THE UNIVERSITY OF MANITOBA

PATHOLOGICAL AND IMMUNOLOGICAL RESPONSES OF WILD MALLARD DUCKLINGS (*ANAS PLATYRHYNCHOS PLATYRHYNCHOS* L.) TO INFECTION WITH THE NEMATODE, *ECHINURIA UNCINATA* (SPIRURIDA: NEMATODA)

by

TERRENCE GENE NERAASEN

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

WINNIPEG, MANITOBA

FALL, 1978

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ABSTRACT

Experimental infections of *Echinuria uncinata* (Rudolphi, 1819), Soloviev, 1912 in male wild mallard ducklings (*Anas p. platyrhynchos* L.) induced the formation of granulomas in the isthmus between the proventriculus and ventriculus. Lymphocytes, macrophages, heterophils and to a lesser extent, eosinophils were important cells involved in granuloma formation. Granulomas were fully formed by 19 days p.i. and were strikingly similar to the schistosome egg granuloma. Three distinct types of granulomas were described: i) Type I, the fully formed *E. uncinata* granuloma was similar to the exudative-productive stage of the schistosome granuloma; ii) Type II, were granulomas containing copious cellular debris and moribund or dead nematodes surrounded by giant cells (involutional stage); and iii) Type III granulomas were characterized by being very large, having thin walls and containing numerous large nematodes. The latter type was found only in breeding hen mallards in the wild and it was suggested that such thin walled granulomas may have developed from Type I granulomas via a mechanism involving deposition of immune complexes in their walls. The presence of such granulomas in breeding hens was considered significant with respect

to propagation of E. uncinata populations.

Granuloma formation was thought to be a manifestation of a delayed (cellular) hypersensitivity reaction. Formation of the granuloma was considered a distinct but not completely separate process from involution of granulomas and consequent death of nematodes. It was suggested that involution may involve an antibody dependent cellular mechanism such as that involved in immunity to schistosomes. Opsonic antibody (perhaps IgE) may have promoted adherence of macrophages (giant cell formation?) to the surface of nematodes, resulting in their death and encapsulation.

Ducklings of intermediate ages at infection were more susceptible to infection than younger and older ducklings. This was due possibly to the effects of greater stress, hormones and/or an immune system challenged at a critical stage in development of competence. Evidence presented suggested that nonspecific Ig and cellular responses protected mallard ducklings from a variety of pathogens in the early post-hatch period. Specific Ig responses were not fully mature until birds were 5-6 weeks of age or more. This arm of the immune system seemed to develop slowly but steadily. Results of this slow maturation were reflected in a decrease in nematode diameter as age at infection increased.

The relationship between *E. uncinata* and responses of the host mallards was complex. Results of neonatal surgical bursectomy and thymectomy were erratic. Neither procedure resulted in greater susceptibility to infection. Antibody responses as detected by immunodiffusion were greater in surgically altered birds relative to those of controls. It was suggested that production of this antibody was controlled by T-cell independent B-cells which were seeded out from the bursa of Fabricius before hatching. However, production of this antibody was thought to be regulated by the level of other antibody produced by B-cells depleted by neonatal bursectomy and dependent on T-cell cooperation.

Though cell mediated responses were not solely responsible for granuloma formation and subsequent involution (and thus protective immunity?), T-cells appeared ultimately to control responses of mallard ducklings to infection with *E. uncinata*.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Dr. H. E. Welch, who accepted me as a student and gave me free rein to proceed at my own pace during the early years of this study, and later when I had accepted a job at Brandon University. I also wish to thank Dr. T.A. Dick who took over as supervisor while Dr. Welch was on sabbatical leave, and offered invaluable criticism of and suggestions to improve the manuscript. His patience and perseverence in examining the thesis in sections is deeply appreciated. Dr. L.C. Graham offered valuable suggestions and made a long-term loan of his MT-1 Ultramicrotome.

I would also like to thank staff and students at the Delta Waterfowl Research Station for the help and friendship they offered while my family and I were resident there. In particular, Dr. Bruce Batt, Dr. Patrick J. Caldwell and Mr. Peter Ward offered much expertise and helpful discussion. Two summer assistants, Ken Walker and Michael Collins were of great help in looking after my ducklings, and assisting with collections and necropsies. I wish to express my most sincere gratitude to my wife Cheryl and my children Sean, Kerri and Wade for enduring so cheerfully, the long wait for the completion of this task and for the numerous sacrifices they made. Without their understanding and encouragement, this thesis would not have been written.

Finally, I wish to thank all members of my committee for so patiently awaiting completion of the manuscript.

I wish to thank the Department of Zoology, Brandon University for keeping the past two summers relatively free of duties so I could get on with this work. Vickie Hanwell-McLean prepared most of the graphs, and Vickie Gelder typed most of the manuscript.

This study was supported by the National Research Council of Canada through Postgraduate Scholarships to the author (1972-1975) and through an Operating Grant to Dr. H.E. Welch. The Delta Waterfowl Research Station provided living quarters, laboratory and office space, and holding facilities for the care and maintenance of ducklings. Funds for these were provided primarily by the North American Wildlife Foundation. Funds for living expenses, equipment and supplies were provided by the Canadian National Sportsmans' Show (Kortwright Memorial Fellowships, 1972-1975). The Department of Zoology, University of Manitoba provided assistance in the form of laboratory space and equipment and optional demonstratorships. Permits for the killing and capturing of migratory birds were kindly provided by the Canadian Wildlife Service.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

Figure Page

I. INTRODUCTION

Echinuria uncinata (Rudolphi, 1819), Soloviev, 1912, has been implicated as a potent pathogen of waterfowl in Manitoba (Cornwell, 1963), the U.S.S.R., and Europe (see review of Austin and Welch, 1972). Little was known of the biology of this nematode in North American until Austin (1970), Austin and Welch (1972) and Ould (1972) examined the occurrence, life-cycle, and pathogenicity of *E. uncinata* in waterfowl in Manitoba, and the effects of various stressors on the host-parasite relationship.

Austin and Welch (1972) reported that *E. uncinata* third stage juveniles were found in *Daphnia magna* Straus, 1820; *D. pulex* Leydig, 1860 emend, Richard, 1896; and *Simocephalus vetulus* (Schodler, 1858) in the Delta Marsh. *Daphnia magna* was the primary natural intermediate host. Experimental infections were established in all of the above crustaceans and seven others, but development to third stage juveniles was obtained only in *D. magna*, *D. pulex*, *S. vetulus* and the conchostracan, *Lynceus brachyurus* (O.F. Müller, 1785). Experimental infections were then established in six of ten species of wild waterfowl exposed to infective larvae at 20 days of age, and in domestic mallards (*Anas platyrhynchos domesticus* L.). Domestic mallards, gadwall (*A. strepera* L.), and pintails (*A. acuta* L.) were the most suitable hosts, while scaup (*Aythya affinis* (Eyton)), ruddy ducks (*Oxyura jamaicensis* (Gmelin)), domestic geese (*Anser domesticus* L.) and Delta mallards (*A. platyrhynchos*) were less suitable in terms of the number of worms recovered 40 days postinfection (p.i.). Blue-wing teal (*A. discors* L.), shovelers (*A. clypeata* L.), redheads (*Aythya americana* (Eyton)), and coots (*Fulica americana* (Gmelin)) were refractory to infection.

Shevtsov and Zabello (1965, in Austin and Welch, 1972) and Austin and Welch (1972) reported that *E. uncinata* infections caused the formation of granulomas at the proventriculus-ventriculus junction. Numerous granulomas were able to occlude the lumen of the alimentary tract (Austin, 1970) and thus cause death of the host (also see Wehr, 1971). Austin and Welch (1972) showed that naturally acquired infections were heavy, induced formation of granulomas, and caused mortality in ducklings released into a pond at the Delta Waterfowl Research Station (DWRS). Twenty days after experimental infection, groups of worms were located in fibrotic submucosal nodules along the isthmus surrounded by a swollen mucosa. By thirty days p.i., the mucosa overlying granulomas was eroded, but by 40 or 50 days p.i., though the granulomas were larger, the mucosa was almost completely healed around a central opening. Granulomas often contained necrotic masses with or without worms. These necrotic masses were further described as "yellow caseous material" which, upon histological examination, were seen to contain large numbers of disintegrating polymorphonuclear eosinophilic cells and debris (Ould, 1972).

Delta mallards (a wild mallard - McGraw mallard cross), aged 1 week or 1 month of age at infection, were more susceptible to infection than older ducklings (2 and 3 months), and harboured larger worms than older birds (Austin and Welch, 1972). Ould (1972) reported that ducklings stressed by crowding or injections of cortisone acetate had more and larger nematodes at necropsy than control birds. Stressed birds also showed thymic and bursal involution; both associated with the General Adaptation Syndrome of Selye (1956). There was less caseous material in the granulomas of stressed birds; the amount of caseous material present was correlated with the degree of stunting and mortality of the contained nematodes.

Neonatal bursectomy had no apparent effect on the number of nematodes recovered and did not prevent production of antibodies to *E. uncinata* as detected by immunodiffusion in agar gel (Ould, 1972). He suggested that this was due to prolonged antigenic stimulation over the course of the infection of 42 days duration. This parallels what is known to occur in bursectomized chickens repeatedly stimulated with antigen injections (Rose and Orlans, 1968; Jankovic and Isakovic, 1966).

This study was initiated as a consequence of the studies cited above and because of the availability of ducklings and infective material. Austin and Welch (1972) and Ould (1972) indicated that third stage juveniles of *E. uncinata* were available from May to August in Station Pond at the DWRS, with peak numbers of 108 larvae/100 cladocerans in August. This *Echinuria*-mallard model at Delta offered a system unavailable elsewhere with which to investigate the development of pathological and immunological responses of a wild waterfowl species to a helminth parasite. The major appeal of the model is that it represents a natural relationship unmodified by laboratory adaptation of either host or parasite.

The major objectives of this study were:

1) to describe the development of the lesion associated with *E. uncinata* infection, particularly with respect to the cells involved;
2) to determine the time of appearance and titre of antibodies during development of the lesions, and to correlate splenic germinal centres with antibody production;
3) to determine the effect of host age at infection on size of lesions, number and size of nematodes, and the humoral response; and
4) to determine if cell-mediated and humoral responses were of differential importance in determining susceptibility of wild mallards to infection, and to development of the lesions and growth of nematodes. Effects of neonatal bursectomy or thymectomy and types of cells involved in development of lesions were to be used as indicators.

II. REVIEW OF THE LITERATURE

Pathology of Helminth Infections

The pathological manifestations of helminth infections have always been of interest to parasitologists for obvious reasons. A variety of host-parasite systems have been investigated and the histology of lesions are as varied as are the investigators. It is difficult to approach the subject from a broad viewpoint so most authors have concerned themselves with particular groups of helminths in particular species of hosts. Few broad generalizations have been attempted.

Many helminths evoke little or no pathological responses in their hosts, though under abnormal conditions pathology may be extensive. *Parelaphostrongylus tenuis* (Nematoda: Metastrongyloidea), the meningeal worm of cervids, induces little histopathological response in white-tail deer or moose during the developmental period (Anderson, 1968; Lankester, 1974) though it causes neurologic disease in moose (Anderson, 1964) and occasionally in white-tail deer (Eckroade *et al.*, 1970). The nematode caused mild eosinophilia, meningitis, and proliferation of glial cells in the central nervous system of moose (Lankester, 1974), and a mild infiltration of lymphocytes and plasma cells around adults in the periventricular white matter of the cerebral hemispheres of white-tail deer, with no proliferation of glial macrophages (Eckroade *et al.*, 1970). When nematodes moved from the neural parenchyma (chiefly dorsal horns of grey matter in the spinal cord) where larvae developed in deer, a heavy infiltration of eosinophils, lymphocytes, and plasma cells into the area vacated was noted (Anderson, 1968). Granulomas and fibroelastic connective tissue proliferation around nematode eggs in the dura mater were also reported. Angiostrongylus cantonensis in the cerebrum, brain stem and cerebellum of rats and mice provoked little response other than a moderate lymphocyte and histiocyte infiltration (Anderson, 1968). However, in monkeys and man where the nematodes were located in the pons, medulla and basal ganglia, there was pronounced eosinophil, neutrophil and lymphocyte infiltration. There was some formation of giant cells particularly around dead worms and cuticles. Perivascular cuffing in these areas was pronounced. John (1974) studied A. cantonensis in the mouse, an abnormal host, and found little histopathology in unsensitized animals. In those animals which were immunized there was an intense inflammatory infiltrate around nematodes and a marked peripheral eosinophilia, neutrophilia and general leucocytosis. Similarly, Stockdale (1970) reported that living Perostrongylus pridhami in the lungs of skunks evoked little reaction, but dead worms were associated with granuloma formation.

Long et al. (1976) indicated that there was almost no histological response by rhesus monkeys (Macaca mullata) to female Anatrichosoma cynamolgi (Trichinellida) which were located within the epithelium of the nasal passages. There was, however, a moderate to extensive eosinophil and macrophage infiltration around male worms found coiled in submucosal cysts.

Finally, Rees (1967) reviewed the pathology of adult cestode infections and noted that such infections were generally nonpathogenic.

The pathological effects of many helminths are usually characterized by moderate to intense inflammation and infiltration resulting in a variable degree of necrosis, encapsulation, fibrosis or cirrhosis. Although the pathology of trichuroid nematode infections of ruminants, rodents, carnivores and primates may be as mild as the stimulation of large amounts of mucous, many species have been shown to provoke intense inflammatory reactions (see review of Beck and Beverley-Burton, 1968). *Trichuris trichura*, *T. suis*, *Capillaria hepatica* and *C. aerophila* usually provoke generalized lymphocyte and leucocyte infiltration of the tissues. Some (*T. suis* in the intestine of pigs, *C. aerophila* in the lungs of carnivores) stimulate the formation of nodules in the small intestine or granulomas around eggs in the lungs. In the latter, pneumonia and bronchitis were noted.

The most extensively studied nematode with respect to pathology is *Trichinella spiralis* in a variety of hosts (mostly in rats, mice, and guinea pigs). Larsh and Race (1975) have recently reviewed the topic, including their original description (Larsh and Race, 1954). In mature mice given a single infecting dose of 400 *T. spiralis* larvae, the pathological response was seen to occur in three distinct phases: i) a mild, early reaction (4 days) with few neutrophils; ii) a moderately severe acute reaction (8 days) characterized by large numbers of neutrophils; and iii) a chronic reaction (day 10) when neutrophils declined and moderate numbers of plasma cells, lymphocytes, and macrophages were evident. In mice previously sensitized, the same reaction was elicited but more quickly and on a larger scale. In immature mice (Larsh et al., 1956), the response was again similar in terms of cellular components but was less severe and took longer to go to completion of the third stage. Other workers using differential staining techniques described similar histopathology in the intestines of mice but indicated that eosinophils were prominent in the infiltrating cells (Walls et al., 1973) or that mast cells were prominent (Karmanska et al., 1972; Kozar, 1973). Walls et al. (1973) noted in addition, that the cellular response to muscle larvae included infiltrates of eosinophils, polymorphs, lymphocytes, and macrophages by day 18 p.i. Plasma cells and basophils were rare and there was no tendency to giant cell formations.

Trichinella spiralis in the small intestine of rats evoked a similar response to that described by Larsh and Race (1954) in mice, but few neutrophils were involved while many eosinophils and lymphocytes were present (Neraasen and Dick, 1974). In addition, plasma cells, mast cells and globule leucocytes were prominent, especially in previously sensitized rats. This response was similar to that described in rats infected with Nippostrongylus brasiliensis (Miller, 1971, a, b).

Intense inflammatory responses in the gut of sheep infected within the trichostrongylid Oesophagostomum columbianum were described by Dobson and Bawden (1974). The response involved lymphocytes, macrophages, mast cells, plasma cells, and globule leucocytes and was markedly influenced by the nutritional regimen of the host sheep. Trichostrongylus axei in the gastric mucosa of sheep and calves evoked a prominent peripheral and tissue eosinophilia and neutrophilia accompanied by hypertrophy of connective tissue (Fitzsimmons, 1969). A similar peripheral neutrophilia, neutrophil infiltration of connective tissue and mucosa of hyperplastic bile ducts and enlarged gall bladder, were noted in sheep infected with T. colubriformis.

Fascioloides magna infections promote a variable tissue response which depends upon the species of host. In the liver of moose (Lankester, 1974), of cervids generally and of cattle (Sinclair, 1967), the response to migrating immature forms is one of infiltration of eosinophils, lymphocytes and macrophages around and in, tunnels in the liver parenchyma. Tunnels or tracts were separated from normal parenchymal tissue, by a layer of fibroblasts, "polymorphs" and mononuclear cells (Lankester, 1974). In sheep, there was no tissue response of this type, so extensive damage to the liver parenchyma resulted from wanderings of immature flukes (Sinclair, 1967). In cattle, deer, and moose, as flukes matured, tracts were fibrosed, worms were encapsulated, and there was a variable degree of bile duct fibrosis. Histopathological responses to *Fasciola hepatica* infections in the livers of cattle, sheep and goats were similar to those of *F. magna* (Sinclair, 1967). Fibrosis and calcification of bile ducts were more prominent in cattle where granulation tissue was noted and extended to the parenchyma of the liver.

Larval cestodes generally evoke an intermediate type of tissue response similar to that described for flukes (see the review of Smyth and Heath, 1970). The tissue response to spargana of *Spirometra* spp. (Pseudophyllidea) is characterized by infiltration of eosinophils, lymphocytes, and plasma cells around larvae which may or may not be encapsulated. Extensive peripheral eosinophilia may also prevail. Cyclophyllidean cysticerci typically evoke an intense inflammatory response in which eosinophils, neutrophils, macrophages and fibroblasts are the predominant cells. The usual result is encapsulation in a cyst which may eventually be calcified, but viable cysts have thin, fibrous walls bounded by a narrow band of eosinophils and macrophages. Peripheral eosinophilia often accompanied the tissue reaction. Ansari and Williams (1976), and Ansari *et al.*(1976) pointed out that cysticercosis often promotes peripheral eosinophilia in man and other animals, and that eosinophils were prominent around cysticerci of *Taenia hydatigena*,

T. saginata and T. ovis. They studied cellular responses of rats to infection with Taenia taeniaformis and found that peripheral eosinophilia occurred in two waves which coincided with marked eosinophilic invasion of the tissues. There were wide zones of eosinophils around cysticerci by 22 days p.i. which further persisted for up to two weeks. After an oral challenge, an anamnestic peripheral eosinophilic response was produced, but not to the tissues. There was no concommitant invasion of tissues by neutrophils.

The early oncosphere of Echinococcus granulosus in the liver of pigs is surrounded by mononuclear leucocytes, and in the liver of rodents by epithelioid cells and leucocytes, including eosinophils (Smyth and Heath, 1970). The developing hydatid is surrounded by an inner layer of radially arranged mononuclear cells and eosinophils, a middle layer of fibroblasts and eosinophils, and an outer layer of fibroblasts. In sheep, eosinophils are conspicuous by their absence. Very early lesions caused by E. multilocularis in rodent livers were described as necrotic foci with numerous leucocytes and macrophages but few polymorphs. Later (79 hours), neutrophils and macrophages were prominent near larvae, with mononuclear cells peripherally. As larval vesicles grew, eosinophils infiltrated the area which was in turn encircled by a dense zone of leucocytes. A dense zone of fibroblasts surrounded the entire developing cyst.

Certain groups or species of helminths always provoke severe histopathological responses resulting in granuloma formation. The muscle worm of cervids, Parelaphostrongylus andersoni, unlike the related P. tenuis, has been shown to provoke granuloma formation around eggs, adults, and larvae located in muscles of the loin and thigh, and around eggs and larvae in the lungs of experimentally infected white-tail deer (Nettles and Prestwood, 1976). Masses of eosinophils and mononuclear cells, and necrosis were characteristic of these lesions.

Although filaroid nematodes were considered essentially non-pathogenic by Nelson (1966) and Denham and Nelson (1976), some species promote extensive histopathology. Living Filaroides martis in the lungs of skunks caused an eosinophilic infiltration of bronchiolar epithelium and connective tissue, mononuclear cell and eosinophil accumulation around bronchioles, and granuloma formation (Stockdale, 1970). Similarly Vincent et al.(1976) reported extensive inflammation and granuloma formation in the lungs of jirds (Meriones unguiculatus) infected with Brugia pahangi, B. patei or B. malayi. Development of granulomas induced by larvae and adults was initiated at about 30 days p.i. Giant cells and endothelial (epithelioid ?) cells were seen adhering to worms. Granulomatous lesions in the lungs of skunks infected with Crenosoma mephitidis characterized by eosinophils, macrophages, and large mononuclear cells with basophilic cystoplasms were described by Stockdale et al.(1974).

Infections of ascarid nematodes may cause severe histopathological and allergic responses, particularly to migration of larvae through the tissues (Sprent, 1954, 1955; Beaver, 1969; Ogilvie and Worms, 1976; Soulsby, 1976). Toxocara canis, T. cati and Ascaris suum provoke remarkable peripheral eosinophilia and eosinophilic granulomatous lesions in the liver and lungs of man and other animals. Neutrophils, eosinophils, phagocytes and pyroninophilic cells were involved in formation of granulomas in the order presented. All of these cell types were adherent to the surface of nematodes (Soulsby, 1976; Ogilvie and Worms, 1976).

Ko (1976) described gastric lesions associated with experimental infections of Echinocephalus sinensis (Gnathostomatidae) in monkeys. Massive accumulations of neutrophils, eosinophils, lymphocytes, macrophages, and plasma cells were described around nematodes. Giant cell formations and layers of epithelioid cells were also described. Similar lesions were described for Dictyocaulus viviparus infections in calves (reviewed in Poynter and Selway, 1966). Pulmonary nodules with central accumulations of eosinophils, a median layer of epithelioid cells, and an outer dense accumulation of eosinophils, lymphocytes and plasma cells were frequently encountered.

The classic example of parasitic granuloma formation is that associated with schistosomiasis (Lichtenberg, 1962). Granulomas are stimulated by the deposition of eggs in the tissues and are characterized by centrally located epithelioid cells and histiocytes (often giant cells) immediately surrounded by concentrically arranged fibroblasts. Peripherally there was a round cell halo with a variable degree of fibroelastic proliferation. Eosinophils were prominent near or in granulomas. This description conforms to the classical picture of chronic granulomatous inflammation (Cameron, 1967). Sadun *et al.* (1970) described lesions in the gut, bladder and ureters of chimpanzees infected with *Schistosoma haematobium*. Large composite, edematous granulomas termed polypoid patches were described which lacked typical peripheral fibrous tissue of the classical granuloma, but which had massive infiltration and lymphoid proliferation at the base. Strands of smooth muscle appeared in the wall; larger bundles showed invasion and replacement by granulation tissue. Granulomas with much more fibroelastic tissue and collagen deposition peripherally were described as fibrous patches. Sandy patches were granulomas devoid of granulation tissue and edema; they contained mainly calcified and degenerating eggs. Erickson *et al.* (1971) compared the pathology of *S. haematobium*, *S. mansoni* and *S. japonicum* infections in the owl monkey (*Aotus trivirgatus*). Granulomas induced by *S. haematobium* eggs were found in a variety of organs and despite being composite (up to 20 eggs/lesion), were the least severe. Eosinophils were most prominent in these lesions. Granulomas around eggs of *S. mansoni* and *S. japonicum* were of a distinctive edematous type but were essentially "classical" in overall apperance. Those of *S. japonicum* were largest and most pathogenic; 4 monkeys died 7 to 10 weeks p.i.

Granuloma formation can be induced by intravenous injection of eggs. Moore et al. (1977) injected S. mansoni eggs into rhesus monkeys and showed that small mononuclear cells (lymphocytes) were found around eggs initially, and that eosinophils accumulated later, especially in sensitized animals (71% of cells were eosinophils 6 days post-injection). Large mononuclear cells were constant (30%) and neutrophils never formed more than 10% of infiltrating cells.

Although considerable work has been carried out concerning the gross and clinical aspects of helminth infections (especially of nematodes) in birds (see review of Wehr, 1971), few detailed histopathological studies are available. Ascaridia columbae larvae in the liver of pigeons provoked eosinophilic granulomatous lesions involving numerous granulocytes in peritoneal lesions in blue herons and egrets (Wehr, 1971).

Capillaria spp. infections often evoke severe pathological lesions in birds (see the review of Beck and Beverley-Burton, 1968). C. annulata in the esophagus and crop of turkeys caused enlargement of mucosal lymphoid follicles, hyperemia, lymphocyte infiltration, and necrosis of the mucosa followed by polymorphonuclear leucocyte invasion. C. obsignata and C. caudinflata in ducks, and C. contorta in ducks and turkeys caused enlargement, inflammation, necrosis and sloughing of the epithelium of the esophagus. Yellow necrotic nodules were evident in addition to the other effects in C. obsignata and C. caudinflata infections.

Granulomas have been described from pheasants infected with *Syngamus trachea* (Fernando *et al*., 1971) in which heterophil invasion and epithelioid and giant cell formations around nematodes were common. There was a general proliferation of lymphoid follicles, granulocytes, and macrophages accompanied by lymphocytic vascular cuffing. Migration of heterophils and lymphocytes out of vessels was also noted. Patnaik *et al*.(1970) described a similar type of pathology in the open-billed stock (*Anastomus oscitans*) caused by the trematode *Chaunocephalus ferox*. Prominent globular nodules opening to the lumen of the intestine, containing flukes and a fibrinous-necrotic exudate were described. There was marked edema and infiltration of the area by lymphocytes and granulocytes with fibroblasts and connective tissue proliferation at the periphery. Less severe lesions, but ones involving chronic inflammation and fibrosis in the heart muscle of swans (*Olor columbianus*) infected with the filaroid, *Sarconema eurycerca* were described by Kluge (1967). Heterophils, lymphocytes and macrophages were moderately abundant in necroptic areas near nematodes.

Rees (1967) noted that in contrast to most adult cestode infections, those in fowl can cause anything from intestinal catarrh to severe hemorrhagic enteritis. Capillary congestion accompanied by lymphocyte and polymorphonuclear leucocyte infiltration around the scolex, fibrosis and epithelial cell proliferation have been observed. Gray (1976) studied the cellular response in the small intestine of fowl infected with *Raillietina cesticillus* and noted an infiltration of mononuclear leucocytes into the tissues near scoleces and a local proliferation of lymphoid tissue in primary infections. There was no evidence of vascular congestion or a neutrophil response, but in birds with long standing primary infections, there was a mast cell and pyroninophil response.

The formation of granulomas in ducks infected with E. uncinata has been well established but the cellular details have not been elucidated.

Immunology of Helminth Infections

The Vertebrate Immune System

Phylogenetically there are three major levels of immunological competence: quasi-immunorecognition; primordial cell-mediated immunity; and integrated cell-mediated and humoral immunity (Cooper, 1976). Quasi-immunorecognition is demonstrable when self, non-self recognition occurs as evidenced by allogeneic incompatibilities (coelenterates, tunicates, mammals). Primordial cell-mediated immunity is demonstrable in annelids and echinoderms by specific allograft incompatibility accompanied by a certain degree of immunological memory. Only vertebrates show integrated cell-mediated and humoral immunity with well developed immunological memory where both cellular and humoral anamnestic responses are exhibited (Cooper, 1976). According to Cooper (1976), immune responses at all levels are considered surveillance systems as suggested by Burnet (1959). Antibody production, and allograft rejection or delayed hypersensitivity, are two measureable characteristics of immune responses; the former has become synonymous with humoral immunity, while the latter is considered the major expression of cell-mediated immunity (Weiss, 1972; Cooper, 1976). Most immunologists have long been concerned with the problem of accounting for these two types of immunity (Park and Good, 1974).

The immune system of vertebrates consists of several cell types found in a variety of lymphoid and myeloid tissues and organs (Cooper, 1976). Central organs include the bone marrow (Weiss, 1972), the thymus and bursa of Fabricius (in birds only), or its equivalent (Park and Good, 1974). Bone marrow is the principal source directly or indirectly, of all immunocompetent cells or their precursors (Weiss, 1972), and once produced there, such cells move via the circulatory system to the thymus or bursa (or equivalent) where they are "educated". Since the early studies of Glick, et al.,(1956) it has been well established that there are two major components of immune responses mediated primarily by two major classes of lymphocytes (Szenburg and Warner, 1962; Cooper et al., 1966). Cells educated in the bursa have been established as being responsible for antibody production or humoral immune responses, and cells processed in the thymus are thought to be responsible for cell-mediated immune responses. However, considerable interaction is known to occur between components of these two lines so that both co-operation and interference or suppression have been documented (see Park and Good, 1974, p. 28).

The thymus is a lymphoepithelial organ which arises as endodermal pharyngeal diverticula in the embryo, and in which mesenchymal cells originating in the yolk sac and bone marrow develop into immunocompetent cells (Weiss, 1972; Park and Good, 1974; Cooper, 1976). In amphibians it has been suggested that such cells do not originate elsewhere but arise de novo (Turpen et al., 1975). During the process of developing competence in the thymus, cells acquire surface antigens (Theta) which are replaced by antigens of a different series (Ly) before the new thymocytes leave to populate peripheral lymphoid organs such as the spleen and lymph nodes. Lymphocytopoiesis is extensive in the thymus and is considered to be independent of antigenic stimulation (Park and Good, 1974). There is a constant production of small lymphocytes possibly stimulated by a hormone produced by epithelial cells within the thymus, and considerable destruction of thymocytes occurs before they leave the thymic environment, especially in older mammals. It has been suggested that 95% of lymphocytes passing through, or being produced in the thymus, are destroyed there (Weiss, 1972). The survivors become long lived thymocytes or T-cells which "seed-out" to peripheral lymphoid organs.

The bursa of Fabricius is a lymphoepithelial organ found only in birds which develops from the dorsal part of the urodeal-proctodeal plate forming the cloaca. By day 12 of incubation in the chicken, the bursa contains numerous epithelial buds, and by 15-16 days, lymphocytes appear and large follicles with a dense cortex and lightly stained medulla develop (Park and Good, 1974). Lymphocytopoiesis, as in the thymus, seems to be independent of antigenic stimulation. The bursal environment provides a special micro-environment in which incoming lymphoid stem cells differentiate and mature into immunocompetent cells capable of antibody production (B-cells). These "educated" cells then migrate to specified regions of lymph nodes and spleen, and form a population of cells which mediate humoral immune responses.

In addition to its inductive function for antibody production, the bursa produces antibody and responds to environmental antigens by serving as a site for sensitization, perhaps in much the same way as do parenteral routes of immunization (Sorvari et al., 1975).

Peripheral lymphoid organs include the lymph nodes, spleen and lymphatic tissues associated with the gut and respiratory tract which receive "educated" lymphocytes from the central lymphoid organs. They are described as vascular filters which filter lymph and blood by means of a three dimensional meshwork of reticular cells and fibers (Weiss, 1972). All trap antigen and immunocompetent cells, thereby providing sites for their interaction, and resultant cellular differentiation and division (Weiss, 1972; Park and Good, 1974).

Lymph nodes are complex chambers through which lymph flows en route to its junction with the vascular system. Nodes are clustered in areas such as the axilla, inguinal region, head and neck, pelvic, pleural and peritoneal cavities, and genital, gastrointestinal and respiratory tracts of man and other mammals (Weiss, 1972). Jankovic and Mitrovic (1967) indicate that in birds, the ileo-caecal junction is an area of concentrated lymphatic tissue. Each node receives lymph, and therefore antigens, from a specific region, and thus each responds to local influences (Weiss, 1972; Park and Good, 1974). Beneath the subcapcular sinus of lymph nodes are located primary and/or secondary lymphoid follicles (germinal centres) in which antibody producing plasma cells are abundant. Such areas, and the development of germinal centres, have been well established as "bursa dependent" (Cooper _et_ _al_., 1966; Weiss, 1972; Park and Good, 1974). Paracortical and deep cortical areas of lymph nodes are said to be thymus dependent, because neonatal thymectomy leads to lymphocyte depletion in those areas.

The spleen acts as a filter, like lymph nodes, but unlike the nodes which filter lymph and respond to regional influences, the spleen filters blood and reacts to blood borne antigens from the systemic circulation. The periarteriolar lymphocyte sheaths (PALS) of the white pulp consist of reticular networks packed with lymphocytes, plasma cells and granulocytes. Development of the PALS is thymus dependent (Weiss, 1972; Park and Good, 1974), while the primary and secondary nodules located in the PALS and marginal zone are bursa dependent, as are their counterparts in lymph nodes (Cooper _et_ _al_., 1967).

Lymphocytes are central cells of immunologic activity (Weiss, 1972; Park and Good, 1974; Cooper, 1976). They constitute a heterogeneous group of cells with considerable capacity for differentiation. On the basis of size they are classified as small (5-8μ dia.), medium (8-12 μ) or large (12-15 μ). The small lymphocyte was once considered to be an effete or terminal cell but such cells are capable of transformation (in culture) to large forms and of division upon stimulation by mitogens such as phytohaemagglutinin (PHA) from red kidney beans, or by concanavalin A (Con A)(Park and Good, 1974; Cooper, 1976). Lymphocytes are motile and frequently traverse endothelial cells in post-capillary venules, invade various organs and tissues and then may recirculate. There are two classes of lymphocytes according to half life; short lived cells with half lives of 13.5 days, and long lived cells with half lives of a few months to many years.

Lymphocytes may also be separated into two major groups depending initially upon the environments in which they differentiate. These two lines are distinguishable on the basis of the presence of Theta (TL) or Ly antigens (thymus "educated" T-cells), or the presence of abundant immunoglobulin receptor molecules (IgM) on their surfaces (bursa "educated" B-cells). It has been suggested that T-cells also bear immunoglobulins on their surfaces but these have not been characterized because they are few and scattered (see Feldmann,1976). T-lymphocytes are specifically stimulated to blast formation and mitosis by PHA and Con A, and are sensitive to antilymphocyte serum (ALS) prepared against histocompatibility antigens on their surfaces. Upon encounter with specific antigen, T-lymphocytes transform, divide, and produce a variety of lymphokines. Mitogens such as bacterial lipopolysaccharides (LPS) specifically stimulate B-cells to blast formation and mitosis. Upon encounter with specific antigen, B-cells differentiate to cells capable of synthesis and secretion of antibody. T-cells are thus considered mediators of cellular immunity while B-cells are mediators of humoral immune responses.

Macrophages have been singled out as immunologically important cells because of their role in antigen capture and processing, and their possible role as effector cells (Weiss, 1972; Park and Good, 1974). According to Weiss (1972), Bloom and Fawcett (1975), and others, macrophages are given many names depending on their location and degree of mobility, but there is general agreement that macrophages are derived from monocytes of bone marrow origin. It is thought that many antigens are initially trapped by macrophages in lymph nodes, spleen and lymphoid tissue generally, and that they are then processed in ways as yet unclear before they effectively contact immunocompetent lymphocytes and stimulate an immune response. Antigens may be retained on or in macrophages for long periods and thus could serve to periodically boost any response resulting from an initial antigenic stimulation (Park and Good, 1974).

The Nature of Immune Responses

Adaptive immune responses are initiated by interaction of antigen with specific receptors on the surface of lymphoid cells (Cohen, 1976a). Antigen was originally defined as any substance which induces an animal to produce antibody. Since immune responses are now known to include those mediated by sensitized T-lymphocytes (cell-mediated immunity, CMI) as well as humoral responses, the more inclusive term immunogen is now generally used (Park and Good, 1974)

Humoral responses are initiated when immunogens bind to the surface receptors (the immunoglobulin molecules) of B-cells, which then stimulates the cells to differentiate, clone, and transform to plasma cells. This stimulation may be direct, or through the co-operation of T-cells or macrophage-like helper cells (Feldmann,1976). Transformed B-cells then produce specific antibody directed against the stimulating immunogen. Antibody (or immunoglobulin) production in response to antigenic stimulation is a unique property of vertebrates and the exquisite specificity of the antibody response to countless numbers of antigens is phenomenal (Cooper, 1976). Equally impressive is the fact that Burnet (1959) predicted that such would be the case. This has been abundantly confirmed since his early book.

Immunoglobulins produced by plasma cells and secreted into the serum of tissue fluids are a heterogeneous group of structurally related proteins mostly associated with the gamma globulin fraction of serum, though some are present in the beta fraction (Sinclair, 1970; Cooper, 1976). There are five major classes of immunoglobulins in mammals, separable on the basis of molecular size, electrophoretic mobility, and the nature of their heavy chains (see Cooper, 1976, Ch. 3). These classes are immunoglobulin M (IgM), IgG, IgD, IgA and IgE.

IgM is a large molecule (macroglobulin) which is found in all vertebrates and is the first produced in an immune response (Weiss, 1972). IgG, a smaller molecule, is more efficient than IgM, has a greater affinity for the stimulating antigen and is associated with secondary or anamnestic responses. IgA, also known as secretory immunoglobulin, is abundant in saliva, lacrimal fluid, and mucous membranes generally. Transepithelial passage of IgA is facilitated by a secondarily associated transport piece and is thought to be associated with protective responses to immunogens entering via mucosal surfaces. IgE molecules (reaginic antibodies) are associated with allergic responses of the immediate type and are characterized by their ability to bind antigen at the surface of basophils and mast cells. Vaso-active amines are thus released from those cells (Weiss, 1972). The importance of IgD is not well understood.

Cooper (1976) reports that chickens have a low molecular weight Ig called IgY because its heavy chain does not conform to any conventional mammalian category. Ducks have conventional IgM and two low molecular weight classes, one of which is similar to IgY of chickens, the other similar to IgN of lungfishes. Pigeons are reported to have a heat labile skin sensitizing antibody similar to human homocytotropic antibody (IgE). Moticka (1975) reported that chickens also have IgA similar to that of humans.

Humoral immunity may be passively transferred to non-sensitized individuals by means of immune sera containing specific antibody, or by sensitized antibody producing cells. It is expressed in a number of ways which were recently characterized by Cheng (1973) and Cohen (1976a) with particular reference to responses to parasites, though they are of general significance. These include: 1) immunoglobulin-mediated specific immunity (precipitation, agglutination, neutralization); 2) complement-immunoglobulin mediated cytolysis; 3) immunoglobulin-macrophage mediated endocytosis (opsonization); 4) immunoglobulin-mediated specific cytotoxicity (cytotoxicity mediated by "killer cells" without endocytosis); and 5) immunoglobulin-E mediated specific hypersensitivity (immediate type). Explanations and details of these reactions are available in Cheng (1973), Park and Good (1974), and Cohen (1976a).

Cell-mediated immunity (CMI) is defined as being mediated primarily by lymphoid cells rather than humoral antibody and is transferrable to recipient animals by lymphoid cells but not serum (Soulsby, 1970; Park and Good, 1974; Cohen, 1976a; Cooper, 1976), or in humans by transfer factor (Lawrence, 1969; Lawrence and Valentine, 1970).

The term delayed hypersensitivity has been used almost synonomously with CMI because reactions characterized by those of delayed skin tests, of which the Mantoux is the best known example, are regarded as the typical manifestations of cell-mediated immune responses. This distinguishes it from antibody-mediated immediate (anaphylactic) hypersensitivity. Delayed hypersensitivity (CMI) plays a major role in solid tissue allograft rejection, in some autoimmune diseases, in defense against some bacteria, viruses, many fungi, and in defense against cancer.

Manifestations of delayed hypersensitivity are complex (see Park and Good, 1974). The entire reaction is initiated by a small number of sensitized T-lymphocytes which upon stimulation by immunogen, either act directly independent of antibody or complement, or which release a variety of lymphokines and other factors (Park and Good, 1974; Cohen, 1976a). A wide variety of lymphokines have been discovered or their existence postulated, including five which affect macrophage activity and resistance to infection, two which affect the transformation of lymphocytes, one which increases and one which suppresses antibody production by B-cells, and two which affect granulocyte migration. A number of others have been characterized which have their effects mostly in culture (see Park and Good, 1974, Table 7.2; Cohen, 1976a, Table 2.4).

The Avian Immune System

According to Cooper (1976), the avian immune system is no more complicated than that of amphibians and reptiles. Central organs are bone marrow, thymus and bursa of Fabricius, while peripheral lymphoid organs or aggregations include spleen, lymph nodes, the glands of Harder, lacrimal glands, and caecal tonsils (Jankovic and Mitrovic, 1967; McGhee, 1970; Cooper, 1976). Because the chicken possesses two discrete central lymphoid organs, the thymus and bursa, it has been widely employed in the study of the ontogeny of immunological responsiveness (reviewed in Moticka, 1975). This led to the concept of a developmental dichotomy between cell-mediated and humoral immune functions (Cooper _et_ _al_., 1966, 1967; Warner, 1967, in Moticka, 1975). Although mammals do not have a bursa and thus have only one discrete central lymphoid organ (thymus), this dichotomy is apparent in the mammalian system and the existence of a "bursa equivalent" has been widely accepted. This simple dichotomy is regarded as generally correct but too simplistic by Moticka (1975), because for instance, thymus dependent cells are mandatory participants in the development of antibody responses to certain antigens (Rouse and Warner, 1972; Droege, 1971), and the role of the bursa in the development of immunoglobulin synthesis has been questioned by several observations. In addition, Manning (1975) suggests that as information from a wide variety of animal models accumulates, the current dogma regarding the role of the thymus as a generator of T-cells only, may need revision. She cites evidence for the fact that B-cells may be influenced there as well, and that T-cells may be educated at other locations.

In the chicken, lymphoid cells first appear at about 10 days of the 21 day incubation period and at this time, only in the thymus. Ackermann (1966, in Moticka, 1975) suggested that these cells were derived from undifferentiated thymic epithelium, but later work indicated that all were derived from blood borne stem cells. That these early lymphocytes could be derived from thymic epithelium is however supported by work with anuran amphibians where it has recently been convincingly demonstrated that lymphocytes are derived from thymic rudiments (Turpen, et al., 1975).

Investigation of the role of the avian thymus in the development of immunological competence has been difficult because of its multilobate structure, and close association with thyroid glands, jugular veins, and brachiocephalic artery. Surgical extirpation followed by sublethal irradiation has been used successfully to eliminate most of the thymus dependent cell line (see Cooper et al., 1967). Chickens so treated have been shown to be markedly deficient in what are considered to be typically cell-mediated immune reactions, and lymphoid cells from such birds are unable to initiate graft versus host reactions when transferred to the chorioallantoic membranes of unrelated chicken embryos.

T-cells are known to function as helper cells in the production of specific antibody to rabbit erythrocytes and Brucella abortus (Droege, 1971) and to horse erythrocytes (Rouse and Warner, 1972). Moticka (1975) suggests that the class of antibody synthesized, rather than the antigen may be thymus dependent. As well, T-cells may function as suppressor cells in that they suppress antibody production to certain antigens as indicated by Droege (1971) who injected 6 week old intact chickens with thymus cells from immunized 6 week old donors and subsequently challenged with B. abortus and rabbit erythrocytes. The ability to synthesize IgM and IgG antibodies was severely depressed, especially to B. abortus. Moticka (1975) suggests that these studies indicate that the helper and suppressor cells to specific antigens exist in different proportions in the thymus. Finally, Moticka (1975) reviewed evidence which indicates that the thymus contains two populations of cells, one of which is bursa dependent.

The bursa of Fabricius is the second organ to become lymphoid in the chicken, and large lymphoid-like cells begin to appear at about 12 to 13 days of incubation. Shortly thereafter, lymphoid cells appear in other areas such as the blood stream, spleen, and along the gastrointestinal tract. The role of the bursa in immunological responses have been studied by surgical or hormonal ablation, and/or irradiation since the initial report of Glick et al.(1956) that the removal of the bursa eliminates the ability of chickens to synthesize immunoglobulins.

Hormonal bursectomy, effected by injection of nortestosterone into embryonated eggs (Meyer *et al*., 1959), or by dipping them into solutions containing testosterone propionate almost entirely eliminated antibody production to a variety of antigens, but had a serious drawback in that it also caused a variable degree of atrophy of the thymic cortex (Szenberg and Warner, 1962). In view of the evidence cited by Moticka (1975) regarding a bursal dependent subpopulation of cells in the thymus, perhaps what Szenberg and Warner (1962) regarded as a problem was in fact more in line with reality. Nonetheless, Cooper *et al*.(1966) combined neonatal bursectomy with sub-lethal irradiation, and others have performed surgical bursectomy *in ovo* as early as 16 days of incubation and have effectively removed the bursal influence without impairing thymic morphology, or hindering what are generally considered to be thymus dependent immune responses (see Moticka, 1975).

In general, the conclusions drawn from a wide variety of studies with bursectomized chickens is that specific antibody production is intimately controlled during embryonic development and in the early post hatching period by the bursa. More specific details are enlightening. Chickens possess at least three classes of Ig's and birds can be rendered almost completely agammaglobulinemic if bursectomized 5 days pre-hatching. Birds bursectomized 3 days pre-hatching were deficient in IgG synthesizing ability with no impairment of IgM synthesis. In addition, evidence has accumulated which indicates that immunization leads to both specific and non-specific antibody synthesis, and that non-specific antibody appears to be independent of the bursa (Toivanen and Toivanen, 1973).

Moticka (1975) suggests that birds possess not two, but three populations of cells which are "educated" or have their immunological origins in different areas, and have different functions. He postulates (Fig. 3, p. 143) a T-cell population which is composed of conventional thymocytes primarily responsible for homograft rejection, delayed hypersensitivity, and graft-versus-host reactivity (CMI), but which also influences the other cell populations. B-cells, or bursa dependent cells are cells "educated" in the bursa and are responsible for specific antibody production, but which are not homologous to mammalian B-cells. The third cell population he calls BM-cells because they are apparently derived directly from bone marrow without the intervention of central lymphoid organs. They are physiologically similar to B-cells but may be capable of synthesizing only IgM. These cells are thought perhaps to be producers of non-specific immunoglobulin and natural antibodies, and are postulated to be completely dependent on T-cells. A further interesting postulate of Moticka (1975) is that specific antibody production is less important to birds than it is to mammals, perhaps because non-specific immunoglobulin responses are so well developed in birds. He suggests that since T-cell functions developed phylogenetically prior to B-cell functions, it is possible that the earliest antibody responses were non-specific consequences of the sensitization of T-cells, which were later replaced (evolutionarily) by highly specific antibody responses as seen in mammals. The BM-cell-dependent, non-specific immunoglobulin response, perhaps better developed in birds than in mammals, is an effective arm of the immune machinery of birds, but it is only an evolutionary remnant in mammals.

The observations of Toivanen and Toivanen (1973) on chickens treated with cyclophosphamide (CY) in the newly hatched period, reconstituted with bursal or bone marrow cells from normal chickens of different ages, and then antigenically challenged, are interesting in this regard. Two discrete populations of postembryonic bursal cells were identified: bursal stem cells (the majority of cells in the bursa up to 2½ to 3½ weeks post hatching) and; post bursal stem cells (majority of cells from 7 weeks onward). Both populations were capable of restoring antibody responses to sheep erythrocytes and _B_. _abortus_ in CY treated, or CY treated-surgically bursectomized chicks. Bursal stem cells were capable of restoring bursal morphology and restoring germinal centres in the spleen; post bursal stem cells were capable of neither. The latter cells began appearing in the involuting bursa and bone marrow at the same time. These authors also showed that specific antibody production was independent of germinal centre formation in the spleen, and that germinal centre formation was independent of the presence of bursal follicles.

Immunity to Helminths

The application of immunological concepts to parasitology is relatively recent and presents problems of large magnitude, especially when dealing with helminths (WHO, 1965). In dealing with immunity to helminth infections, studies must be concerned with a wide range of stages in the course of a helminth's life cycle and the course of an infection, since both are often complex phenomena. A major problem not encountered in protozoan and microbial infections is the antigenic (immunogenic) heterogenicity of helminths, which consist of a wide range of structural, secretion, and excretion antigens (Sinclair, 1970). As well, helminth infections may be of long duration, covering several different stages, which may complicate the assessment of immunological responses of the host (Sandground, 1929; Ogilvie, 1970). Recent years have seen a tremendous surge in the number of studies concerned with parasite immunology as evidenced by the reviews in WHO (1965), PAHO (1967), Jackson *et al.*(1970), and Cohen and Sadun (1976).

Although many animals are able to resist helminth infections and there is some evidence to suggest that natural or innate resistance may play some part in counteracting helminth infections (see Sandground, 1929; Smithers, 1976), most investigations in immunoparasitology have been concerned with acquired immunity (Sinclair, 1970). This phenomen is usually manifested by the appearance of circulating antibodies and/or immunocompetent cells directed against the secretions or tissues of the parasites. The effects of such responses by the host on the parasites involved may take many forms including: inhibition of growth and arrested development; inhibition of morphogenesis and reproduction; inhibition of migrations; and inhibition of parasite enzyme systems or oxygen consumption (WHO, 1965). Kelly (1973) and Cheng (1973) reviewed the mechanisms of immunity to intestinal helminths and provided a similar list of the manifestations of immune responses: i) retardation or inhibition of development (Ostertagia ostertagi, Nematodirus spathiger, Haemonchus contortus); ii) failure to develop anatomical structures (vulval flaps of O. ostertagi); iii) reduction in establishment rate of new infections (Dictyocaulus viviparus); iv) suppression of egg production (Nippostrongylus brasiliensis in rats, Trichostrongylus colubriformis in guinea pigs, N. spathiger in sheep); v) structural or metabolic changes (alterations of n-acetylcholinesterase of N. brasiliensis); and vi) elimination of existing infections ("self-cure" in Haemonchus and Nematodirus infections in sheep, N. brasiliensis in rats, and Trichinella spiralis in the mouse, rat and guinea pig).

Resistance to subsequent infections with the same helminth species has been demonstrated with Trichobilharzia ocellata in black ducks (Rau et al., 1975), Fasciola hepatica in rats (Hayes et al., 1972; Hayes and Mitrovic, 1977), and in cattle but not sheep and goats (in Smithers, 1976), schistosomes in a variety of primate and non primate hosts (Smithers, 1968; Smithers et al., 1969; Smithers, 1976), Toxocara canis in dogs (in Ogilvie and Worms, 1976), Nematospiroides dubuis in mice (Helper et al., 1976), and T. spiralis in rats and mice (Despommier et al., 1977), to cite some recent examples.

Parasitologists have been concerned with understanding the mechanisms controlling immune responses to parasites in general. In this respect it is of interest to note which of the two major efferent arms of immune responses is most effective against particular parasites, or whether they act synergistically as suggested by Soulsby (1970).

All parasites studied have been shown to induce specific antibody synthesis (Cohen, 1976b). Helminth infections generally induce the formation of IgM, IgG and IgE antibodies (Sinclair, 1970), but with the possible exception of complement dependent lethal antibody (IgG) to schistosomes in rat, guinea pig, and human sera (demonstrated in vitro), the direct action of antibodies or for that matter, immunocompetent cells, on metazoan parasites has not been demonstrated. Their effects are therefore indirect and may be complex, multi-phasic phenomena as suggested by Soulsby (1970) and echoed by Kelly (1973). That antibodies have important protective functions is apparent because immunity can be passively transferred via immune sera to a variety of helminths including N. brasiliensis, T. spiralis, D. viviparus, Ascaridia galli, Litomosoides carinii, S. mansoni (Sinclair, 1970) and, T. colubriformis (Connan, 1972). However, there are conflicting reports in this area since Larsh *et al.*(1966, 1975) reported that immunity to *T. spiralis* could not be transferred to recipient mice with immune sera, perhaps because antibodies were stage specific. Similarly, Smithers (1976) cited evidence that lethal antibodies to *Schistosoma* spp. could be passively transferred in rats and mice, but not in rhesus monkeys. Sinclair (1967) reported that *Fasciola hepatica* infections induced an ample antibody response which however, conferred no protection to hosts, though Smithers (1976) reported protection could be passively transferred, but only by massive doses of immune sera injected intraperitoneally.

Helminth infections are remarkable in their ability to stimulate both specific and non-specific IgE (Ogilvie, 1970; Ogilvie and Jones, 1973; Carson *et al.*, 1975; Ogilvie and Worms, 1976). Sinclair (1970) suggested that evidence was relatively strong for a role for antibody in the "self-cure" phenomena associated with *H. contortus* in sheep, *Trichostrongylus* spp. in rabbits, *D. viviparus* in cattle, *N. brasiliensis* in rats, and *Ascaris suum* in pigs. Ogilvie (1970) suggested that reaginic antibodies are perhaps effective in this respect, although the effect would necessarily be indirect. Perhaps the best evidence for a role for reagins is provided by studies of the expulsion of *N. brasiliensis* from the rat gut (Miller, 1971a, b) and *T. spiralis* from the gut of mice and/or rats (Kozar, 1973; Karmanska *et al.*, 1972; Neraasen and Dick, 1974). It is suggested that expulsion is mediated by reaginic antibodies acting in concert with mast cells and their amines, or by other antibody dependent lymphocytic mediators (Kelly, 1973). The latter reviewed the evidence which indicates that specific inhibitors of histamine and serotonin (amines thought to mediate expulsion by virtue of their release by antigen-antibody (IgE) complexes at the mast cell surface), can prevent the expulsion of *H. contortus*, *T. colubriformis* and *N. brasiliensis* from their hosts. However, reports such as those of Keller (1971) which indicated a failure to correlate mast cell numbers and intestinal histamine levels with the expulsive phase of *N. brasiliensis* infections in rats, tend to make the case for reaginic antibodies rather inconclusive.

Cell-mediated immunity (CMI) to helminths has become a productive area of research as evidenced by recent major reviews by Soulsby (1970), Kelly (1973), Larsh and Race (1975) and Larsh and Weatherly (1975). A number of host-parasite models have been investigated in this regard but evidence for the role of CMI in protective immunity is strongest for *Trichinella spiralis* in the mouse, *Trichostrongylus colubriformis* in the guinea pig and *Nippostrongylus brasiliensis* in the in the mouse and rat. Evidence is beginning to accumulate which suggests CMI may also operate in other host-parasite systems such as *Hymenolepis nana* in mice, *Echinococcus multilocularis* in mice, schistosomes in a variety of hosts, and *Fasciola hepatica* in rats, mice and guinea pigs. Soulsby (1970) and Kelly (1973) caution however, that immune responses to helminths are complex, multiphasic phenomena in which no single factor or mechanism may be seen to operate to the exclusion of others.

Larsh and co-workers (see Larsh and Race, 1975; Larsh and Weatherly, 1975) have studied the relationship of CMI to Trichinella spiralis infections for nearly thirty years. They have long held that the expulsion of T. spiralis adults from the gut of mice is a manifestation of a delayed (cellular) hypersensitivity response (CMI) with two components:

1) "an immunologically specific reaction between antigen-sensitive T-cells and the antigen, and
2) an ensuing immunologically non-specific inflammatory reaction (allergic inflammation) to the tissue injury produced by the interaction of the specific elements in the first component".

Evidence in favour of CMI came from several approaches. Firstly, protective immunity was adoptively transferred to recipient mice by lymph node cells (Larsh et al., 1964a) or peritoneal exudate cells (PE) from previously infected donors (Larsh et al., 1964b, 1966), or by spleen cells from artifically sensitized donors (Larsh et al., 1970a, b; Larsh and Weatherly, 1974). Kim (1966) and Kim et al.(1967a, b) showed that delayed dermal hypersensitivity could be transferred to recipient guinea pigs given lymph node or spleen cells from donors sensitized with T. spiralis larval antigens in Freund's complete adjuvant.

Immunosuppressive procedures have provided other evidence for the role of CMI in trichinellosis. Treatment with anti-lymphocyte (ALS) or anti-thymocyte (ATS) serum prolonged the duration of the intestinal phase and reduced the inflammatory response in the gut of infected mice (Kozar _et_ _al_., 1971; Larsh _et_ _al_., 1972, 1974a; Machnicka, 1972; Karmanska _et_ _al_., 1972; Kozar, 1973). Walls _et_ _al_.(1973) reported similar effects on the length of intestinal phase and inflammation in the gut and around muscle larvae in neonatally thymectomized-irradiated mice. Similarly, Grove and Warren (1976) indicated that treatment with a specific suppressant of cell-mediated responses (niridazole) resulted in reduced inflammatory responses to larvae in the muscles. Larsh and Weatherly (1975) cited reports of similar effects with cortisone.

It is interesting to note that Walls _et_ _al_.(1973) found that eosinophils, which were prominent in the inflammatory infiltrate, especially around larvae in the muscles of normal mice, were absent from mice neonatally thymectomized and irradiated. They interpreted this to mean that the eosinophilic response was T-cell dependent, though they could suggest no protective function. However, Larsh _et_ _al_.(1974b, 1975) have suggested that eosinophils may function in the expulsion of _T_. _spiralis_ since they have shown that the primary and secondary (anammestic) eosinophilia in the bone marrow closely parallels the buildup of phospholipase B in the intestinal tissue of mice infected with _T_. _spiralis_. Both factors were shown to parallel the inflammatory and expulsive processes which occurred in the intestine. The authors suggest that eosinophils contain the enzyme, that the eosinophilia is stimulated by the intestinal phase of infection (see also Zaiman and Villaverde, 1964) and that they accumulate at the sites of inflammation where they release the enzyme. It was suggested that enzyme release and its consequent dissolution of cell membranes contributes to the allergic inflammation (see Larsh and Race, 1975) and thus eosinophils are partly responsible for worm expulsion. Basten and Beeson (1970) had shown that a diffusible factor released from sensitized lymphocytes (presumably memory T-cells) in diffusion chambers implanted intraperitoneally in normal rats was capable of inducing eosinophilia. Consequently, Larsh *et al.*(1975) postulated the existence of a lymphokine which is released from sensitized T-cells as a consequence of their contact with worm antigen in the first step of the immune response, which stimulates eosinophilia and migration of eosinophils to the site. They cite a similar report of Colley (1973) who has evidence for the existence of an "eosinophil stimulation promoter" (ESP) produced by sensitized lymph node cells from mice infected with *Schistosoma mansoni*, as support for their hypothesis. Further support is provided by the report of Warren *et al.* (1976) who showed that PE cells from mice infected with *T. spiralis* produced *in vitro*, a lymphokine (ESP) which greatly enhanced the migration of sensitized human and murine eosinophils in the presence of soluble larval antigens.

Evidence of CMI to Trichostrongylus colubriformis has been provided by Wagland and Dineen (1965) who were able to transfer adoptive immunity to recipient guinea pigs with mesenteric lymph node cells from syngeneic infected donors. Dineen and Adams (1971) suggested that the cells responsible for immunity (initiation of rejection) were thymus dependent, long lived, recirculating small lymphocytes (T-cells). They combined neonatal thymectomy with lymph drainage for 8 to 9 days before infection to abolish immune rejection. Neonatal thymectomy alone was ineffective, suggesting that guinea pigs are immunocompetent at birth and that lymph drainage depletes the pool of circulating T-cells. Rothwell et al.(1971) and Rothwell and Dineen (1972, 1973) also implicated T-cells as important in initiating an early, immunologically specific reaction between specific T-cells and antigen, which then stimulates eosinophil and basophil proliferation and accumulation. This specific reaction was then thought to be followed by a non-specific reaction which results in the expulsion of worms as a direct or indirect result of the action of amines released by granulocytes.

Adoptive transfer of immunity to Nippostrongylus brasiliensis with sensitized cells has been accomplished by several workers (Ogilvie and Jones, 1968; Kelly, 1971 ; Kelly and Dineen, 1972 , 1973 ; Keller and Keist, 1972). Neonatal thymectomy (Ogilvie and Jones, 1967) or infections in congenitally athymic (nude) mice (Jacobson and Reed, 1974) greatly extended egg production and the duration of infections, and reduced or abolished resistance to secondary infections. This represents strong evidence for the involvement of CMI, although Keller and Keist (1972) pointed out that the expulsion of adult worms required fully mature lymphocytes. This was supported by Kelly and Dineen (1973) who indicated that sensitized cells from neonatally infected rats were not effective in adoptive transfer of immunity, though those from rats infected later were.

The role of reagins in the immune response to *Nippostrongylus brasiliensis* has been emphasized by Ogilvie and Jones (1967, 1971, 1973) and Kelly (1972) but in each case, the production of IgE antibodies was dependent on T-cell co-operation as evidenced by the effects of thymectomy and/or ATS.

Some evidence has accumulated in favour of the involvement of CMI to *Nematospiroides dubius* in mice. Cypess (1970) showed that sensitized spleen cells could confer adoptive immunity and Hepler *et al.*(1976) reported that prednisone acetate reduced the immunization effect of presensitization to *N. dubius* from 95% protection to only 20%. They suggested that the steroid impaired macrophage function as proposed by Weston *et al.*(1973). The formation of granulomas in mice infected with *Capillaria hepatica* is said to be mediated by CMI (Solomon and Soulsby, 1973, in Larsh and Weatherly, 1975), and Brown *et al.*(1977) reported that a T-cell dependent antibody response acted in resistance to *Ascaris suum* in mice. Finally, a strong correlation between IgE levels and eosinophilia in ascariasis, ancylostomiasis and trichuriasis was reported by Araki _et_ _al_.(1976). Since both eosinophilia and IgE responses have been shown to be T-cell dependent in other systems, CMI appears to be an important but not an exclusive arm of the immune response to these helminths.

Cestodes have been less frequently studied but Larsh and Weatherly (1975) reviewed evidence which suggests that CMI plays a role in resistance of mice to _Hymenolepis_ _nana_. Spleen cells could confer immunity upon unsensitized recipients and thymectomy resulted in the retention of cysticercoids in 65% of 32 operated mice (none of 20 unoperated controls). ATS abolished sensitivity produced by a stimulating infection. In addition, they cited evidence concerning the role of cell-mediated immunity to _Hymenolepis_ _microstoma_ and _H._ _diminuta_ (see Hopkins _et_ _al_., 1972).

Baron and Tanner (1976) reported that CMI controls the dissemination and size of the cyst mass in _Echinococcus_ _multilocularis_ infections in mice because thymectomy and ATS treatment increased the number of cysts in treated mice. Novak (1972, 1974, 1977) reported that splenectomy and cortisone treatment of mice infected with _Mesocestoides_ _corti_ increased the biomass of tetrathyridial masses and that spleen cell homogenates from infected donors were effective in adoptive transfer of immunity. She concluded that CMI was operating in this model.

Work with Taenia taeniaformis in rats has shown that a remarkable eosinophilia is produced by day 22 of a primary infection (Ansari and Williams, 1976) and that immune serum or the IgG_{2a} and IgE fractions of immune serum produced an anamnestic eosinophilic response in nonsensitized animals (Ansari et al., 1976). This parallels studies reported above with regard to T-cell dependent eosinophilia and reaginic antibody responses and is suggestive of the existence of a common synergistic effect of CMI and humoral immunity.

Trematodes, especially schistosomes and Fasciola hepatica have been extensively studied with respect to CMI (see reviews of Smithers and Terry, 1969, 1976; Smithers, 1976; Warren, 1976). There is compelling evidence to suggest that the schistosome egg granuloma is a manifestation of delayed hypersensitivity and that after sensitization by intraperitoneal injection of schistosome eggs, mice exhibit an accelerated and augmented granuloma formation to eggs introduced into the lungs by intravenous injection. This sensitization was shown to be transferrable between mice by lymph node or spleen cells but not with serum (Warren et al., 1967), and that it could only be elicited by soluble material (soluble egg antigen or SEA) which diffused out of intact, fully developed eggs (Boros and Warren, 1970). Sensitization was not produced by irradiated cercariae, all male or all female worms or dead worms injected intraperitoneally (Warren and Domingo, 1970b). Colley (1971) showed that cultures of lymph node cells from mice infected with S. mansoni underwent blastogenesis in response to SEA *in vitro*, and Dunsford *et al*.(1974) reported macrophage migration inhibition of cells from mice sensitized with SEA. Also, bentonite or latex beads coated with SEA produced delayed dermal responses and/or true granulomatous responses in the skin or lungs of mice respectively (Boros and Warren, 1971b; Lichtenberg *et al*., 1971; Dunsford *et al*., 1974).

Further evidence that CMI operates in granuloma formation has been provided by workers using immunosuppressive procedures which specifically inhibit cell mediated responses. These include neonatal thymectomy (Domingo and Warren, 1967), ALS treatment (Domingo and Warren, 1968) and AMS treatment (Boros and Warren, 1971a). Hsu *et al*. (1976) also reported that smaller granulomas were present in athymic (nude) mice at 52 days of infection, than in normal individuals. Few eosinophils and no giant cells, both normally numerous in granulomas, were present.

Granuloma formation around eggs is a major factor in pathology of schistosome infections (Warren, 1968) and is clearly divorced from any protective immunity (Warren, 1972) since immunity is thought to be primarily humoral and directed at the schistosomule (see Warren, 1976). In support of this, Henson *et al*.(1972) produced partial inhibition of hepatic granuloma formation in mice infected with *S. mansoni* with propriomazine (Azium) and found that mean survival time increased in these mice relative to untreated ones, by about 10 days.

Other workers have presented evidence which indicates that delayed hypersensitivity as manifested by granuloma formation may be helpful to the host in coping with schistosome infections. Fine _et al._ (1973) and Buchanan _et al._ (1973) using T-cell depleted mice (thymectomized, ATS treated or thymectomized, irradiated) which were exposed to _S. mansoni_ cercariae following treatment, showed that normal delayed skin responses, the major eosinophilic response, and the IgE response were abolished. Granulomas were inhibited or reduced and mortality rates were greater than in normal mice. This suggests that granuloma formation is a protective mechanism though it does not confer immunity to infection. In this regard, Dunsford _et al._ (1974) suggested that granulomas are formed in response to sustained release of antigen (SEA) which is sequestered in host phagocytes so that this cellular response appears to be important in enabling the host to cope with infection.

There are some reports which indicate that a cellular mechanism(s) may operate to prevent infection, but only in concert with other components of the immune system (see Smithers, 1976; Smithers and Terry, 1976). Maddison _et al._ (1973) showed that natural or specific transfer factor from sensitized monkey leucocytes could confer delayed hypersensitivity on, and reduce the number of _S. mansoni_ in infected rhesus monkeys. The work of Perez and Terry (1974), Perez (1974), Butterworth _et al._ (1974), Capron (1974) and Capron _et al._ (1975) was reviewed by the reviewers noted above. These authors variously indicated that complement independent opsonizing antibody (probably IgE), in the presence of peripheral leucocytes, PE cells, or macrophages could damage or kill schistosomules _in vitro_. Butterworth _et al_.(1976) reported that an effector cell in baboons was associated with the neutrophil-eosinophil fraction of peripheral blood. Smithers and Terry (1976) suggest that these results correlating the co-operation of specific anti-schistosome IgE antibodies and specific cells, with protective immunity may be very significant. They suggest that this particular arm of the immune response should be investigated further as it may have relevance to other helminthoses which are characterized by high levels of IgE antibodies.

Cell mediated immune responses have been strongly implicated in protective immunity to _Fasciola hepatica_ in mice, rats and guinea pigs. Lang _et al_.(1967) showed that PE cells from infected donor mice conferred adoptive immunity on recipient mice. Corba _et al_.(1971) reported that lymph node or spleen cells from infected rats provided 66-100% protection of recipients against primary challenge, while Vernes _et al_.(1972, in Warren, 1976) reported delayed skin reactions and macrophage migration inhibition in infected guinea pigs 15 days postinfections. Hayes and Mitrovic (1977) reported that immunity to _F. hepatica_ was apparent in rats 24 to 48 hours after challenge and postulated that flukes were killed or at least immobilized in the gut wall. They implicated CMI as the major effector mechanism since treatment with an anti-inflammatory/immunosuppressive drug (dexamethazone) reduced the response of sensitized rats to a level comparable to that in unimmunized animals.

Blake (1973) reported that anatids treated with prednisone or thymectomized, showed impaired immunity to *Apatemon gracilis* infections when compared to normal birds. Normally, birds eliminated worms in a two stage process similar to that seen in *Nippostrongylus brasiliensis* infections. This represents one of the few reports concerning CMI to helminths in birds. Evidence for a role of CMI in *Echinuria uncinata* infections in mallards was provided by Ould (1972). Treatment of ducklings with injections of cortisone acetate increased the number and size of nematodes retained by experimentally infected ducklings, and was tentatively correlated with a reduced amount of "caseous material" present in the lumena of granulomas.

III. MATERIALS AND METHODS

Maintenance of Ducklings

Ducklings were raised and held following infection in an outdoor pen supplied with water from an adjacent covered well. Three permanent partitions divided the pen into four equal compartments and moveable partitions were in place so that eight compartments, $9.5m^2$ in area, were available. The walls were plywood to a height of 1.2m and above that, on the sides, ends, and over the top, was chicken wire so that the pen was fully enclosed. The base was of concrete construction with a water tank running down the centre and level concrete pads on both sides. Plywood sheets attached to one wall of the pen by means of hinges and a pulley system provided overhead protection. Screens were placed beneath the overhead panels and brooder lamps were attached to the panels.

All ducklings used in this study were male wild mallards (Anas p. platyrhynchos) obtained from the Delta Waterfowl Research Station (DWRS) hatchery. The DWRS maintains a breeding flock of wild mallards which originated from eggs collected from nests in the Delta Marsh. Ducklings were obtained directly from the drying pans, sexed, and placed in the outdoor pen under brooder lamps. Initially, ducklings were provided access to only enough water for drinking purposes, but more of the water area was gradually provided so that by 7-10 days post-hatching, the maximum amount of water was made available to them. Brooder lamps were removed after 3 or 4 weeks. Sufficient unmedicated Duck and Goose Crumbles (Feed-Rite Mills Ltd.) containing 18% protein was provided twice daily to ensure a slight excess upon cessation of feeding.

Each morning, the overhead panels were raised, the entire pen was thoroughly swept, drained, flushed with clean water, and refilled. Each evening the pen was swept but not drained.

Thirty to forty newly-hatched ducklings were placed in a single compartment, but no more than 20 ducklings older than 10 days were maintained in a single compartment. Ducklings of different ages were never placed in the same compartment.

These procedures were followed in order that stresses related to crowding, dietary factors, and general conditions of habitation could be minimized or at least standardized. Well-water was used to minimize the exposure of ducklings to contaminative organisms which could be transmitted through the water, feces, invertebrates, etc. Wet feces and feed could have provided a rich organic substrate for the growth of micro-organisms which could have influenced the responses of the birds to a variety of infective agents, including *E. uncinata*.

Maintenance and Recovery of Infective *E. uncinata* Larvae

Gravid female *E. uncinata* recovered from wild waterfowl, chiefly mallards, gadwall (*Anas strepera*), and pintail (*A. acuta*), were used to establish laboratory infections in cladocerans.

One or two gravid nematodes were macerated in a small petri dish containing 0.85% saline and ova were pipetted into a large wide-mouth jar containing approximately 2000 freshly collected *Daphnia magna* (preferrably newly released individuals). A pipette attached to an air pump by plastic tubing was inserted into the jar to keep ova in suspension. Cladocerans were allowed to feed in the jar for 2 to 6 hours after which they were gently transferred to 10 or 15 gallon aquaria containing pond-well water.

Aquaria were constantly aerated and maintained at approximately 18-20°C in an isolated room or in environmental chambers. Cladocerans were fed a few millilitres of yeast suspension every two or three days.

When infective larvae were required, Daphnia, which had been infected at least 15 days previously, were removed from the holding tank, transferred to small petri dishes containing a small amount of aquarium water and gently crushed, using the flat surface of a large glass stopper. The resultant mulch was Baermannized for two hours (see Cable, 1977, p. 245) and the larvae removed to small petri dishes.

Larvae were counted on a dissecting microscope and transferred to small, stender dishes containing 0.85% saline. Only intact, active larvae were fed to ducklings.

Infection of Ducklings

Ducklings were starved 18-24 hours prior to infection. Each was weighed to the nearest tenth of a gram, had tarsus and culmen length recorded, was assigned a coded necropsy number, and was fitted with a numbered plastic leg band. If the duckling was too small to retain a band, it was web-clipped and a band applied later.

Forty or sixty larvae (depending on the experiment) were introduced via pipette by extending the neck, opening the bill a moderate distance, and depositing the larvae in a small amount of saline at the back of the pharynx beyond the tracheal opening. Care was taken to prevent regurgitation and an additional one or two ml of saline was given to wash the larvae down the esophagus. Ducklings were held for several hours in covered boxes and then returned to the holding pen where food and water were provided.

Postmortem Examination and Treatment of Specimens

Postmortem Examination

Food was withheld on the day of postmortem examination. Ducklings were killed by intracranial injection of absolute ethanol or by sodium pentobarbital overdose. They were weighed to the nearest 0.1 g, length of culmen, tarsus, and keel recorded and the viscera exposed from the tip of the lower mandible to the cloaca. The stomach was removed, flushed with water and examined for gross lesions. The diameter of each lesion was taken with calipers and recorded. Those used for histological studies were excised and fixed in glutaraldehyde, Stieves, AFA or Bouin's. Lesions to be examined for nematodes were placed in dishes of 0.85% saline and examined as soon as possible.

The length and diameter of the spleen and bursa were recorded and portions to be fixed were placed in glutaraldehyde and AFA or Stieves. The thymus was removed from some birds, blotted on absorbent paper and weighed to the nearest milligram. A small portion of the intestine at the ileo-cecal valve was removed from some birds and fixed in Stieves. All organs from which tissue was removed were placed on paraffin flooded with fixative, and cut into suitably sized pieces with a sharp scalpel.

An index of general condition which takes into account body size and weight, was calculated for each bird from the formula:

$$C = (\text{Keel length(mm)} \times \text{Culmen length(mm)}) / \text{weight(g)}.$$

A similar index has been used for blue-wing teal by Harris (1972).

Examination of Lesions

Lesions were carefully dissected on a dissecting microscope. Worms were removed as intact as possible, counted, and fixed in hot 70% ethanol to which 5% glycerol had been added. They were stored in fixative until they were cleared in lactic acid, mounted temporarily in the same fluid, and measured.

Fixation and Embedding

1. Glutaraldehyde - Methacrylate

Tissues were cut to appropriate size (up to 4mm^2) and fixed for two to three hours at 4°C in 6% glutaraldehyde in 1.12M phosphate buffer (Millonig, 1961) to which was added one drop saturated $CaCl_2$ and

0.3g sucrose per 10 ml of buffer. Tissues were then washed overnight in Millonig buffer containing 5% sucrose (in the refrigerator), rinsed with distilled water, dehydrated quickly to cold 95% ethanol, and then passed through two changes (1 - 2 hours each) of absolute ethanol at RT. Tissues were infiltrated with butoxyethanolglycol methacrylate (three changes, the last overnight) and embedded in Beem's capsules. The embedding medium contained approximately 42 parts fresh infiltration medium and one part polyethylene glycol 400, N, N - dimethylalanine (JB-4 Plastic Embedding Kit, Polysciences Inc., Warrington, Pa.). A small label identifying the type and source of the tissue was inserted into the capsule before the top was sealed. Blocks were allowed to polymerize at RT for 48 hours and then were stored for later sectioning.

2. Sublimate - Paraffin

Portions of some lesions from the proventriculus-ventriculus junction were fixed 4-6 hours in a mixture containing 5.5g $HgCl_2$, 2.7g sodium acetate (0.2 M each) in 100 ml distilled water, transferred to 80% ethanol for 2-6 days, dehydrated in ethanol and embedded in paraffin (Donaldson et al., 1973). Tissue was not treated for the removal of $HgCl_2$ crystals before embedding.

3. Stieves - Paraffin

A variety of tissues were fixed for 24 hours in Stieves fixative (76 ml saturated $HgCl_2$, 20 ml formalin, 4 ml glacial acetic acid) and stored in 70% ethanol (Humason, 1972). Before embedding in paraffin, the tissues were treated for 5-8 hours in iodine alcohol to remove $HgCl_2$ crystals.

4. Other Fixatives - Paraffin

Alcohol - formalin-acetic acid (AFA) and Bouin's were occasionally used. Tissues were fixed for several days and stored in 70% ethanol. Bouin's fixed tissues were washed for several hours in 50% ethanol to remove the picric acid before being embedded in paraffin.

Post-Fixation Histological Techniques

1. Methacrylate blocks

Sections one to two μm thick were cut from methacrylate blocks on a Sorvall MT-1 ultramicrotome and affixed to clean glass slides. Sections were covered with Giemsa blood stain (three parts conc. stock to one part buffered distilled water) and heated over an alcohol burner for approximately 30 seconds. Slides were rinsed in running tap water, drained, and air dried in an upright position. Most were counterstained for a few seconds in 0.5% Thionin (C.I. 5200) in 0.01 M acetate buffer (Humason, 1972), rinsed with tap water and air dried. Sections were permanently mounted in Permount or were temporarily mounted in immersion oil which was removed following examination by dipping the slides in xylene.

2. Paraffin blocks

Parrafin blocks were routinely sectioned at 5-7μm and the sections stained with H and E (Humason, 1972). Other techniques applied to sections from lesions in an attempt to differentiate specific types of cells are briefly outlined below.

a) Toluidine blue

Sections of Sublimate fixed tissues were stained for 20 minutes in 0.1% Toluidine blue in 1% acetic acid, rinsed in distilled water, rinsed in acetone, then xylene, and mounted in Permount. Mast cell granules stained deep blue to purple with little staining of other elements. Iodination was omitted as suggested by Donaldson et al. (1973).

b) Eosin-Thionin

Sections fixed in Sublimate, Stieves or AFA respectively, were stained in eosin for five minutes, rinsed in distilled water and then stained for 30-90 seconds in 0.5% Thionin in 0.01 M acetate buffer. They were then rinsed in distilled water, dehydrated in acetone, and transferred through a series of acetone-xylene mixtures to pure xylene. Finally, they were mounted in Permount. When Sublimate or Stieves was the fixative, the results were comparable to H and E but with better cytoplasmic staining and with differential staining of granulocytes. Mast cell granules were stained only when fixed in Sublimate.

c) Eosin-Giemsa

Tissue fixed in Stieves and stained with H and E followed by staining with Giemsa diluted 1:1 with 2% sodium borate at 70°C demonstrated eosinophilic granulocytes and the cytoplasm of lymphoid cells very well. Sections were dehydrated and cleared as in the eosin-thionin method.

Differential Cell Counts from Lesions

Lesions induced by E. uncinata which had been fixed, sectioned and stained as outlined above were to be used primarily to determine the number and types of infiltrating cells involved in the growth and development of lesions. Consequently, sections were first split into three arbitrary zones which were designated; "Inner", "Outer" and "Middle". The numbers of various types of cells present in these three zones were counted on two slides from each lesion sectioned. Five oil immersion fields from each zone were examined on each slide so that 30 fields were evaluated per lesion. Actual counts were converted to Naperian Logarithms because they varied over a range of 0-149 specific cells/field.

Tissues from the isthmus of uninfected ducklings killed on the first and last days that experimental ducklings were killed, were evaluated for cells in the lamina propria, and submucosa in the same way as outlined above. These counts were to serve as a base line for the counts from experimental birds, and to monitor for any major shift in cellular composition due simply to the seven week experimental period.

Photomicrography

Photomicrographs were taken with a Zeiss Photomicroscope II using Kodak Panatomic X film, followed by routine processing and printing.

Serological and Immunological Procedures

Collection and Storage of Blood and Serum

Immediately, before being killed, ducklings were immobilized on a cardiac puncture board and 20-40 ml of blood were withdrawn into sterile plastic syringes fitted with 20 or 18 gauge needles. A drop of blood was placed on a clean microscope slide and a blood smear made and fixed in absolute methanol for later staining with Giemsa. The blood was then transferred to clean, labelled, disposable culture tubes, stoppered and left at RT for at least two hours. They were then ringed with an applicator stick and placed in the refrigerator overnight to allow separation of serum and clot. Thereafter, sera were removed to clean centrifuge tubes, spun at 2000 G for 10 to 15 minutes and transferred to small, labelled glass or plastic tubes (several per sample). Tubes were stoppered and stored at -10 to -20°C until needed.

Stained blood smears were evaluated for any obvious changes in relative numbers of leucocytes over the experimental periods. Five oil immersion fields containing at least one leucocyte were examined on a smear from each duckling in the experiment concerning development of the lesion.

Serum Proteins

1. Total Protein Content

The total protein content of serum samples was determined by the Lowry technique following the procedure of Searcy and MacInnis (1970). This technique is sensitive to a range of 20-200μg protein/ml.

Serum samples were thawed and diluted 1:400 with distilled water. One ml of 2.68% potassium-sodium tartrate and one ml of 1% copper sulphate ($CuSO_4.5H_2O$) were added to 100 ml 2% sodium carbonate in 0.1 N sodium hydroxide. Four ml of this solution were added to duplicate 1.6 ml diluted serum samples, and the tubes allowed to stand for ten minutes. Then 0.4 ml freshly diluted Folin phenol reagent (3:1, water : conc. stock) was added to each tube, the tubes thoroughly mixed, and let stand for another 30 minutes. A duplicate series of standard solutions containing 220-12.5 μg bovine serum albumin, and a blank containing distilled water were treated in the same way each day that determinations were made. Optical densities were read at 750 Å in a Bausch and Lomb Spectronic 20. A standard curve relating percentage transmission to protein content of the standards was prepared and the protein content of the serum samples read from this curve.

2. Protein Fractions

Serum samples were electrophoresed on cellulose polyacetate membranes (Sepraphore III, Gelman Instrument Company, Ann Arbor, Michigan), for 40 minutes at 300 volts (2.5-3.0 ma/strip). A trisbarbital buffer (pH 8.8, ionic strength 0.06) was routinely used. Buffer was used for no more than 6 electrophoretic runs and stored no longer than a week in the refrigerator.

Electrophoretic bands were fixed and stained for 15 minutes in 0.5% Ponceau S in 5% tricholoroacetic acid, rinsed three times in 5% aqueous acetic acid to remove the background stain, dehydrated and cleared differently. Most were dehydrated in two changes of absolute methanol, transferred to 13% acetic acid in methanol for 60 seconds, placed on 2.5 cm χ 11.2 cm glass slides, and cleared by drying in an oven at 70°C for 15 minutes. Others were transferred from the last 5% acetic acid rinse to 45% dimethylformamide for 10 minutes, placed on glass slides and cleared in an oven at 80-90°C for 20 minutes.

Following clearing, fractions were quantified by scanning with an integrating densitometer-recorder (Gelman Instrument Co.). The percentage of prealbumin, albumin, and alpha, beta, and gamma globulins in the samples was estimated and converted to mg/ml.

Preparation of E. uncinata Antigen

A few ducklings were infected with *E. uncinata* to serve as a source of nematodes for further experimentation, and for the preparation of antigen. Worms from these birds were blotted on filter paper, frozen in glass vials, and stored in a deep freezer until required.

Worms were removed from the freezer, sonicated in distilled water and lyophilized. The dried extract was stored in a freezer and small volumes of antigen prepared by dissolving 10 mg of this material in one ml distilled water. A drop of merthiolate (1:10,000) was added. Such preparations were held in a refrigerator for no more than two weeks before being discarded, and new preparations made. During this interval, a known positive serum sample was included in each set of immunodiffusion slides as a check on the reactivity of the antigen preparation.

Immunodiffusion

Double diffusion of sera from experimental and control ducklings against the crude *E. uncinata* antigen was carried out on agar-coated microscope slides.

Microscope slides were cleaned in potassium dichromate-sulphuric acid followed by a thorough rinsing in distilled water, and stored in acetone until used. Slides were drained, allowed to air dry and placed in an immunoframe (Gelman Instrument Co.) (6 slides per frame) which was clamped onto a levelling table. Slides were coated with an adhesive agar (0.3% Difco Noble Agar in 1% NaCl with 0.05% glycerol added) and allowed to set for 20 minutes. Twenty-four ml of 1% agar in 1% NaCl were pipetted onto each set of 6 slides and allowed to cool. Wells (2mm dia.) were punched into one of two patterns; one with six peripheral wells around a central well (pattern A), or one with four peripheral wells around a central one (pattern B), using a Gelman gel punch assembly.

Into five of the peripheral wells of pattern A slides were pipetted 7µl of undiluted serum from different ducklings. The sixth well received distilled water and into the central well were placed 7µl E. uncinata antigen (10 mg/ml). Slides were placed in a humidity chamber which was sealed with tape, and incubated for a maximum of 96 hours. Slides were examined for the presence of immunoarcs at 10 to 12 hour intervals. After the incubation period, slides were rinsed in two changes of 1% saline (8 hrs, 24 hrs), and distilled water (8 hrs). Slides were removed from the distilled water, the wells completely filled with water, a dampened lintless paper strip applied to the surface of the agar, and the slides allowed to dry completely.

Slides were stained in 1% Amido Schwartz in acetic acid-methanol (45 parts methanol : 45 parts distilled water : 10 parts acetic acid) for 20 minutes, destained in four changes of the stain diluent, and allowed to dry. They were then examined for immunoarcs.

Sera which gave a positive reaction in the undiluted state were then diluted 2, 4, 8 and 16 times and these diluted samples applied to the peripheral wells of pattern B slides. Antigen was applied to the central well and double diffusion allowed to proceed. The presence and time of appearance of immunoarcs were recorded as before.

Relative antibody titre was estimated as follows: sera were assigned a rating according to the length of time to first appearance of an immunoarc according to the schedule in Table 1, and were assigned a further rating, relative to the maximum dilution at which an arc appeared. The ratings relative to the time factor were summed, and this value multiplied by the dilution factor. For example, a serum which reacted within 40 hours undiluted, and within 65 hours at four times dilution was assigned ratings of 6, 3 and 4. The resultant titre was therefore 36. This procedure was adopted in an attempt to provide more precise measures of the relative titres of serum samples since none reacted at a dilution greater than 1:4, though differences were noted in the relative prominence of arcs.

Table 1. Ratings assigned to serum samples with respect to the time an immunoarc was first noted.

Time of appearance in hours	<30	30-40	41-50	51-60	61-70	71-80	>80
Rating	7	6	5	4	3	2	1

Surgical Procedures

Bursectomy

Surgical instruments were sterilized in a hot air sterilizer and stored in 70% ethanol until used.

Day-old ducklings were immobilized and the down feathers clipped from the area around the cloacal opening to the tip of the pygidium. The area was swabbed with 70% ethanol and allowed to dry before a small amount of local anaesthetic was injected subcutaneously. A transverse or longitudinal incision was made just posterior to the cloacal opening and the bursa carefully freed from the cloacal wall and ureters before being pulled through the incision. It was tied off with a length of cat gut suture as close to its junction with the cloaca as possible and excised. The edges of the wound were swabbed with ethanol and closed with three or four square knotted No. 00 cat gut sutures using a number 20 suture needle. A longitudinal incision was easier to close without distorting the cloacal opening but a transverse one was preferred because the bursa was easier to locate and excise.

After surgery, ducklings were held for several hours in a warm, dry box lined with cheesecloth, then placed on a clean, dry screen in the holding pen. Water was provided in small aluminium pans for several days until the incisions healed. Ducklings were then given access to the water tank in the normal way. Within a week, little external evidence of surgery was visible, except for shorter down over the clipped area.

Thymectomy

Day-old ducklings were immobilized and the down clipped from the ventral surface of the upper thorax and the entire length of the neck. The area was swabbed with ethanol and allowed to dry and was then injected in several spots with a small amount of local anaesthetic. The following procedure was performed under a Luxo magnifying lamp. Oblique incisions were made on either side of the neck to expose the lateral aspects of the trachea, esophagus and the chain of thymic lobes which lie along those structures in intimate association with the large jugular veins and the brachiocephalic or carotid arteries.

Lobes of the thymus were carefully freed from the surrounding structures with fine iris forceps and removed. The use of scissors and scalpel was avoided because of the danger of cutting nearby vessels. Bleeding from torn small vessels was controlled by compression with sterile gauze pads. When all, or as much thymic tissue as possible had been removed, incisions were swabbed with 70% ethanol and closed with cat gut sutures. The area was again swabbed with ethanol and allowed to dry. Post-operative treatement was as per bursectomy.

Statistical Analysis

Data were analyzed using an IBM 360 computer and APL Statistical Packages. Standard statistics such as means and standard deviations were determined using the DSTAT function in Statpack 1, Library 2, or a personal program. One way analysis of variance was performed using ANOVA2 while simple and multiple regression analyses were done using the function REG. When T tests were required they were performed by the function TTEST4 from the Library of J. Redfield from the University of Alberta, Edmonton. Standard references were Sokal and Rholf (1969) and Steel and Torrie (1969).

IV. DEVELOPMENT OF THE LESION ASSOCIATED WITH E. UNCINATA INFECTIONS

Histology of the Normal Proventriculus-Ventriculus Junction

The stomach of birds is usually a bipartite structure consisting of a glandular proventriculus and a muscular ventriculus separated by an intermediate isthmus or junction (Ziswiler and Farner, 1972). The isthmus is relatively long in ducks in comparison to that of other birds.

The most striking histological feature of the proventriculus is the presence of compound glands in the tunica mucosa which penetrate the muscularis mucosae, separating it into outer and inner layers (Fig. 1). The epithelium of the mucosa is simple or pseudostratified columnar and extends into the ducts of the compound glands. In the epithelium are numerous goblet cells and extending deep into the lamina propria are numerous simple and compound tubular glands. The lamina propria is relatively extensive and has scattered lymphoid follicles throughout. The submucosa is sparse and because of the intrusion of compound glands between portions of the muscularis mucosae, the relationship of the submucosa to the mucosa is obscurred. The tunica muscularis typically consists of a well developed inner circular layer and a relatively thin outer longitudinal layer (Patt and Patt, 1969; Ziswiler and Farner, 1972).

The histological appearance of the isthmus is similar to, and blends with, the proventriculus. Compound glands are not present, the epithelium is thicker and thrown into folds, and the tubular glands are better developed (Ziswiler and Farner, 1972)(Fig. 2). The lamina propria of the isthmus also has greater numbers of lymphoid follicles, and the muscularis mucosae is undivided. The submucosa, like that of the proventriculus, is sparse. The tunica muscularis is like that of the proventriculus.

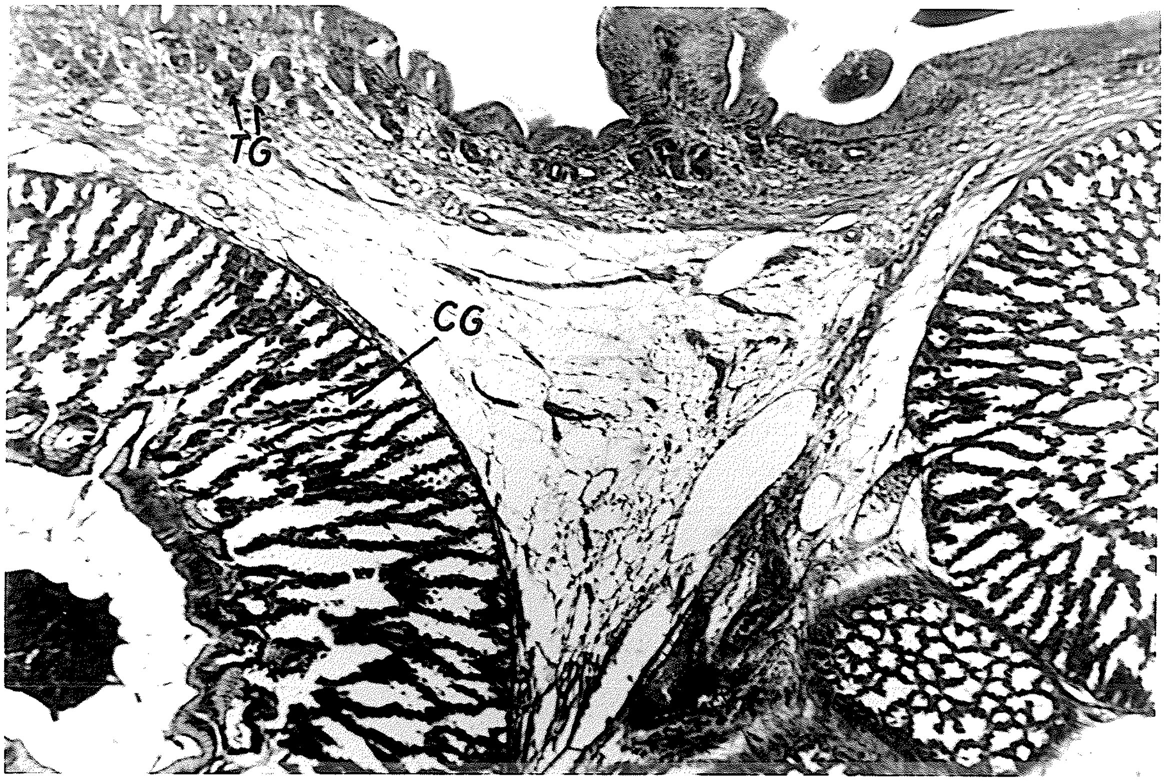
Connective Tissue Cells and Inflammation

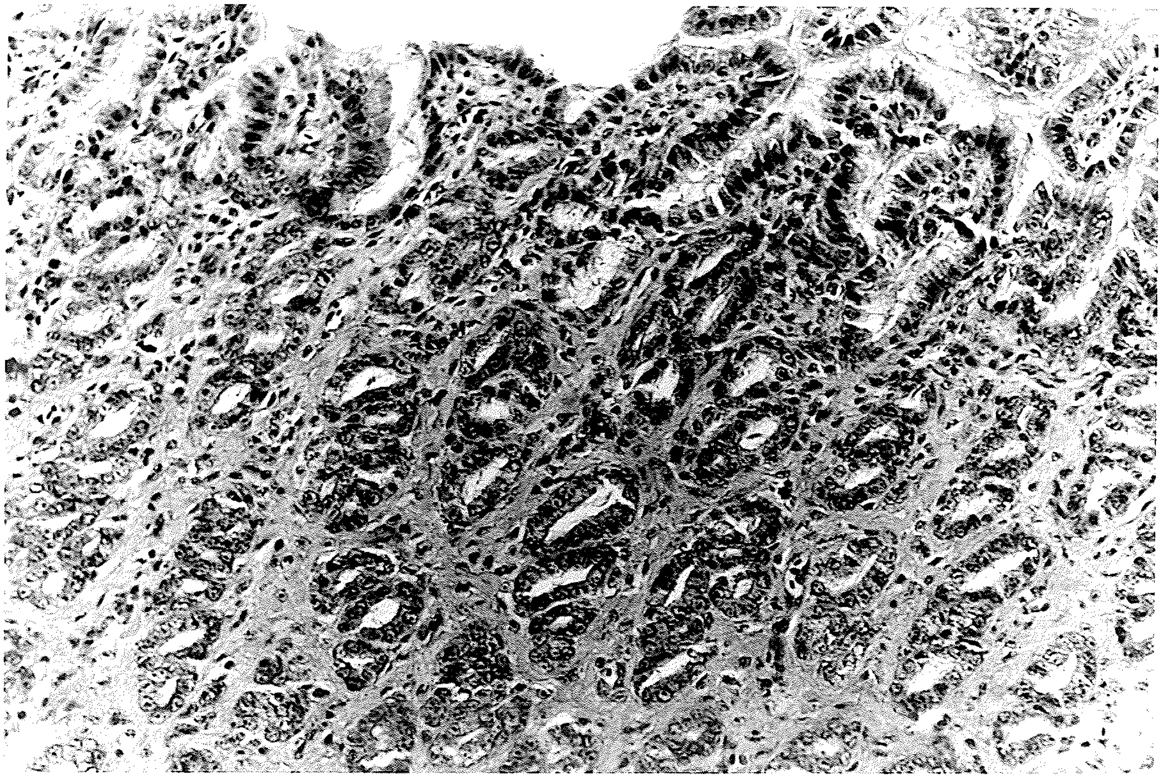
Body tissues respond to injury or invasion by microorganisms or metazoan parasites in a variety of ways. Connective tissues play an important role in protecting an organism from invading foreign material

Figure 1. Section of the mucosa of the proventriculus of an uninfected duckling. Note the small tubular glands (TG), the large compound glands (CG), and the vascular lamina propria. (Hematoxylin-Eosin), 10X.

Figure 2. Section of the isthmus between the proventriculus and the ventriculus of an uninfected duckling. The section is tangential and illustrates the large "villi-like" folds of the epithelium, numerous tubular glands, and the scattered cells of the lamina propria. (Hematoxylin-Eosin), 20X.

69b





(Greep and Weiss, 1973; Bloom and Fawcett, 1975) and include components of lymphoid and vascular tissues. There is constant movement of cells between connective tissues and the blood stream. In addition, cells of the immune system are located and function in connective tissues and lymphoid organs. Cells of the connective, vascular and immune systems are thus inextricably linked by both function and location.

An early and common response to tissue invasion by parasites is an initial nonspecific inflammation which is followed by more specific reactions such as encapsulation, calcification, or granuloma formation (see reviews above). The inflammatory response is a phenomenon of connective tissues mediated by a variety of cells including mast cells, leucocytes, and macrophages (Weiss and Greep, 1977; Cameron, 1967). The process typically begins with injury to tissues and a depolymerization of connective tissue ground substance. Capillaries dilatate, thus increasing blood supply to the area and capillary permeability increases, causing edema. Macrophages, eosinophils, mast cells, and other connective tissue cells accumulate in the immediate area, and circulating neutrophils, monocytes, and lymphocytes pass through the endothelial walls of capillaries to infiltrate the area. Local and infiltrating phagocytes (macrophages and neutrophils) attempt to engulf foreign material. This result is an acute phase of inflammation resulting in the typical signs of inflammation recognized by the ancients as tumor (swelling), rubor (redness), dolor (pain), and calor (yellowish aggregates of dead phagocytes). Following the acute phase, these signs subside and repair or encapsulation of the area proceeds. Concommitant with these processes are those involved in the development of an immune response which is mediated by cells involved in the inflammatory response and others, such as plasma cells.

The following description of the development of the lesions associated with E. uncinata in mallards involved the enumeration of specific types of cells which were prominent in the response. Eosinophils, heterophils, mast cells, lymphocytes, plasma cells, macrophages and blast cells were counted as described in Methods. Descriptions and functions of these cells from mallards are generally similar to the same types of cells described from mammals (Bloom and Fawcett, 1975; Weiss and Greep, 1977). Brief descriptions of these cells follow. Special characteristics of avian cells are taken chiefly from Lucas and Jamroz (1961).

Eosinophil

Eosinophils of birds are homologous to eosinophils of mammals (Lucas and Jamroz, 1961), as they are granulocytes containing numerous uniformly sized spherical granules which stain bright red-orange with eosin. They typically have a lobed nucleus (mostly two or three lobes) which stains well with basic dyes and in which the cromatin is in the form of coarse heterochromatic clumps. In blood smears stained with

Wright's stain, the ctyoplasm stains light blue where the granules are not too concentrated. Lucas and Jamroz (1961) indicate that these two characteristics best differentiate eosinophils from heterophils and that the shape of the granules is somewhat variable.

Eosinophils are relatively rare in differential counts of smears or hemacytometer counts of various avian bloods, ranging from one to seven percent of the total leucocyte count (Twisselman, 1939; Lucas and Jamroz, 1961; Threlfall, 1966). Lucas and Jamroz reported that mallard duck eosinohpils comprised seven percent of differential counts.

Greep and Weiss (1973) indicated that eosinophils are motile phagocytic cells containing lysosome-like specific granules and must therefore have bacterocidal properties. Their granules also contain considerable amounts of myeloperoxidase which could further contribute to bactericidal activities. These cells are often associated with antigen-antibody complexes which they are thought to engulf, thus participating in the processing of antigen and development of an immune response (Deane, 1964).

Greep and Weiss (1973) suggested that eosinophils may dampen effects of the inflammatory process by sequestering the antigen-antibody complex which could initiate the complex and often pathological inflammatory process. They also indicate that eosinophils are attracted to mast cells or their secretions and suggest that this may also indicate that eosinophils act antagonistically to basophils in terms of their action in allergic responses (see below).

Heterophil

Lucas and Jamroz (1961) indicate that heterophils of birds are homologous to neutrophils of mammals. They are easily confused with eosinophils because they contain eosinophilic granules and a multilobed nucleus. They have often been called rod eosinophils because the cytoplasmic granules are most often in the form of distinct fusiform rods which are pointed at both ends and stain brilliantly with eosin. The nucleus is reported to stain poorly in certain regions resulting in typical chromophobic bands of Lucas and Jamroz (1961). The number of nuclear lobes is, on the average, greater than in eosinophils, and the cytoplasm does not stain basophilically. In sections, heterophils and eosinophils were differentiated by the shape and staining characteristics of the granules and the nucleus. Often, because of the plane of section, the granules of heterophils appeared spherical, but rod shapes were usually visible in the same cell. The nucleus of eosinophils usually stained completely and the granules were often less distinct than those of heterophils, stained more orange than red, or were refractory; all important differentiating characteristics as noted by Lucas and Jamroz (1961). The granules of rabbit heterophils stained a deeper red with Giemsa stain than did those of eosinophils (Westermann and Engelbert, 1969a).

Heterophils are numerous in avian bloods, comprising 28-36% of total leucocytes in chickens (Twisselman, 1939), 17-36% in herring gulls (Threlfall, 1966), 48% in the mallard duck and 39% in the Canada goose (Lucas and Jamroz, 1961).

Mast Cell

Mast cells are common in connective tissue and are readily identified by the large, metachromatic granules in the cytoplasm which stain with basic aniline dyes (Bloom and Fawcett, 1975).

Granules of human mast cells contain heparin and histamine and have been shown to release other mediators such as slow reacting substance of anaphylaxis (SRS-A), and eosinophil chemotactic factor of anaphylaxis (ECF-A) (Weiss and Greep, 1977). These mediators are released from mast cells upon combination of surface receptors with specific reaginic antibody (IgE) and antigen. They are thus important in immediate type hypersensitivity reactions.

Mast cells appeared as large cells with large granules which stained deep purple with Giemsa-Thionin or Toluidine Blue, often obscurring the nucleus. Cells were often irregular in outline and granules were often observed outside of cells but in close association with them. No problems were encountered in differentiating between mast cells and eosinophilic granulocytes.

Lymphocyte

Lymphocytes are mononuclear leucocytes usually classified as small, medium, or large, depending on the nuclear/cytoplasmic ratio. The nuclei are generally round, intensely basophilic with numerous blocks of heterochromatin scattered throughout the nucleoplasm, often concentrated near the nuclear membrane. The cytoplasm is usually a narrow rim around the nucleus which stains basophilically and which lacks specific granules (Weiss and Greep, 1977).

Lymphocytes are central cells of the immune system, being involved in aspects of both humoral and cell mediated immunity (see above). Lymphocytes move freely between vascular and tissue compartments, and cells in either area are identical to those from the other. They were relatively easily identified in tissue sections and were similar to cells described from mammals (Weiss, 1972; Bloom and Fawcett, 1975; Weiss and Greep, 1977) and domestic chickens and ducks (Lucas and Jamroz, 1961). Lymphocytes comprised 32-69% of circulating leucocytes from a variety of birds (Twisselmann, 1939; Lucas and Jamroz, 1961; Threlfall, 1966).

Plasma Cell

Plasma cells are essentially unicellular glands concerned with the production of "antibody protein" (Weiss and Greep, 1977). They are mononuclear lymphoid cells of the B or bursa dependent line and are characterized by an abundant basophilic cytoplasm, a large Golgi apparatus lying in the cytocentrum, and an eccentrically placed nucleus. Blocks of heterochromatin are located near the nuclear membrane and are connected to the nucleolus by thin strands of chromatin. The effect of the latter is to give a characteristic "cart wheel" appearance to the nucleus.

Plasma cells may be numerous in the spleen and lymph nodes but are found in variable numbers throughout vertebrate connective tissues. Lucas and Jamroz (1961) indicated that numerous plasma cells could be found in loose connective tissues of the domestic chicken. Their description of mature plasmacytes agrees with that described above as representative of vertebrates in general. They also indicated that immature plasma cells often showed vacuolated regions of cytoplasm near the cytocentrum.

Plasma cells enumerated by myself were identified on the basis of the above descriptions. Cells with the general appearance of immature plasmacytes of Lucas and Jamroz (1961) were also encountered.

Macrophage

Macrophages are large mononuclear phagocytic cells confined to connective tissues (histiocytes) where they may be fixed or freely mobile. The precursors of macrophages are considered to be bone marrow derived monocytes which enter the tissues by way of the blood stream (van Furth, 1975). Kupffer cells of the liver, alveolar macrophages of the lung, free and fixed macrophages of the spleen and lymph nodes, and pleural and peritoneal macrophages of serous cavities are various types of macrophages classified according to location and relative mobility. Monocytes, the macrophages above, plus osteoclasts, and microglial cells of the nervous system are considered by van Furth to belong to the Mononuclear Phagocyte System (MPS) which is a refinement of the old Reticuloendothelial System (RES) of Aschoff (Weiss and Greep, 1977, chapter 4).

Quiescent macrophages are difficult to identify since they can be confused with fibroblasts or lymphoid cells. Activated macrophages however, have abundant, often irregularly dispersed cytoplasms containing vacuoles and phagosomes which often contain exogenous particles. The nucleus is often indented or irregular, has one or more prominent nucleoli and finely dispersed chromatin (Bloom and Fawcett, 1975; Weiss and Greep, 1977). The cytoplasm is less basophilic and more irregular than lymphoid elements, and the nucleus is smaller, more rounded, and contains more chromatin than that of fibroblasts. It was on this basis that macrophages were enumerated.

Macrophages are thought to be the precursors of giant cells; multinucleate masses representing fused macrophages. Such formations are commonly associated with foreign bodies (i.e., in tuberculosis, leprosy, helminth infections) and granulomata. Alternatively, in chronic granulomas, macrophages may establish tight junctions, become less phagocytic, and show fewer lysosomes; they are then called epithelioid cells (Cameron, 1967; Weiss and Greep, 1977, Chapter 4). When such formations were encountered they were not enumerated as macrophages but were simply noted, since it was assumed they were macrophage derivatives.

Blast Cell

There exist two strongly divergent views with regard to the origin of blood cells and their counterparts in the tissues. These views have been formally called the Polyphyletic and Monophyletic Schools of Haemopoiesis (Weiss and Greep, 1977). Proponents of the Polyphyletic School suggest that a separate stem cell exists for each of the cells: erythrocytes, granulocytes, lymphocytes, and monocytes. Those of the Monophyletic School suggest that there exists one stem cell, the haemocytoblast which is capable of differentiating to any of the blood cell types, and that the lymphocyte and haemocytoblast are equivalent. Greep and Weiss (1973, Chapter 11) indicate that the earliest recognizable blood cell precursors are cells which lack cytoplasmic granulation, pigment, or other evidence of belonging to a specific cell line. They closely resemble medium or large lymphocytes. Weiss and Greep (1977) reviewed the recent evidence related to the origin of blood cells and suggested that there is indirect evidence in favour of the existence of a single stem cell which they called a "candidate stem cell" although it has the appearance of a small lymphocyte.

Cells answering the above description and/or were in mitosis were observed in the present work and were classified as blast cells. They were also similar to cells which have been called pyroninophilic blast cells by others (Ruitenberg and Dyzings, 1972; Karmanska *et al*. 1972).

Experimental Design

Fifty ducklings were infected at the age of four weeks with 40 *E. uncinata* larvae and divided into seven groups for necropsy after 5, 12, 19, 26, 33, 40, or 47 days. At necropsy, serum and tissue samples were obtained and processed as described previously. Initially, lesions were cut into small pieces, fixed in glutaraldehyde - osmium tetroxide, and embedded in epon. Other pieces were fixed in AFA, sublimate, Stieves or Bouin's and embedded in paraffin. It was hoped that the plastic embedded tissues could be used to assess the cellular details of the development of the lesions. This proved to be unsatisfactory and impractical so that additional birds were later included in the day 5, 19, 33 and 47 groups in order that methacrylate embedded tissues could be obtained. These could be fixed as much larger pieces than those used for epon embedding and could be more easily sectioned and stained, providing a much better overall picture of the lesions. The short supply of *Echinuria* larvae precluded the complete replication of all groups, and technical problems resulted in methacrylate blocks from the day 47 birds being unsuitable for sectioning.

Results

Histopathology

Day Five

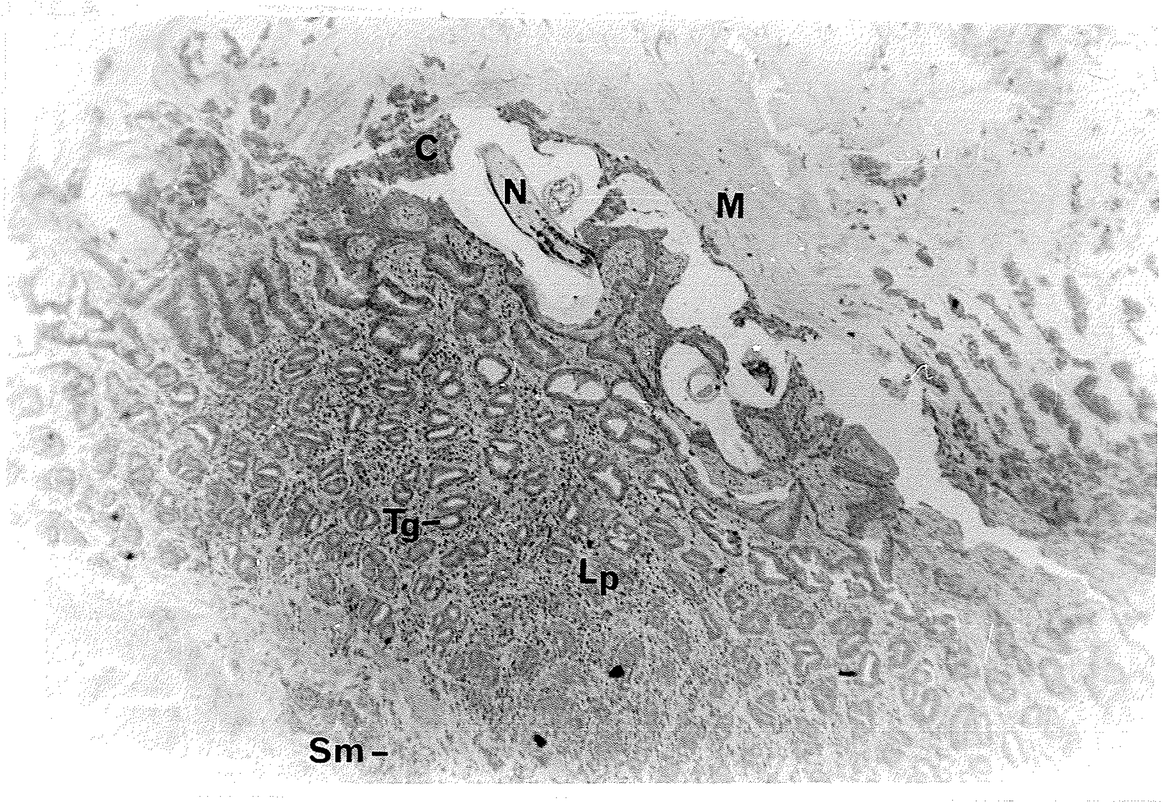
All eight ducklings necropsied five days postinfection (p.i.) had white, slightly raised regions of the mucosa in the proventriculus-ventriculus junction. These areas were usually diffuse, but if compact, were less than a mm in diameter. Some were associated with unidentified fibrous material but most were assumed to be the result of *E. uncinata* infection since nematodes were found in five such lesions from three different birds.

Nematodes were loosely attached to the mucosa and were penetrating the lamina propria between villi-like folds (Fig. 3). Immediately surrounding the worms were necrotic areas full of disintegrating cells and debris. Mucous production was stimulated and the surrounding lamina propria was heavily infiltrated with cells. Figure 4 shows a necrotic area of the mucosa containing nematodes, mucous and disintegrating cells and surrounded by markedly infiltrated "villi" with congested capillaries. Epithelial cells adjacent to the nematodes were undergoing dissolution, contributing to the cellular debris.

Outside the necrotic area the mucosa was markedly inflamed so that a "halo" of cells approximately 0.5mm in diameter surrounded the

Figure 3. Tangential section of the isthmus containing nematodes (N) loosely attached to the mucosa at day 5 postinfection. Note the mucous (M), the cellular debris (C) near the worms and the heavily infiltrated lamina propria (Lp). Sm= submucosa, Tg= tubular mucosal gland. (Giemsa-Thionin), 10X.

Figure 4. Section of a necrotic area of the mucosa 5 days postinfection containing nematodes and surrounded by congested "villi". Note the mucous and cellular debris (MC) next to the worms, the disintegrating epithelial lining of the "villi" (El), and the extensively infiltrated lamina propria (Lp) with dilatated vessels. (Giemsa-Thionin), 20X.


C
N
M
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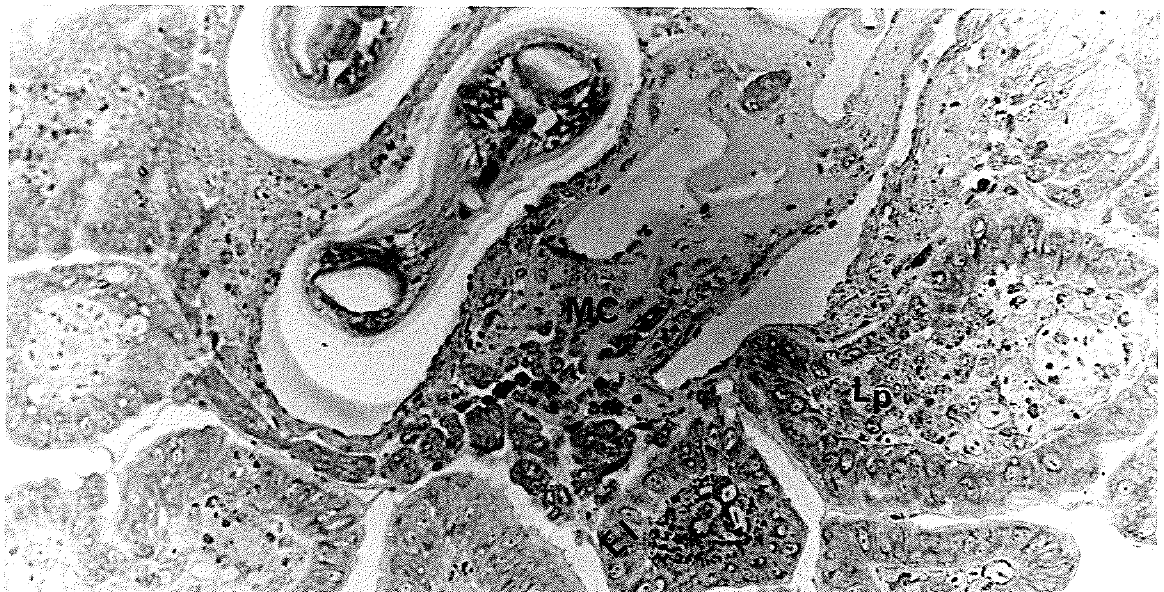
Lp
Sm


MC
Lp
E

nematodes (Fig. 5). Eosinophilic granulocytes were the most common of the infiltrating cells but lymphocytes, mast cells and macrophages were abundant. Note the numerous dilatated and congested vessels thoughout the mucosa.

Vessels in the submucosa and traversing the muscularis were similarly dilatated and arterioles were surrounded by sheaths of lymphoid cells. Numerous aggregations of lymphoid cells occurred in the submucosa and in the connective tissue between muscle bundles. In sections of one lesion, the gastric glands were more numerous around a necrotic, edematous region near the site of worm attachment. The glands were concentrically arranged around this area which was encircled by large numbers of lymphocytes and blast cells (Fig. 6). Epithelial and connective tissue elements appeared to have been transforming to a lymphoid follicle.

The inner zone of lesions at day 5 p.i. included that area immediately adjacent to the worms and the inner portions of the markedly infiltrated halo. The middle zone was the outer regions of the halo and the deep submucosa, while the outer zone included the superficial submucosa and the mucosa at some distance from the primary region of disturbance. Table 2 summarizes the cell counts from four suitable lesions from day 5 birds. One way analysis of variance was performed to determine if the mean number of cells differed significantly between zones. The results (F ratio and associated probability) are presented in Table 2.

Eosinophilic granulocytes (chiefly heterophils), lymphocytes, and macrophages were the most numerous of the cells present. Lymphocytes and macrophages were uniformly distributed throughout the zones while both types of eosinophilic granulocytes were more numerous in the inner zone near the nematodes. Mast cells were less numerous but were uniformly distributed. Blast cells were relatively rare but more numerous in the outer zone. Few recognizeable plasma cells were seen. Cell counts from uninfected controls (Appendix I) showed that eosinophilic granulocytes, lymphocytes and macrophages were much more abundant than normal in lesions at day 5 p.i.

The entire area of marked infiltration as seen in sections was roughly 1.5mm in diameter. The area of intense infiltration, including the halo of cells around the worms, had a mean diameter of 0.50 mm (±0.19mm). Sectioned worms had a mean diameter of 0.08mm (±0.01mm).

Day Twelve

Three of five ducklings had gross lesions in the mucosa of the proventriculus-ventriculus junction. There was a mean of three lesions per bird with a mean external diameter of 5.5mm (Table 3). The lesions were distinct, palpable nodules with centrally located necrotic openings surrounded by an inflammed mucosa. Two such lesions were fixed in sublimate or AFA, embedded in paraffin and sectioned.

Sections of nematodes in these lesions had a mean diameter

Table 2. Mean number of cells from the three zones of day five lesions.

TYPE OF CELL		[1]EG	EOSIN	HETER	MAST	LYMPH	BLAST	MACRO
Zone	[2]N							
Outer	40	[3]1.85 (1.14)	0.21 (0.50)	1.78 (1.13)	0.92 (0.82)	1.78 (1.26)	0.44 (0.70)	1.15 (0.94)
Middle	40	2.60 (1.25)	0.37 (0.63)	2.52 (1.27)	0.87 (0.70)	1.85 (1.11)	0.30 (0.54)	1.21 (1.03)
Inner	40	3.37 (0.59)	1.34 (0.83)	3.05 (0.94)	0.75 (0.85)	1.58 (0.96)	0.28 (0.55)	1.58 (1.27)
Combined	120	2.61 (1.20)	0.64 (0.91)	2.45 (1.23)	0.84 (0.78)	1.74 (1.12)	0.34 (0.60)	1.31 (1.10)
[4]F(2 & 117 df)		21.48	25.47	12.88	0.516	0.629	0.772	1.871
p<		.001	.001	.001	ns	ns	ns	ns

1. EG = eosinophilic granulocyte, EOSIN = eosinophil, HETER = heterophil, LYMPH = lymphocyte, MACRO = macrophage.
2. Number of fields included in the counts.
3. Values given are Naperian logarithms of actual counts with standard deviations in parentheses.
4. Results of one way anovar with probability below.

Table 3. Means of several parameters from day twelve, nineteen, and twenty-six lesions

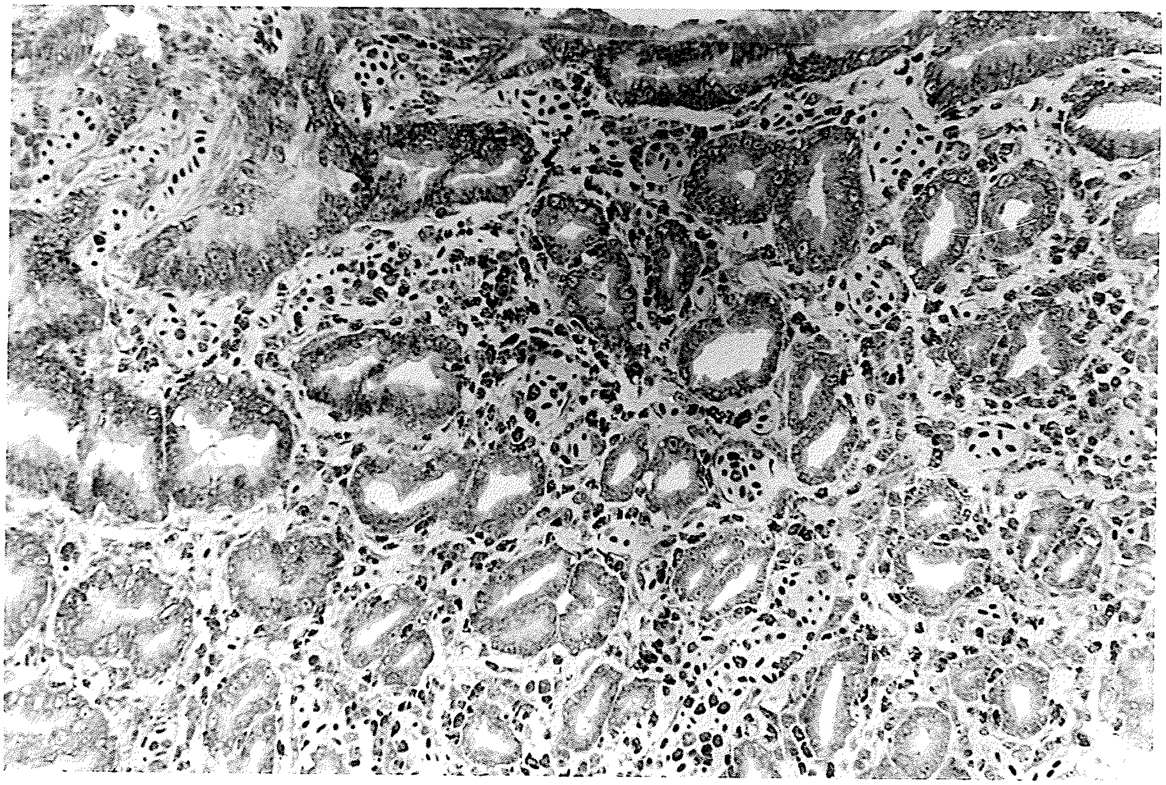
DAYS POSTINFECTION	NUMBER OF LESIONS	EXTERNAL DIAMETER	DIMENSIONS OF SECTIONED LESIONS			DIMENSIONS OF CAVITY		DIAMETER OF NEMATODES
			WIDTH	DEPTH	WALLS	WIDTH	DEPTH	
Twelve	[1]2.3 (1.0)	[2]5.5 (1.0)	0.91 (0.08)	0.85 (0.11)	0.22 (0.06)	0.41 (0.08)	0.58 (0.17)	0.07 (0.01)
Nineteen	2.9 (1.3)	5.1 (1.6)	1.93 (0.52)	1.82 (0.52)	0.38 (0.14)	0.66 (0.58)	0.87 (0.50)	0.10 (0.02)
Twenty-six	2.3 (1.2)	6.7 (2.4)	2.47 (0.21)	2.54 (0.31)	0.70 (0.31)	0.83 (0.14)	1.17 (0.21)	0.10 (0.02)

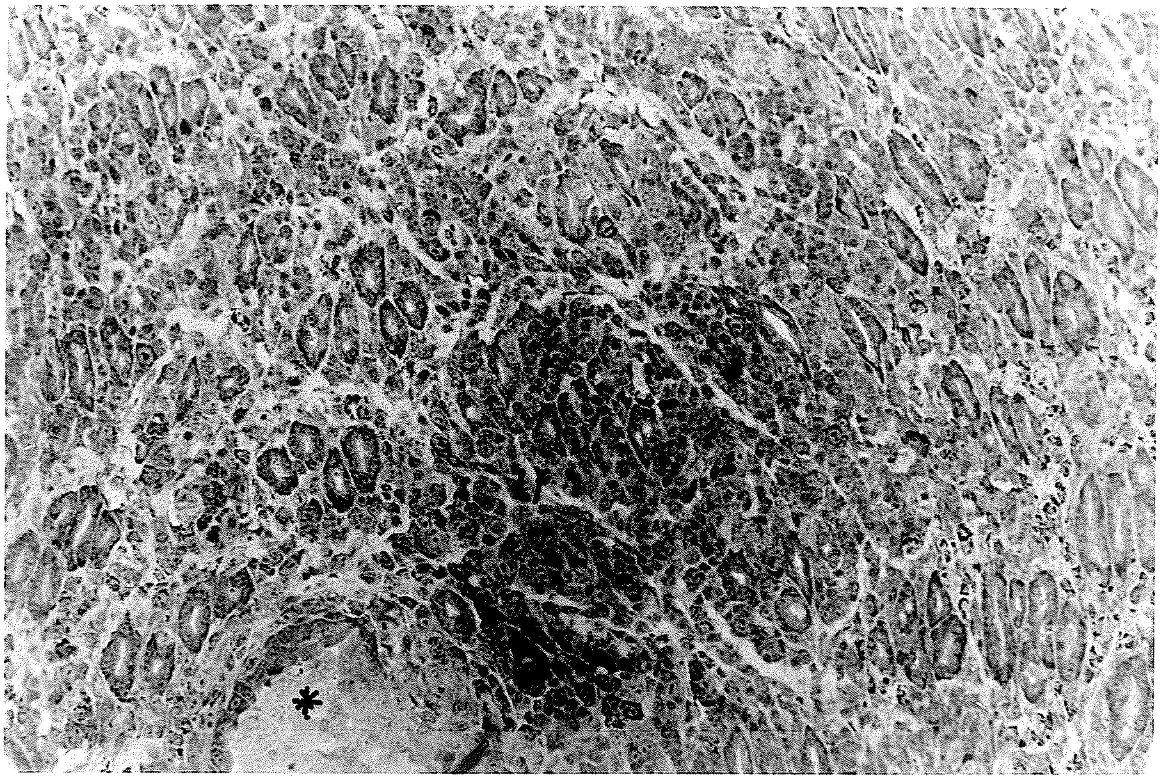
1. Numbers are means with standard deviations in parentheses below.

2. Measurements are in mm.

Figure 5. Tangential section of the epithelium and lamina propria at the edge of a lesion at day 5 postinfection in the markedly infiltrated "halo". Numerous granulocytes are present and there is extensive congestion of dilatated vessels. (Giemsa-Thionin), 20X.

Figure 6. Tangential section of the mucosa near a lesion at day 5 postinfection showing an early lymphoid follicle (Lf) near a necrotic area (*). Note the numerous lymphoid cells and granulocytes in the lamina propria. (Giemsa-Thionin), 20X.



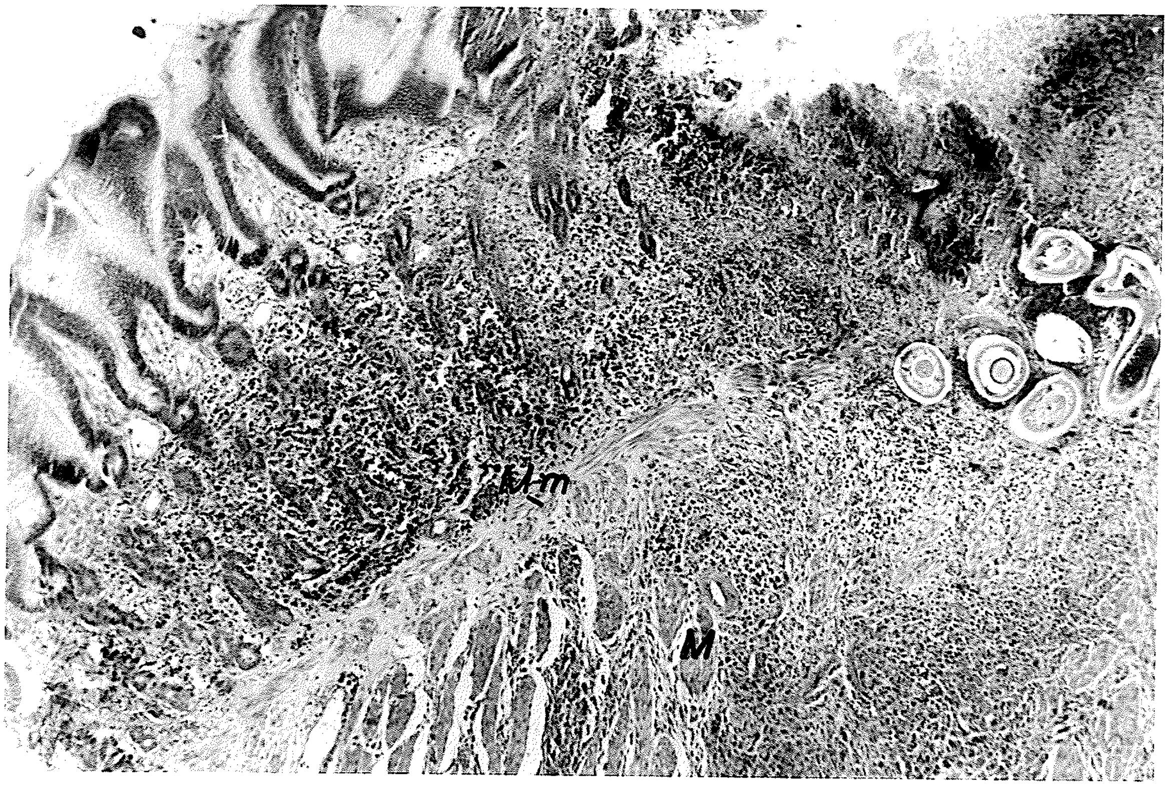


of 0.07mm (maximum 0.08). They were located in a necrotic area in the middle layer of muscle just deep to the muscularis mucosae (Figs. 7 and 8). The mucosa, submucosa and connective tissue between muscle bundles and in the adventitia were markedly infiltrated and contained numerous large lymphoid follicles, and aggregations of infiltrating cells, particularly at the base of the lesions (Figs. 7, 8 and 9). The whole noticeably inflamed area had a mean diameter of 3.26mm (4.72 and 1.80).

Within the general area described above was a relatively distinct fibrotic capsule which measured 0.91mm by 0.85mm (see Table 3), and which contained the nematodes in a relatively well defined cavity (Figs. 7 and 8). The cavity, which measured 0.41mm by 0.58mm, contained a necrotic, semi-solid mass of debris and opened to the mucosal surface via a necrotic channel (Fig. 10). The surface of the mucosa was eroded and the opening to the cavity occluded with a basophilic plug (Fig. 8). Rather indistinct walls 0.22mm thick surrounded the cavity and merged with the capsule and surrounding lymphoid aggregations. The inner portion of the wall was an edematous cellular network with numerous dilatated capilaries and lymph vessels (Fig. 10). Haemorrhage and a general infiltration of cells, particularly eosinophilic granulocytes, into the cavity are apparent in Figure 10. Giant cell formations such as the insert in Figure 10 were present as well but were not abundant.

Figure 7. Cross-section of a lesion at day 12 post-infection containing nematodes which have penetrated through the muscularis mucosae (Mm) into the muscularis (M). Note the basophilic mass extending from the worms to the eroded mucosal surface, and the heavily infiltrated lamina propria. (Eosin-Thionin), 10X.

Figure 8. Tangential section of a lesion at day 12 postinfection from a different bird than in Figure 7. Note the well defined fibrotic appearing wall (W) toward the mucosal surface, the open cavity partially filled with cellular debris and the nematode (*) which appears to be penetrating deeper into the muscularis. (Hematoxylin-Eosin), 50X.


Mm
M

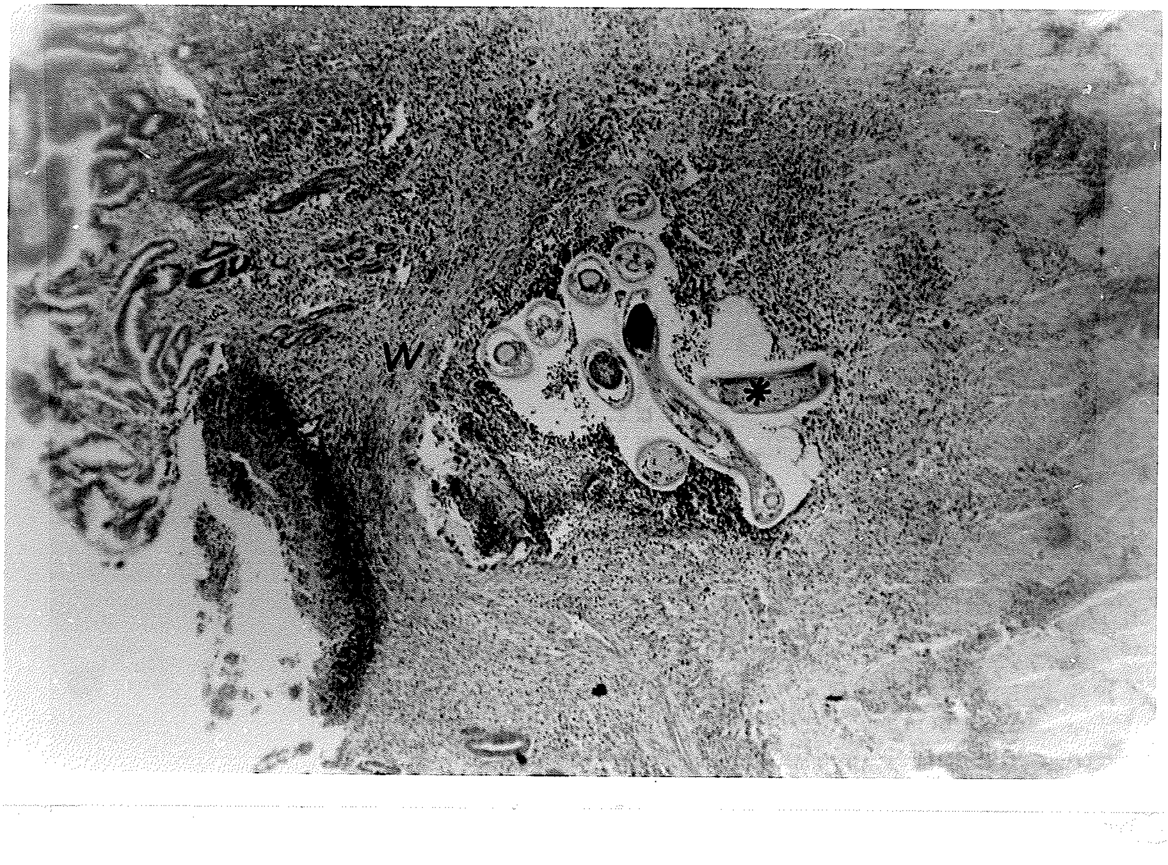
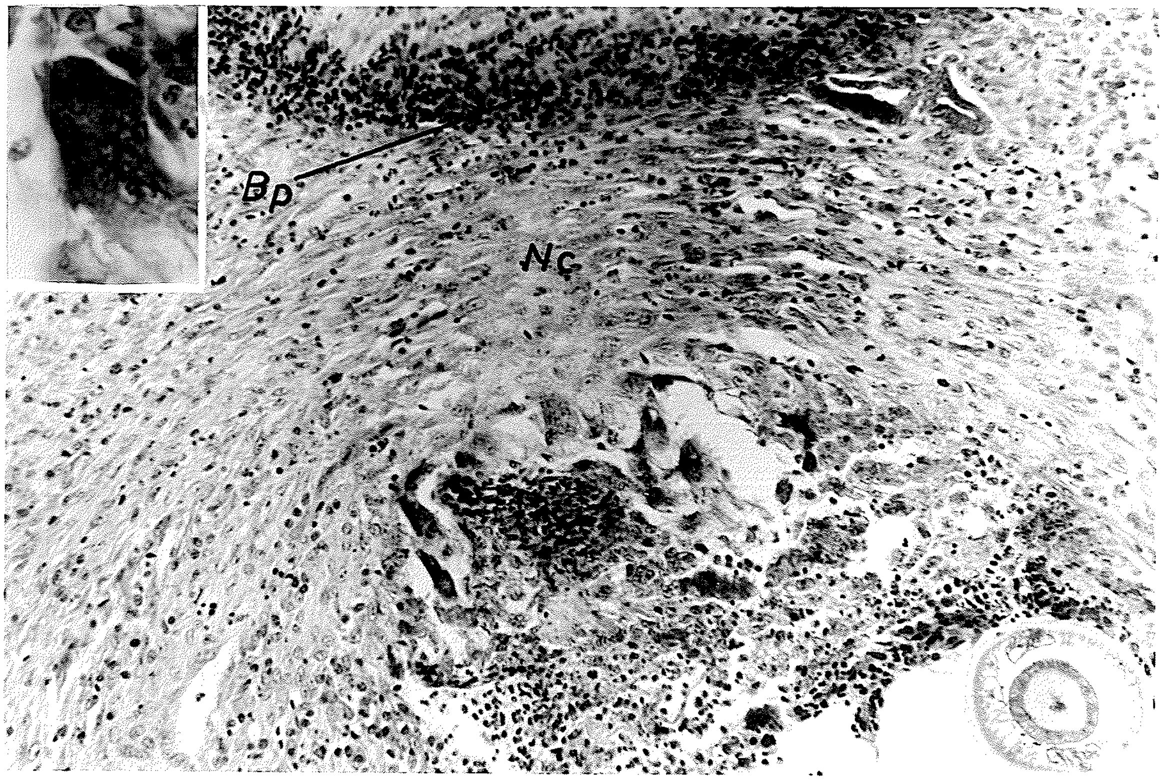

W
*

Figure 9. Lymphocytic proliferation around an artery in the deep muscularis of a lesion at day 12 postinfection. Ln= lymphatic nodule, M= muscle bundle, A= artery. (Eosin-Thionin), 20X.

Figure 10. Fibrotic wall of a lesion at day 12 postinfection showing an edematous, necrotic channel (Nc) extending between the cavity of the lesion and the basophilic plug (Bp) at the surface of the mucosa. Note the cellular infiltrate in the cavity and the dilatated vascular channels (Vc) in the wall. There are numerous fibroblasts, macrophages, and eosinophilic granulocytes throughout. The insert in the upper left is a giant cell formation near a nematode. (Hematoxylin-Eosin), 20X.


M
Ln
A


Bp
Nc

The mean numbers of various types of cells present in each of the three zones of day 12 lesions are presented in Table 4. The inner zone includes the region immediately adjacent to the nematodes and the inner portion of the walls. The middle zone includes the mid region of the walls to the outer region of the capsule where it merged with large lymphoid follicles, and, the deep submucosa. The outer zone includes the more superficial submucosa and the lamina propria at the periphery of the lesions. Large lymphoid follicles were avoided so as to enumerate "mobile cells" or those more closely associated with the lesion. It was not possible to differentiate between eosinophils and heterophils.

Eosinophilic granulocytes, lymphocytes and macrophages were numerous in all zones. Mast cells were less numerous but were present in all zones as well. Blast cells were relatively numerous except in the inner zone, and appreciable numbers of plasma cells were counted. The latter cells could be differentiated from lymphocytes in these paraffin processed tissues by means of their nuclear staining properties, the position of the nucleus in an abundant basophilic cytoplasm, and the presence of a Golgi body ghost. This could not be accomplished with certainty in the methacrylate processed tissues. Hence, those sections were not evaluated for plasma cells. The almost complete absence of plasma cells from day 5 lesions could have been due to this factor.

Table 4. Mean number of cells from the three zones of day twelve lesions[1].

TYPE OF CELL		EG	MAST	LYMPH	BLAST	MACRO	PLASMA
Zone	N						
Outer	20	3.36 (0.95)	1.17 (0.69)	3.26 (0.42)	0.94 (0.72)	2.28 (0.40)	0.38 (0.57)
Middle	20	3.04 (0.74)	1.12 (0.65)	3.66 (0.40)	1.34 (0.93)	2.06 (0.80)	0.52 (0.65)
Inner	20	2.44 (1.02)	0.97 (0.55)	2.67 (0.52)	0.04 (0.16)	2.94 (0.25)	0.04 (0.16)
Combined	60	2.95 (0.97)	1.09 (0.39)	3.20 (0.60)	0.77 (0.87)	2.43 (0.65)	0.31 (0.54)
F(2 & 57 df)		5.164	0.550	24.300	19.078	14.720	4.863
p<		.03	ns	.001	.001	.001	.025

[1] For an explanation see Table 2.

In terms of zonal distribution, plasma cells, lymphocytes, and blast cells were more numerous in the middle zone than elsewhere (see F ratios, Table 4). Macrophages were most numerous in the inner zone next to the nematodes. The means for eosinophilic granulocytes indicated that they were more numerous in the outer zone, but analysis of variance showed that there were no significant differences between zones.

Day Nineteen

All of nine ducklings necropsied at 19 days p.i. had gross lesions similar to those of day 12 birds. There were 2.9 lesions per bird, with a mean external diameter of 5.1mm (Table 3).

Five lesions from four different birds were fixed in glutaraldehyde, embedded in methacrylate, and sectioned. All were suitable for at least some measurements and four were suitable for the enumeration of specific infiltrating cells.

In sections, the lesions were similar to those of day 12 except that the lesion proper (capsule) was large ($F=15.166$, $p<.005$), averaging 1.93mm by 1.82mm (Table 3). Because of their size and the fact that they were embedded in methacrylate (necessitating smaller blocks), the full extent of the area of general infiltration is not known. Because the mean external diameters were not different I concluded that the inflamed areas were probably similar. The entire area visible in sections, including large lymphoid follicles, averaged 2.69mm by 2.40mm.

Lesions were located deeper in the circular muscle layer than at day 12 and were pushing out to the external longitudinal layer (Fig. 11). Large lymphoid follicles surrounded the lesion, particularly in an arc at the base near the outer layer of muscle (Figs. 11 and 12). These follicles often appeared to extend from areas of massive proliferation right to the cavity containing nematodes.

Between the peripheral follicles and the lesion proper was a layer of muscle which apparently encircled the capsule, some muscle bundles were seen in the wall itself (Fig. 11). Vessels with large lymphocytic sheaths also encircled the capsule and sent branches into the wall and to the mucosa; vessels in all areas were dilatated and congested. The tissue was generally edematous and villi near the opening of the lesion were broad, flattened and congested (Fig. 11), similar to those of earlier lesions (Figs. 4 and 7). Relatively distinct walls averaging 0.38mm thick surrounded an irregular cavity or cavities, which measured 0.66mm by 0.87mm (Table 3). The cavity was either fluid filled as in Figure 11, or contained a semi-solid mass of debris consisting chiefly of macrophages and granulocytes (Fig. 13). Worms present had a mean diameter of 0.10mm. Opening to the mucosal surface was an irregular channel containing a necrotic plug similar in composition to the contents of the cavity, and much like that of day 12 lesions (Fig. 10).

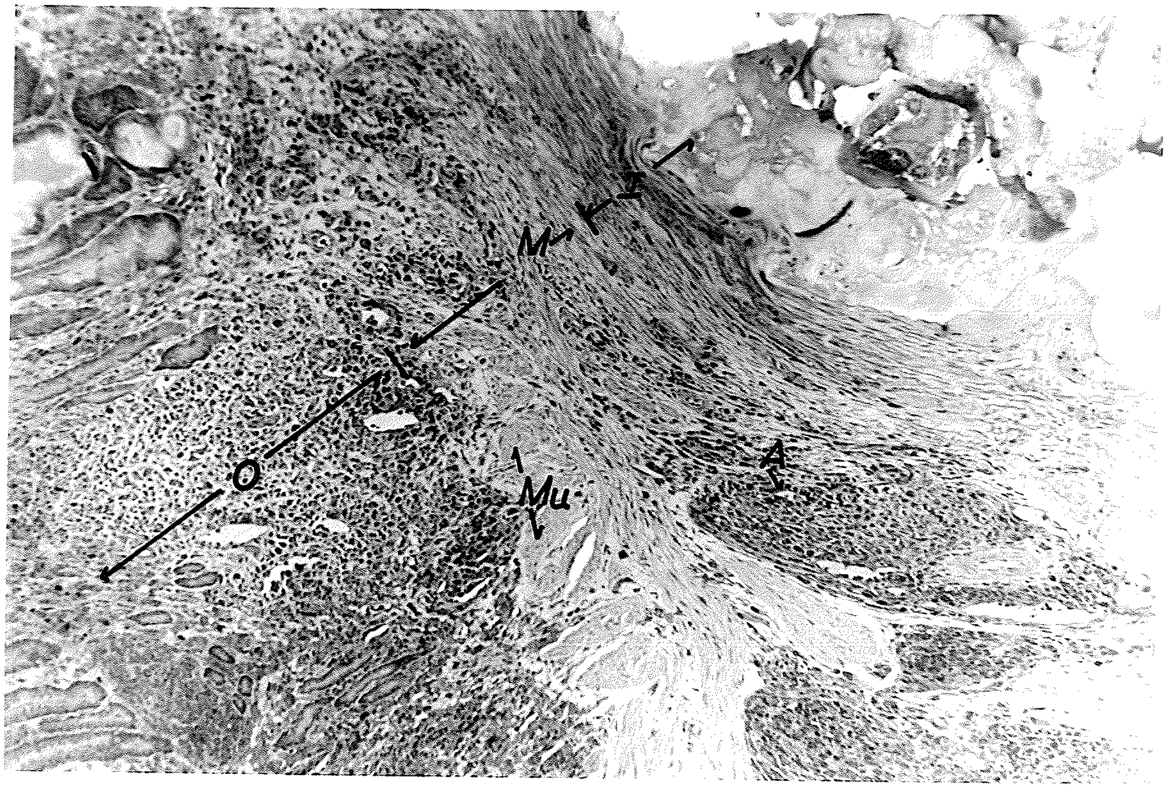
The inner portion of the wall immediately adjacent to the cavity was characterized by a narrow band of giant cells, individual macrophages, and numerous eosinophils (Figs. 13 and 14). External to this was an edematous region with apparently fluid filled spaces and expanded capillaries which had often haemorrhaged into the cavity (Figs. 13 and 14). Capillaries were frequently clogged with eosinophils. In some lesions this region was a cellular network having many heterophils, macrophages, fibroblasts and a few mast cells as in Figure 14. In others it appeared necrotic with few recognizeable cells. The two areas just described constitute the inner zone (see Fig. 11), which was similar to that of day 12 lesions except that at day 19, the distinct layer of giant cells illustrated in Figure 14 was present.

The inner zone blended with a much more cellular middle zone (Fig. 11), characterized by concentric sheets of leucocytes which appeared compressed or flattened (Fig. 13). The outermost region of this zone merged with large peripheral lymphoid follicles (Fig. 12) and contained remarkable numbers of granulocytes (chiefly heterophils), lymphocytes, blast cells and some plasma cells (Figs. 15 and 16). Isolated bundles of muscle and numerous small blood vessels were present in the middle zone as well.

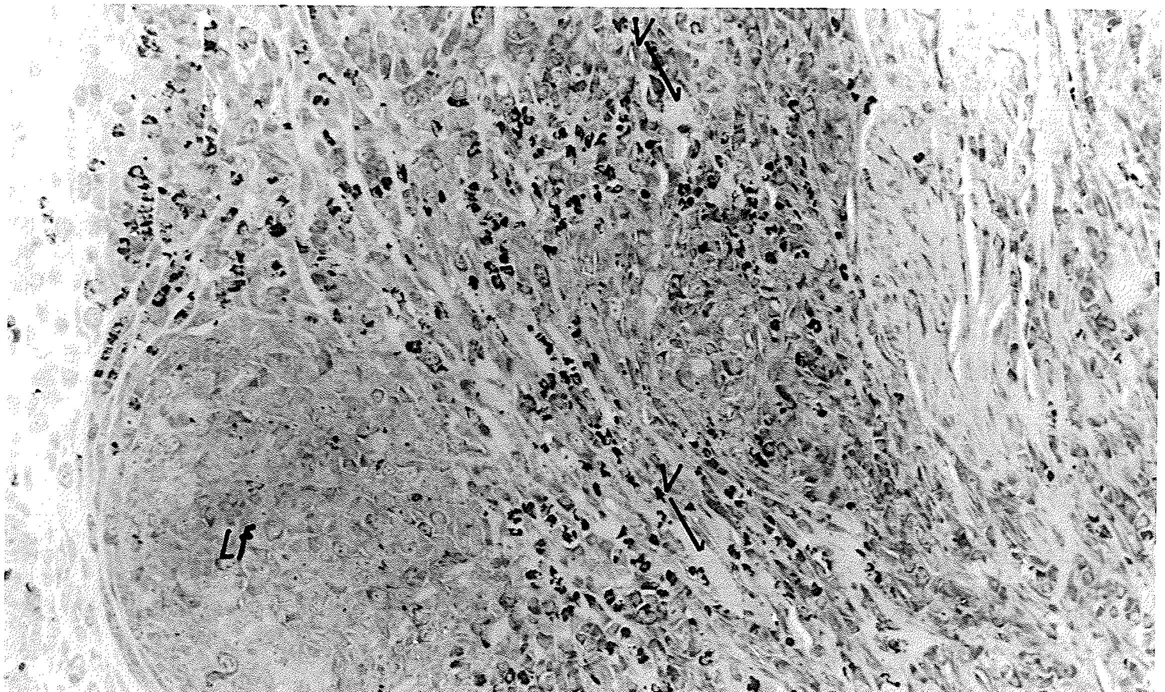
The outer zone of day 19 lesions, which included the submucosa and mucosa immediately adjacent to the opening of the lesion, was

Figure 11. A portion of a lesion at day 19 postinfection near the mucosal surface. Note the Inner (I), Middle (M), and Outer (O) zones, and the edge of the large open cavity containing a section of a nematode. Arterioles (A) are sheathed with lymphocytes, vessels are dilatated and congested, and the tissue appears generally edematous. The darkly staining cells are mostly heterophils. Note that muscle bundles (Mu) surround the lesion and form part of the wall in the Middle zone. (Giemsa-Thionin), 10X.

Figure 12. Outer region of the Middle zone of a lesion at day 19 postinfection. Two lymphoid follicles (Lf) are present, numerous granulocytes are visible, and the whole area is edematous. Numerous dilatated vessels are indicated (V). (Giemsa-Thionin), 20X.



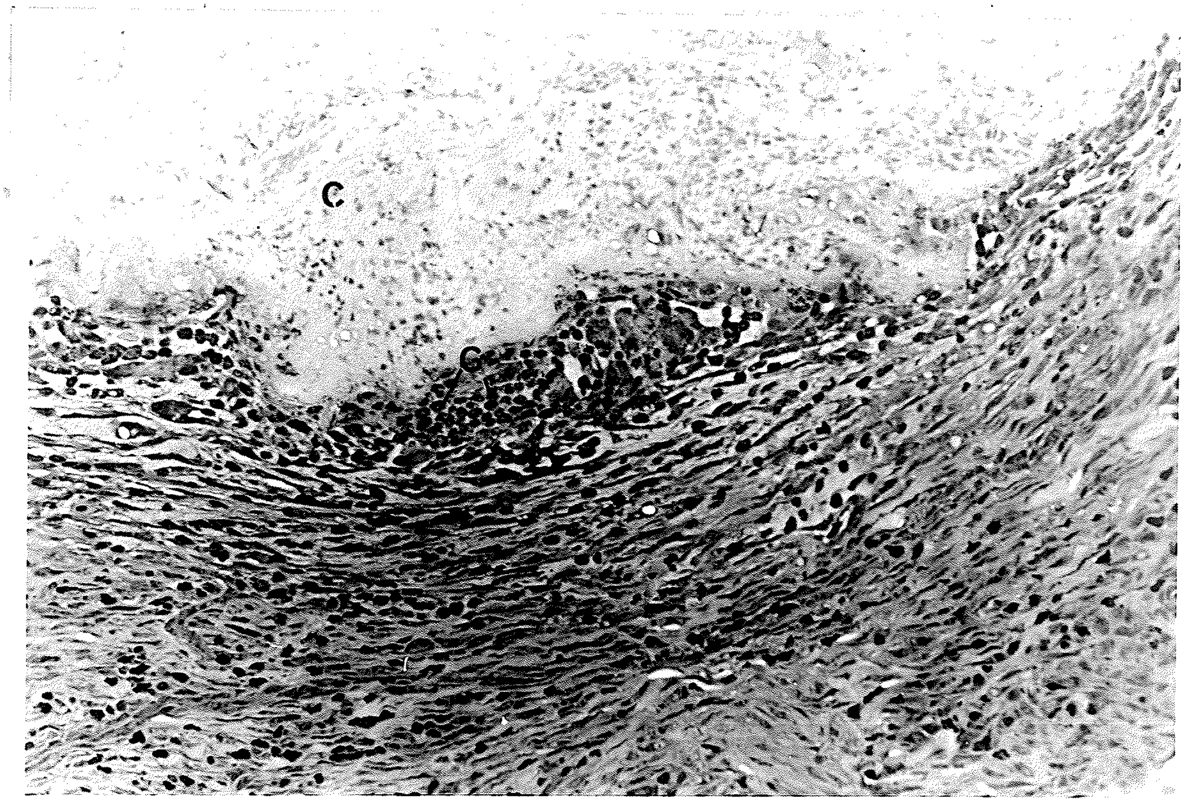
I
M
O
Mu
A



V
V
Lf

Figure 13. Cavity (C), Inner zone and part of the Middle zone of a lesion at day 19 post-infection. Note the cellular infiltrate in the fluid-filled cavity, the large number of granulocytes (G), particularly next to the cavity, and the edematous appearing wall. (Giemsa), 10X.

Figure 14. Inner zone of a lesion at day 19 postinfection showing epithelioid and giant cells (Gc) lining the periphery of the cavity. Eosinophils (E) are numerous in the cavity and an erythrocyte is also present (*), indicating that vessels had hemorrhaged into the lumen. (Giemsa-Thionin), 50X.


C
G

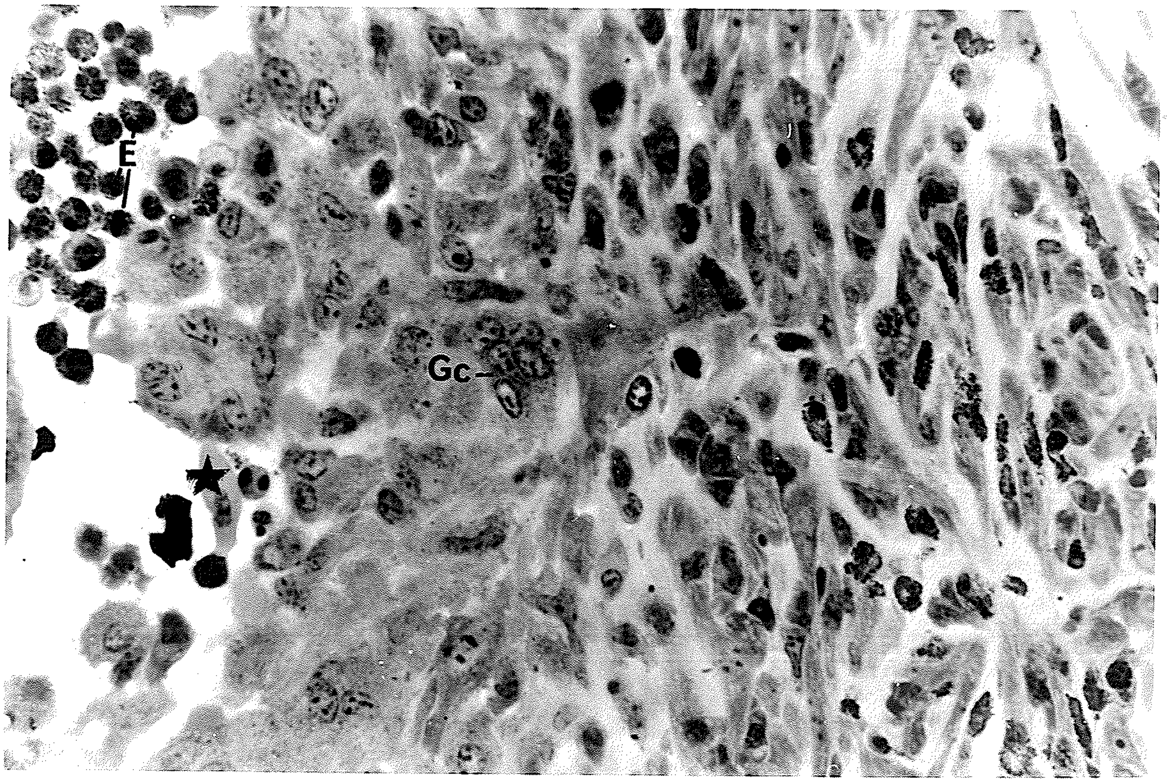
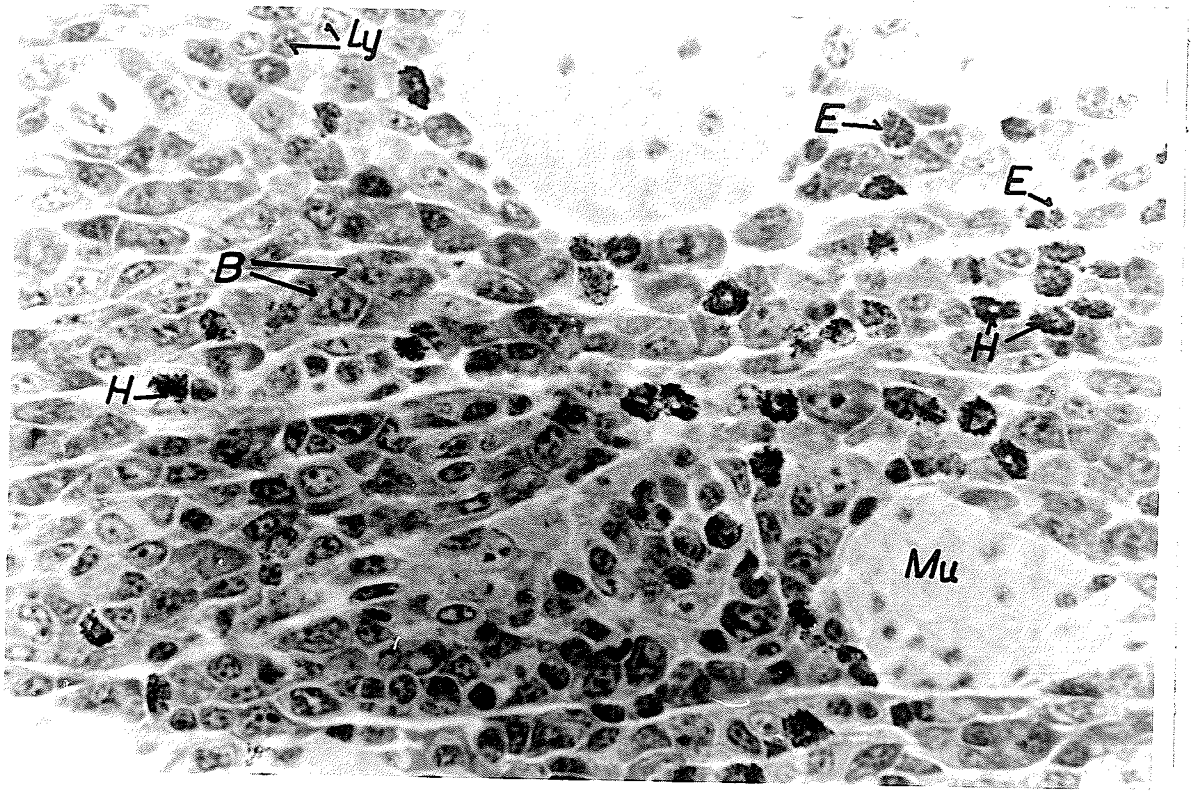
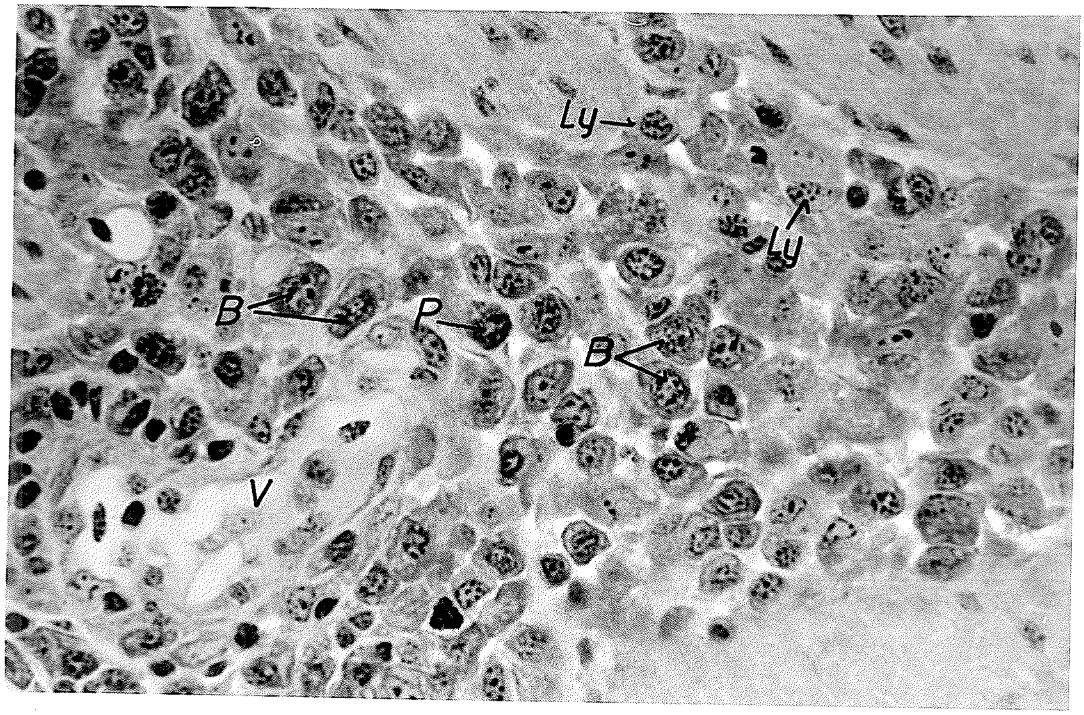

E
Gc
★

Figure 15. Middle zone of a lesion at day 19 post-infection. Eosinophils (E), heterophils (H), lymphocytes (Ly), and blast cells (B) are abundant. Mu= muscle bundles. (Giemsa-Thionin), 50X.

Figure 16. Middle zone of a lesion at day 19 post-infection showing large blast cells (B), lymphocytes (Ly) and plasma cells (P) around a vessel (V). (Giemsa-Thionin), 50X.


Ly
E
E
B
H
H
Mu


Ly
Ly
B
P
B
V

characterized by large numbers of heterophils, abundant mast cells and numerous congested blood vessels (Fig. 17). The epithelial cells of tubular glands showed degenerative changes, particularly vesiculation of nuclei.

Table 5 summarizes the numbers of various types of cells counted in the three zones of lesions from day 19. The most numerous cells were eosinophilic granulocytes (mostly heterophils), lymphocytes, and macrophages. Heterophils were uniformly distributed while eosinophils were much more numerous in the inner zone. Lymphocytes were more numerous in the middle and outer zones while macrophages were uniformly distributed. The number of macrophages reported in Table 5 for the inner zone does not include those nuclei associated with giant cell formations so that the distribution was really more to the inner zone than indicated. Mast cells were much less abundant that the other cells, but were uniformly distributed throughout the lesions. Blast cells were rare in the inner zone, moderately abundant in the outer zone and were significantly more numerous in the middle zone. Few plasma cells were counted, but as pointed out earlier, they could not be readily differentiated from other lymphoid cells in methacrylate sections. Most were perhaps included in the lymphocyte or blast cell categories.

Day Twenty-Six

Four of five ducklings necropsied on day 26 p.i. had gross

Figure 17. Outer zone of a lesion at day 19 postinfection. Portions of three tubular glands (Tg) are present showing vesiculated nuclei in epithelial cells. Congestion of vessels is marked (Cv). Most of the darkly staining cells are heterophils; a few are mast cells (Ma). (Giemsa-Thionin), 50X.


TG
Ma
CV
CV

Table 5. Mean number of cells from the three zones of day nineteen lesions[1].

TYPE OF CELL		EG	EOSIN	HETER	MAST	LYMPH	BLAST	MACRO
Zone	N							
Outer	40	2.55 (1.30)	0.22 (0.63)	2.49 (1.32)	0.51 (0.55)	2.38 (1.04)	0.58 (1.02)	1.77 (0.48)
Middle	40	2.13 (1.23)	0.46 (0.79)	1.94 (1.33)	0.40 (0.57)	2.70 (1.09)	1.67 (1.09)	1.99 (0.69)
Inner	40	2.80 (1.19)	2.11 (1.18)	1.85 (1.21)	0.44 (0.61)	1.69 (1.22)	0.17 (0.46)	1.82 (0.93)
Combined	120	2.50 (1.26)	0.93 (1.23)	2.09 (1.31)	0.45 (0.57)	2.26 (1.19)	0.81 (1.10)	1.86 (0.73)
F(2 & 117 df)		3.024	53.180	2.853	0.427	8.488	29.860	0.944
p<		ns	.001	ns	ns	.001	.001	ns

1. For an explanation see Table 2.

lesions with a mean of 2.3 per bird. The external diameters varied between 3.0 and 10.0mm, with a mean of 6.7mm (Table 3).

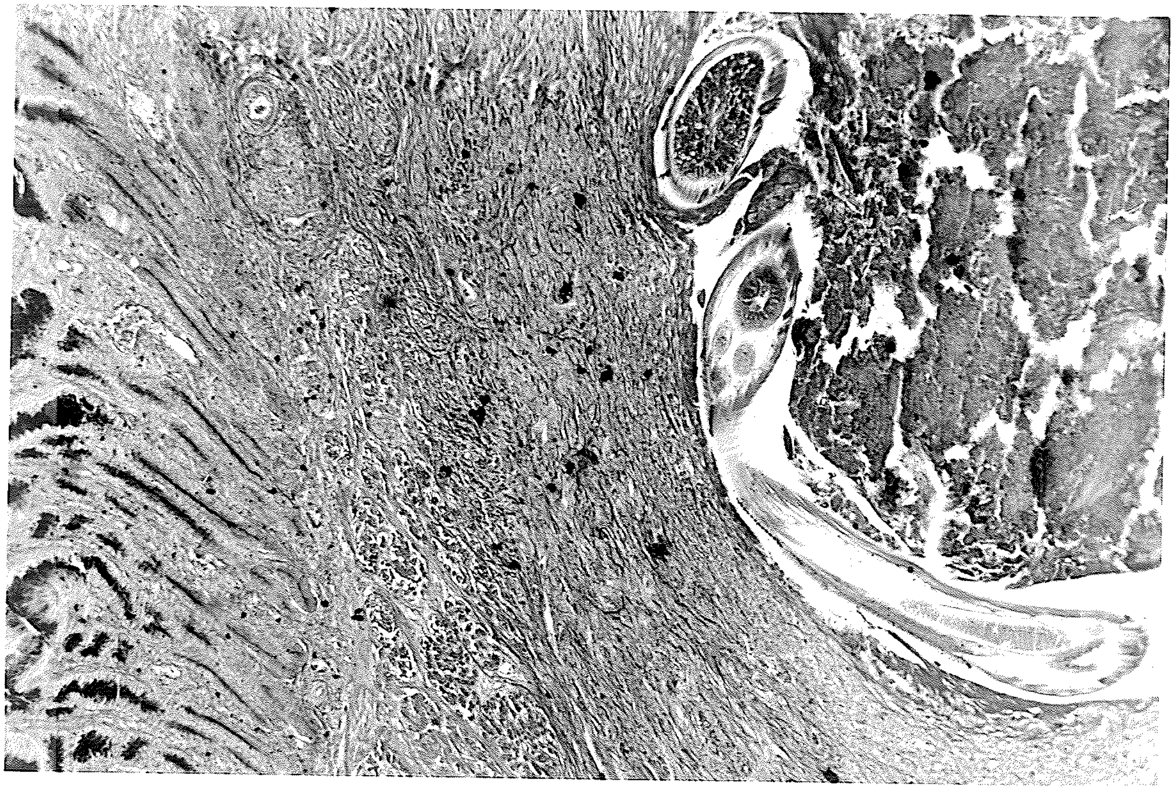
Two lesions were fixed in sublimate or AFA, embedded in paraffin and sectioned. The general histological features of these lesions were similar to those of day 19, being cellular, fibrotic, and located in the circular muscle layer (Figs. 18 and 19). Peripherally, there were large lymphoid follicles which appeared to have originated in the epimysium and other connective tissue sheaths between the circular and longitudinal muscle layers. Such aggregations are apparent in Figure 19, extending into the wall of the lesion.

The lesions of day 26 were larger than those of day 19 ($F=5.844$, $p<.05$), and had much thicker walls ($F=10.036$, $p<.01$)(Table 3). The distinct walls were fibrotic peripherally and punctuated by numerous arterioles and small sinuses (Fig. 19). The cavity containing the nematodes was slightly larger than the average at day 19, but not significantly so. It contained, besides nematodes, necrotic cellular debris and numerous eosinophilic granulocytes. It was surrounded by numerous giant cell formations as illustrated by Figure 20.

The mean diameter of sectioned worms was 0.10mm, not different from those of day 19. However, the larger of the two lesions examined had much thicker walls (1.02mm compared to 0.50mm) and a smaller cavity (0.73 by 1.02mm) compared to the other lesion (0.93 by 1.32mm). Nematodes in the smaller cavity (larger lesion) were of small diameter

Figure 18. Cross-section of a lesion at day 26 postinfection. The mucosa is to the left and the lesion is within the circular layer of the muscularis, bundles of which are sectioned at the lower left. Note the fibrotic wall and the almost solid contents of the cavity. (Mallory's), 10X.

Figure 19. A portion of a different lesion at day 26 postinfection. The section is through the base of the lesion parallel to the mucosal surface. Note the cellular infiltrate in the inner Middle zone (M), the vascular channels there and in the muscularis (Mu), and the lymphoid follicles at the periphery (Lf). (Mallory's), 10X.



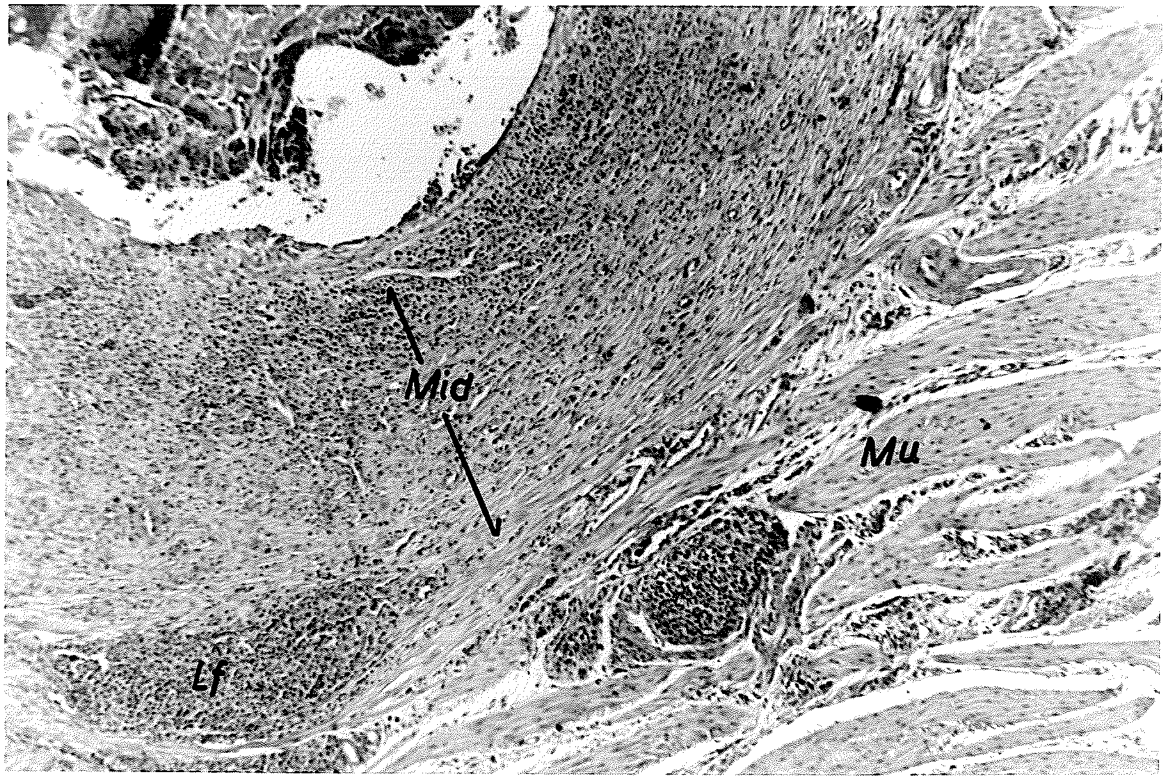
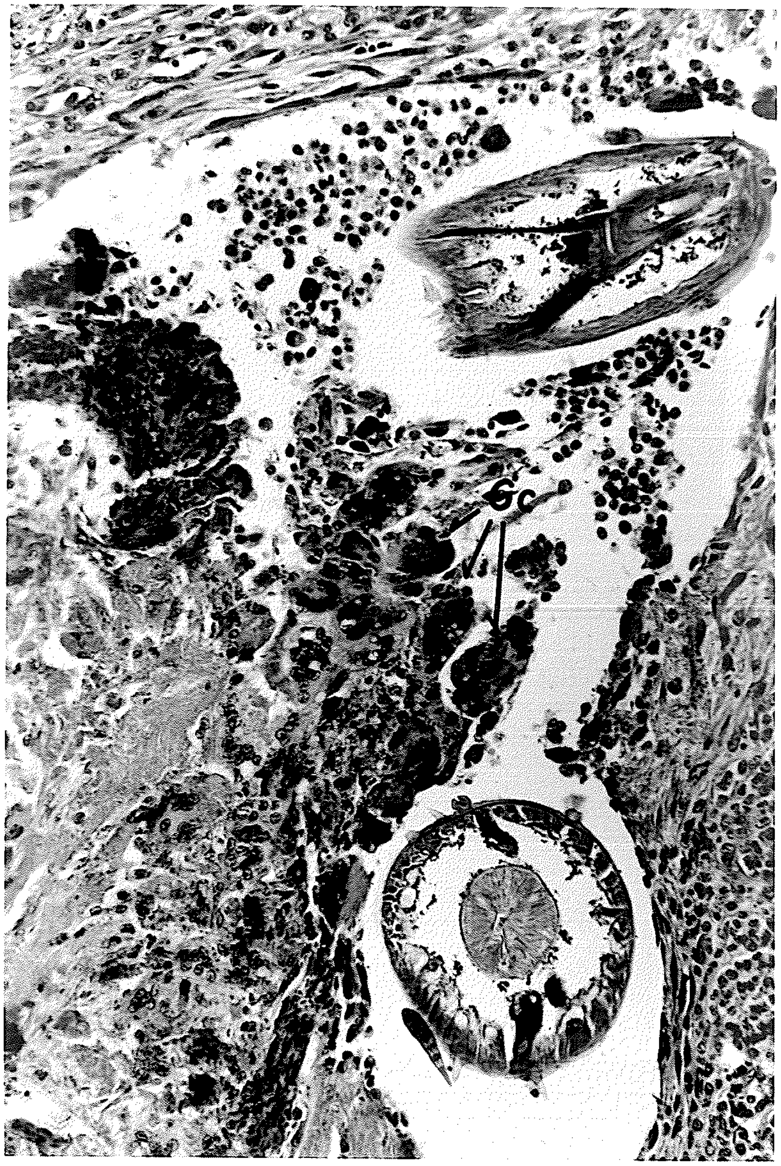

Mid
Mu
Lf

Figure 20. Nematodes in the cavity of a lesion at day 26 postinfection, with numerous eosinophilic granulocytes and giant cell formations (Gc) around them. The worm in the upper right appears to be in poor condition. (Eosin-Thionin), 20X.


Gc

(0.08mm) compared to those in the larger cavity (0.11mm). The lesion with the smaller worms opened to the surface via a wide necrotic channel and the cavity itself was packed solidly with debris. It appeared to be in the process of dissolution.

The mean numbers of various types of cells in the zones of these lesions are presented in Table 6. Eosinophilic granulocytes, lymphocytes, and macrophages were again abundant with lesser numbers of mast cells, plasma cells and blast cells. Eosinophilic granulocytes were much more numerous in the inner zone than elsewhere, as were macrophages. Mast cells were more numerous in the outer zone, while blast cells were more numerous in the middle zone. Plasma cells were moderately abundant but were essentially uniformly distributed. Any distributional tendencies that may have been present were masked by large deviations from the means in all zones.

Day Thirty-Three

All of ten ducklings necropsied at day 33 had gross lesions with a mean of 2.1 per duckling and a mean external diameter of 5.5mm (Table 7).

Five lesions from four different birds were fixed in glutaraldehyde and embedded in methacrylate. All yielded some measurements and four were useful for enumerating the cells involved.

Although the external diameters of lesions were not different

Table 6. Mean number of cells from the three zones of day twenty-six lesions.[1]

TYPE OF CELL		EG	MAST	LYMPH	BLAST	MACRO	PLASMA
Zone	N						
Outer	20	1.50 (1.20)	1.35 (0.48)	2.76 (0.90)	0.06 (0.25)	1.91 (0.54)	0.47 (0.58)
Middle	20	2.83 (0.75)	0.56 (0.65)	2.69 (0.82)	0.84 (0.76)	2.49 (0.46)	0.19 (0.42)
Inner	20	3.60 (0.53)	0.42 (0.55)	2.57 (0.46)	0.37 (0.78)	2.89 (0.36)	0.31 (0.57)
Combined	60	2.64 (1.23)	0.78 (0.69)	2.68 (0.74)	0.42 (0.71)	2.43 (0.61)	0.32 (0.53)
F(2 & 57 df)		29.720	15.890	0.335	7.552	23.050	1.353
p<		.001	.001	ns	.005	.001	ns

1. For an explanation see Table 2.

from those of day 26, the dimensions of sectioned lesions were greater (F=8.897, p<.025)(Tables 3 and 7).

Lesions were centrally located in the middle layer of muscle and were forcing the outer layer to bulge outward. Walls of lesions were not thicker than those of day 26, and though the cavities appeared larger, analysis of variance indicated there were no significant differences in these measurements. Sections of worms had a much greater mean diameter in day 33 lesions (F=122.096, p<.001). In one lesion, a female Echinuria contained shelled, embryonated ova measuring 0.031 by 0.019mm.

The outer zones of lesions were surrounded by lymphoid follicles, muscle bundles, large sheathed arteries and dilatated vessels, as described for days 19 and 26 p.i.

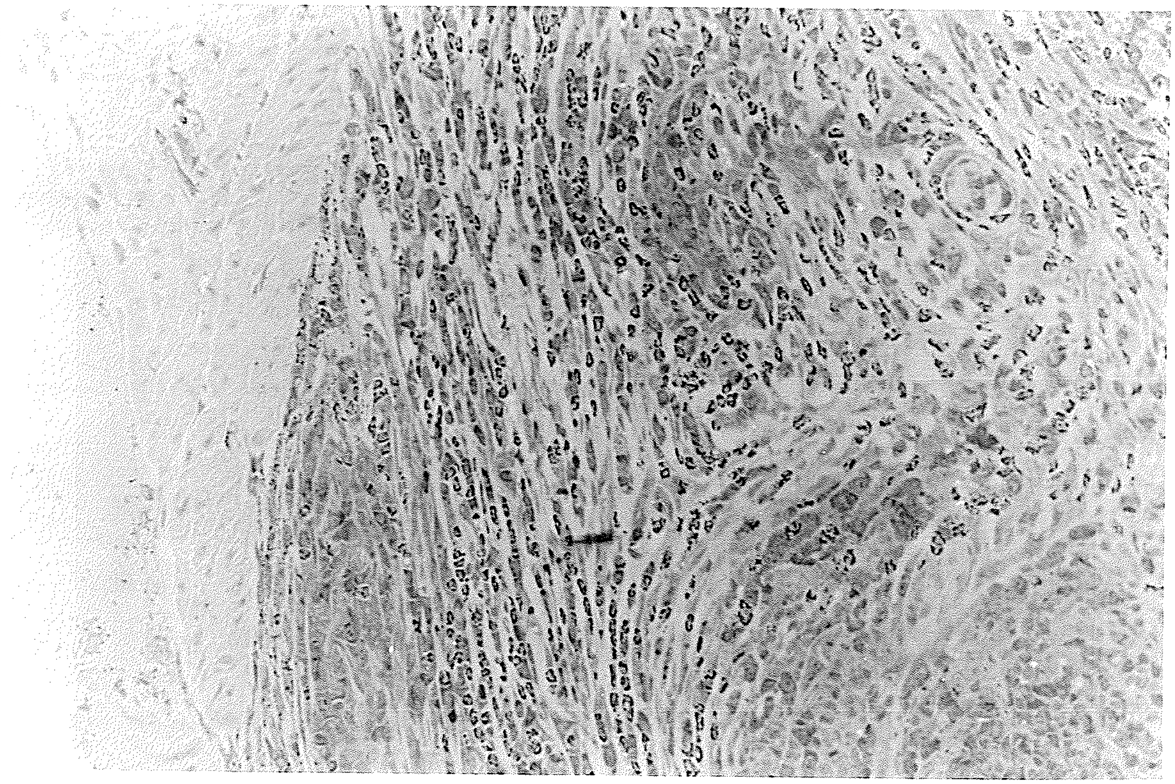
The middle zone of most lesions was characterized by an outer relatively fibrous area in which large numbers of cells (especially granulocytes) were arranged in concentric sheets (Fig. 21); these appeared compressed as though an outward pressure was being exerted from the inner regions of the wall or cavity. The major part of the middle zone in most lesions was composed of an edematous, highly vascular network of cells and sinuses as described for earlier lesions, and the spaces were filled with blood, or at least with fluid.

The inner zone was variable, but in most lesions, blended with the edematous, cellular middle zone with no marked change in cellular arrangement. Figure 22 shows the irregular edge of the wall of one lesion bordering on a fluid filled cavity. Clusters of macrophages, lymphocytes and heterophils are shown separated by numerous vascular channels which appear to drain into the lumen of the cavity. In another lesion, the inner zone consists of similar clusters of lymphocytes, granulocytes, macrophages and some plasma cells in a more compact arrangement (Fig. 23). One lesion (not shown), containing dead or moribund worms, had a cavity packed with cellular debris and completely surrounded by giant cells, similar to that shown in Figure 20 from day 26; exceptions were areas where vessels had haemorrhaged to the lumen. A second lesion with a cavity full of debris had a few giant cells around the cavity and contained normal, healthy appearing nematodes.

The number and distribution of various types of cells were similar to day 26 (Table 8). Heterophils were the dominant granulocyte and were most abundant in the middle and inner zones. Eosinophils were not numerous and were distributed uniformly throughout all three zones. Lymphocytes were most numerous in the middle zone, while macrophages were most numerous in the inner zone. Again, the macrophage counts do not include those cells associated with giant cell formations so the value in Table 8 for inner zone macrophages is minimal. Mast cells were uniformly distributed throughout the lesions.

Figure 21. Outer region of the Middle zone from a lesion at day 33 postinfection showing compressed, concentrically arranged tracts of cells (chiefly granulocytes and lymphocytes). (Giemsa-Thionin), 20X.

Figure 22. Inner zone of a lesion at day 33 postinfection showing clusters of macrophages, lymphocytes and heterophils. There appear to be numerous channels between the cavity (**) and vessels entering the wall (V). (Giemsa-Thionin), 50X.



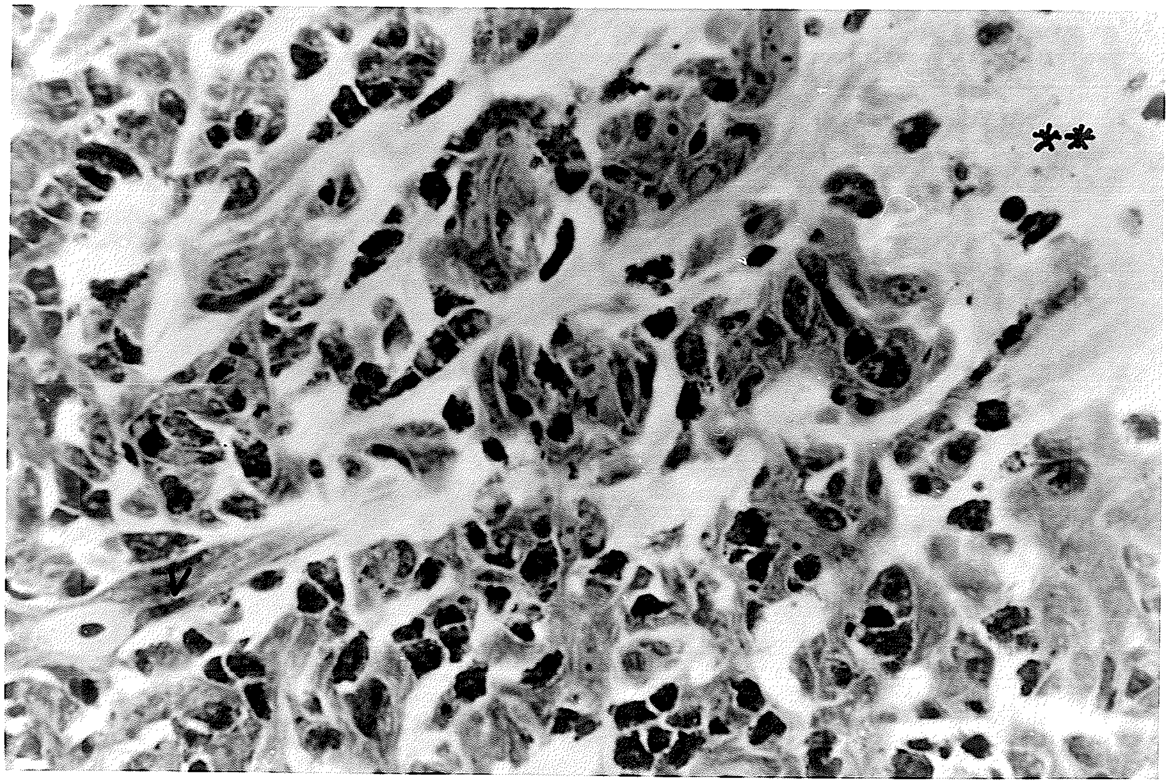


Figure 23. Inner zone of a lesion at day 33 post-infection showing very large accumulations of lymphocytes (Ly), plasma cells (P), macrophages (Mc), and granulocytes (G). (Giemsa-Thionin), 50X.

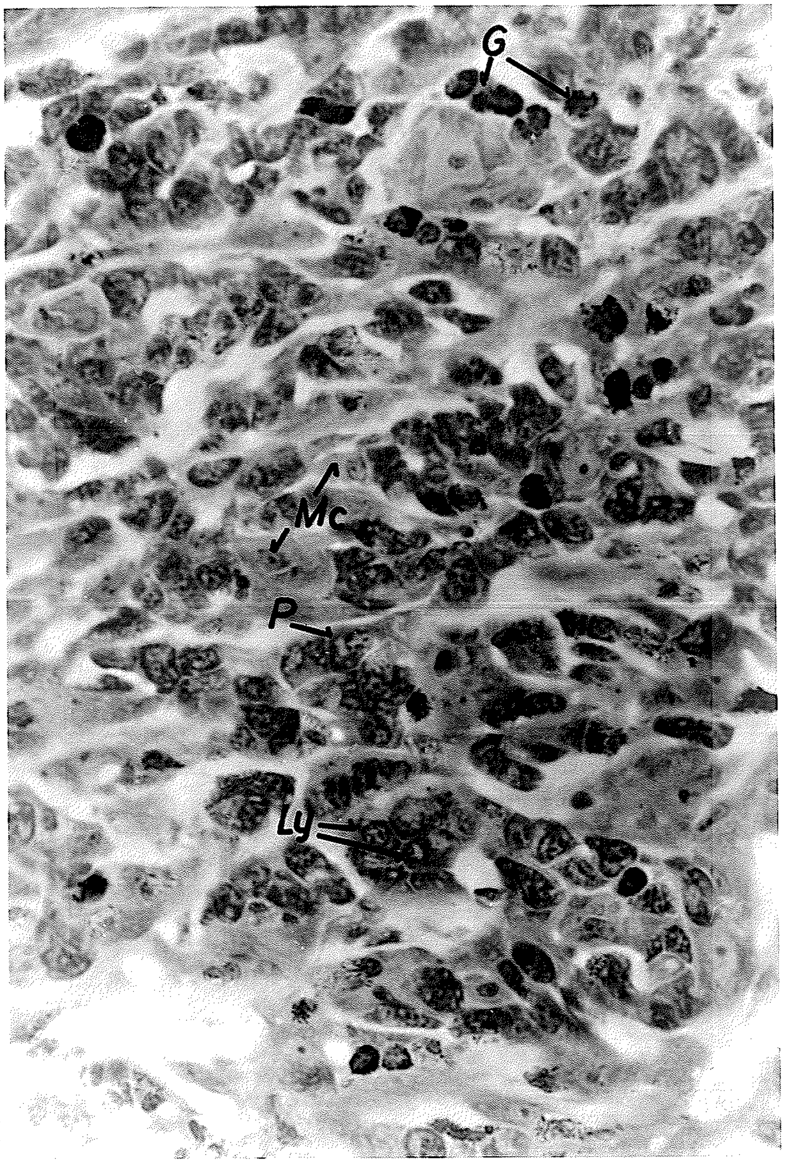

G
Mc
P
Ly

Table 7. Means of several parameters from day thirty-three and forty-seven lesions.

DAYS POSTINFECTION	NUMBER OF LESIONS	EXTERNAL DIAMETER	DIMENSIONS OF SECTIONED LESIONS			DIMENSIONS OF CAVITY		DIAMETER OF NEMATODES
			WIDTH	DEPTH	WALLS	WIDTH	DEPTH	
Thirty-three	[1]2.1	[2]5.5	4.50	3.80	0.81	1.28	1.95	0.23
	(1.6)	(1.8)	(1.23)	(0.93)	(0.19)	(0.58)	(0.80)	(0.06)
Forty-seven	2.1	8.6	5.39	3.97	1.05	2.48	1.33	0.39
	(0.6)	(2.99)	(1.92)	(0.63)	(0.36)	(1.77)	(0.52)	(0.06)

1. Numbers are means with standard deviations in parentheses below.

2. Measurements are in mm.

Table 8. Mean number of cells from the three zones of day thirty-three lesions.[1]

TYPE OF CELL		EG	EOSIN	HETER	MAST	LYMPH	BLAST	MACRO
Zone	N							
Outer	40	2.75	0.23	2.73	1.04	2.20	0.06	1.45
		(1.05)	(0.53)	(1.03)	(0.48)	(1.07)	(0.36)	(0.68)
Middle	40	3.83	0.34	3.80	1.16	3.21	0.09	1.81
		(1.02)	(0.57)	(1.06)	(0.84)	(0.53)	(0.36)	(0.64)
Inner	40	3.14	0.35	3.05	0.91	2.78	0.04	2.16
		(0.77)	(0.59)	(0.83)	(1.00)	(0.82)	(0.22)	(0.71)
Combined	120	3.24	0.31	3.19	1.04	2.73	0.06	1.81
		(1.05)	(0.56)	(1.07)	(0.83)	(0.93)	(0.32)	(0.73)
F(2 & 117 df)		13.200	0.512	12.640	0.925	14.660	0.338	10.880
p<		.001	ns	.001	ns	.001	ns	.001

[1] For an explanation see Table 2.

Day Forty

All of five ducklings necropsied on day 40 had gross lesions with a mean of 1.8 per bird. The external diameter varied from 7.0 to 15.0mm with an average of 8.9mm. Three lesions were examined; two contained yellow, caseous material with no worms, while the third contained nine partially gravid nematodes.

Only two partial lesions were fixed in sublimate and embedded in paraffin so that data regarding dimensions of the lesions is limited; no measureable cavity nor worms were seen, though both were present. Lesions were similar to those of day 33 in size (4.36 χ 3.27mm) and in histological details. Notably, the walls were edematous and reticular and the outer zone and adventitia had massive lymphoid follicles. Large lymphocytic and leucocytic sheaths surrounded arteries and vessels throughout the lesions.

Although no sections examined contained enough of a cavity or of whole worms for measurements to be taken, there was enough material present to note that giant cells were present around the entire edge of the cavity and that the cavity was filled with a solid mass of cellular debris.

Mean numbers of various types of cells in the three zones of day 40 lesions are presented in Table 9. There were some notable changes in the distribution and abundance of various types of cells from

Table 9. Mean number of cells from the three zones of day forty lesions[1]

TYPE OF CELL		EG	EOSIN	HETER	MAST	LYMPH	BLAST	MACRO	PLASMA
Zone	N								
Outer	20	2.03 (1.18)	1.97 (1.16)	0.25 (0.63)	0.88 (0.67)	3.05 (0.61)	0	1.94 (0.43)	0.65 (0.66)
Middle	20	3.18 (0.49)	3.03 (0.57)	1.00 (0.66)	0.61 (0.75)	2.26 (0.48)	0.25 (0.63)	2.17 (0.63)	1.32 (0.72)
Inner	20	2.50 (0.78)	1.95 (1.05)	1.35 (0.65)	0.57 (0.55)	2.03 (0.960)	0	2.48 (0.45)	0.78 (0.73)
Combined	60	2.57 (0.98)	2.32 (1.07)	0.87 (0.79)	0.69 (0.66)	2.44 (0.83)	0.08 (0.27)	2.20 (0.55)	0.56 (0.76)
F(2 & 57 df)		8.967	8.354	14.910	1.265	11.270	3.080	5.593	5.084
p<		.004	.005	.001	ns	.001	ns	.01	.01

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[1] For an explanation see Table 2.

earlier lesions. Eosinophils were much more numerous than before, while heterophils were less abundant. Eosinophils were most numerous in the middle zone and heterophils were most numerous in the inner zone. Lymphocytes were more numerous in the outer zone than elsewhere, and macrophages were again most abundant in the inner zone. Mast cells were uniformly distributed throughout the lesions and blast cells were noticeably less abundant than before, being entirely absent from all but the middle zone. Plasma cells were more abundant than in earlier lesions and were most numerous in the middle zone.

Day Forty-Seven

Eight of nine ducklings necropsied on day 47 had gross lesions in the junction area with a mean of 2.1 per bird. Lesions varied in diameter from 4.7 to 15.0mm with a mean of 8.6mm (Table 7).

Six lesions fixed in Stieves, AFA or Bouin's and embedded in paraffin were sectioned and stained. They were from five different ducklings. Most were useful for obtaining measurements and two fixed in Stieves were useful for enumerating the cellular composition.

Dimensions of sectioned lesions were similar to those of day 33. No significant differences were noted when lesions from these two periods were compared, except that the diameter of sectioned worms from day 47 was greater (F=22.292, p<.001)(see Table 7 for all measurements).

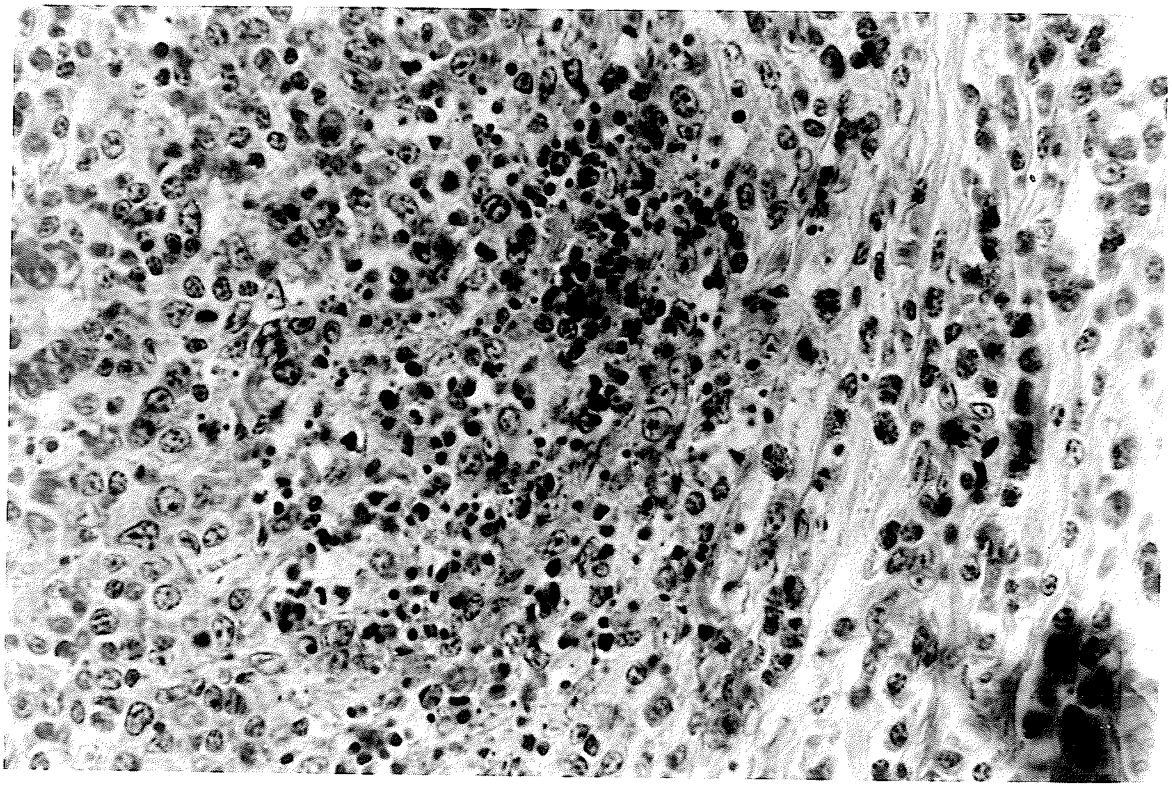
Two types of lesions were seen as in days 33 and 40; those that contained large healthy appearing nematodes (Type I, as discussed on page 137), and those containing dead or moribund worms and much cellular debris (Type II). Type I lesions were very cellular with large worms containing eggs with well developed first stage larvae. They were surrounded by muscle and large lymphoid follicles in which considerable macrophage activity was apparent, giving the follicles a "starry sky" appearance (Fig. 24 (Wolke and Wyand, 1969). The walls of these lesions (middle zone) were characteristically edematous with reticular networks of cells and many small vessels, some of which haemorrhages to the lumen of the cavity. In addition, there were several foci of intense phagocytic activity in the central region of the middle zone which contained numerous macrophages and lymphocytes (Fig. 25). The inner zone was ephithelioid in nature, tending to giant cell formation. Two of the five lesions were Type I.

In Type II lesions containing portions of dead or nearly dead worms, the most striking characteristics were the solid, mineralized contents of the cavities and the giant cells, which completely surrounded them (Fig. 26).

Counts of cells indicated that most types of cells were distributed non-randomly, with eosinophils, lymphocytes and plasma cells being most abundant in the middle zone (Table 10). Blast cells were relatively common in the middle zone but were entirely absent from the outer zone. As in day 40 lesions, heterophils were less abundant than eosinophils but were evely distributed throughout the lesions. Mast

Figure 24. Lymphoid follicle from a lesion at day 47 postinfection illustrating a "starry sky" effect where macrophage activity is evident. The follicle has the appearance of a germinal centre. M= macrophage. (Hematoxylin-Eosin), 20X.

Figure 25. Area of intense phagocytic activity in the Middle zone of a lesion at day 47 postinfection. (Hematoxylin-Eosin), 20X.



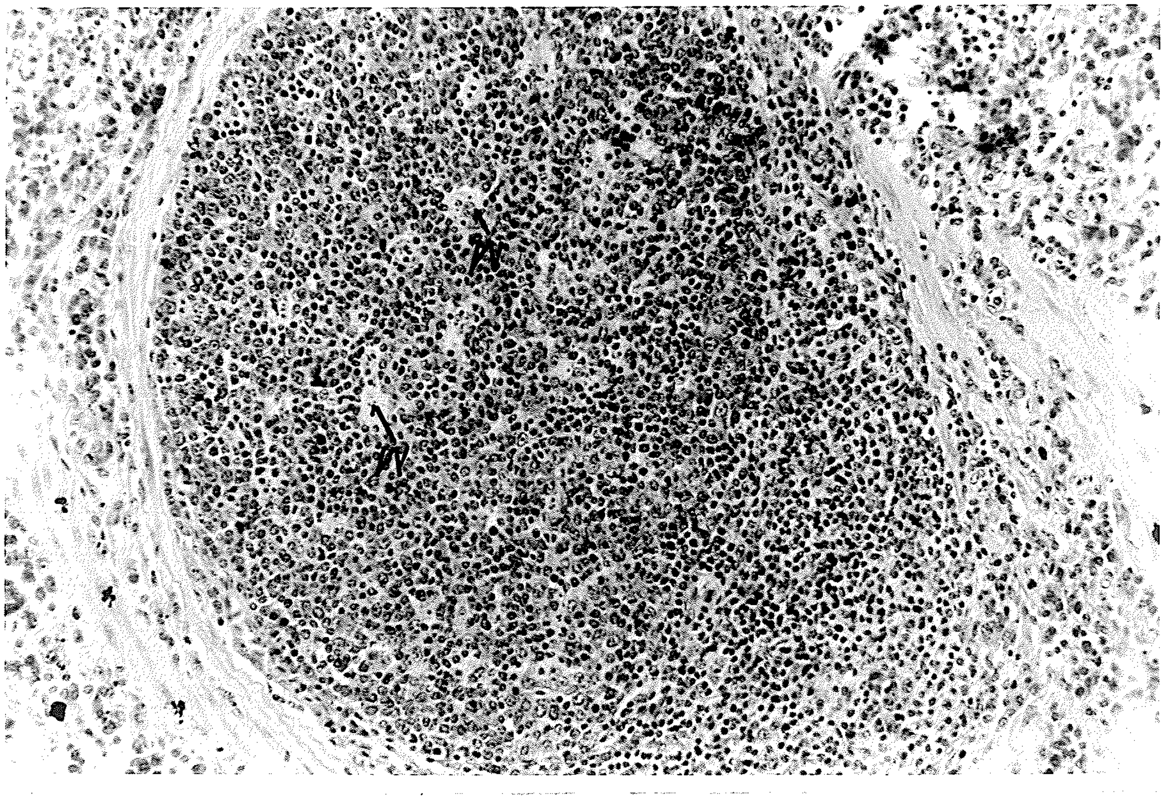
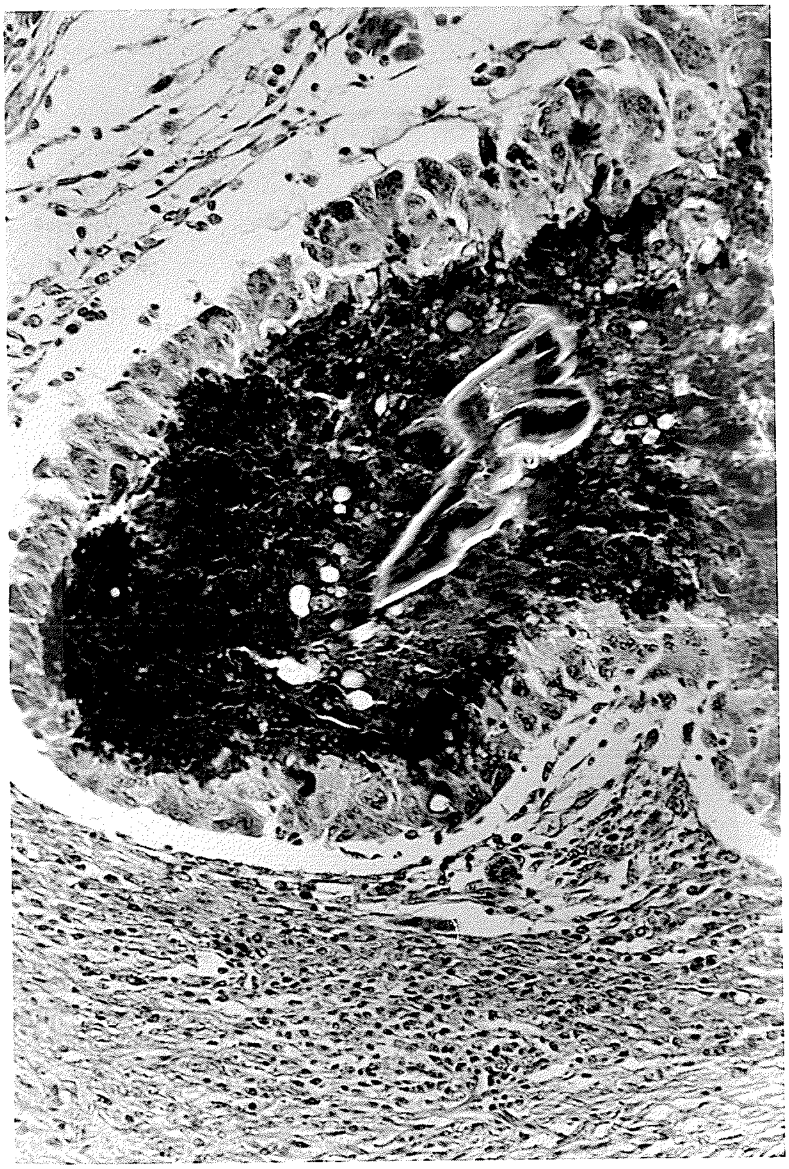


Figure 26. Portion of a lesion at day 47 postinfection with a dead worm and a mass of debris completely surrounded by giant cells. (Eosin-Thionin), 20X.

1176



cells were not counted at day 47 p.i. because I only had tissues which had been fixed in Stieves and post-treated by iodination. This is known to interfere with the staining of mast cell granules (Donaldson *et al.*, 1973). Plasma cells were notably more abundant than in earlier lesions.

Counts of specific cells in the lamina propria and submucosa of the isthmus region of uninfected control ducklings killed at the same age and time as the day 47 birds are presented in Appendix I. There was little difference between those counts and the comparable ones from controls killed at day 5 p.i. They were considerably lower than those from lesions, indicating that variations noted in the number of specific types of cells over the course of the experiment were due to infection and were not simply related to the age of ducklings.

Growth in diameter of the lesions and the nematodes over the period day 5 to 47 presented in Figure 27, and growth in the width and depth of the cavity in lesions is represented in Figure 28. Data are from Tables 3 and 7. All measurements increased slowly until day 26 and all had increased sharply by day 33, as indicated by the increase in the slope of the lines. As indicated previously, differences in overall diameter of lesions, depth of cavities and nematode diameters between days 26 and 33 were significant. This suggests that the growth of lesions and the nematodes were strongly correlated. After day 33, the diameter of the lesions increased slightly but not significantly

Table 10. Mean number of cells from the three zones of day forty-seven lesions.[1]

TYPE OF CELL		EG	EOSIN	HETER	LYMPH	BLAST	MACRO	PLASMA
Zone	N							
Outer	20	2.95 (0.56)	2.77 (0.78)	0.64 (1.12)	3.01 (0.46)	0	1.71 (0.59)	1.38 (0.93)
Middle	20	4.08 (0.45)	4.01 (0.52)	0.69 (0.94)	3.72 (0.35)	0.40 (0.68)	2.00 (0.40)	2.27 (0.61)
Inner	20	3.09 (0.67)	2.70 (0.81)	1.34 (1.11)	2.58 (1.21)	0.05 (0.25)	2.41 (0.59)	0.93 (0.97)
Combined	60	3.38 (0.81)	3.16 (0.93)	0.90 (1.09)	3.10 (0.89)	0.15 (0.45)	2.04 (0.60)	1.53 (1.01)
F(2 & 57 df)		18.360	21.220	2.695	10.980	5.211	8.758	12.770
p<		.001	.001	ns	.001	.01	.001	.001

1. For an explanation see Table 2.

Figure 27. Mean diameters of sectioned lesions and nematodes from ducklings infected with 40 _E_. _uncinata_ larvae at four weeks of age and necropsied at intervals post-infection.

●——● Diameter of lesions
○------○ Diameter of nematodes

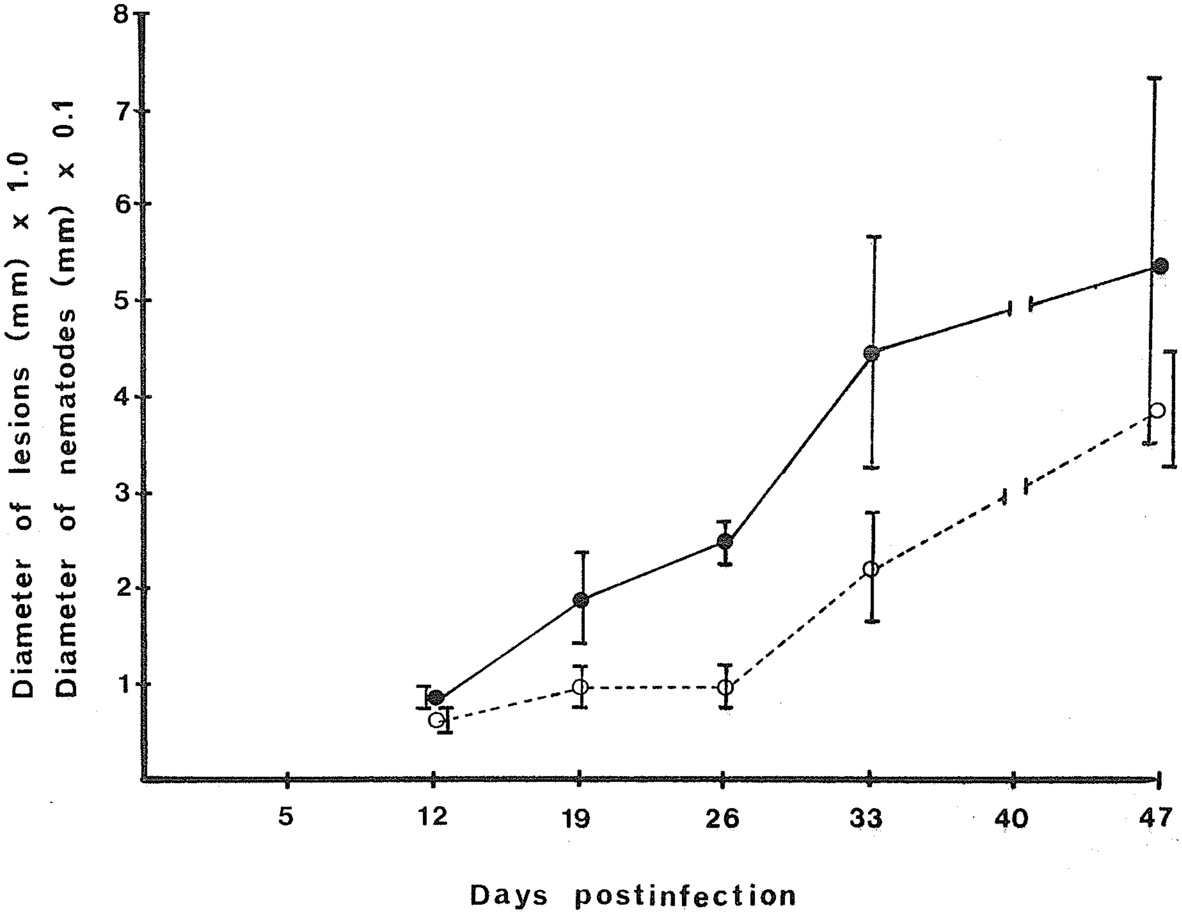
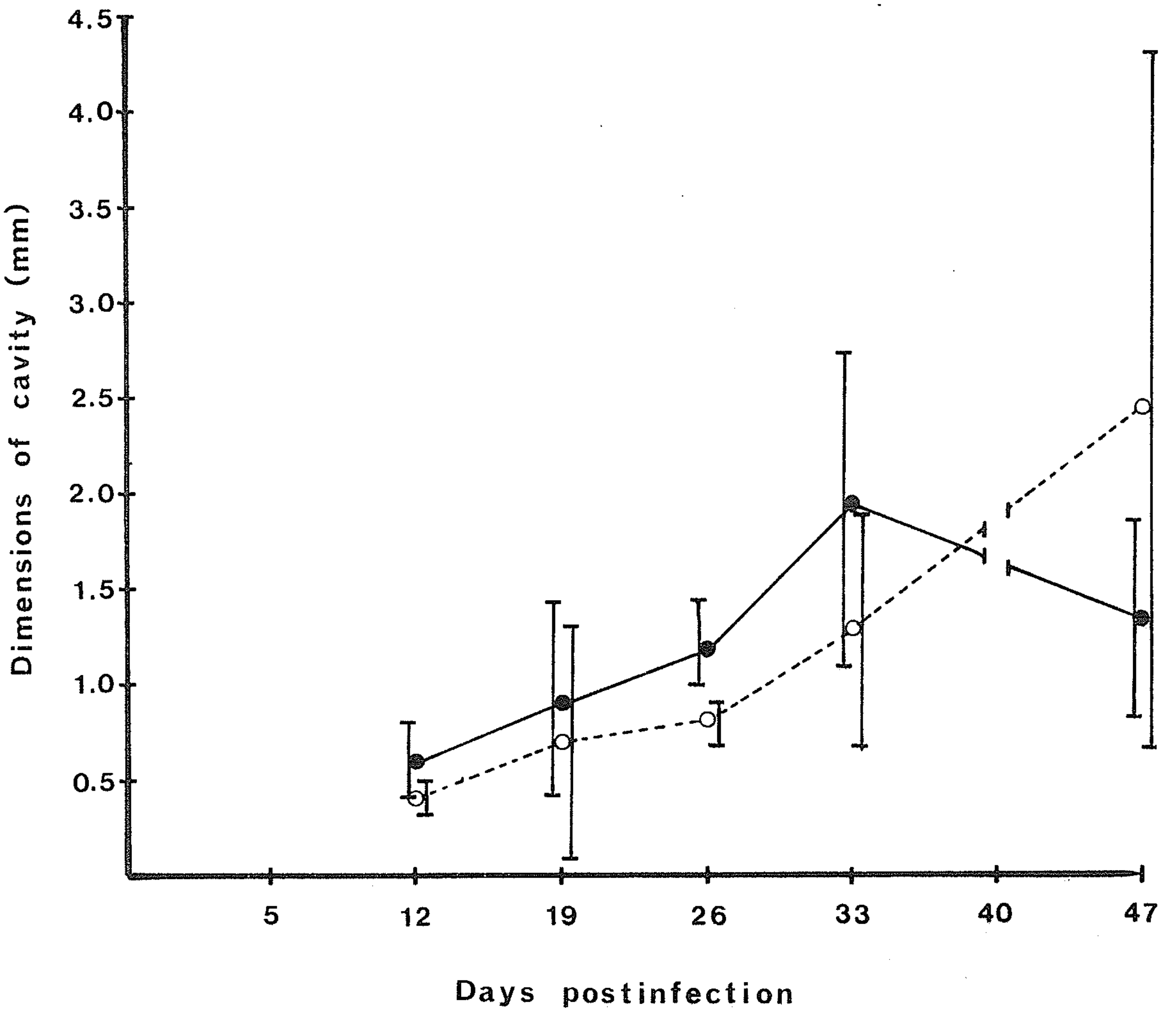

Diameter of lesions (mm) x 1.0
Diameter of nematodes (mm) x 0.1
8
7
6
5
4
3
2
1
5
12
19
26
33
40
47
Days postinfection

Figure 28. Dimensions of the cavity within lesions from ducklings infected with 40 *E. uncinata* larvae at four weeks of age and necropsied at intervals postinfection.

●——● Depth of cavity
○- - - - -○ Width of cavity



4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Dimensions of cavity (mm)
5
12
19
26
33
40
47
Days postinfection

so, while the cavity dimensions changed in opposite directions (Fig. 28). Neither depth nor width of the cavities of day 33 and 47 lesions were significantly different. The mean diameter of nematodes at day 47 was however, significantly greater than that of nematodes at day 33.

Changes in the numbers of specific types of cells from day 5 to 47 p.i. are summarized in Figures 29 and 30. Eosinophilic granulocytes, lymphocytes and macrophages were abundant in lesions throughout the course of the experiment but numbers of these cells fluctuated considerably (Fig. 29). Mast cells, plasma cells and blast cells were less numerous but also fluctuated considerably (Fig. 30). Differences between means from one necropsy day to the next with respect to particular cell types were tested by analysis of variance; where a ▲ appears below a day in a figure, the mean number of cells in lesions from that day is significantly different from that in lesions from the day immediately preceeding it.

An examination of Figures 29 and 30 reveals that the numbers of most types of cells were significantly different from one necropsy day to the next. Eosinophilic granulocytes, lymphocytes, macrophages, mast cells and blast cells increased in number from day 5 to day 12 and fluctuated from then on. In contrast, the controls (Table 1, Appendix I) revealed no significant trends with age.

Of the two types of eosinophilic granulocytes, heterophils (Fig. 29c) were the most prominent until day 33 and were generally most abundant in the middle zone of lesions. On days 40 and 47, heterophils were less numerous and were most abundant in the inner zone. Eosinophils (Fig. 29b), on the other hand, were less prominent until day 33 and were most abundant in the inner zone. Thereafter they became much more numerous and were more abundant in the middle zone. Of leucocytes observed on blood smears only eosinophils showed a significant increase in number to day 47 (Table 2, Appendix I).

Lymphocytes (Fig. 29d) were very abundant in all lesions, particularly on days 12, 33 and 47, and were usually most abundant in the middle zone. The number of macrophages from different days fluctuated over the course of the experiment. All of the peaks in Figure 29e (macrophages) are significantly different from those they preceeded except that between days 40 and 47.

The number of mast cells (Fig. 30a) was never great and except for day 26, they were evenly distributed throughout the lesions. Two peaks are apparent in Figure 30a; one at day 12 and one at day 33, with lows at days 19 and 40.

Plasma cells (Fig. 30c) were uniformly rare until day 40; their numbers peaked on day 47. These cells were usually most abundant in the middle zone. Blast cells (Fig. 30b) were most abundant early in the course of infection, especially on days 12 to 26. Thereafter, the number of these cells was low and they were virtually absent from all zone except the middle zone.

Serum Proteins and Antibody Production

Serum samples from all experimental ducklings and a number

Figure 29. Changes in mean numbers of cells in lesions from ducklings infected with 40 *E. uncinata* larvae at four weeks of age and necropsied at intervals postinfection.

a) Eosinophilic granulocytes
b) Eosinophils
c) Heterophils
d) Lymphocytes
e) Macrophages
▲ Mean number of cells/field significantly different from that of the preceding day.

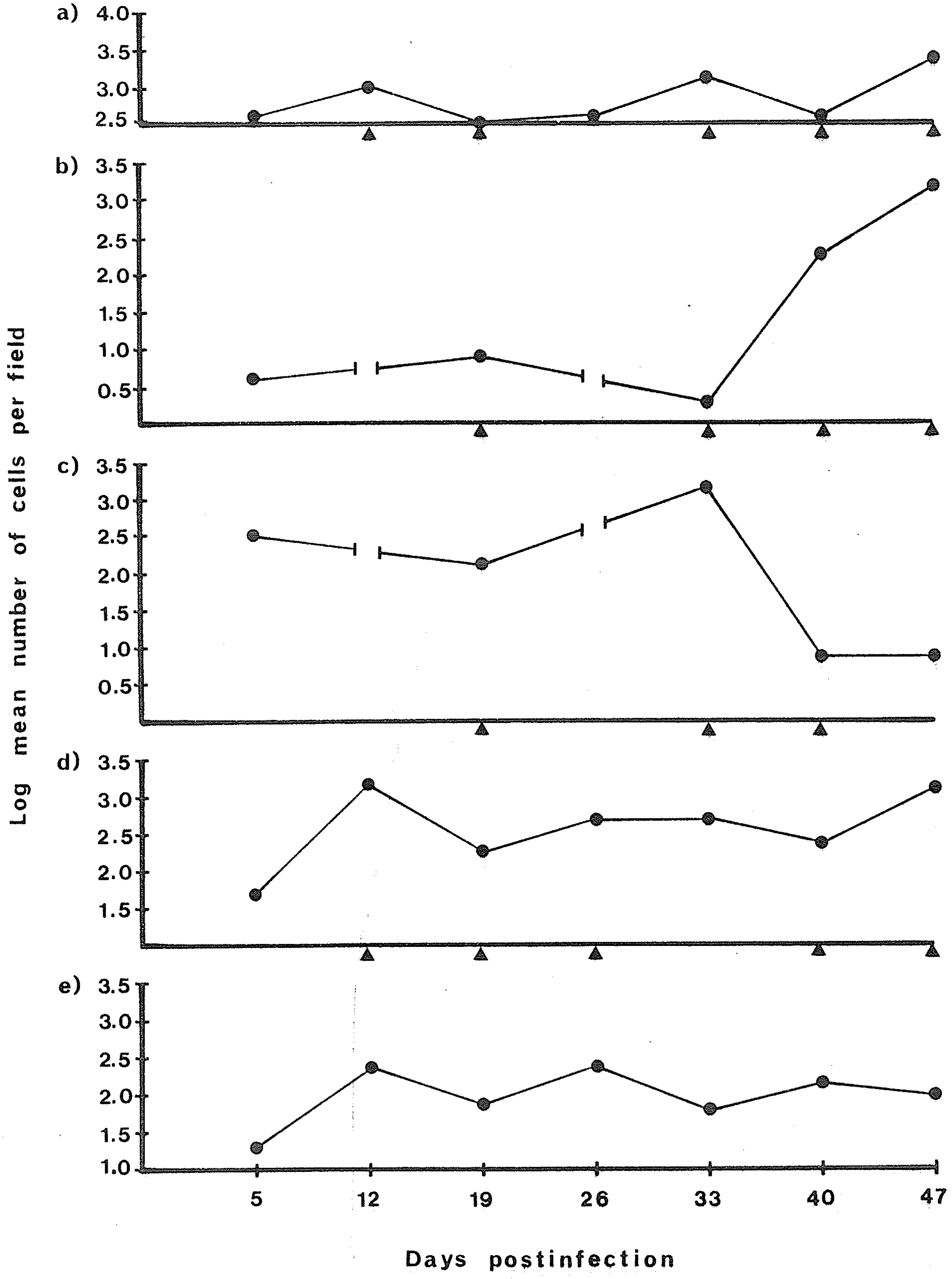
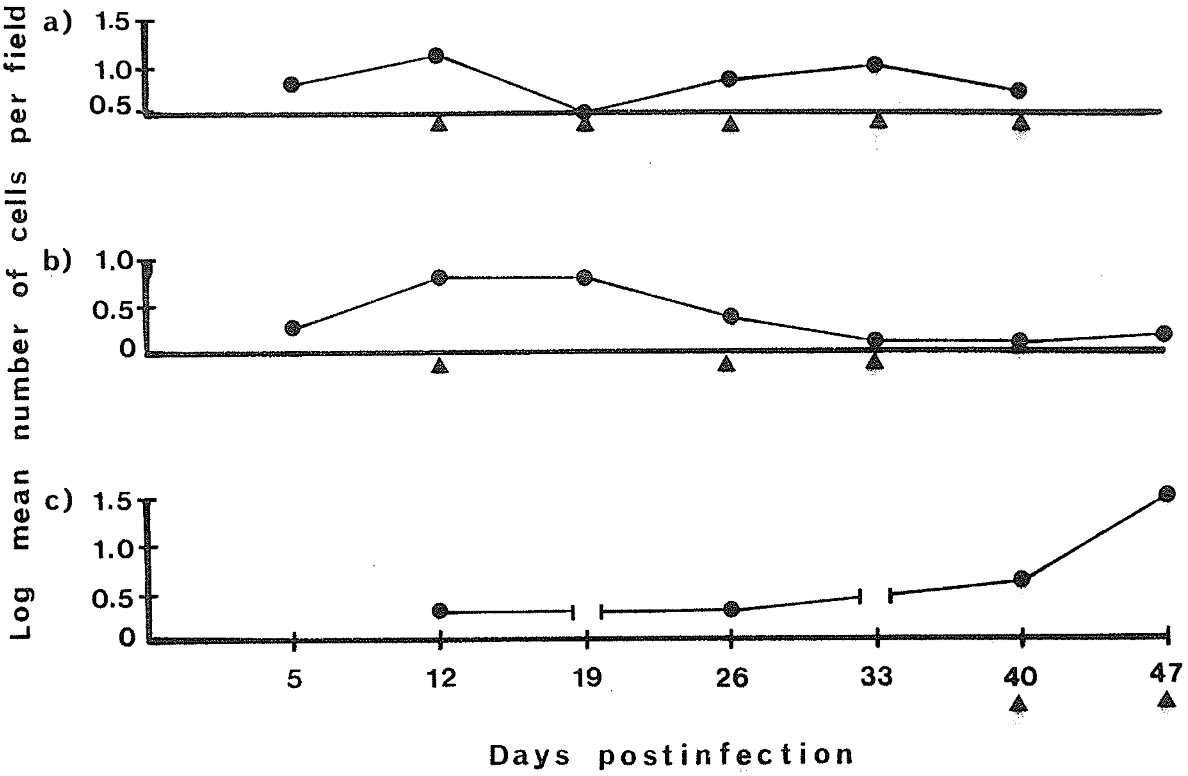

a)
4.0
3.5
3.0
2.5
b)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
c)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
d)
3.5
3.0
2.5
2.0
1.5
e)
3.5
3.0
2.5
2.0
1.5
1.0
5
12
19
26
33
40
47
Log mean number of cells per field
Days postinfection

Figure 30. Changes in mean numbers of cells in lesions from ducklings infected with 40 *E. uncinata* larvae at four weeks of age and necropsied at intervals postinfection.

a) Mast cells
b) Blast cells
c) Plasma cells
▲ Mean number of cells/field significantly different from that of the preceding day.



a)
1.5
1.0
0.5
b)
1.0
0.5
0
c)
1.5
1.0
0.5
0
Log mean number of cells per field
5
12
19
26
33
40
47
Days postinfection

of uninfected ducklings of various ages were treated as outlined above and the data compiled in Tables 11 and 12. Total serum protein varied from a low of 42.7 mg/ml to a high of 56.5 mg/ml in samples from experimental birds. This constituted a significant amount of variation. Samples from days 12, 26 and 40 had greater amounts of protein than did other samples.

Serum samples were electrophoretically separated into their component fractions and the amount of each fraction present was estimated. Analysis of variance on each of these fractions over the course of the experiment indicated that only the albumin fraction varied significantly. Linear regression analysis showed that a significant correlation existed between total protein and serum albumin. The equation $Y = 0.502X + 6.1$, where Y is albumin and X is total protein, describes the relationship which is significant at the 0.1% level (F=106.192, 1 and 48 df). The square of the multiple correlation coefficient ($r^2= 0.68$) indicated that 68% of the variation in serum albumin could be explained by its regression on total protein. The converse is probably a more significant relationship given that albumin was the major component of the serum protein. The regression of gamma globulin on total protein was also significant, but only at the 5% level and little of the variation was explained by the relationship ($r^2=0.082$).

Uninfected controls were not available for all groups of experimentals but the only significant differences noted were in total

Table 11. Serum protein fractions from ducklings infected at four weeks of age with 40 *E. uncinata* larvae and necropsied at intervals postinfection.

SERUM FRACTION		PRE-ALBUMIN	ALBUMIN	GLOBULIN			TOTAL PROTEIN
				ALPHA	BETA	GAMMA	
DAYS POSTINFECTION	N						
Five	8	[1]2.0 (0.62)	27.43 (2.92)	5.64 (1.82)	5.45 (2.38)	2.12 (1.27)	42.65 (6.98)
Twelve	5	1.79 (0.42)	35.08 (2.93)	8.00 (2.52)	7.89 (2.02)	3.71 (2.10)	56.46 (3.54)
Nineteen	9	1.72 (1.09)	27.06 (5.06)	6.57 (1.98)	6.18 (2.66)	2.63 (1.22)	44.16 (9.61)
Twenty-six	4	3.07 (0.85)	31.23 (5.56)	6.20 (0.77)	7.53 (2.11)	3.71 (2.38)	51.75 (5.19)
Thirty-three	10	2.13 (1.12)	27.90 (4.88)	4.49 (1.64)	5.50 (2.47)	3.89 (2.09)	43.92 (7.31)
Forty	5	4.64 (1.10)	33.01 (4.17)	5.02 (2.97)	6.39 (1.12)	2.65 (1.77)	51.71 (3.90)
Forty-seven	9	0.38 (0.62)	29.02 (5.12)	6.77 (2.92)	5.21 (1.66)	2.01 (0.68)	43.39 (8.23)
[2]F(6 & 43 df)			2.782	1.945	1.334	1.813	3.317
p<			.05	ns	ns	ns	.01

1. Means expressed as mgm/ml with standard deviations in parentheses below.
2. Results of one way anovars on columns.

Table 12. Serum protein fractions from uninfected control ducklings.

SERUM FRACTION		PRE-ALBUMIN	ALBUMIN	GLOBULIN			TOTAL PROTEIN
				ALPHA	BETA	GAMMA	
AGE IN WEEKS	N						
Four	5	0.74 (0.47)	23.25 (5.01)	6.25 (1.56)	4.43 (1.39)	1.59 (0.65)	36.26 (6.88)
Five	5	[1]1.96 (0.31)	28.23 (1.66)	4.86 (1.36)	7.28 (0.88)	1.96 (0.74)	44.29 (3.63)
Seven	5	2.17 (0.54)	22.82 (1.89)	5.89 (0.91)	5.09 (1.28)	5.18 (0.49)	41.16 (3.82)
Eleven	10	0.98 (0.62)	25.17 (2.54)	6.26 (3.15)	5.74 (2.89)	2.73 (2.96)	40.87 (9.75)
Thirteen	5	0.89 (0.21)	24.85 (1.18)	4.91 (0.70)	4.80 (0.45)	1.91 (0.44)	37.36 (1.12)
[2]F(4 & 24 df)			3.018	0.639	1.701	3.153	1.134
p<			.05	ns	ns	.05	ns

1. Means expressed as mgm/ml with standard deviations in parentheses below.
2. Results of one way anovars on columns.

protein between day 12 birds and controls of similar age, and in gamma globulin between day 19 birds and the controls. In the first case, more total protein was present in the sera of experimentals; in the second, less gamma globulin was present in the sera of experimental ducklings. Controls were available for the day 5 and 47 birds as well but no significant differences were noted between their respective serum fractions.

Antibody production, as measured by semi-quantitative immunodiffusion, did not occur until after 26 days of infection and was limited to a few birds. Only three of ten ducklings at day 33, and three of nine at day 47, produced detectable amounts of precipitating antibody against reconstituted lyophilized whole worm _E. uncinata_ antigen. Estimates of the titre of these sera were relatively low, varying from one to eight. None of the serum samples from control ducklings was positive.

Discussion

A diagrammatic representation of the sequential development of lesions induced by _E. uncinata_ in the isthmus between the proventriculus and ventriculus is presented in Figure 31 (days 5, 12 and 19). The early response to invading larvae (day 5) was one of intense inflammation. Nematodes were loosely attached to the mucosal surface and some were actively penetrating the epithelium into the lamina propria between "villi"(Fig. 31a). There was localized infiltration of the area immediately adjacent to groups of nematodes, presumably as a consequence of tissue damage and perhaps the release of lytic secretions by the nematodes. Vessels in the lamina propria and submucosa were markedly dilatated and congested and cells were passing from these vessels into the tissues near nematodes. The response at day 5 p.i. was typical of inflammatory reactions in damaged tissue as described by Cameron (1967) and Weiss and Greep (1977).

Eosinophilic granulocytes, especially heterophils (neutrophils), were abundant and were concentrated near the worms in the "halo" (Figs. 3-5, 31a). This response was similar to the phase of acute inflammation described for T. spiralis infections in mice (Larsh and Race, 1954), and for Nippostrongylus brasiliensis infections in mice and rats (Ogilvie and Jones, 1971, 1973), but was more localized than the panmucosal inflammation associated with T. spiralis infections. In addition, lymphoid cuffs (see Fig. 9) were present around vessels in the lamina propria and in the muscularis and proliferation of localized lymphoid follicles was apparent (Fig. 6). These observations are strikingly similar to those of Fernando et al.(1971) concerning the early stages of Syngamus trachea infections in the lungs of pheasants (days 0-7).

By day 12 p.i., lesions were discrete nodules consisting of large accumulations of infiltrating cells and large lymphoid follicles (Fig. 31b). Nematodes had penetrated through the lamina propria and muscularis mucosae and were entering the thick circular layer of the muscularis (Figs. 7, 8). They were completely surrounded by masses of cells and cellular debris. The mucosal surface was eroded as Austin and Welch (1972) had indicated, and between the worms and the mucosal surface was a distinct basophilic plug (Fig. 8). Although no definite walls separated nematodes from the surrounding cellular mass, fibrotic walls appeared to be forming in the submucosa (Figs. 8, 10). These were relatively distinct only laterally and toward the mucosal surface (see Figs. 8, 10, 31b). Some giant cells were present at the periphery of the cavity as were numerous individual macrophages (Fig. 10). Eosinophilic granulocytes were prominent throughout the lesion, but were especially numerous in the cellular mass immediately next to nematodes. As the lesion was edematous and well vascularized, this was probably the source of many of the cells present. Because blast cells and large lymphoid follicles were numerous, it is likely that local proliferation of cells, including lymphocytes, granulocytes and possibly plasma cells, contributed significantly to the cell population as well.

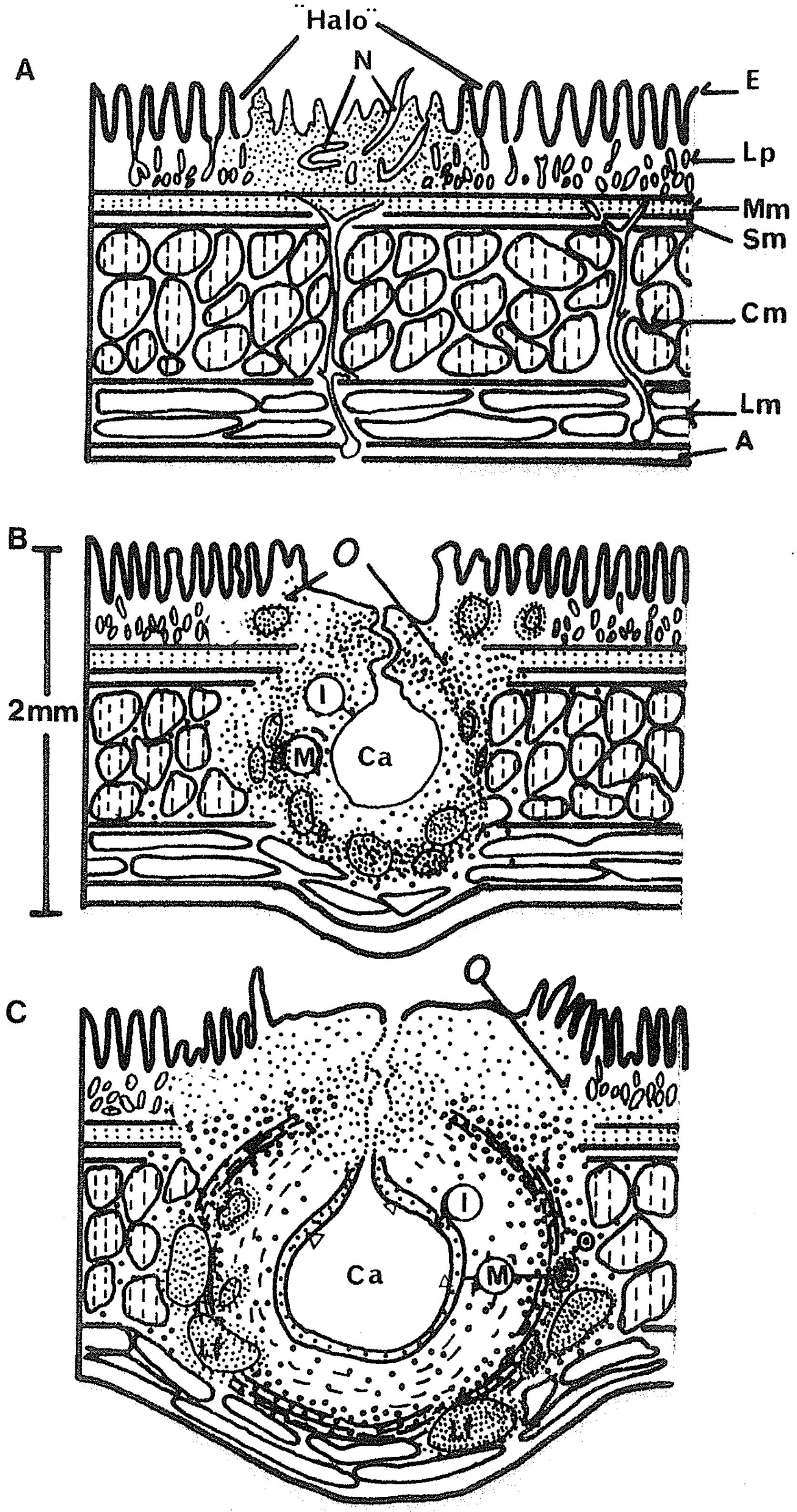
By day 19 p.i., the lesions were fully formed with distinct inner, middle (wall) and outer zones (Figs. 11, 31c). Nematodes had penetrated deep into the layer of circular muscle and the lesions had increased significantly in dimensions compared to those of day 12

Figure 31. Diagrammatic representation of the sequential development of the *E. uncinata* granuloma in male wild mallard ducklings infected with 40 larvae at the age of four weeks and necropsied at intervals postinfection.

a) Five days postinfection
b) Twelve days postinfection
c) Nineteen days postinfection

Legend:

A= adventitia; Ca= cavity; Cm= circular muscle; I= Inner zone; Lm= longitudinal muscle; Lp= lamina propria; M= Middle zone; O= Outer zone; Sm= submucosa. ▷= region of giant cells.


A
"Halo"
N
E
Lp
Mm
Sm
Cm
Lm
A
B
2mm
O
I
M
Ca
C
O
I
M
Ca

(Figs. 27, 28), as had the contained nematodes (Fig. 27). Prominent in the inner zone were eosinophils, macrophages, epithelioid and giant cells (Fig. 14). Peripheral to this, the major portion of the wall (middle zone) was an edematous, reticular network of lymphocytes, heterophils, blast cells and some plasma cells (Figs. 13, 15, 16). The outermost region of the middle zone had large numbers of heterophils, fibroblasts and connective tissue which merged with massive lymphoid follicles of the outer zone (Fig. 12). Muscle bundles and blood vessels were intimately associated with the walls of the lesion, integrating the whole structure into the wall of the gut (Figs. 11, 15, 31c).

At this stage the lesion can be called a granuloma as described by Cameron (1967) (see Section II, Pathology of Helminth Infections). It closely resembles the granulomas induced by *S. trachea* in pheasants (Fernando *et al*., 1971), *Chaunocephalus ferox* in the gut of storks (Patnaik *et al*., 1970) and *Ascaridia columbae* in the liver of pigeons (Wehr, 1971). The granuloma of *E. uncinata* also resembles the eosinophilic granuloma described by Ko (1976) in the stomach of rhesus monkeys infected with *Echinocephalus sinensis*, and the schistosome egg granuloma (Lichtenberg, 1962; Hsu *et al*., 1969; Sadun *et al*., 1970; Erickson *et al*., 1971). Granulomas described here were most similar to the polypoid patches of Sadun *et al*. (1970) formed around groups of *Schistosoma haematobium* eggs in chimpanzees. These were large, edematous granulomas with limited peripheral fibrous tissue but with extensive lymphoid infiltration in the walls and massive proliferating lymphoid follicles at the bases of the granulomas.

Details of the cellular response revealed that heterophils had become very abundant near invading larvae by day 5 p.i. Since they are non-specific phagocytes, homologous to mammalian neutrophils, their early invasion of the tissue in response to penetrating E. uncinata larvae is a manifestation of an acute inflammatory reaction. Vasodilatation was pronounced, leading to edema and accumulation of leucocytes, including large numbers of heterophils. This accumulation continued to day 19 p.i. when the granuloma was completely formed, but it was obvious that many cells were entering the cavity and eventually dying (Figs. 13, 14). Lymphocytes and macrophages reached peak numbers later (day 12) than heterophils (day 5) (see Fig. 29) and their numbers fluctuated near these high levels from then on. Both types of cells are important components of granulomatous responses (see Section II, Pathology of Helminth Infections) and their involvement in immune responses is well known (Weiss, 1972; Park and Good, 1974; Weiss and Greep, 1977). Lymphocytes entering the area of tissue injury in the first few days probably included immunocompetent T-cells whose stimulation and subsequent responses augmented the non-specific inflammatory response. This led to invasion and accumulation of more heterophils, lymphocytes, macrophages, and to a lesser extent, eosinophils. Macrophages began to form giant cells early and these were relatively abundant by day 19 p.i. (Figs. 14, 31c). These were typical foreign body giant cells formed in response to foreign material which is either too complex or too large to be engulfed by individual cells (see Weiss and Greep, 1977, Ch. 4). They are also typical of granulomas. Thus, in terms of a well defined structure, and in terms of the cellular components, the E. uncinata granuloma was completely formed by day 19 p.i. (Fig. 31c).

The number of reports in the literature regarding the possible function of eosinophils makes the late (day 40 and 47) prominence of eosinophils in E. uncinata granulomas particularly interesting. Peripheral eosinophilia has frequently been associated with a variety of helminth infections (see above review) and as indicated in Table 2, Appendix I, was also observed in this study. Ansari et al. (1976) showed that the peripheral eosinophilia in Taenia taeniaformis infections in rats was coincident with a marked eosinophil invasion of tissues around cysticerci. As well, eosinophils are constant features of granulomas formed in response to helminths or their products. The evidence reported by Colley (1973), Larsh et al. (1974b, 1975), and Warren et al. (1976) which indicated that eosinophil production and migration to sites of tissue injury are mediated by a lymphokine (ESP) produced by sensitized lymphocytes (T-cells), may explain these phenomena including the peripheral eosinophilia and abundance of eosinophils in late E. uncinata granulomas. The involvement of eophisophils may be important in the protection of hosts from parasites since Larsh et al. (1947b, 1975) suggested that they may be effector cells mediating the expulsion of T. spiralis from the gut of mice. Similarly, Butterworth et al. (1976) implicated neutrophils and/or eosinophils in co-operation with immune serum as mediators of in vitro damage to schistosomules.

Other reports concerning the possible functions of eosinophils are particularly interesting with respect to E. uncinata granulomas. Westermann and Engelbert (1969a, b, c) studied the distribution, numbers and development of eosinophilic and heterophilic granulocytes in the rabbit thymus and reviewed the possible functions of eosinophils. They suggested that since eosinophils contain peroxidase (thought to stimulate cell division in plant cells, Rytömaa, 1962), they may promote high rates of cell division in the thymus. This could have been the case with eosinophils in late E. uncinata granulomas as the lymphoid follicles showed evidence of great activity though blast cells were not abundant elsewhere.

The rise in numbers of plasma cells at day 40 p.i. correlates with the first detection of antibody at day 33. Their peak at day 47 also correlates with the occurrence of slightly greater antibody titres at that time.

Granulomas and the cavity containing nematodes increased in dimensions from day 19 to day 33 p.i., particularly between days 26 and 33 p.i. (Figs. 27, 28). The latter rapid increase in size was coincident with a rapid increase in nematode size as measured by mean diameter of sectioned worms (Fig. 27). Echinuria uncinata fourth stage juveniles are known to undergo the final moult at day 20 p.i. in Delta mallards, after which growth in length was rapid until day 40 p.i., when eggs with fully formed first stage larvae were shed (Austin and Welch, 1972). Except for the fact that nematodes in my material grew more slowly to day 26 p.i., the growth pattern of nematodes exhibited by my material is comparable to that shown by Austin and Welch (1972). I first noted eggs in female worms on day 33 p.i. while Austin and Welch noted that 30 day old females had uteri about 70% full of unembryonated eggs. Thus maturation of nematodes in my material was apparently normal as well.

As the nematodes and the granulomas enlarged, so did the cavity. It seems likely that nematodes enlarged the cavity by actively abraiding the cellular walls with the aid of their cuticular cordons as suggested by Kennedy _et_ _al_.(1973). Cellular material was observed in the canals of cordons by myself and by Kennedy _et_ _al_. (1973), who also observed the same type of material in the esophageal lumen. However, although granulomas and the cavity within grew progressively from day 19 to day 47 p.i. (Figs. 27, 28), there were both qualitative and quantitative differences which appeared to represent stages beyond the fully developed state.

Granulomas at day 26 p.i. were of two basic types. All had a debris clogged cavity, but the size of cavity and size of nematodes, and thickness of the walls were different. One type, hereinafter called Type I (Fig. 31c), was similar to those of day 19, having a spacious cavity with large, healthy appearing nematodes and relatively thin walls (Figs. 18, 19). Few giant cells were apparent. A second type of granuloma (Type II) differed from Type I by: i) being less deeply set in the muscularis; ii) having much thicker walls but a smaller cavity; iii) containing smaller nematodes; and iv) having numerous giant cells around the cavity and nematodes (Figs. 20, 32(1)). This granuloma probably represents an early rejection of worms by the particular duckling involved. It may represent the result of a host response to a small number of nematodes which failed to penetrate into the muscularis and were unable to cope with the tremendous cellular response which occurs in the mucosa. Alternatively, such granulomas could be manifestations of a rapid and effective protective response by a very competent host. In any event, Type I granulomas are interpreted to be ones which persist for some time, while Type II granulomas are thought to be ones which had changed from a well established state to a stage of involution (i.e., from Type I to Type II). However, at this stage (day 26 p.i.), it is possible that Type II granulomas represent ones which had never become well established because of their superficial location.

All granulomas at days 33, 40 and 47 p.i. were well established deep in the muscularis and both Type I and II were represented. These Type II granulomas were in the process of involution, and because of their nature and location, had probably changed from an earlier Type I stage. The sequence: i) establishment of groups of worms in the mucosa; ii) development of Type I granulomas (Fig. 31); and iii) change to Type II granulomas (Fig. 32(2)), was the normal sequence of events associated with experimental *E. uncinata* infections in most male wild mallard ducklings. No sections of granulomas older than 47 days were examined in this study, but a number of ducklings were infected for other uses and were examined at intervals from 90 days to 6 months p.i. Few showed any signs of having been infected beyond 90 days, suggesting that most experimentally infected male wild mallard ducklings are capable of rapid and complete dissolution of *E. uncinata* granulomas.

Because giant cells were apparent in early granulomas and became numerous as the nematodes became necrotic (Fig. 26), these giant cells are thought to be partly responsible for immobilization and eventual death of the nematodes. Their involvement in phagocytosis of dead worms was obviously extensive (see Fig. 26). Anderson (1968) indicated that giant cells were formed after death of *Angiostrongylus cantonensis* in man or monkeys. Fernando *et al.*(1971) and Ko (1976) described similar formations around the anterior end of *S. trachea* and *E. sinensis* respectively, some of which were associated with dead nematodes but most of which were not. Fernando *et al.*(1971) were convinced that giant cells were formed by fusion of monocyte derived macrophages. I feel that such was the case in the granulomas induced by *E. uncinata* as well.

Hsu *et al.*(1969, in Smithers and Terry, 1976) described five stages in the formation of the schistosome egg granulomas: "(1) *the non reactive or weakly negative stage* describes the lesion around living immature eggs; (2) *the exudative stage* is formed around mature eggs and is characterized by an accumulation of neutrophils among the acidophilic PAS-positive radiating filaments which originate from the egg shell periphery; (3) *the exudative-productive stage* is manifested by a granulomatous transformation, including an arrangement of epithelioid cells in a palisade around the egg and the formation of giant cells; (4) *the productive stage* is seen when the miracidium shows signs of degeneration and when the egg is surrounded by giant cells outside of which are epithelioid cells, histiocytes, fibroblasts and neutrophils; (5) *the involutional stage* is seen when giant cells invade and destroy the egg, the layers of epithelioid cells become thinner, fibroblasts become more prominent and there is a formation of collagen fibres" (p. 416). A similar progression from polypoid patches (exudative-productive stage ?) to fibrous patches (productive stage ?) to sandy patches (involutional stage ?) was described for *S. haematobium* granulomas by Sadun *et al.*(1970).

In this study, the early inflammatory response to invading *E. uncinata* larvae at day 5 (Fig. 31a) approximately corresponds to the *non reactive or weakly negative stage* of Hsu *et al.*(1969), and by day 12 p.i. (Fig. 31b) the lesion had progressed to a stage comparable to their *exudative stage*. Their *exudative-productive stage*, which is manifested by a granulomatous transformation including epithelioid and giant cells corresponds to the *E. uncinata* granuloma in its early, fully formed state (Type I) at day 19 p.i. (Figs. 13-17, 31c). Such Type I granulomas were also similar to the polypoid patches of Sadun *et al.* (1970), being extremely vascular and edematous, having only occasional giant cells, and having an epithelioid cell layer interrupted by vascular or lymph channels which apparently contributed cells and fluid to the cavity. Type II *E. uncinata* granulomas represent stages in the change from Type I granulomas through *the productive* to *the involutional stage*. The presence of giant and epithelioid cells around the cavity and around moribund or dead nematodes (see Figs. 20, 26), as well as the densely stained accumulations of cellular debris, are indicative of this transformation.

Because both Type I and Type II granulomas were present beyond 19 days p.i., it is apparent that the rapidity of involution was variable depending on the duckling involved. Consequently, I believe that well developed *E. uncinata* granulomas in mallards develop along three possible lines (see Fig. 32). The first of these (Fig. 32(1)), shows that they can change rapidly to the involutional stage and are resolved before the nematodes become ovigerous. Such granulomas may involute rapidly as a result of a rapid response on the part of the host, or as a result of few worms initiating the infection. The second

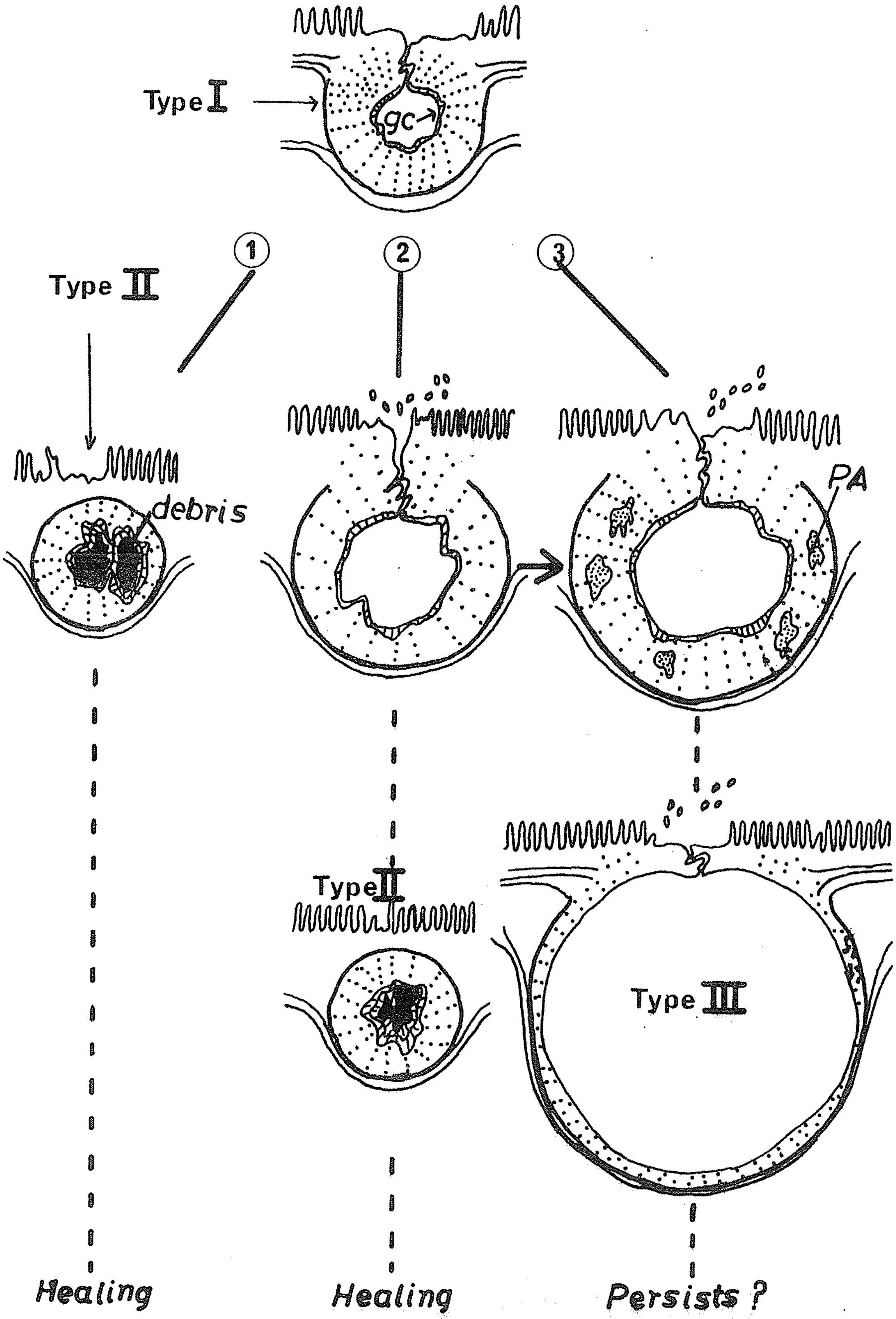
Figure 32. Highly diagrammatic representation of possible routes of development of *E. uncinata* granulomas after their initial establishment as Type I at day 19 postinfection.

1, 2, and 3 - possible routes

gc = layer of giant cells bordering the cavity.

debris = debris clogged cavity and nematodes surrounded by confluent giant cells.

PA = areas of intense phagocytic activity in the wall.


Type I
gc
Type II
1
2
3
debris
PA
Type II
Type III
Healing
Healing
Persists ?

possibility (Fig. 32(2)), is that once established, the granulomas and their nematodes may grow and persist for a variable length of time; long enough for the nematodes to mature and shed eggs, thus completing the life cycle. Most such granulomas eventually involute (become Type II). Their slow change to Type II may be due to the presence of a larger number of worms than in the first case. The large number of worms may be able to remove accumulated giant and epithelioid cells almost as fast as they are formed. Both of the first two lines of development were demonstrated in my material from experimentally infected male wild mallard ducklings.

The third proposed line of development of E. uncinata granulomas leads to a distinct Type III granuloma. This is illustrated as a separate sequence (Fig. 32(3)) though it may represent a continuation along line 2 as indicated by the broken arrow. It is included here because of the nature of granulomas recovered from mallards in the field. Birds collected from the wild population whose worms served as the source of eggs to establish laboratory infections in Daphnia magna were rarely infected, but one adult male of 49 collected and three of eight breeding females, harboured Type III granulomas. These were large and had very thin walls consisting almost entirely of tough fibrous connective tissue. Large worms (much larger than experimentally grown ones) were contained in a cavity with a clear fluid and little cellular debris. When these granulomas were punctured, the fluid was under some pressure as it squirted from the opening. Although Type III granulomas were not sectioned, their thin walls differed from those from experimentally infected birds. How does a large, thin walled Type III granuloma develop in birds in the wild, in contrast to experimental infections where a thick walled granuloma develops, becomes occluded with cells, and the nematodes destroyed?

Assuming that the development of granulomas described here from experimental infections is representative of what occurs in birds in the wild, such a transformation would involve some mechanism whereby the wall is reduced to a thin layer of fibrous tissue in the outer region of the middle zone (see Fig. 31c). The characteristics of Type I granulomas at day 47 are interesting in this regard. They were distinctive in that there were centres of what appeared to be intense, phagocytic activity in the middle zone (the major part of the wall, Fig. 25) and abundant macrophage activity was apparent in large lymphoid follicles surrounding the granulomas, reminiscent of hyperplastic lymph nodes (Fig. 24). In addition, eosinophils and plasma cells were much more abundant than in previous granulomas; both began to increase in number on day 40 p.i. (Fig. 29b and 30c). Nemotodes in them were large and contained eggs with well developed third stage larvae. These characteristics may be of significance with respect to the formation of the Type III granulomas seen in wild birds.

Allison and Houba (1976) discussed immunopathology due to complexes of antigen and antibody (Type II or Arthus reactions) in parasitic infections. They indicated that antigen-antibody complexes in granulomas are one of the factors contributing to the pathogenesis of chronic inflammation. Such formations are chemotactic for mononuclear phagocytes and this is said to be one of the ways in which mononuclear phagocyte infiltration can be produced. As well, immune complexes can bring about lysosomal enzyme release from phagocytes, resulting in local tissue injury. Mononuclear phagocytes (macrophages) and heterophils (neutrophils) were prominent in E. uncinata granulomas. The latter type of cell was very abundant in the middle zone of granulomas at day 47 p.i. Antibody was produced by day 33 p.i. and was detected in the serum of three birds at day 47. It is possible that the areas of intense phagocytic activity in the middle zone of day 47 granulomas were areas where immune complexes had stimulated lysosomal enzyme release and the resultant tissue destruction, thus creating openings in the wall of the granuloma. If such areas were extensive enough, the thin wall of Type III granulomas may have been formed. The abrupt rise in number of eosinophils in granulomas at days 40 and 47 (Fig. 29b) provides additional support for this hypothesis.

Sabesin (1963) and Litt (1964) suggested that eosinophils phagocytose antigen-antibody complexes. If, as suggested above, areas of intense phagocytic activity observed in the walls of Type I granulomas at day 47 represented sites of tissue lysis mediated by such complexes, then the rise in number of eosinophils and their prominence in the wall rather than the inner zone could be explained. The significance of this association is not clear however, since Deane (1964) suggested that by engulfing antigen, eosinophils were in some way participating in the afferent arm of an immune response, while Greep and Weiss (1973) and Dunsford et al. (1974) suggested that such phagocytosis represents sequesteration of the potentially damaging complex or antigen. This sequesteration was thought to modulate the pathological effects of infection.

Either interpretation is possible with respect to my material but in any case, the coincidence of high eosinophil numbers, areas of tissue damage and significant antibody titres, provides circumstantial evidence to support the suggested mechanism regarding reduction in thickness of the walls of Type I granulomas, to form those characteristic of Type III.

Another mechanism involving Type II reactions as discussed by Coombs (1976) could have been involved. Antigens of worm origin may have become attached to or picked up by host cells. Complement fixing antibody, if present, may then have unleashed the tissue damaging lytic effects of complement and attendant non specific effects, resulting in localized breakdown of the granuloma wall. In the absence of complement fixing antibody, "K or Killer cells", if present, are able to lyse antibody sensitized target cells in vitro and may function in vivo (Coombs, 1976). Killer cells are thought to be normal macrophages which become activated to kill target cells. Since macrophages were abundant, some may have been active in this manner, though their involvement here is merely speculation.

The last possibility is that if infections are initiated by numerous menatodes, large Type II granulomas may initially develop but the combined feeding and/or abraisive activity of a large number of nematodes may create a thin walled Type III granuloma.

Whatever the mechanism involved in their formation, Type III granulomas were not observed in experimentally infected mallard ducklings but their presence in mallards from the wild population may be significant for a number of reasons. Type III granulomas may be the only type of granuloma which persists for a long period, assuring that infections initiated on the breeding grounds are: i) present in other areas encompassed by the distributional range of the major definitive hosts (mallards, pintails) and/or, ii) present in birds returning to the breeding grounds in the spring. Development of such granulomas would be of adaptive significance in terms of propagation of the species of nematode. Type III granulomas are, perhaps, a better source of infective material than are Type I granulomas for reasons other than their possible longevity. They have numerous large nematodes which contain large numbers of eggs. It is also likely that eggs are more easily shed, and are therefore shed more frequently from Type III granulomas because their walls are thin and their cavities are full of fluid under pressure. They are, therefore, more likely to be compressed and to expel eggs due to muscular activity of the host's stomach than are thick walled, Type I granulomas.

Finally, the presence of Type III granulomas in breeding female mallards may be their most significant characteristic. Female mallards in the wild harbouring these granulomas would serve as a very good source of infective stages for cladocerans in the ponds utilized by the hen. By the time ducklings hatched there would be a significant number of third stage larvae available to infect them. Ducklings from numerous broods utilizing such ponds could thus become infected early in life. If only a few of those infections resulted in Type III granulomas, a persistent source of infective material would be assured. This has obvious adaptive value for the nematode.

The occurrence of Type III granulomas in a greater proportion of female mallards than in males, suggests that host sex may affect their development. It may be that female sex hormones influence the responses of the host so that the change from Type I to Type III (rather than to Type II) occurs at sexual maturity. There are conflicting reports in the literature regarding the effects of sex hormones on parasites. Gray (1973) reported that estrogens were at least partially responsible for reduced resistance of adult female chickens to the tapeworm *Raillietina cesticillus* while Nadakal *et al.*(1973) reported that stilboestrol inhibited the growth of *R. tetragona* in chickens. Breeding female rabbits were reported to harbour more *Trichostrongylus retortaeformis* and *Graphidium strigosum* than did males (Dunsmore, 1966, 1971). On the other hand, estradiol was reported to decrease susceptibility of mice to *Echinococcus granulosus* infection, and females had fewer and smaller cysts than males as a result of intraperitoneal injections of protoscoleces (Frayha, 1971). Thus, it is known that female sex hormones can increase susceptibility to parasites so that some similar effect may operate in the case of *E. uncinata* in mallards.

It is also possible that stresses associated with the breeding period may serve to reduce the ability of the host to contain and destroy granulomas. Ould (1972) has shown that stressors of various kinds increased susceptibility of Delta mallards to *E. uncinata* and resulted in greater growth of nematodes. The breeding period is a time of great stress, particularly for female ducks. An hormonal influence, complicated by the effects of such stress may be important in causing the development of Type III granulomas in females.

Plasma proteins were consistently separated into five fractions including prealbumin, albumin, and alpha, beta and gamma globulins. More than one class of alpha or beta globulin were occasionally encountered, but not consistently enough to warrant their documentation. Electropherograms were similar to those obtained by Deutsch and

Goodloe (1945) from the plasma of muskovy ducks and mallard-muskovy hybrids except that no fibrinogen was present in my material as I used serum. They also noted variation in the number of globulin classes. The proportions of albumin in sera of ducklings from this study were greater, and the proportions of globulin classes correspondingly less, than those reported by Deutsch and Goodloe (1945). They used adult birds as the source of their material which may explain these discrepancies since albumin content is known to decrease with age while the globulin content increases (Medway and Kare, 1959; Threlfall, 1966). Infected ducklings were sampled between the ages of five and thirteen weeks and uninfected birds were sampled anywhere from four to thirteen weeks of age in this study. No consistent trend to a reduction in the albumin content or an increase in the globulin content of their sera was noted (Tables 11 and 12).

Total protein was measured by the Lowry technique which is similar to the Biuret method but enables the use of smaller volumes of serum. Sturkie and Newman (1951) compared the Biuret and Micro-Kjeldahl methods of protein estimation and found them comparable so that the estimates I obtained were reasonably accurate. Total protein fluctuated significantly over the experimental period due mainly to fluctuations in the amount of albumin present, but there was no overall trend to a reduction in total protein or albumin. Hypoproteinemia due to a loss of albumin through mucosal surfaces has been reported for several nematode infections including: *Ostertagia ostertagii* in cattle; *Trichostrongylus axei* in calves, sheep and lambs; *Hyostrongylus rubidus* and *Strongyloides ransomi* in pigs; and *Nippostrongylus brasiliensis* in rats (see review of Nielsen, 1976). Batte *et al.*(1976) associated a decrease in the percentage of albumin in the sera of pigs infected with *Trichuris suis*, with albumin loss by the gut mucosa. They also noted an increase in the globulin fraction at 7 days p.i. (gamma), 14 days p.i. (alpha) and 24 days p.i. (beta), which they suggested was due to the production of immunoglobulins. Georgieva and Vladimirova (1975) reported similar observations on *Haemonchus contortus* infections in lambs. Nielsen (1976) also indicated that fascioliasis and infections with *Schistosoma matheei* were reported to cause hypoalbuminemia and hyperglobulinemia where, at least in fascioliasis, the hyperglobulinemia was attributed to large amounts of IgG_1.

None of the above effects were observed in primary *E. uncinata* infections due, perhaps, to the small numbers of nematodes involved. The early stages of infection could conceivably cause some loss of plasma proteins through the mucosal surface because considerable congestion, edema and tissue damage was apparent. This would not be significant unless infections involved very large numbers of worms and much more of the mucosal surface than involved in this study. Similarly, large numbers of well developed, fluid-filled granulomas could cause a loss of plasma proteins due to slow leakage of fluid from them, or the spurting of large amounts of fluid during periods of ventricular activity. Such a mechanism is undoubtedly involved in the shedding of eggs into the lumen of the gut. Again, only in heavy infections would such a loss be significant, and as indicated by Austin and Welch (1972), would be less important pathologically than other effects such as blockage of the alimentary canal. As pointed out by Symons and Fairbairn (1962), physiological effects of infection such as reduced absorptive capacity in one part of the gut may be compensated for by an increase in absorptive capacity in another, resulting in no measureable change. Losses of plasma proteins on a small scale could similarly be compensated for by an increase in plasma protein production. Nielsen (1976) indicated that such is the case where the rate of plasma protein catabolism (particularly albumin catabolism) is directly related to the rate of plasma protein anabolism in a variety of animals studied.

The proportion of birds which produced antibody to E. uncinata antigen was low (30%) as were the titres obtained. Ould (1972) reported that antibody was detectable in Delta mallards at 50 days p.i. but did not test earlier samples. I found that antibody was present in detectable amounts by day 33 p.i. but not at day 26 or earlier. This was correlated with an increase in the number of plasma cells in granulomas and with what could have been immune complexes in the walls of granulomas.

The detection of antibody is not unexpected in view of

Cohen's assertion that antibodies have been detected in most, if not all, parasitic infections studied (Cohen, 1976a). It should be borne in mind that generally only precipitins are detected by immunodiffusion so that other types of antibodies may have been produced and gone undetected (Crowle, 1975). Tanner (1968) pointed out that each serological test employed may detect the antibody response to a small group of antigens, and that the timing and magnitude of the responses may vary depending on the technique employed. He reported that the precipitating antibody response to T. spiralis in rabbits was unrelated to the antibody response as detected by the complement fixation test. The whole-worm extract antigen used in this study was probably a heterogeneous mixture of antigens but only one definite arc was observed on the plates, indicating that a limited number of antibody molecules were reacting. Other tests may have shown a greater variety of antibodies.

Antibodies (agglutinins) have been detected as early as two days postinfection in pigs infected for the first time with Hyostrongylus rubidus (Smith and Herbert, 1976) but most responses are not detectable until considerably later. Houba et al.(1976) reported that in baboons infected with S. mansoni, different types of worm antigens (gut associated, membrane associated) were detected from 2 to 5 weeks p.i., and the corresponding antibodies were detected one to two weeks later (3 to 7 weeks p.i.). Maddison et al.(1970) detected antibody to S.

mansoni by means of indirect haemagglutination and PCA 4 weeks p.i., while titres obtained by five other tests rose to high levels only after worm burdens declined at approximately 8 weeks. Sadun et al. (1970) detected antibody in chimpanzees one month after infection with S. haematobium, and Ruitenberg and Duyzings (1972) detected antibody to T. spiralis in rats three weeks after a primary infection. These results are comparable to the timing of antibody production to E. uncinata in wild mallards reported here.

The first appearance of antibody at day 33 may also suggest that antibody production was correlated with the attainment of sexual maturity and to the production of eggs. As indicated earlier, sexual maturity is attained by day 20 and eggs are produced by day 30 p.i., shortly before antibody was detected. Gravid female worms were used to prepare antigen so that egg associated antigens were undoubtedly present in the preparation. This would parallel the situation with schistosome infections where, at the time of egg deposition in the tissues, there is a great increase in antibody levels (Smithers, 1976).

V. EFFECTS OF AGE AT INFECTION ON THE HOST-PARASITE RELATIONSHIP

Experimental Design

Seventy, one-day-old ducklings were divided into seven equal groups and infected with 60 E. uncinata larvae each at one of the ages listed in Table 13. Each duckling was identified with a coloured leg band or web clip at the time of infection. Half of each group was examined 20 days later; the other half of each group was examined at 50 days postinfection. At the time of necropsy various growth parameters were noted, the number and diameters of the granulomas taken, and the granulomas removed to dishes of saline for later examination. Splenic tissue and blood samples were taken and processed for lymphoid follicles, or total protein, protein fractions and antibody titre respectively. Splenic follicles were assessed in order to determine if there was any relationship between their number and antibody titre.

Results

Twenty Days Postinfection

Parameters related to growth of the ducklings, their general condition as measured by the condition index, and the number of follicles per mm^2 of splenic tissue are summarized in Table 13. In terms of growth in body size and weight, these ducklings may have been slightly behind uninfected birds of comparable age when they were infected during the first two weeks after hatching, but thereafter the fact that they were given a nematode infection did not seem to alter their growth pattern (see Table 14). The exception to this generalization are those ducklings infected at the age of four weeks. All parameters were considerably lower when compared to birds infected at three weeks and to

Table 13. Weight, size, condition index and splenic follicles from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 20 postinfection.

PARAMETER	WEIGHT gm	CULMEN mm	KEEL mm	[1]CONDITION INDEX	[2]SPLENIC FOLLICLES
AGE AT INFECTION					
One day	[3]258.9	43.1	43.5	7.3	0.22
	(33.8)	(1.9)	(2.4)	(0.8)	(0.13)
One week	405.6	49.0	51.4	6.3	0.14
	(44.9)	(2.2)	(1.9)	(0.9)	(0.09)
Two weeks	434.2	49.8	52.8	6.2	0.32
	(103.0)	(1.8)	(4.9)	(0.84)	(0.26)
Three weeks	755.7	59.5	75.5	6.0	0.14
	(101.1)	(3.9)	(6.2)	(0.4)	(0.10)
Four weeks	450.0	53.2	63.4	7.65	0.76
	(23.5)	(3.0)	(4.5)	(1.2)	(0.35)
Five weeks	903.3	62.6	93.4	6.5	0.44
	(102.6)	(2.2)	(5.4)	(0.6)	(0.26)
Six weeks	810.2	63.8	93.3	7.3	0.64
	(19.5)	(1.3)	(2.1)	(0.4)	(0.38)

1. Calculated from (Culmen χ Keel) / Weight.
2. Number of splenic follicles per mm^2 of splenic tissue.
3. Numbers are means with standard deviations in parentheses below.

Table 14. Weight, size, condition index and splenic follicles from uninfected control ducklings of different ages.

PARAMETER		WEIGHT gm	CULMEN mm	KEEL mm	[1]CONDITION INDEX	[2]SPLEEN DIAMETER	SPLENIC FOLLICLES
AGE GROUP	N						
Five weeks	5	[3]526.2	56.6	63.0	6.88	4.5	0.52
		(90.0)	(1.1)	(4.4)	(0.82)	(0.6)	(0.27)
Seven weeks	5	816.5	62.8	88.2	6.83	5.0	0.29
		(112.4)	(3.3)	(6.8)	(0.6)	(0.3)	(0.12)
Eleven weeks	10	858.3	64.2	90.8	6.83	5.4	0.54
		(98.6)	(2.8)	(5.5)	(0.6)	(1.3)	(0.2)
Thirteen weeks	5	990.4	62.6	100.4	6.39	7.0	0.64
		(128.3)	(1.5)	(6.6)	(0.5)	(0.7)	(0.25)

1. Calculated from (Culmen χ Keel) / Weight.
2. Number of splenic follicles per mm^2 of splenic tissue.
3. Numbers are means with standard deviations in parentheses below.

uninfected controls of the same age (Table 14, seven weeks). This particular group was noted at necropsy to have Aspergillus fumigatus infections which may account for their relatively small size and low overall condition.

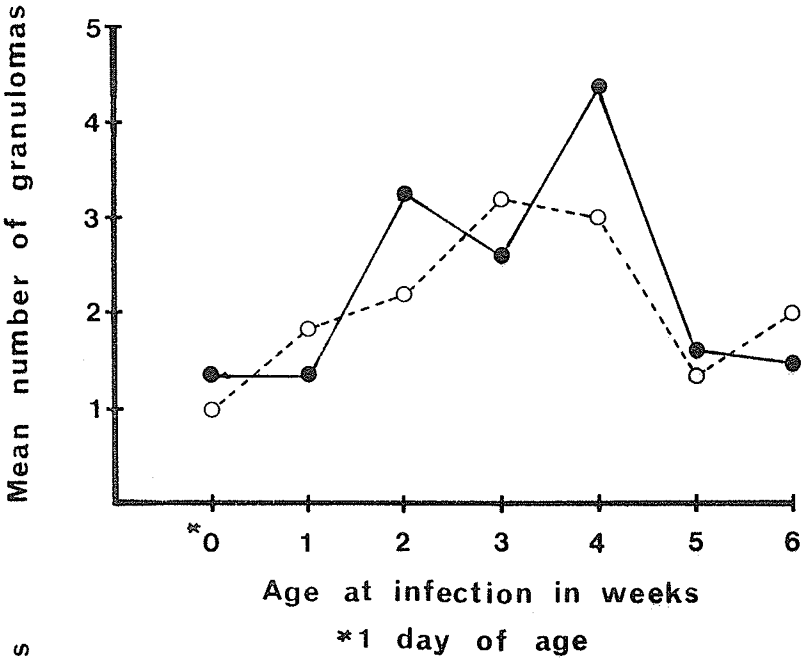
The number and diameters of lesions, and the number of nematodes recovered from each of the age groups at 20 days postinfection are presented in Appendix II (Table 1). All of the parameters analysed varied significantly between groups (see the F ratios) but no particular patterns related to the chronological ages of the birds were established. The number of granulomas per bird was higher in the two to four week groups when compared to the older and younger age groups (Fig. 33). Except for the one and two week groups, birds infected at early ages generally had more nematodes than ones infected later (Fig. 34). However, one way analysis of variance showed that the number of nematodes recovered from the one day, and two to four week groups were not statistically different, due in part to the large variances associated with these means (Fig. 35). The trellis diagram in Figure 35 consists of the F ratios (from analyses of variance comparing pairs of samples) on the right hand side, and the probability associated with the ratios on the left. The first row represents the F ratios which compare the one day group to all of the others. The corresponding probabilities are presented in the first column. The one week group had fewer worms than the one day and three week groups, and significantly

Figure 33. Mean number of granulomas/bird recovered from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 20 or 50 postinfection.

●——● day 20
○-----○ day 50

Figure 34. Mean number of worms/bird recovered from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 20 or 50 postinfection.

●——● day 20
○-----○ day 50


Mean number of granulomas
5
4
3
2
1
*0
1
2
3
4
5
6
Age at infection in weeks
*1 day of age

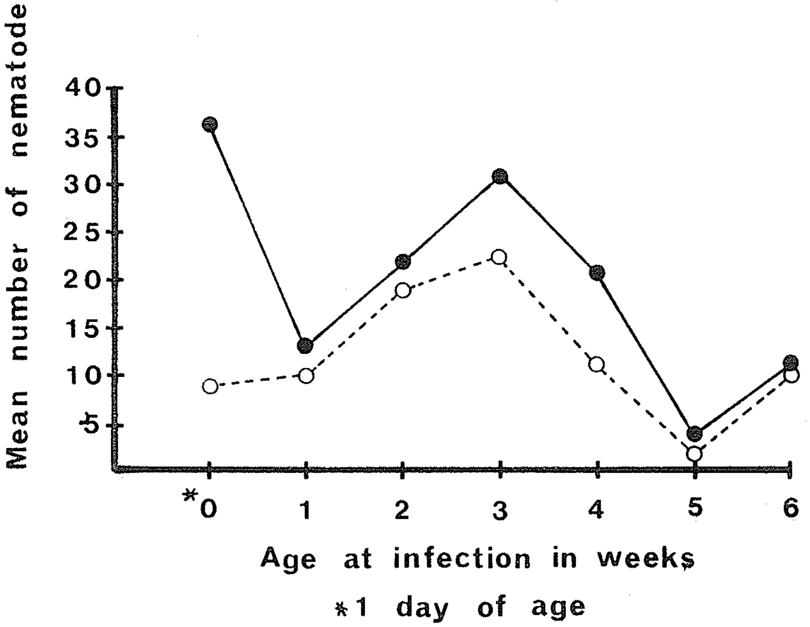

Mean number of nematodes
40
35
30
25
20
15
10
5
*0
1
2
3
4
5
6
Age at infection in weeks
*1 day of age

Figure 35. Trellis diagram illustrating the results of one way analysis of variance of the number of worms recovered from groups of ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 20 postinfection. (Unbracketed numbers in the compartments on the right side of the diagram are F ratios, bracketed numbers below are the degrees of freedom for each comparison. Numbers on the left side of the diagram are the probabilities associated with the corresponding F ratios.)

	*1	2	3	4	5	6	7
**	36.6	13.0	22.0	30.6	20.9	4.1	11.5
*1		16.12 (1,8)	4.88 (1,7)	0.93 (1,8)	5.00 (1.8)	36.90 (1,8)	10.51 (1,7)
2	.005		5.08 (1,7)	18.05 (1,8)	2.20 (1,8)	11.2 (1,8)	0.07 (1,7)
3				3.47 (1,8)	0.04 (1,7)	38.25 (1,6)	2.71 (1,8)
4		.005			2.99 (1,8)	62.31 (1,8)	9.71 (1,7)
5						12.74 (1,8)	1.70 (1,7)
6	.001	.01	.001	.001	.01		2.25 (1,7)
7	.025			.025			

* 1= 1 day; 2= 1 week; 3= 2 weeks; 4=3 weeks; 5= 4 weeks; 6= 5 weeks; 7= 6 weeks at infection.

** Mean numbers of worms/bird in each group more than the five week group. The five week group had significantly fewer nematodes than all groups except the six week one.

Linear regression of the number of worms on the number of granulomas showed that there was no significant relationship between the two in ducklings examined at 20 days postinfection (F=1.96, 1 & 31 df)(see Appendix III, Table 1).

The mean diameter of granulomas fluctuated considerably from group to group. They were generally larger in birds with large numbers of worms but no good relationship was discernable.

The mean length and diameter of nematodes recovered from these ducklings are presented in Appendix II (Table 2). The total amount of variation of both parameters was highly significant and showed a consistent age related trend; the younger the age at infection, the larger the nematodes at 20 days postinfection (Fig. 36). The exception to this trend was the three week group. The worms from the five week group were not measured but were noted to have been smaller than usual at the time they were fixed.

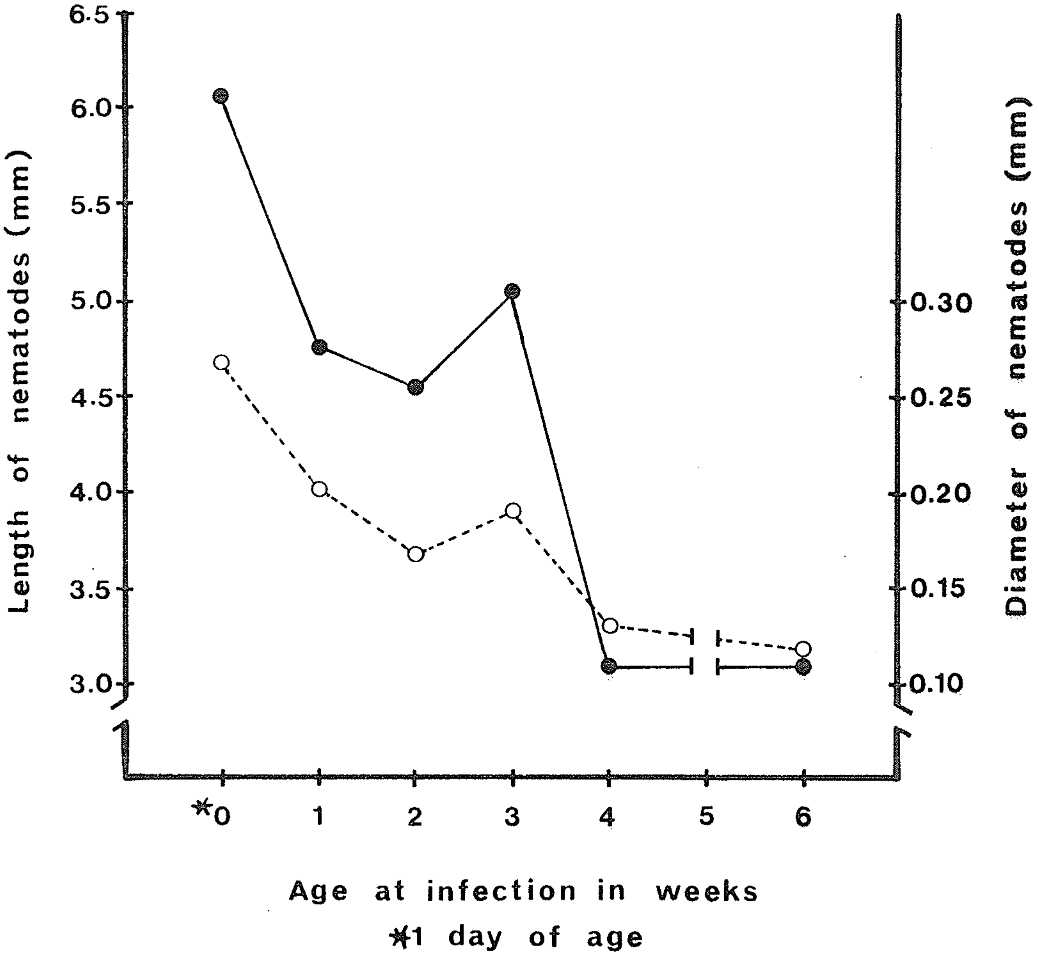
The sera of all groups were analysed for total protein content and for the amounts of each of the major fractions present (Table 15). The total protein content varied considerably from group to group due to significant variations in all fractions except the alpha globulin. The four week group had considerably greater amounts of most

Figure 36. Mean lengths and diameters of nematodes recovered from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 20 postinfection.

●——● Length in mm
○----○ Maximum diameter in mm

Figure 37. Relationship between the number of granulomas and the number of worms recovered from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 50 postinfection.

$t = 2.69,\ p < .05$
$F = 12.737,\ p < .005$


6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Length of nematodes (mm)
0.30
0.25
0.20
0.15
0.10
Diameter of nematodes (mm)
*0
1
2
3
4
5
6
Age at infection in weeks
*1 day of age

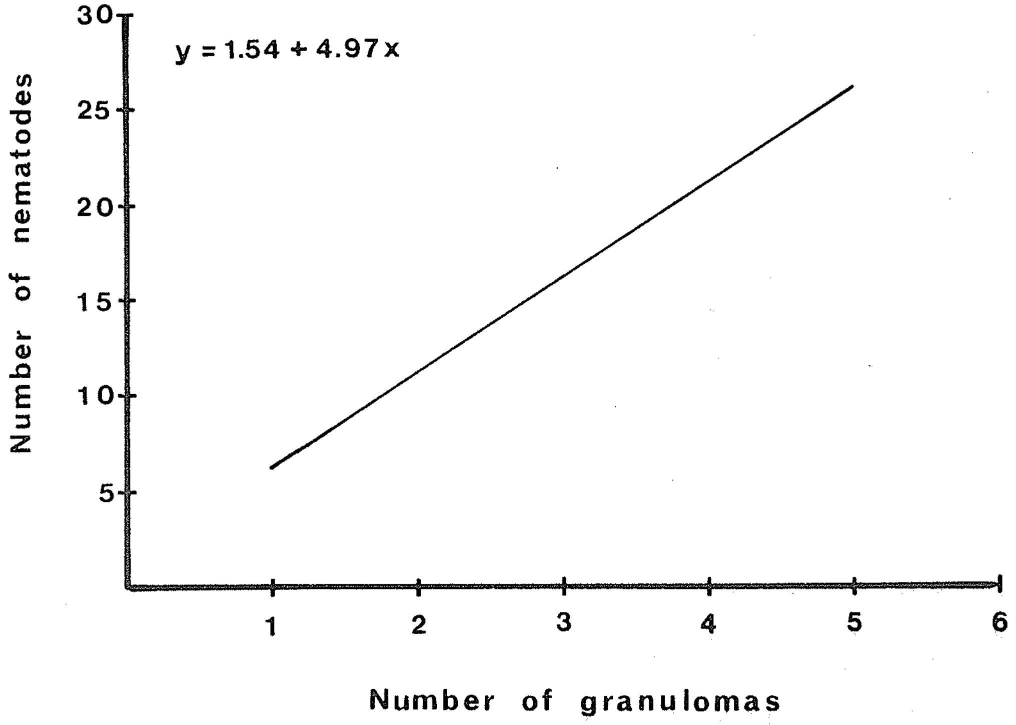

30
25
20
15
10
5
Number of nematodes
y = 1.54 + 4.97x
1
2
3
4
5
6
Number of granulomas

Table 15. Means of serum protein fractions from ducklings infected with 60 E. uncinata larvae at various ages and necropsied at day 20 postinfection.

SERUM FRACTION		PRE-ALBUMIN	ALBUMIN	GLOBULIN			TOTAL PROTEIN
				ALPHA	BETA	GAMMA	
AGE AT INFECTION	[1]N						
One day	4	[2]1.55 (0.63)	27.43 (4.92)	6.63 (2.84)	5.95 (2.62)	3.14 (1.22)	44.70 (4.84)
One week	5	0.88 (0.51)	25.42 (2.22)	5.62 (2.74)	3.15 (1.03)	1.84 (0.71)	36.92 (3.22)
Two weeks	4	1.27 (1.16)	24.85 (1.51)	5.54 (1.03)	6.84 (1.15)	2.07 (1.18)	40.57 (3.05)
Three weeks	5	0.74 (0.44)	23.84 (1.77)	4.71 (0.51)	4.27 (1.17)	2.16 (0.33)	35.72 (2.60)
Four weeks	5	0.40 (0.29)	38.75 (7.80)	6.80 (1.94)	8.34 (2.89)	4.81 (0.39)	59.10 (8.35)
Five weeks	5	1.71 (0.53)	23.38 (1.31)	5.08 (1.12)	5.29 (0.78)	2.04 (0.35)	37.51 (1.98)
Six weeks	5	0.69 (0.22)	25.87 (0.76)	6.03 (1.17)	5.92 (1.13)	2.50 (0.47)	41.00 (1.61)
[3]F(6 & 26 df)		2.859	10.03	0.869	4.838	15.54	17.740
p<		.05	.001	ns	.005	.001	.001

1. Number of birds.
2. Numbers are means with standard deviations in parentheses below.
3. Results of one way anovar with probability (p) below.

fractions than the other groups, accounting for most of the variation.

The sera of these birds were also subjected to immunodiffusion against E. uncinata antigen. Virtually no antibody was detected. The only exception was one sample from one bird infected at the age of one day. A very weak precipitin arc was produced; the estimated titre was 1.0.

A number of parameters which were thought intuitively to be related, were tested for correlation by means of linear regression. It was thought that if splenic follicles were involved in antibody production, there might be some relationship between the number of those structures in spleens and the number of worms, the gamma globulin content of the sera and perhaps the condition of the birds. All of these parameters were used in pairs for simple linear regression procedures, or in various combinations for multiple regression analyses.

There was a significant linear relationship between the number of splenic follicles (Table 13) and condition index (Appendix III, Table 1; $F=6.096$, $p<.025$). Since larger values of condition index represent poorer condition, this relationship suggests that birds in poorer overall condition had more splenic follicles. Only 17% of the variability in number of splenic follicles was accounted for by this relationship since the square of the correlation coefficient (r^2) was only 0.169.

Gamma globulin content of the serum samples from these birds was linearly related to the number of splenic follicles alone (Appendix III, Table 1), but this accounted for only approximately 18% of the variability ($r^2=0.179$). When the number of nematodes present was added as another variable (which was not significantly correlated with gamma globulin or splenic follicles alone), the relationship was improved in that 28% of the variability in gamma globulin content could be accounted for. The equation, $Y=1.84X_1 + 0.03X_2 + 1.38$ (where Y is gamma, X_1 is splenic follicles, and X_2 represents the number of worms), describes the relationship ($F=5.599$, $p<.03$).

Gamma globulin was also correlated with the number of granulomas ($F=8.316$, $p<.001$) with 22% of the variability in gamma globulin being accounted for ($r^2=0.217$) (Appendix III, Table 1).

Fifty Days Postinfection

Data related to size, weight, condition and number of splenic follicles for birds necropsied after 50 days are presented in Table 16. None of the groups appeared to have been retarded in growth at this time; even members of the four week group, which were small and in poor condition at 20 days postinfection, were of normal size and in good condition as measured by the condition index. They did have a considerable number of splenic follicles per mm^2 of splenic tissue. There was a significant variation in splenic follicles from group to group

Table 16. Weight, size, condition index and splenic follicles from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 20 postinfection.[1]

PARAMETER	WEIGHT gm	CULMEN mm	KEEL mm	CONDITION INDEX	SPLENIC FOLLICLES
AGE AT INFECTION					
One day	808.5	63.4	87.0	6.9	0.61
	(108.3)	(2.0)	(8.2)	(0.2)	(0.41)
One week	768.4	61.7	88.5	7.1	0.36
	(90.7)	(2.1)	(5.7)	(0.4)	(0.13)
Two weeks	791.9	63.5	89.3	7.2	1.01
	(110.5)	(1.3)	(7.0)	(0.5)	(0.33)
Three weeks	911.3	65.0	98.4	7.0	0.65
	(48.7)	(2.7)	(3.5)	(0.4)	(0.52)
Four weeks	927.9	62.4	97.6	6.6	1.46
	(123.0)	(1.8)	(4.3)	(0.7)	(0.37)
Five weeks	934.3	66.6	100.8	7.3	0.76
	(105.4)	(1.8)	(4.6)	(0.8)	(0.41)
Six weeks	988.2	69.3	105.5	7.5	1.11
	(134.9)	(3.0)	(4.4)	(1.1)	(0.70)

1. For an explanation see Table 13.

(F=14.22, 6 & 125 df; p<.001) with the two week, four week, and six week groups all having large numbers.

Data regarding the number of granulomas, diameter of granulomas, and numbers of nematodes recovered from all age groups at 50 days postinfection are summarized in Appendix II (Table 3). Only the mean number of granulomas per bird varied significantly from group to group as indicated by one way analysis of variance (see the F ratios, Table 3, Appendix II). Birds in the two, three and four week groups had the greatest number of granulomas and the greatest number of worms per bird (Figs. 33, 34). However, when each of the age groups was compared to the others in this regard, the only significant differences noted were between the five week group and the one and two week groups. The large variances associated with the means for most groups are undoubtedly responsible for the inability to detect real differences where they at least superficially appear to exist.

When the raw data for number of granulomas per bird and the number of worms per bird were pooled and then subjected to linear regression analysis, it was found that the two parameters were significantly and positively correlated (F=12.737, p<.005) (Appendix III, Table 2). Figure 37 illustrates the relationship, but only 29% of the variability was accounted for by this relationship as the square of the correlation coefficient (r^2) was estimated at 0.291.

Figures 33 and 34 illustrate that the variability in these two parameters, from age group to age group , at day 50 was consistent with the patterns established by day 20. For example, the mean number of worms recovered from each group was lower at 50 days, but the trends described by the curves are generally comparable (Fig. 34). This suggests that these parameters were influenced by factors which were, perhaps, more characteristic of the particular groups of birds than by factors associated with age at infection *per se*.

The lengths and diameters of the nematodes recovered from each group (Appendix II, Table 4) are illustrated in Figure 38 and the means were tested for differences by analysis of variance. Mean length of worms differed significantly overall because there were differences between individual groups. Nematodes from the three week group were obviously shorter than those from all other groups (Fig. 38) and this was confirmed by comparing them to those of all other groups (Fig. 39). Worms from the six week group were also shorter than those from the one and four week groups. There was a significant trend to a reduction in the mean diameter of worms with increasing age at infection (Fig. 38) which was substantiated by comparing each of the groups to all the others (Fig. 40). This was most apparent when the one day and one week groups were compared to each other and to all other groups. The reduction in mean diameter of worms was also circumstantially correlated with a less extensive development of the uterus.

Figure 38 . Mean length and diameter of nematodes recovered from ducklings infected at various ages with 60 *E. uncinata* larvae and necropsied at day 50 postinfection.

●——● Length
○-----○ Diameter

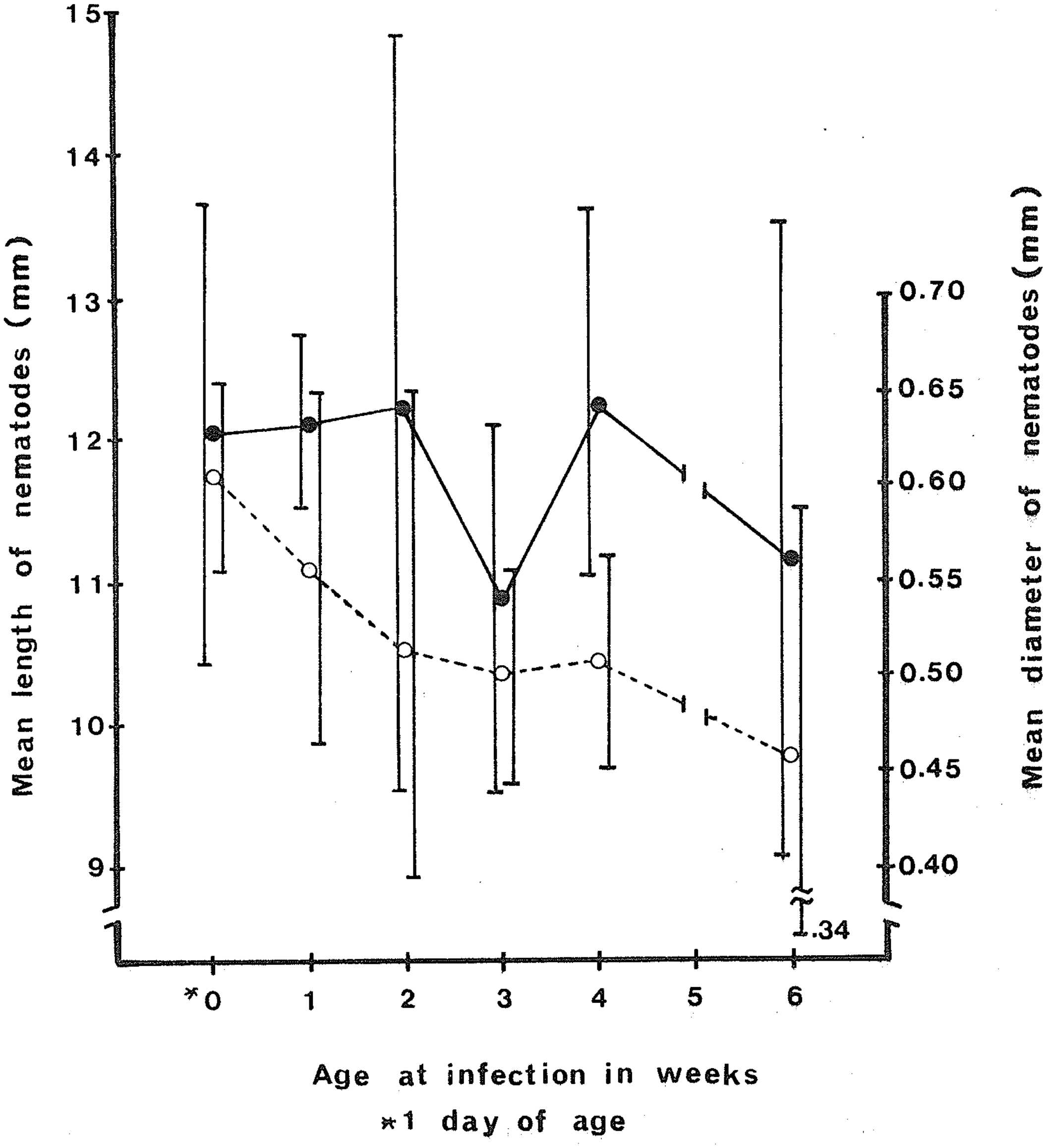

15
14
13
12
11
10
9
Mean length of nematodes (mm)
0.70
0.65
0.60
0.55
0.50
0.45
0.40
.34
Mean diameter of nematodes (mm)
*0
1
2
3
4
5
6
Age at infection in weeks
*1 day of age

Figure 39. Trellis diagram illustrating the results of one way analysis of variance comparing the mean lengths of nematodes from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 50 post-infection. (Numbers in the compartments on the right side of the diagram are F ratios. The minimum degrees of freedom are 1&31. Numbers on the left side of the diagram are the probabilities associated with the corresponding F ratios.)

	*1	2	3	4	5	6
	**12.04	12.11	12.20	10.81	12.22	11.22
*1		0.02	0.06	8.51	0.18	1.84
2			0.02	12.65	0.11	2.29
3				6.66	0.001	2.09
4	.001	.001	.01		15.95	0.72
5				.001		3.67
6		.05			.01	

* 1= 1 day; 2= 1 week; 3= 2 weeks; 4= 3 weeks; 5= 4 weeks; 6= 6 weeks at infection.

** Mean length of worms in mm from each of the groups.

Figure 40. Trellis diagram illustrating the results of one way analysis of variance comparing the mean diameters of nematodes from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 50 postinfection. (Numbers on the right side of the diagram are F ratios. The minimum degrees of freedom are 1&31. Numbers on the left side of the diagram are the probabilities associated with the corresponding F ratios.)

	*1	2	3	4	5	6
	**0.65	0.56	0.52	0.50	0.51	0.46
1		3.06	7.78	46.04	31.23	22.25
2	.05		1.43	9.25	5.31	7.23
3	.001			0.68	0.14	2.78
4	.001	.001			0.40	2.00
5	.001	.001				2.55
6	.001	.001	.05		.05	

*1= 1 day; 2= 1 week; 3= 2 weeks; 4= 3 weeks; 5= 4 weeks; 6= 6 weeks at infection.

** Mean diameter of worms in mm from each group.

Serum proteins were analysed as before and immunodiffusion against E. uncinata antigen was carried out. The results of these procedures are presented in Table 17 and in Appendix II (Table 5). There was significant variation in the protein content of the sera from different groups due mainly to variation in the albumin and alpha globulin fractions (Table 17). Variation was less pronounced than at day 20 (see Table 15). Although there was no significant variation overall in the gamma globulin fraction, its mean level fluctuated with that of total protein. Because of the importance of the gamma fraction as that which carries most of the antibody, the gamma and albumin fractions were run in a regression analysis against total protein. This showed that there was a significant linear regression of both fractions on total protein (F=24.580 and 48.674 respectively, $p<.001$ in each case) (Appendix III, Table 2). Forty-four percent of the variation in gamma globulin and sixty-one percent of the variation in albumin could be accounted for by this relationship.

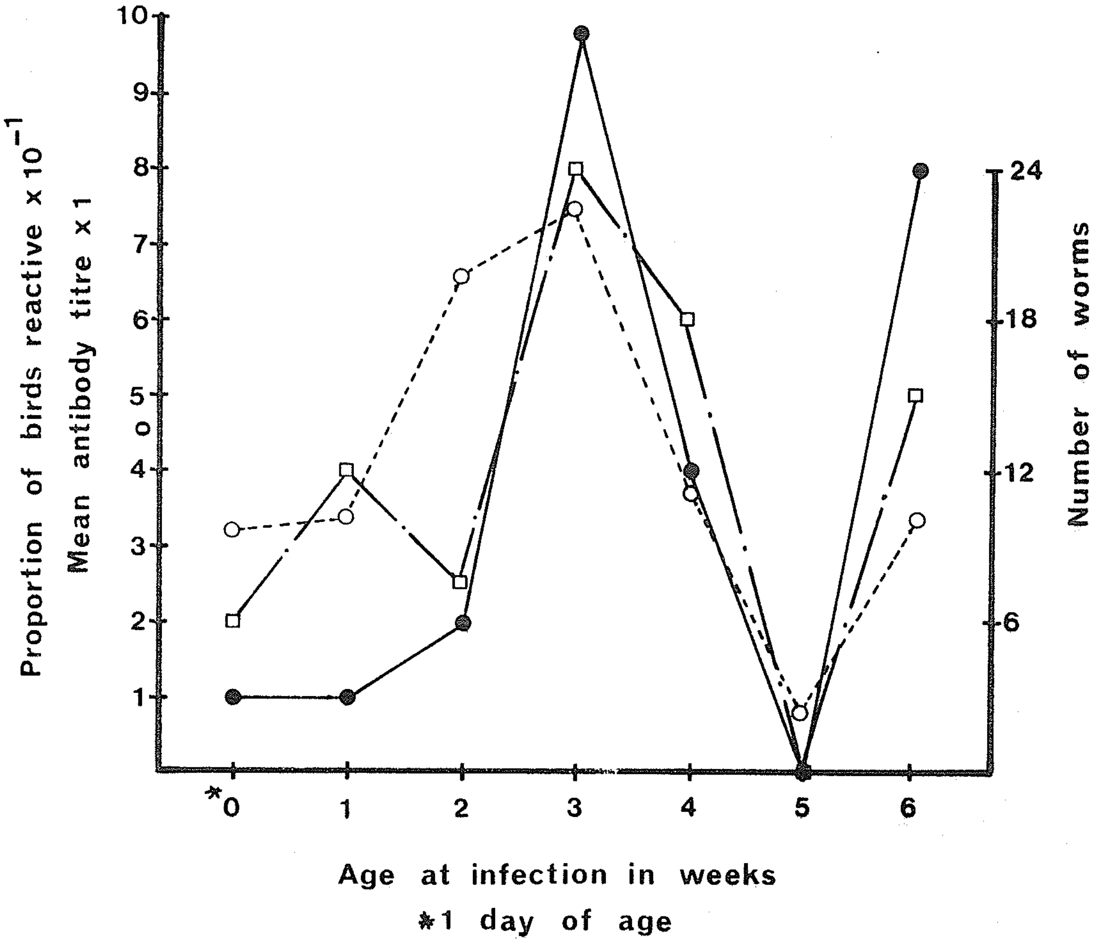
Table 5 in Appendix II presents the results of the semi-quantitative immunodiffusion procedures; some of these data are illustrated in Figure 41. The proportion of birds which had detectable antibody in their serum (proportion of birds reactive in Fig. 41) and the mean titre of the sera were low in the one day to two week groups, were high in the three and four week groups and were intermediate in the six week group (mean antibody titre in Fig. 41). There were no

Table 17. Serum protein fractions from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 50 postinfection.

SERUM FRACTION		PRE-ALBUMIN	ALBUMIN	GLOBULIN			TOTAL PROTEIN
				ALPHA	BETA	GAMMA	
AGE AT INFECTION	N						
One day	5	1.29 (1.20)	34.09 (7.67)	5.72 (0.90)	5.00 (1.09)	3.50 (1.93)	49.58 (5.48)
One week	5	1.14 (0.46)	25.91 (2.12)	5.12 (1.35)	6.56 (2.61)	2.19 (1.32)	40.92 (3.74)
Two weeks	5	2.16 (1.60)	34.63 (3.38)	7.13 (2.20)	6.75 (1.27)	4.50 (1.61)	55.15 (7.01)
Three weeks	5	1.57 (1.51)	35.03 (3.09)	7.09 (1.31)	6.89 (2.92)	6.46 (4.34)	57.04 (5.51)
Four weeks	5	0.98 (0.73)	27.11 (2.86)	4.43 (1.75)	4.63 (2.60)	3.40 (1.02)	40.55 (2.54)
Five weeks	5	0.98 (0.39)	28.22 (2.71)	4.96 (0.73)	4.81 (1.38)	2.52 (0.83)	41.48 (4.04)
Six weeks	3	0.84 (0.50)	26.5 (1.97)	5.24 (1.69)	4.55 (1.60)	3.14 (0.37)	40.27 (2.01)
F(6 & 26 df)		---	5.207	2.518	1.249	2.309	11.320
p<		---	.005	.05	ns	ns	.001

Figure 41. Mean number of nematodes, proportion of birds reactive, and mean antibody titre of serum samples from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 50 postinfection.

○-----○ Number of nematodes
□—·—□ Proportion of birds reactive
●——● Titre


Proportion of birds reactive x 10⁻¹
Mean antibody titre x 1
○
10
9
8
7
6
5
4
3
2
1
24
18
12
6
Number of worms
*0
1
2
3
4
5
6
Age at infection in weeks
*1 day of age

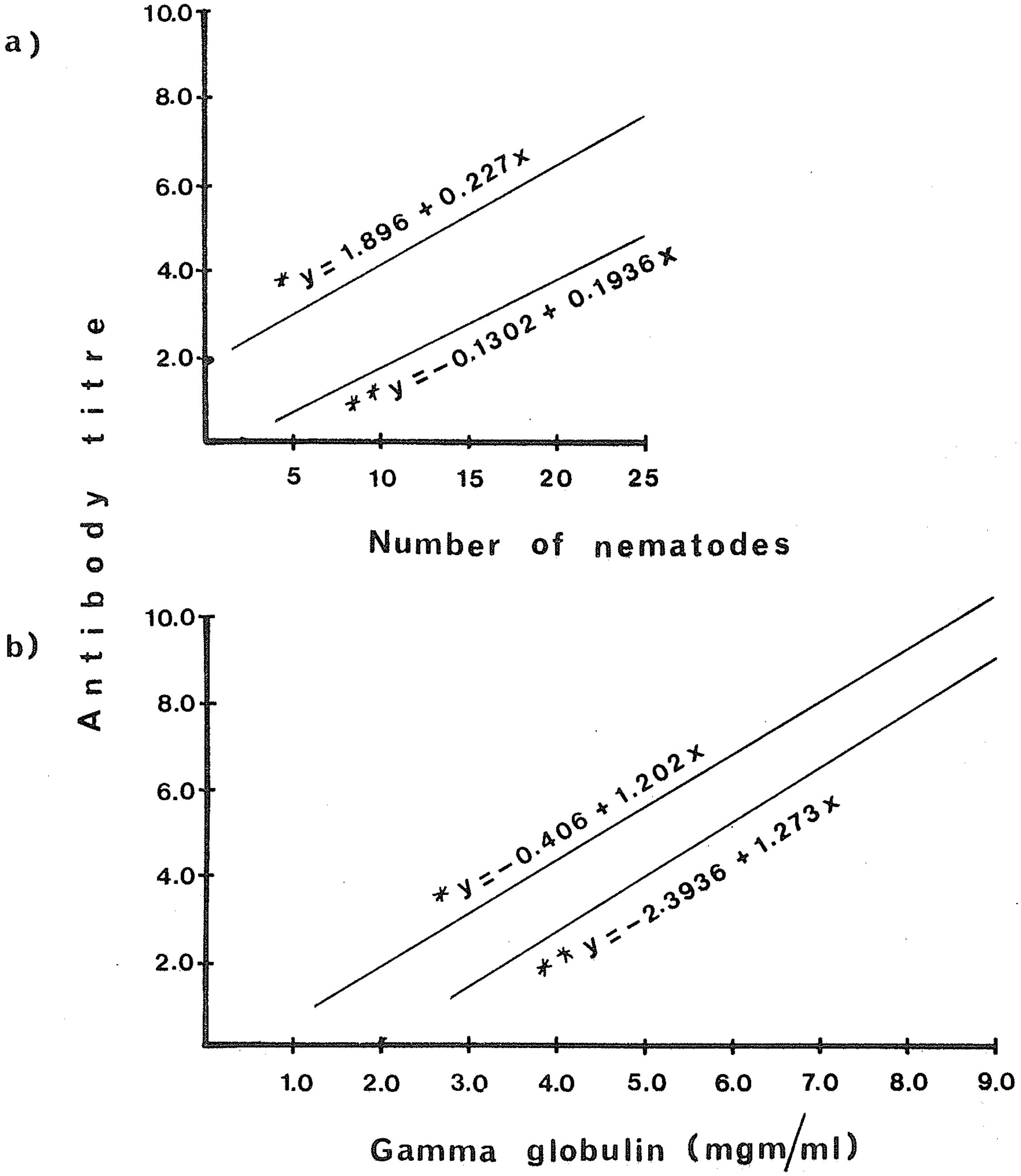
reactors in the five week group. Figure 41 also relates these parameters to the number of worms present at 50 days in each of the age groups. The patterns of the curves are remarkably similar, and all patterns are similar to that plotted for gamma globulin (not shown).

Linear regression analysis (Appendix III, Table 2; Fig. 42) showed that when all ducklings were considered regardless of whether or not they produced antibody, titres of their sera were directly correlated with both the number of worms and gamma globulin content. Regression of titre on number of worms from all birds (Fig. 42a) accounted for 29% of the variability in titre (r^2=0.293), while regression on gamma globulin (Fig. 42b) accounted for 48% of the variability in titre (r^2=0.476). This indicates that the number of nematodes present was important in determining the amount of specific antibody produced by individual birds, and also that a significant amount of the gamma globulin present was specific antibody directed at _E_. _uncinata_. A second set of regression lines were run in which only those birds in which specific antibody had been detected were included (13 birds, see Appendix II, Table 5). When this was done, the amount of variation in titre attributable to the number of worms was 38% (r^2=0.38, reactors only in Fig. 42a). There was only a small difference in dependency of titre on gamma globulin (r^2=0.43, reactors only in Fig. 42b). The effect of removing non reactors from the analysis was to remove those birds which had nematodes but in which no antibody could be detected -

Figure 42. Relationship between antibody titre of serum samples and either the number of worms present or the gamma globulin content of serum samples from ducklings infected with 60 _E_. _uncinata_ larvae and necropsied at day 50 postinfection.

a) Titre vs number of worms.
* Only reactors included.
(t= 2.616, $p<.05$; F= 6.848, 1&11df ,
p<.025)
** All birds included.
(t= 3.584, $p<.01$; F= 12.846, 1&31df ,
p<.001)

b) Titre vs gamma globulin.
* Only reactors included.
(t= 2.87, $p<.02$; F= 8.249, 1&11df ,
p<.005)
** All birds included.
(t= 5.311, $p<.001$; F= 28.27, 1&31df ,
p<.001)


a)
b)
Antibody titre
10.0
8.0
6.0
4.0
2.0
* y = 1.896 + 0.227 x
** y = −0.1302 + 0.1936 x
5
10
15
20
25
Number of nematodes
10.0
8.0
6.0
4.0
2.0
* y = −0.406 + 1.202 x
** y = −2.3936 + 1.273 x
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
Gamma globulin (mgm/ml)

thus the improvement in the relationship between titre and number of worms. The small change in the titre - gamma globulin relationship further supports the suggestion that a significant amount of the gamma globulin present was antibody specific to E. uncinata.

Discussion

Infection of wild mallard ducklings with 60 E. uncinata larvae at weekly intervals between one day and 6 weeks post-hatching did not result in a consistent trend to a reduction in the numbers of granulomas or nematodes recovered 20 to 50 days p.i. as the age at infection increased (see Figs. 33, 34, 35). Birds in the 2 to 4 week groups had significantly more granulomas than other groups at 50 days and the 2 and 3 week groups appeared to have more nematodes at 50 days p.i., though these numbers were not significantly different from those of most other groups. The small samples involved and the great variability within groups, especially with respect to numbers of worms, perhaps obscurred what may have been significant differences. Trends illustrated for these parameters indicated that one day, one week, five and six week-old-birds may have been less susceptible to infection. Austin and Welch (1972) reported that one-week-old Delta mallards given 50 E. uncinata larvae were more susceptible to infection than 8 or 12 week-old birds. Their data indicated that 4 week-old birds were about as susceptible as those 1 week-old, though they did not indicate that the four week group had more worms than the older groups. Those reports and the present data suggest that susceptibility is variable between hatching and six weeks of age and that at about five or six weeks, mallards develop a response of sufficient magnitude to either prevent the establishment of nematodes initially, or to reject them by 40 or 50 days postinfection. This response is apparently well developed by eight weeks of age.

Sinclair (1970) suggested that there is a certain minimum level of antigenic stimulus required to completely mobilize the immune response of a host animal. Larger infecting doses (constituting a greater antigenic stimulus) in the present experiment may have caused a sharper differentiation of susceptibility with respect to age at infection. However, most reports of greater susceptibility of young animals to helminth infections differentiate between immature (young) and mature (old) groups only (see Sandground, 1929). According to Sandground (1929), age resistance had been demonstrated for Syngamus trachea and Ascaridia lineata in chickens. Similarly, Kauker (1941) and Shevtsov and Zabello (1965) reported that young ducks were more susceptible to E. uncinata infection, and Threlfall (1966) reported young herring gulls and chickens were more susceptible to C. lari infections. Differences in susceptibility as measured by number of worms surviving are less clear cut when age classifications are most specific (Austin and Welch, 1972; this study). Stoimenov (1976) fed chickens from one to seven months of age with up to 2000 Heterakis gallinae eggs and found that three and four month old birds harboured the greatest number of nematodes and that pathology was greatest in those birds.

There was a direct correlation between the number of granulomas and the number of worms present at 50 days p.i. (Fig. 37), corroborating the findings of Austin and Welch (1972). This relationship was not apparent at day 20 because the number of worms/bird and number of granulomas in those birds was more variable than at 50 days (see F ratios, Tables 1, 3 in Appendix II). This suggests that groups of nematodes of variable numbers were able to establish themselves in the mucosa and to remain there until day 20, but that only those groups which were composed of some minimum number of worms were able to resist the granulomatous response and persist until day 50. This interpretation is supported by the fact that the mean number of worms/granuloma varied significantly from group to group at day 20 but not at day 50 (Tables 1, 3 in Appendix II). This also further supports my earlier contention (Section IV) that Type II granulomas were in the process of involution because they were initiated by a small number of nematodes.

Although no clear cut age related effects with respect to number of granulomas and worms were found in this study, more subtle but perhaps as important, effects were noted. The length and diameter of nematodes at 20 days p.i. were markedly influenced by the age of ducklings at the time of infection (Table 2 in Appendix II; Fig. 36), with younger birds having consistently larger worms than older ones. By 50 days p.i. this relationship was less significant but held for at least the diameter of nematodes (Table 4 in Appendix II; Figs. 38, 40). Austin and Welch (1972) found this to be the case when the length of worms from birds infected at one, four, eight or twelve weeks were compared 40 days postinfection. This effect in the present study could not be correlated with antibody detected because virtually none was detected at 20 days p.i. regardless of the age at infection. There is a possibility that the length of nematodes may have been affected by precipitating antibody as detected by immunodiffusion at 50 days p.i. because those birds with the greatest titres had the shortest worms (3 week group, Tables 4, 5 in Appendix II; Fig. 41). However, antibody titre was linearly related to the number of nematodes present (Fig. 42a) which I interpret as an antigenic dose - antibody response relationship. Thus, I conclude that antibody detected had no apparent effect on the number of nematodes surviving to day 50 and probably was not the mechanism whereby nematode size was reduced in older birds. If, in fact, antibody had an effect on the size of nematodes, it was not apparent below a minimum level and that level was attained only by birds in the three week group. It also follows that since the number of nematodes was not linearly related to the age of birds at infection, the production of antibody to E. uncinata was not either.

There is some circumstantial evidence which suggests that non precipitating antibody was produced in response to infection, which may have been specific to E. uncinata. The number of splenic follicles per mm^2 at 20 days p.i. was significantly correlated with the gamma globulin content of serum samples which indicates that some of those follicles were germinal centres having plasma cells capable of producing immunoglobulin. Further, when the number of worms was included as a second independent variable in the regression analysis, the amount of variation in gamma globulin accounted for by the relationship increased from 18 to 28%. This suggests that antibody specific to E. uncinata was produced by 20 days p.i. but was not detected. This could have been complement fixing, agglutinating, or at least a type of antibody not detectable by immunodiffusion. Secondly, if the antibody was directed at antigens specific to immature worms, it would not have been detected because only adult worm antigen was employed.

As indicated earlier (Section IV), the first appearance of antibody at day 33 p.i. and the reported attainment of sexual maturity and production of ova by day 30 p.i., suggested that antigens associated with mature worms, perhaps the eggs, were responsible for initiating the antibody response. If such was the case, then specific antibody would not have been present at day 20. If, on the other hand, there was antibody specifically aimed at immature worms, it would not have been detected even at day 50.

A third interpretation is that the Ig response associated with worm burden and splenic follicles at day 20 represented a nonspecific one, or one directed at unrelated antigen(s). The apparent adjuvant effect of Nippostrongylus brasiliensis infection on IgE production to unrelated antigens has been recently documented (Carson et al., 1975 and references therein) so something similar may have occurred in the present case. It is unlikely that IgE levels would have been pronounced enough to have been indirectly detected, even if nonspecifically stimulated. However, Moticka (1975) has shown that chickens at least, characteristically produce non-antibody immunoglobulin (non-specific Ig) in response to antigenic stimulation which peaks five days after stimulation and persists for several weeks. Specific Ig on the other hand, does not peak until about 15 days post-stimulation and begins to fall by 20 days. If this occurred with E. uncinata infection, it could explain and support the observation that at least non-specific Ig was present in the sera of birds necropsied at 20 days postinfection.

At 50 days p.i. specific antibody was detected in all groups of birds and the level of the response was strongly correlated with number of nematodes and gamma globulin (Fig. 42). Titre was about equally correlated with gamma globulin and number of nematodes in birds which responded, indicating that a large proportion of the gamma fraction was specific Ig directed at adult E. uncinata antigens. There were no detectable relationships between the number of splenic follicles and number of worms, titre or gamma globulin at day 50 p.i. There was a great increase in the number of splenic follicles over the number at day 20, probably reflecting the greater variety of antigenic stimuli afforded birds having lived a further four weeks. Also, many follicles may have been inactive at that time so that their presence was not reflected in gamma globulin levels.

The results presented in this section serve to emphasize that host responses to helminths are complex phenomena. Although all groups of ducklings (except, perhaps the five and six week groups) were essentially equal in the statistical sense in terms of susceptibility to infection, they showed great variability in their more subtle responses to infection. Immunological competence can be expressed in ways other than the prevention of infection. Threlfall (1966) indicated that although young gulls and chicks were more susceptible to C. lari infection, they lost most of their worms at about five weeks of age, presumably due to a maturation of their immune responses at that time. Ogilvie and Jones (1967) and Jones and Ogilvie (1971) indicated that the immune rejection mechanism for N. brasiliensis was not fully developed in rats until nine weeks of age, but that the first step in the two stage process (antibody mediated damage to the worms) was well developed even in very young rats. Thus, infections were retained much longer than normal in young rats because of a deficiency in the second stage of the rejection mechanism. Similarly, Cypess et al. (1973) indicated that mice are not fully mature immunologically in terms of their responses to Nematospiroides dubius until the age of 3 or 4 weeks. Wild mallard ducklings are able to reject E. uncinata infection at least partially, as indicated by the involuting granulomas in Section IV, and the general decline in numbers of granulomas and numbers of nematodes/bird from day 20 to day 50 in this section. All groups, but perhaps the five and six week old ones especially, showed signs of being immunocompetent in this regard. Once a granuloma became established, the rejection mechanism could conceivably take a considerable time, the length of which might depend upon the number of nematodes involved. Consequently, if observations had been made for a longer period of time, perhaps more dramatic age related effects (i.e. differential survival time of nematodes) might have been recorded. Future studies might well consider this approach.

There were obviously a great many physiological and other changes occurring in ducklings over the course of this experiment related to maintenance of homeothermy, rapid growth, and attainment of juvenal plumage, including flight feathers. These physiological changes would include rising and perhaps fluctuating levels of hormones such as thyroxine and corticosteroids, (especially glucocorticoids), and changes in metabolic rate. Ducklings attained nearly maximum body weight at seven to eight weeks of age in this study. Although growth rates were not documented, the rate of increase in body weight to a similar level and over a similar period of time was relatively constant in gadwall ducklings (Oring, 1968), suggesting that metabolic drain or stress due to growth was relatively constant as well. Ducklings in the present study were fully feathered by six to seven weeks, but plumage development was accelerated between three and six weeks of age. This was shown to be true of gadwall ducklings as well (Oring, 1968). This factor could constitute a further stress in addition to that imposed by rapid growth in body size, which would be most apparent between the ages of three and six weeks. In addition, brooder lamps were removed at about three weeks post-hatching so that the need to expend energy to thermally regulate could have imposed a further stress. Untergasser and Hayward (1972) showed that although mallard ducklings thermoregulate within two days of hatching, they do so at the expense of a high metabolic rate. Ducklings kept under brooder lamps would not expend energy in this way. Finally, thyroxine levels may have been higher during this three to six week period in conjunction with the high growth rate and accelerated plumage development. These factors may have influenced the course of infection with _E. uncinata_ in different ways, depending on the timing of infection.

High thyroxine levels have been shown to favourably affect the growth of _Ascaridia galli_ in chickens (Noble and Noble, 1976, p. 426). This may have been true in mallards infected with _E. uncinata_ in this study and in that of Austin and Welch (1972). Thyroxine levels are generally higher in young animals than in older ones and if _E._

uncinata infections were enhanced in the presence of high thyroxine levels, the generally greater susceptibility of young mallards to infection could be partially explained. If, as suggested above, thyroxine levels were highest in birds three to six weeks of age, then in those birds infected between two and four weeks, the effect of thyroxine would have been most pronounced when their nematodes were in the process of establishing granulomas (a period encompassing up to three weeks). Thus, they may have retained slightly greater numbers of worms than younger or older birds (one day, one week, five week and six week groups). Birds in the other groups would presumably have had lower levels of thyroxine and have been under less stressful conditions. Ould showed that stresses induced by crowding or cortisone injections had a beneficial effect on E. uncinata in Delta mallards. Thus, it is reasonable to speculate that ontogenetically mediated stresses as proposed could have affected susceptibility. The overall result in this study was an apparently greater susceptibility of ducklings infected at two to four weeks of age (Figs. 33, 34). Though few statistically significant differences in mean number of worms/bird were demonstrated in support of this, the relationship between number of worms and antibody titre was indicative of a biologically important difference in this parameter between age groups. This relationship did support the suggestion that intermediate aged birds were more susceptible.

The mechanism of this increased susceptibility must involve a transitory suppression of the immunological system of the hosts. A complication which makes interpretation difficult is that over the course of the experiment, immunological competence of the ducklings was being developed. Infections were superimposed upon this process at progressively later stages as age at infection was increased.

Moticka (1975) emphasized the possible importance of non specific Ig production in the immunological responses of birds. Evidence was presented above which suggested that non specific Ig was produced by mallard ducklings in response to E. uncinata infection. Moticka (1975) suggested that these responses, perhaps mediated by cells processed in bone marrow only (BM-cells), were important effector mechanisms in birds, but were less important in mammals because of their highly developed humoral responses. These ideas can be interpreted to suggest that birds are at an intermediate stage in the evolution of the vertebrate immune system; they have well developed cellular immune responses (phylogenetically early) mediated by T-cells, well developed non specific Ig responses mediated by cells which are perhaps intermediate between T-cells and highly specific B-cells, and are beginning to develop cells responsible for specific Ig production (fully differentiated B-cells). Ontogenetically these effector arms could be expected to mature at different rates, perhaps in the sequence outlined. Moticka (1978) has, in fact, shown that the non specific response (called polyclonal hyperimmunoglobulinemia) is well developed in chickens within the first week and that the specific immunoglobulin system does not fully develop until chicks are four to six weeks of age. If this is true of mallard ducklings, then interactions between hormone levels, stresses associated with prefledging, and development of immunological competence could be expected to influence responses to parasites such as E. uncinata.

Ducklings infected at one day and one week of age would be expected to have relatively well developed cellular and non specific responses (also see Section VI). If they were relatively unstressed as suggested above during the period of worm establishment, these responses may have been of sufficient magnitude to reduce the number of nematodes relative to intermediate aged birds. Birds of intermediate ages at infection, being under greater ontogenetically mediated stresses but without a fully developed specific Ig system would be more susceptible to infection. Finally, birds infected at five or six weeks of age may have reached the stage where their immunocompetence was almost fully developed and were, at the same time, subject to less stress. Their susceptibility to E. uncinata was thus lower. As well, as pointed out earlier, immunological competence can be expressed in ways other than prevention of infection. Effects such as reduction in growth rate and/or reproductive potential are good examples (Kelly, 1973; Cheng, 1973). In this study, a consistent trend to a reduction in the diameter of nematodes with increasing age at infection was noted

(Figs. 36, 38). This trend was not correlated with titres of precipitating antibody but it could represent the result of undetected specific antibody directed at antigens other than those employed. If so, this effect may be indicative of a steady maturation of the specific Ig response with age, as suggested above.

VI. EFFECTS OF SURGICAL BURSECTOMY AND THYMECTOMY

Experimental Design

Nine ducklings were bursectomized at one day of age and infected with 60 E. uncinata larvae at the age of two weeks. Four of these ducklings were to be used to assess the number of worms after 50 days and the other five to assess the details of the histopathological responses of bursectomized birds. Unfortunately, problems were encountered with the methacrylate embedding medium in that the supplier had problems with the manufacturing process and faulty blocks were produced. It was, therefore, not possible to meet the latter objective and the small number of birds from which the numbers data were taken was consequently a more serious alternative than had been envisaged.

Fifteen ducklings were thymectomized at the age of one day; thirteen survived to the age of two weeks, at which time they were infected with 60 E. uncinata larvae. All were necropsied after 50 days.

Granulomas from five birds were fixed in order to assess the nature of the histological response of thymectomized birds, while the remainder were examined to obtain nematode numbers and sizes.

Results

Table 18 summarizes data regarding the numbers of granulomas, numbers of worms, and diameters of granulomas which were recovered from surgically altered and control birds 50 days postinfection. Bursectomized ducklings had an average of 3.3 granulomas and a mean number of 25.8 nematodes per bird. The mean number of worms per granuloma was 9.6, and the mean diameter of the granulomas was 6.6mm. None of these parameters differed significantly from those of control ducklings which were not bursectomized but which were infected at two weeks of age (tested by means of one way anovars and Student's "t" tests). Although all parameters in Table 18, except the number of granulomas, were somewhat lower for thymectomized ducklings than for controls, none were significantly different. Nor were they significantly different from those for bursectomized ducklings.

Serum proteins were analysed as previously described and the results summarized in Table 19. Bursectomized ducklings had less serum protein than infected controls, due mainly to smaller albumin fractions. Despite this, the serum gamma globulin levels of these groups were not significantly different (see the F ratios in Table 19). Gamma globulin levels were also compared to those of uninfected control ducklings

Table 18. Mean numbers of granulomas and worms, and mean diameters of granulomas from surgically altered and normal ducklings infected with 60 E. *uncinata* larvae at two weeks of age and necropsied at day 50 postinfection.

PARAMETER		NUMBER GRANULOMAS		NUMBER WORMS/BIRD		NUMBER WORMS PER INF. BIRD		NUMBER WORMS/GR.		DIAMETER GRANULOMAS mm
TREATMENT	[1]N		N		N		N		N	
Bursectomy	9	3.3 (1.1)	4	25.8 (10.9)	4	25.8 (10.9)	4	9.6 (6.2)	30	6.6 (1.1)
Thymectomy	13	2.5 (1.2)	8	10.0 (13.4)	5	16.0 (13.9)	5	4.2 (4.5)	33	6.4 (2.1)
Normal	5	2.2 (1.5)	5	19.4 (10.9)	4	24.3 (1.7)	5	7.6 (4.9)	11	7.4 (2.3)
[2]F		0.890		2.419				1.616		1.094
[3]df		2 & 24		2 & 14				2 & 13		2 & 71
[4]p<		ns		ns				ns		ns

1. N = number of samples.
2. F = F ratio one way anovar
3. Degrees of freedom numerator and denominator.
4. Probability.

aged seven and eleven weeks (Table 12). Controls aged seven weeks had significantly more of this fraction than did bursectomized birds (t= 3.212, p<.05) while there was no difference between the infected controls and the uninfected ones.

Total protein content of sera from thymectomized ducklings was considerably lower than that of infected control ducklings, due to smaller amounts of all fractions including gamma globulin (Table 19). There were no differences in this regard between thymectomized and bursectomized birds. Compared to seven week uninfected controls, thymectomized birds had less gamma globulin (t=5.861, p<.05).

The sera of surgically altered birds were also subjected to immunodiffusion against _E. uncinata_ antigen, the results of which are presented in Table 20. Seven of nine bursectomized ducklings had demonstrable antibody against the antigen preparation with a mean titre for reactors of 9.1. This was considerably greater than that of controls. The difference was significant at the 5% level when the mean titres for all birds (column 4) were tested (t=2.841). Eight of 13 serum samples from thymectomized ducklings were positive for antibody to _E. uncinata_ antigen; mean titre for reactors was 7.4. Mean titre for all birds (column 4, Table 20) was greater than for uninfected controls (normal)(t=2.584, p<.05) and about the same as that for the bursectomized ducklings (no significant difference, t test).

Table 19. Serum protein fractions (mg/ml) from surgically altered or normal ducklings infected with 60 E. uncinata larvae at two weeks of age and necropsied 50 days postinfection.

SERUM FRACTION		PRE-ALBUMIN	ALBUMIN	GLOBULIN			TOTAL PROTEIN
				ALPHA	BETA	GAMMA	
TREATMENT	N						
Bursectomy	[1]9	[2]2.13 (1.46)	24.97 (3.17)	5.80 (1.64)	4.96 (1.58)	3.34 (1.59)	41.20 (4.08)
Thymectomy	13	1.21 (0.60)	26.72 (1.68)	5.27 (1.55)	5.01 (1.07)	3.10 (1.01)	41.31 (4.06)
Normal	5	2.16 (1.60)	34.63 (3.38)	7.13 (2.20)	6.75 (1.27)	4.50 (1.61)	55.15 (7.01)
[3]F1		---	24.943	---	---	1.991	17.873
[4]p<			.001			ns	.001
F2		---	28.490	---	---	1.665	---
p<			.001			ns	
F3		---	45.490	---	---	4.494	---
p<			.001			.05	
F4		---	2.845	0.594	0.008	0.190	0.004
p<			ns	ns	ns	ns	ns

1. Number of samples.
2. Means with standard deviation in parentheses.
3. F1 is one way anovar, all treatments with 2 & 24 df.
   F2 is one way anovar, bursectomy vs normal with 1 & 12 df.
   F3 is one way anovar, thymectomy vs normal with 1 & 16 df.
   F4 is one way anovar, thymectomy vs bursectomy with 1 & 20 df.
4. Probability

Table 20. Mean antibody titre of serum samples from surgically altered and normal ducklings infected with 60 *E. uncinata* larvae at two weeks of age and necropsied at day 50 postinfection.

PARAMETER	NUMBER OF DUCKLINGS	NUMBER POSITIVE	MEAN TITRE (REACTORS)	MEAN TITRE (ALL BIRDS)
Treatment				
Bursectomy	9	7	9.3 [1](4-20)	7.2 [2](7.1)
Thymectomy	13	8	7.4 (1-12)	4.5 (5.6)
Normal	4	1	2.0	0.5 (1.0)

1. Range.
2. Standard deviation.

The mean length and diameter of nematodes recovered from these ducklings are presented in Table 21. Nematodes from bursectomized birds averaged 12.07mm in length and had a mean diameter of 0.50 mm; neither measurement was significantly different from that of nematodes from controls (see F2, Table 21). Nematodes recovered from thymectomized ducklings were considerably shorter than those from either infected controls or bursectomized birds, averaging 10.5mm in length (see F3 and F4, Table 21). The mean diameter of this group of worms was not significantly different from the other groups.

Mean values for several morphological or condition factors from surgically altered and infected control ducklings are presented in Table 22. Comparable measures for uninfected control ducklings are in Table 14. Bursectomized birds were larger at necropsy than controls, were in better overall condition (lower condition index), and had fewer splenic follicles per mm^2 of splenic tissue. None of these differences was statistically significant. The mean diameter of spleens from bursectomized birds was far greater than that for any of the control groups. Thymectomized birds did not differ from any of the groups in weight or condition index. They had more splenic follicles than seven week old uninfected controls ($t=2.462$, $p<.05$) but fewer than infected controls ($t=2.754$, $p<.05$). They had larger spleens than both seven and eleven week old uninfected controls ($t=7.621$, 4.397; $p<.01$ and $p<.05$ respectively) but the diameter of their spleens were not different from

Table 21. Mean total length and mean maximum diameter of *E. uncinata* recovered from surgically altered or normal ducklings infected with 60 *E. uncinata* larvae at two weeks of age and necropsied at day 50 postinfection.

MEASUREMENT		LENGTH mm	DIAMETER mm
TREATMENT	N		
Bursectomy	17[1]	12.07[2]	0.50
		(1.09)	(0.07)
Thymectomy	27	10.51	0.52
		(1.13)	(0.05)
Normal	38	12.20	0.52
		(2.68)	(0.13)
[3]F1		6.186	0.396
[4]p<		.005	ns
F2		.035	0.386
p<		ns	ns
F3		9.549	0.041
p<		.001	ns
F4		20.522	2.245
p<		.001	ns

---

1. Number of nematodes.
2. Means with standard deviation in parentheses.
3. F1 is one way anovar, all treatments, 2 & 79 df.
   F2 is one way anovar, bursectomy vs normal, 1 & 53 df.
   F3 is one way anovar, thymectomy vs normal, 1 & 63 df.
   F4 is one way anovar, bursectomy vs thymectomy, 1 & 42 df.
4. Probability of level of significance of the difference.

Table 22. Means of morphometric and associated parameters measured or calculated for surgically altered or normal ducklings infected with 60 _E. uncinata_ larvae at two weeks of age and necropsied at day 50 postinfection.

PARAMETER		WEIGHT gm	CULMEN mm	KEEL mm	[1]CONDITION INDEX	[2]SPLENIC FOLLICLES	[3]THYMUS WEIGHT	DIAMETER SPLEEN mm
TREATMENT	[4]N							
Bursectomy	9	[5]924.9 (110.5)	65.3 (3.1)	97.7 (6.3)	6.93 (0.30)	0.76 (0.68)	2.71 (0.77)	10.27 (2.0)
Thymectomy	13	825.9 (154.0)	61.8 (4.7)	93.4 (7.7)	7.09 (0.70)	0.61 (0.45)	0.69 (0.53)	8.6 (1.7)
Normal	4	791.9 (110.5)	63.5 (1.3)	89.3 (7.0)	7.20 (0.49)	1.01 (0.34)	2.86 (1.13)	6.9 (1.3)

1. Calculated from (Culmen χ Keel) / Weight.
2. Number of splenic follicles/mm^2 of splenic tissue.
3. Mgm/gm body weight.
4. Number of birds.
5. Means with standard deviations in parentheses below.

those of normal infected or bursectomized groups. It was obvious that thymectomized birds had less thymic tissue than all other groups for which these data were taken.

Simple and multiple linear regression analyses were performed in an attempt to delineate and characterize the relationships, if any, between the various parameters measured or otherwise observed.

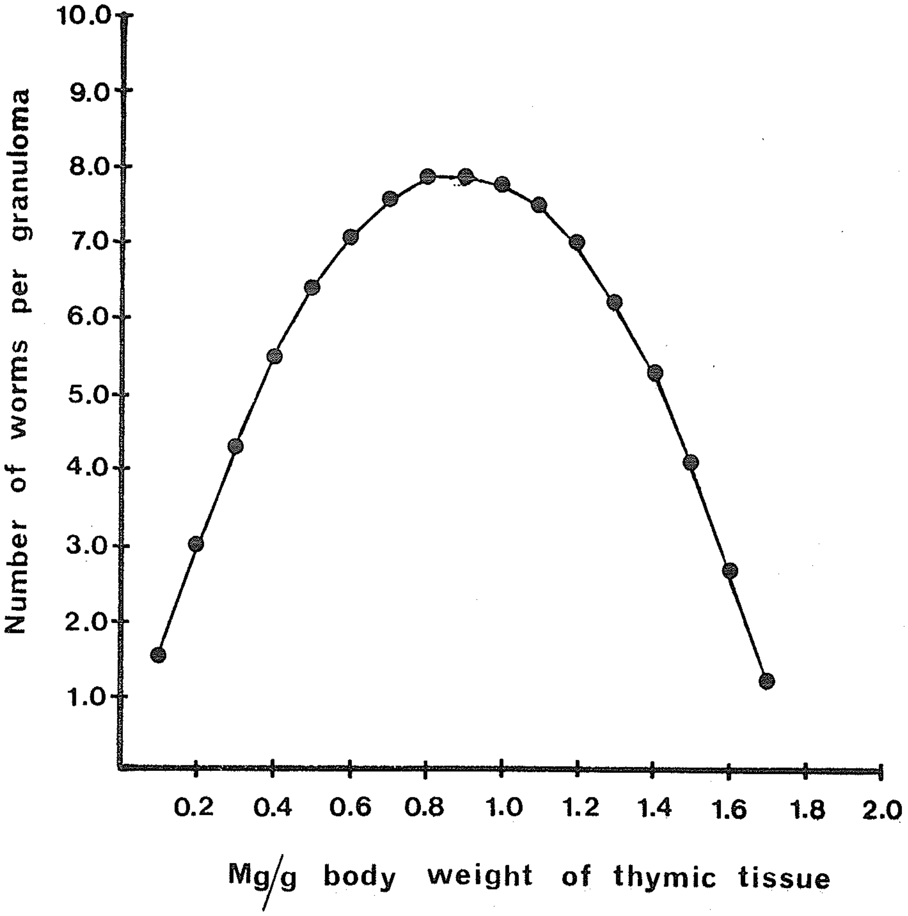
When either bursectomized or thymectomized birds were considered, there were no significant linear relationships between the number of worms recovered and any of the parameters including: the number of granulomas, diameter of granulomas, antibody titre, gamma globulin content of sera, number of splenic follicles, condition index or thymus weight. Nor was there any significant parabolic regression of number of worms per bird on thymus weight. The number of bursectomized ducklings available for these analyses was small (4) so that any significant results which might have been noted would have been of doubtful value. Eight thymectomized ducklings were included in this series of analyses so the results are more confidently regarded as indicative that no relationships exist between the number of worms/bird and the other measures listed.

Although no linear or parabolic relationship could be demonstrated between the number of worms/bird and thymus weight in thymectomized ducklings, parabolic regression of the number of nematodes/granuloma against thymus weight (Appendix III, Table 3(1)) indicated there was a trend in the data to low numbers of worms in birds with little thymic tissue, higher numbers in birds with intermediate amounts of thymic tissue, and low numbers again in ducklings with greater amounts of tissue. This is illustrated in Figure 43. The associated F ratio (2&5 df) of 6.975 is significant at the 5% level and the r^2 of 0.74 indicated that 74% of the variability in number of worms/granuloma was attributable to variation in thymus weight. The same procedure was performed on these data from normal ducklings infected at one day, one week and three weeks of age. There was a significant parabolic relationship between the number of worms recovered and the relative weight of thymic tissue for the one day group but not for the others (Appendix III, Table 3(2)). This may perhaps indicate that if there exists a relationship between thymus weight and the number of nematodes surviving until day 50 p.i., it holds only for very young ducklings, or those deficient in thymic tissue as in thymectomized birds.

All bursectomized and thymectomized ducklings were included in regression analyses in attempts to correlate such parameters as number and diameters of granulomas with each other and with other parameters such as thymus weight, diameter of spleen, number of splenic follicles and antibody titre. Since splenic enlargement may be a function of enlargement of the bursa and/or thymus dependent areas of the white pulp, spleen size was run in regression analyses against thymus weight, number of splenic follicles and condition index. Spleens of

Figure 43. Parabolic relationship between the number of worms per granuloma and weight of thymic tissue per unit of body weight for ducklings thymectomized at one day of age, infected with 60 E. *uncinata* larvae at two weeks of age, and necropsied at day 50 postinfection.

$F = 6.975$ (2&5 df), $p < .05$
$r^2 = 0.74$


Number of worms per granuloma
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
Mg/g body weight of thymic tissue

bursectomized birds were of greater diameter than those of ducklings from all other groups (Table 22).

When all nine bursectomized birds were included in the analysis, there was no simple linear regression of the number of granulomas or the diameter of granulomas on thymus weight or on splenic measurements. There was, however, a significant linear regression (negative) of the diameter of the granulomas on the number of granulomas which accounted for 47% of the variability associated with the diameter of granulomas ($r^2=0.4699$) (Appendix III, Table 3(3)). When thymus weight was added as a third variable the relationship was improved so that 57% of the variability could be accounted for, but the F ratio (3.91, 2 & 6 df) was of borderline significance. When all thymectomized ducklings were considered, the number and diameter of granulomas were neither linearly correlated with each other, nor to any of the parameters thymus weight, condition index or spleen diameter.

In bursectomized birds there was no significant regression of either length or diameter of spleen on thymus weight; nor was there when all three parameters were included in a multiple regression procedure. Similarly, there was no simple linear relationship between spleen diameter or length, and the number of splenic follicles. However, spleen diameter was significantly correlated with spleen length ($F=53.87$, $p<.001$; $r^2=0.86$) (Appendix III, Table 3(4)), and when the number of splenic follicles was added as a second independent variable, the amount of variability in spleen diameter accounted for by the relationship increased to 95% ($F=56.5$, $p<.001$; $r^2=0.9495$) (Appendix III, Table 3(5)). This indicates that although spleen length was the major factor affecting the diameter of the spleens of bursectomized birds, splenic follicles were also important, accounting for over half of the remaining variability.

Spleen diameter of thymectomized ducklings was correlated only with thymus weight ($F=7.981$, $p<.025$; $r^2=0.42$) (Appendix III, Table 3(6)). There were no correlations between spleen diameter and spleen length, number of splenic follicles or condition index.

When all bursectomized ducklings were considered, there was a significant linear regression of antibody titre on gamma globulin ($F=11.588$, $p<.01$; $r^2=0.62$) (Appendix III, Table 3(7)), and condition index ($F=11.63$, $p<.025$; $r^2=0.62$) (Appendix III, Table 3(8)). Both relationships improved when the number of splenic follicles was added as a second independent variable in that the square of the multiple correlation coefficients increased to 0.67 and 0.70 respectively. The best relationship of all was that between titre and total protein and gamma globulin ($F=9.298$, $p<.025$; $r^2=0.76$) (Appendix III, Table 3(9)). Though total protein and titre were not linearly related alone, they seemed to act synergistically with other factors to result in changes in titres. Other factors undoubtedly included the number of nematodes present, but too few birds were available to make the necessary analysis meaningful. Both total protein and gamma globulin levels in bursectomized birds were linearly correlated with condition index so that I concluded that those parameters are different measures of the same things. Thus, they all seemed to have an effect on the ability of the birds to produce gamma globulin generally, and antibody to E. uncinata antigen specifically.

Mean antibody titre of thymectomized ducklings was not linearly related to gamma globulin level, number of splenic follicles, thymus weight or condition index, when all birds were included in the analysis. When only those ducklings having positive serum samples were included, titre was significantly correlated with gamma globulin alone, and in combination with total protein (Appendix III, Table 3(10, 11)). The latter was the best relationship with 61% of the variability in titre accounted for ($r^2=0.61$). There was no correlation between titre and the number of worms recovered.

Sectioned lesions were not available from normal ducklings infected at two weeks of age, but were available from normal birds infected at the age of four weeks; these were compared with those from thymectomized birds. The granulomas from normal birds had much thicker walls than those from thymectomized ones (1.05mm compared to 0.54mm) as seen in sections ($p<.001$). Whether or not this reflects an effect of the age at infection, or the effect of reduced thymic tissue cannot be stated. It is most likely a reflection of their combined effect.

Discussion

Surgical removal of the bursa of day-old wild mallard ducklings followed by infection with 60 E. uncinata larvae had no apparent effect upon the number or size of granulomas, or upon the number of worms recovered 50 days p.i. as compared to normal unoperated birds. Nor was the size of nematodes significantly different from those of unoperated controls. This corroborates the report of Ould (1972) who recorded similar results from bursectomized ducklings infected at 35 days of age, and further suggests that the intact bursa plays no important role in the early response of mallards to a primary infection with E. uncinata. A comparable number of worms was able to establish in granulomas and to persist until at least 50 days p.i. in birds with or without a bursa. It is possible that infections may have persisted longer, or that responses to secondary infections may have been curtailed in bursectomized birds. Neither of those possibilities was investigated.

Neonatal bursectomy did not abolish the ability of ducklings to produce antibody to E. uncinata in this study, a finding which confirms the report of Ould (1972). It follows that cells responsible for the humoral response detected in this study and that of Ould (1972), must have received bursal processing before hatching so that removal of the organ at one or two days had no effect on responses to E. uncinata antigens. Paradoxically, bursectomized birds had significantly greater antibody titres than unoperated ones in this study, and proportionately more bursectomized birds produced antibody. This is in contrast to what has generally come to be expected of bursectomized birds. Graetzer et al. (1963) reported that titres of natural hemagglutinins and precipitating antibody to bovine serum albumin were drastically reduced in chickens bursectomized at one or two days of age. It is possible that the surgical procedure, or the possible attendant exposure to infectious agents during the procedure, may have served to prime the immune system nonspecifically, resulting in a greater response. Due to the difficulty encountered in obtaining infective materials, unoperated and sham-bursectomized controls were not run at exactly the same time as the experimental birds, though unoperated controls infected at the same age were available from a previous experiment. Ould (1972) reported no differences between sham-bursectomized and unoperated controls, and there were no differences in condition or other morphological parameters in this study between unoperated and bursectomized birds. Thus, nonspecific stimulation cannot be ruled out.

It is possible that bursectomy resulted in a delay in peak antibody production to E. uncinata so that an apparently greater response may have been a temporal anomaly, in that control ducklings may have responded with peak antibody production before 50 days p.i. when samples were taken, and bursectomized birds did not produce peak titres until later. This is unlikely to have occurred however, since no antibody was detected at 20 days p.i. in the control group, and in an earlier section of this thesis (Section IV), it was noted that antibody was not produced in four week old birds until at least 33 days p.i. and was higher at 47 days p.i. Larger samples and more sensitive techniques may be necessary to explain the results presented here.

Park and Good (1974) outlined a number of factors which can influence the antibody response including: adjuvants, immunosuppressive agents, immune paralysis (tolerance), antigenic competition, desensitization and antibody itself. The latter may be of interest in the present case where bursectomized ducklings had an enhanced antibody response compared to infected controls. Park and Good (1974) point out that passively administered specific antibody appears to regulate the production of antibody via a feedback mechanism; IgG antibody given shortly after immunization may suppress IgG, IgM, IgA and even CMI responses. It may be that in normal ducklings, antibodies of different specificities than those detected are produced (there was some indication of this in Section V) which normally suppress the production of the antibodies detected. If bursectomy resulted in a depletion of specific B-cells capable of producing the suppressing antibodies, a greater response could be expected.

Few studies have been done concerning the effects of bursectomy on the responses of birds to helminth parasites. Blake (1973) reported that bursectomy did not increase the number of Apatemon gracilis recovered from experimentally infected ducks and did not affect egg production. Johnson et al. (1974) reported that hormonal bursectomy or surgical bursectomy at 3-14 days post-hatching increased the incidence and intensity of infection with Ascaridia galli in chickens. Herd and McNaught (1975) reported that the histiotrophic stage of arrested development of Ascaridia galli in chickens was suppressed if chickens were treated with cyclophosphamide. Both studies suggest that humoral immunity is important in responses to that nematode. Johnson et al. (1974) also showed that treatment with cortisol, cortisone and prednisone (all inhibitors of CMI) also caused an increase in incidence and intensity of infection, indicating that both humoral and cellular responses were involved in immunity to A. galli in chickens. Davis et al. (1974) reported that neonatal bursectomy had no effect on the granulomatous response of chickens to eggs of Schistosoma mansoni. The results of Ould (1972) and those of this study indicate that such is the case with E. uncinata infections in wild mallards, though further studies using more birds and more refined techniques are warranted.

The fact that in this study, splenic germinal centre formation was unimpaired in bursectomized mallard ducklings, suggests that bursal stem cells which are capable of mediating the formation of splenic germinal centres, seed out from the mallard bursa before hatching and do not need the bursal micro-environment for further maturation. This is in contrast to what Toivanen and Toivanen (1973) propose for bursal stem cells in chickens which are found in the bursa up to 3½ weeks of age and which require the bursal micro-environment for maturation. This suggests that mallards may mature immunologically more quickly than chickens, and may have a greater degree of immunocompetence upon hatching. Toivanen and Toivanen (1973) found that antibody production was independent of splenic germinal centres as well, so that the fact that antibody titre to _E. uncinata_ was not directly correlated with the number of splenic follicles in my birds is perhaps understandable. There was a synergistic effect of splenic follicles and other factors, suggesting that titre was not completely independent of splenic follicle formation. Splenic follicles may be involved in the production of non specific immunoglobulin (polyclonal hyperimmunoglobulinemia of Moticka, 1978) and thus may be independent of the bursa by virtue of being entirely T-cell dependent. The indirect correlation between titre and splenic follicles may thus reflect their common stimulation.

Few studies have been concerned with the effects of thymectomy on the responses of birds to helminth infections. Blake (1973) reported that thymectomy prolonged the intestinal phase of _Apatemon gracilis_ infection in ducks and permitted greater egg production by the trematode. This possibility was not investigated in this study though one of the subtle effects noted (see Section V) was that the diameter of the worms and presumably the egg containing volume was greatest in nematodes from young birds. If thymectomy could be seen as a procedure which would mimic the effects of an immature immune system, some similar effect might have been noted here. No indication of this was apparent.

Total thymectomy was not attained in this study as evidenced by the fact that some thymic tissue was present in all birds, albeit much reduced when compared to normal or bursectomized ducklings of the same age. This may explain the equivocal results obtained. There were no differences in the number or diameters of granulomas, or the number of worms in thymectomized ducklings compared to unoperated controls or to bursectomized birds. It is possible that the small amounts of thymic tissue present were enough to mediate any cellular responses elicited by _E_. _uncinata_ infections. Alternatively, ducklings may have been immunocompenent to _E_. _uncinata_ at hatching. Irradiation or longterm lymphatic drainage has been found necessary following thymectomy to deplete the pool of circulating T-cells which are present at birth in guinea pigs subsequently infected with _Trichostrongylus_ _colubriformis_ (Dineen and Adams, 1971), and in mice subsequently infected with _E_. _multilocularis_ (Baron and Tanner, 1976). Such post-surgical procedures were necessary to abolish normal immune responses of these hosts to the respective helminths. More dramatic results may have been obtained had these procedures been employed in this study.

The striking resemblance of the _E_. _uncinata_ granuloma to that associated with schistosome eggs in a variety of hosts was pointed out earlier (p. 133) and there is compelling evidence in favour of thymus-dependent cell-mediated responses in the induction of the schistosome egg granuloma in mammals. As well, Davis et al. (1974) have shown that neonatal bursectomy had no effect on experimentally induced granulomas around S. mansoni eggs in chickens, but that thymectomy markedly reduced the granulomatous response. They thus indicated that cellular hypersensitivity was the major, if not the sole, mediator of granuloma formation. The fact that the walls of E. uncinata granulomas were thinner in thymectomized ducklings than those in normal birds (though the two groups were of different ages at infection), provides additional evidence that granuloma formation is thymus dependent and is a manifestation of delayed cellular hypersensitivity. In addition, Ould's (1972) report that cortisone acetate injections seemed to reduce the granulomatous response (less caseous material) and perhaps increased the number of nematodes which survived also favours the implication of delayed hypersensitivity.

In schistotome infections, although granuloma formation has been attributed to CMI, it could not be correlated with protective immunity as it had no apparent effect upon the number of adult worms (Warren, 1972, 1976) and was solely a response to soluble egg antigens (Boros and Warren, 1970). Other reports indicated that granuloma formation was perhaps a mechanism which allowed the host animal to cope with schistosome infections by alleviating pathological effects (Buchanan et al., 1973; Fine et al., 1973; Dunsford et al., 1974). These reports emphasize the fact that host-parasite relationships are complex phenomena and that rarely are there clear cut relationships between effector arms of the immune response and the helminths which induce them. This appears to be true of the mallard - Echinuria model.

The indication of a parabolic relationship between the number of worms/granuloma and thymus weight in thymectomized birds, and between the number of worms/bird and thymus weight in very young birds, is intriguing. This relationship can be interpreted to mean that a certain degree of cellular response is necessary for the initial establishment of Echinuria in the mucosa of mallards, and that very young birds, or those with little thymic tissue, are incapable of responding. In the absence of a cellular response, larvae may not aggregate and may therefore not be able to establish granulomas. In view of the suggestion of Kennedy et al.(1973) that E. uncinata may feed on host cells, the cellular response may be necessary for at least their early growth and development. The nonsusceptibility of other ducks to infection with E. uncinata may be attributable to a lack in their ability to respond to larvae, thus passively preventing the establishment. Austin and Welch (1972) reported a number of waterfowl species which were refractory to infection. One of those species, the shoveler, was refractory to infection in an ancillary experiment conducted by myself, even when bursectomized at hatching. These birds showed no signs of having been infected while mallards often had scars as remanents of early granulomas which had involuted and healed.

However, unless there is an absolute requirement on the part of the nematode for granuloma formation, any inflammatory response which could eventually result in encapsulation of groups of nematodes in an abcess or cyst would seem to serve as an adequate site for the growth and development of worms. Furthermore, where granuloma formation around schistosome eggs has been effectively abolished in T-cell depleted mice, the result was increased pathology and mortality because eggs were surrounded by zones of liquefactive necrosis and cellular debris (Buchanan *et al.*, 1973; Fine *et al.*, 1973). There is, thus, no good reason to believe that the absence of similar responses to *E. uncinata* in mallards would result in complete unsusceptibility to infection and little pathology as suggested for athymic birds. Some other explanation must be sought to explain the parabolic relationship between number of worms and amount of thymic tissue in thymectomized birds and the greater antibody responses of all surgically altered ducklings.

There are really two separate facets of the response of mallard ducklings to *E. uncinata* infections: i) granuloma formation; and ii) involution of granulomas and consequent destruction of the nematodes. It is likely that granuloma formation is a manifestation of a delayed hypersensitivity reaction mediated almost exclusively by T-cells. This has been strongly suggested for granuloma formation around schistosome eggs in mammals (see Smithers and Terry, 1976) and in chickens (Davis *et al.*, 1974). Ould's (1972) work with stress and cortisone, the nature of the cellular details of the developing granuloma (see Section IV), and the reduction in thickness of the walls of granulomas from thymectomized ducklings presented in this section, all tend to support the same conclusion with respect to the *E. uncinata* granuloma. Also, several authors have indicated that T-cell mediated responses are phylogenetically old (Park and Good, 1974; Moticka, 1975, 1978; Cooper, 1976). Thus one might expect an early ontogenetic maturation of such responses, perhaps *in ovo*, so that all ducklings, including "athymic" ones, could be expected to produce granulomatous responses at hatching, or shortly thereafter.

The mechanism which causes rapid involution of granulomas, or which prevents the initial establishment of nematodes in birds with little thymic tissue may involve a synergistic effect on non specific Ig (polyclonal hyperimmunoglobulin - PHIg)(Moticka, 1975, 1978) and early cell-mediated responses, both of which are T-cell dependent. It is possible that very young birds are protected from a variety of pathogens for a short period after hatching by virtue of co-operation between PHIg and a small population of T-cells. This protective system may be transitory and suppressed as immunological competence develops.

If so, such a situation may be artificially produced in birds by almost complete thymic ablation, resulting in the unsusceptibility of athymic birds in Figure 43, or of young birds in Figures 33 and 34. Such a reaction would operate to prevent infection, rather than to cause involution of granulomas. As this early protective response became senescent or was suppressed, worms would be able to establish infections so that intermediate aged birds (or those with intermediate amounts of thymic tissue) had well established granulomas but were unable to mediate their involution.

Involution of granulomas may involve antibody-dependent cell mediated responses as the immune system of ducklings matures. This may include macrophages and opsonizing antibody which act in concert to envelop worms in giant cells and to destroy them. The antibody involved may have been directed at T-cell dependent antigens while the antibody detected by immunodiffusion may have been independent of T-cell co-operation but was regulated by the presence or absence of the other antibody (see above). Capron (1974) and Capron _et_ _al_.(1975) have presented strong evidence for the existence of specific anti-schistosome IgE antibodies (T-cell dependent) which are involved in promoting adherence of macrophages to _S_. _mansoni_ schistosomules, resulting in significant damage _in_ _vitro_. This is similar to the proposal outlined above. Finally, Droege (1971) indicated that in chickens, the production of antibodies to certain antigens is controlled by both helper and suppressor cells T-cells. Moticka (1975) suggested that these helper and suppressor cells exist in different proportions in the avian thymus. Interactions between these cell populations and changes in their relative proportions, perhaps as a result of the process of immunological maturation, may explain some of the effects seen in the experiments outlined in this section.

If one visualizes the mallard thymus as initially developing helper and suppressor cells relatively early posthatching, birds with moderate amounts of thymic tissue may have had relatively equal proportions of helper and suppressor cells. As the specific Ig responses began to mature, suppressor cells may have functioned initially to "weed-out" B-cells which responded with relatively low specificity to antigenic stimulation. This may have thus permitted proper interaction of helper cells and B-cells of greater specificity, or may have directed the differentiation of those B-cells to a stage of development whereby they could produce antibody of great specificity and avidity. As this happened, helper cells may have been stimulated to proliferate, perhaps as a result of some feed-back mechanism resulting from differentiation of target B-cells. These helper cells may then have caused selective cloning of specific B-cells and promoted secretion of large amounts of specific Ig. Thus, birds with relatively normal amounts of thymic tissue may have a preponderance of helper cells with respect to particular antigens. At that point, their humoral responses may have been almost fully mature.

In this study, if such a system were operative, thymectomized birds with intermediate amounts of thymic tissue (or normal birds of intermediate ages) may have been at a critical point of development of immunological competence, Their PHIg-cellular responses may have been suppressed or senescent, while the integration of specific cellular and humoral reactions was not fully completed. Thus, they may have been capable of granuloma formation, but not of involution of those granulomas. In thymectomized birds with adequate thymic remanents (or in older ducklings), B-cells may have been sufficiently differentiated under the influence of suppressor cells to have stimulated helper cell proliferation to the extent that a fully developed humoral response was possible. The result was the relatively rapid involution of granulomas so that fewer remained at day 50 p.i. The fact that nematodes were smaller in diameter in older ducklings may be indicative that undetected antibody (perhaps the opsonic IgE involved in granuloma involution proposed above), was being produced in ever increasing quantities as birds matured.

Depending on the nature of the cells involved, the system suggested above may be extended to explain the surprisingly great antibody responses detected by immunodiffusion in surgically altered birds. If the antibody response detected was not dependent on T-cell co-operation but was suppressed by the presence of other antibodies which were produced by T-cell dependent B-cells, then thymectomy may have prevented the interaction and thus antibody response was higher than in normal birds. Similarly, if bursectomy resulted in the depletion of those same T-cell dependent B-cells (responsible for production of regulatory antibody) then the effects would have been the same - no suppression and a resultant greater response.

If such was the case, one might expect to see rather erratic responses in thymectomized ducklings, depending upon the degree to which the individuals in the group were deficient in thymic tissue. This was the case in this study although some semblance of order was suggested by the parabolic relationship shown in Figure 43. Graetzer _et al._ (1963) indicated that thymectomy of chickens during the first three days after hatching did not consistently affect precipitin production to bovine serum albumin when challenged at six to nine weeks of age. In some birds the response was significantly higher and in general, responses were more erratic than in normal birds. This may suggest a similar situation to that proposed for the _E. uncinata_ - mallard system.

VII. CONCLUDING DISCUSSION

E. uncinata infections in wild mallard ducklings induced the formation of granulomas located in the thick circular muscle layer of the isthmus between the proventriculus and ventriculus. Granulomas were initiated by inflammatory reactions to larval nematodes burrowing into and through the mucosa to their final site in the muscularis. The response by five days p.i. was similar to the non reactive or weakly negative stage of development of the schistosome egg granuloma (Hsu *et al.*, 1969). Tissue destruction, vasodilatation, edema and infiltration of heterophils, lymphocytes and macrophages were characteristic of this early stage. By day 12 p.i. the lesion was comparable to the exudative stage and by 19 days p.i. the *E. uncinata* granuloma was fully formed and comparable to the exudative-productive stage of Hsu *et al.* (1969) (Fig. 31). It also closely resembled the composite granulomas around *Schistosoma haematobium* eggs, called polypoid patches (Sadun *et al.*, 1970). Fully formed *E. uncinata* granulomas, called Type I were completely integrated into the wall of the stomach; they were encircled and penetrated by proliferating lymphoid follicles and blood vessels, and by bundles of muscle. Muscle bundles appeared to be passively involved as a result of their replacement by lymphoid cells proliferating in connective tissue around bundles and arterioles supplying them. A band of connective tissue was present in this peripheral zone which served to complete the integration of the granuloma into the gut wall. This area and the lamina propria of the mucosa bordering on the lumen of the gut constituted the outer zone. A distinctive middle zone made up the major portion of granuloma wall. It was characteristically an edematous, reticular network of lymphocytes, heterophils, macrophages and a few plasma cells with numerous small vascular sinuses and expanded capillaries. Scattered fibroblasts and muscle bundles as well as encroaching lymphoid follicles were also present in the middle zone. A narrow band of epithelioid cells, macrophages and giant cells bordered on the cavity containing the nematodes. This constituted a distinct inner zone. The cavity contained a variable amount of necrotic cellular debris and cells (chiefly eosinophils and lymphocytes) and opened to the surface of the mucosa via an irregular necrotic channel (Fig. 31c). In Type I granulomas giant cells were relatively rare, cellular debris was minimal, and the cavity appeared to be fluid filled.

Once the granuloma was formed there seemed to be three possible pathways that could be followed (see Fig. 32). The first of these routes (Fig. 32 (1)) is that granulomas did not undergo further development but proceeded through a series of changes to a stage where the cavity was clogged with cellular debris and the worms and their cavity surrounded by giant cells (Type II granulomas). The result was death of the nematodes before they matured and involution of the granuloma. Such involuted granulomas (Type II) were similar to the involutional stage of Hsu _et_ _al_. (1969) and sandy patches described by Sadun _et_ _al_. (1970) for _S_. _haematobium_ granulomas.

The second pathway (Fig. 32 (2)) was the most common route of development observed in experimental infections of male wild mallard ducklings in this study. Growth of the granuloma and its cavity beyond the fully formed state at day 19 p.i. (Fig. 31c) was correlated with growth of the nematodes (see Figs. 27, 28). A rapid increase in size of nematodes was accompanied by a concommitant increase in dimensions of the granuloma and its cavity. It was suggested that nematodes actively abraided the granuloma walls, perhaps in feeding on cells, and thereby enlarged the cavity (see Kennedy _et_ _al_., 1973). The results were the maturation of nematodes and commencement of egg production by day 33 p.i. Eggs passed from the vulva of female _E_. _uncinata_ are presumed to be deposited in the fluid filled cavity and passed from there to the lumen of the gut by way of the necrotic channel (Figs. 10, 31c). Since the granuloma seemed to be well incorporated into the gut wall, muscular activity of the stomach, particularly of the ventriculus, would serve to expel large numbers of ova. Such activity would be associated with active feeding, permitting ova to enter the lumen of the gut at a time when food passage would assure their deposition in the external environment along with the feces of the host. Eggs would thus be deposited in the feeding areas utilized by the host, assuring that an infective source was available in an area to which hosts habitually returned. The significant difference in route 2 as opposed to route 1 (Fig. 32 (1,2)), was that granulomas persisted for a longer period of time, permitting the nematodes to reproduce and providing the possibility that the life cycle of the worms could be completed. The end result in most cases, however, appeared to be a change to Type II granulomas (involution) and subsequent death of nematodes followed by healing. It was suggested that slow progression of granulomas to the involutional stage may have been due to the fact that a relatively large number of worms was involved in initiating them, and that a larger number of worms may have been able to remove epithelioid and giant cells as they formed, preventing involution until reproduction had been accomplished. It was pointed out (p. 179) that some minimum number of worms was required to establish granulomas that could persist to 50 days p.i., a fact which is supportive of the suggested effect of a large number of worms on the slow rate of involution seen in route 2 (Fig. 32 (2)).

A third possible result of infection of mallards with E. uncinata is the formation of large, thin-walled granulomas such as those observed in breeding female mallards in the wild (see Fig. 32 (3)). It was assumed that such granulomas initially developed to Type I as seen in experimentally infected ducklings but that further development resulted in distinct Type III granulomas with numerous, large nematodes, large fluid filled cavities and very thin wall composed primarily of fibrous connective tissue. It was suggested that Type III granulomas may develop as they do if initiated by large numbers of worms. Alternatively, it was suggested that they develop from thick-walled Type I granulomas. It was suggested that Type III granulomas may develop as a result of interaction of immune complexes and lysosomal enzyme release by mononuclear phagocytes in the walls of Type I granulomas. Large areas of phagocytic activity were observed in the middle zone of Type I granulomas at day 47 p.i., suggesting that phagocytes had been attracted to localized areas where immune complexes may have been formed. Allison and Houba (1976) indicated that such reactions are contributory factors to the pathology of chronic granulomatous inflammation generally. That such reactions are involved in reducing the thick walls of Type I granulomas to thin walls of Type III is entirely possible. Further support for this interpretation was provided by the abrupt increase in the number of eosinophils and a shift in their center of abundance from the inner zone to the middle zone beginning at day 40 p.i. (Fig. 29b). Eosinophils are said to phagocytose antigen-antibody complexes (Sabesin, 1963; Litt, 1964; Greep and Weiss, 1973; Dunsford _et al_., 1974) so their sudden abundance near areas of intense phagocytic activity and tissue destruction suggests that eosinophils were involved in that activity in _E. uncinata_ granulomas. Coincident with these effects were increased numbers of plasma cells (Fig. 30 c) and antibody production at day 33 p.i. No direct evidence for this mechanism exists, though indirect evidence is suggestive.

Regardless of the mechanism of their formation, Type III granulomas may be extremely important in the maintenance of _E. uncinata_ in wild waterfowl populations. Because most experimentally induced granulomas underwent involution, it was suggested that Type III granulomas may represent the only type which could persist for a long period. Their development in a few birds would ensure that infective material (ova) could be transported from the breeding to the wintering grounds, thereby establishing foci of infections throughout the range of the definitive host. Perhaps more importantly, Type III granulomas, if they persist long enough, could be brought back to the breeding grounds in the spring, to serve as a source of infective material for numerous young ducklings. As well, it was suggested that large, compressible Type III granulomas with large numbers of ova may be superior sources of eggs when compared to thick-walled Type I granulomas. Finally, Type III granulomas were found in a large proportion of breeding female mallards, and a very low proportion of breeding males. This could be their most significant characteristic, since infective larvae would be available to ducklings as soon as they hatched, as the hen would have shed large numbers of ova into areas where she fed during the incubation period. The drakes abandon the hen mallards after incubation begins so infections in males would not serve as good sources of infective material for ducklings. Females of waterfowl species return to their natal areas to breed, while males generally do not unless they return with a female from the same area (see Bellrose, 1976). Thus, an infective source, once established in female mallards could be a long term source of infection, and thus of great significance to the maintenance of *E. uncinata* in wild mallard populations.

The response of mallards to infection with *E. uncinata* appears to be divisible into two distinct but not entirely separate processes: i) formation of the granuloma and; ii) involution of the granuloma and death of nematodes. Granuloma formation appears to be a manifestation of T-cell dependent delayed hypersensitivity similar to that mediating the formation of the schistosome egg granuloma (Warren *et al.*, 1967; Boros and Warren, 1971 b; Domingo and Warren, 1967, 1968; Hsu *et al.*, 1976). The striking resemblance of the *E. uncinata* granuloma to the schistomsome egg granuloma (Sadun *et al.*, 1970; Hsu *et al.*, 1969) and the involvement of lymphocytes, macrophages, heterophils and eosinophils all argue in favour of this interpretation. The dependence of the eosinophil response on T-cells in *T. spiralis* and *T. taeniaformis* infections (Walls *et al.*, 1973; Ansari *et al.*, 1976) suggests that the eosinophil response in *E. uncinata* infections may also be T-cell dependent. Other workers have shown that granuloma formation around schistosome eggs injected into chickens was unimpaired by bursectomy but was reduced in thymectomized chickens (Davis et al., 1974), suggesting that granuloma formation was T-cell dependent. Furthermore, Ould (1972) showed that cortisone injections of ducklings infected with E. uncinata reduced the granulomatous response (less caseous material), and in this study, walls of granulomas from thymectomized birds were thinner than those from normal birds. All of these observations support the contention that granuloma formation is mediated by T-cell dependent delayed hypersensitivity.

Mallard ducklings appear to be capable of granuloma formation at hatching as evidenced by the fact that birds infected at one day of age and thymectomized ducklings with little thymic remnants were able to form granulomas in response to infection. This suggests that a circulating pool of T-cells capable of responding to E. uncinata antigens is present at hatching in male wild mallard ducklings which are not depleted by thymectomy. This is reported to be the case with guinea pigs and Trichostrongylus colubriformis as well (Dineen and Adams, 1971). Furthermore, cell-mediated responses are said to be phylogenetically old, so that one could expect them to be developed early in ontogeny (Park and Good, 1974; Cooper, 1976; Moticka, 1975).

The second aspect of the response of mallards to E. uncinata infections is involution of granulomas once formed. The mechanism of involution has not been elucidated but a cellular response involving massive accumulations of heterophils and lymphocytes, and a build-up of epithelioid and giant cells at the periphery of the cavity appeared to be involved. This was followed by envelopment of the nematodes by giant cells resulting in the death of the worms. Whether these events were mediated by purely cellular mechanisms, or were partly antibody dependent is not known. However, evidence from similar systems suggests a predominantly cellular mechanism in cooperation with opsonizing and/or complement-fixing antibody may be involved (see Smithers and Terry, 1976).

Smithers (1976) reviewed recent evidence which indicates that complement independent opsonizing antibody acting in concert with macrophages may be responsible for direct effects of host immune responses on schistosomules of *S. mansoni*. Perez (1974) demonstrated an opsonizing antibody in the sera of rats immune to *S. mansoni*. This sera, when added to an *in vitro* system containing peritoneal exudate cells from normal rats, resulted in complement independent damage to the integument of schistosomules. Antibody titre correlated well with immunity in the rat and that portion of fractionated serum containing opsonizing antibody activity was able to passively confer immunity to normal rats. Capron (1974) and Capron *et al.* (1975) showed that opsonizing anti-schistosome IgE and macrophages could damage *S. mansoni* schistosomules *in vitro*. In this regard Smithers (1976) suggested that since high levels of IgE antibody have been associated with a number of helminth infections, a mechanism whereby opsonizing antibodies of that type could promote adherence of macrophages to helminths was very significant and could have general application to a variety of systems. Opsonizing antibody was not demonstrated in the present system but it is interesting to speculate that if produced, it could have promoted the adherence of numerous macrophages to the surface of nematodes followed by their fusion as giant cells. The eventual result would have been the death of worms and involution of the granuloma. If indeed granuloma formation around _E. uncinata_ in wild mallards is T-cell dependent as suggested above, this and the demonstrated dependence of IgE formation on T-cell cooperation argues in favour of such a possibility. Another T-cell dependent phenomenon is the eosinophil response to _T. spiralis_ and _T. taeniaformis_, and a late eosinophil invasion of the middle zones of granulomas was noted in this study. Larsh _et al_. (1974 b, 1975) suggested that eosinophils are effector cells which help mediate the expulsion of _T. spiralis_ from the gut of mice, a phenomenon which is primarily cell-mediated. All of these observations imply that close cooperation between cell-mediated and humoral responses (particularly those involving IgE, Smithers, 1976) is important in protective immunity to helminths. Perhaps the _Echinuria_-mallard system is another example of such a relationship.

Results presented here with respect to the effects of age at infection and surgical bursectomy or thymectomy suggested that young mallards are perhaps less susceptible to infection (at least the retention of granulomas) due to an effective nonspecific immunoglobulin response (polyclonal hyperimmunoglobulinemia) (Moticka, 1975; 1978) acting in concert with a cellular response which was well developed at hatching. As well, it was suggested that birds infected at one day and one week of age were subject to less ontogenetically associated stress (rapid growth, plumage development, maintenance of homeothermy) and their relatively nonspecific responses enabled them to suppress infections. Intermediate aged birds were thought to be under greater stresses, their PHIg responses were perhaps senescent (or at least inadequate in the face of suppression by stresses or hormone levels) and their specific Ig responses were in developmental stages such that they were ineffective. It was suggested that since Droege (1971) and Rouse and Warner (1972) found that T-cells acted as both suppressor and helper cells in antibody production to certain antigens, as specific Ig producing ability was developing in mallard ducklings, perhaps a critical stage was reached between the ages of two and four weeks. In this period it was suggested that suppressor cells were relatively more abundant than helper cells and may have suppressed antibody production which would eventually be able to mediate involution of granulomas. The interaction of thymus dependent suppressor cells and B-cells was suggested as a mechanism whereby specific Ig producing B-cells are selected, resulting in maturation of humoral immune responses. In birds infected at five and six weeks of age, it was suggested that the process of maturation of humoral immune responses was almost complete and that helper cells were more numerous than suppressor cells, resulting in greater production of antibody which could mediate granuloma involution in cooperation with macrophages. Data which indicated that some factor was produced in greater quantity as age at infection increased was presented in Section V; it was noted that worm diameter decreased as age at infection increased. This factor could have been steadily increasing titres of antibody (opsonizing IgE) or antibody of greater avidity. Moticka (1975, 1978) reported that chickens do not have fully mature humoral responses until about 4 to 6 weeks of age but that their cellular responses mature much earlier. This could have been the case with wild mallard ducklings.

Antibody detected in this study was correlated with the number of nematodes present and was apparently not responsible for the decrease in nematode diameter as age at infection increased. In addition, bursectomized and thymectomized ducklings had greater amounts of this antibody. It was suggested that this antibody was produced by T-cell independent B-cells that were not depleted by neonatal surgical bursectomy. Higher titres of this antibody in bursectomized birds was possibly due to the absence of T-cell dependent B-cells which in normal birds produce antibodies which suppress their production as suggested by Park and Good (1974). If thymectomy resulted in depletion of helper T-cells, these regulatory antibodies would not be produced, and an augmented response could again be produced. This is of course pure speculation, but it does help explain the paradoxical situation of greater antibody responses seen in supposedly immunosuppressed ducklings. If the regulatory antibodies above were involved in granuloma involution, one would expect thymectomy and bursectomy to result in infections of greater duration than normal. This was not tested in this study but could be done in the future.

Finally, it is interesting to note that bursectomy did not interfere with splenic germinal center formation, suggesting that unlike chickens (Toivanen and Toivanen, 1973), mallard ducklings at hatching (or before) produce bursal stem cells which do not require the bursal environment in order to mediate splenic germinal center formation. This suggests that post-bursal stem cells seed out from the mallard bursa earlier than they do in chickens, or that splenic germinal centers are independent of the bursa; perhaps mediated by Motickas (1975) BM-cells. If this latter is the case, germinal centers in the spleen of birds may be involved in PHIg production. The generally erratic responses of thymectomized birds confirms the same observation made by Graetzer _et al_. (1963) with thymectomized chickens. It is also consistent with the hypothesis that responses to _E. uncinata_ are chiefly mediated by T-cells and T-cell dependent antibody. Differential depletion of T-cell populations caused by partial thymectomy could result in suppression of different effector mechanisms. Taken as a group, the responses of the birds involved could be expected to be erratic.

In conclusion, evidence presented here and in the literature suggests that granulomatous responses are T-cell dependent, cell-mediated immune responses which may act synergistically with T-cell dependent antibody responses (IgE) as suggested by Smithers (1976), to protect hosts against invading helminths. Larsh and Weatherly (1975) suggested that all evidence favours the role of CMI in immunity to helminths but the cooperation of humoral responses is becoming more and more important.

CONCLUSIONS

1. The lesion associated with E. uncinata infections in wild mallard ducklings was initiated by penetration of juvenile nematodes into the mucosa of the isthmus. As nematodes penetrated into the thick, circularly arranged muscle layer of the muscularis, distinct walls and a cavity formed. The lesion was a fully developed granuloma, well integrated into the gut wall (Type I) by day 19 p.i. E. uncinata granuloma closely resembled those around schistosome eggs; their developmental stages were also similar. Heterophils, lymphocytes, and macrophages were important types of cells involved in granuloma formation.

Once granulomas were fully formed, three possible routes of further development were noted: i) progression to an involutional stage (Type II) with no maturation of nematodes; ii) growth of granulomas and nematodes with maturation of, and reproduction by the nematodes, and; iii) formation of large, thin-walled granulomas as seen in breeding hen mallards in the wild. In addition to heterophils, lymphocytes and macrophages, eosinophils, plasma cells, and giant cells were important components of granulomas beyond the fully developed Type I stage at day 19 p.i.

2. Antibodies detected by immunodiffusion of sera from infected ducklings against reconstituted lyophillized adult whole worm antigen, appeared by day 33 p.i. Titres were positively correlated with the number of nematodes present. No direct correlation between titre of these antibodies and the number of splenic germinal centers was detected. Indirect correlations between these parameters, and between them and gamma globulin content suggested that splenic germinal centers were involved in production of undetected specific antibody or nonspecific immunoglobulin (PHIg). Formation of splenic germinal centers was unimpaired by neonatal bursectomy, suggesting that cells responsible for splenic germinal center formation in the mallard (bursal stem cells) "seed out" from the bursa before hatching, and do not require the bursal environment for maturation.

3. Very young ducklings (one day or one week of age) and other ducklings (5-6 weeks of age) were less susceptible to infection with E. uncinata than were ducklings of intermediate ages at infection (2-4 weeks). It was suggested that young ducklings have well developed nonspecific Ig responses and well developed cellular responses which protected them from infection in the absence of stress associated with rapid growth and maintenance of homeothermy. Birds of intermediate ages were thought to be subject to more stress and were at the same time at a critical stage in development of immunological competence; nonspecific

Ig responses were perhaps senescent and their specific Ig responses were at a stage of development where cell populations capable of maximally efficient responses were being selected, perhaps by their interaction with thymus dependent suppressor cells. At the same time, their cellular responses were suppressed by stresses associated with rapid growth, maintenance of homeothermy and perhaps hormonal influences. Birds infected at 5 or 6 weeks of age were again less susceptible, perhaps because their specific Ig responses were almost completely developed.

As age at infection increased the diameter of nematodes recovered decreased. This was attributed to a progressive age-related maturation of specific Ig producing ability. It was suggested that the observed effect was an effect of undetected antibody directed at the nematodes which was produced in greater quantity (or was more efficient) with age.

4. Formation of the E. uncinata granuloma is a manifestation of a delayed hypersensitivity reaction mediated by T-cells. Mallard ducklings are capable of mounting this cellular response upon hatching, though some impairment was noted in thymectomized ducklings as evidenced by slightly thinner-walled granulomas. It was suggested that involution of granulomas and death of nematodes was the result of antibody dependent cellular responses involving opsonic antibody (IgE?), macrophages

(giant cells), and perhaps eosinophils. Results of bursectomy and thymectomy procedures were not unequivocal but did suggest that production of antibody responsible for initiating granuloma involution was dependent upon T-cell cooperation. Thus, responses of mallard ducklings to *E. uncinata*, though not completely cellular in nature, were ultimately controlled by thymus dependent cells. It should be emphasized that the relationship between *E. uncinata* and its mallard host is complex and worthy of further investigation.

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APPENDIX I

Number of various types of cells from control ducklings and number of leucocytes in blood smears from ducklings in Section IV.

Table 1. Mean number of various types of cells per field in the lamina propria and submucosa of the isthmus of uninfected ducklings necropsied at the same age as infected ducklings necropsied on days five and forty-seven postinfection.

TYPE OF CELL	DAYS POSTINFECTION	
	Five	Forty-seven
Eosinophilic granulocyte	[1] 0.67 (0.64)	0.69 (0.61)
Lymphocyte	1.57 (0.60)	2.28 (0.68)
Macrophage	1.31 (0.41)	1.93 (0.29)
Mast cell	0.29 (0.51)	0.28 (0.48)
Plasma cell	0.08 (0.24)	0.22 (0.35)
Blast cell	[2] 0 -	0.08 (0.23)

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[1] Values are Naperian logarithms of actual counts with standard deviations in parentheses below.

[2] A few blast cells noted, but never more than 1/field.

Table 2. Number of various types of leucocytes per field in blood smears from ducklings infected with 60 *E. uncinata* larvae at four weeks of age and necropsied at intervals postinfection.

		TYPE OF LEUCOCYTE				
DAYS POSTINFECTION	*N	[1]Eo	Hetero	Lympho	Mono	Baso
FIVE	60	[2]0.15	0.55	1.13	0.08	0.03
		[3](0.40)	(0.75)	(0.85)	(0.28)	(0.18)
		[4]8	28	58	4	2
NINETEEN	80	0.25	0.70	1.33	0.08	0
		(0.54)	(0.92)	(1.16)	(0.27)	-
		11	30	56	3	-
THIRTY-THREE	50	0.08	0.56	1.54	0.04	0.02
		(0.27)	(0.84)	(0.81)	(0.20)	(0.14)
		4	25	69	2	1
FORTY-SEVEN	90	0.52	0.57	1.54	0.14	0
		(0.67)	(0.72)	(1.02)	(0.38)	-
		19	20	56	5	-

[1] Eo= eosinophil, Hetero = heterophil, Lympho = lymphocyte, Mono = monocyte, Baso = basophil

[2] Mean number of cells/field

[3] Standard deviation

[4] Per cent of total

*Number of fields counted

APPENDIX II

Mean numbers of granulomas and worms, mean diameters of granulomas, mean length and mean maximum diameters of nematodes, and mean antibody titres from ducklings infected with 60 E. *uncinata* larvae at various ages and necropsied at day 20 or 50 postinfection.

Table 1. Mean numbers of granulomas and worms, and mean diameters of granulomas from ducklings infected with 60 E. uncinata larvae at various ages and necropsied at day 20 postinfection.

PARAMETER		NUMBER GRANULOMAS	NUMBER WORMS/BIRD	NUMBER WORMS/GR.	DIAMETER GRANULOMAS mm	
AGE AT INFECTION	[1]N					[2]N
One day	5	[3]1.4 (0.6)	36.6 (11.8)	29.6 (14.9)	6.8 (2.3)	7
One week	5	1.4 (0.6)	13.0 (5.7)	9.7 (4.8)	5.5 (1.2)	7
Two weeks	4	3.3 (1.0)	22.0 (6.3)	7.4 (3.3)	3.9 (0.9)	13
Three weeks	5	2.6 (0.6)	30.6 (7.3)	11.9 (2.0)	5.5 (0.7)	13
Four weeks	5	4.4 (1.5)	20.9 (10.4)	6.4 (2.1)	3.5 (1.5)	22
Five weeks	5	1.6 (0.6)	4.1 (1.8)	4.0 (2.6)	4.5 (2.8)	8
Six weeks	4	1.5 (0.6)	11.5 (11.1)	6.3 (5.2)	5.3 (0.8)	6
F(6 & 26 df)		9.575	7.645	8.379	6.406*	
p<		.001	.001	.001	.001	

1. Number of birds.
2. Number (total) of granulomas.
3. Means with standard deviations in parentheses below.

* 6 & 69 df.

Table 2. Mean total length and maximum diameter of E. *uncinata* from ducklings infected with 60 E. *uncinata* larvae at various ages and necropsied at day 20 postinfection.

AGE AT INFECTION	N	LENGTH mm	DIAMETER mm
One day	40	[1]6.05 (0.70)	0.27 (0.04)
One week	30	4.74 (1.42)	0.20 (0.06)
Two weeks	30	4.51 (0.72)	0.17 (0.03)
Three weeks	25	5.09 (1.03)	0.19 (0.03)
Four weeks	49	3.12 (0.20)	0.13 (0.01)
Six weeks	21	3.14 (0.64)	0.12 (0.02)
F(5 & 185 df)[2]		72.893	87.174
p<		.001	.001

1. Numbers are means with standard deviations in parentheses below.
2. Results of one way anovar with probability (p) below.

Table 3. Mean numbers of granulomas and worms, and mean diameters of granulomas from ducklings infected with 60 E. uncinata larvae at various ages and necropsied at day 50 postinfection.[1]

PARAMETER		NUMBER GRANULOMAS	NUMBER WORMS/BIRD	NUMBER WORMS PER INF. BIRD	NUMBER WORMS/GR.	DIAMETER GRANULOMAS mm	
AGE AT INFECTION	N						N
One day	5	1.0 (0.0)	9.6 (7.7)	12.0 (6.4)	9.6 (7.7)	8.7 (1.6)	5
One week	5	1.8 (0.5)	10.0 (4.0)	10.0 (4.0)	5.6 (1.7)	6.2 (1.7)	9
Two weeks	5	2.2 (1.5)	19.4 (10.9)	24.3 (1.7)	7.6 (4.9)	7.4 (2.3)	11
Three weeks	5	3.2 (0.8)	22.4 (20.9)	37.3 (6.0)	6.1 (5.6)	6.9 (2.6)	16
Four weeks	5	3.0 (1.6)	11.1 (11.2)	13.9 (10.7)	7.2 (8.0)	6.3 (2.4)	15
Five weeks	5	1.4 (1.5)	2.4 (5.4)	12.0 (0.0)	1.1 (2.4)	8.6 (3.6)	7
Six weeks	4	2.0 (1.4)	10.0 (12.0)	20.0 (5.7)	3.3 (4.0)	5.8 (1.6)	8
F(6 & 27 df)		2.290	1.693	---	1.332	1.743	
p<		.025	ns		ns	ns	

1. For an explanation see Table 1.

Table 4. Mean total length and maximum diameter of *E. uncinata* from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 50 postinfection[1]

AGE AT INFECTION	N	LENGTH mm	DIAMETER mm
One day	19	12.04 (1.60)	0.61 (0.05)
One week	14	12.11 (0.57)	0.56 (0.09)
Two weeks	38	12.20 (2.68)	0.52 (0.13)
Three weeks	29	10.81 (1.30)	0.50 (0.06)
Four weeks	22	12.22 (1.18)	0.51 (0.06)
Six weeks	21	11.22 (2.12)	0.46 (0.12)
F(5 & 137 df)		2.699	5.677
p<		.025	.001

1. For an explanation see Table 2.

Table 5. Antibody titre of sera from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 50 postinfection.

AGE AT INFECTION	N	NUMBER POSITIVE	MEAN TITRE POSITIVES	MEAN TITRE OVERALL
One day	5	1	1.0	0.2 [1](0.5)
One week	5	2	1.0	0.4 (0.6)
Two weeks	4	1	2.0	0.5 (1.0)
Three weeks	5	4	9.8 [2](4-16)	7.8 (6.9)
Four weeks	5	3	4.0 (1- 8)	2.4 (3.4)
Five weeks	5	0	---	---
Six weeks	4	2	8.0 (2-14)	4.0 (6.7)

[1] Standard deviation

[2] Range.

APPENDIX III

Results of regression analyses of data from Sections V, and VI.

Table 1. Results of simple linear regression analysis of pooled data from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 20 postinfection.[1]

REGRESSION LINE	Y Dependent variable	X Independent variable	a	b	^{2}F	r^2	df
1	Number of worms	Number of granulomas	14.41	2.42	1.96 (ns)	0.059	1, 31
2	Number of splenic follicles	Condition index	-0.512	0.134	6.096 (.025)	0.169	1, 31
3	Gamma globulin	Splenic follicles	2.028	1.605	6.552 (.025)	0.179	1, 31
4	Gamma globulin	Number of granulomas	1.719	0.396	8.316 (.001)	0.217	1, 31

1. Where $Y = a + bX$.
2. F ratio regression anovar with probability in parentheses below.

Table 2. Results of simple linear regression analysis of pooled data from ducklings infected with 60 *E. uncinata* larvae at various ages and necropsied at day 50 postinfection.[1]

REGRESSION LINE	Y Dependent variable	X Independent variable	a	b	F	r^2	df
1	Number of worms	Number of granulomas	1.54	4.97	12.737 (<.005)	0.291	1, 31
2	Gamma globulin	Total protein	-5.219	0.1906	24.580 (<.001)	0.44	1, 31
3	Albumin	Total protein	6.772	0.5056	48.674 (<.001)	0.61	1, 31
4*	Titre	Number of worms	1.896	0.227	6.848 (<.025)	0.38	1, 11
5**	Titre	Number of worms	-0.1302	0.1936	12.846 (<.001)	0.29	1, 31
6*	Titre	Gamma globulin	-0.406	1.202	8.249 (<.005)	0.43	1, 11
7**	Titre	Gamma globulin	-2.3936	1.273	28.27 (<.001)	0.48	1, 31

1. For an explanation see Table 1.
* Reactors only.
**All birds included.

Table 3. Results of simple and multiple linear regression and parabolic regression analyses of data from surgically altered ducklings infected with 60 *E. uncinata* larvae at two weeks of age and necropsied at day 50 postinfection.[1]

REGRESSION	Dependent Variable Y	Independent Variables X_1	X_2	a	b	c	F	r^2	df
1	Number of worms per granuloma	Thymus weight	$(\text{Thymus weight})^2$	-0.258	18.42	-10.34	6.975 ($<.05$)	0.7361	2, 5
2	Number of worms per bird	Thymus weight	$(\text{Thymus weight})^2$	-635.1	473.8	-85.35	37.13 ($<.05$)	0.9738	2, 2
3	Diameter of granulomas	Number of granulomas	---	7.82	-0.656	---	-6.205 ($<.05$)	0.4699	1, 7
4	Spleen diameter	Spleen length	---	3.05	0.570	---	53.87 ($<.001$)	0.860	1, 7
5	Spleen diameter	Number of splenic follicles	Spleen length	2.617	0.729	0.559	56.50 ($<.001$)	0.9495	2, 6
6	Spleen diameter	Thymus weight	---	7.195	2.05	---	7.98 ($<.025$)	0.420	1, 11
7	Titre	Gamma globulin	---	-4.52	3.52	---	11.558 ($<.01$)	0.620	1, 7
8	Titre	Condition indix	---	135.4	-18.50	---	11.63 ($<.025$)	0.620	1, 7
9	Titre	Total protein	Gamma globulin	-30.89	0.702	2.751	9.298 ($<.025$)	0.760	2, 6
10	Titre	Gamma globulin	---	-4.710	4.096	---	15.578 ($<.005$)	0.5861	1, 6
11	Titre	Total protein	Gamma globulin	11.903	-0.447	4.757	7.995 ($<.05$)	0.61	2, 5

1. $Y = a + bX_1$ for simple linear regression; $Y = a + bX_1 + cX_2$ for multiple linear regression; $Y = a + bX_1 + c(X_1)^2$ for parabolic regression. See Table 1 for other explanations.