, 1974), and Listeria monocytogenes in mice (Cypess et al., 1974a). T. spiralis also increases the length of incubation period in mice and survival time of the host following a subsequent administration of sarcoma 180 ascitis cells (Lubiniecki and Cypess, 1975b). Results presented here are in agreement with their data.

# CHAPTER 6

## Effect of T. spiralis on antibody catabolism

### Introduction

This study was initiated to determine if the catabolism of IgM was increased in mice infected with T. spiralis, as this could contribute to the observed depression of circulating antibody levels against SRBC.

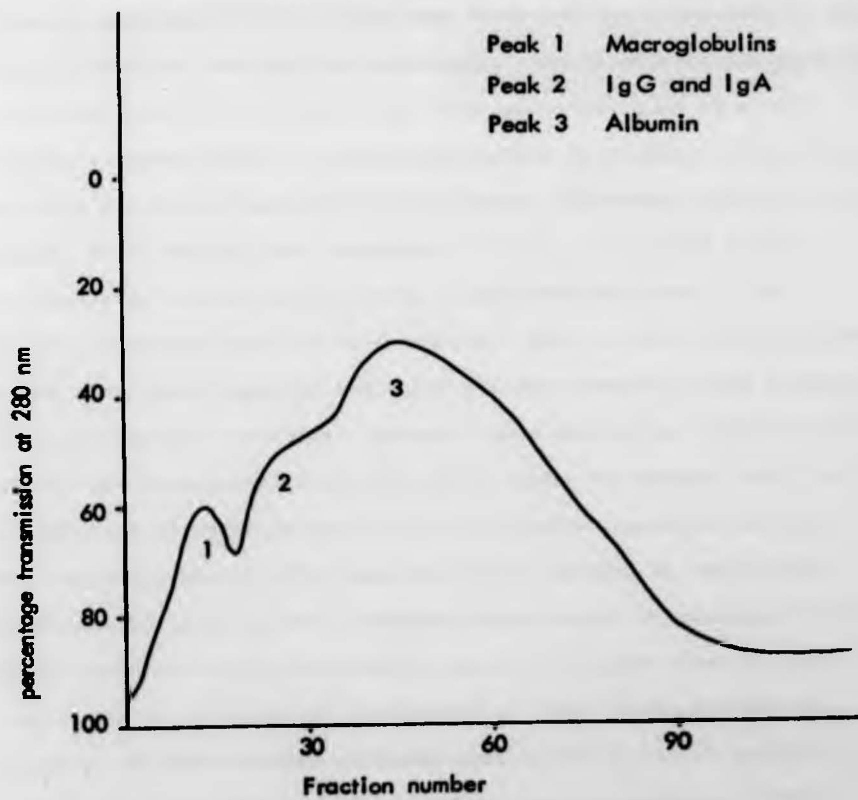
The principle of this experiment was to compare the rate of disappearance of passively transferred $^{125}I$ macroglobulins in a group of normal mice and in groups of mice infected with T. spiralis of different ages. Macroglobulins, which were IgM, $\alpha 2$ macroglobulins and some lipoproteins, were used in this study since it is difficult to prepare pure IgM.

### Preparation of macroglobulins:

20 normal mice were exsanguinated and their sera collected and pooled. The collected serum was centrifuged at 1000 g to remove any sediment. The top layer of the serum containing the lipids was discarded. 7 ml of the clear serum was fractionated by gel filtration on G-200 Sephadex which excludes proteins over 800,000 molecular weight and so is extremely useful for the isolation of macroglobulins. The flow rate in the column was adjusted to 18 ml $h^{-1}$. Figure 48. shows the elution profile of serum proteins. The proteins eluted with phosphate buffered saline pH 6.8 prepared as equal volumes of $Na_2HPo_4$ (0.01M) and $NaH_2Po_4$ (0.01M). The fractions which contained the first peak (containing the macroglobulins) were pooled and precipitated with

Figure 48.

Elution profile of serum proteins from a column of G-200 Sephadex



an equal volume of saturated ammonium sulphate solution. The precipitate was dissolved in a minimum amount of PBS. The resultant solution was centrifuged and the precipitate discarded. The dissolved protein was dialysed against 5 litres of PBS twice overnight. The protein content of the solution measured by determining the absorbence at 280 nm using a UV Spectrophotometer was 2.4 mg $ml^{-1}$. Once the macroglobulins had been prepared they were radiolabelled with $^{125}I$-lactoperoxidase by the method of David (1972).

Radiolabelling has been used to detect or quantitate many protein and peptide preparations in as small as picogram quantities. Many chemical iodination methods have been used such as chloramine-T, iodine monochloride and electrolytic iodination. These methods had some disadvantages such as the presence of high concentrations of strong oxidising agents which can cause alterations in protein structure and activity due to non-specific side reactions (McConahey and Dixon, 1966; Hunter, 1970; Jornval and Zeppezauer, 1972), or can lead to low efficiency of iodine incorporation (Zappacosta and Rossi, 1967). Recently enzymatic methods of iodination, such as the lactoperoxidase method, have been reported and these are more sensitive and yield products with high specific activity. However, this method has some disadvantages, such as the incorporation of radioiodine into the enzyme itself and the introduction of contaminants into the iodination reaction mixture. More recently, David (1972) described a new method of radioiodine labelling, by coupling the lactoperoxidase enzyme to cyanogen bromide (CNBr)-activated sepharose-4B which helps the iodination of protein without the introduction of contaminants. This technique has the advantage of being capable of iodinating a wide range of proteins using different iodine and KI concentrations, temperature, and pH conditions. The sepharose-bound enzyme can readily be removed by

centrifugation.

Lactoperoxidase-linked CNBr sepharose 4B was prepared as follows: 0.5 g CNBr activated sepharose 4B (Pharmacia Fine Chemicals) was washed 5 times in a total volume of 100 ml HCl (0.001 M) the sepharose was swollen and formed 1.5 ml of sediment. The sepharose was suspended in 10 ml bicarbonate buffer (0.1 M, pH 8.3 contained 0.5 M NaCl) then left to settle. 1 ml of the settled beeds was added to lactoperoxidase (prepared as 2.3 mg in 0.46 ml bicarbonate buffer). 0.54 ml bicarbonate buffer was added to the whole suspension to make a total of 2 ml. The suspension was mixed by rotation at 4°C overnight. The sepharose was then washed thoroughly with 0.01 M phosphate buffer pH 7.5 containing 0.2 glycine at 4°C for 4 hours for the glycine to block any unoccupied sites on the sepharose. The prepared sepharose beeds were washed in PBS pH 7.4 and stored in PBS containing $10^{-5}$ M merthiolate at 4°C. Sepharose-bound lactoperoxidase in the buffer is stable over long periods of time at 4°C.

Iodination was carried out at room temperature as follows: 250 μℓ of the suspension which contained 60 μg of lactoperoxidase linked CNBr-activated sepharose 4B (LP-4B) was washed in PBS. 5 ml of the protein solution in PBS was added to the LP-4B (50 μℓ of LP-4B for each 1 ml protein solution containing 1-10 mg protein). 10 μℓ of $10^{-3}$M KI $ml^{-1}$ was added to give a final concentration of $10^{-5}$ M KI. 100 μci $^{125}I$ NaCl for each 10 mg protein was added to the protein solution under the protective sterile hood. The reaction was initiated by the addition of 10 μℓ 0.03% $H_2O_2$ per ml protein solution. The labelled solution was incubated at room temperature for 10-15 minutes under continuous agitation, then the LP-4B was spun out. The labelled protein was dialysed against 5 litres of PBS twice overnight at 4°C to remove the free iodine. To check for the absence of free iodine

5 $\mu\ell$ of $^{125}I$ labelled protein solution was added to 95 $\mu\ell$ PBS + 100 $\mu\ell$ of 20% Trichloroacetic acid (TCA) then incubated for 30 minutes at 4°C. 100 $\mu\ell$ of the supernatant was separated and counted in an auto gamma counter. The rest of the supernatant and precipitated protein was counted separately and the percentage of radioactivity in the precipitate was calculated. If it was less than 95%, then the solution was dialysed and checked again. To check for protein aggregation 5 $\mu\ell$ of $^{125}I$ protein solution was added to 195 $\mu\ell$ PBS and incubated for 30 minutes at 4°C, then centrifuged and radioactivity in the 100 $\mu\ell$ of the supernatant was counted separately from the rest of the supernatant plus the precipitate. The percent of radioactivity in the precipitate should be less than 10%.

Experiment 6.1

This experiment was designed to assess the effect of T. spiralis infection on the rate of catabolism of IgM. 55 Simpson mice were divided into 4 groups. The first three groups of 15 mice each were infected with 400 T. spiralis larvae per mouse for 7, 14 and 30 days. The fourth group of 10 mice was left as an uninfected control. 5 mice of each of the infected groups were autopsied 3 days post infection and 209 (SE 45), 227 (SE 27) and 228 (SE 17) adult worms recovered respectively. 0.1 ml of the prepared $^{125}I$ labelled macroglobulins was injected into the tail vein of each mouse of the experiment on the same day. 50 $\mu\ell$ of blood was taken from the retro-orbital sinus of each mouse and radioactivity measured in a gamma counter 24 hours after injection then every day for 9 days. Radioactivity of the blood was expressed as the percentage of the original activity measured 24 hours after inoculation (Fig. 49-51). The mean counts are presented

Figure 49.

Effect of a 7 day old _T. spiralis_ infection on $^{125}$I macroglobulin clearance

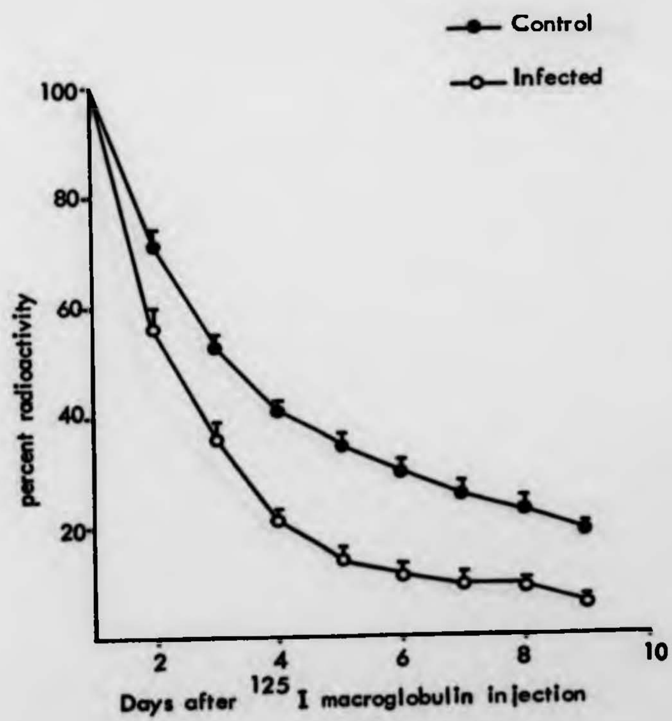


Figure 50.

Effect of a 14 day old T. spiralis infection on $^{125}I$ macroglobulin clearance

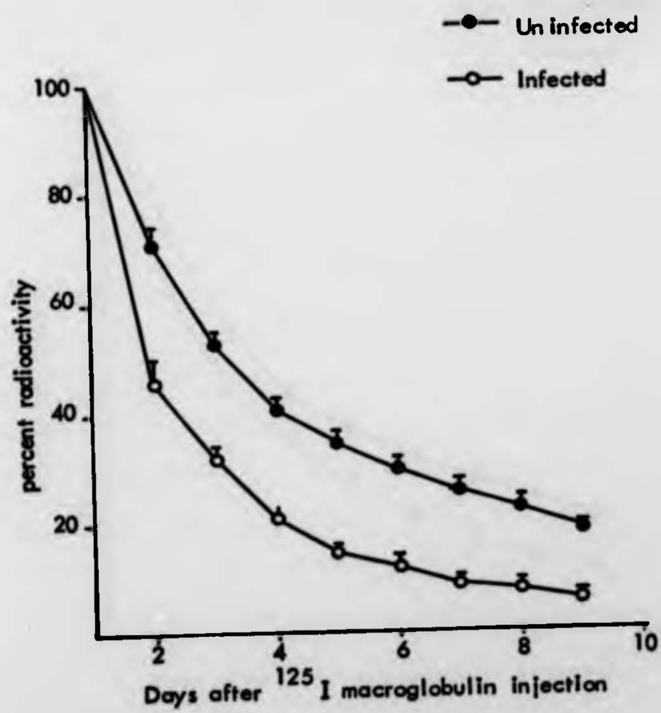
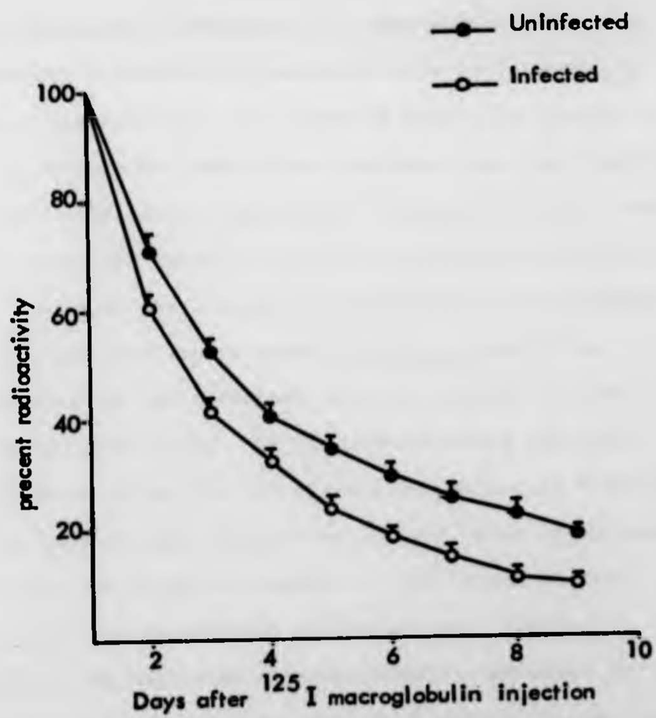


Figure 51.
Effect of a 30 day old T. spiralis infection on $^{125}$I macroglobulin clearance



in Table 44. A consistent significant decrease in blood radioactivity was noticed in the 3 groups of infected animals when compared to controls at all days starting from day 2. These findings indicate increased catabolism of macroglobulins in T. spiralis infected animals. T ½ in the control mice was 3.3 days and in mice with 7, 14 and 30 day old T. spiralis infections the T ½ was 2.9, 1.9, 2.5 days respectively.

## Discussion

The results obtained in experiment 6.1 demonstrated increased catabolism of passively transferred macroglobulins in T. spiralis infected animals. The fact that the 3 ages of infection studied (7, 14 and 30 day old) showed the same effect suggests that the 3 phases of T. spiralis infection, either separately or conjointly, are responsible for the increased catabolism of the transferred macroglobulins.

The normal result of any antigenic stimulation is the synthesis of immunoglobulins and this occurs after T. spiralis infection. Differential elevation of the different antibody classes can be observed in parasitic infections. It has been reported that anti-T. spiralis antibodies of IgM and IgA classes were found in high titres for comparatively long periods (Ljungström, 1974). Also an increase in IgM-containing cells was found in mesenteric lymph nodes of mice infected with T. spiralis (ljungström and Ruitenberg, 1976).

The decrease in the half life of macroglobulins observed in experiment 6.1 and the increased IgM levels in T. spiralis reported by Ljungström (1974) suggests that elevated IgM levels may be a major cause of increased IgM catabolism in T. spiralis infected animals.

Table 44

Catabolism of $^{125}I$ macroglobulins in blood of normal mice and mice infected with T. spiralis

| Group | No. of mice | Mean $^{125}I$ counts $min.^{-1}$ after injection of $^{125}I$ macroglobulin ± SE and p value | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 h | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 |
| 7 day old infection | 10 | 2563<br>± 174<br>(n.s.) | 1451<br>± 149<br>$<0.01$ | 959<br>± 135<br>$<0.005$ | 574<br>± 90<br>$<0.0005$ | 373<br>± 60<br>$<0.0005$ | 305<br>± 62<br>$<0.0005$ | 256<br>± 57<br>$<0.0005$ | 230<br>± 46<br>$<0.0005$ | 167<br>± 33<br>$<0.0005$ |
| 14 day old infection | 10 | 3182<br>± 303<br>$<0.05$ | 1400<br>± 58<br>$<0.0005$ | 991<br>± 59<br>$<0.0025$ | 649<br>± 37<br>$<0.0005$ | 473<br>± 33<br>$<0.0005$ | 372<br>± 53<br>$<0.0005$ | 274<br>± 27<br>$<0.0005$ | 250<br>± 26<br>$<0.0005$ | 185<br>± 12<br>$<0.0005$ |
| 30 day old infection | 10 | 2547<br>± 236<br>(n.s.) | 1520<br>± 119<br>$<0.01$ | 1071<br>± 109<br>$<0.01$ | 835<br>± 98<br>$<0.025$ | 600<br>± 56<br>$<0.0005$ | 472<br>± 48<br>$<0.0005$ | 382<br>± 46<br>$<0.0005$ | 295<br>± 41<br>$<0.0005$ | 251<br>± 29<br>$<0.0005$ |
| Uninfected controls | 10 | 2682<br>± 122 | 1870<br>± 55 | 1433<br>± 88 | 1107<br>± 58 | 940<br>± 58 | 782<br>± 51 | 691<br>± 49 | 588<br>± 55 | 505<br>± 36 |

n.s. = not significant ($p > 0.05$)

Increased catabolism would result in lower circulating IgM antibody levels to heterologous antigens in T. spiralis infected animals even if they were synthesizing antibody at the same rate as uninfected mice. Similar effects were reported in H. polygyrus infection (Brown et al., 1976). They showed that increased IgG levels due to H. polygyrus infection are a major cause of increased $IgG_1$ catabolism in mice infected with H. polygyrus. This increased IgG catabolism resulted in lower circulating IgG antibody levels in infected animals.

# CHAPTER 7

## Effect of T.spiralis infection on the affinity of antibody for Human serum albumin (HSA).

### Introduction

The term antibody affinity refers to the strength of interaction between an antigenic determinant and the homologous antibody binding site. Thus a high affinity antibody is one which forms a strong bond with an antigenic determinant to give an antibody-antigen complex with low tendency to dissociate.Low affinity antibodies, conversely form weak bonds which easily dissociate. T. spiralis infection in mice depressed PFC responses to SRBC, antibody levels to SRBC and LPS antigens and increased macrophage activity and in this chapter the affinity of the antibody produced was studied.

### Measurement of antibody affinity:

Bascially, the measurement of antibody affinity depends upon the determination of free and antibody-bound antigen at equilibrium (Steward, 1974) and requires the separation of free and bound antigen. Because of antibody heterogeneity, determination of antibody affinity is carried out over a range of antigen concentrations.

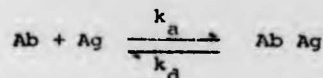
Among the methods for determining antibody affinity, ammonium sulphate precipitation has been used extensively to determine the amount of antibody to certain antigens, affinity of anti-hapten antibodies and relative affinity of anti-protein antibodies. This method has the advantage that it does not need prior purification of antibody so that whole serum can be used as a source of antibody, also only

small volumes of serum are required and several samples may be conveniently analysed at a time. However in systems other than those involving hapten antigens, the application of this method is limited to antigens which are soluble in 50% saturated ammonium sulphate.

Method:

Human serum albumin (HSA) (Miles Laboratories) was labelled by $^{125}I$ lactoperoxidase method (David, 1972) as described in Chapter 6. Radiolabelled HSA ($^{125}I$ HSA), at a range of 2.5 - 25 μg antigen concentration in 40 μl PBS (pH 7.2) contained 1 μl of 1:10 $Na^{22}$ was added to each 10 tubes (Hawksley Microfuge tubes, capacity 400 μℓ). Ten μℓ of antiserum was added to each tube, gently mixed and incubated at 4°C for 1 hour. 50 μℓ of saturated ammonium sulphate (SAS) was then added to each tube, gently mixed and left at 4°C for 30 minutes. Following incubation, the tubes were spun for 5 minutes at 4°C in a Beckman Microfuge. 2/3 of the supernatant was taken off and the rest of the supernatant plus the precipitate were then counted in auto gamma counter. Total radioactivity of each antigen concentration without serum was also counted, so that the percentage of the added radioactivity which is globulin bound was determined. In order to correct for non-specific binding of antigen to ammonium sulphate precipitable globulin at each antigen concentration, negative sera were included in the assay. Free antigen and bound antigen were then calculated from the data from the gamma counter using a computer programme written in Fortran.

The quantitative relationship of interaction between antibody and antigen at equilibrium is represented as follows:



$$Ab + Ag \underset{k_d}{\overset{k_a}{\rightleftharpoons}} Ab\ Ag$$

where Ab represents free antibody, Ag = free antigen, Ab Ag = the antibody-antigen complex, $k_a$ and $k_d$ = the association and dissociation constants respectively.

The equilibrium constant k, or affinity of anti antigen antibody can be determined by measuring the free and antibody bound antigens at equilibrium over a range of concentrations. Fig. 52 shows Langmuir plot of ideal antibody-antigen binding.

The plot of 1/b (bound antigen) versus 1/c (free Ag) allows both affinity K and antibody sites $Ab_t$ to be determined, according to the following equation:

$$1/b = 1/Ab_t \times 1/c \times 1/K + 1/Ab_t$$

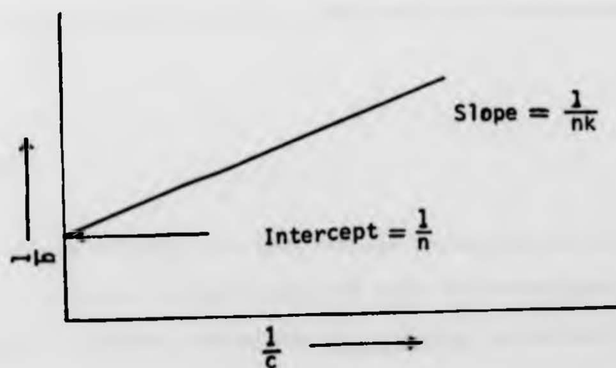
where $Ab_t$ = antibody binding sites, c = free antigen concentration. When 1/c = 0 (in the case of extreme antigen excess) then $1/b = 1/Ab_t$ thus $Ab_t$ may be determined by extrapolation to 1/c = 0. Affinity K = 1/c when half the total Ab sites ($Ab_t$) are bound. Antibody affinity is therefore expressed as the equilibrium constant K, with units of litres/mole.

Experiment 7.1

9 Simpson mice were infected with 400 *T. spiralis* larvae each. An immunization protocol with HSA was started 7 and 14 days after infection on infected and a comparative number of normal animals. HSA immunization was carried out as i.p. injection of 1 mg HSA in saline weekly for 4 weeks. Infected and normal mice were exsanguinated on day 14 after the end of immunization and the affinity of their antibody determined. At the end of the experiment, 5 infected mice

Figure 52 :

Langmuir plot of ideal antibody-antigen binding. Bound antigen (b) , Free antigen (c) , Antibody-valence (n) and Affinity (K) .



were digested and a mean of 150,400 (SE 26,100) larvae obtained. The affinity of antibody (Table 45 and 46 and Fig. 53 and 54) from infected animals were higher than that from the controls ($p < 0.025$ and $p < 0.0005$) for the group infected for 7 days and 14 days respectively.

Experiment 7.2

This experiment was essentially a repeat of experiment 7.1, with the inclusion of a third group of animals infected for 28 days. Groups of animals were infected on separate days with 400 *T. spiralis* larvae each. The number of muscle larvae obtained 35 days later was 176,800 (SE 11,400), 1274 (SE 109), and 120,500 (SE 33,176) respectively from animals immunized 7, 14 and 28 days after infection. The results of this experiment (Table 47 and Fig. 55) showed increased affinity of antibody in the three groups of *T. spiralis* infected animals when compared to controls confirming the results of experiment 7.1.

## Discussion

Antibody affinity for HSA antigen (experiments 7.1 and 7.2) was found to be higher in mice infected with different ages of *T. spiralis* infection. It seems likely that *T. spiralis* infection leads to alterations of the population of immunologically competent cells favouring the selection by antigen of B cells with high affinity receptors.

Changes in either quantity or quality (affinity) of antibody, or both, may occur in response to inoculation of foreign agents. For

Table 45

Effect of a 7 day old T. spiralis infection on affinity of antibody for HSA antigen

| Group | Mouse no. | Affinity of antibody (litres per mole) x $10^6$ |
|---|---|---|
| 7 day old infection | 1 | 1.80 |
| | 2 | 3.10 |
| | 3 | 3.40 |
| Mean, SE and p value | | 2.80<br>0.49<br>$< 0.025$ |
| Uninfected controls | 1 | 0.05 |
| | 2 | 0.90 |
| | 3 | 1.25 |
| Mean, and SE | | 0.73<br>0.35 |

Figure 53.

Effect of 7 day old T. spiralis infection on the affinity of antibody for HSA

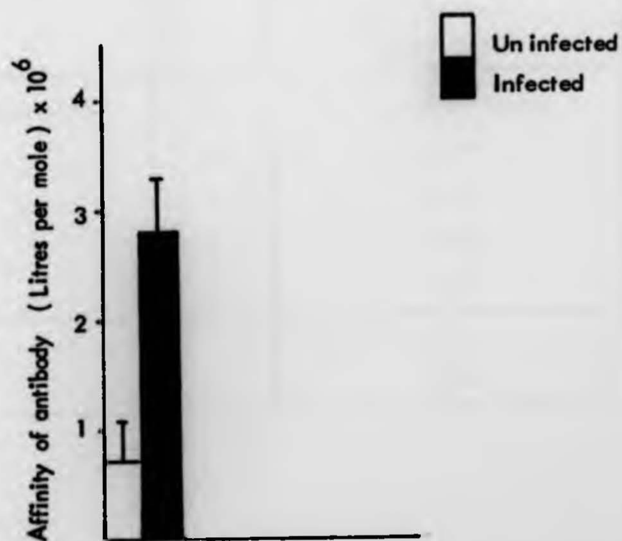


Table 46

Effect of a 14 day old T. spiralis infection on affinity of antibody for HSA antigen

| Group | Mouse no. | Affinity of antibody (litres per mole) x $10^6$ |
|---|---|---|
| 14 day old infection | 1 | 2.50 |
| | 2 | 2.90 |
| | 3 | 3.00 |
| | 4 | 3.50 |
| | 5 | 4.00 |
| | 6 | 4.15 |
| Mean, SE and p value | | 3.34 |
| | | 0.30 |
| | | $< 0.0005$ |
| Uninfected controls | 1 | 0.05 |
| | 2 | 0.35 |
| | 3 | 0.55 |
| | 4 | 0.60 |
| | 5 | 1.00 |
| Mean and SE | | 0.50 |
| | | 0.20 |

Figure 54.

Effect of 14 day old T. spiralis infection on the affinity of antibody for HSA

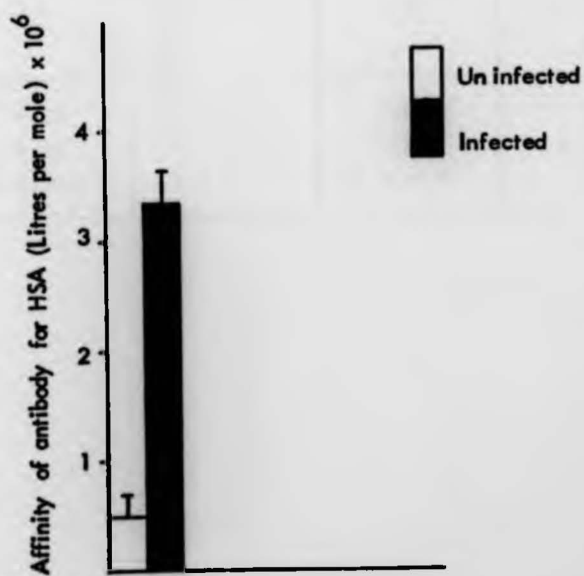


Table 47

Effect of different ages of T. spiralis infection on affinity of antibody for HSA antigen

| Mice no. | Affinity of antibody for HSA (litres per mole) x $10^6$ | | | |
|---|---|---|---|---|
| | 7 day old infection | 14 day old infection | 28 day old infection | Uninfected controls |
| 1 | 2.60 | 0.47 | 1.52 | 0.17 |
| 2 | 3.45 | 0.85 | 1.90 | 0.37 |
| 3 | 3.75 | 1.30 | 2.70 | 0.55 |
| 4 | 5.5 | 1.60 | 3.20 | 0.75 |
| 5 | 6.5 | 3.60 | 3.90 | 0.87 |
| 6 | - | - | - | 1.00 |
| Mean | 4.36 | 1.56 | 2.64 | 0.62 |
| SE | 0.71 | 0.54 | 0.43 | 0.13 |
| p value | $< 0.0005$ | $< 0.05$ | $< 0.0005$ | - |

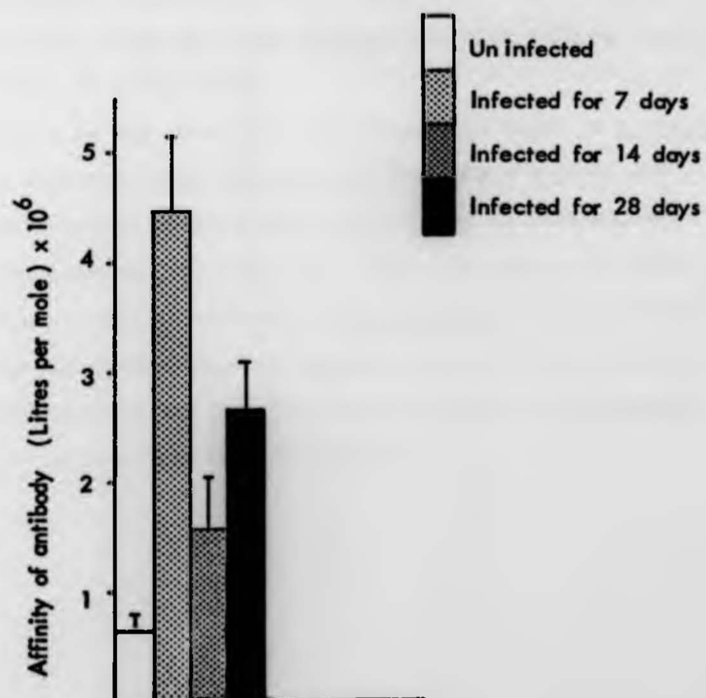
Table 47

Effect of different ages of T. spiralis infection on affinity of antibody for HSA antigen

| Mice no. | Affinity of antibody for HSA (litres per mole) x $10^6$ | | | |
|---|---|---|---|---|
| | 7 day old infection | 14 day old infection | 28 day old infection | Uninfected controls |
| 1 | 2.60 | 0.47 | 1.52 | 0.17 |
| 2 | 3.45 | 0.85 | 1.90 | 0.37 |
| 3 | 3.75 | 1.30 | 2.70 | 0.55 |
| 4 | 5.5 | 1.60 | 3.20 | 0.75 |
| 5 | 6.5 | 3.60 | 3.90 | 0.87 |
| 6 | - | - | - | 1.00 |
| Mean | 4.36 | 1.56 | 2.64 | 0.62 |
| SE | 0.71 | 0.54 | 0.43 | 0.13 |
| p value | $< 0.0005$ | $< 0.05$ | $< 0.0005$ | - |

Figure 55.

Effect of different ages of T. spiralis infection on affinity of antibody for HSA



example carbon reduces the affinity of mouse anti-protein antibody but does not affect the quantity (Passwell et al., 1974), whereas Freund's complete adjuvant increases both (Soothill and Steward, 1971).

Macrophages may influence antibody affinity by antigen removal, a process that favours selection of high affinity B cells (Siskind and Benacerraf, 1969). In addition, it has been suggested that variations in macrophage function underlies differences in affinity of antibody response (Passwell et al., 1974; Soothill and Steward, 1971; and Morgan and Soothill, 1975a). They suggested that poor macrophage function leads to a low affinity antibody response because of poor selection of lymphocytes.

In Chapter 5 it was shown that the intestinal phase of *T. spiralis* increases the activity of macrophages and this could explain why although total antibody to a heterologous antigen is reduced, the affinity of the antibody is increased. Thus, the enhanced ability to produce antibodies of high affinity in *T. spiralis* infected animals would be an important compensatory mechanism to overcome quantitative defects in PFC response and antibody levels induced by *T. spiralis* infection and thus help the host to survive.

# GENERAL DISCUSSION AND CONCLUSION

The results presented in this study show that the effect of *T. spiralis* on the host immune response to heterologous antigens is of great complexity. This is not surprising considering the complexity of both the hosts immune response and the life-cycle of the parasite.

Antibody responses to the majority of multideterminant antigens appear to depend on interactions between, at least, 3 functionally distinct cell types, T cells, B cells and macrophages (Unanue, 1972).

In adult animals, the bone marrow produces stem cells, some of which migrate to the thymus where they multiply and differentiate into thymic lymphocytes (T cells). Eventually after antigenic stimulation with a T-dependent antigen these become immunocompetent, leave the thymus and give a population of T lymphocytes. The other stem cells differentiate to produce B lymphocytes which, after antigenic stimulation differentiate into blast cells which in turn develop to plasma cells capable of secreting antibodies. B and T cells sometimes act synergistically (Playfair, 1971), thus although T cells are unable to produce antibodies, they may cooperate with B cells to help them to produce antibodies against the so-called T-dependent antigens. The immunoglobulin classes and subclasses of antibody formed are characteristic of the B cell. Playfair and Purves (1971) suggested that there were two populations of B cells; $B_1$ cells are prevalent in the bone marrow and respond to SRBC independently of T cells and secrete only IgM antibody, while $B_2$ cells are more numerous in the spleen and require the cooperation of T cells to respond to SRBC and eventually switch to producing IgG antibody.

A third cell, the macrophage, also plays an important role in both induction and expression of the immune response.

Other factors of relative importance which may play a part in the control of the immune response are genetic factors such as the immune response (Ir) genes (Munro and Bright, 1976) and back ground genes (Biozzi et al., 1975). Feed back control may occur with the products of immune reaction affecting the rates of synthesis and catabolism of antibodies and endocrine hormones (growth hormones, thyroxine and insulin) may play a metabolic role. Activation of cell bound $C_3$ may also play a part of the events leading to stimulation of the resting B lymphocytes (Hartmann, 1975 and Pepys, 1972).

Depression of the antibody response induced by T. spiralis would occur if there was any defect or damage to any of the 3 cell types or in the cooperation between them, i.e. block at various stages of immune induction or expression. Results presented here, suggest that several mechanisms may be involved in the immunodepression induced by T. spiralis infection and that they may be affected in different ways with the different phases of T. spiralis infection.

3 phases of T. spiralis infection (intestinal, migrating and developing and the mature muscle phase) depressed the humoral response to the T-dependent antigen, SRBC, although a mixture of 2 or more of these life cycle phases was more effective in depressing the immune response than was any phase on its own. Both the number of PFC and the antibody levels in the sera of mice were depressed by T. spiralis infection and the induction and maintenance of this immunodepression was positively related to the level of infection. This was found with both the intestinal phase and the full infection.

The intestinal phase of T. spiralis depressed the antibody response to the T-dependent antigen, SRBC, but the fact that there was a normal

response to the T-independent antigen, LPS, suggests that the B lymphocytes are normal during this phase of infection and therefore the defect which causes the depressed response to SRBC may be either in the T cell population or in macrophages or in the cooperation between them and the B cells.

IgG responses were normal, or even raised, during the intestinal phase of T. spiralis infection and as it is known that the IgG response needs T cell cooperation this may be evidence that T-B cell cooperation is normal during this phase of infection. Therefore it may be suggested that those T cells involved in helper activity are normal while other T cell populations were defective. In support of this suggestion is the depressed CMI responses, which are T-dependent phenomena, during the intestinal phase of T. spiralis infection as measured by heart allograft technique (Ljungstrom and Huldt, 1977).

Increased phagocytic activity was found during the intestinal phase of T. spiralis infection as evidenced by increased clearance of $^{125}$I PVP and $^{51}$Cr-SRBC which indicates that the recognition and phagocytosis of the second antigen are not defective.

At this point we are in a situation which shows normal B cell population, normal T-B cell helper function and enhanced macrophage function. This implies that immunodepression during intestinal phase of T. spiralis may affect the T cell function or there is lack of cooperation between macrophage and lymphocyte population or both mechanisms are acting together.

"Antigenic competition" or antigen induced suppression is when antibody response to one antigen is reduced by prior injection of an unrelated antigen. This phenomenon is observed with a wide variety of antigens (see review by Adler, 1964). Recently 2 models for antigenic competition have been suggested. The action of non-specific T cell

suppressor cells and competition on the macrophage surface. Waksmann (1977) showed that, following antigenic stimulus, suppressor T cells release a non-specific glycoprotein mediator (IDS) which inhibits DNA synthesis. The production of IDS appears to be linked to cell division; and occurs within a few hours of stimulation by adherent spleen cells and by adherent cortisone-sensitive (cortical) thymocytes. IDS acts only at short range and its target action is limited to the $G_1$ phase of mitosis and it shows no antigenic specificity.

Taussig and Lackmann (1972) and Schrader and Feldmann (1973) have suggested that antigenic competition occurs at the macrophage level. The explanation for this is that when an antigen is introduced into an animal it forms a complex on the surface of T cells with antibody-like receptors (IgT) and that this complex become attached to the macrophage surface and is then presented to the T cell in a form suitable for activation of B lymphocytes which begin antibody production. When a second antigen is presented during the response to the first antigen the macrophage receptors will be less available for attachment of the second antigen-IgT complex leading to the diminished triggering of B cells to the second antigen.

T. spiralis is antigenic to its hosts and functional antigens of T. spiralis are T-dependent (Walls et al., 1973; Ruitenberg and Steerenberg, 1974; Ruitenberg et al., 1977 and Ljungström and Ruitenberg, 1976), their antigens may compete with other non-cross reacting antigens introduced to the host during the response to T. spiralis infection. This may be supported by the presence of normal B cell population and undefective macrophage function. Jones et al. (1976) showed that splenocytes from infected mice, when added to cultures of normal cells, actively suppress the in vitro antibody response to SRBC. This in vitro suppression is T-lymphocyte dependent since it was abolished by

treatment of the splenocytes with anti-thy-1 antiserum and c' and was enhanced by treatment with anti-Ig antiserum and c'. These results support the hypothesis that there is antigenic competition in *T. spiralis* infection. However the fact that IgG responses were normal during *T. spiralis* infection in the present study implies that antigenic competition between *T. spiralis* and SRBC may not be the only explanation of this immunodepression.

The induction phase of antibody production depends on the presentation of antigen in an immunogenic form suitable for the activation of the lymphocytes. SRBC antigen is processed by macrophages (Argyris, 1967) and hence a functional defect could be envisaged, either in antigen uptake or in the processing and presentation of antigen to lymphocytes. In the present study, the results of PVP and SRBC clearance tests demonstrated that the intestinal phase of *T. spiralis* infection stimulated rather than depressed the phagocytic activity. It is possible that the defect in the immune response may be in handling and processing of the antigen by these, overactive, macrophages after initial antigen uptake and before presentation of the antigen to the lymphocytes. Because IgG responses were normal in this study and according to Playfair and Purves (1971) who suggested the presence of 2 populations of B cells, it may be suggested that *T. spiralis* infection affects the presentation by the macrophages of antigen to $B_1$ cells. This is supported by the work of Loose *et al.* (1972) who showed that although peritoneal macrophages from mice infected with *P. berghei* could phagocytose SRBC they were less effective in inducing an immune response to SRBC in recipient mice than those from uninfected animals. Further work is needed to confirm the possibility of this happening in *T. spiralis* infection. This could be done by exposing macrophages from normal and *T. spiralis* infected animals to SRBC either *in vivo* or *in*

vitro and the degree of phagocytosis could be measured before transferring them to recipient mice. Studying the immune response in recipient mice would show the role of sensitized macrophages in handling SRBC antigens in infected animals. At this point, a question therefore arises whether macrophage populations are all of one nature, and capable of phagocytosis and presentation of antigens, or whether 2 populations of macrophages are present in which one population is responsible for antigen recognition and phagocytosis and the other population is responsible for antigen presentation to lymphocytes.

Lubiniecki et al. (1974b) reported a defect in the uptake of SRBC by splenic macrophages and increased uptake by liver macrophages in mice infected with T. spiralis larvae for 7 days. From his results and results presented here, it seems probable that altered distribution of SRBC may be an additional contributing factor in the immunodepression induced by T. spiralis infection.

Biozzi et al. (1975) have selectively bred 2 lines of mice which are high and low antibody producers. They found that the rate of clearance and distribution in the body organs of radiolabelled SRBC were identical in both low and high responders, but the SRBC persisted in splenic macrophages for much longer in high responders than in low responders. However, other antigens such as Pneumococcus polysaccharide (SIII), Keyhole limpet haemocyanin (KLH) and Levan were phagocytized faster by low responder strain macrophages. They suggested that the increase in KLH degradation and presentation is a major factor in the regulation of the antibody synthesis in the low responding lines. They also noticed important morphological differences and high lysosomal enzyme activities in peritoneal macrophages of low responders. It is possible that the increased clearance rates of PVP and SRBC induced by T. spiralis infection lead to increased degradation of the antigens and hence less antibody is

produced although it is recognized that the macrophages of both high and low responders Biozzi mice handled SRBC in the same way.

Argyris (1967) has shown that phagocytosed SRBC are as immunogenic as non phagocytosed ones. In addition, some experiments indicate that breakdown by macrophages of SRBC may be an essential prerequisite for immune induction (Shortman and Palmer, 1971; Feldmann and Palmer, 1971). It is therefore, possible that over activity of macrophage function in T. spiralis infected animals alters the immunogenicity of SRBC.

Several studies have demonstrated that the ratio of macrophages to lymphocytes is critical for the induction of an immune response. It has been shown that stimulated mouse macrophages produce a factor(s) which is depressive to the immune response (Nelson, 1973 and Chene and Hirsch, 1972). This may provide a further explanation of immunodepression induced by T. spiralis infection contrasted with apparently enhanced phagocytic activity.

A 30 day old T. spiralis infection, that is when larvae are in the muscles of the host, depressed the humoral immune response to both the T-dependent and T-independent antigens, SRBC and LPS which indicates that later stages of T. spiralis infection also affect the B-cell population. However, during this stage of infection IgG responses to SRBC are still normal. It is possible that in the late stages of T. spiralis infection there is selective depression of the $B_1$ population of Playfair and Purves (1971) leading to a reduced IgM response to SRBC.

Macrophage function in a 30 day old T. spiralis infection was normal in ordinary infection but was activated with a higher level of infection. This again would favour the role of macrophages in immunodepression induced by later stages of T. spiralis infection in a way similar to the intestinal phase.

produced although it is recognized that the macrophages of both high and low responders Biozzi mice handled SRBC in the same way.

Argyris (1967) has shown that phagocytosed SRBC are as immunogenic as non phagocytosed ones. In addition, some experiments indicate that breakdown by macrophages of SRBC may be an essential prerequisite for immune induction (Shortman and Palmer, 1971; Feldmann and Palmer, 1971). It is therefore, possible that over activity of macrophage function in T. spiralis infected animals alters the immunogenicity of SRBC.

Several studies have demonstrated that the ratio of macrophages to lymphocytes is critical for the induction of an immune response. It has been shown that stimulated mouse macrophages produce a factor(s) which is depressive to the immune response (Nelson, 1973 and Chene and Hirsch, 1972). This may provide a further explanation of immunodepression induced by T. spiralis infection contrasted with apparently enhanced phagocytic activity.

A 30 day old T. spiralis infection, that is when larvae are in the muscles of the host, depressed the humoral immune response to both the T-dependent and T-independent antigens, SRBC and LPS which indicates that later stages of T. spiralis infection also affect the B-cell population. However, during this stage of infection IgG responses to SRBC are still normal. It is possible that in the late stages of T. spiralis infection there is selective depression of the $B_1$ population of Playfair and Purves (1971) leading to a reduced IgM response to SRBC.

Macrophage function in a 30 day old T. spiralis infection was normal in ordinary infection but was activated with a higher level of infection. This again would favour the role of macrophages in immunodepression induced by later stages of T. spiralis infection in a way similar to the intestinal phase.

Evidence for T cell defect during late stages of T. spiralis infection were reported as delayed allograft rejection (Svet-Moldavsky et al., 1970; Ljungström and Huldt, 1977) and as depression of rosette-forming cells (Faubert and Tanner, 1974a).

The migrating and developing phase (NBL) of T. spiralis represents an intermediate stage between the intestinal phase and the encysted muscle phase. This phase caused depression of the immune response to SRBC, indicating a T cell defect, but did not affect the response to LPS, indicating a normal B cell population, and normal macrophage function determined by PVP clearance. It is possible that the T cell population is first depressed by early stages of T. spiralis infection (intestinal phase and migrating phase) helped by lack of coordination between the 3 cells (namely B cell, T cell and macrophages) then later in infections when the larvae reach the host muscles, the B cell population are also affected.

All stages of T. spiralis produced increased catabolism of passively transferred macroglobulins. This was explained by the presence of high levels of anti-T. spiralis IgM antibodies produced during T. spiralis infection which may provide a homeostatic or feed back mechanism which controls or limits the production of antibodies to heterologous antigens even if infected animals were synthesizing antibody at the same rate as uninfected controls. This mechanism may provide another explanation for lowered serum anti-SRBC antibodies in T. spiralis infection.

Non specific polyclonal B cell activation leading to the exhaustion of B cell potential, as judged by increased background PFC in infected animals has been suggested as one of the mechanisms of immunodepression induced by T. brucei infection (Urquhart et al., 1973 and Hudson et al., 1976) and by S. mansoni infection (Ramalho pinto et al., 1976). In this study an attempt was made to study this possibility in T. spiralis

infection, but under the conditions of the experiment, results did not support this possibility. Further *in vivo* and *in vitro* study may be useful to clear up this point in this parasite model.

It has been shown that bone marrow cells may be the cellular site for unresponsiveness (Playfair, 1969). In *T. spiralis* infection, Faubert and Tanner (1974a) showed that bone marrow cells from *T. spiralis* infected animals were unable to reconstitute normal thymectomized irradiated mice. This indicates that defective BM cells may play a role in immunodepression induced by *T. spiralis*.

Faubert and Tanner (1971 and 1975) have presented evidence that serum of animals infected with *T. spiralis* contains a factor which agglutinates and is cytotoxic to lymphoid cells and can delay skin homograft rejection. They also showed that *T. spiralis* larvae have lymphocytotoxic properties. They claimed that these effects are due to substances secreted by the *T. spiralis* parasite. However their work showed no correlation between the agglutination titres and the number of inoculating larvae or to the intensity of infection. There are also indications that serum and larval factors may differ in molecular weight and specificity. In addition, Barriga (1978a) found no evidence of cytotoxicity for spleen cells *in vivo* in experiments of cell transfer from parental mice to $F_1$ hybrids. This suggests that the factor present in the sera of infected animals is different from the larval factor and may be of host origin. The present study showed activated macrophage function in animals infected with *T. spiralis* and it is possible that the factor present in the sera of infected animals may originate from the activated macrophages.

Molinari *et al*. (1975) showed that *T. spiralis* infection produces a marked histopathologic change within the thymic cortex and medulla reflected as increase in total cell number, quantity, distribution and

proportion of histiocytes and lymphoblasts and number of mitotic figure in thymic cortex, which could be related to the alteration of the immunological expression during infection. However Ljungström and Huldt (1977) have demonstrated the cortical depletion of lymphocytes in the thymus which might lead to a reduction in the traffic of cortical lymphocytes to the medulla where maturation occurs.

Eosinophilia is a common feature of T. spiralis infection. The role of eosinophils in immunodepression is unknown. However, eosinophils modulate allergic inflammatory responses by synthesis and release of prostaglandins (Hubscher, 1975), therefore additional modulatory roles in antibody production cannot be excluded.

In conclusion, from this study and other worker's studies, we are now in a situation where we can understand some of the happenings during T. spiralis infection. It is possible that during early stages of T. spiralis infection (intestinal and migrating phase) proliferation of lymphoid cells both in Peyer's patches, intestinal mucosa (Larsh and Race, 1954 and Walls et al., 1973) and in mesenteric lymph nodes occur (Faubert and Tanner, 1974b) but this was not seen in T cell deprived mice (Walls et al., 1973 and Faubert and Tanner, 1974b) which suggests that the cells migrated from the thymus. This will, therefore, be followed by depletion of lymphocytes from the thymus (Ljungström and Huldt, 1977). Simultaneously, the intestinal phase increases the macrophage activity (present study). When a second T-dependent antigen is introduced during this phase of T. spiralis infection, a deficient T cell population helped by the mechanism of antigenic competition at the macrophage function, therefore may lead to depressed immune response to the second antigen. This mechanism does not affect the immune response to T-independent antigen LPS (present study). Meanwhile

activated macrophages may influence antibody affinity by antigen removal leading to the production of high affinity antibodies (present study) as a compensatory mechanism to overcome quantitative defects in PFC response and antibody levels induced during T. spiralis infection and thus help the host to survive.

During late stages of T. spiralis infection B cells are also affected leading to depressed antibody response to both T-dependent and T-independent antigens (present study).

The increase in anti T. spiralis IgM antibody levels (Ljungström, 1974 and Ljungström and Ruitenberg, 1976) in turn, may limit IgM production to a second antigen due to a feed back mechanism or lead to increased catabolism (present study) of antibodies in T. spiralis infection even if they were synthesized at the same rate as in uninfected mice.

The increase in the affinity of the antibody to HSA antigen (present study) may be a compensatory mechanism for the various defects discussed above.

Although this study has shed more light on the effect of T. spiralis infection on the immune response and to the mechanism of immunodepression induced by T. spiralis infection, it is probably premature to attempt a rational synthesis of all the facts. More study is needed to confirm the postulations mentioned and to study further points which can modulate the immune response in this parasite model.

# REFERENCES

ABDALLAH, A. (1946). Bacteriological flora in urinary schistosomiasis. J. Egypt. Med. Ass., 29, 33-37.

ABO GABAL, I., EL SAID, W. M. and HASIB, N. (1970). Urinary bilharziasis and chronic pyelonephritis. Ain Shams Med. J., 21, 167-176.

ACKERMAN, S. B., and SEED, J. R. (1976). Effects of Trypanosoma brucei gambiense infections in Microtus montanus on susceptibility to Ehrlich's tumours. Infect. Immunity, 13, 388-391.

ADLER, F. L. (1964). "Competition of antigens", In: Progress in Allergy, 8, 41-57, Kallós, P. and Waksman, B. H. (Editors). S. Karger.

AITKEN, M. M., JONES, P. W., HALL, G. A. and HUGHES, D. L. (1976). The effect of fascioliasis on the susceptibility of cattle to Salmonella dublin. Br. Vet. J., 132, 119-120.

ALBRIGHT, J. F., ALBRIGHT, J. W., and DUSANIC, D. G. (1977). Trypanosome-induced splenomegaly and suppression of mouse spleen cell responses to antigen and mitogens. J. Reticuloendothel. Soc., 21, 21-31.

ALLT, G., EVANS, E. M. E., EVANS, D. H. L. and TARGETT, G. A. T. (1971). Effect of infection with trypanosomes on the development of experimental allergic neuritis in rabbits. Nature (London), 233, 197-199.

ANDERSSON, J., SJÖBERG, O. and MÖLLER, G. (1972). Induction of immunoglobulin and antibody synthesis in vitro by Lipopolysaccharides. Eur. J. Immunol., 2, 349-353.

ARAUJO, F. G., COELHO, P. M. Z., PEREIRA, L. H. and PELLEGRINO, J. (1977). Schistosoma mansoni: impairment of the cell-mediated immune response in mice. Clin. exp. Immunol., 28, 289-291.

ARGYRIS, B. F. (1967). Role of macrophages in antibody production. Immune response to sheep red blood cells. J. Immunol., 99, 744-750.

BARKER, L. R. (1971). Experimental malaria: Effects upon the immune response to different antigens. J. infect. Dis., 123, 99-101.

BARRIGA, O. O. (1975). Selective immunodepression in mice by Trichinella spiralis extracts and infections. Cell. Immunol., 17, 306-309.

BARRIGA, O. O. (1978a). Depression of cell-mediated immunity following inoculation of Trichinella spiralis extract in the mouse. Immunology, 34, 167-173.

BARRIGA, O. O. (1978b). Modification of immune competence by parasitic infections. 1. Responses to mitogens and antigens in mice treated with Trichinella spiralis extract. J. Parasit., 64, 638-644.

BECH, V., GODTHAAB, M. D. and GREENLAND (1962). Measles epidemics in Greenland. Am. J. Dis. Child., 103, 252-253.

BENDINELLI, M., KAPLAN, G. S. and FRIEDMAN, H. (1975). Reversal of leukemia virus-induced immunosuppression in vitro by peritoneal macrophages. J. Nat. Cancer Inst., 55, 1425-1432.

BENNETT, I. L. and HOOK, E. W. (1959). Infectious diseases (Some aspects of salmonellosis). Ann. Rev. Med., 10, 1-20.

BERKOVICH, S. and STARR, S. (1966). Effects of live type 1 poliovirus vaccine and other viruses on the tuberculin test. New Engl. J. Med., 274, 67-72.

BIOZZI, G., STIFFEL, C., MOULTON, D. and BOUTHILLIER, Y. (1975). Selection of lines of mice with high and low antibody response to complex immunogens. In: Immunogenetics and immunodeficiency, pp. 180-227. Benacerraf (Editor), MTP Lancaster.

BOMFORD, R. and WEDDERBURN, N. (1973). Depression of immune response to moloney leukemia virus by malarial infection. Nature (London), 242, 471-473.

BRADLEY, S. G. and WATSON, D. W. (1964). Suppression by endotoxin of the immune response to actinophage in the mouse. Proc. Soc. Exp. Biol. Med., 117, 570-572.

BRITO, I. V., PEEL, MARGARETT M., and REE, G. H. (1976). Immunological response to tetanus toxoid during a schistosomal infection in mice. J. trop. Med. Hyg., 79, 161-163.

BRODY, J. A. and McALISTER, R. (1964). Depression of tuberculin sensitivity following measles vaccination. Am. Rev. resp. Dis., 90, 607-611.

BRO-JØRGENSEN, K., GÜTTLER, F., JØRGENSEN, P. N. and VOLKERT, M. (1975). T lymphocyte function as the principal target of lymphocytic choriomeningitis virus-induced immunosuppression. Infect. Immunity, 11, 622-629.

BROWN, A. R., CRANDALL, R. B. and CRANDALL, C. A. (1976). Increased IgG catabolism as a possible factor in the immunosuppression produced in mice infected with Heligmosomoides polygyrus. J. Parasit., 62, 169-171.

BRUCE, R. G. and PHILIPS, R. S. (1974). The effect of concurrent malaria and trypanosome infections on immunity to Trichinella spiralis in mice. Proc. 3rd Int. Congr. Parasit., 3, 1188.

BRYCESON, A. D., BRAY, R. S. and DUMONDE, D. C. (1974). Experimental cutaneous leishmaniasis. IV. Selective suppression of cell-mediated immunity during the response of guinea-pigs to infection with Leishmania enriettii. Clin. exp. Immunol., 16, 189-201.

CAMPBELL, W. C. and YAKSTIS, J. J. (1969). Mating success and fecundity of pairs of Trichinella larvae administered to mice. Proc. 2nd Int. Conf. Trichinellosis, Wiadomości Parazytologiczne, 15, 526-532.

CASSIMOS, C., LAZANAKIS, S. and THOMAIDIS, T. (1966). Antibody response after immunization with typhoid-paratyphoid A and B vaccine in Kala azar. Acta Paed. Scand., 55, 301-304.

CHEN, C. and HIRSCH, J. G. (1972). The effects of mercaptoethanol and of peritoneal macrophages on the antibody-forming capacity of non-adherent mouse spleen cells in vitro. J. exp. Med., 136, 604-617.

CHERNYAKHOVSKAYA, Yu., SHAGIYAN, G. Sh., and SVET-MOLDAVSKII, G. Ya. (1971). Correlation between the degree of invasion of mice by Trichinella spiralis and inhibition of transplantation immunity. Akademiya Nauk SSSR, Doklady, Biological Sciences Section, 196-198, 85-87.

CHIMISHKYAN, K. L. and OVUMYAN, G. Sh. (1975). The effect of Trichinella spiràlis on the susceptibility and antibody production to vaccinia virus. Vop. virus, 4, 438-441.

CHIMISHKYAN, K. L., OVUMYAN, G. Sh., TRUBCHENINOVA, L. P., SOROKINA, E. V. and SVET-MOLDAVSKY, G. J. (1974). Immunosuppressive effect of Trichinella spiralis. 3rd Int. Congr. Parasit., 2, 669-670.

CHISARI, F. V., NORTHRUP, R. S. and CHEN, L. C. (1974). The modulating effect of cholera enterotoxin on the immune response. J. Immunol., 113, 729-739.

CHRISTENSON, R. O. (1950). Nemic ova, In: Introduction to nematology, pp. 175-187. Chitwood, B. G. and Chitwood, M. B. (Editors). Baltimore, Monumental.

CHUNG, H. L. and REIMANN, H. A. (1930). Quoted from Freeman, J. C. Immunodepression in Trypanosomiasis, Ph.D. Thesis (1975). School of Biological Sciences, Brunel University, Uxbridge, Middlesex, England.

CLINTON, B. A., STAUBER, L. A. and PALCZUK, N. C. (1969). Leishmania donovani: antibody response to chicken ovalbumin by infected golden hamsters. Expl Parasit., 25, 171-180.

COLEMAN, R. M., BRUCE, A., RENCRICCA, N. J. (1976). Malaria: macrophage migration inhibition factor (MIF). J. Parasit., 62, 137-138.

COLLINS, F. M. and SCOTT, M. T. (1974). Effect of *Corynebacterium parvum* treatment on the growth of *Salmonella enteritidis* in mice. Infect. Immunity, 9, 863-869.

CORSINI, A. C., CLAYTON, C., ASKONAS, B. A., and OGILVIE, B. M. (1977). Suppressor cells and loss of B-cell potential in mice infected with *Trypanosoma brucei*. Clin. exp. Immunol., 29, 122-131.

COX, F. E. G. (1975). Enhanced *Trypanosoma musculi* infections in mice with concommitant malaria. Nature, 258, 148-149.

COX, F. E. G. (1976). Increased virulence of trypanosome infections in mice with malaria or piroplasmosis : immunological considerations. In: Biochemistry of parasites and host-parasite relationships, pp. 421-426. Van den Bossche, H. (Editor). Amsterdam, The Netherlands: North Holland Publishing Company.

COX, F. E. G. (1977). Interactions between trypanosomes and piroplasms in mice. Protozoology, 3, 129-134.

COX, F. E. G., WEDDERBURN, N. and SALAMAN, M. H. (1974). The effect of Rowson-parr virus on the severity of malaria in mice. J. Gen. Microbiol., 85, 358-364.

CRANDALL, C. A. and CRANDALL, R. B. (1976). *Ascaris suum*: Immuno-suppression in mice during acute infection. Expl Parasit., 40, 363-372.

CRANDALL, R. B., CRANDALL, C. A. and FRANCO, J. A. (1974). *Heligmosomoides polygyrus* (= *Nematospiroides dubius*) : Humoral and intestinal immunologic responses to infection in mice. Expl Parasit., 35, 275-287.

CUNNINGHAM, A. J. and SZENBERG, A. (1968). Further improvements in the plaque technique for detecting single antibody-forming cells. Immunology, 14, 599-600.

CYPESS, R. H., LUBINIECKI, A. S. and HAMMON, W. (1973). Immunosuppression and increased susceptibility to Japanese B Encephalitis virus in Trichinella spiralis infected mice. Proc. Soc. exp. Biol. Med., 143, 469-473.

CYPESS, R. H., LUBINIECKI, A. S. and SWIDWA, D. M. (1974a). Decreased susceptibility to Listeria monocytogenes in mice after infection with Trichinella spiralis. Infect. Immunity, 9, 477-479.

CYPESS, R. H., MOLINARI, J. A., EBERSOLE, J. L. and LUBINIECKI, A. S. (1974b). Immunological sequelae of Trichinella spiralis infection in mice. II. Potentiation of cell mediated response to BCG after infection with Trichinella spiralis. Infect. Immunity, 10, 107-110.

CYPESS, R. H., SWIDWA, D. W., KENNY, J. F. and YEE, R. B. (1974c). Influence of a metazoan infection in the mouse on enteric colonization and immune response to Escherichia coli. J. Infect. Dis., 130, 534-538.

DALESANDRO, D. A. and KLEI, T. R. (1976). Evidence for immunodepression of Syrian hamsters and Mongolian jirds by Dipetalonema viteae infections. Trans. R. Soc. trop. Med. Hyg., 70, 534-535.

DAVID, G. S. (1972). Solid state lactoperoxidase : A highly stable enzyme for simple, gentle iodination of proteins. Biochem. Biophys. Res. Commun., 48, 464-470.

DENHAM, D. A. (1965). Studies with methyridine and Trichinella spiralis. 1. Effect upon the intestinal phase in mice. Expl Parasit., 17, 10-14.

DENHAM, D. A. (1968). Immunity to Trichinella spiralis. 3. The longevity of the intestinal phase of the infection in mice. J. Helminth., 42, 257-268.

DENHAM, D. A., and MARTINEZ, A. R. (1970). Studies with methyridine and Trichinella spiralis. 2. The use of the drug to study the rate of larval production in mice. J. Helminth., 44, 357-363.

DENT, P. B. (1972). Immunodepression by oncogenic viruses. Prog. Med. Virol., 14, 1-35.

DESSAINT, J. P., CAMUS, D., and CAPRON, A. (1977). Depression of lymphocytes proliferation by a factor released by Schistosoma mansoni. Annals d'Immunologie, 128c, 57-58.

DIAMANTSTEIN, T., KEPPLER, W. and BLITSTEIN-WILLINGER, E. (1976). Suppression of the primary immune response in vivo to sheep red blood cells by T-cell mitogens. Immunology, 30, 401-407.

DRESSER, D. W. and WORTIS, H. H. (1965). Use of antiglobulin serum to detect cells producing antibody with low haemolytic efficiency. Nature (London), 208, 859-861.

DRESSER, D. W. and WORTIS, H. H. (1967). Localised haemolysis in gel. In: Handbook of experimental immunology, pp. 1054-1067. Weir, D. M. (Editor). Blackwell, Oxford.

DUNMIRE, C., RUCKDESCHEL, J. C. and MARDINEY, M. R. (1975). Suppression of in vitro lymphocyte responsiveness to purified protein derivative by measles virus. A re-exploration of the phenomenon. Cell. Immunol., 20, 205-217.

DUSZYNSKI, D. W., RUSSEL, D., ROY, S. A. and CASTRO, A. (1978). Suppressed rejection of Trichinella spiralis in immunized rats concurrently infected with Eimeria nieschulzi. J. Parasit., 64, 83-88.

EARDLEY, D.D., Jayawardena, A.N. (1977). Suppressor cells in mice infected with Trypanosoma brucei. J. Immunology, 119 (3) 1029 - 1033.

EITZMAN, D. V. and SMITH, R. T. (1959). Antibody response to heterologous protein in rabbits of varying maturity. Proc. Soc. Exp. Biol. Med., 102, 529-531.

EVANS, T. C. (1970). Biologic factors in studies of ionizing radiation. IN: Trichinosis in man and animals, pp. 109-128. Gould, S. E. (Editor). Charles, C., Thomas Publisher.

FAHEY, J. L. and SELL (1965). The immunoglobulins of mice. V. The metabolic (catabolic) properties of five immunoglobulin classes. J. Exp. Med., 122, 41-58.

FAUBERT, G. M. (1976). Depression of the plaque-forming cells to sheep red blood cells by the new-born larvae of Trichinella spiralis. Immunology, 30, 485-489.

FAUBERT, G., TANNER, C. E. (1971). Trichinella spiralis : Inhibition of sheep haemagglutinins in mice. Expl Parasit., 30, 120-123.

FAUBERT, G. M., and TANNER, C. E. (1974a). The suppression of sheep rosette-forming cells and the inability of mouse bone marrow cells to reconstitute competence after infection with the nematode Trichinella spiralis. Immunology, 27, 501-505.

FAUBERT, G. M. and TANNER, C. E. (1974b). Enlargement of lymph nodes during infection with Trichinella spiralis: A preliminary histological study. In: Trichinellosis. Proc. 3rd Int. Conf. on Trichinellosis, pp. 353-366. Kim, C. W. (Editor). Intext Educational Publishers, New York.

FAUBERT, G. M., and TANNER, C. E. (1975). Leucoagglutination and cytotoxicity of the serum of infected mice and of extracts of Trichinella spiralis larvae and the capacity of infected mouse sera to prolong skin allografts. Immunology, 28, 1041-1050.

FELDMANN, M. and PALMER, J. (1971). The requirement for macrophages in the secondary immune response to antigens of small and large size in vitro. Immunology, 21, 685-699.

FINGER, H. M., BARTOSCHEK, M and EMMERLING, P. (1972a). Time relationships between injection of antigen and adjuvant. II. Immunosuppression induced by Bordetella pertussis vaccine when given before secondary antigenic stimulation. Pathol. Microbiol., 38, 93-102.

FINGER, H., EMMERLING, P., HOF, H. and PLAGER, L. (1973). Time relationships between injection of antigen and adjuvant. V. Primary and secondary immune responses to sheep erythrocytes in mice pretreated with incomplete Freund's adjuvant. Z. Immun. Forsch. Exp., 145, 227-241.

FINGER, H., EMMERLING, P. and PLAGER, L. (1972b). Time relationships between injection of antigen and adjuvant. III. Adjuvancy of Bordetella pertussis given at various times after the primary antigenic stimulus. Infect. Immunity, 5, 783-791.

FIREMAN, P., FRIDAY, G. and KUMATE, J. (1969). Effect of measles vaccine on immunologic responsiveness. Paediatrics, 43, 264-272.

FLOERSHEIM, G. L. and SZESZAK, J. J. (1972). Poly 1 poly C and endotoxins share immunosuppressive properties and increase the toxicity of alpha-Amanitin and Hexobarbital. Agents Actions, 2, 150-155.

FRANZL, R. E. and McMASTER, P. D. (1968). The primary immune response in mice. 1. The enhancement and suppression of haemolysin production by a bacterial endotoxin. J. Exp. Med., 127, 1087-1107.

FREEMAN, J. (1975). Immunodepression in Trypanosomiasis, Ph.D. Thesis, School of Biological Sciences, Brunel University, Uxbridge, Middlesex, England.

FREEMAN, J., HUDSON, K. M., LONGSTAFFE, J. A. and TERRY, R. J. (1973). Immunodepression in trypanosome infections. Parasitology, 67, xxiii.

FRIDAY, G. A., KUMATE, J. and FIREMAN, P. (1968). Suppression of delayed hypersensitivity and atopic manifestations by measles vaccine. J. Allergy, 41, 111.

GANGULY, R., CUSUMANO, C. L. and WALDMAN, R. H. (1976). Suppression of cell-mediated immunity after infection with attenuated Rubella virus. Infect. Immunity, 13, 464-469.

GOLENSER, J., SPIRA, D. T. and ZUCKERMAN, A. (1975). Dynamics of thymidine incorporation by spleen cells from rats infected with Plasmodium berghei. Clin. Expl Immunol., 22, 364-371.

GOLUB, E. S. and WEIGLE, W. O. (1969). Studies on the induction of immunologic unresponsiveness. III. Antigen form and mouse strain variation. J. Immunol., 102, 389-396.

GOOD, A. H., and MILLER, K. L. (1976). Depression of the immune response to sheep erythrocytes in mice infected with Taenia crassiceps larvae. Infect. Immunity, 14, 449-456.

GOODWIN, L. G. (1970). The pathology of African trypanosomiasis. Trans. R. Soc. trop. Med. Hyg., 64, 797-812.

GOODWIN, L. G., GREEN, D. G., GUY, M. W. and VOLLER, A. (1972). Immunosuppression during trypanosomiasis. Br. J. Exp. Path., 53, 40-43.

GOOSE, J. (1977). Studies on immunity to Fasciola hepatica in the rat. Ph.D. Thesis, School of Biological Sciences, Brunel University, Uxbridge, Middlesex, England.

GORCZYNSKI, R. M. (1974). Immunity to murine sarcoma virus-induced tumours. II. Suppression of T cell mediated immunity by cells from progressor animals. J. Immunol., 112, 1826-1838.

GOULD, S. E. (1945). Quoted from Villella, J. B. (1970). Life cycle and morphology. In: Trichinosis in man and animals. Gould, S.E. (Editor), pp. 19-60. Charles C. Thomas, Publisher.

GREENWOOD, B. M. (1968). Autoimmune disease and parasitic infections in Nigerians. Lancet, 2, 380-382.

GREENWOOD, B. M. (1974a), Possible role of a B-cell mitogen in hypergammaglobulinaemia in malaria and trypanosomiasis. Lancet, 1, 435-436.

GREENWOOD, B. M. (1974b). Immunosuppression in malaria and trypanosomiasis, in parasites in the immunized host: mechanism of survival. Ciba Foundation Symposium 25 (New series), 137-146. Elsevier. Excerpta Medica. North Holland, Associated Scientific Publishers.

GREENWOOD, B. M., BRADLEY-MOORE, A. M., PALIT, A. and BRYCESON, A. D. M. (1972). Immunodepression in children with malaria. Lancet, 1, 169-172.

GREENWOOD, B. M., PLAYFAIR, J. H. L. and TORRIGIANI, G. (1971). Immunosuppression in murine malaria. 1. General characteristics. Clin. Exp. Immunol., 8, 467-478.

GREENWOOD, B. M. and VOLLER, A. (1970a). Suppression of autoimmune disease in New Zealand mice associated with infection with malaria. 1. (NZB x NZW) $F_1$ hybrid mice. Clin. Exp. Immunol., 7, 793-803.

GREENWOOD, B. M. and VOLLER, A. (1970b). Suppression of autoimmune disease in New Zealand mice associated with malaria. II. NZB mice. Clin. Exp. Immunol., 7, 805-815.

GREENWOOD, B. M., WHITTLE, H. C. and MOLYNEUX, D. H. (1973). Immunosuppression in gambian trypanosomiasis. Trans. R. Soc. trop. Med. Hyg., 67, 846-850.

GURSCH, O. F. (1949). Intestinal phase of Trichinella spiralis. J. Parasit., 35, 19-26.

HAMMERBERG, B., MUSOKE, A. J., HUSTEAD, S. T. and WILLIAMS, J. F. (1976). Anticomplementary substances associated with Taeniid metacestodes. In: Pathophysiology of parasitic infection, pp. 233-240. Soulsby, E. J. L. (Editor). Academic Press.

HARLEY, J. P. and GALLICCHIO, V. (1971). Growth of Trichinella spiralis larvae from birth to day 13 post inoculation in the male albino rat. J. Parasit., 57, 781-786.

HARTMANN, K. U. (1975). Possible involvement of $C_3$ during stimulation of B lymphocytes. Transplantation Review, 23, 98-104.

HENDERSON-BEGG, A. (1946). Heterophile antibodies in trypanosomiasis. Trans. R. Soc. trop. Med. Hyg., 40, 331-339.

HENNEY, C. S., LITCHENSTEIN, L. M., GILLESPIE, E. and ROLLEY, R. T. (1973). In vivo suppression of the immune response to allo-antigen by cholera enterotoxin. J. Clin. Invest., 52, 2853-2857.

HIGAZI, A. M., EL-EBRASHY, N., EISA, A. A., EL-MAHDY, H., SABRY, Y., TORKY, H. and ELSHAWAF, A. (1972). Pyelonephritis predisposed to by urinary bilharziasis. J. Egypt Med. Ass., 55, 439-455.

HOLMES, P. H., MAMMO, E., THOMSON, A., KNIGHT, P. A., LUCKEN, R., MURRAY, P. K., MURRAY, M., JENNINGS, F. W. and URQUHART, G. M. (1974). Immunosuppression in bovine trypanosomiasis. Vet. Record, July 27, 86-87.

HOUBA, V., BROWN, K. N. and ALLISON, A. C. (1969). Heterophile antibodies, M-antiglobulins and immunoglobulins in experimental trypanosomiasis. Clin. Exp. Immunol., 4, 113-123.

HOWARD, J. G., CHRISTTE, G. H. and SCOTT, M. T. (1973). Biological effects of Corynebacterium parvum. IV. Adjuvant and inhibitory activities on B lymphocytes. Cell Immunol., 7, 290-301.

HUBSCHER, T. (1975). The role of the eosinophil in the allergic reactions. II. Release of prostaglandins from human eosinophilic leukocytes. J. Immunol., 114, 1389-1393.

HUDSON, K. M., BYNER, C., FREEMAN, J. and TERRY, R. J. (1976). Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis. Nature (London), 264, 256-258.

HUGHES, W. T., SMITH, J. S., KIM, M. H. and LOUISVILLE, M. D. (1968). Suppression of the histoplasmin reaction with measles and small pox vaccines. Am. J. Dis. Child., 116, 402-406.

HULDT, G., GARD, S. and OLOVSON, S. G. (1973). Effect of Toxoplasma gondii on the thymus. Nature, 244, 301-303.

HULL, R. M. (1971). Laboratory studies on a South American strain of Trypanosoma vivax. Trans. R. Soc. trop. Med. Hyg., 65, 258.

HUNTER, R. (1970). Standardization of the chloramine-T method of protein iodination. Proc. Soc. Exp. Biol. Med., 133, 989-992.

JAMES, E. R. (1974). Studies on immunity to Trichinella spiralis in mice. Ph.D. Thesis, London University.

JARRETT, W. F. H., JENNINGS, F. W., McINTYRE, W. I. H., MULLIGAN, W., SHARP, N. C. C. and URQUHART, G. M. (1959). Immunological studies on Dictyocaulus viviparus infections in calves - double vaccination with irradiated larvae. Am. J. Vet. Res., 20, 522-526.

JAYAWARDENA, A. N., TARGETT, G. A. T., LUCHARS, E., CARTER, R. L., DOENHOFF, M. J. and DAVIES, A. J. S. (1975). T cell activation in murine malaria. Nature, 258, 149-151.

JAYAWARDENA, A. N. and WAKSMAN, B. H. (1977). Suppressor cells in experimental trypanosomiasis. Nature (London), 265, 539-541.

JERNE, N. K. and NORDIN, A. A. (1963). Plaque formation in agar by single-antibody-producing cells. Science, 140, 405.

JERUSALEM, C. (1968). Relationship between malaria infection (Plasmodium berghei) and malignant lymphoma in mice. Z. Trop. Parasit., 19, 94-108.

JOHNSON, A. G., JACOBS, A., ABRAMS, G. and MERRITT, K. (1967). Comparative changes in the mouse spleen during immunostimulation or immunosuppression. In: Germinal centres in immune responses, pp. 234-239. Cottier, H., Odartchenko, N., Schindler, R. and Congden, C. C. (Editors). Springer-Verlag, New York.

JONES, J. F., CRANDALL, C. A. and CRANDALL, R. B. (1976). T-dependent suppression of the primary antibody response to sheep erythrocytes in mice infected with Trichinella spiralis. Cell Immunol., 27, 102-110.

JORNVALL, H. and ZEPPEZAUER, M. (1972). Iodination of horse liver alcohol dehydrogenase. Biochem. Biophys. Res. Comm., 46, 1951-1955.

KASUYA, S., OHTOMA, H. and ISHIZAKI, T. (1977). Suppressing effects of purified eosinophils derived from guinea pigs sensitized with Ascaris antigen on lymphocyte blastformation. Jap. J. Med. Sci. Biol., 30, 297-307.

KAUFFMAN, C. A., PHAIR, J. P., LINNEMANN, C. C. and SCHIFF, G. M. (1974). Cell-mediated immunity in humans during viral infection. 1. Effect of rubella on dermal hypersensitivity, phytohemagglutinin response, and T-lymphocyte numbers. Infect. Immunity, 10, 212-215.

KAYE, D., MERSELIS, J. G. and HOOK, E. W. (1965). Influence of Plasmodium berghei infection on susceptibility to Salmonella infection. Proc. Soc. Exp. Biol. Med., 120, 810-813.

KILHAM, L. and OLIVER, L. (1961). The promoting effect of trichinosis on encephalomyocarditis (EMC) virus infection in rats. Am. J. trop. Med. Hyg., 10, 879-884.

KIRCHNER, H., CHUSED, T. M., HERBERMAN, R. B., HOLDEN, H. T. and LAVIN, D. H. (1974). Evidence of suppressor cell activity in spleens of mice bearing primary tumours induced by Moloney sarcoma virus. J. Exp. Med., 139, 1473-1487.

KLOETZEL, K., FALEIROS, J. J., MENDES, S. R., STANLEY, C. T. and ARIAS, H. S. (1973). Concommitant infection of albino mice by Trypanosoma cruzi and Schistosoma mansoni. Parasitological parameters. Trans. R. Soc. trop. Med. Hyg., 67, 652-658.

KONGSHAVN, P. A. L., HO, A. and SEBALDT, J. (1977). Suppression of in vitro antibody responses by Listeria primed spleen cells. Cell Immunol., 28, 284-297.

KUPERS, T. A., PETRICH, J. M., HOLLOWAY, A. W. and ST. GEME, J. W. (1970). Depression of tuberculin delayed hypersensitivity by live attenuated mumps virus. J. Pediat., 76, 716-721.

LARSH, J. E. (1970). Immunology. In: Trichinosis in man and animals, pp. 129-146. Gould, S. E. (Editor). Charles C. Thomas, Publisher.

LARSH, J. E. and RACE, G. J. (1954). A histopathologic study of the anterior small intestine of immunized and non immunized mice infected with Trichinella spiralis. J. Infect. Dis., 94, 262-272.

LEHMAN, J. S., FARID, Z., SMITH, J. M., BASSILY, S. and EL-MASRY, N. A. (1973). Urinary schistosomiasis in Egypt: clinical, radiological, bacteriological and parasitological correlations. Trans. R. Soc. trop. Med. Hyg., 67, 384-399.

LEIPER, R. T. (1939). Quoted from Denham, D. A. (1965). Studies with methyridine and Trichinella spiralis. 1. Effect upon the intestinal phase in mice. Expl Parasit., 17, 10-14.

LJUNGSTRÖM, I. (1974). Antibody response to Trichinella spiralis. In: Trichinellosis, Proc. 3rd Internat. Conf. on Trichinellosis, pp. 449-460. Kim, C. W. (Editor). Intext Educational Publisher, New York.

LJUNGSTRÖM, I. and HULDT, G. (1977). Effect of experimental trichinosis on unrelated humoral and cell mediated immunity. Acta Path. Microbiol. Scand., Section C, 85, 131-141.

LJUNGSTRÖM, I. and RUITENBERG, E. J. (1976). A comparative study of the immunohistological and serological response of intact and T cell-deprived mice to Trichinella spiralis. Clin. Exp. Immunol., 24, 146-156.

LONGSTAFFE, J. A., FREEMAN, J. and HUDSON, K. M. (1973). Immunosuppression in trypanosomiasis: some thymus dependent and thymus independent responses. Trans. R. Soc. trop. Med. Hyg., 67, 264-265.

LOOSE, L. D., COOK, J. A. and DILUZIO, N. R. (1972). Malarial immuno-depression - A macrophage mediated defect. Proc. Helminth. Soc. Wash., 39, 484-491.

LOOSE, L. D. and DILUZIO, N. R. (1976). A temporal relationship between reticuloendothelial system phagocytic alterations and antibody responses in mice infected with Plasmodium berghei (NYU - 2 strain). Am. J. trop. Med. Hyg., 25, 221-228.

LUBINIECKI, A. S. and CYPESS, R. H. (1975a). Immunological sequelae of Trichinella spiralis infection in mice : Effect on the antibody responses to sheep erythrocytes and Japanese B encephalitis virus. Infect. Immunity, 11, 1306-1311.

LUBINIECKI, A. S. and CYPESS, R. H. (1975b). Quantitative study of the effect of previous Trichinella spiralis infection on sarcoma 180 ascitis tumour formation in mice. Tropenmed. Parasit., 26, 329-334.

LUBINIECKI, A. S., CYPESS, R. H. and LUCAS, J. P. (1974a). Synergistic interaction of two agents in mice: Japanese B encephalitis virus and Trichinella spiralis. Am. J. trop. Med. Hyg., 23, 235-241.

LUBINIECKI, A. S., CYPESS, R. H. and LUCAS, J. P. (1974b). Immune response to and distribution of sheep erythrocytes in Trichinella spiralis infected mice. Tropenmed. Parasit., 25, 345-349.

MACKANESS, G. B. (1964). The immunological basis of acquired cellular resistance. J. Exp. Med., 120, 105-120.

MACKANESS, G. B. (1969). The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J. Exp. Med., 129, 973-992.

MACKENZIE, P. K. I., BOYT, W. P., ESMLIE, V. W., LANDER, K. P. and SWANEPOEL, R. (1975). Immunosuppression in ovine trypanosomiasis Vet. Record, 97, 452-453.

MAHMOUD, A. F., STRICKLAND, T. and WARREN, K. (1977). Toxoplasmosis and the host-parasite relationship in murine Schistosomiasis mansoni. J. Infect. Dis., 135, 408-413.

MALAKIAN, A. and SCHWAB (1968). Immunosuppressant from group A streptococci. Science, 159, 880-881.

MALAKIAN, A. H. and SCHWAB (1971). Biological characterization of an immunosuppressant from group A streptococci. J. Exp. Med., 134, 1253-1265.

MANSFIELD, J. M. and KREIER, J. P. (1972a). Autoimmunity in experimental Trypanosoma congolense infections of rabbits. Infect. Immunity, 5, 648-656.

MANSFIELD, J. M. and KREIER, J. P. (1972b). Tests for antibody and cell mediated hypersensitivity to trypanosome antigens in rabbits infected with Trypanosoma congolense. Infect. Immunity, 6, 62-67.

MANSFIELD, J. M. and WALLACE, J. H. (1974). Suppression of cell-mediated immunity in experimental African trypanosomiasis. Infect. Immunity, 10, 335-339.

MASIHI, K. N. and WERNER, H. (1977). Suppression and enhancement of humoral immune response to Toxoplasma gondii by passive antibody. Experientia, 33, 1586-1587.

McBRIDE, J. S. and MICKLEM, H. S. (1977). Immunosuppression in murine malaria. II. The primary response to bovine serum albumin. Immunology, 33, 253-259.

McBRIDE, J. S., MICKLEM, H. S. and URE, J. M. (1977). Immunosuppression in murine malaria. I. Response to type III pneumococcal polysaccharide. Immunology, 32, 635-644.

McCONAHEY, P. J. and DIXON, F. J. (1966). A method of trace iodination of proteins for immunologic studies. Int. Arch. Allergy, appl. Immun., 29, 185-189.

McGREGOR, I. A. and BARR, M. (1962). Antibody response to tetanus toxoid inoculation in malarious and non-malarious Gambian children. Trans. R. Soc. trop. Med. Hyg., 56, 364-367.

McMASTER, P. D. and FRANZL, R. E. (1968). The primary immune response in mice. II. Cellular responses of lymphoid tissue accompanying the enhancement or complete suppression of antibody formation by a bacterial endotoxin. J. Exp. Med., 127, 1109-1126.

MEEROVITCH, E. and ACKERMAN, S. J. (1974). Trypanosomiasis in rats with trichinosis. Trans. R. Soc. trop. Med. Hyg., 68, 417.

MEEROVITCH, E. and BOMFORD, R. (1977). Macrophage potentiation by *Trichinella spiralis*. Ann. trop. Med. Parasit., 71, 245-247.

MELLMAN, W. J. and WETTON, R. (1963). Depression of the tuberculin reaction by attenuated measles virus vaccine. J. Lab. Clin. Med., 61, 453-458.

METCHNIKOFF, E. (1905). Quoted from Nathan, C. F., Karnovsky, M. L. and David, J. R. (1971). Alterations of macrophage functions by mediators from lymphocytes. J. Exp. Med., 133, 1356-1373.

MITCHELL, G. F. and HANDMAN, E. (1977). Studies on immune responses to larval cestodes in mice: a simple mechanism of non-specific immunosuppression in *Mesocestoides corti*-infected mice. Aust. J. Exp. Biol. Med. Sci., 55, 615-622.

MITCHELL, G. F., MARCHALONIS, J. J., SMITH, P. M., NICHOLAS, W. L. and WARNER, N. L. (1977). Studies on immune responses to larval cestodes in mice: immunoglobulins associated with the larvae of *Mesocestoides corti*. Aust. J. Exp. Biol. Med. Sci., 55, 187-211.

MOLINARI, J. A., CYPESS, R. H. and APPEL, B. N. (1975). Effect of infection with *Trichinella spiralis* and BCG on thymic histology. Int. Arch. Allergy appl. Immun., 48, 776-783.

MÖLLER, G. and MICHAEL, G. (1971). Frequency of antigen-sensitive cells to thymus-independent antigens. Cell Immunol., 2, 309-316.

MÖLLER, G. and WIGZELL, H. (1965). Antibody synthesis at the cellular level. J. Exp. Med., 121, 969-989.

MORGAN, A. G. and SOOTHILL, J. F. (1975a). Relationship between macrophage clearance of PVP and affinity of anti-protein antibody response in inbred mouse strains. Nature (London), 254, 711-712.

MORGAN, A. G. and SOOTHILL, J. F. (1975b). Measurement of the clearance function of macrophages with $^{125}$I-labelled polyvinylpyrolidone. Clin. Exp. Immunol., 20, 489-498.

MORTENSEN, R. F., CEGLOUSKI, W. S. and FRIEDMAN, H. (1974). Leukemia virus-induced immunosuppression. X. Depression of T cell mediated cytotoxicity after infection of mice with Friend leukemia virus. J. Immunol., 112, 2077-2086.

MOTA-SANTOS, T. A., GAZZINELLI, G., RAMALHO-PINTO, F. J., PELLEGRINO, J. and DIAS DA SILVA, W. (1976). Immunodepression in mice following Schistosoma mansoni infection. Rev. Inst. Med. Trop. Sao Paulo, 18, 246-250.

MOTA-SANTOS, T. A., TAVARES, C. A. P., GAZZINELLI, G., PELLEGRINO, J. (1977). Immunosuppression mediated by adult worms in chronic Schistosomiasis mansoni. Am. J. trop. Med. Hyg., 26, 727-731.

MOULTON, J. E. and COLEMAN, J. L. (1977). Immunodepression in deer mice with experimentally induced trypanosomiasis. Am. J. Vet. Res., 38, 573-579.

MUNOZ, J. (1964). Effect of bacteria and bacterial products on antibody response. Adv. Immunol., 4, 397-440.

MUNOZ, J. J. and COLE, R. L. (1977). Effect of Trichinella spiralis infection on massive cutaneous anaphylaxis in mice. Infect. Immunity, 15, 84-90.

MUNRO, A. and BRIGHT, S. (1976). Products of the major histocompatibility complex and their relationship to the immune response. Nature, 264, 145-152.

MURRAY, P. K., JENNINGS, F. W., MURRAY, M. and URQUHART, G. M. (1974). The nature of immunosuppression in Trypanosoma brucei infections in mice. II. The role of the T and B lymphocytes. Immunology, 27, 825-840.

MUSCHEL, L. H., SIMONTON, L. A., WELLS, P. A. and FIFE, E. H. (1961). Occurrence of complement-fixing antibodies reactive with normal tissue constituents in normal and disease states. J. Clin. Invest., 40, 517-524.

NELSON, D. S. (1973). Production by stimulated macrophages of factors depressing lymphocyte transformation. Nature (London), 246, 306-307.

NELSON, G. S., BLACKIE, E. J. and MUKUNDI, J. (1966). Comparative studies on geographical strains of Trichinella spiralis. Trans. R. Soc. trop. Med. Hyg., 60, 471-480.

NEVES, J. and DA LUZ LOBO MARTINS, N. R. (1967). Long duration of septicaemic salmonellosis: 35 cases with 12 implicated species of Salmonella. Trans. R. Soc. trop. Med. Hyg., 61, 541-552.

NEVEU, T., BRANELLEC, A. and BIOZZI, G. (1964). Propriétés adjuvantes de Corynebacterium parvum sur la production d'anticorps et sur l'induction de l'hypersensibilité retardée envers les protéines conjuguées. Annls Inst. Pasteur, Paris, 106, 771-777.

NOTKINS, A. L., MERGENHAGEN, S. E. and HOWARD, R. J. (1970). Effect of virus infections on the function of the immune system. Annual Rev. Microbiol., 24, 525-538.

OGILVIE, B. M. and WILSON, R. I. M. (1976). Evasion of the immune response by parasites. Br. Med. Bull., 32, 177-181.

OLSON, P. R., WEIBLEN, B. J., O'LEARY, J. J., MOSCOWITZ, A. J. and McCULLOUGH, J. (1976). A simple technique for the inactivation of IgM antibodies using Dithiothreitol. Vox. Sang., 30, 149-159.

OTTESEN, E. A., WELLER, P. F. and HECK, L. (1977). Specific cellular immune unresponsiveness in human filariasis. Immunology, 33, 413-421.

PASSWELL, J. H., STEWARD, M. W. and SOOTHILL, J. F. (1974). Inter-mouse strain differences in macrophage function and its relationship to antibody responses. Clin. Exp. Immunol., 17, 159-167.

PELLEY, R. P., RUFFIER, J. J. and WARREN, K. S. (1976). Suppressive effect of a chronic helminth infection, Schistosomiasis mansoni, on the in vitro responses of spleen and lymph node cells to the T cell mitogens phytohemagglutinin and con canavalin A. Infect. Immunity, 13, 1176-1183.

PEPYS, M. B. (1972). Role of complement in induction of the allergic response. Nature, New Biol., 237, 157-159.

PERRUDET-BADOUX, A. and BINAGHI, R. A. (1977). Adhérence, sous dépendance d'anticorps de cellules péritonéales de rat aux larves de Trichinella spiralis. Ann. d'Immunol., 128c, 243-244.

PERRUDET-BADOUX, A., BINAGHI, R. A., BOUSSAC-ARON, Y. (1976). Production of different classes of immunoglobulins in rats infested with Trichinella spiralis. Immunochemistry, 13, 443-445.

PERRUDET-BADOUX, A., BINAGHI, R. A. and BOUSSAC-ARON, Y. (1977). Enhancement of antibody production on an unrelated antigen in rats infested with Trichinella spiralis. Ann. d'Immunol., 128c, 965-966.

PHILLIPS, R. S., SELBY, G. R. and WAKELIN, D. (1974). The effect of Plasmodium berghei and Trypanosoma brucei infections on the immune expulsion of the nematode Trichuris muris from mice. Int. J. Parasit., 4, 409-415.

PHILLIPS, R. S. and WAKELIN, D. (1976). Trichuris muris: Effect of concurrent infections with rodent piroplasms on immune expulsion from mice. Expl Parasit., 39, 95-100.

PLAYFAIR, J. H. L. (1969). Specific tolerance to sheep erythrocytes in mouse bone marrow cells. Nature (London), 222, 882-883.

PLAYFAIR, J. H. L. (1971). Cell cooperation in the immune response. Clin. Exp. Immunol., 8, 839-856.

PLAYFAIR, J. H. L. and PURVES, E. C. (1971). Antibody formation by bone marrow cells in irradiated mice. 1. Thymus-dependent and thymus-independent responses to sheep erythrocytes. Immunology, 21, 113-121.

POELS, L. G. and VAN NIEKERK, C. C. (1977). Plasmodium berghei: Immunosuppression and hyper immunoglobulinaemia. Expl Parasit., 42, 235-247.

PORTARO, J. K., BRITTON, S. and ASH, L. R. (1976). Brugia pahangi: depressed mitogen reactivity in filarial infections in the jird, Meriones unguiculatus. Expl Parasit., 40, 438-446.

PURVIS, A. C. (1977). Immunodepression in Babesia microti infections. Parasitology, 75, 197-205.

RAMALHO-PINTO, F. J., DE SOUZA, J. B. and PLAYFAIR, J. H. L. (1976). Stimulation and suppression of response of mouse T cells to the schistosomules of Schistosoma mansoni during infection. Nature, 259, 603-604.

REED, S. G., LARSON, C. L., and SPEER, C. A. (1977). Suppression of cell-mediated immunity in experimental Chagas disease. Z. Parasit. Kde, 52, 11-17.

REED, W. R., OLDS, J. W. and KISCH, A. L. (1972). Decreased skin-test reactivity associated with influenza. J. Infect. Dis., 125, 398-402.

ROSENSTREICH, D. L. and OPPENHEIM, J. J. (1976). Role of macrophages in the activation of T and B lymphocytes *in vitro*. In: Immunobiology of the macrophages, pp. 161-199. Nelson, D. S. (Editor). Academic Press, New York, San Francisco and London.

RUITENBERG, E. J. and KRUYT, B. C. (1975). Effect of intestinal flagellates on immune response of mice. Parasitology, 71, xxx.

RUITENBERG, E. J., LEENSTRA, F. and ELGERSMA, A. (1977). Thymus dependence and independence of intestinal pathology in a *Trichinella spiralis* infection: A study in congenitally athymic (nude) mice. Br. J. Exp. Path., 58, 311-314.

RUITENBERG, E. J. and STEERENBERG, P. A. (1974). Intestinal phase of *Trichinella spiralis* in congenitally athymic (nude) mice. J. Parasit., 60, 1056-1057.

SALAMAN, M. H., WEDDERBURN, N. and BRUCE-CHWATT, L. J. (1969). The immunodepressive effect of a murine plasmodium and its interaction with murine oncogenic viruses. J. Gen. Microbiol., 59, 383-391.

SCHRADER, J. W. and FELDMANN, M. (1973). The mechanism of antigenic competition. 1. The macrophage as a site of a reversible block of T-B lymphocyte collaboration. Eur. J. Immunol., 3, 711-717.

SCHWAB, J. H. (1975). Suppression of the immune response by microorganisms. Bact. Rev., 39, 121-143.

SCOTT, M. T. (1972). Biological effects of the adjuvant *Corynebacterium parvum*. 1. Inhibition of PHA, mixed lymphocyte and GVH reactivity. Cell Immunol., 5, 459-468.

SEED, J. R. and GAM, A. A. (1967). The presence of antibody to a normal rabbit liver antigen in rabbits infected with *Trypanosoma gambiense*. J. Parasit., 53, 946-950.

SENGERS, R. C. A., JERUSALEM, C. R. and DOESBURG, W. H. (1971). Murine malaria. IV. Disturbed immunological responsiveness during Plasmodium berghei infection. Expl Parasit., 30, 41-53.

SEWELL, M. M. H. (1963). The immunology of Fascioliasis. 1. Autofixation of guinea pig complement. Immunology, 6, 453-461.

SHARMA, S. C., and RAMACHANDRAN, M. (1976). Suppression of immune response to sheep erythrocytes in Litomosoides carinii infected albino rats. Indian J. Exp. Biol., 14, 188-189.

SHIMP, R. G., CRANDALL, R. B. and CRANDALL, C. A. (1975). Heligomosomoides polygyrus (= Nematospiroides dubius) : suppression of antibody response to orally administered sheep erythrocytes in infected mice. Expl Parasit., 38, 257-269.

SHOKEIR, A. A., IBRAHIM, A. M., HAMID, M. Y., SHALABY, M. A., HUSSEIN, H. E. and BADR, M. (1972). Urinary schistosomiasis in upper Egypt. II. A bacteriological study. East African Med. J., 49, 312-326.

SHORTMAN, K. and PALMER, J. (1971). The requirement for macrophages in the in vitro immune response. Cell Immunol., 2, 399-410.

SISKIND, G. W. and BENACERRAF, B. (1969). Cell selection by antigen in the immune response. Adv. Immunol., 10, 1-50.

SMITH, R. T. and BRIDGES, R. A. (1958). Immunological unresponsiveness in rabbits produced by neonatal injection of defined antigens. J. Exp. Med., 108, 227-250.

SMITH, J. H., KAMEL, I. A., ELWI, A. and VON LICHTENBERG, F. (1974). A quantitative post mortem analysis of urinary schistosomiasis in Egypt. 1. Pathology and pathogenesis. Am. J. trop. Med. Hyg., 23, 1054-1071.

SMITHERS, S. R. and TERRY, R. J. (1976). The immunology of schistosomiasis. Adv. Parasit., 14, 399-422.

SOOTHILL, J. F. and STEWARD, M. W. (1971). The immunopathological significance of the heterogeneity of antibody affinity. Clin. Exp. Immunol., 9, 193-199.

STARR, S. and BERKOVICH, S. (1964). Effects of measles, gamma-globulin modified measles and vaccine measles on the tuberculin test. New Engl. J. Med., 270, 386-391.

STEWARD, M. W. (1974). Outline studies in biology. Immunochemistry. Chapman and Hall, London.

STEWARD, M. W. and VOLLER, A. (1973). The effect of malaria on the relative affinity of mouse antiprotein antibody. Br. J. Exp. Path., 54, 198-202.

STRICKLAND, G. T., AHMED, A. and SELL, K. W. (1975). Blastogenic response of Toxoplasma infected mouse spleen cells to T and B cell mitogens. Clin. Exp. Immunol., 22, 167-176.

STRICKLAND, G. T., PETTITT, L. E. and VOLLER, A. (1973). Immunodepression in mice infected with Toxoplasma gondii. Am. J. trop. Med. Hyg., 22, 452-455.

STRICKLAND, G. T., and SAYLES, P. C. (1977). Depressed antibody responses to a thymus dependent antigen in toxoplasmosis. Infect. Immunity, 15, 184-190.

STRICKLAND, G. T., VOLLER, A., PETTITT, L. E. and FLEK, D. G. (1972). Immunodepression associated with concommitant toxoplasma and malaria infections in mice. J. Infect. Dis., 126, 54-60.

SUTER, E. and RAMSEIER, H. (1964). Cellular reactions in infection. Adv. Immunol., 4, 117-173.

SVET-MOLDAVSKY, G.J., SHAGHIJAN, G.S., CHERNYAKHOVSKAYA, I.Y., MKHEIDZE, D.M., LITOVSCHENKO, T.A., OZERETSKOVSKAYA, N.N. and KADAGHIDZE, Z.G. (1970). Inhibition of skin allograft rejection in Trichinella infected mice. Transplantation, 9, 69-71.

TANABE, K., WAKI, S., TAKADA, S. and SUZUKI, M. (1977). Plasmodium berghei: suppressed response of antibody-forming cells in infected mice. Expl Parasit., 43, 143-152.

TANNER, C. E. (1968). Relationship between infecting dose, muscle parasitism, and antibody response in experimental trichinosis in rabbits. J. Parasit., 54, 98-107.

TANNER, C. E. and LIM, H. C. (1974). The effect of Trichinella spiralis on the thymus, spleen and lymph nodes. 3rd Internat. Congr. Parasitology, 2, 672.

TARZAALI, A., VIENS, P. and QUEVILLON, M. (1975). Immunodepression after whooping cough vaccination in mice infected with Plasmodium berghei yoelii. Arch. Inst. Pasteur d'Algérie, 50/51, 163-170.

TAUSSIG, M. J. and LACKMANN, P. J. (1972). Studies on antigenic competition. II. Abolition of antigenic competition by antibody against or tolerance to the dominant antigens: A model for antigenic competition. Immunology, 22, 185-197.

TAYLOR, D. W. and SIDDIQUI, W. A. (1978). Effect of falciparum malaria infection on the in vitro mitogen responses of spleen and peripheral blood lymphocytes from owl monkeys. Am. J. trop. Med. Hyg., 27, 738-742.

TURK, J. L. and BRYCESON, A. D. M. (1971). Immunological phenomena in leprosy and related diseases. Adv. Immunol., 13, 209-266.

UNANUE, E. R. (1972). The regulatory role of macrophages in antigenic stimulation. Adv. Immunol., 15, 95-165.

URQUHART, G. M., MURRAY, M. and JENNINGS, F. W. (1972). The immune response to helminth infection in trypanosome infected animals. Trans. R. Soc. trop. Med. Hyg., 66, 342-343.

URQUHART, G. M., MURRAY, M., MURRAY, P. K., JENNINGS, F. W. and BATE, E. (1973). Immunodepression in Trypanosoma brucei infections in rats and mice. Trans. R. Soc. trop. Med. Hyg., 67, 528-535.

VICKERMAN, K. (1978). Antigenic variation in trypanosomes. Nature (London), 273, 613-617.

VOLLER, A., GALL, D. and MANAWADU, B. R. (1972). Depression of the antibody response to tetanus toxoid in mice infected with malaria parasites. Z. Tropenmed. Parasit., 23, 152-155.

WAKSMAN, B. H. (1977). Tolerance, the thymus, and suppressor T cells. Clin. Exp. Immunol., 28, 363-374.

WALLS, R. S., CARTER, R. L., LEUCHARS, E. and DAVIES, A. J. S. (1973). The immunopathology of trichinasis in T-cell deficient mice. Clin. Exp. Immunol., 13, 231-242.

WARREN, K. S., MAHMOUD, A. F., BOROS, D. L., RALL, T. W., MANDEL, M. A. and CARPENTER, C. J. (1974). In vivo suppression by cholera toxin of cell-mediated and foreign body inflammatory responses. J. Immunol., 112, 996-1007.

WARREN, H. S., and WEIDANZ, W. P. (1976). Malarial immunodepression in vitro: Adherent spleen cells are functionally defective as accessory cells in the response to horse erythrocytes. Eur. J. Immunol., 6, 816-819.

WATERSTON, R. H. (1970). Antigenic competition: A paradox. Science, 171, 1108-1109.

WEDDERBURN, N. (1970). Effect of concurrent malarial infection on development of virus-induced lymphoma in BALB/c mice. Lancet, 2, 1114-1116.

WEDDERBURN, N. (1974). Immunodepression produced by malarial infection in mice. In: Parasites in the immunized host - mechanism of survival. Ciba Foundation Symposium 25 (New series), 123-135. Elsevier. Excerpta Medica. North Holland, Associated Scientific Publisher.

WEDDERBURN, N. and DRACOTT, B. N. (1977). The immune response to type III pneumococcal polysaccharide in mice with malaria. Clin. Exp. Immunol., 28, 130-137.

WEIDANZ, W. P. and RANK, R. G. (1975a). Immunosuppressive effects of experimental infection with Plasmodium gallinaceum. Proc. Soc. Exp. Biol. Med. 148, 725-728.

WEIDANZ, W. P. and RANK, R. G. (1975b). Regional immunosuppression induced by Plasmodium berghei yoelii infection in mice. Infect. Immunity, 11, 211-212.

WEIGLE, W. O. (1973). Immunological unresponsiveness. Adv. Immunol., 16, 61-122.

WILKIE, B. N., CAOILI, F. and FRIEND, S. (1976). Nonspecific immunosuppression induced in mice with killed Pasteurella haemolytica in Freund's complete adjuvant. Int. Archs. Allergy appl. Immun., 50, 745-750.

WOODRUFF, A. W. (1968). Helminths as vehicles and synergists of microbial infections. Trans. R. Soc. trop. Med. Hyg., 62, 446-452.

YOELI, M. (1956). Some aspects of concommitant infections of plasmodia and schistosomes. 1. The effect of Schistosoma mansoni on the course of infection of Plasmodium berghei in the field vole (Microtus guentheri). Am. J. trop. Med. Hyg., 5, 988-999.

ZABRISKIE, J. (1967). Mimetic relationships between group A streptococci and mammalian tissues. Adv. Immunol., 7, 147-188.

ZAPPACOSTA, S. and ROSSI, G. (1967). An improved method for radioiodination of γ G-immunoglobulin. Immunochemistry, 4, 122-125.

ZAUNDERER, M. and ASKONAS, B. A. (1976). Several proliferative phases precede maturation of IgG-secreting cells in mitogen stimulated cultures. Nature, 260, 611-613.

ZWEIMAN, B., PAPPAGIANIS, D., MAIBACH, H. and HILDRETH, E. A. (1971). Effect of measles immunization on tuberculin hypersensitivity and *in vitro* lymphocyte reactivity. Int. Arch Allergy appl. Immunol., 40, 834-841.