IMMUNITY AND PRIMARY REACTION ASSAY OF ANTIBODIES TO *Ascaridia galli*

by

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INTRODUCTION

An immune phenomenon to helminth parasites has been reported as early as 1929 when Stoll described the "self cure" response in Haemonchus contortus infections. The immune response has been reported by; Sarles and Taliaferro (1936) for Nippostrongylus muris, Lawler (1940) for Strongyloides ratti, Mauss (1941) for Trichinella spiralis, and others. Sarles' work in 1938 with N. brasiliensis suggested that antibodies were formed to the secretions and/or excretions from the body openings of this nematode. Working with Ascaridia galli, Todd and Hansen (1951) theorized that an expenditure of energy against the parasitic infection prevented maximum efficient weight gains. They observed that chickens harboring the greatest number of worms, within the range from 0 to 15 worms, also made the greatest weight gains.

The interest generated by these workers has initiated an in-depth investigation of the immune mechanisms involved in the host-parasite interplay. The challenge is viewed from two directions: the effects of the parasite upon antigenic stimulation of the host and the effects of the host on the parasite in warding off the infection. There is an extensive literature on immunologic studies of parasitic systems; therefore, this review is limited primarily to the work involving nematode infections.

The purpose of this study is twofold. Firstly, to review the literature dealing with the immune responses to nematode invasions in hopes of better understanding the mechanisms involved, and secondly, to assay specific antibodies by a primary reaction measurement. The experimental system chosen was the domestic chicken and its intestinal roundworm, Ascaridia galli, whose morphology and life cycle are known (Ackert, 1931). The direct life cycle of this parasite and the ease of rearing and handling

the chicken host are distinct advantages of this host-parasite system.

REVIEW OF LITERATURE

Intricacies of the host-parasite interaction are not well known. Presently, accurate and reliable diagnostic techniques by the recovery of eggs or demonstration of the parasites are expensive, time consuming, and offer only presumptive evidence of infection. Sadun (1963) emphasized the need for immunobiologic methods in the diagnosis of parasitic infections. Recent advances in methodology, increasing interest in host-parasite relationships, and the hope that greater knowledge of immunology might aid in the diagnosis and control of many parasitic infections of man and animals have instigated rapid expansions in immunologic research in helminthology.

Immunodiagnosis of parasitic infections seeks to elicit effective methods for producing active protective immunity in the host. The classical method is subjecting the host to infection; however, this method assumes the risk that the pathology caused by parasitic infection may endanger the life of the host or disturb its physiology.

Many abortive attempts to develop vaccines containing dead worms have been made and are reviewed by Thorson (1963a). It is generally accepted that the helminth parasite contains a wide variety of potential antigens many of which do not stimulate protective immunity. The effective antigens would then exist in very low quantities with respect to the total bulk of injected vaccine; thus, not stimulating an appropriate antibody response (Jackson et al., 1970). Identification of antigens crucially involved in host-parasite systems would contribute to the understanding of the immune

phenomenon.

Antigen Characterization Studies:

The nematode contains a wide variety of potential antigens. In addition, the involvement of a series of larval stages further complicates the study as different components of these stages may function at different times. Isolation and characterization of the "functional" antigen(s) defined as eliciting specific protective antibodies, is the goal of many investigators.

No satisfactory standard fractionation procedure of whole-worm antigens has been made. Melcher (1943) initiated one of the first attempts by describing an extraction procedure which consisted of delipidization with ether, extraction of the residue with buffered saline, precipitation of acid-insoluble components, and harvesting acid-soluble constituents from the supernate. The acid-insoluble fraction was found to be albumin-like; the acid-soluble fraction was globulin-like. Working with Ascaris, Campbell (1936) isolated an antigenically active polysaccharide by borate buffer extraction and repeated cold alcohol precipitations.

More recent extraction studies of somatic antigens have been reported by Kent (1960, 1963) who separated defatted whole-worm extracts by DEAE-Sephadex column chromatography. Further fractions were obtained by eluting with NaCl solutions of increasing normalities which yielded five antigenically different fractions with varying protein/carbohydrate ratios. The author emphasized the necessity for using extraction methods which preserve protein-polysaccharide complexes because some extraction methods denatured protein antigens. Tanner (1965) cautioned investigators to avoid physiochemical changes in the antigens during fractionation. He observed that saline extracts of Trichinella larvae treated with

2-mercaptoethanol to cleave disulfide bridges and then alkylated in order to maintain cleavage, produced an antigen which resembled normal human serum albumin in double diffusion studies. Whole worm crude extracts have also been studied by Jeska (1967) for Toxocara canis, and by Soulsby (1957), Kagan (1957), and Tormo and Chordi (1965) for Ascaris suum.

A different approach was taken by Oliver-Gonzalez (1943) and Kagan (1957) who demonstrated different antigens in tissue isolates such as cuticle, muscle, and eggs. Williams and Soulsby (1970) using gel diffusion and immunoelectrophoresis reported that there was a progressive increase of antigens with developmental growth of A. suum. This further supported Soulsby's (1963) "stage-specific" antigens described for larvae of A. suum.

In addition to somatic (structural) antigens which may stimulate antibody production are the so-called physiologic antigens which include the secretions and excretions of the parasite. These antigens are produced generally by the incubation of various stages of the nematode life cycle in different media. The idea of the excretory and secretory products of parasites acting as functional antigens originated with Sarles (1938) who observed that larvae of Nippostrongylus muris formed precipitates at the mouth, excretory pore, and anus when placed in homologous immune serum. Similar observations have been reported by Campbell (1955), Thorson (1953), Sadun and Norman (1957), Chipman (1957), and Levine and Kagan (1960). Isolated purified malic dehydrogenase and aminopeptidase from A. suum demonstrated protection in pigs (Rhodes et al., 1964, 1965, 1966). A discussion of "metabolic antigens" is given by Thorson (1963a, b).

Other substances which may stimulate protective immunity are the hatching and exsheathing fluids. Studies by Stewart (1953), Soulsby

et al. (1959) and Soulsby and Stewart (1960) showed that the molt from the third to fourth larval stage of Haemonchus was essential for attaining patency. The release of appreciable quantities of molting fluid was presumed to contain essential antigens in initiating the "self cure" phenomenon. Sommerville (1957) and Rogers and Sommerville (1960) reported the release of Haemonchus exsheathing fluid to be stimulated by high CO_2 tensions (low O_2 tensions) and Rogers (1963) characterized the fluid as containing a leucine aminopeptidase. Alger (1968) used Ouchterlony techniques to compare somatic and metabolic antigens with exsheathing fluid components of Haemonchus contortus. The active fractions were reported to be somatic antigens as no visible reaction with metabolic antigens was observed. The immunologic functions of hatching and exsheathing fluids in nematode-host relations have not been defined adequately.

It is apparent that molting fluids served as antigenic stimuli and it has been suggested by Thorson et al. (1968) that hormones may be the physiologically active agent in some parasitic infections however studies in this direction have been few.

A review of the literature on antigen characterization studies strengthens the view that just the surface has been scratched and much work needs to be done. The development of sophisticated biochemical and immunological techniques gives encouragement to the parasitologist. Work conducted by Ozerol and Silverman (1969, 1970) included amino acid composition and infrared studies of Haemonchus antigens which resulted in a precise evaluation of the antigens involved. Detailed analyses such as these will undoubtedly provide valuable information in elucidating the immune mechanisms in the host-parasite interaction.

Defense Mechanisms of the Host:

The degree to which the host responds to a parasitic stimulation depends on many factors. The quantity of antigen given the host, the route of administration, and the parental association established exert considerable influence upon the antibody response. Ackert (1931) found that larvae of Ascaridia galli bury their anterior ends deeply between intestinal villi and into the glands of Brunner but they seldom pass through the wall of the intestine. The antigenic stimulation of this nematode would not appear to elicit antibody production to the same degree as an Ascaris infection with concomitant systemic migratory phase.

In conjunction with the effects of antigens on the host, the defense mechanisms of the host play an important role in the immune response. It is well to remember that although the host is stimulated to produce antibodies to most if not all of the parasite antigens, all the antibodies formed may not be protective in nature. Some may be simple responses to antigenic stimulation.

The host has an innate ability to resist parasite infections. Emphasis in this paper is on the chicken host which has been found to be an excellent precipitin producer to animal serum proteins (Goodman et al., 1951; Makinodan et al., 1952), and a reasonably good producer of agglutinins and neutralizing antibodies (Wolfe and Dilks, 1949). Factors which influence the host's innate immune response include age, sex, nutrition, and genetic constitution.

Age immunity studies almost unequivocally show that young animals are more susceptible to nematodes than older ones. Herrick (1928), Sarles (1929), and Ackert and Edgar (1939) were among the pioneers in this field of study.

Working with portions of the duodenum of chickens, Ackert and co-workers found increasing numbers of goblet cells (secretory cells in the intestinal epithelium) as the chickens grew older to 124 days. This corresponds to the development of age immunity as observed by Herrick (1926). Similar findings that globule leukocytes increase with age in host animals has recently been observed by Whur (1966). These leukocytes were characterized as resembling that of a plasmocyte. The author believed that the worms were responsible for the presence of the globule leukocytes; however, the actual function of these cells was not suggested. Dobson (1966a) reported that the globules of these leukocytes contained condensed globulins and suggested that similar globules are found in plasma cells.

In the case of the skin-penetrating nematode, Strongyloides ratti, Lewert and Lee (1957) noted changes in the basement membrane of the skin of the older host which prevented entrance of the parasite. Histochemical studies of the skin revealed that acellular elements of the basement membrane became more highly polymerized with age and resisted the enzymes facilitating penetration of the larvae (Lewert and Lee, 1954).

The sex of the host may influence the fate of the invading parasite. Mathies (1959) infected mice with Aspiculuris tetraptera, a mouse pinworm, and found twice the number of worms in males. The females showed resistance at first estrus. Jackson et al. (1970) stated that immunity in the male developed gradually and increased with age, whereas the female showed a dramatic surge of resistance just before the onset of puberty. This view is not held by all investigators and the issue remains controversial.

The effects of nutrition upon immunity may be profound. Ackert et al. (1927) and Zimmerman et al. (1927) found that chickens with vitamins A, B,

and D deficiencies harbored more and longer worms than the control chickens. Lack of vitamin A affects the normal functioning of epithelial membranes and thus the gastro-intestinal tract lining may be altered. Krone (1963) found an increased resistance to Heterakis with vitamin A supplements and Leutskaya (1964) confirmed earlier work that vitamin A increased antibody response to Ascaridia. Ackert and Nolf (1931) reported diets low in vitamin B caused partial paralysis of the muscles of the digestive tract and resulted in an increased number of worms. Vasilev (1961) found that vitamin B_{12} enchanced resistance to Ascaridia.

According to Reidel and Ackert (1951), the type and source of proteins have a significant effect on the resistance of chickens to ascarids. Ackert and Beach (1933) supplemented skim milk and meat meal to a cereal based ration which was found to increase resistance of chickens to A. galli, whereas, a peanut meal supplement significantly lowered resistance to the nematodes. Also, pteroylglutamic acid with a liver extract had a suppressive effect on the development of Ascaridia as reported by Sadun et al. (1950) and Reidel (1954) found that glycine and leucine had no value in increasing resistance to this nematode. Diets low in phosphorus and calcium reduced the growth and number of Ascaridia in chickens (Gaafar and Ackert, 1953).

Genetic influences on the immune response of the host is generally accepted. Ackert and Wilmoth (1934) introduced cockerels highly susceptible to ascarids to a resistant flock which resulted in the filial generation being less resistant. An inverse relationship between breed weight and morbidity has been reported by Ackert et al. (1935) and Darski (1962). The lighter breeds, White Leghorns and White Minorcas, were more susceptible to Ascaridia infections than the heavier breeds and varieties, White Plymouth

Rock and Rhode Island Red (Ackert et al., 1935). Jaffe (1966) indirectly confirmed earlier work by observing a greater antibody response in the heavier birds with larger bursas. This further intensifies the importance of the immune mechanisms in host-parasite relationships.

The immune response of the chicken host is not well established; however, avian immunology has been developing in recent years. It is beyond the scope of this paper to review every publication concerning avian immunity and only pertinent references are cited.

Good and Papermaster (1964) reported the antibody-producing capability of chickens up to four weeks of age was very low, but a sudden development occurred between four to five weeks. They determined that maturation of immunoglobulins in the chicken was reached in about five weeks. Maternal transfer of antibodies in avian species has been established and such antibody probably aids in protection immediately after hatching until the young bird reaches immunologic maturity (Patterson et al., 1962).

The major lymphoid organs of chickens include the thymus and spleen, plus one unique to avian species - the bursa of Fabricius. The elucidation of the biologic role of these immunocompetent organs is credited numerous investigators (Metcalf, 1960; Miller et al., 1965; Good et al., 1965; Glick, 1958; Glick et al., 1956). It is generally believed that the bursa is principally responsible for competence to produce humoral antibody and the thymus for competence to produce delayed sensitivity reactions and cellular immunity. The original concept of a dissociation of immunologic processes was suggested by Warner et al. (1962). An excellent discussion of these organs and their role in the immune response is presented by Warner and Szenberg (1964).

In the chicken the thymus is a primary lymphoid organ in which lymphocytogenesis is completed by the 14th day of incubation (Papermaster and Good, 1962). The organ consists of several lobes with each lobe subdivided by septa of connective tissue into many lobules. The texture of the organ makes complete removal in newborn chicks a difficult procedure, however, complete thymectomy causes a depression of small lymphocytes in the blood and spleen (Abramoff and LaVia, 1970).

The extensive work of Warner and Szenberg (1962) suggested a cellular role of the thymus organ. They noted that neonatal thymectomy in chicks destroyed the birds' capacity to normally reject homografts; however, when injected with human gamma-globulin there was no effect on the production of antibody. Aspinall and co-workers (1963) found similar results, whereas, Cooper et al. (1965, 1966) reported inconsistent results. Cooper and associates presented a more thorough approach by making use of thymectomy followed by irradiation which prolonged the process of homograft rejection.

Okamoto (1970), working with Hymenolepis nana, a dwarf tapeworm of mice, depressed and restored acquired immunity in neonatally thymectomized mice by placing thymus grafts subcutaneously in the recipients. The grafts, even in small quantities, restored their ability to reject cysticercoids from the intestinal villi.

These findings, among many others, gave evidence for the function of the thymus as the primary responsible lymphoid organ in cell mediated immune responses. Thymectomized birds did not show normal delayed hypersensitivity responses and the population of small lymphocytes was reduced. These birds possessed the normal levels of germinal centers, plasma cells and immunoglobulins (Abramoff and LaVia, 1970).

The bursa of Fabricius has sometimes been called the "cloacal thymus". The bursa is a blind, plicated, sac-like lympho-epithelial structure which arises from the dorsal diverticulum of the cloaca (Calhoun, 1933). It becomes a lymphoid organ around the 15th day of incubation and is fully developed in the young bird in one to four months (Ackerman and Knouff, 1959). In most older birds, the bursa atrophies and eventually involutes completely.

The relationship between the bursa and antibody formation was discovered accidentally by Glick and co-workers (1956) when adult chickens failed to produce antibodies to Salmonella typhimurium after surgical removal of the bursa during the first two weeks of age. These findings have since been verified repeatedly by numerous workers. St. Pierre and Ackerman (1965) implanted cell-impermeable Millipore diffusion chambers containing portions of the bursa which restored the antibody producing capacity of the chickens. They suggested that the bursa of Fabricius produced a non-cellular agent capable of restoring immunologic activity in bursectomized chicks.

Attempts were made by Sadler (1965) to infect bursectomized and non-bursectomized White Leghorn chickens with Ascaridia galli; however, inconsistent results were obtained. In one trial, a marked increase of worms occurred in the bursectomized birds as compared to the non-bursectomized chickens while in a subsequent trial the difference was not significant. Johnson (1968) studied the effects of bursectomy on worm burdens in A. galli infections and found a greater number of worms in either hormonal or surgically bursectomized chickens than in non-bursectomized birds. Surgical removal of the bursa on three day post-hatched birds resulted in an average worm burden of 9.4 A. galli and embryonic hormonal bursectomy with

testosterone proprionate resulted in an average worm burden of 9.9. The control non-bursectomized chickens harbored an average of 2.3 worms and 1.9 worms, respectively.

Abramoff and LaVia (1970) and Jackson et al. (1970) discuss extensively bursectomy studies. Detectable antibody following antigenic stimulation in such birds was lacking; however, homograft rejections and other delayed hypersensitivity reactions were still evident. Bursectomized birds were markedly deficient in IgG (Ortega and Der, 1964; Pierce et al., 1966) and IgM innumoglobulins (Pierce et al., 1966).

Cooper et al. (1966) expressed the view that the thymus and bursa of Fabricius were closely integrated; the thymus recognizing the foreigness of an antigen and transferring this information to the immunoglobulin molecule to give specific binding capacity. This view merits consideration; however, it is generally accepted that in the chicken there is a rather distinct separation of the functional components of the immune system.

There are two basic approaches to the field of immunology: the humoral antibody aspects and the study of cellular mechanisms. Recent evidence has suggested that the stimulation of certain types of antibodies may be closely related to the stimulation of cell mediated immunity (Ogilvie, 1970).

Ogilvie and Jones (1967) and Brown et al. (1968) showed immunity to both Nippostrongylus brasiliensis and Plasmodium berghei (protozoan) in the rat to be thymus dependent yet immunity was predominantly antibody mediated. The formation of reagin (IgE) was reported and Claman et al. (1966) and Mitchell and Miller (1968) indicated that cells derived from bone marrow and thymus are vital for production of this type of antibody. Jones and associates (1970) showed immunity to N. brasiliensis to be

stimulated in the intestine but protective antibodies found in circulation were associated with electrophoretically fast IgG. In general, IgA is important in immunity in the intestine (Tomasi and Bienstock, 1968), and other secretions.

Similar findings were reported earlier by Douvres (1962) who isolated antibodies from the cecum and colon of cattle infected with Oesophagostomum radiatum. Thses antibodies precipitated secretions and excretions of the nodular worm, whereas, sera from these animals did not. He believed the presence of worms in the tissues triggered the infiltration of specific antibodies from the sera and that the antibodies were not formed by cells in the local area.

A discussion of aspects of humoral versus cellular acquired immunity is presented by Jackson et al. (1970) and Ogilvie (1970).

Humoral Antibody Studies:

This study deals primarily with humoral immune responses of the chicken to parasitic antigenic stimulation by the intestinal roundworm, Ascaridia galli. Keep in mind that in vivo, the defense mechanism must act as a continuum and that various components of the immune response may function at different times during a parasitic infection.

Methods of detecting antibodies are based on the primary, secondary or tertiary reaction of the antigen-antibody molecule. By definition, the primary reaction is the initial interaction between antigen and antibody. In vitro visual manifestations of primary interactions comprise the secondary reactions while in vivo observations of antigen-antibody complexes make up the tertiary tests (Abramoff and LaVia, 1970).

Immunologic studies of parasitic systems have been based on secondary

and tertiary reactions with varying degrees of reliability. These tests have been developed primarily for diagnostic purposes of detecting parasitic infections.

Among the tertiary tests are the passive cutaneous anaphylaxis (PCA) reactions for diagnosis of Oesophagostomum columbianum (Dobson, 1966b) and for Toxocara canis and Trichinella spiralis (Ivey and Slanga, 1965; Ivey, 1965). Reliable skin tests for detection of hydatid cysts (Kagan et al., 1966), for Entamoeba histolytica (Maddison et al., 1968), and for leishmaniasis (Southgate and Manson-Bahr, 1967) have been developed. However, basic to these tests was the in vivo manifestation of the primary antigen-antibody interaction and for many parasitic systems these tests would not be feasible or applicable.

Most of the serological tests used in parasite studies are secondary type reactions. Haemonchus contortus infections can be measured effectively by indirect hemagglutination (IHA) and agar-gel diffusion methods as discussed by Soulsby (1960). Mucosal extracts of sheep infected with Oesophagostomum columbianum formed specific precipitates with an extract of third stage larvae of this parasite in agar-gel plates (Dobson, 1966c). Ascaridia galli cultured in intestinal mucus extracts were investigated by Ackert (1942). He reported a direct relationship between the age of the chicken host, the development of resistance against this nematode parasite, and the number of goblet cells in the intestinal epithelium.

A microprecipitin phenomenon described by Sarles and Taliaferro (1936) has been observed with larvae of Nippostrongylus brasiliensis (Sarles, 1938), A. galli (Sadun, 1949), and others. Sadun incubated eggs, larvae, and adult Ascaridia worms separately with immune serum from previously infected chickens and observed the formation of oral precipitates with the larvae

only. He deduced that the larval stage was the active agent involved in A. galli immunity.

In vitro manifestations of immune sera on parasites are reviewed by Weinstein (1967) and Jackson et al. (1970).

Keep in mind that these serological tests did not explicitly attribute immunity to the production of specific antibodies but evaluated the resistant status of the host on the basis of an immune serum. There are several non-antibody components which are important in immunity and represent nonspecific mechanisms of acquired defense such as the interferon and properdin systems (Abramoff and LaVia, 1970; Weiser et al., 1969).

A multitude of antibodies may be produced by parasitic stimulation; however, immunity to the infection is not necessarily mediated by these antibodies. The protective capacity of antibodies in parasitic systems has not extensively been studied. Most antibodies concerned with immunity are of the 7S immunoglobulin class (IgG) (Lee and Lewert, 1960; Kagan et al., 1968). An antiserum fraction containing predominantly $7S_{\gamma_1}$ globulin was found by Wilson (1966) to protect guinea pigs against Dictyocaulus viviparus while the $7S_{\gamma_2}$ fraction completely failed to protect. Similar findings were reported by DiConza (1969) with Hymenolepis nana (tapeworm) in mice; however, the immunoglobulin class was not determined.

Jones et al. (1970) demonstrated protective immunity to N. brasiliensis in rats to involve $7S_{\gamma_2}$ globulins and in 1 of 4 antisera tested, with $7S_{\gamma_2}$ globulins. The protective antibodies in an Ascaridia infection have not been characterized.

Secondary reaction tests may be misleading. Farr (1958) observed that some populations of antibodies had the capacity to form precipitates in the presence of antigen while other populations of antibodies did not.

Thus, the binding of antigen to antibody in the primary reaction may or may not result in a secondary or tertiary reaction. This suggests that a negative secondary manifestion test only indicates that the test used did not detect antibodies to a given antigen and the actual presence or absence of specific antibodies in the system was not determined. Secondary reaction tests do not necessarily measure total antibody content but are useful tools in determining the capacity of the antibody to precipitate antigens or agglutinate antigen-coated particles.

Coombs et al. (1945) developed a sensitive antiglobulin test for detecting functionally "univalent" specific antibodies. It was an indirect measurement of the primary antigen-antibody reaction. Coombs and associates observed that in numerous cases of suspected haemolytic disease of the newborn and of reactions to blood transfusions, that the crucial demonstration of Rh antibody was lacking even in cases that strongly suggested that immunization had taken place. The method they devised showed that red cells sensitized with weak or "incomplete" antibodies were strongly agglutinated when a rabbit anti-human-globulin serum was added. Diamond and Abelson (1945) reported "incomplete" forms of the antibodies anti-C, anti-E, and anti-c while Levine and Gilmore (1945) called attention to an "incomplete" form of the anti-sheep cell agglutinin found in cases of infectious mononucleosis. It would seem that the principle of the test could be applied to a number of antigen-antibody systems where for some reason the complex could not be observed by any other characteristic reaction.

MATERIALS AND METHODS

Experimental Birds

One-day-old Babcock cockerels were obtained from a commercial hatchery and vaccinated intranasally against Newcastle disease.[1] The birds were kept in electric brooders and fed a commercial ration.

Embryonated Ascaridia galli eggs were cultured using modifications of the methods of Hansen et al. (1954, 1956). Female A. galli worms were thoroughly ground using a mortar and pestle. To this mixture was added a warm solution (40°C) of artificial digestive juices (1.0% pepsin and 0.5% HCl), which was stirred every 5 minutes for 15 minutes. The digested contents were poured through an 80-mesh screen into small petri dishes which had been kept moist with egg culture solution (100 drops of 1:1000 dilution merthiolate solution/1000 ml water). The eggs were allowed to settle and adhere to the glass at which time the supernate was decanted carefully. Two additional washings with egg culture fluid removed the artificial digestive juices and any remaining debris. The eggs were covered with egg culture solution and kept at 30°C in a dry air incubator for 14 days and then refrigerated until needed.

The refrigerated eggs were scraped free from the glass with a rubber policeman and the mixture centrifuged 750-800 rpm for 5 minutes. The supernate was replaced with 10 to 15 ml of a 1.25 M sucrose solution to suspend the eggs before pouring into a small desicoated container. Desicoat is a silicone material which prevents the eggs from adhering to surfaces of the glassware. With a micropipetter, 0.1 ml of the egg-sugar

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[1] Live virus, B, Type, Salsbury Laboratories, Charles City, Iowa.

mixture was placed on a glass slide and covered with a cover slip in order to count the number of eggs. The volume of the egg suspension was adjusted with egg culture solution to attain the desirable concentration of 100 ± 10 *A. galli* eggs/0.1 ml. Chickens were infected at 14 days of age with 100 ± 10 larvated eggs by means of a calibrated micropipette inserted into the crop. The birds were marked with a wing band at the time of infection.

The birds were killed 75 days postinfection by cervical dislocation before removing the small intestine. An adaption of the hydraulic method of Ackert and Nolf (1929) was used to flush the worms from the intestine.

Collection of Serum

Approximately 5 ml samples of blood taken from the left wing vein of each bird were allowed to clot at room temperature. The clot was quartered, incubated one hour at 37°C, then refrigerated overnight. The clot was removed and the serum centrifuged for 15 minutes, 2000 rpm, at 4°C, before storing at -25°C. The bleedings were scheduled for various days of the infection with day 0 being preinfection. Serum samples were collected on days 7, 15, 21, 33, 37, and 50 postinfection, respectively, for a total of 42 samples.

Salt Precipitation and Gel Filtration

The lipids in 100 ml normal chicken serum[1/] were removed by centrifugation at 10,000 rpm, 4°C, for 45 minutes. The globulins were precipitated by the drop-wise addition of 25% Na_2SO_4 to a final concentration of 0.18 gm/ml. After stirring at room temperature for 1 hour, the precipitate was recovered by centrifugation (10,000 rpm, 25°C, 15 minutes) and dissolved

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[1/] Industrial Biological Laboratories Inc., Rockville, Maryland.

to 60% the original serum volume in borate saline buffer (BSB), pH 8.0. The mixture was dialyzed overnight at 10°C on a stirring apparatus against BSB. The dialyzate was diluted with BSB to 75% the original serum volume and then the precipitation procedure was repeated to a final concentration of 0.14 gm/ml. After again dialyzing, the mixture was precipitated a third time. The dialyzate was diluted to 50% the original serum volume with BSB and precipitated to a final concentration of 0.14 gm/ml and dialyzed 72 hours at 10°C. Following dialysis, the preparation was cleared by centrifugation (10,000 rpm, 25°C, 40 minutes). The globulin concentration was 16.84 mg/ml as determined by ultraviolet absorption on a Gilford Spectrophotometer 240 at 280 nM. To determine the concentration of a protein solution, the optical density reading is divided by 1.500. This is based on the assumption that a 1% gamma-G solution has a spectrophotometric reading of 15.000 and therefore, a 1 mg/ml protein preparation has an optical density of 1.5000.

The salt-precipitated globulins were separated by Sephadex G-200 filtration with borate-buffered saline in a column 60 x 2.2 cm. A Mariotte flask system was used to maintain a constant column pressure. A 2.0 ml (33.68 mg) aliquot of the globulin preparation was passed through the column and 35-minute fractions (3.4 ml) were collected. The protein concentration of each tube was determined by spectrophotometric readings at 280 nM and the peak gamma-G fractions pooled. Total protein content was calculated to be 23.3 mg (approximately 1 mg/ml). Desirable doses of vaccine require high concentrations of gamma-globulin so a twofold concentration by pervaporation was done, followed by overnight dialysis against BSB at 10°C. The final concentration of the chicken gamma globulin was 2.61 mg/ml.

Preparation of Antisera

Two New Zealand White rabbits each were injected subcutaneously in multiple sites with 4.2 mg chicken gamma globulin emulsified in an equal volume of Freund's complete adjuvant[1/] followed with a second injection of 2.61 mg two weeks later. The quality of a good emulsion was ascertained by allowing a drop to fall onto the surface of water in a beaker and if it remained a droplet the emulsion was satisfactory. Two weeks after the last injection, the rabbits were bled from a marginal ear vein and sera collected and processed as previously described for chicken serum. Subsequent bleedings (about 40 ml/rabbit) were done every five days until enough antisera was collected for later serologic tests.

Qualitative assay of the presence or absence of antibodies for each serum collection was determined by a ring test. Normal chicken serum was layered over an aliquot of each antiserum sample in small precipitin tubes and incubated at room temperature for 15 minutes. A sharp, solid line, where the two sera interacted, indicated a response. Monospecificity was determined by immunodiffusion tests and immunoelectrophoresis. All antisera was pooled and 1.0% sodium azide added to prevent bacterial growth before storing at -25°C.

Preparation of Antigen

Freshly collected adult A. galli males were kept alive at room temperature for 3 hours in several changes of physiologic saline in order to evacuate their intestinal contents (Wharton, 1915). The worms were then washed with tap water until turbidity was no longer apparent, rinsed

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[1/] Difco Laboratories, Detroit, Michigan.

in distilled water, blotted dry, and frozen at -20°C.

The frozen worms were weighed (80.0 mg) and placed in 25 ml trishydrochloride buffer (0.05 M, pH 8.0) and homogenized with a VirTis "23" blender in an ice bath for 30 minutes. This homogenate was stored at -20°C.

Iodination of Antigen

The whole worm antigen was prepared for radioactive labelling by centrifuging the thawed crude homogenate for 10 minutes, 10,000 rpm at 4°C. A spectrophotometric scan of the supernatant between 220 nM and 310 nM resulted in a presumptive nucleic acid peak near 260 nM. Therefore, a sodium sulfate fractionation step was performed to further purify the preparation. To 10 ml crude worm extract was added slowly 2 gm Na_2SO_4 then centrifuged at 3000 rpm, 25°C for 15 minutes. The precipitate was dissolved in 4 ml borate saline buffer (BSB) and dialyzed 1 hour against BSB at 10°C. The dialyzate was centrifuged (2500 rpm, 25°C, 10 minutes), and optical densities of the supernate between 250 and 290 nM were recorded. A protein peak near 280 nM was confirmed. By pervaporation the preparation was concentrated to 1.5 ml then dialyzed overnight in buffered (pH 8.6) 0.02 M glycine. The resulting concentration of the antigen was approximately 1.0 mg/ml.

The McFarlane (1958) iodination technique was used to label the antigen. This technique substitutes 1 to 5 atoms of iodine ortho to the hydroxyl group on the tyrosine ring. The free iodine and tracer iodine are incorporated on the same percentage basis.

Free iodine was liberated from ICl with the addition of 1.0 N HCl and the volume ICl needed for satisfactory incorporation was calculated as:

$$\frac{\text{mg protein}}{\text{mol. wt. protein}} \times \text{no. of atoms per molecule of iodine substitution desired} \times 163 \text{ (mol. wt. ICl)}$$

= ml ICl (0.1 ml ICl : 330 ml 1.0 N HCl)

In this study, 10 ul ICl was used.

One drop of 1 millicurie (mc)/ml radioactive iodine-125[1/] from a Pasteur pipette has 6.7×10^7 DPM (disintegrations per minute). To obtain a final count rate of 2000 counts per minute (cpm) per microgram (ugm) of protein, the number of drops of radioactive iodine was determined as:

$$\text{umg protein} \times 2000 \text{ cpm} \times 10 \text{ (efficiency of scintillation counter)} = \frac{\text{DPM to add}}{6.7 \times 10^7 \text{ DPM/drop}}$$

= no. of drops of radioactive iodine

Two drops were sufficient for this experiment.

The calculated amounts of ICl and ^{125}I (in the form of carrier free NaI), were added to a small beaker containing 0.5 ml glycine buffer (0.2 M, pH 8.6) and an equal volume of 0.1 M NaOH. The NaOH prevented denaturation of the protein in an acidic solution. The radioactive iodine preparation was added dropwise to the protein dialyzate (1.5 ml, 1.0 mg, in 0.2 M glycine buffer, pH 8.6) while mixing constantly for 10 minutes on a magnetic stirrer. The solution was then transferred to dialysis tubing with a Pasteur pipette and dialyzed against 1.5 ml 1.0 M KI in 300 ml BSB at room temperature on a magnetic stirrer for 5 to 6 hours. The 0.005 M KI helps prevent unlabelling of the iodine bound to tyrosine. The buffer was changed and dialysis continued for another 5 to 6 hours. The final change

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[1/] New England Nuclear, Boston, Massachusetts.

used 4 liters of buffer followed by dialysis for 48 hours at 10°C. The dialyzate contained 1 mg protein/1.2 ml; thus the concentration was 0.833 mg/ml. A 10 ul aliquot of the dialyzate in 1.0 ml BSB was counted in a Nuclear Chicago automatic gamma well scintillation counter Model 4230 which resulted in approximately 8000 cpm. To obtain 1000 cpm, a 1:10 dilution of the dialyzate was used throughout the experiment (100 ul labelled antigen in 0.9 ml BSB equal to 83 ugm/ml concentration). The labelled antigen was kept refrigerated in a small test tube covered with parafilm and designated ^{125}I WAg (iodinated worm antigen).

The per cent iodine incorporation in the protein molecule was determined by rinsing the Pasteur pipette used to transfer the labelled antigen to dialysis tubing with 1.0 ml of 4.5 mg/ml bovine gamma globulin (BGG). The BGG served as a protein carrier so that when 1.0 ml of 10% trichloroacetic acid (TCA) was added, all protein was precipitated. The mixture was incubated for 15 minutes at room temperature then centrifuged at 2500 rpm, 4°C for 10 minutes. The supernate was pipetted into a scintillation counter tube and the precipitate was suspended in 2.0 ml 5% TCA and recentrifuged. After pipetting the supernate into a second scintillation counter tube, the precipitate was dissolved in 2.0 ml of 1.0 M NaOH and transferred to a third counter tube. The volumes in each scintillation counter tube were equal for each test throughout the experiment. One minute counts were recorded for each tube and the per cent iodine incorporation was determined by dividing the counts in the precipitate by the total counts in all three tubes. It was found that 37% of the tagged iodine was incorporated into the protein antigen molecule.

Standardization of the Antisera

An experiment was conducted to determine the minimum volume of antiserum required for maximum precipitation of chicken gamma globulins. To six test tubes containing 10 ul normal chicken serum in 0.1 ml BSB was added anti-chicken gamma globulin from 0.1 ml in 0.1 ml increments to 0.6 ml. The tubes were incubated 30 minutes at room temperature and refrigerated overnight. Following centrifugation (2500 rpm, 4°C, 15 minutes), the supernates were removed and discarded and the precipitates were suspended in 1.0 ml BSB and centrifuged again. The washing procedure was repeated twice. The precipitates were subsequently dissolved in 1.0 ml 0.05 M NaOH and optical densities recorded for each tube at 280 nM. Optical densities plotted against the volume of antisera in each test tube revealed a plateau which indicated that maximum precipitation of 10 ul chicken gamma globulin had been reached. The minimum amount of anti-chicken gamma globulin used in this study was 0.6 ml.

Primary Reaction Test

In Experiment 1, antibodies against Ascaridia galli in each chicken serum sample were assayed by adaptions of the indirect Coombs test. Using forty-two 10 x 75 mm test tubes, 10 ul from each of the 42 chicken serum samples was added respectively to 10 ul of 83 ugm/ml ^{125}I WAg in 0.1 ml BSB. Following incubation at room temperature for 15 minutes, 0.6 ml anti-chicken gamma globulin was added to precipitate all chicken 7S immunoglobulins (IgG); thus, labelled antigen bound to antibody would be precipitated. Each stoppered tube was agitated by wrist action then placed in a 37°C water bath for 1 hour and refrigerated overnight. After centrifugation (25000 rpm, 4°C, 15 minutes), the supernates were pipetted

into scintillation counter tubes appropriately designated for each serum sample. The precipitates were suspended in 0.5 ml BSB and agitated on a Vortex Jr. mixer and recentrifuged. The wash step was repeated and the supernates pipetted into the proper counter tube of the first set. The precipitates were dissolved in 0.9 ml 0.05 M NaOH, agitated on a Vortex Jr. mixer, and transferred by pipette to a second set of marked counter tubes and the washing of the precipitates was repeated. The volume in each counter tube was 1.8 ml. Two minute counts were recorded for each tube and the per cent radioactivity in the precipitates calculated.

A TCA control was used to determine the amount of unbound iodine free in the system. In a small test tube the following were added in order: 0.5 ml DEAE-BGG (9mg/ml) in BSB, 0.5 ml distilled water, 10 ul ^{125}I WAg, and 1.0 ml 10% TCA. The mixture was agitated, incubated at room temperature for 30 minutes, and refrigerated overnight. After centrifugation (2500 rpm, 4°C, 15 minutes), the supernate was transferred by pipette to a scintillation counter tube marked S_1. The precipitate was suspended in 1.0 ml 5% TCA, agitated on Vortex Jr. mixer and recentrifuged. The supernate was pipetted into a second counter tube marked S_2 and the wash step was repeated with the third supernate being added to the tube marked S_2. The precipitate was dissolved in 1.0 ml 1.0 M NaOH and pipetted to a counter tube marked P and this step was repeated so that the volume in all tubes was equal. Two minute counts were recorded and per cent radioactivity in the precipitate was 78%. This indicated that there was 22% unbound iodine free in the system which may be a source of error in future experiments. To reduce the free iodine, the ^{125}I WAg was dialyzed against 0.005 M KI in BSB for 48 hours with several changes of buffer. A second TCA was run and the radioactivity in the precipitate was 93.1% which was acceptable. In all

subsequent tests, two TCA controls were included to check the amount of unbound iodine free in the system.

Results from the control sera in Experiment 1 indicated a high per cent radioactivity in the precipitates. Protein in minute quantities, if positively charged, tend to adsorb onto negatively charged glassware; therefore, attempts were made to coat the glassware with egg albumin prior to each test.

In order to determine a satisfactory concentration of egg albumin, five test tubes were coated with 100 ul egg albumin of different concentrations ranging from 1 mg/ml to 5 mg/ml in 1 mg increments. The test was conducted as described in Experiment 1 with a few modifications as follows. The 10 ul chicken sera was added before the 10 ul ^{125}I WAg and an extra wash step was included to obtain a more thorough and complete washing of the precipitate. To keep the volumes in the scintillation counter tubes equal, the amount of 0.05 M NaOH needed to dissolve the precipitate was increased to 2.3 ml. For all subsequent tests, 100 ul of 3 mg/ml egg albumin was used to coat the test tubes.

Based on presumptive evidence from Experiment 1, serum samples from the infected chickens on days 0, 15, 21, and 37 were further studied. As refinements of Experiment 1, the test tubes were coated with egg albumin prior to the primary reaction test, and each serum sample was tested in triplicate. The procedure was modified as described in the egg albumin studies. To obtain more accurate counts per minute in the gamma well scintillator, 20-minute counts were recorded and the per cent radioactivity in the precipitates determined.

RESULTS AND DISCUSSION

Experiment 1 (Table I)

A review of the literature indicated that some secondary and tertiary reactions used in parasitic systems have failed to yield exacting results concerning specific antibodies present. It is possible that specific antibodies were produced but the initial antigen-antibody complexes were not precipitated and negative results were recorded. The primary reaction test was favored over secondary and tertiary type reactions for several reasons. The primary reaction test does not rely on a visual manifestation as do all secondary and tertiary type reactions.

The primary reaction test is designed to measure the binding of a radioactively labelled protein antigen to specific antibody by precipitating the antigen-antibody complexes with an anti-IgG antiserum. The anti-chicken gamma globulin used throughout the experiment was found to be monospecific for IgG as determined by ring tests and would therefore precipitate all 7S gamma globulins in the chicken sera. The 7S fraction includes those IgG antibodies bound to the labelled worm antigen and those unbound in the chicken sera. It follows that the per cent radioactivity in the precipitates corresponds to the labelled antigen bound to specific 7S gamma globulins.

In this experiment all sera from control and infected birds showed an increase in binding capacity for radiolabelled antigen when day 0 is compared with day 21. The greatest per cent change (18.9) was in the infected bird harboring no worms at necropsy while the bird with the most worms at necropsy showed the least per cent change (2.9) in antibody response. The data are only suggestive that an immune response was elicited by *A. galli*. Because

a high per cent (37.4 to 49.5) of radioactive precipitates was recorded on day 0 when no worm antigens were present in control and experimental birds, it was necessary to attempt refinement of the primary reaction test. Factors affecting the results on day 0 are; 1) unbound radioactive iodine free in the system, 2) nonspecific protein binding to free iodine and the complex then combining to negatively charged glassware, and 3) nonspecific protein antigen binding to negatively charged glassware.

A TCA (trichloroacetic acid) test revealed that 22% of the radioactive iodine was unbound. The free iodine would not be precipitated by the antiserum thus producing a high radioactive count in the supernatants. The free iodine may also bind to protein nonspecifically and then bind to negatively charged glassware thereby producing a high radioactive count in the precipitates. In effect, this would overshadow the true per cent antigen binding in the precipiates as precipitate values are determined as a per cent of the total radioactivity in the system. To reduce this source of error, the labelled worm antigen preparation was dialyzed two days in several changes of borate saline buffer (BSB). In all subsequent experiments, a TCA control was included and the per cent of unbound iodine free in the system never exceeded 10%. The effectiveness of the primary reaction test is determined by comparing the degree per cent change on different days of the infection to the values on day 0 which is the control for each experimental bird. Day 0 value should by zero. The closer day 0 values are to zero the more significant will be a 10% change.

Another possible source of error is nonspecific protein binding to negatively charged glassware. Labelled protein worm antigen in microgram quantities may adhere to the sides of the test tubes thus preventing specific antibodies in the chicken serum from combining with those antigen molecules

already bound to the glassware. After the labelled antigen-antibody complexes are precipitated with the addition of anti-chicken gamma globulin, the supernates are removed leaving antigen molecules bound to the glassware. The two washings of the precipitate with BSB do not affect these nonspecifically bound worm antigens. However, when the precipitate is dissolved with 0.05 M NaOH, the nonspecifically glass-bound labelled worm antigen would also be dissolved. Accordingly, the radioactive count in the precipitates would not be an accurate measure of antigen binding to specific IgG in the chicken serum. To eliminate nonspecific binding to the glassware it seemed feasible to coat the test tubes with a non-cross-reacting protein prior to the experiment. Egg albumin was chosen to adsorb the negative charges on the glassware and the optimum concentration for maximum coating was determined as 3 mg/ml.

Experiment 2 (Table II)

Using egg albumin coated tubes and TCA controls, the protocal of Experiment 1 was repeated in triplicate. The "background" count was reduced in all sera. This is significant in evaluating the accuracy and sensitivity of the primary reaction method in the assay of antibodies to a specific nematode antigen. The chicken with the most worms at necropsy (Bird 5) showed the greatest increase in binding capacity for radiolabelled antigen. It is noteworthy that the antibody response in the chicken with the most worms varied directly with time while the chicken with no worms at necropsy (Bird 4) had a peak response on day 21 postinfection followed by a 15.5 per cent drop on day 37.

It could be reasoned that the chicken harboring worms at necropsy continued to form specific antibodies in an attempt to rid itself of

nematodes, whereas, the chicken having no worms at necropsy attested to the success of the immune system in eliminating the nematode. It is possible that tissue phase antigens may play an active part in the success of the immune system in warding off the infection. Soulsby (1963) presented a review of literature concerning antigenic differences in life cycle stages and he emphasized that antigens prepared from one developmental stage may be more valuable in a diagnostic test than those of another stage. This might be related to the "functional" antigen(s) being a metabolite excreted or secreted by the living nematode during a tissue and/or molting stage. If such antibodies were successful in neutralizing the stage-specific antigen(s), the worms would be eliminated and not recovered at necropsy. The data from Bird 4 suggest that this mechanism may have been functioning at day 21.

The Ascaridia galli involvement with the chicken host is not a new host-parasite relationship and it is reasonable to assume that long term parasitism tends to commensalism. Perhaps this would explain why the experiments do not detect sharp changes in antibody response.

Experiment 3

This experiment was designed to obtain more statistically accurate data by increasing the number of birds to 25. The experiment was abandoned when the infected chickens had no worms at necropsy (50 days postinfection). There are several factors to consider when attempting to explain these negative results. There are several reports on the effects of dosage on worm burdens. Most reports indicated an optimum dosage range for maximum worm burden. The results varied with each investigator but most reports showed that tolerance was induced with a dose of larvated A. galli over

500 ± 50. Another influencing factor was reported by Shults and Daugalieva (1968) who found that 1 to 5-day old chickens fed Ascaridia galli eggs developed immunologic tolerance which lasted the entire experiment irrespective of infective dosage.

Egerton and Hansen (1955) reported the distribution of Ascaridia galli recovered at necropsy followed a negative binomial curve. The distribution was characterized by most of the hosts having none or a few worms and only a few hosts having a large number of worms. An immunologic defense mechanism was suspected as being operative in resisting the nematode. The results of this study support the immunological implications reported by Egerton and Hansen (1955). The data suggested that the few birds harboring large number of worms at necropsy may show an increasing antigen binding with duration of the infection which indicates an existing immune response while the majority of chicken hosts may be able to combat immunologically the invading nematodes earlier in the infection, thus, no worms are recovered at necropsy.

The method proposed in this study is a relatively quick and simple technique for an effective measure of antigen binding. This test could be a valuable tool in early diagnosis of an ascarid infection and a projected use for diagnosing other parasitic infections, especially those cases where sacrificing the host is the only reliable diagnostic method for determining worm burden. A purified worm antigen preparation would improve the accuracy of this test as it would help eliminate nonspecific protein binding. The primary reaction test may prove valuable in detecting the antigen or antigens which will elicit functional antibody formation. Passive transfer studies could then determine if the antibody was protective

against reinfection.

A further area of study with the primary reaction test is the possible correlation of day 0 values with the number of worms recovered at necropsy. Tables I and II indicated that the chicken with the most worms at necropsy had the largest per cent radioactivity in the precipitate. If this correlation would hold true on a random sample of a large number of birds, the parasitologist could predict with reasonable accuracy, the worm burden for a particular chicken. For laboratory purposes, the chickens with high per cent values on day 0 (preinoculation) would be chosen if a high burden of worms was desired.

TABLE I

Per Cent Radioactivity in Serum Precipitates Following Primary Reaction Test, Experiment 1

Bird		Days postinfection							Degree change at day 21 (%)	Worms recovered (No.)
		0	7	15	21	33	37	50		
Control	1	39.5	40.0	46.8	51.3	50.5	47.8	50.0	11.8	0
	2	42.4	41.1	51.9	51.5	43.3	40.9	52.6	9.1	0
Infected	3	44.5	46.6	52.9	51.4	59.2	52.1	55.8	6.9	3
	4	37.4	43.0	50.9	56.3	52.8	51.5	52.9	18.9	0
	5	49.5	49.2	53.1	52.4	50.0	50.0	46.5	2.9	7
	6	42.8	43.2	50.0	52.8	53.4	49.2	49.9	10.0	3

TABLE II

Per Cent Radioactivity in Serum Precipitates Following Primary Reaction Test, Experiment 2

Bird		% Reduction radioactive ppts., Exps. 1 vs 2 at day 0	Days postinfection[a]				Degree change at day 21 (%)	Worms recovered (No.)
			0	15	21	37		
Control	1	25.3	14.2 ± 2.36	37.9 ± 5.04	27.3 ± 4.54	41.2 ± 1.39	13.1	0
	2	19.4	23.0 ± 3.22	30.2 ± 2.25	28.0 ± 1.59	32.6 ± 1.78	5.0	0
Infected	3	17.1	27.4 ± 4.46	15.7 ± 2.32	19.7 ± 2.35	42.9 ± 1.77	(-)7.7	3
	4	9.5	27.9 ± .91	32.7 ± 2.07	37.2 ± 1.12	21.7 ± 1.17	9.3	0
	5	19.9	29.6 ± .28	47.2 ± .80	52.3 ± 1.42	53.3 ± 1.86	22.7	7

[a] ± = one standard deviation.

SUMMARY

The immunologic responses of a host to parasitic infections have been observed by many investigators but intricacies of the mechanisms are not well known. A review of the literature revealed that immunobiologic methods involved in parasite systems have not been investigated extensively and efforts have concentrated on developing standard techniques which will yield accurate and repeatable results. Most tests studied thus far rely on visual evidence of a secondary or tertiary type antigen-antibody reaction.

A primary reaction test was chosen for this study because of its sensitivity in measuring microquantities of specific antibody bound to radiolabelled protein antigen. A crude antigen homogenate of adult male Ascaridia galli was labelled with iodine-125 and tested with serum samples from A. galli infected Babcock cockerels at different days of the infection. The radiolabelled worm antigen-antibody complexes were precipitated with an anti-chicken gamma globulin and radioactivity counted in a gamma well scintillator to determine the per cent antigen binding that had occurred. All chickens were sacrificed 50 days postinfection and examined for A. galli.

A high per cent radioactivity in the precipitates was observed in all control chickens and preinoculation sera of the experimental birds in Experiment 1 so attempts were made to reduce this nonspecific precipitation. Microgram quantities of protein worm antigen may adhere to negatively charged glassware until denatured with 0.05 M NaOH resulting in greater radioactivity in the precipitates. Unbound iodine free in the system was 22% as determined by a trichloroacetic acid test. The unbound iodine may nonspecifically bind to protein and/or the glassware; thus, contributing to the high per cent radioactivity in the precipitates. Dialyzing the

unbound iodine free in the system and coating the test tubes with a non-cross-reacting protein (3 mg/ml egg albumin) reduced the per cent values in the precipitates significantly.

Experiment 2 indicated specific antibody formation to occur in chickens infected with 100 ± 10 embryonated A. galli eggs. The infected chicken with the most worms at necropsy showed the greatest antigen binding. The response was characterized by an increasing per cent change in the precipitate with duration of the infection. The infected chicken with no worms at necropsy showed the least per cent change and a peak response on day 21 postinfection.

The primary reaction test is a sensitive and relatively simple technique for measuring antigen binding to specific antibody. The method proposed in this study showed promise as a good clinical indicator of an Ascaridia galli infection and a projected use in diagnosing other parasitic infections. A possible correlation between worm burden 50 days postinfection and preinoculation values was postulated.

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IMMUNITY AND PRIMARY REACTION ASSAY OF ANTIBODIES TO *Ascaridia galli*

by

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The immunologic responses of a host to parasitic infections have been observed by many investigators but intricacies of the mechanisms are not well known. A review of the literature revealed that immunobiologic methods involved in parasite systems have not been extensively investigated and efforts have concentrated on developing standard techniques which will yield accurate and repeatable results. Most tests studied thus far rely on visual evidence of a secondary or tertiary type antigen-antibody reaction.

A primary reaction test was chosen for this study because of its sensitivity in measuring microquantities of specific antibody bound to radiolabelled protein antigen. A crude antigen homogenate of adult male Ascaridia galli was labelled with iodine-125 and tested with sera from A. galli infected Babcock cockerels at different days of the infection. The radiolabelled worm antigen-antibody complexes were precipitated with an anti-chicken gamma globulin and radioactivity counted in a gamma well scintillator to determine the per cent antigen binding that had occurred. All chickens were sacrificed 50 days postinfection and examined for A. galli.

A high per cent radioactivity in the precipitates was observed in all control chickens and preinoculation sera of the experimental birds in Experiment 1 so attempts were made to reduce this nonspecific precipitation. Microgram quantities of protein worm antigen may adhere to negatively charged glassware until denatured with 0.05 M NaOH resulting in greater radioactivity in the precipitates. Unbound iodine free in the system was 22% as determined by a trichloroacetic acid test. The unbound iodine may nonspecifically bind to protein and/or the glassware; thus, contributing to the high per cent radioactivity in the precipitates. Dialyzing the unbound iodine free in the system and coating the test tubes with a non-cross-reacting protein (3 mg/ml egg albumin) reduced the per cent values in the precipitates

significantly.

Experiment 2 indicated specific antibody formation to occur in chickens infected with 100 ± 10 embryonated A. galli eggs. The infected chicken with the most worms at necropsy showed the greatest antigen binding. The response was characterized by an increasing per cent change in the precipitate with duration of the infection. The infected chicken with no worms at necropsy showed the least per cent change and a peak response on day 21 postinfection.

The primary reaction test is a sensitive and relatively simple technique for measuring antigen binding to specific antibody. The method proposed in this study showed promise as a good clinical indicator of an Ascaridia galli infection and a projected use in diagnosing other parasitic infections. A possible correlation between worm burden 50 days postinfection and preinoculation values was postulated.