THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

STUDIES OF ADULT AND LARVAL ASCARID ANTIGENS USING HOMOLOGOUS PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIGS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

RICHARD FRANCIS COLLINS

Oklahoma City, Oklahoma

1973

STUDIES OF ADULT AND LARVAL ASCARID ANTIGENS USING HOMOLOGOUS PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIGS

APPROVED BY

Michael H. Ivey

Philip E. Smith

J. F. W. Jackson

William A. Lam

Wallace Fridberg

DISSERTATION COMMITTEE

ACKNOWLEDGEMENTS

The author wishes to acknowledge the helpful assistance of Mr. Attway Ausbie during various phases of this study. His help was invaluable during the infection and care of dogs, collection of worms, and collection of blood from infected guinea pigs.

Sincere thanks is given to my advisory committee, Drs. M. H. Ivey, W. A. Cain, J. L. W. Jackson, P. E. Smith, and W. Friedberg. I would also like to thank Dr. G. A. Castro, who served on my advisory committee at its inception, for his interest and help during much of my graduate program.

The helpful suggestions and assistance of Dr. Don Parker concerning the statistical analyses is gratefully acknowledged.

Special appreciation is expressed to Dr. M. H. Ivey for his guidance, advice, knowledge, and most especially, his help in the preparation of this manuscript. I am also indebted to Dr. Ivey, who, through his research grant with the National Institutes of Health, furnished the financial assistance which enabled me to continue my graduate program.

Finally, I wish to thank my wife, Beth, for her help in proofreading and preparing the manuscript. Her patience and understanding is also very much appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES--Continued

STUDIES OF ADULT AND LARVAL ASCARID ANTIGENS USING HOMOLOGOUS PASSIVE CUTANEOUS ANAPHYLAXIS IN GUINEA PIGS

CHAPTER I

INTRODUCTION

Prior to 1950 clinicians observed and reported a large number of eosinophilic disorders, variously described as chronic eosinophilia, eosinophilia leukemoides, eosinophilia and splenomegaly, extreme eosinophilia and leukocytosis, Loeffler's syndrome, visceral lesions associated with extreme eosinophilia, and eosinophilic leukemia (Zinkham, 1968). Although a parasitic etiology was frequently considered, it was not until 1950 that Mercer et al. and Juniper and Beach identified nematode larvae in their respective patients. Subsequently Beaver et al. (1952) discovered that the larvae of the dog ascarid, Toxocara canis, caused visceral lesions and eosinophilia in abnormal hosts. They introduced the term visceral larva migrans to describe such cases.

It is now fairly well established that visceral larva migrans is a more common disease than was previously suspected. In addition to T. canis, other helminth species have been implicated in the etiology of this disease. However, T. canis is still considered to be the primary etiologic agent. The parasite may cause serious damage to various tissues, especially those of the eye and nervous system (Ashton, 1960;

Duguid, 1961; Moore, 1962; Braun-Vallon, 1963; Greer, 1963; De Haan, 1964; Beautyman et al., 1966; Danis et al., 1966; De Buen et al., 1966; Engel et al., 1971). For these reasons, it has assumed a significant place in the glossary of infectious diseases.

Various workers have studied experimental Toxocara canis infections. Animals which have been studied include baboons, monkeys, dogs, rabbits, rats, mice, and guinea pigs. For the most part, these workers have studied massive infections. The host response has been of an acute rather than a chronic nature. The converse, a chronic host response rather than an acute one, occurs in human infections.

Clinical diagnosis of human infections with T. canis is difficult since the symptoms mimic those of other diseases. At present the demonstration of larvae provides the only means for proof of a T. canis etiology. This is most commonly done by liver biopsy, a risky procedure that may give inconclusive results in mild cases. This lack of a convenient diagnostic tool has generated much interest and study of the value of various immunological tests for the presumptive diagnosis of T. canis infections.

Most of the common immunological techniques have been used. These include intradermal tests, agglutination, gel diffusion, bentonite flocculation, fluorescent antibody, complement fixation, Schultz-Dale, passive cutaneous anaphylaxis, larval precipitin, and ring precipitin tests. Although the presence of Toxocara antibodies has been shown with all of the aforementioned tests, the presence of common determinant groups, especially of Ascaris, complicates the interpretation of positive tests.

Important problems to be considered in visceral larva migrans research include: (1) the replication of the chronic host response of humans in an experimental animal, thereby establishing a model that more closely resembles humans; (2) the study of the experimental host response to a low-dose infection, which is the more probable type of infection among humans; and (3) assessment of the sensitivity and specificity of T. canis antigens and the counterpart Ascaris suum antigens in immunological tests.

The objectives of this study were as follows:

1. To investigate the eosinophilic response of the guinea pig to low-dose infections of T. canis.
2. To evaluate the sensitivity and specificity of adult and larval somatic antigens, and perienteric fluid of T. canis and A. suum by intradermal skin tests on infected guinea pigs.
3. To evaluate the sensitivity and specificity of adult and larval somatic antigens, and perienteric fluid of T. canis and A. suum by passive cutaneous anaphylaxis in guinea pigs.

CHAPTER II

LITERATURE REVIEW

Although significant progress has been made in the immunodiagnosis of helminthic infections in recent years, most of the manifestations of immunity to helminths are not completely understood. A number of reviews dealing with the immunologic aspects of helminthic infections have been published in the past decade (Tromba, 1962; Urquhart *et al.*, 1962; Soulsby, 1962; Jachowski, 1963; Soulsby, 1966; Pan American Health Organization, 1967; Soulsby, 1967a, 1967b; Kozar, 1967; Ogilvie and Jones, 1969; Sinclair, 1970; Valdivieso and Tamsitt, 1970; Fife, 1971; Jarrett and Urquhart, 1971).

The nematodes as a group constitute a serious threat to the health and vitality of man and other animals in many parts of the world. Their antigenic complexity and the presence of common antigens among taxonomically distinct species complicate attempts at serologic diagnosis. *Toxocara canis*, an important etiologic agent of visceral larva migrans (VLM), has been studied extensively since it was first reported from a human by Beaver *et al.* (1952). This literature review will be confined to the objectives of the present study, i.e., eosinophil and antibody responses, and the evaluation of the sensitivity and specificity of three different *Toxocara* antigens with their couterpart

Ascaris antigens in serological tests. Eosinophilia occurs rather frequently in parasitic infections. *T. canis* was first incriminated as a cause of eosinophilia in humans by Beaver *et al.* (1952). There have been many case reports of suspected and proven VLM since that time and eosinophilia is a common characteristic of many of these (Brill *et al.*, 1953; Dent *et al.*, 1956; Karpinski *et al.*, 1956; Chaudhuri and Saha, 1959; Krauss, 1960; Molina Pasquel and Diaz Munoz, 1960; Van Thiel, 1960; Bourke and Yeates, 1961; Harris, 1961; Molina Pasquel, 1961; Williams and Menning, 1961; Lewis *et al.*, 1962; Lorentz, 1962; Rey, 1962; Richards *et al.*, 1962; Brett, 1963; Marchal *et al.*, 1963; Baldone *et al.*, 1964; Brain and Allan, 1964; Bratanov and Konstantinova, 1964; Appelmans *et al.*, 1965; Frankish, 1965; Szczepanska, 1965; Escobar-Melguizo and Little, 1966; Bonnette *et al.*, 1967; Reekie and Miller, 1969; Rodriguez de la Vega and Zamora Ubieta, 1969; Haddow and Gall, 1970; Moreau *et al.*, 1971).

In contrast to the chronic eosinophilia that is generally observed in human VLM, the eosinophilia observed in experimental animals infected with *Toxocara* is of a more acute nature. This difference may or may not be related to the number of infective eggs given to the animals under study. Sadun *et al.* (1957) infected rabbits and monkeys with large numbers of *T. canis* eggs (13,000 to 40,000 and 900,000 respectively). The eosinophil level in the rabbits increased until 2 to 4 weeks after infection and then gradually decreased. The monkeys had slightly elevated counts 20 months after infection. Chaudhuri and Saha (1959) found that the eosinophilia peaked by the third week following infection of guinea pigs with large numbers (1,000

to 1,400) of T. canis eggs. The levels then declined by the sixth week but were still elevated. Another group of guinea pigs given 450-500 eggs daily for 2 weeks showed elevated eosinophil levels which lasted 8 weeks. Sharp and Olson (1962b) observed a persistent eosinophilia for 4 weeks in guinea pigs infected with large numbers (2,500) of T. canis eggs. Olson and Schultz (1963) showed that the onset and the extent of eosinophilia were dose-dependent in guinea pigs infected with T. canis. The eosinophilia was maximal approximately 2 weeks after infection. It declined during the following 2 weeks but remained at abnormal levels in all infection groups. Dhar et al. (1966) observed conflicting results in six rats and two monkeys, each of which were given 1,500 eggs. Three of the six rats' eosinophilia persisted for 10 weeks. In the other rats and the monkeys the eosinophil counts returned to normal levels within 5 weeks following infection. Two monkeys given 1,000 and 500 eggs respectively exhibited marked eosinophilia at 3 weeks post-infection (Wiseman and Woodruff, 1967). The levels declined to normal by the twelfth week. Bisseru (1969) infected three mice and a monkey with 200, 300, 500, and 2,500 T. canis eggs, respectively. The mice exhibited a maximum eosinophilia by 1 week following infection; the level declined to normal by the third week. Eosinophilia developed in the monkey and reached its highest peak about 2 weeks after infection. The eosinophil level then fell but remained elevated for about 4 months. A reinfection with 1,000 eggs at that time did not noticeably affect the eosinophil level. Wiseman et al. (1969) infected four monkeys with 200, 100, 50, and 20 eggs, respectively. The mean eosinophil counts of all four monkeys were normal

until the fifth week following infection. The counts then increased, reaching a broad peak from the ninth to thirteenth weeks. A slow decline then occurred but the mean count was still elevated at the nineteenth week. Aljeboori and Ivey (1970) infected groups of baboons with various numbers of T. canis eggs (1, 5, or 20 eggs per gram of body weight). The animals had peak eosinophilic responses the second or third week after infection. The peak intensity was apparently dose-dependent. The eosinophilia declined but remained above preinfection levels for 12 weeks. A reinfection at that time caused a secondary rise in eosinophils. However, the responses never reached the first infection levels.

A substantial amount of work has been done on the serological diagnosis of T. canis infections in experimental animals and in humans. Many workers have observed cross-reactions between Toxocara and Ascaris. Precipitin tests have often been employed. Heiner and Kevy (1956) described the syndrome of VLM in three young siblings. Reacting their sera against adult Toxocara antigen in Ouchterlony plates, zones of precipitation developed near the reservoirs containing these patients' sera but not near the reservoirs containing sera from other persons. Sadun et al. (1957) reported that specific antibodies are produced by rabbits following active infection or artificial immunization with T. canis. They detected antibodies with either a crude antigen consisting of extracts of adults and eggs or an acid-soluble protein fraction of adults. Falkinburg and Kay (1962) reported the use of the gel diffusion test for a suspected case. An extract of T. canis cuticle formed precipitin bands with this patient's serum and the sera from positive

controls. Pina et al. (1962) detected a high percentage of reactors (18.9 per cent) to T. canis adult antigen among persons with eosinophilia. However, there were also high percentages of reactors to A. lumbricoides (16.6 per cent) and to A. suum (16.2 per cent) adult antigens. Sharp and Olson (1962a) studied the responses of guinea pigs infected with either Toxocara, Ascaris, or Trichinella. Extracts of infective Toxocara and Ascaris eggs and Trichinella larvae were used. Precipitation could be shown by agar-gel diffusion tests between anti-Ascaris serum and all three antigens, between anti-Toxocara serum and Toxocara antigen, and between anti-Trichinella serum and Trichinella and Toxocara antigens (but not between the other combinations). Ouchterlony analyses of sera from three cases of suspected VLM revealed precipitins to Toxocara whole-worm extract in two of the cases (Huntley and Moreland, 1963). One of these also reacted with Ascaris whole-worm extract. Huntley et al. (1965) studied the sera of 51 Puerto Rican patients of between 1 and 5 years of age who had sustained eosinophilia and clinical evidence of visceral involvement. Ouchterlony analyses revealed high percentages of reactors to Toxocara and Ascaris extracts (22 per cent and 19 per cent respectively). Cross reactions occurred frequently. Ivey (1965) detected antibodies to Toxocara larval antigen with sera from T. canis-infected guinea pigs. Precipitin bands did not occur with antigens of Ascaris larvae or adults, Trichinella larvae, or Toxocara adults. Rahman (1966) studied experimental VLM in rabbits. Agar gel diffusion failed to differentiate Toxocara infection from Ascaris infection when antigens of adults were used. Fernando (1968a) also studied the antibody responses of rabbits infected

with T. canis. Saline extracts of adults or infective eggs formed precipitin bands with these animals' sera. Agar gel diffusion of serum from a suspected case of VLM demonstrated the presence of antibody to T. cati but not to T. canis (Perlmutter et al., 1968). Fernando et al. (1970) demonstrated specific antigens in an extract of T. canis infective eggs. Sera from T. canis-infected monkeys failed to react with the corresponding antigens of A. lumbricoides in Ouchterlony plates. These workers also observed common antigens in adult worm extracts in both gel diffusion and immunoelectrophoresis. Cross-reactions were also observed when sera from infected guinea pigs were reacted against adult worm extracts (Lamina, 1970a). Ouchterlony analyses of the sera from suspected cases were positive (Engel et al., 1971; Moreau et al., 1971).

The measurement of agglutination due to antigen-antibody interaction has been used by many workers. Fellers (1953) studied the ability of sera from various patients to cause agglutination of a 5 per cent suspension of adult worms. No agglutination of Ascaris or Toxocara occurred with sera of five patients who had ascariasis or in three patients who had eosinophilia. In a suspected case of VLM an increase in the T. canis titer from 1:8 to 1:160 was demonstrated over a five and one-half week period. However, the A. lumbricoides titer was markedly elevated, 1:640. The applicability of the agglutination reaction has been extended to a wide variety of soluble antigens by attaching them to the surface of particles. The particles most widely used have been erythrocytes (passive hemagglutination), bentonite (bentonite flocculation), and latex.

Sadun et al. (1957) detected antibodies to a purified adult Toxocara antigen by the bentonite flocculation test. Results using sera from infected rabbits indicated that this test can detect specific antibodies. The results of flocculation tests with sera from humans and animals infected with different helminths failed to indicate any extensive degree of cross-reactivity. Kagan et al. (1959) prepared Toxocara and Ascaris antigens by several techniques. They tested these antigens against sera from suspected VLM cases and sera from infected rabbits but were unable to demonstrate any specificity in the bentonite flocculation test. Huntley et al. (1965) stated that hemagglutinin and/or flocculation tests were positive with Dirofilaria antigens in 30 per cent and with Toxocara antigens in 30 per cent of 27 Puerto Rican patients with the VLM syndrome. Four sera reacted with both antigens. The tests were performed at the Center for Disease Control, Atlanta, Georgia. The presence of antiglobulins in sera from children with the VLM syndrome was observed by Huntley et al. (1966). Twenty-six of 59 children's sera were found to agglutinate gamma globulin-coated latex particles. Antisera produced by infection of rabbits with either Ascaris or Toxocara cross-reacted with Ascaris and Toxocara antigens in hemagglutination tests (Kagan, 1958). Jung and Pacheco (1958a) prepared Toxocara whole-worm extract and Ascaris polysaccharide fraction. They considered the indirect hemagglutination test to be demonstrably diagnostic in rabbits that were repeatedly heavily infected with ascarids. Four rabbits infected with Toxocara developed high titers of antibodies (1:320 to 1:1,280) against Toxocara antigen, but only low titers (1:20 to 1:80) against Ascaris antigen. The

converse was observed in four rabbits infected with Ascaris, i.e., high titers (1:5,120) against Ascaris antigen, but low titers (1:20 to 1:80) against Toxocara. However, they were unable to observe any conclusive results when these antigens were tested with batteries of human sera. These workers used this test with extracts of adult Ascaris or Toxocara and sera from various categories of persons, including suspected VLM, contacts of suspected cases, cases of known infection with helminths other than Toxocara, siblings and parents of these known cases, and individuals not known to have parasitic infection or eosinophilia (Jung and Pacheco, 1960). Suspected cases usually showed higher titers for Toxocara and/or Ascaris antibody. Sera from persons lacking symptoms of VLM generally had low titers with both antigens. Also, low titers for both antigens were generally observed with sera of persons having other helminthiases. However, persons with strongyloidiasis frequently gave high titers with Toxocara antigen (20 per cent) and with Ascaris antigen (51.4 per cent). Kagan et al. (1959) prepared Ascaris and Toxocara antigens by several techniques. They tested these antigens against sera from suspected VLM cases and sera from infected rabbits. No specificity was demonstrated with the hemagglutination test. This test yielded high titers with sera from persons with eosinophilia (Biagi et al., 1961). However, in addition to Ascaris and Toxocara antigens, there were elevated titers with antigenic extracts of other helminths. Lewis et al. (1962) reported high serum titers (1:2,560) with both Ascaris and Toxocara antigens in a confirmed case. No details of the antigen preparations were given. Pina et al. (1962) detected a high percentage of reactors (16.6 per cent) to T. canis

adult antigen among persons with eosinophilias. However, there were also high percentages of reactors to antigenic extracts of adults of A. lumbricoides (13.9 per cent) and A. suum (17.6 per cent). Vinke et al. (1963) examined the sera of 197 patients but gave no description of the test or the antigens which were used. They concluded that the test was not reliable due either to lack of infection or to nonspecificity of the reaction. A later publication (Vinke et al., 1964) discussed the lack of specificity of the hemagglutination test and referred to data in their earlier paper. This test yielded a negative result with a proven case of VLM but demonstrated antibody to T. canis in a suspected case (Baldone et al., 1964). Appelmans et al. (1965) observed a positive hemagglutination reaction with serum from a suspected case but gave no details concerning the test. Extracts of adult Ascaris or Toxocara demonstrated nonspecific reactions with sera of normal adults, children of various ages without clinically apparent nematode infection, and suspected VLM cases (Wood et al., 1965). Escobar-Melguizo and Little (1966) and Lampkin and Mauer (1970) reported positive hemagglutination tests with sera from their suspected cases. The tests were done at the Center for Disease Control, Atlanta, Georgia, and detailed information was not given. Gelpi and Mustafa (1967, 1968) reported considerable cross-reactivity among Ascaris, Toxocara and Dirofilaria. Reactions to adult antigens occurred with the sera of persons with Loeffler's syndrome as well as with the sera from control subjects. An antigenic preparation isolated from the body fluid of A. suum was highly reactive with sera from suspected VLM cases (Oliver-Gonzalez et al., 1969). Although high reactivity was also observed in persons

from an Ascaris endemic area, there did not appear to be any relationship between increased titers and the presence of adult worms in the intestine. Haddow and Gall (1970) reported a positive VLM titer in a young child with suspected VLM. The test was negative 2 years later. Although no description of the test was given, it is assumed that it was a hemagglutination test since it was done at the Center for Disease Control. Cross-reactivity between Toxocara and Ascaris antigens was reported by Aljeboori and Ivey (1970). Baboons that received varying numbers of T. canis eggs exhibited antibody titers to larval and adult antigens. The antibody response to A. suum larval extract paralleled the response to T. canis larval extract but was of a lower titer.

Olson (1960) reported the use of an in vitro larval precipitate test. He used antisera prepared from infected rabbits and sera from children with suspected VLM. He observed cross-reactions with larvae of Ascaris and Toxocara and antisera from infected rabbits. The results with sera from suspected cases were also inconclusive. In a later study Richards et al. (1962) tested 70 clinically negative children; two were strongly positive with Toxocara larvae. Olson and Schultz (1963) used this test with the sera of guinea pigs which had been given varying numbers of infective T. canis eggs. The test did not become positive until day 28 and it was positive only with sera from heavily infected animals. Todorov and Stoyanov (1966) and Lamina (1970b) reported that this test was specific and gave no reaction with sera of guinea pigs infected with Ascaris. Engel et al. (1971) tested the serum from a suspected case. The in vitro larval precipitate test was positive with Toxocara larvae.

Mitchell (1964) used formalin-fixed second-stage larvae in the indirect fluorescent antibody technique. Cross-reactions with sera from A. suum- or T. canis-infected animals and the heterologous larvae were questionable or weak. Sera from five of 25 children and 17 of 49 normal adults gave significant reactions with Toxocara but no mention was made concerning Ascaris. Hogarth-Scott (1966) used the direct fluorescent antibody (Coons) technique. There were some cross-reactions with T. canis and T. cati second-stage larvae in sera from infected rabbits. However, the second-stage larvae of Toxascaris leonina did not react. He obtained similar results using human sera. A later study (Hogarth-Scott et al., 1969) demonstrated fluorescent antibodies to second-stage larvae of T. canis in the sera of suspected and proven VLM cases. Bisseru and Woodruff (1968), using the indirect fluorescent antibody test, found that T. leonina showed cross-reacting fluorescence in tests with Toxocara immune sera from puppies. They also observed cross-reactions with sera from patients with A. lumbricoides infections. Wiseman et al. (1971) used this test to evaluate anthelminthic efficacy in suspected and proven cases of VLM. Serial examinations of the sera showed that the Toxocara antibody levels gradually declined following treatment with diethylcarbamazine. Baufine-Ducrocq et al. (1971) observed increased antibody titers in three suspected cases.

Complement-fixing antibodies have been detected in many parasitic infections. The complement fixation (CF) procedure is one of the more sensitive, versatile techniques in general use. Sadun et al. (1957) were unable to consistently detect antibodies in the sera of

rabbits infected or passively immunized with T. canis. Pacheco and Danaraj (1963) tested sera from 14 patients with tropical eosinophilia. Ethanol extracts of a number of helminths, including Toxocara and Ascaris, failed to indicate infection with any particular helminth. Fernando (1968a) studied the antibody responses of rabbits infected with T. canis. Using saline extracts of adults and infective eggs, he found a correlation between the test results and the resistance of the animals to reinfection. He also studied the serologic response of puppies to large numbers of worms (1968b). There was a rise in CF antibody titer that was directly related to resistance to superinfection. The presence of a heterophile antigen complex in T. canis was confirmed by Fernando (1968c). Reinfection of rabbits with large numbers of eggs (100,000 or 200,000) produced a marked rise in heterophile antibody that resembled the classic Forssman type. Rabbits that received these egg doses for the first time did not show an increased titer. The corresponding antigen was present mainly in the infective eggs and the adult intestine.

Schultz-Dale tests have demonstrated considerable cross-reactions between Ascaris, Toxocara, and Trichinella (Sharp and Olson, 1962a). Extracts of infective Toxocara and Ascaris eggs and Trichinella larvae were used. The ilea from guinea pigs infected with these parasites were hypersensitive both to homologous and heterologous antigens. Systemic anaphylaxis with homologous and heterologous antigens was also demonstrated. A later study (Olson and Schultz, 1963) showed that there was a dose-dependency between the post-infection time for positive tests and the number of eggs given an animal. Schultz-Dale

tests were positive in heavily infected guinea pigs as early as day 8, while the lightly infected animals were not positive until day 20. Ivey (1965) observed cross-reactions with ilea of guinea pigs infected with either Toxocara, Ascaris, or Trichinella. However, considerably less homologous antigen was required to elicit reactions in the respective animals. In addition, he found that Toxocara larval antigen was more useful for the detection of Toxocara infections.

Although it is clear that specific antibody is a significant factor in acquired immunity, there is uncertainty as to the types of antibody involved in natural infections with Toxocara. In recent years, studies with laboratory animals have contributed markedly to the characterization of helminth-induced homocytotropic antibodies (reagins). This class of antibody has been demonstrated in experimental infections with various helminths (Ogilvie, 1964, 1967; Zvaifler et al., 1966, 1967; Sadun et al., 1967, 1968; Mota et al., 1968, 1969a, 1969b; Hogarth-Scott, 1969; McAninch and Patterson, 1970; Strejan and Campbell, 1970; Barratt and Herbert, 1970; Dobson et al., 1971; Williams and Perez Esandi, 1971; Keller and Jones, 1971).

Antibodies mediating the Schultz-Dale and passive cutaneous anaphylaxis (PCA) reactions in guinea pigs were reported by Ivey (1965) and Ivey and Slanga (1965). The tests indicated increased sensitization with increased numbers of worms and duration of infection. Results of the two tests correlated. They found that PCA antibodies may differ from those involved in hemagglutination, bentonite flocculation, or gel diffusion. Ivey (1967a) was unable to demonstrate positive PCA reactions in guinea pigs sensitized with sera from Toxocara-infected

rabbits and challenged with T. canis adult antigen (heterologous PCA). However, this antigen was reactive at sites sensitized with sera from infected guinea pigs (homologous PCA). Kagan (1968) published the results of PCA tests with sera from suspected and proven VLM cases. The tests were performed by Ivey with T. canis larval antigen. The tests detected antibody in five of the six VLM sera but in none of the controls. Routine serologic tests detected antibody in only one of the six VLM sera. Homologous PCA reaction occurred in rats challenged with adult T. canis antigen (Dobson et al., 1967). Hogarth-Scott (1967a) demonstrated the presence of common antigens in extracts of adult A. suum, T. canis, T. cati, and Toxascaris leonina. Homologous PCA reactions occurred in rabbits sensitized with sera from infected rabbits and challenged with wholeworm saline extracts or fractionated antigens of these helminths. Homologous PCA reactions also were detected in man, rat, and rabbit (Hogarth-Scott, 1967b, 1967c). Sera from clinical cases of ascariasis and sera from laboratory workers sensitized to Ascaris were tested in baboons. Sera from rabbits infected with Toxocara, Toxascaris, or Ascaris were assayed in rabbits, and sera from rats infected with Nippostrongylus were assayed in rats. In a later study sera from suspected and proven cases of VLM gave positive PCA reactions in baboons (Hogarth-Scott et al., 1969). A fractionated antigen from adult T. canis produced positive reactions with sera from the proven cases and some of the suspected cases. They observed a correlation between these reactions and elevated levels of IgE in these persons' sera. Johansson et al. (1968) found marked elevation of IgE levels in sera from Ethiopian children in comparison to a similar group of Swedish children. They considered this difference

to be a reflection of the higher incidence of parasitic infections in the Ethiopian children. Their hypothesis is strengthened by their finding of even higher IgE levels in Ethiopian children with verified Ascaris infections. Heiner and Rose (1970) reported elevated levels of IgE in each of five patients with VLM. They also found elevated levels in persons with various non-parasitic disorders. Homologous PCA reactions were reported by Kobayashi et al. (1970) and Takeuchi (1970) using sera from Toxocara-infected rabbits. The latter author observed irregular patterns of reaginic antibody formation. There was no correlation between infection dose and presence or absence of antibody. There was also a lack of correlation between infection dose and persistence of antibody in the serum.

Intradermal tests with a given antigen will indicate presence or absence of sensitivity in the test subject. The method is a simple, straightforward means for diagnosis if cross-reactivity with other antigens can be ruled out. Brunner et al. (1944) reported positive skin tests in dogs. He used extracts of A. suum and T. canis adults. Twelve of 24 dogs had positive reactions with these extracts and most of these positive reactors were infected with nematodes. Jung and Pacheco (1958a, 1958b) found that intradermal testing with aqueous extracts of adults or larvae of these species was of little value for VLM diagnosis in humans. A cutaneous scratch test using adult T. canis antigen was described by Sprent and English (1958). They stated that preliminary trials showed that the test may have some use in diagnosis. However, they gave no additional information. Woodruff et al. (1961) reported a negative skin test in a young girl who had a T. canis larva

in her eye. They failed to give any details of the antigen used. Duguid (1961) prepared a mixture of T. canis first-stage larvae and adults. He used a saline extract of these for skin tests. The antigen was injected intradermally into healthy rabbits and into rabbits previously infected with T. canis. All infected rabbits gave positive reactions while the normal rabbits gave no reactions. Intradermal tests in humans paralleled these results. Positive skin tests occurred in six of 65 selected patients but no other details were given (Woodruff et al., 1962). Shrand (1964) used the antigen preparation of Duguid (1961). A suspected case (a 19-month-old girl) gave a strongly positive test but was negative with various other antigen preparations, including Ascaris. Woodruff et al. (1964) observed positive reactions in 11 of 35 patients having conditions possibly due to previous Toxocara infection (asthma, urticaria, chorioretinitis, or unexplained eosinophilia). The antigen, a preparation from dried adult worms, did not cross-react in other helminth infections. Otten et al. (1966) and Danis et al. (1966) reported positive skin tests in their respective patients with suspected VLM. They stated that the antigen was a preparation of T. canis larvae that was furnished by Woodruff.

Woodruff et al. (1966) tested groups of persons with the T. canis adult antigen. They found higher percentages of positive reactors in persons with epilepsy (7.5 per cent) and poliomyelitis (13.6 per cent) than in apparently health persons (2.1 per cent). They considered that there was a causal relationship between these diseases and infection with T. canis, i.e., the larvae distribute the viruses to the central nervous system. Brain and Allan (1964) considered a

positive test in an epileptic patient to be indicative of T. canis infection. A high incidence of positive tests among epileptic patients was also reported by Haddock (1968) and Dada (1970). Khalil et al. (1971) studied the incidence of positive skin tests among Egyptian infants with chronic poliomyelitis and normal controls. They used an extract of larvae and adult T. canis. The incidence in the polio cases was 5.8 per cent whereas in normal controls it was 1.4 per cent. Wiseman et al. (1970) found that there was no apparent relationship between the incidence of positive Toxocara skin tests and positive Toxoplasma dye tests.

Strongly positive skin tests were reported in two siblings with suspected VLM (Kuzemko, 1966). The antigen was supplied by Woodruff. Todorov and Stoyanov (1966) found that skin tests were of low sensitivity in guinea pigs infected with T. canis. In addition the tests were non-specific as judged from results with antigens of both T. canis and Ascaris. Positive tests occurred in rats that had been infected with T. canis (Dobson et al., 1967). However, they apparently did not test normal animals. Wiseman and Woodruff (1967) tested mice and monkeys prior to and following infection with T. canis. Negative skin tests occurred prior to infection but positive reactions occurred following infection. Normal animals remained negative. Conversion of skin tests was also reported in monkeys following infection with T. canis (Wiseman et al., 1968, 1969). The time interval between infection and skin test conversion was inversely related to the number of T. canis eggs given the animals.

Wiseman and Woodruff (1968) reported a high incidence (12.7

per cent) of positive skin tests in patients with clinically suspected VLM. Bisseru (1968) found a high incidence (5.52 per cent) of positive reactors in an apparently healthy population in West Malaysia. He attributed this to poor environmental hygiene and consequent infection of humans with T. canis. Reekie and Miller (1969) reported a positive test in a young boy with suspected VLM. Wiseman (1969) and Wiseman and Woodruff (1971) found a rough correlation between numbers of positive reactors and dog ownership in Africa. Those tribes in which dog ownership was more common generally had higher percentages of positive skin tests. Woodruff (1970) reviewed the data from skin tests and concluded that the test using T. canis adult antigen was reliable for both survey and diagnostic purposes.

Further evidence on the sensitivity and specificity of the skin test was presented by Wiseman and Woodruff (1970). The test was positive in each of nine persons with proved VLM but in only three of 122 persons with other helminthiases, and two of 156 apparently healthy persons. Four of 200 individuals tested at the British Military Hospital in Cyprus were positive (Bradford, 1970). Ball et al. (1971) studied skin reactions to antigens derived from A. suum, T. canis, and Necator americanus. A laboratory worker with atopic reactions to A. suum had very severe immediate reactions to all three antigens and a subject who had been repeatedly infected with Necator also had severe immediate reactions to all three antigens. However, a subject who had received a single experimental infection of 25 Necator did not respond to any of the antigens. Infection of mice with T. canis and subsequent skin testing with T. canis antigen gave inconclusive results (Takeuchi,

1970). Moreau *et al.* (1971) reported positive intradermal reactions with *T. canis* antigen in two suspected cases.

To summarize, different classes of antibodies are evidently produced in response to *T. canis* infections. Various serologic tests will detect these antibody responses. However, the high degree of cross-reactivity that has been demonstrated among ascarid antigens makes interpretation of positive tests difficult. The reported success of intradermal tests using *T. canis* adult antigens seems to contradict the results obtained using other serologic tests. It is evident that further studies must be done on the sensitivity and specificity of ascarid antigens before positive serologic tests can be considered as reliable indicators of *T. canis* infection.

CHAPTER III

MATERIALS AND METHODS

Source of Adult Toxocara and Ascaris

Each puppy was given syrup of Antepar[1] *per os* in a dosage of 70 mg per pound body weight at 7 to 10 days of age. This removed congenitally acquired *Toxocara canis* and *Toxascaris leonina*. The puppies were also given Disophenol Parenteral 4.5 per cent[2] for removal of hookworms acquired *in utero*. Puppies were experimentally infected at 2 to 3 weeks of age with 120 to 140 infective *Toxocara canis* eggs. Each puppy was killed at 10 weeks of age by intracardiac injection of an aqueous solution of pentobarbital sodium (120 mg/cc). Each dog was injected with 1 cc per each 5 pounds of body weight. The small intestine was removed and adult worms recovered. The worms were washed in cold running tap water prior to dissection for antigen preparations, i.e., removal of uteri, cuticles, and perienteric fluid.

Adult *Ascaris suum* were collected from a local slaughter house. The worms were washed in cold running tap water prior to dissection for antigen preparations, i.e., removal of uteri, cuticles, and perienteric fluid.

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[1]Burroughs Wellcome and Company, Tuckahoe, New York.

[2]American Cyanamid Company, Princeton, New Jersey.

Maintenance and Routine Immunization of Dogs

Puppies were obtained from bitches purchased from various sources. The physical condition of these dogs and their puppies varied considerably. Certain medications were used at various times for these animals. Ambex[3] was used for replacement of depleted fluids, protein, and electrolytes in certain dogs. Five ml per pound body weight were administered subcutaneously at weekly intervals in affected animals. Panalog ointment[4] was used for local therapy of eye and skin infections. It was administered once or twice per week. Mychel[5] was used for therapy of respiratory ailments. It was administered in capsules at daily intervals in a dosage of 50 mg per kg body weight.

Each puppy was given intracutaneous injections of Canine Distemper-Hepatitis-Leptospira Antiserum[6] in a dosage of 0.2 cc per pound of body weight. The first injection was given at 2 weeks post-infection and subsequent injections were given every other week for 10 weeks.

Incubation and Hatching of Toxocara and Ascaris Eggs

The terminal one-third of the uteri of mature female worms were resected. The uterine sections were placed in 50 ml conical centrifuge tubes. Two per cent sodium hydroxide was added to these and the preparation was placed under refrigeration. The uteri were dissolved after three changes of sodium hydroxide over a 24 hour period.

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[3]Elanco Products Company, Indianapolis, Indiana.

[4]E. R. Squibb and Sons, Inc., New York, New York.

[5]Rachelle Laboratories, Inc., Long Beach, California.

[6]Bio-Ceutic Laboratories, Inc., St. Joseph, Missouri.

The eggs were washed repeatedly with distilled water following slow centrifugation (100 G) and decantation until a neutral pH was reached. A 1:1 suspension of the washed eggs was made using 0.1 N sulfuric acid solution containing aqueous merthiolate (1:10,000). One-half ml of a Toxocara egg suspension, suspended in 12-15 ml of acid-merthiolate solution was placed in each of several siliconized 250 ml erlenmeyer flasks. The flasks were stoppered with cheesecloth and incubated in a humidity chamber for 4 weeks at 24 C. The humidity chamber was simply an inverted box placed on a flat surface. A 500 ml beaker of water containing a large sponge provided the necessary humidity. One ml aliquots of an Ascaris egg suspension, suspended in 8 ml of acid-merthiolate solution were placed in each plastic petri dish. The petri dishes were incubated in a humidity chamber for 4 weeks at 30 C. At the end of the incubation period the eggs were examined microscopically to determine the percentage of infective eggs. Generally, a 90 to 95 per cent development rate of Ascaris eggs and a 50 to 60 per cent development rate of Toxocara eggs were obtained. The eggs of each species were pooled in 50 ml conical centrifuge tubes centrifuged at 100 G, decanted, and washed once with distilled water.

The method of Fairbairn (1961), with slight modifications, was used for the in vitro hatching of the eggs. Ten ml or less eggs were diluted with three to five volumes of fresh 6 per cent sodium hypochlorite (Purex Bleach) for removal of the shells. The suspension was placed in erlenmeyer flasks and agitated in a water bath shaker maintained at 35 C. Deshelling of Ascaris eggs normally was complete within 2 hours but occasionally required 3 to 4 hours. Deshelling of Toxocara eggs never required more than 1 to 2 hours. When microscopic

observation revealed the complete removal of shells, the sodium hypochlorite was diluted at least 1:1 with distilled water and the eggs were centrifuged for 3 minutes at 100 G. The eggs were washed twice with distilled water using a stream of water from a squeeze bottle to suspend them. Each wash was followed by centrifugation at 100 G for 3 minutes and aspiration of the supernatant fluid. They were then either dialyzed overnight against cold running tap water or careful washings and centrifugations were continued (ten times) to remove the sodium hypochlorite.

Hatching was accomplished in erlenmeyer or side arm flasks equipped with holed-rubber stoppers and glass and rubber tubing which enabled gas to circulate through the flasks. Five hundred ml flasks were used for Ascaris eggs and 25 ml flasks were used for Toxocara eggs. No more than 10 ml of the washed deshelled Ascaris eggs or 2 ml of the washed, deshelled Toxocara eggs were placed in each flask along with four volumes of a 0.25 M sodium chloride - 0.1 M sodium bisulfite solution. This was then incubated at 38-39 C for about 5 minutes in a water bath shaker. A 0.1 M sodium bicarbonate solution was gassed by bubbling with a 95 per cent nitrogen - 5 per cent carbon dioxide mixture until the pH was between 8.0 and 8.5. A volume of the gassed sodium bicarbonate solution equivalent to that of the sodium chloride - sodium bisulfite solution was added to each hatching flask. The complete hatching medium was then flushed with the gas mixture for 5 minutes at which time all tubes were pinched closed, trapping the gas inside the flasks. Shaking was then begun and continued until hatching was completed. Hatching was usually completed within 2 hours.

The hatched larvae were then collected by centrifugation at 100 G for 3 minutes. The hatching medium was drawn off with a Pasteur pipette. The larvae were then resuspended in 0.85 per cent saline, centrifuged at 100 G for 3 minutes, and the saline was drawn off. This washing procedure was repeated five times.

Antigen Preparations

Adult *Toxocara canis*

Adult female worms were slit open and the internal organs were removed. The remaining cuticles with attached muscle layers were washed in 0.85 per cent saline. Twenty to 30 cuticles were transferred to fresh 0.85 per cent saline and disrupted with a Sorvall Omni-Mixer[7] at a setting of "5" for 10 minutes. The Omni-Mixer container was immersed in an ice-water bath during the procedure.

After extraction of the resultant preparation overnight in 0.85 per cent saline at 6 C, the preparation was subjected to ultrasonic vibration with a Biosonik III[8]. The container with the preparation was immersed in an ice-water bath during disruption. Three 5 minute periods at full power with a 5 minute period between each disruption period were used. The suspension was kept overnight at 6 C and then centrifuged at 1,000 G for 5 minutes to remove the larger particles. The supernatant fluid was then centrifuged at 20,000 G for 2 hours at 5 C and the supernatant fluid was collected. After dialysis against four 2 liter changes of distilled, deionized water

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[7]Ivan Sorvall, Inc., Norwalk, Connecticut.

[8]Bronwill Scientific, Inc., Rochester, New York.

over a period of 36 hours at 5 C, the dialysate was lyophilized and stored at -20 C until used.

Prior to its being used for serologic tests, the lyophilized material was dissolved in sterile 0.85 per cent saline and passed through a 0.45 μ Millipore filter. The protein content of the antigen preparation was then estimated by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Perienteric Fluid of Toxocara canis

Adult female worms were slit open and placed in a stainless steel pan immersed in ice. The perienteric fluid which accumulated was decanted. This was then centrifuged at 20,000 G for 2 hours at 5 C and the supernatant fluid was collected. The remaining steps were identical with those used in the preparation of the adult antigen.

Toxocara canis Larval Antigen

Hatched second-stage larvae were placed in 0.85 per cent saline and the suspension subjected to a pressure of 20,000 psi in a French pressure cell[9] precooled at 6 C. The resulting suspension was subjected to ultrasonic vibration and treated in a manner identical to that used for adult antigen. However, a white pellicle formed on the surface following centrifugation. This was removed by careful suction with a Pasteur pipette and discarded.

Various Ascaris suum Antigens

Similar methods were used for the preparation of adults,

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[9]American Instrument Company, Inc., Silver Spring, Maryland.

perienteric fluid, and larvae of A. suum.

Purified Perienteric Fluid of Ascaris suum

The purified perienteric fluid was obtained from Dr. Irving G. Kagan, Division of Parasitology, Center for Disease Control, Atlanta, Georgia. It was prepared according to the method of Oliver-Gonzalez et al. (1969).

Infection of Guinea Pigs with Toxocara or Ascaris

Infective eggs were washed several times with saline and stored at 6 C until used. The eggs were assayed for viability and infectivity before use. Viability was determined by the extent of hatching in vitro. Counts of hatched larvae were then made to estimate the percentage of eggs which contained viable larvae. Infectivity of Toxocara eggs was determined by infecting five mice with 1,000 eggs per mouse and subsequently digesting them at four days post-infection. The method of Olson et al. (1960), with slight modifications, was used for digestion. The skin, tails, feet, and gastrointestinal tracts were removed. The carcasses were ground for 20 seconds in a blendor with 100 ml of a 0.75 per cent pepsin solution in 0.85 per cent saline to which 1 ml of concentrated hydrochloric acid had been added. The resulting suspension was further diluted with 300 ml of the pepsin solution and placed in a 37 C water bath with continuous stirring for 1 hour. Infectivity of Ascaris eggs was determined by infection of two guinea pigs with 300 eggs each. The livers and lungs of these animals were removed and digested at 3 days post-infection. Aliquots of the resulting suspensions were examined microscopically for the

presence of larvae.

Female albino guinea pigs weighing 500 to 700 grams were maintained on a balanced diet ad libitum in air conditioned quarters. These animals were divided into eight groups of 12 each. Two groups were infected with 0.01 egg per gram body weight with Toxocara and Ascaris eggs, respectively. Two groups were infected with 0.1 egg per gram body weight with Toxocara and Ascaris eggs, respectively. Two groups were infected with 1.0 egg per gram body weight with Toxocara and Ascaris eggs, respectively. The remaining two groups were not infected and served as control animals.

Several of the guinea pigs were found to be pregnant following the initiation of the experiment. Additional animals were then infected to serve as replacements for these animals. However, no apparent differences in blood counts or antibody responses between the pregnant and non-pregnant animals were detected. Both of these groups were therefore included in the experiment. This accounts for the larger number of T. canis-infected guinea pigs in the 1.0 egg per gram infection group.

Collection of Blood from Infected Guinea Pigs

Approximately 4 cc of blood was obtained from each animal by cardiac puncture at each bleeding. All animals were bled one time prior to infection. Thereafter, each group of 12 guinea pigs was divided into subgroups of six animals each. Members of one subgroup were bled at 4, 15, 28, 42, 56, 70, and 84 days after infection, while members of the other subgroup were bled at 8, 21, 35, 49, 63, and 77 days after infection. This schedule permitted each animal to be bled

at approximately 2 week intervals throughout most of the study.

Following each bleeding, the blood samples were left at room temperature for 1-2 hours. At that time the clots were separated from the sides of the collection tubes with applicator sticks and the blood was placed under refrigeration at 6 C for 20-24 hours. The sera were then separated from the clotted blood samples and stored separately at -20 C.

Blood Cell Counts

Blood samples were obtained by cardiac puncture between 9:00 and 12:00 A.M. Blood smears were made on cover slips, allowed to air dry, covered with Wright's stain, and allowed to stand for 2 minutes. An equal volume of phosphate buffer, pH 6.4, was then added and mixed by blowing. The buffer-stain mixture was allowed to remain on the smear for 6 minutes. The smears were then rinsed with deionized water and air dried. The stained smears were mounted with Permount on pre-cleaned microscope slides. Differential counts were made microscopically under oil immersion. One hundred white blood cells were counted per slide and the percentages of the cell types were calculated. The intervals for differential counts corresponded to the protocol for collection of blood from infected guinea pigs.

Procedure for Intradermal Tests

Two ml of a 0.5 per cent solution of Evans blue dye in sterile 0.85 per cent sodium chloride solution was injected into the heart of each guinea pig immediately prior to direct skin tests. The back of the animal was shaved and the area then delineated with a

fiber-tipped marker into 16 equal-distance test sites. The sites were arranged in four parallel columns, two on each side of the spine, with 2 cm spaces between the sites. Direct skin tests were made on each animal by inoculating intradermally 0.1 ml of each antigen solution in the desired concentration. Control inoculations of 0.1 ml sterile 0.85 per cent sodium chloride solution were also made on each animal. One ml tuberculin syringes and 26 gauge, ½ inch long needles[10] were used for all intracutaneous injections. Both infected and non-infected animals were tested. The test-site delineations permitted replicate inoculations of each antigen and the control. The larger of the two replicate sites for each antigen on each animal was taken as the lesion size for the particular antigen on that animal.

Each animal was killed 30 minutes after the final injection. The dorsal skin was then removed and the extent of dye diffusion on the inside of each test site was measured. The area of dye diffusion was measured using a transparent stencil patterned after that recommended by Kagan and Pellegrino (1961). In instances in which the areas to be measured were noncircular, the largest diameter circle whose area did not include non-blued skin was recorded. The cecum and large intestine of each guinea pig were removed and the presence or absence of nematode infection (Paraspidodera uncinata (Rudolphi, 1819), Travassos, 1914) was determined.

Procedure for Passive Cutaneous Anaphylaxis (PCA)

Female albino English short-hair guinea pigs[11] weighing 600

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[10]Jelco Laboratories, Raritan, New Jersey.

[11]Camm Research, Wayne, New Jersey.

to 700 g were used in all experiments. The test sites were arranged in four parallel columns, two on each side of the spine, with 2 cm spaces between the sites. As many as 24 test sites were marked on the shaved back of each guinea pig. A modification of the PCA procedure of Ivey (1967b) was used. Each marked site was injected intracutaneously with 0.1 ml of guinea pig serum for homologous PCA or human serum for heterologous PCA. Each guinea pig was given an intracardiac injection of 2 ml of a 0.5 per cent Evans blue dye solution in 0.85 per cent sterile, sodium chloride solution at either 48 hours after serum injection (homologous PCA) or 18 hours after serum injection (heterologous PCA). This was followed at once by a 0.1 ml intracutaneous antigen challenge into each previously marked site. One ml tuberculin syringes and 26 gauge, ½ inch long needles were used for all intracutaneous injections.

Each animal was killed 30 minutes after the final injection. The dorsal skin was then removed and the extent of dye diffusion on the inside of each test site was measured. The area of dye diffusion was measured using a transparent stencil patterned after that recommended by Kagan and Pellegrino (1961). In instances in which the areas to be measured were noncircular, the largest diameter circle whose area did not include non-blued skin was recorded.

Quantitation of Antibody

Positive control sera were obtained from guinea pigs repeatedly infected with eggs of Toxocara canis or Ascaris suum. The antibody content of these sera was determined by the modified PCA procedure described previously. Serial dilutions of the sera were challenged

with 0.5 µg of the corresponding larval antigen. The reciprocal of the highest serum dilution that provoked a positive PCA reaction was taken as the number of PCA units in the undiluted serum.

CHAPTER IV

RESULTS

The eosinophil responses of guinea pigs given 0.01, 0.1, or 1.0 Toxocara canis egg per gram body weight were determined (Table 1). Only the animals receiving one egg per gram of body weight developed an eosinophilia. In these animals the percentage of eosinophils reached a peak at 15 to 21 days after infection. The eosinophil percentage then returned to approximately normal levels within 14 days.

The intradermal responses to various Toxocara and Ascaris antigens were determined in guinea pigs infected with varying numbers of Toxocara canis for 84 to 98 days (Tables 2, 3, and 4). Visualization and measurement of the intradermal response was aided by the use of Evans blue dye as described in Materials and Methods. Adult (TA) and perienteric fluid (TP) antigens of T. canis as well as the adult (AA) and larval (AL) antigens of Ascaris suum did not provoke marked skin reactions in Toxocara-infected animals. However, T. canis larval antigen (TL) as well as the perienteric fluid antigen (AP) of Ascaris elicited the largest intradermal reactions in these guinea pigs. TL consistently elicited the largest reactions at all the concentrations employed. In general, AP provoked larger reactions than all the antigens except TL. The relationship between the lesion sizes elicited by TL and AP generally remained the same at all concentrations

TABLE 1

EOSINOPHILIC RESPONSE OF GUINEA PIGS GIVEN DIFFERENT LEVELS OF INFECTIVE *TOXOCARA CANIS* EGGS

Observation Period (days)	Eosinophilic Response[a] at Different Infection Levels[b]			
	1.0 Egg/g	0.1 Egg/g	0.01 Egg/g	Uninfected
0[c]	3.0 ± 0.7	3.2 ± 1.0	3.0 ± 0.7	5.7 ± 2.2
4[d]	3.8 ± 1.2	2.4 ± 0.7	4.4 ± 1.9	3.2 ± 1.4
8	5.4 ± 1.3	1.0 ± 0.4	4.5 ± 1.7	4.4 ± 2.2
15	10.0 ± 2.1	4.4 ± 2.1	5.4 ± 3.4	3.2 ± 0.9
21	10.4 ± 4.3	1.8 ± 0.3	2.8 ± 0.5	3.6 ± 1.5
28	7.2 ± 3.2	4.8 ± 2.2	6.6 ± 3.0	3.0 ± 1.1
35	3.3 ± 0.8	1.2 ± 0.5	5.3 ± 2.6	4.8 ± 1.9
42	4.2 ± 1.5	3.2 ± 2.5	5.7 ± 3.1	5.8 ± 1.8
49	5.2 ± 1.6	3.8 ± 0.9	5.3 ± 1.8	3.0 ± 0.8
56	2.7 ± 1.4	5.6 ± 2.4	7.6 ± 2.7	5.6 ± 1.8
63	3.9 ± 1.1	2.8 ± 1.1	5.8 ± 1.5	3.6 ± 1.5
70	4.3 ± 1.8	4.8 ± 3.1	5.0 ± 2.4	4.0 ± 1.1
77	6.6 ± 1.7	3.8 ± 1.3	6.3 ± 2.0	5.0 ± 2.7
84	2.0 ± 1.7	4.8 ± 2.6	7.6 ± 4.0	5.6 ± 0.9

[a]Percentage (mean ± S.E.).

[b]Infective dose is in terms of number of eggs per gram body weight.

[c]At 0 days (preinfection) all of the animals in the uninfected, 0.01 egg/g, 0.1 egg/g, and 1.0 egg/g groups were bled (10, 12, 11, and 15 animals respectively).

[d]At 4 days and at alternate observation periods 5, 7, 5, and 6 animals in each group were bled (uninfected, 0.01 egg/g, 0.1 egg/g, and 1.0 egg/g respectively). The remaining animals in each group (5, 5, 6, and 9 animals respectively) were bled at the other observation periods.

TABLE 2

THE RESPONSE TO INTRADERMAL INJECTION OF *ASCARIS* AND *TOXOCARA* ANTIGENS IN GUINEA PIGS INFECTED WITH *TOXOCARA CANIS* (0.01 EGG/GRAM BODY WEIGHT)

Antigen	Mean Lesion Size (mm^2) at Different Antigen Concentrations (ug)[a]			
	1.0	0.5	0.1	0.01
Ascaris suum:				
Adult (AA)	5.0	6.3	2.0	3.0
Larva (AL)	4.5	6.3	2.0	2.0
Perienteric Fluid (AP)	8.0	6.6	3.0	2.0
Toxocara canis:				
Adult (TA)	4.5	5.6	3.0	2.0
Larva (TL)	12.0	10.3	10.0	6.0
Perienteric Fluid (TP)	5.5	6.3	5.0	2.0
Saline Solution	0.7	3.0	2.0	2.0
Difference between:				
TL mean and AL mean	7.5[b]	4.0[b]	---	---
TL mean and AP mean	4.0[c]	3.6[b]	---	---
AA mean and TA mean	0.5	0.7	---	---
Error mean square	0.0148	0.0098	---	---

[a]The number of animals tested with 1.0, 0.5, 0.1, and 0.01 ug were 2, 3, 1, and 1 respectively.

[b]Significant at $p < 0.01$.

[c]Significant at $p < 0.05$.

TABLE 3

THE RESPONSE TO INTRADERMAL INJECTION OF *ASCARIS* AND *TOXOCARA* ANTIGENS IN GUINEA PIGS INFECTED WITH *TOXOCARA CANIS* (0.1 EGG/GRAM BODY WEIGHT)

Antigen	Mean Lesion Size (mm^2) at Different Antigen Concentrations (ug)[a]			
	1.0	0.5	0.1	0.01
Ascaris suum:				
Adult (AA)	6.6	4.5	3.5	1.0
Larva (AL)	6.6	5.0	4.0	1.7
Perienteric Fluid (AP)	9.6	7.5	5.0	1.5
Toxocara canis:				
Adult (TA)	5.3	3.5	3.5	1.5
Larva (TL)	12.0	9.0	9.5	2.5
Perienteric Fluid (TP)	8.0	4.0	3.2	1.5
Saline Solution	2.0	2.0	1.5	1.5
Difference between:				
TL mean and AL mean	5.4[b]	4.0[b]	5.5[b]	0.8
TL mean and AP mean	2.4[c]	1.5	4.5[b]	1.0
AA mean and TA mean	1.3	1.0	0.00	0.5
Error mean square	0.0135	0.0095	0.0068	0.0022

[a]The number of animals tested with 1.0, 0.5, 0.1, and 0.01 ug were 3, 2, 2, and 2 respectively.

[b]Significant at $p < 0.01$.

[c]Significant at $p < 0.05$.

TABLE 4

THE RESPONSE TO INTRADERMAL INJECTION OF *ASCARIS* AND *TOXOCARA* ANTIGENS IN GUINEA PIGS INFECTED WITH *TOXOCARA CANIS* (1.0 EGG/GRAM BODY WEIGHT)

Antigen	Mean Lesion Size (mm^2) at Different Antigen Concentrations (ug)[a]			
	1.0	0.5	0.1	0.01
Ascaris suum:				
Adult (AA)	5.6	4.2	4.0	1.1
Larva (AL)	6.6	6.0	5.6	1.3
Perienteric Fluid (AP)	10.3	9.7	7.6	1.1
Toxocara canis:				
Adult (TA)	6.0	4.1	3.6	1.3
Larva (TL)	11.0	10.0	9.3	3.3
Perienteric Fluid (TP)	7.3	6.0	4.6	1.1
Saline Solution	1.5	1.6	1.3	1.1
Difference between:				
TL mean and AL mean	4.4[b]	4.0[b]	3.7[c]	2.0[b]
TL mean and AP mean	0.7	0.3	1.7	2.2[b]
AA mean and TA mean	0.4	0.1	0.4	0.2
Error mean square	0.0163	0.0138	0.0370	0.0024

[a]The number of animals tested with 1.0, 0.5, 0.1, and 0.01 ug were 3, 4, 3, and 3 respectively.

[b]Significant at $p < 0.01$.

[c]Significant at $p < 0.05$.

in all three infection groups. However, among the other antigens, this relationship was variable. The general tendency was for a direct relationship between the concentration of antigen injected and the lesion size, i.e., those animals injected with a larger concentration of a given antigen exhibited a larger lesion size than did other animals tested with smaller concentrations of the same antigen.

For statistical purposes those skin-test groups which contained more than a single animal were each analyzed separately as a single factor experiment with repeated measure (Winer, 1962). The tests for significance of differences in selected pairs of means were performed as preplanned single degree of freedom comparisons. The reactions provoked by TL were significantly different from AL reactions at the three infection levels. However, the differences between TL and AP reactions were not always statistically significant, especially at the higher infection levels. There was no significant difference between TA and AA reactions at any infection level. The intradermal reactions to the Ascaris and Toxocara antigens elicited in uninfected animals, in general, corresponded to the reactions provoked by saline in the infected animals. Ten uninfected guinea pigs were injected with these antigens. Two of these ten animals had large lesions at several of the injection sites. Data derived from two guinea pigs in the 0.01 egg per gram group were omitted since one of these animals did not react to any of the antigens and the other over-reacted to the saline injection.

The responses of guinea pigs infected with 0.01, 0.1, or 1.0 egg of A. suum per gram of body weight to intradermal injections with

Toxocara and Ascaris antigens are summarized in Tables 5 through 7, respectively. Although these animals reacted to all six of the antigens, none of the antigens demonstrated any consistent effectiveness for detection of A. suum infection. As was also found with the T. canis-infected animals, the general tendency was for a direct relationship between lesion size and the amount of antigen injected at the particular site. However, the largest mean lesion sizes in the 1.0 egg per gram group were with the 0.3 µg antigen concentrations. The lesions elicited with the 0.5 µg and 0.1 µg antigen concentrations were very similar in size in this infection group. In general, AL and AP elicited the largest intradermal reactions in all three groups of infected guinea pigs. However, the relationship between the lesion sizes due to these two antigens was not always consistent at the different antigen concentrations employed. The relationships between the lesion sizes due to the other antigens at the different concentrations were also inconsistent.

Statistical analyses were performed as described previously for the T. canis-infected animals. There was a significant difference between the reactions to AL and AP in both the 0.01 egg per gram- and 0.1 egg per gram-infected guinea pigs (Tables 5 and 6, respectively). However, this difference was significant at only the 0.3 µg antigen concentration, and not at higher or lower concentrations. The difference between AL and TL lesion sizes was significant only in the 0.1 egg per gram-infected guinea pigs, and at only the 0.5 µg antigen concentration (Table 6). The difference between these was not significant at lower antigen concentrations in this infection group. In addition,

TABLE 5

THE RESPONSE TO INTRADERMAL INJECTION OF *ASCARIS* AND *TOXOCARA* ANTIGENS IN GUINEA PIGS INFECTED WITH *ASCARIS SUUM* (0.01 EGG/GRAM BODY WEIGHT)

Antigen	Mean Lesion Size (mm^2) at Different Antigen Concentrations (ug)[a]		
	0.5	0.3	0.1
Ascaris suum:			
Adult (AA)	6.0	2.6	1.7
Larva (AL)	9.0	5.3	3.0
Perienteric Fluid (AP)	8.0	7.6	4.0
Toxocara canis:			
Adult (TA)	5.0	2.1	1.2
Larva (TL)	6.5	4.6	1.2
Perienteric Fluid (TP)	5.0	3.6	1.0
Saline Solution	1.0	1.3	1.0
Difference between:			
AL mean and TL mean	2.5	0.7	1.8
AL mean and AP mean	1.0	2.3[b]	1.0
AA mean and TA mean	1.0	0.5	0.5
Error mean square	0.0288	0.0081	0.0077

[a]The number of animals tested with 0.5, 0.3, and 0.1 ug were 2, 3, and 2 respectively.

[b]Significant at $p < 0.01$.

TABLE 6

THE RESPONSE TO INTRADERMAL INJECTION OF *ASCARIS* AND *TOXOCARA* ANTIGENS IN GUINEA PIGS INFECTED WITH *ASCARIS SUUM* (0.1 EGG/GRAM BODY WEIGHT)

Antigen	Mean Lesion Size (mm^2) at Different Antigen Concentrations (ug)[a]		
	0.5	0.3	0.1
Ascaris suum:			
Adult (AA)	5.5	1.6	1.5
Larva (AL)	8.0	2.5	2.7
Perienteric Fluid (AP)	7.5	6.0	3.0
Toxocara canis:			
Adult (TA)	3.5	2.0	2.5
Larva (TL)	5.0	2.6	2.5
Perienteric Fluid (TP)	4.0	1.8	1.5
Saline Solution	1.0	1.3	1.5
Difference between:			
AL mean and TL mean	3.0[c]	0.1	0.2
AL mean and AP mean	0.5	3.5[b]	0.3
AA mean and TA mean	2.0	0.4	1.0
Error mean square	0.0068	0.0084	0.0048

[a]The number of animals tested with 0.5, 0.3, and 0.1 ug were 2, 3, and 2 respectively.

[b]Significant at $p < 0.01$.

[c]Significant at $p < 0.05$.

TABLE 7

THE RESPONSE TO INTRADERMAL INJECTION OF ASCARIS AND TOXOCARA ANTIGENS IN GUINEA PIGS INFECTED WITH ASCARIS SUUM (1.0 EGG/GRAM BODY WEIGHT)

Antigen	Mean Lesion Size (mm^2) at Different Antigen Concentrations (ug)[a]		
	0.5	0.3	0.1
Ascaris suum:			
Adult (AA)	1.8	4.0	1.5
Larva (AL)	2.5	7.3	2.5
Perienteric Fluid (AP)	3.1	7.0	3.0
Toxocara canis:			
Adult (TA)	1.8	3.1	1.5
Larva (TL)	2.5	6.3	1.7
Perienteric Fluid (TP)	2.0	4.0	1.5
Saline Solution	1.1	1.1	1.2
Difference between:			
AL mean and TL mean	0.00	1.0	0.8
AL mean and AP mean	0.6	0.3	0.5
AA mean and TA mean	0.00	0.9	0.00
Error mean square	0.0144	0.0079	0.0082

[a]The number of animals tested with 0.5, 0.3, and 0.1 ug were 3, 3, and 2 respectively.

there were no significant differences between the lesions due to these larval antigens in the other infection groups. No statistically significant differences between the lesion sizes due to AA and TA were observed in any of the infection groups. The reactions to the various antigens elicited in uninfected animals were similar to those elicited by saline in the infected animals. Two animals in the 0.01 egg per gram group and two animals in the 0.1 egg per gram group were omitted from Tables 5 and 6, respectively. None of these animals reacted to any of the antigens. In addition, there was a single uninfected animal which reacted to both larval antigens and Ascaris perienteric fluid. This data was also omitted from the tabulated data.

Forty of the guinea pigs used in this study were infected with the common intestinal nematode, Paraspidodera uncinata. The presence of this infection had no apparent effect on the outcome of skin tests. There was no indication that active infection with this nematode caused sensitization to Ascaris or Toxocara antigens.

Preliminary passive cutaneous anaphylaxis (PCA) tests indicated that a 0.5 μg concentration of crude antigen gave maximum specific skin reactions with minimum non-specific reactions. The maximum concentration of the purified perienteric fluid of A. suum which was satisfactory with these criteria was 0.1 μg.

PCA tests for homocytotropic antibody were done with the sera collected at scheduled intervals from Toxocara-infected or Ascaris-infected animals. The pooled sera were tested against the homologous larval antigen (TL and AL for sera from Toxocara and Ascaris infections, respectively). Pooled sera from guinea pigs at each T. canis

infection level provoked positive PCA reactions beginning at 15 days post-infection. Sera collected 21 days after infection provoked maximum-sized PCA reactions. The lesion sizes at sites sensitized with sera from subsequent bleedings through 84 days post-infection were of comparable size.

Pooled sera from guinea pigs infected with the above dosage levels but with A. suum showed a different pattern of responses. The sera of animals given 1.0 egg per gram elicited positive PCA reactions at 21 days post-infection. Positive reactions also occurred at sites sensitized with sera from animals infected for 28 and 35 days with 1.0 egg per gram. Sera from animals infected for 42 days failed to elicit positive reactions. The lesion sizes elicited by sera from this and later bleedings had decreased to sizes comparable to sites sensitized with normal sera. The sera from guinea pigs infected with 0.1 egg per gram showed a similar pattern of response except that the positive reactions were not quite as large. Sera from animals infected with 0.01 egg per gram provoked no apparent cytotropic antibody response.

Besides the differences in timing and duration cited above, there were distinct differences between the lesion sizes elicited with the different sera. The positive reactions at sites sensitized with the sera from T. canis-infected guinea pigs were four to five times larger than sites sensitized with pre-infection sera. Positive reaction sites with the sera from A. suum infections were only about twice as large as those observed at sites sensitized with pre-infection sera.

Pooled guinea pig sera obtained 21 days after infection with

0.01, 0.1, or 1.0 egg per gram of body weight were used to determine the comparative sensitivity and specificity of the different antigens in PCA tests (Tables 8, 9, and 10, respectively). Toxocara larval antigen (TL) was obviously the most reactive antigen with sera obtained from T. canis-infected animals. The largest mean lesion size for TL was observed with the sera obtained from guinea pigs infected with 0.01 egg per gram. For statistical purposes the response variables were considered to be normally distributed. The preplanned comparisons were not a set of orthogonal comparisons. Hence, the probability level reported is not appropriate for joint considerations. However, it is appropriate for each comparison considered alone. The mean lesion size with TL was significantly different from the mean lesion size due to any of the other antigen-antibody reactions. This relationship was true at all three infection levels. None of the other antigens was reactive with sera from these infected animals. The other antigens elicited similar PCA reactions with both the sera from infected animals and normal sera.

Ascaris larval antigen (AL) was the most reactive antigen with sera obtained from A. suum-infected animals. However, the sera from these animals were not as reactive with the homologous larval antigen as were the sera from T. canis-infected animals with their homologous larval antigen. As was also observed with the T. canis sera-sensitized sites, those sites sensitized with sera from 0.01 egg per gram infections with Ascaris were more reactive than sites sensitized with sera from more heavily infected animals. The difference between the mean lesion size due to AL and those due to each of the

TABLE 8

HOMOLOGOUS PCA REACTIONS USING POOLED SERA FROM GUINEA PIGS INFECTED FOR 21 DAYS WITH 0.01 EGG/GRAM OF *TOXOCARA CANIS* OR *ASCARIS SUUM* AND CHALLENGED WITH *ASCARIS* AND *TOXOCARA* ANTIGENS

Antigen[b]	Mean Lesion Size (mm^2)[a]		
	Toxocara Canis Sera[c]	*Ascaris Suum* Sera[c]	Normal Sera
Ascaris suum:			
Adult (AA)	1.6	1.7	1.4
Larva (AL)	1.6	4.1	1.4
Perienteric Fluid (AP)	1.6	3.6	1.4
Perienteric Fluid, Purified (APP)	2.6	3.7	1.6
Toxocara canis:			
Adult (TA)	1.8	1.1	1.2
Larva (TL)	9.0	3.4	1.9
Differences Between Selected Means			
	TL vs. TA: 7.2[d]	AL vs. AA: 2.4[d]	---
	TL vs. AA: 7.4[d]	AL vs. TA: 3.0[d]	---
	TL vs. AP: 7.4[d]	AL vs. AP: 0.5	---
	TL vs. APP:6.4[d]	AL vs. APP:0.4	---
	TL vs. AL: 7.4[d]	AL vs. TL: 0.7	---
	Error mean square: 0.0079	0.0095	---

[a]Each mean is based on an observation on each of eight different animals.

[b]0.5 ug of AA, AL, AP, TA, or TL and 0.1 ug of APP.

[c]1:5 dilutions of pooled sera from 5 *T. canis*- or 5 *A. suum*-infected animals.

[d]Significant at $p < 0.01$.

TABLE 9

HOMOLOGOUS PCA REACTIONS USING POOLED SERA FROM GUINEA PIGS INFECTED FOR 21 DAYS WITH 0.1 EGG/GRAM OF *TOXOCARA CANIS* OR *ASCARIS SUUM* AND CHALLENGED WITH *ASCARIS* AND *TOXOCARA* ANTIGENS

Antigen[b]	Mean Lesion Size (mm^2)[a]		
	Toxocara Canis Sera[c]	*Ascaris Suum* Sera[c]	Normal Sera
Ascaris suum:			
Adult (AA)	1.1	1.5	1.0
Larva (AL)	1.0	2.3	1.1
Perienteric Fluid (AP)	1.2	1.9	1.1
Perienteric Fluid, Purified (APP)	1.7	1.4	1.3
Toxocara canis:			
Adult (TA)	1.4	1.0	1.1
Larva (TL)	5.4	1.5	1.1
Differences Between Selected Means			
TL vs. TA: 4.0[d]	AL vs. AA: 0.8[d]	---	
TL vs. AA: 4.3[d]	AL vs. TA: 1.3[d]	---	
TL vs. AP: 4.2[d]	AL vs. AP: 0.4	---	
TL vs. APP: 3.7[d]	AL vs. APP: 0.9[d]	---	
TL vs. AL: 4.4[d]	AL vs. TL: 0.8[d]	---	
Error mean square: 0.0058	0.0020	---	

[a]Each mean is based on an observation on each of eight different animals.

[b]0.5 ug of AA, AL, AP, TA, or TL and 0.1 ug of APP.

[c]1:5 dilutions of pooled sera from 6 *T. canis*- or 5 *A. suum*-infected animals.

[d]Significant at $p < 0.01$.

TABLE 10

HOMOLOGOUS PCA REACTIONS USING POOLED SERA FROM GUINEA PIGS INFECTED FOR 21 DAYS WITH 1.0 EGG/GRAM OF *TOXOCARA CANIS* OR *ASCARIS SUUM* AND CHALLENGED WITH *ASCARIS* AND *TOXOCARA* ANTIGENS

Antigen[b]	Mean Lesion Size (mm^2)[a]		
	Toxocara Canis Sera[c]	*Ascaris Suum* Sera[c]	Normal Sera
Ascaris suum:			
Adult (AA)	1.3	1.3	1.3
Larva (AL)	1.7	4.3	1.8
Perienteric Fluid (AP)	1.0	1.8	1.2
Perienteric Fluid, Purified (APP)	1.8	1.6	1.3
Toxocara canis:			
Adult (TA)	1.6	1.0	1.5
Larva (TL)	6.8	2.4	1.7
Differences Between Selected Means			
TL vs. TA: 5.2[d]	AL vs. AA: 3.0[d]		---
TL vs. AA: 5.5[d]	AL vs. TA: 3.3[d]		---
TL vs. AP: 5.8[d]	AL vs. AP: 2.5[d]		---
TL vs. APP:5.0[d]	AL vs. APP:2.8[d]		---
TL vs. AL: 5.1[d]	AL vs. TL: 1.9[d]		---
Error mean square: 0.0054		0.0089	---

[a]Each mean is based on an observation on each of eight different animals.

[b]0.5 ug of AA, AL, AP, TA, or TL and 0.1 ug of APP.

[c]1:5 dilutions of pooled sera from 9 *T. canis*- or 5 *A. suum*-infected animals.

[d]Significant at $p < 0.01$.

other antigens was significant with sera obtained from animals infected with 1.0 egg per gram. This relationship was also true at sites sensitized with sera obtained from animals infected with 0.1 egg per gram except that the difference between sites challenged with AL and Ascaris perienteric fluid (AP) was not significant. The only differences which were significant at sites sensitized with 0.01 egg per gram infection sera were those between AL and each of the adult antigens (AA and TA).

Sera from hyperinfected guinea pigs diluted to contain 50 PCA units were tested against dilutions of the various antigens in homologous PCA tests. Tables 11 through 13 show the mean lesion sizes with 0.5, 0.1, and 0.02 µg antigen concentrations, respectively. If a given antigen was not reactive with one of the antisera at one concentration, it was removed from further testing at lower concentrations. This eliminated needless duplication of negative reactions.

TL was the most reactive antigen with T. canis antiserum at the 0.5 µg antigen concentration. However, AL was more reactive than TL with Toxocara antiserum at lower antigen concentrations. Interestingly, AL elicited larger reactions at sites sensitized with T. canis antiserum than it did at sites sensitized with the homologous antiserum. The lesion sizes at sites challenged with either larval antigen were consistently larger than the lesion sizes provoked by the adult antigens at comparable sites. Ascaris perienteric fluid (AP) elicited larger reactions than did the perienteric fluid of Toxocara (TP) at T. canis-sensitized sites. The two adult antigens (AA and TA) showed comparable reactivity with T. canis antiserum. The purified perienteric fluid of A. suum (APP) was reactive with T. canis antiserum

TABLE 11

HOMOLOGOUS PCA REACTIONS IN GUINEA PIGS SENSITIZED WITH *TOXOCARA CANIS* OR *ASCARIS SUUM* ANTISERA AND CHALLENGED WITH 0.5 µg AMOUNTS OF *ASCARIS* AND *TOXOCARA* ANTIGENS

Antigen	Mean Lesion Size (mm^2)[a]		
	Toxocara Canis Antiserum[b]	*Ascaris Suum* Antiserum[b]	Normal Serum
Ascaris suum:			
Adult (AA)	2.5	4.5	2.3
Larva (AL)	5.7	6.7	1.7
Perienteric Fluid (AP)	3.5	8.7	2.1
Toxocara canis:			
Adult (TA)	2.8	2.2	2.2
Larva (TL)	7.7	3.5	2.1
Perienteric Fluid (TP)	2.3	3.0	2.0
Difference between:			
AA mean and TA mean	0.3	2.3[c]	---
AL mean and TL mean	2.0[c]	3.2[c]	---
AP mean and TP mean	1.2[d]	5.7[c]	---
AL mean and AP mean	2.2[c]	2.0[c]	---
Error mean square	0.0084	0.0113	---

[a]Each mean is based on an observation on each of eight different animals.

[b]50 PCA units of the respective antiserum.

[c]Significant at $p < 0.01$.

[d]Significant at $p < 0.05$.

TABLE 12

HOMOLOGOUS PCA REACTIONS IN GUINEA PIGS SENSITIZED WITH *TOXOCARA CANIS* OR *ASCARIS SUUM* ANTISERA AND CHALLENGED WITH 0.1 µg AMOUNTS OF *ASCARIS* AND *TOXOCARA* ANTIGENS

Antigen	Mean Lesion Size (mm^2)[a]		
	Toxocara Canis Antiserum[b]	*Ascaris Suum* Antiserum[b]	Normal Serum
Ascaris suum:			
Adult (AA)	1.7	1.7	1.6
Larva (AL)	6.1	5.2	1.2
Perienteric Fluid (AP)	3.3	7.8	1.4
Perienteric Fluid, Purified (APP)	4.6	1.8	1.2
Toxocara canis:			
Larva (TL)	4.9	3.7	1.3
Perienteric Fluid (TP)	2.0	1.6	1.1
Difference between:			
AL mean and TL mean	1.2[d]	1.5[d]	---
AL mean and AP mean	2.8[c]	2.6[c]	---
AL mean and APP mean	1.5[d]	3.4[c]	---
TL mean and APP mean	0.3	1.9[c]	---
TL mean and AP mean	1.6[c]	4.1[c]	---
Error mean square	0.0135	0.0139	---

[a]Each mean is based on an observation on each of eight different animals.

[b]50 PCA units of the respective antiserum.

[c]Significant at $p < 0.01$.

[d]Significant at $p < 0.05$.

TABLE 13

HOMOLOGOUS PCA REACTIONS IN GUINEA PIGS SENSITIZED WITH *TOXOCARA CANIS* OR *ASCARIS SUUM* ANTISERA AND CHALLENGED WITH 0.02 µg AMOUNTS OF *ASCARIS* AND *TOXOCARA* ANTIGENS

Antigen	Mean Lesion Size (mm^2)[a]		
	Toxocara Canis Antiserum[b]	*Ascaris Suum* Antiserum[b]	Normal Serum
Ascaris suum:			
Larva (AL)	5.5	3.8	1.5
Perienteric Fluid (AP)	2.5	5.2	1.1
Perienteric Fluid, Purified (APP)	2.5	1.3	1.2
Toxocara canis:			
Larva (TL)	3.4	1.3	1.0
Difference between:			
AL mean and TL mean	2.1[c]	2.5[c]	---
AL mean and AP mean	3.0[c]	1.4[d]	---
AL mean and APP mean	3.0[c]	2.5[c]	---
TL mean and APP mean	0.9	0.0	---
TL mean and AP mean	0.9	3.9[c]	---
Error mean square	0.0070	0.0119	---

[a]Each mean is based on an observation on each of eight different animals.

[b]50 PCA units of the respective antiserum.

[c]Significant at $p < 0.01$.

[d]Significant at $p < 0.05$.

but not with A. suum antiserum. The lesions elicited with APP and TL at sites sensitized with T. canis antiserum were similar in size.

The PCA reactions at sites sensitized with A. suum antiserum showed some marked differences from the reactions observed at sites sensitized with T. canis antiserum. AP was the most reactive antigen with A. suum antiserum. It was significantly more reactive than any of the other antigens at all three antigen concentrations. AL was more reactive than the remaining antigens. AA was significantly more reactive than was TA; this contrasted with the similar reactivities of these two antigens at sites sensitized with T. canis antiserum. APP was not reactive at sites sensitized with A. suum antiserum.

Additional studies were done with a lower antigen concentration, i.e., 0.004 µg. AL, TL and AP did not elicit very large reactions, indicating that this concentration of the crude antigen preparations did not contain enough of the reactive antigens for the elicitation of meaningful PCA tests. The PCA reactions elicited at this concentration were not significantly different from reactions that occurred at sites sensitized with normal sera. However, AP was slightly more reactive than the other antigens with either antiserum.

Heterologous PCA reactions using 1:5 serum dilutions from six suspected cases of visceral larva migrans were observed in guinea pigs. None of these sera reacted with any of the antigen preparations used during this study. Twelve human sera obtained from Dr. Irving G. Kagan, Center for Disease Control, Atlanta, Georgia failed to give any conclusive results. Two of these sera did elicit slightly larger lesion sizes with T. canis larval antigen. However, this increase in

size as compared to sites sensitized with normal sera was too slight to be considered indicative of infection. In addition, some sera elicited nonspecific blueing reactions. These reactions were apparently due to complement activity since heating at 56 C for 20 minutes eliminated these reactions.

CHAPTER V

DISCUSSION

Among the Toxocara-infected guinea pigs only those receiving the highest dose (1.0 egg per gram body weight) showed an elevated eosinophilic response. The cell count reached a peak the second or third week after infection and abruptly declined to normal levels by the fifth week (Table 1). Since the other groups exhibited no apparent eosinophilia, it appeared that there was a relationship between the size of the infective dose and the intensity of the response. Olson and Schultz (1963) found that the intensity of the host response in guinea pigs was dose-related. Aljeboori and Ivey (1970) also observed a similar relationship in baboons. The results of the present study support these workers' findings.

Olson and Schultz (1963) reported slight eosinophilia in guinea pigs infected with low doses (50 larvae) of T. canis. This number approximates the number of infective eggs given to the 0.1 egg per gram infection group in the present study. The lack of correlation with their results may be due to the techniques employed. They used absolute eosinophil counts whereas differential counts were employed during the present study. However, Aljeboori and Ivey (1970) reported close agreement between cell counts in baboons obtained by the two methods.

The timing of the response generally agreed with previous observations in experimental animals. A number of workers have shown that the maximum eosinophilic response usually occurred within 2 to 3 weeks after infection in various hosts: rabbit (Sadun et al., 1957); guinea pig (Chaudhuri and Saha, 1959; Olson and Schultz, 1963); rat (Dhar et al., 1966); monkey (Wiseman and Woodruff, 1967; Bisseru, 1969); baboon (Aljeboori and Ivey, 1970). In the present study as well as in those cited above, the eosinophil level declined rather markedly following the peak at 21 days. This contrasts with the chronic eosinophilia observed in humans infected with T. canis. There is no widely accepted explanation for the observed differences in eosinophilic responses between humans and other animal species infected with T. canis.

Although eosinophilia is a frequently described occurrence in diseases due to tissue-invasive nematodes, the function of the eosinophil in these infections is largely unknown. Its relationship to antibody formation has been studied. Litt (1968) and Kay (1970) have found eosinophilia to be correlated with the presence of γ 1 antibody (passive cutaneous anaphylaxis (PCA) producing) rather than with γ 2 antibody (precipitin) in the guinea pig. The eosinophilia of helminth infections appears to parallel the formation of reaginic antibody (IgE) in humans (Smithers, 1967; Rosenberg et al., 1971). The discrepancy between these findings and those of the present study, i.e., the lack of eosinophilia and presence of PCA (homocytotropic) antibody, may be due to quantitative differences in the amounts of antigenic stimulation. It is possible that a correlation between eosinophilia and homocytotropic

antibody titer may be demonstrated only when larger amounts of stimulating antigen are present. The presence of significant PCA antibody without an accompanying rise in number of blood eosinophils would seem to preclude the possibility that these cells are produced in greater number as a response to, or as a result of, the presence of this type of antibody.

There are various other explanations for quantitative differences in numbers of eosinophils. Possibly the larvae provoke histamine release by direct cell damage during migration. Vaughn (1953) reported that eosinophils contain an antihistamine substance. He advocated the view that histamine is eosinophilotactic. If this is true, then a direct relationship should exist among number of larvae, free histamine, and eosinophils.

It is also possible that antigen-antibody complexes act as an eosinophilotactic stimulus (Litt, 1961; Cohen et al., 1963). Possibly these complexes also act as some sort of trigger mechanism for increased eosinophilopoiesis. In either case, the quantity of antigen-antibody complexes formed in response to low-dose helminth infection may be insufficient for the elicitation of an eosinophilia.

In the present studies specific antibodies detectable by intradermal tests were produced by guinea pigs infected with either T. canis or A. suum (Tables 2-7). Larval extracts of each of these species were more reactive in guinea pigs infected with the homologous nematode species. The greater sensitivity of larval extracts in detecting antibody in intradermal tests was expected since both these species develop only as far as the larva stage in the guinea pig.

High reactivity to the adult antigens would not be expected since the adult stages are not present. However, common antigens are no doubt present in different stages of each species and some reactivity to these antigens would be expected. Apparently, the skin reactivity resulting from T. canis or A. suum infections was quantitatively independent of the size of the infective dose. Similar-sized lesions were produced in the 1.0 egg per gram, 0.1 egg per gram, or 0.01 egg per gram infections. The antigens involved were evidently highly immunogenic since large lesions occurred with both the homologous and heterologous antigens.

Ascaris perienteric fluid was reactive in Toxocara or Ascaris-infected guinea pigs. Somewhat surprisingly this antigen provoked slightly larger reactions in the T. canis-infected animals than in the Ascaris-infected animals. Such reactions emphasize the lack of specificity encountered. Oliver-Gonzalez et al. (1969), using a fractionated antigen from Ascaris perienteric fluid in the hemagglutination test, observed significantly elevated titers in sera from suspected VLM cases and lower titers with sera from individuals with ascariasis.

A considerable body of literature has accumulated regarding the claimed specificity of Toxocara adult antigen in intradermal tests. Wiseman, Woodruff, and others have claimed that Toxocara adult antigen was specific for the detection of VLM in humans, rabbits, mice, and monkeys. Todorov and Stoyanov (1966) and Takeuchi (1970) reported inconclusive skin test results in T. canis-infected guinea pigs and mice, respectively. The results of the present study using guinea pigs indicated that the adult antigen does not elicit specific reactions.

The reactions to intradermal injections of Ascaris or Toxocara adult antigens were very similar in either Ascaris- or Toxocara-infected animals. With the exception of the above-mentioned skin test reports, other authors have noted a high degree of cross-reactivity between extracts of adult Ascaris and Toxocara in various tests (Wodehouse, 1956; Kagan, 1957, 1958; Jung and Pacheco, 1960; Huntley and Moreland, 1963; Nash, 1964; Biguet et al., 1965; Rahman, 1966; Jeska, 1967; Capron et al., 1968; Aljeboori and Ivey, 1970).

It has been known for many years that the antibodies detected in helminthic infections in many cases showed little correlation with the symptoms, course of the disease, or the immunity which developed. The symptomatology of helminthic infections, particularly ascariasis, frequently includes host responses which are allergic in nature. In 1967, Ishizaka and Ishizaka reported the detection of IgE as the carrier of reaginic activity in humans. This finding has prompted many workers to study this type of immunoglobulin in humans and experimental animals. Reagins are characterized as heat-labile, non-precipitating antibodies that possess the property of prolonged tissue fixation to homologous skin or to the skin of closely related species. Homocytotropic antibodies with these characteristics have been discovered in many species, including guinea pigs.

The production of homocytotropic antibody is now considered to be a common occurrence in helminthic infections. Hogarth-Scott et al. (1969) and Heiner and Rose (1970) reported raised levels of IgE in humans presumably infected with Toxocara. High levels of IgE were also reported by Johansson et al. (1968) for children with ascariasis.

Homocytotropic antibody is also produced in experimental Toxocara or Ascaris infections (Hogarth-Scott, 1967a; Takeuchi, 1970; Dobson et al., 1971) as well as with artificial immunization with Ascaris extracts (Strejan and Campbell, 1970; McAninch and Patterson, 1970). The actual function of these antibodies is still a matter for conjecture and is beyond the scope of the present study.

Passive cutaneous anaphylaxis (PCA) tests using sera from guinea pigs infected with low doses of T. canis or A. suum indicated that specific homocytotropic antibodies are produced (Tables 8-10). These antibodies appeared approximately 15 days after infection and apparently produced maximum PCA reactions by 21 days after infection. It is not possible to say with complete certainty whether the same or different antigen-antibody systems were involved in the intradermal and PCA tests. However, the homocytotropic antibody from T. canis-infected animals exhibited almost complete specificity for the homologous larval antigen in PCA tests. This contrasted with the skin reactivity of the infected animals to several of the antigens. The sera of these infected animals probably contained different populations of antibodies at different times. The PCA tests used sera taken at 21 days after infection while the skin tests were performed at 84 to 98 days after infection. Nevertheless, an important consideration is that in either intradermal or PCA tests the homologous larval antigen was the most reactive antigen.

The presence of high concentrations of homocytotropic antibody in response to nematode infections has been demonstrated by several investigators. However, the high titers noted in the present

study in guinea pigs infected with small numbers of Toxocara is of interest. Apparently, the larvae of this species possess an immunogen(s) which stimulates a high cytotropic antibody response. The larvae of Ascaris were apparently not as immunogenic for this type of antibody. However, direct comparisons are not possible since infection with Ascaris is acute in the guinea pig while T. canis infection is chronic. Takeuchi (1970) observed a lack of correlation between PCA titer and infective dose in massive Toxocara infections in rabbits. An independency of titer with low-dose infections has not before been shown. If the response of humans to low-dose infections of T. canis is similar to that observed in the guinea pigs in this study, then the presence of an elevated level of IgE may be an important aid to diagnosis.

The considerable cross-reactivity of antigens tested with sera from guinea pigs infected repeatedly with large numbers of eggs (Tables 11-13) differed from the specificity of the PCA antibody response of guinea pigs given low-dose infections (Tables 8-10). Hogarth-Scott (1967a), using rabbits for detection of homocytotropic antibody, showed extensive cross-reactivity between Ascaris and Toxocara antigens. It is known that there is an inverse relationship between the amount of antigen necessary to provoke a maximal PCA reaction and the amount of IgG antibody (Ovary, 1964). Ivey (1967a) found that more Toxocara antigen was required for provocation of positive reactions with T. canis antiserum at a low level of sensitization (64 PCA units of rabbit IgG antibody). His results also indicated that the homologous larval antigen was more sensitive for detection of T.

canis antibody of this type. In the present study a low level of sensitization with T. canis antiserum (50 PCA units of guinea pig homocytotropic antibody) was sufficient for the production of positive reactions with several of the antigens employed. The Toxocara larval antigen was more reactive than the other antigens at the highest antigen concentration. This relationship only existed at the high concentration; Ascaris larval antigen was more reactive at the lower concentrations. Presumably different amounts of the cross-reactive and species-specific antigens were present at a given concentration of each crude antigen preparation. Although the quantity of the different preparations was decreased a corresponding amount by dilution, the relative reactivities of the different antigens may have been changed. Relatively speaking, more of the species-specific reactivity could have been removed by dilution. Since the species-specific antigen was presumably present in smaller quantity, dilution may have reduced it past the threshold amount necessary for production of a discernible PCA reaction. The cross-reactive antigen may still be present in sufficient quantity to induce a PCA reaction at this lowered concentration.

The reactivity of purified Ascaris perienteric fluid with the T. canis homocytotropic antibody but not with the A. suum homocytotropic antibody supports the findings of Oliver-Gonzalez et al. (1969) using this antigen in hemagglutination tests. They suggested that the serum reactivity with this antigen was related to the presence of common antigens in migrating nematode larvae. A diminished response in old Ascaris infections is therefore due to a lack of larvae

in the tissues. Since the larvae of T. canis persist in the host's tissues for extended periods, serum from a chronic infection with this species would be expected to retain its reactivity to the antigen. The similar reactivities observed with homocytotropic antibodies from low-dose Ascaris or Toxocara infections support their suggestion. Sera from 0.01, 0.1, and 1.0 egg per gram infections with these species reacted poorly to the antigen. It is possible that antibody production for this antigen is negligible in low-dose infections. If antibody production (homocytotropic or otherwise) is related to the presence of a sufficiently large amount of the stimulating larval antigen, then one would expect some threshold of antigenic stimulation above which detectable antibody is produced. Another very real possibility in this particular antigen-antibody system is that the purification procedure enables a heretofore hidden antigen (common to Toxocara and Ascaris) to be exposed. Thus the antigen is available to react with antibody in serologic tests.

The antigenic "insult" to the host as a result of a massive helminth infection may be the reason for the increased reactivity to heterologous antigens. A helminth is a mosaic of antigens; these surely differ in immunogenicity. In addition, there are many common antigens among closely related species. These two conditions in combination help to explain the high degree of cross-reactivity and differing serum reactivities to different antigens. As an example, a given species-specific antigen may be quantitatively more immunogenic than a given common antigen. This specific antigen in a low-dose infection would be the primary immunogen. However, in a high dose

infection the common antigen, although less immunogenic, could then be present in high enough quantity to evoke a significant antibody response. This response may be greater than the response elicited by the species-specific antigen. A more likely explanation may be simply that there is a finite limit to a host's antibody response to a particular antigen.

Earlier work by Ovary (1958) clearly demonstrated a direct correlation between lesion size and antibody concentration in the PCA test, especially when the challenge antigen was given intravenously. This system allowed uniform diffusion of the antigen from the circulation into all areas of the sensitized skin sites. The greater the antibody concentration used for sensitization, the greater was the area sensitized as the antibody diffused away from the injection site. The modified PCA procedure used in the present study involved injection of the challenge antigen intracutaneously into the previously sensitized site. This system may not accurately measure antibody concentration since it is unlikely that the antigen can diffuse as uniformly throughout the sensitized site as in the case of intravenously administered antigen challenge. Antigen injected directly into sensitized sites probably reacts avidly with antibody nearest the injection site and thereby does not have an opportunity to diffuse outward. Thus the antigen may never reach the outer region of the sensitized site. This same occurrence may account for the differing serum reactivity of T. canis antiserum with high and low concentrations of Ascaris and Toxocara larval antigens. The species-specific Toxocara antigen, when present in lower quantities, may react immediately upon injection

without diffusing outward to the extent permitted with higher quantities. This could result in the appearance of a smaller lesion size which does not reflect the antibody content actually present.

The heterologous PCA test detects IgG (precipitating) antibody rather than IgE. Human sera from suspected VLM cases was not reactive to any of the antigens with this test. It would have been useful to have had serum from a known case. This would have permitted helpful comparisons. However, a known positive serum was not available. Ivey (cited in Kagan, 1968) obtained promising results with the heterologous PCA test using Toxocara larval antigen. It is possible that none of the sera tested in the present study had significant precipitating antibody titers to this antigen. This could have been due to a lack of infection or to an infection that was too light for elicitation of a precipitating antibody response.

The PCA test in guinea pigs is a useful method for detecting antibody. It is also a valuable method for the evaluation of antigens for diagnostic purposes. However, it does have certain limitations. There is variability in the reactivity of different guinea pigs; some animals will produce larger reactions than will others. It is necessary, therefore, to use at least several animals for each experiment. This may not be financially feasible for serological diagnosis.

The modified PCA procedure of Ivey (1967b) may have a limitation for the detection of IgG. The optimal latent period between sensitization of skin sites with human serum and subsequent antigen challenge is 6 hours (Ovary et al., 1960). This latent period is not long enough to permit healing of the wound resulting from the intracutaneous

antibody injections. Consequently, intracutaneous antigen challenge 6 hours later is invariably accompanied by leakage of the antigen solution from the skin site. This can be avoided by waiting 18-20 hours after sensitization before making the intracutaneous antigen challenge. Possibly the longer time interval permits diffusion of the IgG antibody away from the sensitized site. As a result, the sensitivity of the test will be lessened. However, Ovary (1958), using the conventional PCA test with intravenous antigen challenge, observed little difference between the reactions detected at 6 hour or 24 hour latent periods when an optimal amount of antigen was used. The primary advantage of the modified procedure is that an extremely small quantity (less than 1 microgram) of the antigen is required, whereas the conventional procedure often requires several hundred micrograms of antigen. As a consequence, many more tests can be carried out with a given amount of antigen using the modified procedure. When the antigen is not readily obtainable, as is especially true for T. canis larval antigen, the test is very practical.

CHAPTER VI

SUMMARY

The sensitivity and specificity of adult and larval somatic antigens, and perienteric fluid of Toxocara canis and Ascaris suum were investigated. Intradermal skin tests and homologous passive cutaneous anaphylaxis were utilized in guinea pigs.

Forty-eight guinea pigs were divided equally into four groups. The animals of three groups were infected with either 1.0, 0.1, or 0.01 infective T. canis egg per gram body weight; the animals of the fourth group were not infected and served as controls. An identical protocol was followed for infection of 48 guinea pigs with A. suum. Each group of 12 guinea pigs was divided into two subgroups of six animals. Each subgroup was bled at alternate intervals following infection. The intervals were 4 days, 8 days, 15 days, and 21 days, followed by bleedings each week for a period of 63 more days. Sera were saved for subsequent antibody analyses and antigen evaluations. Differential cell counts were made on each blood sample for the determination of relative numbers of eosinophils.

Only those animals receiving the heaviest infection (1.0 egg per gram) of T. canis eggs developed an eosinophilia. The percentage of eosinophils in these animals reached a peak at 15 days to 21

days after infection and then returned to approximately normal level by 35 days after infection. The absence of an eosinophilic response in the other animals indicated that the response may be dose-dependent.

Antigenic extracts of adults, larvae, and perienteric fluid of T. canis and A. suum were prepared. Intradermal tests of the Toxocara-infected guinea pigs with these antigens indicated that Toxocara larval antigen (TL) and Ascaris perienteric fluid antigen (AP) provoked the largest skin reactions. None of the antigens provoked reliable and predictable skin reactions in Ascaris-infected animals. However, Ascaris larval antigen (AL) generally elicited larger reactions than did the other antigens. Adult antigens of Toxocara (TA) and Ascaris (AA) were ineffective for detection of a specific infection with either helminth.

Passive cutaneous anaphylaxis (PCA) tests with sera from the infected guinea pigs detected distinct differences in the patterns of homocytotropic antibody response. Pooled sera collected at scheduled intervals after infection were tested against the homologous larval antigen (TL and AL for sera from Toxocara and Ascaris infections, respectively). PCA antibody was first detected 15 days after infection with T. canis. Maximum sensitization with the pooled sera, as measured by lesion size, occurred 21 days after infection and persisted for the duration of the study, i.e., 84 days. The pooled sera from all three T. canis infection groups showed this pattern.

Guinea pigs infected with A. suum showed a different pattern of responses. Using sera from animals infected with 1.0 or 0.1 egg per gram, positive PCA reactions occurred 21, 28, and 35 days after infection

but not with sera from later bleedings. The sera from animals infected with 0.01 egg per gram failed to sensitize guinea pigs.

Sera collected at 21 days after infection with either A. suum or T. canis were used to determine the comparative sensitivity and specificity of Toxocara and Ascaris antigens in homologous PCA tests. TL was highly reactive in sites sensitized with sera from all three infection levels of T. canis; none of the other antigens were particularly reactive with these sera. AL was the most reactive antigen in sites sensitized with sera obtained from A. suum-infected animals. In addition, some reactivity to a crude preparation (AP) and to a purified preparation (APP) of Ascaris perienteric fluid was observed with sera obtained from animals infected with 0.01 egg per gram of T. canis or A. suum.

Sera from guinea pigs hyperinfected with A. suum or T. canis were diluted to contain 50 PCA units. These sera were tested with 0.5, 0.1, and 0.02 µg concentrations of the above antigens in homologous PCA tests. TL was the most reactive antigen with T. canis antiserum at the 0.5 µg antigen concentration but AL was more reactive than TL at the lower antigen concentrations. AP elicited larger reactions than did Toxocara perienteric fluid (TP) at the sites sensitized with T. canis antiserum. APP was reactive at sites sensitized with T. canis antiserum but not at sites sensitized with A. suum antiserum. The two adult antigens (AA and TA) exhibited similar reactivities at sites sensitized with T. canis antiserum.

The PCA reactions at sites sensitized with A. suum antiserum showed some marked differences from the reactions observed at sites

sensitized with T. canis antiserum. AP was the most reactive antigen with A. suum antiserum at all three antigen concentrations. AL was more reactive than the remaining antigens. AA was significantly more reactive than TA.

Heterologous PCA reactions in guinea pigs using sera from suspected human cases of visceral larva migrans failed to give any further insight into the relative usefulness of these antigens. None of the antigens were able to elicit distinctly positive reactions with these sera.

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