EXPERIMENTAL STUDIES OF EOSINOPHILIA IN RATS INFECTED WITH TAENIA TAENIAEFORMIS

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ABSTRACT

EXPERIMENTAL STUDIES OF EOSINOPHILIA IN RATS INFECTED WITH *TAENIA TAENIAEFORMIS*

By

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Rats were dosed with eggs of *Taenia taeniaeformis* and hematologic parameters were measured throughout the course of primary infection. There was no evidence of anemia, but differential leukocyte counts revealed distinct and reproducible patterns of white blood cell changes. A lymphocytosis developed at the end of the first and fifth weeks post infection (p.i.). Neutrophil counts peaked 8 days p.i., although at that time there was no marked neutrophilic infiltration of the tissues. Eosinophil counts began to rise during the second week p.i. and reached a peak during the third week, followed by a decline and then another peak during the fifth week p.i. Eosinophilic infiltration of the tissues was remarkable during the period of peripheral eosinophilia. A wide zone of eosinophils surrounded the developing larvae at 22 days p.i. and persisted in some cases for a further 2 weeks. Eosinophils remained in lesser numbers in the connective tissue capsule throughout the infection, often in association with plasma cells.

After oral challenge with 1000 eggs infected rats showed brisk secondary eosinophilic responses 3 to 7 days later but other

hematologic parameters were unaffected. Average peripheral eosinophil counts at 3 and 4 days post challenge were significantly higher than those in unchallenged controls ($P<0.05$ and $P<0.01$, respectively). There was no detectable increase in eosinophilic infiltration of small intestinal tissues in challenged rats.

These results are discussed in relation to current understanding of the mechanisms of eosinophil chemotaxis *in vitro* and the possible causes of local eosinophil accumulation in parasitic infections *in vivo*.

Normal rats were given intravenous doses of either immune serum or immunoglobulin fractions 24 hr before oral challenge with 1000 eggs of *Taenia taeniaeformis*. Total eosinophil counts per cubic millimeter were performed for 3 days prior to and 6 days after challenge. Sensitized rats showed sharp peaks of eosinophilia 2 to 6 hr after this dose. The pattern of eosinophilic response was similar to that which occurs after challenge of immune infected rats. The differences in peripheral eosinophil levels in passively immunized and normal rats were statistically significant. Immunoglobulin fractions containing protective IgG_{2a} were most effective, but a fraction containing reaginic antibody activity also transferred the secondary eosinophilic response. The findings are discussed in relation to the probable contribution of antigen-antibody reactions to the production of secondary eosinophilic responses in experimental cysticercosis.

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Dedicated to my family and to

the spirit of my brother

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INTRODUCTION

Cysticercosis and hydatidosis are cyclozoonotic diseases caused by members of the Order *Cyclophyllidae*, Family *Taeniidae* (Phylum: *Platyhelminthes*, Class: *Cestoda*). They are cosmopolitan in their distribution and have been recognized as disease entities in man and animals for many years. The tapeworms of man, *Taenia saginata* and *Taenia solium*, and their relationship to the larval stages in the musculature of cattle and swine, respectively, were recognized in the 19th century. Present evidence suggests that infection with these two parasites occurs in man and domestic cattle and swine in many countries of the world and that in developing countries infection rates are high and contribute significantly to losses in animal production (Abdussalam, 1974; Soulsby, 1974).

Taeniid metacestodes are not susceptible to any chemotherapeutic agents and surgical removal or excision is required if their locations are identified and accessible. Since there is evidence of acquired immunity in both natural and experimental infections, immunological prophylaxis represents a realistic approach to effective control of cysticercosis.

The experimental model of *Taenia taeniaeformis* infection in the rat has been used in this study to investigate certain pathological and immunological aspects of cysticercosis. The literature review has therefore been assembled to provide a background on clinical and

experimental cysticercosis and to introduce some aspects of the host-parasite relationship which have been experimentally explored in the laboratory.

The first section concerns the biological characteristics of taeniiasis in relation to the clinical and economic importance of the principal zoonotic cysticercoses. The second section refers to pathological and ultrastructural features of infection with *T. taeniaeformis* in the rat. The third section is specifically concerned with the immune response in rodent cysticercosis and the role of the eosinophil leukocyte. A discussion is presented on the relationship of this cell to the immune response in parasitic infections.

In the research reports which follow, an analysis is made of some of the hematological and pathological consequences of primary and secondary exposure to *T. taeniaeformis* in the rat. Experiments are described in which the eosinophilic response to challenge infection is shown to be a consequence of antigen-antibody reactions during larval migration and development.

LITERATURE REVIEW

Cestodes (Phylum: *Platyhelminthes*, Class: *Cestoda*) are common parasites of all vertebrate animals, including man. Members of the Order *Cyclophyllidae*, Family *Taeniidae* have both medical and veterinary importance. In this family the adult forms are found in the intestinal tract of the definitive host and the larval stages occur in various intermediate host tissues.

Biological Aspects of Taeniid Diseases

Taenia saginata Goeze, 1782: Life Cycle and Clinical Importance

Man is the only definitive host for *T. saginata*. The mature worm normally measures 4-10 meters in length and possesses between 1,000 and 2,000 segments. The scolex has four suckers without hooks. The gravid segments each contain about 80,000 eggs and each worm may liberate many segments daily. Longevity of the worm is usually limited only by the death of the host. Cattle are the main intermediate hosts, but buffalo, giraffe and other ruminants may also become infected (Soulsby, 1965).

Cysticercus bovis is the name applied to larvae of *T. saginata* and it is commonly referred to as the "beef bladderworm." It is normally found in skeletal and cardiac muscles but occasionally occurs in viscera and other tissues. McIntosh and Miller (1960) studied the

maturation of the cysticercus and reported that development was complete by 16 weeks of age. The factors influencing cyst distribution in beef carcasses are not fully known, but parasites tend to lodge in muscles of the body which are in constant use, such as the masseters and diaphragm. *Cysticercus bovis* generally begins to degenerate several months after reaching maturity, and many are destroyed by 9-12 months (Soulsby, 1965). However, Froyd (1964) has shown that in some cattle cysticerci remain viable for several years. Man becomes infected by eating raw or uncooked meat containing viable cysticerci.

Infection in man may result in varied clinical manifestations. Pawlowski and Schultz (1972) recently provided a comprehensive review of the clinical significance of taeniiasis and they have listed many symptoms associated with enteric dysfunction (e.g., diarrhea, cramps). However, there are others (pruritus, dizziness) which are probably related to systemic allergic responses. Taeniid proglottids occasionally enter the appendix and reproductive tract and cause local inflammatory responses (Berry and Burrows, 1955; Schacher and Hajj, 1970). Cysticercosis of man, due to *T. saginata*, is still an open question, but many authors have reported that man is susceptible. Abuladze (1964) found nine cysticerci in the heart and one in the meninges of a 40-year-old man in the U.S.S.R. who was infected with *T. saginata*. There are other comparable reports from Chile (Delard *et al.*, 1958), India (Dixon and Lipscomb, 1961), U.S.S.R. (Prokopenko, 1968), Poland (Kalawski and Pawlawski, 1970) and Egypt (Abdou, 1959).

Cysticercosis in cattle does not normally produce clinical signs and antemortem diagnosis is difficult. However, Dewhirst *et al.*

(1960) indicate that hemoglobin levels in the blood of infected animals may decline and Evranova and Mosina (1966) showed that glycogen synthesis is decreased in the liver and skeletal muscles of infected calves.

Taenia solium Linnaeus, 1758: Life Cycle and Clinical Importance

Adults of *T. solium* live only in the small intestine of man. The mature tapeworm is usually 3-5 meters long and each proglottid may contain from 30,000 to 90,000 eggs. The scolex has an armed rostellum and four suckers. Human and porcine cysticercosis are produced by *Cysticercus cellulosae,* the larval form of *T. solium* commonly known as the "pork bladderworm." Infection in pigs is referred to as "pork measles." Infection in man and pigs is acquired by ingestion of eggs, although autoinfection is believed to occur in man following reverse peristalsis. A number of other animals have been mentioned in the literature as intermediate hosts, including ruminants, horses, dogs, bears and monkeys, although the identification of the cysticerci was probably erroneous in many cases (Soulsby, 1965). Cysticerci occur in hogs most frequently in striated muscles, particularly in the tongue and diaphragm, but the heart, abdominal wall, liver, lungs, brain and eye may also be infected (Soulsby, 1965).

The clinical picture associated with the adult tapeworm in man is similar to that seen in *T. saginata*. However, cysticercosis in man results in a serious and sometimes fatal disease. Schenone (1974) reported that the most frequent location sites are the central nervous system and the eye and its appendages, but other organs may be involved less frequently. Larvae which reach the brain develop in

the ventricles and frequently become racemose in character, then being referred to as *Cysticercus racemosus*. When the cysticercus is alive there is little reaction around it, but on its death there is a marked tissue reaction and a great variety of neurological symptoms may develop including epilepsy, incoordination, transient paresis, meningoencephalitis and failing vision (Faust, Russel and Jung, 1970).

In pigs cysticerci rarely cause clinically recognizable illness. However, paralysis of the tongue and epileptic convulsions have been described in infected hogs. In dogs, a rabies-like syndrome may appear in cases of cerebral cysticercosis.

Epidemiology of Cyclozoonoses

Transmission of *T. saginata* from man to cattle may be direct or indirect. Direct transmission is more common in Africa and in Latin America, where raw stools from dry latrines are used as fertilizer, and promiscuous defecation in fields, hedges and bushes is practiced (Abdussalam, 1974). It can occur also when the hands of workers who feed and handle calves are contaminated with eggs (Urquhart, 1961). Surveys in Kenya suggested that the majority of cattle are exposed to infection within a few months of birth (Soulsby, 1974). The indirect method of transmission is much more common, especially in Europe and North America, where it is largely attributed to contaminated sewage. Sewage treatment is nonexistent in many areas, and where it exists it may break down from overloading of sewage works resulting from rapidly increasing urban populations. Bovine infections from contaminated sewage are generally of low density due to the disperson of eggs of the parasite over herbage by irrigation or flooding. Birds (gulls) and insects (*Sarcophagina* and *Chrysomyia*)

have been found to carry eggs of *T. saginata* and spread the infection (Silverman and Griffiths, 1955; Round, 1961).

Consumption of raw or undercooked beef depends on complex behavioral factors such as individual and group food habits, dietary and religious beliefs and reituals. Various undercooked meat dishes have been associated with taeniid infections such as 'Shashlik' in the U.S.S.R., 'Tikka' in India and Pakistan, 'Pasterma' and 'Kebab' in Egypt, Turkey and the Middle East, and 'Larb' in Thailand. Undercooked steaks or roasts are consumed under different names in Europe, the Middle East and the Americas.

Many studies on the viability of taeniid eggs have been carried out. Silverman (1955b) indicated that eggs do not survive longer than 14 days in the absence of surface moisture and this is the most important factor controlling the survival of eggs of *T. saginata*. He pointed out that eggs of *T. saginata* can survive for 335 days at 4-5°C. The eggs will survive when kept in saline at room temperature for 60 days. They are inactivated after 10 min exposure to 59°C. Under natural conditions they are long-lived and in Denmark eggs were found to survive for up to 159 days on grass (Jepsen and Roth, 1952), but in the highlands of Kenya eggs survived for at least a year (Duthy and Van Someren, 1948).

Transmission of *T. solium* from man to swine is largely limited to the direct infection process. Infection in hogs is usually due to unhygienic human practices, such as defecation in pig pens or grazing areas, or the spreading of human feces from dry latrines onto pig pastures.

Epidemiological data on the prevalence of human taeniiasis (*T. saginata* and *T. solium*) and cysticercosis in various parts of the world are grossly incomplete. In 1947, Stoll published his estimations regarding the prevalence of human taeniiasis. He calculated that some 49 million persons were infected with *T. saginata* and 2.5 million with *T. solium*. Unfortunately, there are not enough statistical data at the moment to explore the prevalence of cysticercosis and taeniiasis infection in most of the world. However, it is known that after the Second World War there was a steady increase in the prevalence of tapeworms and cysticercosis infection in man and domestic animals, respectively, in Europe (Pawlowski and Schultz, 1972). Human infections with *T. saginata* generally do not exceed 0.5% of the population and in western Europe the prevalence is much lower. Endemic foci of infection occur in certain areas in eastern Europe, Poland, U.S.S.R. and Yugoslavia (Pawlowski and Schultz, 1972; Soulsby, 1974). In the majority of European countries, despite improvements in meat inspection services, prevalence rates of bovine cysticercosis are increasing.

Several epizootics of bovine cysticercosis in the United States have been attributed to a variety of poor sanitary practices, especially in southwestern feed lots (Miller, 1956; Slonka *et al.*, 1975).

Economic Significance of Cysticercosis

It is difficult to assess the economic importance of taeniiasis and cysticercosis in man due to the immense variety of clinical manifestations and the lack of specific data on the direct and indirect

costs incurred as a result of these diseases. However, financial losses caused by condemnation of animal carcasses can be carefully quantitated and reflect the public health significance of taeniid infections. For example, Pawlowski and Schultz (1972) have concluded that in developing countries cysticercosis results in a loss of $25 per animal, while in industrialized countries the loss is of the order of $75 per animal. They estimated that cysticercosis causes losses of at least $176 million/year to the South American meat industry. Added to this are the costs of treatment of infected patients and the control programs which are necessary in endemic areas.

Infection with *Taenia taeniaeformis* (Batsch, 1786) Wolffhugel, 1911 in the Rat

Biological Characteristics

Extensive experimental study of the complex disease entities which occur in man and domestic animals infected with taeniid parasites cannot be justified at this time. There is insufficient basic information on host-parasite relationships in these infections to warrant undertaking such expensive research. Fortunately, however, there exist several experimental models for cysticercosis which can be used for laboratory research purposes. Infection with *T. taeniaeformis* in the rat provides a particularly suitable system for work on the immunology and pathology of cysticercosis.

Adult *T. taeniaeformis* occur in the small intestine of the cat, and the larval form, known as *Cysticercus fasciolaris*, develops in the liver of rats and other small rodents. Rodents become infected by ingesting the eggs which then hatch in the small intestine. The

released oncosphere penetrates the villus within 15 min (Banerjee and Singh, 1969), and the embryo migrates via intestinal venules to the portal circulation and is carried to the liver parenchyma. The larvae continue development until the parasite reaches a fully infective metacestode stage by 60 days after infection (Hutchison, 1958). After oral consumption of the mature *C. fasciolaris* by the definitive host, the scolex attaches to the mucous membrane of the intestine and the tapeworm reaches the adult form in about 6 weeks.

Histopathological Changes in Rodent Cysticercosis

The development of the cysticerci in rodent livers has been studied by Leuckart (1886), Young (1908), Lewert and Lee (1955), Hutchison (1958), Singh and Rao (1967) and Banerjee and Singh (1969). Histopathological and histochemical studies have been described by several workers and much attention has been given to the histogenesis of the "cysticercus sarcoma" (Curtis, Dunning and Bullock, 1933, 1934a and 1934b; Dunning and Curtis, 1946; Wantland, 1953; Lewert and Lee, 1955, 1956; Sweatman and Plummer, 1957; Banerjee and Singh, 1969). However, there has been little study of the host response and the inflammatory reaction surrounding the cysticercus at various developmental stages.

Silverman and Maneely (1955) suggested that penetration of the duodenal wall is accomplished by the combined action of hooks and penetration glands. The hooks are used for initial attachment to the mucosa and for movement through the tissue after cytolysis, caused by the secretions of the penetration glands, has occurred. There have been no studies of histopathological changes in the intestine of rats

during penetration and migration of embryos of *T. taeniaeformis*. However, Barker (1970) concluded that no detectable changes occurred in the intestinal mucosae of rabbits following penetration by oncospheres of *T. pisiformis*. He felt that movement was achieved through the intercellular spaces and that cytolysis was not necessary for penetration and migration.

Pathological responses to early post-embryonic stages in the rat liver have been described by Singh and Rao (1967). They reported evidence of hemorrhage in the vicinity of the embryo by 24 hours after infection and observed extensive hemorrhagic tracts developing between 96 and 120 hours. Similar hemorrhagic tracts have been seen by other workers in experimental infections with *T. pisiformis* and *T. hydatigena* in rabbits and lambs, respectively (Sweatman and Plummer, 1957).

The subsequent development of *C. fasciolaris* has been detailed by Hutchison (1958). He found that growth proceeded rapidly until the 40th day of infection, then slowed and ceased altogether by 22 weeks. Lewert and Lee (1956) attributed the rapid growth to production of a collagenase-like enzyme which facilitated the absorption of nutrients by these early developmental stages. Later, enzyme production dropped and this was considered likely to result in slowing of the growth rate.

Singh and Rao (1967) reported that multiple hemorrhagic tracts developed during this rapid growth phase which caused disruption of the liver architecture and extensive necrosis. They referred to the developing host connective tissue capsules as "spindle cell sarcomas" and felt that these were neoplastic masses. Thereafter, infiltration

by mononuclear and eosinophilic cells was said to increase, though the pattern and sequence of cell infiltration was not described in any detail. They also observed extensive proliferation of the bile ducts by the 55th day of infection. Varute (1971) showed that mast cells were irregularly distributed in the cyst wall of *C. fasciolaris*.

An interesting sequel to the pathologic changes caused by *C. fasciolaris* is the high frequency of hepatic sarcomas in chronically infected rats. This was described 50 years ago by Bullock and Curtis (1924a,b; 1925a,b; 1926, 1928, 1930), and Dunning and Curtis (1946) demonstrated that an active agent is present in washed, freshly ground *T. taeniaeformis* larvae which is capable of initiating multiple peritoneal sarcomas when injected intraperitoneally in rats.

Clinical Pathology of Rodent Cysticercosis

There is very little information available on clinical pathology in rodents infected with *T. taeniaeformis*. Miller and Dawley (1928) studied some erythrocyte parameters in infected rats but only 2 rats were included in each group and it is difficult to assess the significance of the decline in erythrocyte counts which they reported. They recorded a peak in peripheral eosinophil counts from 16-25 days after egg dosing in rats, although not all their animals became infected. Control animals were not included in their first experiment, although in another experiment there was a single control animal which was found to be infected at autopsy. Freeman (1964) studied the hematological responses in mice and guinea pigs after either oral or intraperitoneal inoculation with *Taenia crassiceps*. He observed marked eosinophilia following intraperitoneal injection of these parasites and felt that there was a relationship between the degree of

eosinophilia and resistance. His data on animals infected orally are difficult to interpret since few became infected and control animals were not included. Eosinophilia occurs in human cysticercosis but the pattern of these responses has not been well studied (Dixon and Hargreaves, 1944).

Ultrastructural Characteristics of Rodent Cysticercosis

Microscopic studies of the structure of many taeniid metacestodes have been published (Moniez, 1880; Leuckart, 1886; Young, 1908; Crusz, 1948) and, more recently, the electron microscope has been used to clarify ultrastructural characteristics (Siddiqui, 1963; Voge, 1963; Nieland and Weinbach, 1968; Jha and Smyth, 1969; Bortoletti and Ferretti, 1971). Cysticerci typically consist of a fluid-filled bladder, the external surface of which is referred to as a tegument. It is this surface which contacts the host tissue, and it has been known for many years that it bears multiple hair-like processes, now characterized by electron microscopy as microvilli or microtrichs (Crusz, 1948; Siddiqui, 1963; Jha and Smyth, 1969). Similarities between the mucosal surfaces of mammalian intestinal epithelial cells and the tegumental cells of taeniid larvae have been suggested (Jha and Smyth, 1969). Both probably serve to increase absorptive surface areas although no workers have investigated this phenomenon experimentally in *T. taeniaeformis* infections.

Electron microscopy observations on *T. taeniaeformis* (Voge, 1963; Nieland and Weinbach, 1968) have shown that it shares many common ultrastructural characteristics with other cestodes and bears readily demonstrable microtrichs on the outer surface. These develop

as early as 14 days after infection (Bortoletti and Ferretti, 1971). Little information is available on the ultrastructure of the developing host tissue response, although Byram (1974) recently described inflammatory cells of all types in the connective tissue capsule. He feels that the primary contact between host and parasite is made by highly modified macrophages lining the inner capsule wall, though his criteria for identification of these cells are not clear at this time.

Immunity in Rodent Cysticercosis and the Possible Role of the Eosinophil Leukocyte

Evidence for Acquired Immunity in Cysticercosis

There is a considerable body of evidence, derived from both field and laboratory studies, for the development of a high degree of acquired resistance to cysticercosis. Observations made by several groups of research workers in Australasia indicate that sheep become immune to infection with *T. hydatigena* and *T. ovis* and that this immunity is transferred from ewes to lambs (Blundell-Hasell, Gemmell and Macnamara, 1968; Rickard and Bell, 1971a). Some success has now been achieved in efforts to vaccinate domestic animals against cysticercosis using antigens of the embryonic and post-embryonic stages (Gemmell, 1969; Rickard and Bell, 1971a,b).

Comprehensive reviews of evidence for immunity in experimental cysticercosis in laboratory animals have been prepared by Leid (1973) and Musoke (1975). It has been known for many years that rats and rabbits become highly resistant to infection with *T. taeniaeformis* and *T. pisiformis*, respectively. This protective response was shown by

Miller and Gardiner (1932) and Campbell (1938a) to be antibody mediated and both experimental and natural passive immunization have been demonstrated (Miller, 1931; Campbell, 1938a). Comparable results have been achieved in sheep by Blundell-Hasell, Gemmell and Macnamara (1968), who were able to transfer immunity to *T. hydatigena* with serum from immunized lambs.

Characterization of the Immune Response to *T. taeniaeformis*

A thorough investigation of the characteristics of the protective antibody response in experimental cysticercosis in the rat has recently been pursued in our laboratory (Leid and Williams, 1974a,b; Musoke and Williams, 1975a,b; Musoke, Williams, Leid and Williams, 1975). It appears that the primary protective antibody is in the IgG_{2a} immunoglobulin class and that as infection proceeds protective antibodies in other immunoglobulin classes appear. However, the principal activity is constantly associated with IgG_{2a} and these antibodies act on the invading parasite in a complement dependent manner. There is also a marked antibody response of the IgE or reagin type, though the role which these antibodies play in protection is not clear at this time. Both IgG_{2a} and IgE antibodies in the rat are known to sensitize mast cells and cause the liberation of vasoactive substances. This is a mechanism whereby immediate allergic responses are mediated in rats and other animals. It is not known whether comparable allergic events occur in the protective response to *T. taeniaeformis*, although it seems likely that some cellular processes follow interaction between parasite antigens and these antibodies.

Immediate allergic reactions characterize cysticercosis in man and domestic animals but the significance of these events at the local tissue level is difficult to assess. Nothing is currently known regarding possible cell-mediated attack on invading metacestodes but the characteristic involvement of eosinophils in allergic reactions and parasitic diseases points to their having some role in immunity in this infection.

The Eosinophil Leukocyte-Morphology and Ultrastructure

The eosinophil has a long history of association with helminth infections and allergic disorders. Wharton Jones (1846) gave the first recognizable description of an eosinophil leukocyte which he called the "coarse granular cell" of blood. Paul Ehrlich established in 1879--in his "Farbenanalytischen Untersuchungen"--a full description of the "acedophil leukocyte" as a result of the discovery that these cells had great avidity for acidic dyes. Since then, innumerable data on the occurrence of eosinophils have been collected. As early as 1914 Emil Schwarz provided a bibliography of 2758 references on the eosinophil. Yet while Wharton, Ehrlich, Schwarz and others defined the occurrence of eosinophils under various clinical and experimental conditions, they added little to our knowledge of their function. The association of eosinophilia with a variety of unrelated disorders is still not well understood.

The eosinophils of all mammals are similar, although differences occur in the size, shape and number of granules. In morphological character, except for the granules, the eosinophil appears similar to the neutrophil leukocyte. The nuclei of the two cell lines are

virtually indistinguishable, though the eosinophil nucleus tends to have fewer lobes (Zucker-Franklin, 1968; Basten, Boyer and Beeson, 1970). However, the two cells behave differently in response to infection, antigenic challenge, and adrenal corticosteroid administration. Eosinophils show a marked tendency to accumulate beneath surfaces exposed to the environment such as the skin, bronchi and gut.

Archer (1960; 1963) described the eosinophil as having a bilobed nucleus and a cytoplasm filled with spherical granules which were biconcave discs surrounded by a membrane and containing a dense crystalline core. Very similar descriptions have been presented for human eosinophils (Miller, DeHarven and Palade, 1966; Zucker-Franklin, 1968, 1971) and those of many animal species (Vercauteren, 1951, 1953, 1955; Archer, 1960; Archer and Hirsch, 1963a,b; Zucker-Franklin and Hirsch, 1964; Cotran and Litt, 1969). The eosinophil granules of rats are similar to those in human eosinophils, but the nucleus is generally elongated and coiled to form a U-shaped or ring-like pattern. The granules of mature eosinophils have been shown to be of a single type with a crystalline structure surrounded by a homogeneous matrix. These elongated crystalloids in mature granules are derived by transition from larger, spherical, homogeneous granules that are elaborated at an earlier stage of eosinophil development (Archer and Hirsch, 1963a,b; Scott and Horn, 1970). Miller, DeHarven and Palade (1966) described the eosinophil granules of the rat as elliptical or, more rarely, circular structures which measure $\sim$0.3 to 1.2 μ in diameter. They are limited by a well-defined unit membrane, $\sim$95 Å thick, and contain an amorphous or finely granular

matrix. Embedded in this matrix is a dense core or "internum" which consists either of parallel bands or a square lattice structure.

The isolated granules can be disrupted by freezing and thawing, by exposure to weak acid, or by treatment with surface active agents. Archer and Hirsch (1963a,b) and Hirsch (1965) indicate that large amounts of proteins are liberated from disrupted granules. Qualitative studies reveal the presence of various enzymes including B-glucuronidase (Vercauteren, 1953; Archer and Hirsch, 1963a), aryl sulfatase (Tanaka, Valentine and Fredericks, 1962; Archer and Hirsch, 1963a), alkaline phosphatase (Vercauteren, 1951, 1955) and peroxidase (Vercauteren, 1951, 1955; Gross, 1962; Archer and Hirsch, 1963a; Miller, DeHarven and Palade, 1966). Cotran and Litt (1969) showed that peroxidase was limited to the matrix of the granule. This is in agreement with other experiments, which showed that peroxidase is present only in the external matrix of the granules of rabbits (Bainton and Farquhar, 1967), humans (Enomoto and Kitani, 1966), and rats (Kelenyi, Zombai and Nemeth, 1965; Yamada and Yamauchi, 1966). The overall impression from all these histochemical studies is that the noncrystalloid matrix is an enzymatic storehouse.

Eosinophil granules differ from those of neutrophils in their high content of peroxidase and the absence of lysozyme and phagocytin (Archer and Hirsch, 1963b). Zucker-Franklin (1971) observed that in contrast to the specific granules of mature neutrophils, which are believed to be devoid of peroxidase, the granule of mature eosinophils is particularly rich in this enzyme. Gedigk and Gross (1962) discussed the component of the granule which reacted with acidic dyes, and

concluded that the eosinophilic properties are dependent primarily on the presence of basic amino acids (e.g., arginine).

Physiological Characteristics

Eosinophils are produced in the bone marrow from a primitive stem cell or myeloblast which differentiates to a promyelocyte. This cell contains reddish azurophilic cytoplasmic granules, and differentiates further into myelocytes of the neutrophil, eosinophil or basophil series. Precursors of eosinophils are therefore recognizable as eosinophilic myelocytes, metamyelocytes and band forms. The cytoplasm of the myelocyte eosinophil is basophilic and the granules are distinctly orange. The metamyelocyte eosinophil is no longer capable of division. The nucleus is kidney bean shaped, the cytoplasm is pale blue as in the mature cell of the series, and there are eosinophilic granules. The band form has a non-segmented nucleus, which becomes segmented into 2 or 3 lobes on maturation in most species other than the rat or mouse.

The hypothesis proposed by Ehrlich in 1879 that the bone marrow serves as the only source of eosinophils has since been confirmed by many other investigators (e.g., Homma, 1921; Biggart, 1932). Spry (1971a,b) suggested that in normal rats eosinophils matured without further division in the spleen after leaving the bone marrow. This process of maturation was shown to take approximately 3-6 days (Hudson, Chin and Moffat, 1972). Quantitative studies on eosinopoiesis by Hudson (1968) suggest that for each circulating blood eosinophil in the normal animal, there is a marrow reserve of 300 juvenile (band) and mature cells in 100 to 300 tissue eosinophils. However, the

eosinophil does not seem to be a true "blood cell"; its life in the circulating blood is very short.

Tissue distribution of eosinophils has been studied by Biggart (1932), Godlowski (1952, 1953), Vaughn (1953), and Samter (1965). Many of the cells accumulate in the lung, lamina propria of the gut, and spleen. After circulating in the blood they apparently enter tissues in a random manner (Basten, Boyer and Beeson, 1970). Their life span from the time of bone marrow release to their destruction or loss from mucosal surfaces is calculated to range from 8-15 days.

Osgood (1937) and Archer (1963) hypothesized that eosinophils enter the tissues from the circulation and disintegrate there. Intravascular lysis of eosinophils has not been demonstrated but has only been implied from other observations (Padawer and Gordon, 1952a,b). Thevathasan and Gordon (1958) have described degenerating eosinophils in the circulating blood of rats, but this observation has not been made in other species. The fate of the eosinophil in tissue is therefore not clear. Some loss from mucous surfaces is generally accepted because there is evidence that they may lose their granules and perhaps become phagocytized by other cells of the reticuloendothelial system (Speirs, 1958a,b).

Eosinophils in man normally constitute 4% of the circulating leukocytes. This represents 50-400 (ave. 200)/cu.mm (Sunderman and Boerner, 1949). In domestic animals the figures are usually higher, ranging from 3-9% (375-700 cu.mm) (Schalm, Jain and Carrol, 1975), but there is some variation with age, sex, and strain or breed of animal. In rats (Sprague-Dawley) Schalm *et al.* (1975) give a range from 0.3-1.9% (22-152 cu.mm). Eosinophil counts as high as 4000

cu.mm have been observed in parasitic infections in the rat (Despommier, Weisbroth and Fass, 1974) and comparable levels have been recorded in other animals.

Eosinophils are capable of locomotion and phagocytosis. Most observers have stated that their movement is rather sluggish (Hirsch and Cohn, 1960), although it has been reported that they are active in phagocytosis. They have been reported to phagocytize both gram-negative and gram-positive bacteria, fungi and antigen-antibody (Ag-Ab) complexes, among other things (Sabesin, 1963; Zucker-Franklin and Hirsch, 1964; Litt, 1964a). The eosinophilic granules adjacent to the particles being engulfed are disrupted with discharge of the contents into or alongside the phagocytic vacuoles (Archer and Hirsch, 1963b). In the event that the particle is too large for complete engulfment, eosinophils appear capable of pinching off fragments (Litt, 1963, 1964a,b).

Antigen-antibody complexes are particularly effective in attracting eosinophils from the circulation *in vivo* and the cells migrate into the tissue through intercellular spaces of the endothelial wall (Marchesi and Florey, 1960). Specific antisera to particulate antigens have been shown to enhance the extent of phagocytosis and Archer and Hirsch (1963b) indicated that specific immune serum was required for phagocytosis of erythrocytes and zymosan particles by horse eosinophils. Ishikawa, Yu and Arbesman (1972) demonstrated that the phagocytic activity of eosinophils for *Candida albicans* parallels serum antibody IgG titers in human blood. Eosinophils have been seen to engulf sensitized foreign particles either in the circulation or in the tissues.

Eosinophilia in Allergic and Parasitic Diseases

The term "eosinophilia" is usually accompanied by a descriptive word indicating the location of the eosinophil increase, e.g., peripheral blood eosinophilia, tissue eosinophilia, peritoneal eosinophilia. Conditions associated with an increase of eosinophils in man and animals are numerous and include allergies, parasitic infections, chronic skin diseases (Miale, 1967) and some types of lymphatic tumors (Archer, 1963). Eosinophilic leukemia has been reported, but is extremely difficult to distinguish from other hypereosinophilic syndromes (Bentley, Reardon, Knoedler and Krivit, 1961; Ackerman, 1964). The degree of eosinophilia in response to parasites is related to many of the same conditions upon which the immune response is dependent, i.e., nature of the parasite (antigen), location in the host and the intensity (dose) of the infection (Donohugh, 1966; Basten, Boyer and Beeson, 1970). Parasites which migrate or reside in the tissues cause a more extensive eosinophilia than those which reside in the lumen of the intestinal tract. This appears to be related to the degree of cellular inflammation which accompanies the parasitic infection (Walls and Beeson, 1972).

Foreign proteins have been known for quite some time to cause circulating and tissue eosinophilia. Little or no eosinophil accumulation results from the initial protein contact, although eosinophilia of the draining lymph node is seen when protein is injected into the footpads of guinea pigs. Subsequent injection of homologous protein produces marked eosinophilia at the site of the injection (Litt, 1962; 1963; 1964a,b). Tissue eosinophilia also appears in the retest

reaction at sites of delayed hypersensitivity responses (Arnason and Waksman, 1963). However, antibody appears to be the prime determinant of eosinophilia and the type of antibody formed is very important. Litt (1968) and Kay (1970) have found eosinophilia correlated with 7 S γ_1 antibody (responsible for cutaneous anaphylaxis [PCA]) rather than 7 S γ_2 in the guinea pig. In humans and in rats IgE but not rat IgM or IgA are apparently most responsible for eosinophilia (Zolov and Levine, 1969). Litt (1961) demonstrated that the eosinophilotactic stimulus was the Ag-Ab complex rather than antigen alone, and this led Litt (1961) and Parish (1970) to conclude that eosinophilia is a consequence of Ag-Ab interaction and that the eosinophil is not involved in Ab production. Skin sensitizing antibodies, able to mediate PCA responses, are a particularly prominent feature of the immune response to tissue helminth infections.

Histamine is liberated from sensitized mast cells during Ag-Ab reactions *in vivo* and is responsible for many of the symptoms of the immediate type hypersensitivity reaction. It has been associated with an increase of eosinophils and differing viewpoints about this association have abounded for many years. Histamine does not appear to be directly chemotactic for eosinophils (Parish, 1970) and the current view is that other substances released along with histamine are the chemotactic agents for this cell. These factors have been partially characterized as eosinophilotactic factor of anaphylaxis (ECF-A) (Kay, Stechschulte and Austen, 1971; Wasserman, Goetzl and Austen, 1974), and eosinophilotactic factor of complement (ECF-C) (ward, 1969; Kay, 1970; Lachmann, Kay and Thompson, 1970; Kay, Stechschulte and Austen, 1971).

Kay, Stechschulte and Austen (1971) have isolated ECF-A from sensitized guinea pig lung following specific antigen challenge. The conditions for its release appear identical to those required for the release of histamine and slow reacting substance of anaphylaxis (SRS-A). Boyden (1962), Ward (1969), Lachmann, Kay and Thompson (1970) studied eosinophil chemotaxis *in vitro* using micropore filters (Boyden chamber). Their work indicated that Ag-Ab complexes attracted eosinophils by activating complement and the trimolecular complex of complement C_{567} appears to be responsible. Another complement component generated by immune complexes is C5A, which is specifically chemotactic for eosinophils and is now known as ECF-C. It is not clear, however, if such factors are generated during parasite infections and in the absence of experimental evidence associations between these immunologically mediated mechanisms and the occurrence of eosinophilia in parasitic diseases must be largely speculative.

In one of the few attempts to determine the mechanism of eosinophilia in a parasitic infection, Basten and Beeson (1970) and Basten, Boyer and Beeson (1970) showed that the eosinophil response of the rat to *Trichinella spiralis* was dependent on thymus derived lymphocytes. They were able to show that secondary type eosinophil responses occurred on challenge of immune rats, and that this type of response could be transferred to normal rats using lymphocytes. Evidence for the involvement of lymphocytes in the development of eosinophilia has also been presented by Cohen and Ward (1971). It is therefore clear that immunological mechanisms which are cell dependent can lead to the production of factors attracting eosinophils.

A great deal more work will be required in order to develop a dynamic account of the occurrence of eosinophil responses in helminth infections. Until that time the role of this cell in parasitic diseases will remain obscure.

REFERENCES

REFERENCES

Abdou, A. E. H. 1959. Cysticercosis - A public health problem in Egypt. J. Egypt. Pub. Health Ass. 34: 205-215.

Abdussalam, M. 1974. The problem of taeniiasis-cysticercosis. *World Health Organization*. Panel on the health and economic importance of cysticercosis to the countries. 7th Inter-American meeting at the ministerial level, Foot and Mouth Disease and Zoonoses Control.

Abuladze, K. I. 1964. [*Principles of Cestology*, Vol. IV, *Taeniata*.] Izd. Nauka, Moscow.

Ackerman, G. A. 1964. Eosinophilic leukemia. Blood 24: 372-388.

Archer, G. T. and Hirsch, J. G. 1963a. Isolation of granules from eosinophil leukocytes and study of their enzyme content. J. Exp. Med. 118: 277-286.

Archer, G. T. and Hirsch, J. G. 1963b. Motion picture studies on degranulation of horse eosinophils during phagocytosis. J. Exp. Med. 118: 287-294.

Archer, R. K. 1960. The biology of the eosinophil leucocyte. Vet. Rec. 72: 155-158.

Archer, R. K. 1963. *The Eosinophil Leucocytes*. Blackwell Scientific Publications, Oxford.

Arnason, B. G. and Waksman, B. H. 1963. The retest reaction in delayed sensitivity. Lab. Invest. 12: 737-747.

Bainton, D. F. and Farquhar, M. G. 1967. Segregation and packing of granule enzymes in eosinophil. J. Cell. Biol. 35 Abt. Seventh Annual Meeting of the American Society for Cell Biology: A6.

Banerjee, D. and Singh, S. K. 1969. Studies on *Cysticercus fasciolaris* 1. Studies on the early stages of infection in cysticerciasis in rats. Indian J. Anim. Sci. 39: 149-154.

Barker, I. K. 1970. The penetration of oncospheres of *T. pisiformis* into the intestine of the rabbit. Can. J. Zoology 48: 1329-1332.

Basten, A. and Beeson, P. B. 1970. Mechanism of eosinophilia. II. Role of the lymphocyte. J. Exp. Med. 131: 1288-1305.

Basten, A., Boyer, M. H. and Beeson, P. B. 1970. Mechanism of eosinophilia: 1. Factors affecting the eosinophil response of rats to *Trichinella spiralis*. J. Exp. Med. 131: 1271-1287.

Bentley, H. P., Reardon, A., Knoedler, J. P. and Krivit, W. 1961. Eosinophilic leukemia. American J. Med. 30: 310-322.

Berry, L. R. and Burrows, R. B. 1955. Appendicitis with cestodes. Arch. Path. 59: 587-593.

Biggart, J. H. 1932. Some observations on the eosinophile cell. J. Path. Bact. 35: 799-816.

Blundell-Hasell, S. K., Gemmell, M. A. and Macnamara, F. N. 1968. Immunological responses of the mammalian host against tapeworm infections. VI. Demonstration of humoral immunity in sheep by the activated embryos of *Taenia hydatigena* and *T. ovis*. Exptl. Parasit. 23: 78-82.

Bortoletti, G. and Ferretti, G. 1971. Observations on the ultrastructure of the tegument in the larval forms of *Hydatigera* (=Taenia) *taeniaeformis* and considerations on the development of the cyclophyllidean cestode larvae. Rivista di Parasitologia XXXII: 249-271.

Boyden, S. 1962. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J. Exp. Med. 115: 453-466.

Bullock, F. D. and Curtis, M. R. 1924a. Cysticercus sarcoma. J. Cancer Res. 8: 152-155.

Bullock, F. D. and Curtis, M. R. 1924b. A study of the reactions of tissues of the rat liver to the larvae of *Taenia crassicollis* and the histogenesis of cysticercus sarcoma. J. Cancer Res. 8: 446-481.

Bullock, F. D. and Curtis, M. R. 1925a. Types of cysticercus tumors. J. Cancer Res. 9: 425-443.

Bullock, F. D. and Curtis, M. R. 1925b. On the transplantability of the larvae of *Taenia crassicollis* and the probable role of the liver in cysticercus disease of rats. J. Cancer Res. 9: 444-452.

Bullock, F. D. and Curtis, M. R. 1926. Further studies on the transplantation of the larvae of *Taenia crassicollis* and the experimental production of subcutaneous cysticercus sarcomata. J. Cancer Res. 10: 393-421.

Bullock, F. D. and Curtis, M. R. 1928. A cysticercus carcino-osteochondrosarcoma of the rat liver with multiple cysticercus sarcomata. J. Cancer Res. 12: 326-334.

Bullock, F. D. and Curtis, M. R. 1930. Spontaneous tumors of the rat. J. Cancer Res. 14: 1-115.

Byram, J. E. 1974. Rodent cysticercosis: morphology of the capsule. 49th Annual Meeting, The American Society of Parasitologists. Kansas City, Missouri. (Abstracts) 18: 23.

Campbell, D. H. 1938a. The specific protective property of serum from rats infected with *Cysticercus crassicollis*. J. Immunol. 35: 195-204.

Cohen, S. and Ward, P. A. 1971. *In vitro* and *in vivo* activity of a lymphocyte and immune complex-dependent chemotactic factor for eosinophils. J. Exp. Med. 122: 133-146.

Cotran, R. S. and Litt, M. 1969. The entry of granule-associated peroxidase into the phagocytic vacuoles of eosinophils. J. Exp. Med. 129: 1291-1299.

Crusz, H. 1948. Further studies on the development of *Cysticercus fasciolaris* and *Cysticercus pisiformis*, with special reference to the growth and sclerotization of the rostellar hooks. J. Helminth. 12: 179-198.

Curtis, M. R., Dunning, W. F. and Bullock, F. D. 1933. Genetic factors in relation to the etiology of malignant tumors. Amer. J. Cancer 17: 894-923.

Curtis, M. R., Dunning, W. F. and Bullock, F. D. 1934a. Duration and extent of irritation versus genetic constitution in the etiology of malignant tumors. Amer. J. Cancer 21: 554-570.

Curtis, M. R., Dunning, W. F. and Bullock, F. D. 1934b. Further evidence in support of the somatic mutation hypothesis of the origin of malignancy. Amer. J. Cancer 21: 86-98.

Delard, G., *et al*. 1958. Estudio epidemiologico/parasitologico de la communidad de Schwager. Revista del Servicio Nacional de salud Santiago 3: 383-397.

Despommier, D., Weisbroth, S. and Fass, C. 1974. Circulation eosinophils and trichinosis in the rat: the parasitic stage responsible for induction during infection. J. Parasit. 60: 280-284.

Dewhirst, L. W., Trautman, R. J., Pistor, W. J. and Reed, R. E. 1960. Studies on ante-mortem diagnostic procedures in bovine cysticercosis infections (abstract). J. Parasit. 46: 10-11.

Dixon, H. B. F. and Hargreaves, W. H. 1944. Cysticercosis (*Taenia solium*). A further ten years' clinical study, covering 284 cases. Q. J. Med. 13: 107-121.

Dixon, H. B. F. and Lipscomb, F. M. 1961. Cysticercosis. An analysis and follow-up of 450 cases. Privy Council, Medical Research Council Special Report Series No. 299, Her Majesty's Stationery Office, London.

Donohugh, D. L. 1966. Eosinophils and eosinophilia. Calif. Med. 104: 421-427.

Dunning, W. F. and Curtis, M. R. 1946. Multiple peritoneal sarcoma in rats from intraperitoneal injection of washed ground *Taenia* larvae. Cancer Res. 6: 668-670.

Duthy, B. L. and van Someren, V. D. 1948. The survival of *T. saginata* eggs on open pasture. E. Afr. Agric. J. 13: 147-149.

Ehrlich, P. 1879. Uber die specifischen Granulationen des Blutes. Arch. Anat. Physiol. Lp2. Physiol. Abt. 571-579.

Enomoto, T. and Kitani, T. 1966. Electron microscopic studies on peroxidase and acid phosphatase reaction in human leukocytes. Acta Haematol. Jap. 29(Nihon Ketsueki Gakkai): 554-570.

Evranova, V. G. and Mosina, S. K. 1966. Histochemical changes in liver and lung experimental hydatidosis in sheep and cysticerciasis in cattle. Uchen. Zap. Kazan. Vet. Inst. 96: 218-223.

Faust, E. C., Russell, P. F. and Jung, R. C. 1970. *Craig and Faust's Clinical Parasitology*, 8th ed. Lea and Febiger, Philadelphia, p. 529-557.

Freeman, R. S. 1964. Studies on responses of intermediate hosts to infection with *Taenia crassiceps* (Zedar, 1800) (Cestoda). Can. J. Zool. 42: 367-385.

Froyd, G. 1964. The longevity of *Cysticercus bovis* in bovine tissues. Br. Vet. J. 120: 205-211.

Gedigk, P. and Gross, R. 1962. *The Physiology and Pathology of Leukocytes*. Ed. Braunsteiner, H. Grune and Stratton, New York and London, p. 5-45.

Gemmell, M. A. 1969. Immunological responses of the mammalian host against tapeworm infections. X. Immunization of sheep against *T. hydatigena* and *T. ovis* with chemically or physically treated embryos. Exptl. Parasit. 26: 58-66.

Godlowski, Z. Z. 1952. The fate of eosinophils in hormonally induced eosinopenia and its significance. J. Endocrinol. 8: 102-125.

Godlowski, Z. Z. 1953. *Enzymatic Concept of Anaphylaxis and Allergy*. Edinburgh, 1953.

Gross, R. 1962. *The Eosinophils. The Physiology and Pathology of Leukocytes*. Ed. Braunsteiner, H. and Zucker-Franklin, D. Grune and Stratton, New York.

Hirsch, J. G. 1965. *The Eosinophil Leucocyte in the Inflammatory Process*. Ed. B. W. Zweifach, L. Grant and R. T. McCluskey. Academic Press, New York.

Hirsch, J. G. and Cohn, Z. A. 1960. Degranulation of polymorphonuclear leukocytes following phagocytosis of microorganisms. J. Exp. Med. 112: 1005-1014.

Homma, E. 1921. Pathologische und biologische Untersuchungen Uber die Eosinophilzellen und die Eosinophilie. Virchews Arch. Path. Anat. 233: 11-51.

Hutchison, W. M. 1958. Studies on *Hydatigera taeniaeformis*. 1. Growth of the larval stage. J. Parasit. 44: 574-582.

Hudson, G. 1968. Quantitative study of the eosinophil granulocytes. Semin. Hematol. 5: 166-186.

Hudson, G., Chin, K. N. and Moffatt, D. J. 1972. Marrow reserve of eosinophils: estimation of transit time in normal guinea pigs. Cell. Tissue Kinet. 5: 191-192.

Ishikawa, T., Yu, M. C. and Arbesman, C. E. 1972. Electron microscopic demonstration of phagocytosis of *Candida albicans* by human eosinophilic leukocytes. J. Allergy Clin. Immunol. 50: 183-187.

Jepsen, A. and Roth, H. 1952. Epizootiology of *Cysticercus bovis*: resistance of the eggs of *Taenia saginata*. Intern. Vet. Congr., 14th, London, Proceedings 2: 43-50.

Jha, R. K. and Smyth, J. D. 1969. *Echinococcus granulosus*: ultrastructure of microtriches. Exptl. Parasit. 25: 232-244.

Jones, T. W. 1846. The blood corpuscle considered in its different phases of development in the animal series. Memoir I. Vertebrata, Phil. Trans. Roy. Soc. Lond. 136: 63.

Kalawski, K. and Pawlowski, Z. S. 1970. Inwazje tasiemca nieuzbrojonego (*Taenia-rhynchur saginatur*) na terenic misasta Poznania w latach 1954-1968. Przegl. epidem. 24: 337-380 or Epid. Rev. 24: 258-261.

Kay, A. B. 1970. Studies on eosinophil leucocyte migration. II. Factors specifically chemotactic for eosinophils and neutrophils generated from guinea-pig serum by Antigen-Ab complex. Clin. Exp. Immunol. 7: 723-737.

Kay, A. B., Stechschulte, D. J. and Austen, K. F. 1971. An eosinophil leukocyte chemotactic factor of anaphylaxis. J. Exp. Med. 133: 602-619.

Kelenyi, Von G., Zombai, E. and Nemeth, A. 1965. Histochemische und elektronenmikrosopische Beobachtungen an der spezifischen Granulation der eosinophilen Granulozyten. Acta Histochem. 22: 77-89.

Lachmann, P. J., Kay, A. B. and Thompson, R. A. 1970. The chemotactic activity for neutrophil and eosinophil leukocytes of the trimolecular complex of the fifth, sixth and seventh components of human complement prepared in free solution by the "reactive lysis" procedure. Immunol. 19: 895-900.

Leid, R. W. 1973. Ph.D. Thesis, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan, U.S.A.

Leid, R. W. and Williams, J. F. 1974a. Immunological responses of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobin classes involved in passive transfer of resistance. Immunol. 27: 195-208.

Leid, R. W. and Williams, J. F. 1974b. Immunological responses of the rat to infection with *Taenia taeniaeformis*. II. Characterization of reaginic antibody and an allergen associated with the larval stage. Immunol. 27: 209-225.

Leuckart, R. 1886. *The Parasites of Man*. Edinburgh: Young J. Pentland.

Lewert, R. M. and Lee, C. L. 1955. Studies on the passage of helminth larvae through host tissues. III. The effects of *Taenia taeniaeformis* on the rat liver as shown by histochemical techniques. J. Infect. Dis. 99: 177-186.

Lewert, R. M. and Lee, C. L. 1956. Quantitative studies of the collagenase-like enzymes of cercariae of *Schistosoma mansoni* and *Strongyloides ratti*. J. Infect. Dis. 9y: 18-51.

Litt, M. 1961. Studies in experimental eosinophilia. III. The induction of peritoneal eosinophilia by the passive transfer of serum antibody. J. Immunol. 87: 522-529.

Litt, M. 1962. Studies in experimental eosinophilia. IV. Determinants of eosinophil localization. J. Allergy 33: 532-543.

Litt, M. 1963. Studies in experimental eosinophilia. V. Eosinophils in lymph nodes of guinea pigs following primary antigenic stimulation. Amer. J. Path. 42: 529-549.

Litt, M. 1964a. Eosinophils and antigen-antibody reactions. Anns. N.Y. Acad. Sci. 116: 964-985.

Litt, M. 1964b. Studies in experimental eosinophilia. VII. Eosinophils in lymph nodes during the first 24 hr following primary antigenic stimulation. J. Immunol. 93: 807-813.

Litt, M. 1968. Studies in experimental eosinophilia. VIII. Induction of eosinophilia by homologous $7S\gamma_1$ antibody and by extremely minute doses of antigen. Allergology, Proc. 6th Cong. Intern. Assoc. Allergol. (ed. by B. Rose, M. Richter, A. Sehon and A. W. Frankland), p. 38. Excerpta Medica International Congress Series No. 162. Excerpta Medica Foundation, Amsterdam.

Marchesi, V. T. and Florey, H. W. 1960. Electron micrographic observations on the emigration of leucocyte. Quant. J. Exptl. Physiol. 45: 343-348.

McIntosh, A. and Miller, D. 1960. Bovine cysticercosis with special reference to the early developmental stages of *T. saginata*. Amer. J. Vet. Res. 11: 169-177.

Miale, J. B. 1967. *Laboratory Medicine: Hematology*, Third Edition. C. V. Mosby Co., St. Louis.

Miller, D. 1956. Tapeworm hazard in dry lands. Publ. Hlth. Rep. 71: 1239-1240.

Miller, H. M. 1931. The production of artificial immunity in the albino rat to a metazoan parasite. J. Prev. Med. 5: 429-452.

Miller, H. M. and Gardiner, M. L. 1932. Passive immunity to infection with a metazoan parasite *Cysticercus fasciolaris* in the albino rat. J. Prev. Med. 6: 479-496.

Miller, F., DeHarven, E. and Palade, G. E. 1966. The structure of eosinophil leukocyte granules in rodents and in man. J. Cell Biology 31: 349-362.

Miller, M. and Dawley, W. 1928. An experimental study of some effects of *Cysticercus fasciolaris* Rud. on the white rat. J. Parasit. 15: 87-103.

Moniez, R. 1880. Essai monographique sur les cysticerques. Travaux Inst. Zool. Lille Station Maritime Wimereux. Paris 3: 1-190.

Musoke, A. J. 1975. Ph.D. Thesis, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan, U.S.A.

Musoke, A. J. and Williams, J. F. 1975a. Immunoglobulins associated with passive transfer of resistance to *Taenia taeniaeformis* in the mouse. Immunol. 28: 97-102.

Musoke, A. J. and Williams, J. F. 1975b. The immunological response of the rat to infection with *Taenia taeniaeformis*. V. Mechanism of action of immunoglobulins involved in passive transfer of resistance. Immunol. (in press).

Musoke, A. J., Williams, J. F., Leid, R. W. and Williams, C. S. F. 1975. The immunological response of the rat to infection with *Taenia taeniaeformis*. IV. Immunoglobulins involved in passive transfer from mothers to offspring. Immunol. (in press).

Nieland, M. L. and Weinbach, E. C. 1968. The bladder of *Cysticercus fasciolaris*: electron microscopy and carbohydrate content. Parasit. 58: 489-496.

Osgood, E. E. 1937. Culture of human marrow: length of life of neutrophils, eosinophils and basophils of normal blood. J.A.M.A. 109: 933-937.

Padawer, J. and Gordon, A. S. 1952a. A mechanism for the eosinopenia induced by cortisone and by epinephrine. Endocrinology 51: 52-58.

Padawer, J. and Gordon, A. S. 1952b. Eosinopenia and degenerating eosinophilic leukocytes in blood. Proc. Soc. Exp. Biol. N.Y. 80: 581-582.

Parish, W. E. 1970. Investigations on eosinophilia. The influence of histamine, antigen-antibody complexes containing γ_1 or γ_2 globulins, foreign bodies (phagocytosis) and disrupted mast cells. Br. J. Derm. 82: 42-64.

Pawlowski, Z. and Schultz, M. G. 1972. Taeniiasis and cysticercosis (*T. saginata*). Advances in Parasit. 10: 269-343.

Prokopenko, L. I. 1968. Approaches and methods of taeniarhynchosis eradication in the U.S.S.R. Int. Congr. Trop. Med. Malar. 8th, Teheran, 233-237.

Rickard, M. and Bell, K. J. 1971a. Immunity produced against *Taenia ovis* and *T. taeniaeformis* infection in lambs and rats following *in vivo* growth of larvae in filtration membrane diffusion chambers. J. Parasit. 57: 571-575.

Rickard, M. and Bell, K. J. 1971b. Successful vaccination of lambs against infection with *Taenia ovis* using antigens produced during *in vitro* cultivation of the larval stages. Res. Vet. Sci. 12: 401-402.

Round, M. C. 1961. Observations on the possible role of filth flies in the epizootiology of bovine cysticercosis in Kenya. J. Hyg. Camb. 59: 505-513.

Sabesin, S. E. 1963. A function of the eosinophil: phagocytosis of antigen-antibody complexes. Proc. Soc. Exp. Biol. Med. 112: 667-670.

Samter, M. 1965. *Eosinophils. Immunological Diseases.* Ed. Samter, M. Little Brown and Company, Boston, p. 242-245.

Schacher, J. F. and Hajj, S. N. 1970. *Taenia* proglottide in the human uterus. Amer. J. Trop. Med. Hyg. 19: 626-627.

Schalm, O. W., Jain, N. C. and Carrol, E. J. 1975. *Veterinary Hematology*, 3rd edition. Lea and Febiger, Philadelphia.

Schenone, H. 1974. Cysticercosis as a public health and animal health problem. W.H.O. Provisional agenda Item 9, Panel on the health and economic importance of cysticercosis to the countries. 7th Inter-American meeting at the Ministerial Level on Foot and Mouth Disease and Zoonoses Control.

Schwarz, E. 1914. Die Lehre von der allgemeinen und ortlichen "Eosinophilie". Engebn. Allg. Path. 17: 137.

Scott, R. E. and Horn, R. G. 1970. Fine structural features of eosinophile granulocyte development in human bone marrow. Ultrastructure Research 33: 16-28.

Siddiqui, E. H. 1963. The cuticle of cysticerci of *Taenia saginata, T. hydatigena* and *T. pisiformis*. Quart. J. Micr. Sci. 104: 141-144.

Silverman, P. H. 1955. The biology of sewers and sewage treatment. The survival of the egg of the "beef tapeworm" *Taenia saginata*. Advancement of Science, London 12: 108-111.

Silverman, P. H. and Griffiths, R. I. 1955. A review of methods of sewage disposal in Great Britain, with special reference to the epizootiology of *Cysticercus bovis*. Ann. Trop. Med. Parasit. 49: 436-450.

Silverman, P. H. and Maneely, R. B. 1955. Studies on the biology of some tapeworms of the genus *Taenia*. III. The role of the secreting gland of the hexacanth embryo in the penetration of the intestinal mucosa of the intermediate host, and some of its histochemical reactions. Ann. Trop. Med. Parasit. 49: 326-330.

Singh, B. B. and Rao, B. V. 1967. On the development of *Cysticercus fasciolaris* in albino rat liver and its reaction on the host tissue. Ceylon Vet. J. XV: 121-129.

Slonka, G. F., Moulthrop, J. I., Dewhirst, L. W., Hotchkiss, P. M., Vallaza, B. and Schultz, M. G. 1975. An epizootic of bovine cysticercosis. J. Amer. Vet. Ass. 166: 678-681.

Soulsby, E. J. L. 1965. *Textbook of Veterinary Clinical Parasitology*, Vol. I, *Helminths*. Blackwell Scientific Publications, Oxford.

Soulsby, E. J. L. 1974. Taeniasis and cysticercosis: the problem in the old world. Panel on the health and economic importance of cysticercosis to the countries. 7th Inter-American Meeting at the Ministerial Level on Foot and Mouth Disease and Zoonoses Control.

Speirs, R. S. 1958a. A theory of antibody formation involving eosinophils and reticuloendothelial cells. Nature 181: 681-682.

Speirs, R. S. 1958b. Advances in the knowledge of the eosinophil in relation to antibody formation. Ann. N.Y. Acad. Sci. 73: 283-306.

Spry, C. J. F. 1971a. Mechanism of eosinophilia. I. Kinetics of normal and accelerated eosinopoiesis. Cell. Tiss. Kinet. 4: 351-364.

Spry, C. J. F. 1971b. Mechanism of eosinophilia. VI. Eosinophil mobilization. Cell. Tiss. Kinet. 4: 365-374.

Stoll, N. R. 1947. This wormy world. J. Parasit. 33: 1-18.

Sunderman, F. W. and Boerner, F. 1949. *Normal Values in Clinical Medicine*. W. B. Saunders Company, Philadelphia and London.

Sweatman, G. K. and Plummer, P. J. G. 1957. The biology and pathology of the tapeworm *Taenia hydatigena* in domestic and wild hosts. Can. J. Zool. 35: 93-109.

Tanaka, K. R., Valentine, W. N. and Fredericks, R. E. 1962. Human leucocyte arylsulfatase activity. Brit. J. Haemat. 8: 86-92.

Thevathasan, O. I. and Gordon, A. S. 1958. Adrenocortical-medullary interactions on the blood eosinophils. Acta Haemat. 19: 162-172.

Urquhart, G. M. 1961. Epizootiological and experimental studies on bovine cysticercosis in East Africa. J. Parasit. 47: 857-869.

Varute, A. T. 1971. Mast cells in cyst wall of hydatid cyst of *Taenia taeniaeformis* (Batsch). Ind. J. Exp. Biol. 9: 200-203.

Vaughn, J. 1953. The function of the eosinophile leukocyte. Blood 8: 1-14.

Vercauteren, R. 1951. A cytochemical approach to the problem of the significance of blood and tissue eosinophilia. Enzymologia 14: 340-346.

Vercauteren, R. 1953. The properties of the isolated granules from blood eosinophiles. Enzymologia 16: 1-13.

Voge, M. 1963. Observations on the structure of cysticerci of *Taenia solium* and *Taenia saginata* (Cestoda: *Taeniidae*). J. Parasit. 49: 85-90.

Walls, R. S. and Beeson, P. B. 1972. Mechanism of eosinophilia. VIII. Importance of local cellular reactions in stimulating eosinophil production. Clin. Exp. Immunol. 12: 111-119.

Wantland, W. W. 1953. *Cysticercus fasciolaris* in the wild rat and the development of this strobilocercus in the white rat. J. Parasit. 39: Suppl. 27.

Ward, P. A. 1969. Chemotaxis of human eosinophils. J. Exp. Med. 122: 121-128.

Wasserman, S. I., Goetzel, E. J. and Austen, K. F. 1974. Preformed eosinophil chemotactic factor of anaphylaxis (ECF-A). J. Immunol. 112: 351-358.

Yamada, E. and Yamauchi, R. 1966. Some observations on the cytochemistry and morphogenesis of the granulocytes in rat bone marrow as revealed by electron microscopy. Acta Haematol. Jap. (Nihon Ketsueki Gakkai) 29: 530-541.

Young, R. T. 1908. The histogenesis of *Cysticercus pisiformis*. Zool. Jahrb. Abt. Fur Anat. 26: 183-254.

Zolov, D. M. and Levine, B. B. 1969. Correlation of blood eosinophilia with antibody classes. Int. Arch. Allergy Appl. Immunol. 35: 177-193.

Zucker-Franklin, D. 1968. Electron microscopic studies of human granulocytes: structural variation related to function. Semin. Hematol. 5: 109-133.

Zucker-Franklin, D. 1971. Eosinophilia of unknown etiology: a diagnostic dilemma. Hospital Practice March, 1971: 119-127.

Zucker-Franklin, D. and Hirsch, J. G. 1964. Electron microscopic studies on the degranulation of rabbit peritoneal leukocytes during phagocytosis. J. Exp. Med. 120: 569-576.

ARTICLE 1

THE EOSINOPHILIC RESPONSE OF THE RAT TO INFECTION WITH *TAENIA TAENIAEFORMIS*

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Running title: Eosinophilic Response to *Taenia taeniaeformis*

Abstract: Rats were dosed with eggs of *Taenia taeniaeformis* and hematologic parameters were measured throughout the course of primary infection. There was no evidence of anemia but differential leukocyte counts revealed distinct and reproducible patterns of white blood cell changes. A lymphocytosis developed at the end of the first and fifth weeks post infection (p.i.). Neutrophil counts peaked 8 days p.i., although at that time there was no marked neutrophilic infiltration of the tissues. Eosinophil counts began to rise during the second week p.i. and reached a peak during the third week, followed by a decline and then another peak during the fifth week p.i. Eosinophilic infiltration of the tissues was remarkable during the period of peripheral eosinophilia. A wide zone of eosinophils surrounded the developing larvae at 22 days p.i. and persisted in some cases for a further 2 weeks. Eosinophils remained in lesser numbers in the connective tissue capsule throughout the infection, often in association with plasma cells.

After oral challenge with 1000 eggs infected rats showed brisk secondary eosinophilic responses 3 to 7 days later but other hematologic parameters were unaffected. Average peripheral eosinophil counts at 3 and 4 days post challenge were significantly higher than those in unchallenged controls ($P<0.05$ and $P<0.01$, respectively). There was no detectable increase in eosinophilic infiltration of small intestinal tissues in challenged rats.

These results are discussed in relation to current understanding of the mechanisms of eosinophil chemotaxis *in vitro* and the possible causes of local eosinophil accumulation in parasitic infections *in vivo*.

INTRODUCTION

Cysticercosis in man and animals often results in eosinophilia in peripheral blood (Dixon and Smithers, 1934; Beltran, 1962; Soule, Calamel, Chevrier and Pantaleon, 1971) although the pattern of this response has not been well studied. Eosinophils are prominent cells in the inflammatory infiltrate surrounding developing cysticerci of *Taenia hydatigena* in the livers of lambs (Pullin, 1955; Sweatman and Plummer, 1957) and there is a local accumulation of eosinophils around the established cysticerci of *Taenia ovis* (Sweatman and Henshall, 1962). After death of cysticerci of *Taenia saginata* in cattle eosinophils enter the lesions in large numbers (Silverman and Hulland, 1961). The mechanisms by which these cells are called into play in taeniid metacestode infections are unknown, but experimental cysticercosis in laboratory rodents provides an excellent opportunity to study this phenomenon.

Miller and Dawley (1928) followed the hematologic changes in rats after infection with eggs of *Taenia taeniaeformis* and concluded that there was some evidence of anemia accompanied by a peripheral eosinophilia which peaked between 16 and 25 days post exposure. There was no secondary eosinophilic response to challenge infection. However, they used small groups of rats (generally 2 or 3 per group) and their experiments were poorly controlled. Freeman (1964) dosed mice orally with eggs of *Taenia crassiceps* and observed a gradual increase in peripheral eosinophils over the first six weeks after exposure. Few of the mice became infected with cysticerci and no differences were observed between eosinophilic responses in those which were found to be infected at autopsy and those which were not.

These experiments do not provide a clear picture of the pattern of eosinophilia in experimental cysticercosis, and in order to investigate some of the factors which mediate eosinophilia in rats we have therefore examined the sequence of blood and tissue changes following primary and secondary exposure to *T. taeniaeformis*. Observations are presented here which show that reproducible patterns of eosinophilia occur in the peripheral blood and liver after primary infection and that a brisk secondary eosinophilic response follows challenge in immune animals.

MATERIALS AND METHODS

The strain of *T. taeniaeformis* used in these experiments was obtained from the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland, and has been propagated in our laboratory for 3 years using the methods described by Leid and Williams (1974a). Spartan [Spb:(SD)BR] female rats 28 days old were purchased from Spartan Research Animals, Haslett, Michigan. Fischer 344 female rats 28 days old were obtained from A. R. Schmidt, Co., Madison, Wisconsin. All animals were given proprietary brand food and water *ad libitum* throughout this study.

Oral infections with 500 eggs were established by gastric intubation in groups of 5 rats. This dose resulted in approximately 80 hepatic cysts in each animal. Groups of 5 uninfected rats were used as controls and none of them was infected at autopsy. In subsequent experiments groups of animals which had been infected for 3-5 months were challenged orally with 1000 eggs and serial bleedings were made.

Blood samples were obtained by snipping the tip of the tail and, after discarding the first few drops, blood was collected directly into standard red and white cell pipettes and microhematocrit capillary tubes. Samples were taken for 2 days prior to infection and then daily for 12 days, followed by collections at 2- to 4-day intervals for prescribed periods. In order to avoid variations derived from the normal daily fluctuation in blood parameters in rats (Jakobson and Hortlung, 1954) samples were collected between the hours of 11 a.m. and 3 p.m. Basten, Boyer and Beeson (1970) observed minimal variation in blood values in samples taken from rats during this period.

Total erythrocyte (RBC) and leukocyte (WBC) counts were made according to standard hematologic procedures (Schalm, 1965). Packed cell volume percentage estimates (PCV) were made following centrifugation of heparinized samples in an Adams Autocrit centrifuge. Differential leukocyte counts were made on 400 cells in Giemsa-stained blood smears.

Samples of liver tissue were obtained at necropsy of rats killed 2, 5, 7, 11, 22, 37, 62 and 90 days after primary infection, and fixed in buffered formalin for 48 hours. Processed tissues were sectioned and stained with hematoxylin and eosin, Giemsa and Masson's trichrome. Samples of liver and small intestine were taken 1, 2 and 4 days after challenge infection with 1000 eggs and processed similarly. Normal control rats of the same age as experimentals were included in all these histopathologic studies.

Ultrastructural observations were made on sections from the host capsule surrounding 62-day-old cysticerci. Tissue samples

(1 cu.mm) from several parts of the cyst wall were fixed in 3% glutaraldehyde in 0.05 M s-Collidine buffer at pH 7.4 for 4 hr at 4 C, washed in sucrose buffer and then immersed in 1% osmium tetroxide at pH 7.4 for 2 hr. After alcohol dehydration the tissue was embedded in Epon 812 and sections were cut in a Sorvall ultramicrotome and stained with Reynolds lead citrate (Reynolds, 1963). These preparations were examined in a Zeiss E M9S-2 electron microscope.

RESULTS

The hematologic changes in Spartan rats during the first 52 days following primary infection are shown in Figure 1A. No differences were observed in total RBC counts and PCV between the infected and control animals (Figure 1B). There was a marked increase in total WBC counts which peaked in infected rats 8 days post infection (p.i.). This increase was followed by a sharp fall then a further increase which was sustained until the end of the third week. There was another peak of leukocytosis at about the fifth week p.i. All infected rats showed this pattern of WBC fluctuation. The second peak of leukocytosis varied in individuals from day 14 to 18, and was equally as high as the first, although the average values plotted on the graph do not adequately show this.

The differential WBC count revealed the distribution of each cell type in these responses (Figure 1C). Coincidental increases in lymphocytes and neutrophils were responsible for the onset of leukocytosis at the end of the first week p.i. Thereafter there was a slight absolute neutrophilia in infected rats which persisted for an

Figure 1. Hematologic parameters in Spartan rats exposed to 500 eggs of *Taenia taeniaeformis* on day 0 (A,C) and uninfected control rats of the same age and strain (B,D). Abbreviations are listed in text.

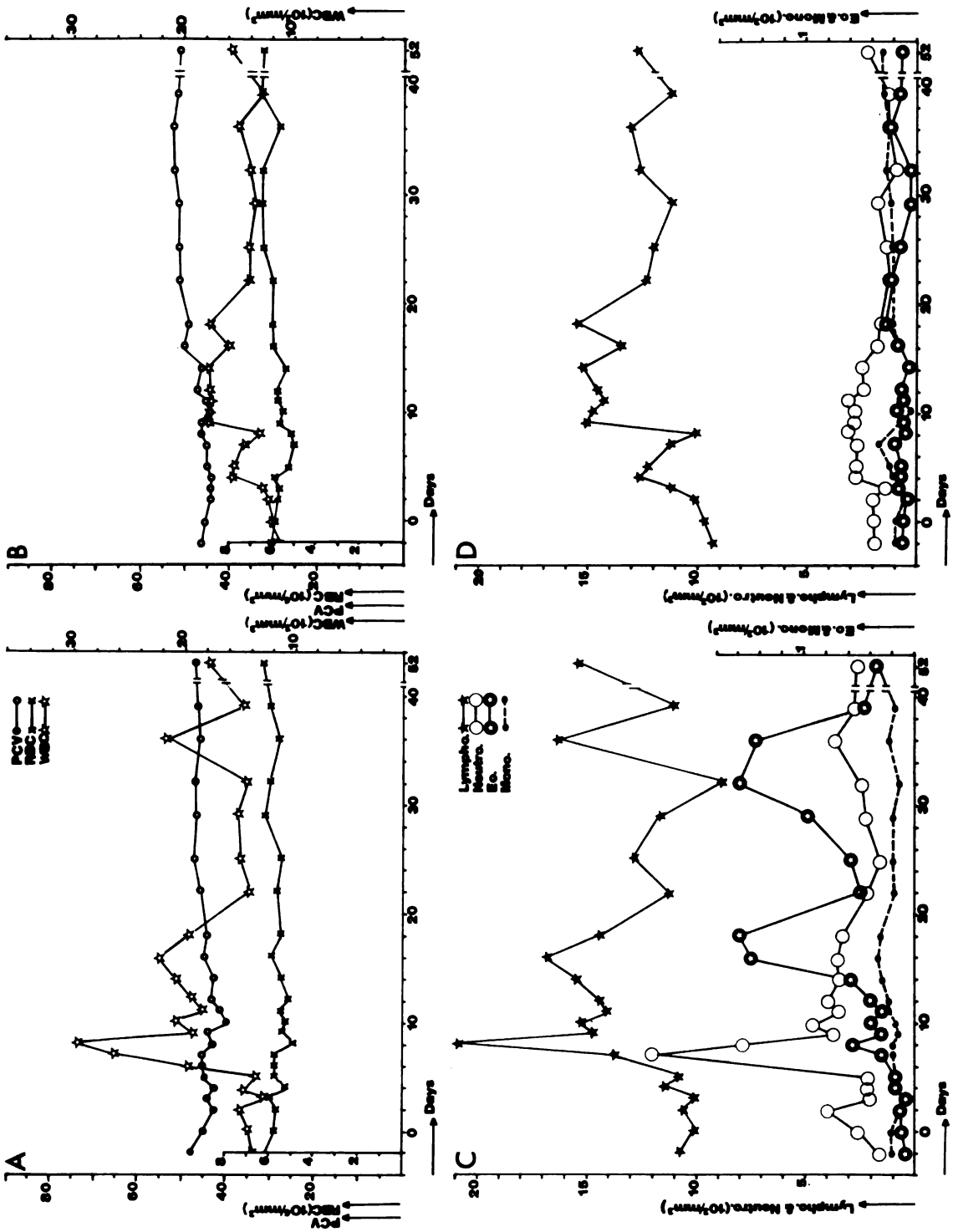

A
B
C
D
PCV
RBC(10⁶/mm³)
WBC(10³/mm³)
Lympho.& Neutro.(10³/mm³)
Eo.& Mono.(10³/mm³)
Days
Lympho.
Neutro.
Eo.
Mono.

Figure 1

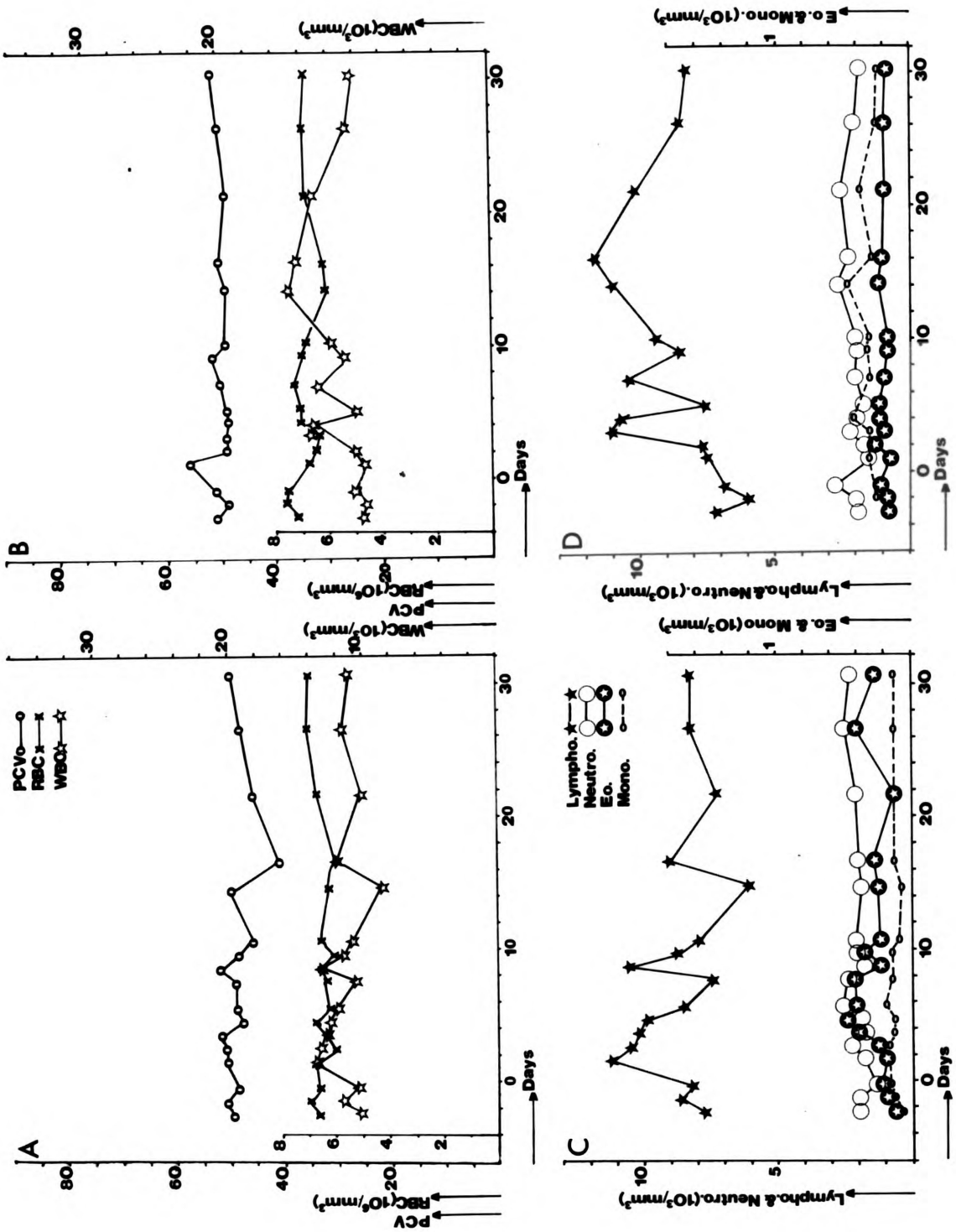
additional 6 weeks before the counts returned to the normal range. After day 8 lymphocyte numbers fell to the levels in control rats, but there was a second peak of lymphocytosis in infected rats at 36 days.

No differences were observed in absolute monocyte numbers in infected and control rats, but the pattern of the eosinophilic response was quite distinct. All infected rats began to show an absolute increase in numbers of eosinophils at the beginning of the second week p.i., and after a slight fall this was continued until the middle of the third week. At that time there was an average 8-fold increase in circulating eosinophils as compared to controls, and peaks as high as $2280/mm^3$ were observed. This was followed by a decline, though counts were sustained well above the control level until a further peak occurred during the fifth week, when values over $3700/mm^3$ were recorded. Thereafter the counts declined sharply before falling gradually to control levels over the next 3 months.

The pattern of eosinophilia in response to primary infection was very similar in a second experiment employing Fischer 344 inbred rats. However, peak values at 32 days p.i. were approximately 25% higher than in the Spartan strain, and the peripheral eosinophil count did not fall to normal levels over the succeeding months but hovered slightly above the control values.

After oral challenge of infected Spartan rats with 1000 eggs, complete hematologic pictures were followed for 30 days. At the time of challenge all blood parameters in infected rats were within the normal range. The results are shown in Figures 2 and 3. No changes in PCV, RBC or WBC counts were observed (Figure 2A).

Figure 2. Hematologic parameters in Spartan rats infected with *Taenia taeniaeformis* for 5 months and challenged on day 0 with an oral dose of 1000 eggs (A,C), and control unchallenged rats of the same age and strain (B,D). Abbreviations are listed in the text. The differences between eosinophil counts on days 3 and 4 in the challenged animals (C) were significantly different from those in controls ($P<0.05$ and $P<0.01$, respectively, Student's 't' test).


A
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WBC(10³/mm³)
Days
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Neutro.
Eo.
Mono.
Lympho.& Neutro.(10³/mm³)
Eo.& Mono.(10³/mm³)

Figure 2

Figure 3. Secondary eosinophilic response in an individual Spartan rat following oral challenge with 1000 eggs of *Taenia taeniaeformis* on day 0. The peak value of 650/mm^3 was reached on day 4. The shaded area indicates the normal range $\pm$ 2 standard deviations for the eosinophil counts in control rats.

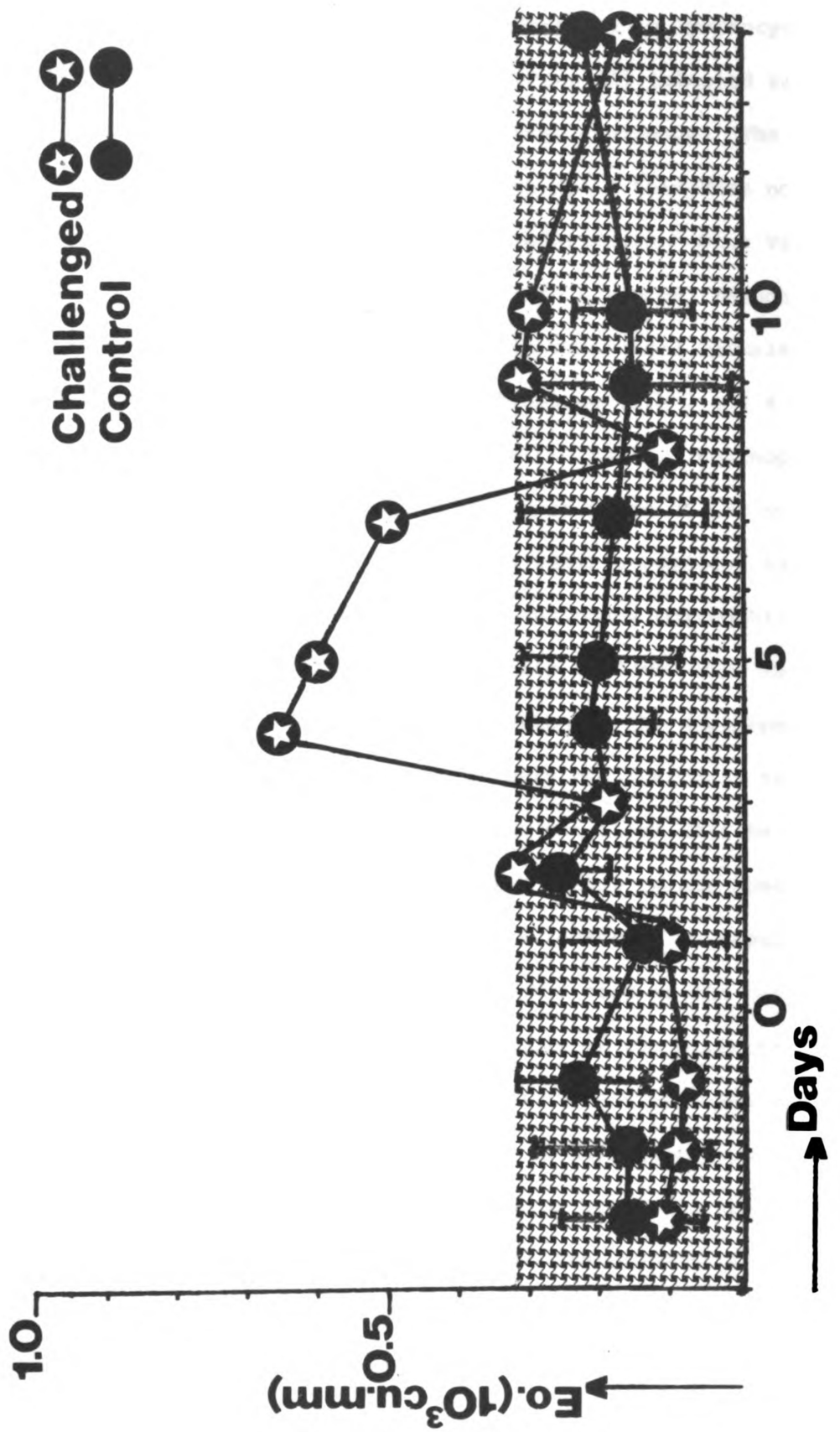

Figure 3

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Differential counts showed no remarkable increases in lymphocytes, neutrophils or monocytes (Figure 2C). However, all infected rats showed a peak of eosinophilia 3 to 7 days post challenge. The average peak value was 500/mm^3 on day 4, but again this does not reflect the sharp, transient increases in individuals. Peak values in individual rats were as high as 840/mm^3 and a typical response is depicted in Figure 3. The average counts in challenged animals were significantly different from those in controls on days 3 and 4 ($P<0.05$ and $P<0.01$, respectively, Student's 't' test). Eosinophil counts quickly fell but fluctuated in the upper range of the normal levels for several weeks. This pattern was again reproduced in Fischer 344 rats, although peak values for secondary eosinophilia were somewhat higher and individual peaks over 1000/mm^3 were recorded.

A complete histopathological description of the development of the inflammatory cell infiltrate in primary cysticercosis in the rat and the formation of the host capsule in the liver will be published elsewhere. The observations which follow relate specifically to the participation of the eosinophil leukocyte in these events. Eosinophils were first detected in the zone of the cellular infiltration surrounding postoncospheral stages of the parasite at 11 days p.i. (Figure 4a), although increased numbers of these cells were present in portal areas by 7 days. By 22 days there was a remarkable zone up to 120 μ wide and consisting almost entirely of eosinophils (Figure 4b, c) directly apposed to the tegument of the vesicular metacestode. This zone was widest at the pole where scolex formation had begun. It extended over approximately a quarter of the surface of the parasite effectively separating the latter from

Figure 4. a - e Histologic appearance of cysticerci of *Taenia taeniaeformis* at 11, 22, 37 and 62 days post infection, respectively. There were few inflammatory cells surrounding the organism at 11 days (4a - X 100) but by 22 days a wide zone of eosinophils had developed in immediate contact with the tegument (4b - X 100). This zone was especially broad at the point of scolex differentiation (4c - X 400). By 37 days the tegument readily separated from the eosinophil layer (arrowed) which had become more tenuous (4d - X 100). Eosinophils were no longer in direct contact with the parasite tegument by day 62 (4e - X 100), but were plentiful in the connective tissue capsule. Electron micrographs of polymorphonuclear cells in this capsule revealed the characteristic eosinophil granules (4F - X 7920). Eosinophils (Eo) were often present in association with plasma cells (P). f is a fibroblast.

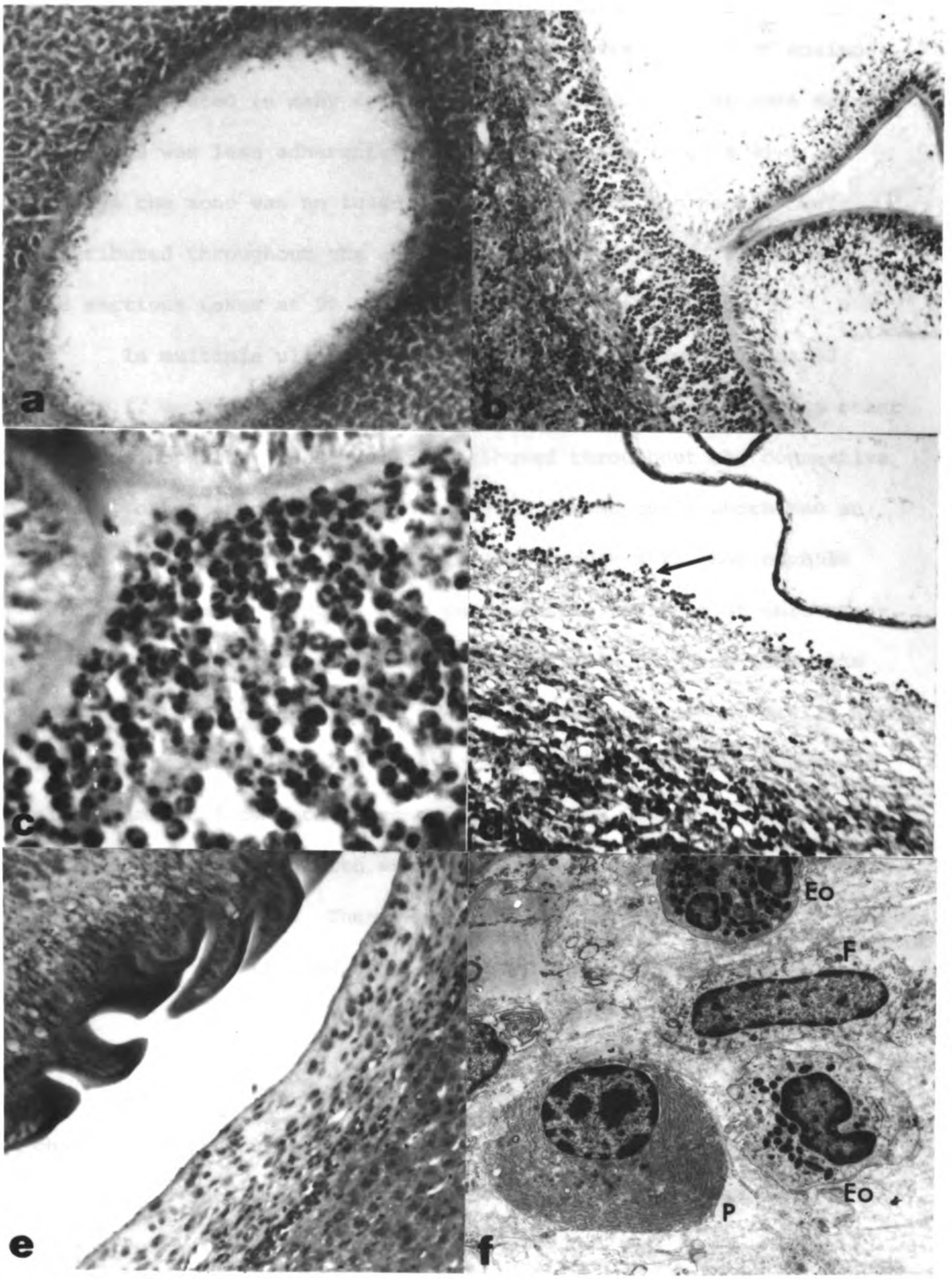

Figure 4

the fibroblastic layer. By 37 days there was much variation in the stage of cyst development within a single liver. Zones of eosinophilia persisted in many cysts, and the tegument of the more mature parasites was less adherent to the host capsule (Figure 4d). By 62 days the zone was no longer present although eosinophils were distributed throughout the connective tissue capsule (Figure 4e), and sections taken at 90 days had a similar appearance.

In multiple ultrathin sections of the host capsule at 62 days p.i. we were unable to detect any granulocytic leukocytes other than eosinophils. These were distributed throughout the connective tissue, often in close association with plasma cells which had an extremely active endoplasmic reticulum (Figure 4f). Eosinophils were never seen in contact with the parasite tegument at this stage.

Sections of liver and small intestine were taken from rats which had been infected for 2 months and then challenged orally with 1000 eggs either 1, 2 or 5 days before being killed. Tissues were also examined from unchallenged infected rats, and from uninfected rats of the same age which were killed 1, 2 or 5 days after being dosed with 1000 eggs. There were no detectable secondary eosinophilic responses in the tissues of challenged rats.

DISCUSSION

In these hematologic studies there was no indication of any change in erythrocyte parameters after either primary or secondary infection. Total RBC counts and PCV values closely followed those in control animals. Miller and Dawley (1928) described the onset of anemia in rats after the first week of infection with *T.*

taeniaeformis, and total RBC counts did not return to normal for several weeks. This observation was derived from 2 experiments in which the control groups consisted of 1 rat each and there were either 1 or 2 infected rats as principals. It is therefore difficult to assess the significance of the differences which they recorded. Anemia does not appear to be a common clinical feature of cysticercosis in man and domestic animals although there is one record of a decline in hemoglobin and PCV values in the blood of cattle experimentally exposed to *T. saginata* (Dewhirst, Trautman, Pistor and Reed, 1960).

Total WBC counts increased abruptly approximately 1 week after primary infection and this reflected absolute increases in circulating neutrophils and lymphocytes. It is difficult to account for the single sharp peak of neutrophilia. At this stage of infection we did not observe any marked tissue reaction in the liver and the few infiltrating polymorphonuclear cells which began to appear during the second week were mainly eosinophils. The short period of intense neutrophilia probably resulted from a transient stimulation of neutrophil release from the bone marrow pool, but the fate of these cells is not at all clear. Lymphocytes, on the other hand, began to appear in the liver by 11 days and were present throughout the infection in the host capsule surrounding the parasite. Their local accumulation may reflect the rapid development of an immune response and it seems likely that some differentiate into plasma cells which are numerous in the connective tissue layer later in infection. High levels of protective antibodies appear in the serum by 14 days p.i. and these are exclusively in the IgG_{2a}

immunoglobulin class (Musoke and Williams, 1975), but it is not yet known if antibody is produced locally in the capsule. After the first 28 days of infection antibody activity begins to appear in a broad spectrum of immunoglobulin classes (Musoke and Williams, 1975) and it is possible that the increase in lymphocyte numbers during the fifth week of infection is correlated with this shift in the pattern of antibody production.

The course of the eosinophilic response which we observed following primary infection did not follow that described by Miller and Dawley (1928). They reported that the percentage of circulating eosinophils increased by 16 days after exposure and was maintained at an elevated level for 10 to 20 days depending on the degree of infection. In the 2 experiments from which this conclusion was drawn, the control groups consisted of 1 rat each, and in one instance the rat became accidentally infected. The eosinophil counts were made on small groups of infected rats and, in view of the fact that they are expressed as percentage values and absolute leukocyte counts were not made, we do not consider that their results provide clear evidence of eosinophilia.

Peaks of peripheral eosinophilia in our experiments occurred approximately 2-1/2 weeks and 5 weeks after infection, although a slow rise in peripheral counts was evident during the second week. At that time we observed eosinophils accumulating at the interface between the metacestode and the developing capsule, and the intensity of eosinophilic infiltration was remarkable in tissue sections taken during the third week. This zone of eosinophils was not described by Bullock and Curtis (1924) or Singh and Rao (1967) in their accounts

of histopathological changes in infected livers. It seems likely that some chemotactic mechanism attracts eosinophils to the area surrounding the parasite at this stage and that the mechanism operates to a lesser extent throughout the subsequent course of the infection. The eosinophilic zone was less marked in tissues examined at 37 days, but eosinophils were consistently present in the host capsule even at later stages.

The sustained eosinophilia in peripheral blood must come about as a result of an increase in bone marrow production (Basten, Boyer and Beeson, 1970), although release of a marrow reserve may have contributed to the initial rise. The factors which modulate eosinophil production *in vivo* are not well understood but recent research on eosinophilotactic mechanisms provides some basis for explaining our observations. Immunologic mediation of chemotaxis is known to occur via the generation of C3A, C5A and C_{567} during complement fixation (Ward, 1969; Kay, 1970) and also by the release of a small peptide, eosinophil chemotactic factor of anaphylaxis (ECF-A), from mast cells sensitized with antibodies in the IgE or IgG classes (Kay and Austen, 1972; Kay, Stechschulte and Austen, 1971). Antibodies in the IgE and IgG_{2a} classes are both demonstrable in the serum of rats by the third week of infection with *T. taeniaeformis* (Leid and Williams, 1974a, 1974b) and the IgG_{2a} antibodies are complement fixing (Musoke and Williams, 1975). Reaginic antibodies increase in titer reaching peak values during the fifth week of infection (Leid and Williams, 1974b) and they have been shown to fix to rat mast cells *in vitro* (Leid, personal communication). It is therefore possible that antigen-antibody reactions occurring

around the developing cysticercus could be responsible for the production of eosinophilotactic substances via the complement system and/or via the release of ECF-A from reagin-sensitized mast cells. Reagin titers decline to low levels or disappear after the fifth week of infection and it may be that this is associated with the declining intensity of peripheral and tissue eosinophilia after that time.

Another possible mechanism of eosinophilia must be considered in attempting to account for our observations. We have recently detected factors released *in vitro* by cysticerci of *T. taeniaeformis* which are able to initiate complement fixation via the alternate pathway and generate anaphylatoxin activity from normal rat serum (Hammerberg, Musoke, Hustead and Williams, 1975). These factors are detectable in the parasite cyst fluid by 21 days after infection. It is therefore reasonable to suppose that chemotactic substances are produced locally by the interaction of the host complement system and parasite products. We have as yet no quantitative data on the production of these factors, but variations in the rate of release might influence the intensity of the local chemotactic response. Similarly the intense accumulation of eosinophils around the pole of the cysticercus at which scolex formation begins could be related to the local release of complement-interacting factors. It is also possible that at this point there may be qualitative or quantitative differences in antigen production and release.

Miller and Dawley (1928) did not detect any increase in the percentage of eosinophils in peripheral blood following oral challenge

of infected rats, and concluded that there was no secondary eosinophilia. In our experiments secondary peaks of peripheral eosinophilia were seen 3 to 7 days after challenge with 1000 eggs but other leukocyte parameters were unaffected. We did not detect any secondary eosinophilic responses in the tissues of infected challenged rats, although this may have been due to selection of inappropriate time intervals for tissue examination or to the limitation of eosinophilic infiltration to local areas surrounding the invading oncospheres. Since the challenge dose was quite low it is difficult to detect early parasitic stages in the tissues. Nevertheless, we feel that it is likely that tissue infiltration does occur reflecting the peripheral eosinophilia, although more detailed tissue examinations may be necessary in order to demonstrate this process.

Secondary eosinophilia has been reported in a variety of helminthiases including dictyocaulosis (Weber and Rubin, 1958; MacKenzie and Michel, 1964; Weisman, 1971), and trichostrongylosis (Rothwell and Dineen, 1972; Rothwell, 1975), and an anamnestic eosinophilic response has been shown to occur in sensitized rats challenged intravenously with larvae of *Trichinella spiralis* (Basten, Boyer and Beeson, 1970). Most authors have speculated that secondary responses are immunologically mediated and involve immediate hypersensitivity mechanisms, but there have been no experimental analyses of the phenomenon. Boyer, Basten and Beeson (1970) and Basten and Beeson (1970) attributed the eosinophilic responses in their experimental system to a lymphocyte dependent immunologic process. However, it has been pointed out that intravenously administered larvae of *T. spiralis* rapidly die in the lungs and the sequence of events which

they observed in rats after primary and secondary exposure bears little relationship to that which occurs in trichinellosis (Despommier, Weisbroth and Fass, 1974). Basten and Beeson (1970) were unable to transfer eosinophilic responses passively with plasma and considered that antibody played no role in the induction of peripheral eosinophilia. In a later paper (Ansari, Williams and Musoke, 1975) we present evidence that secondary eosinophilic responses to *T. taeniaeformis* can be passively transferred with immune serum.

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LITERATURE CITED

ANSARI, A., J. F. WILLIAMS, and A. J. MUSOKE. 1975. Antibody mediated secondary eosinophilic responses to *Taenia taeniaeformis* in the rat. J. Parasit. (submitted).

BASTEN, A., and P. B. BEESON. 1970. Mechanism of eosinophilia. II. Role of the lymphocyte. J. Exp. Med. 131:1288-1305.

_____, M. H. BOYER, and P. BEESON. 1970. Mechanism of eosinophilia. 1. Factors affecting the eosinophil response of rats to *Trichinella spiralis*. J. Exp. Med. 131:1271-1287.

BELTRAN, P. G. 1962. Cysticercosis of the nervous system. III. Clinical findings and treatment. J. Neurosurgery 19:641-643.

BOYER, M. H., A. BASTEN, and P. B. BEESON. 1970. Mechanism of eosinophilia. III. Suppression of eosinophilia by agents known to modify immune responses. Blood 36:458-469.

BULLOCK, F. D., and CURTIS, M. R. 1924. A study of the reaction of the tissues of the rat liver to the larvae of *Taenia crassicollis* and the histogenesis of cysticercus sarcoma. J. Cancer Res. 8:446-481.

DESPOMMIER, D., S. WEISBROTH, and C. FASS. 1974. Circulating eosinophils and trichinosis in the rat: the parasitic stage responsible for induction during infection. J. Parasit. 60:280-284.

DEWHIRST, L. W., R. J. TRAUTMAN, W. T. PISTOR, and R. E. REED. 1960. Studies on *ante-mortem* diagnostic procedures in bovine cysticercosis infections. (Abstract). J. Parasit. 46:Sect. II, 10-11.

DIXON, H. B. F., and D. W. SMITHERS. 1934. Epilepsy in cysticercosis (*Taenia solium*). Qu. J. Med. 3:603-616.

FREEMAN, R. S. 1964. Studies on responses of intermediate hosts to infection with *Taenia crassiceps* (Zeder, 1800) (Cestoda). Can. J. Zool. 42:367-385.

HAMMERBERG, B., A. J. MUSOKE, S. T. HUSTEAD, and J. F. WILLIAMS. 1975. Anticomplementary factors associated with taeniid metacestodes. *In* E. J. L. Soulsby (Ed.) *Pathophysiology of Parasitic Infections*. Academic Press (in press).

JAKOBSON, T., and J. HORTLUNG. 1954. Studies on the endocrine regulation of the number of circulating eosinophil cells in the rat. Acta Endocrinol. 15:265-281.

KAY, A. B. 1970. Studies on eosinophil leucocyte migration. II. Factors specifically chemotactic for eosinophils and neutrophils generated from guinea pig serum by antigen-antibody complexes. Clin. Exp. Immunol. 7:723-737.

_____, and K. F. AUSTEN. 1972. Antigen-antibody induced cutaneous eosinophilia in complement deficient guinea-pigs. Clin. Exp. Immunol. 11:37-42.

_____, D. J. STECHSCHULTE, and K. F. AUSTEN. 1971. An eosinophil leukocyte chemotactic factor of anaphylaxis. J. Exp. Med. 133:602-619.

LEID, R. W., and J. F. WILLIAMS. 1974a. The immunological response of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobulin classes involved in passive transfer of resistance. Immunology 27:195-208.

_____, _____. 1974b. The immunological response of the rat to infection with *Taenia taeniaeformis*. II. Characterization of reaginic antibody and an allergen associated with the larval stage. Immunology 27:209-225.

MACKENZIE, A., and J. F. MICHEL. 1964. Observations on the eosinophil leucocyte response and its specificity after vaccination and infection of cattle with *Dictyocaulus viviparus*. Vet. Rec. 76:1493-1497.

MILLER, M., and W. DAWLEY. 1928. An experimental study of some effects of *Cysticercus fasciolaris* Rud. on the white rat. J. Parasit. 15:87-103.

MUSOKE, A. J., and J. F. WILLIAMS. 1975. The immunological response of the rat to infection with *Taenia taeniaeformis*. V. The sequential appearance of protective immunoglobulins and the mechanism of action of IgG_{2a} antibodies. Immunology (in press).

PULLIN, J. W. 1955. Observations on liver lesions in lambs experimentally infected with the cysticercus of *Taenia hydatigena*. Can. J. Comp. Med. 14:17-25.

REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-212.

ROTHWELL, T. L. W. 1975. Studies of the responses of basophil and eosinophil leucocytes and mast cells to the nematode *Trichostrongylus colubriformis*. 1. Observations during the expulsion of first and second infections by guinea-pigs. J. Path. 116: 51-60.

_____, and J. K. DINEEN. 1972. Cellular reactions in guinea-pigs following primary and challenge infections with *Trichostrongylus colubriformis* with special reference to the roles played by eosinophils and basophils in rejection of the parasite. Immunology 22:733-745.

SCHALM, O. W. 1965. *Veterinary Hematology*, Second Edition. Lea and Febiger, Philadelphia, p. 167-172.

SILVERMAN, P. H., and T. J. HULLAND. 1961. Histological observations on bovine cysticercosis. Res. Vet. Sci. 2:248-252.

SINGH, B. B., and B. V. RAO. 1967. On the development of *Cysticercus fasciolaris* in albino rat liver and its reaction on the host tissue. Ceylon Vet. J. 15:121-129.

SOULE, P. C., CALAMEL, M., CHEVRIER, L., and PANTALEON, J. 1971. La cysticercose bovine expérimentale: aspects parasitologique, immunologique et hématologique. Rec. Med. Vet. 147:1247-1257.

SWEATMAN, G. K., and P. J. G. PLUMMER. 1957. The biology and pathology of the tapeworm *Taenia hydatigena* in domestic and wild hosts. Can. J. Zool. 35:93-109.

_____, and T. C. HENSHALL. 1962. The comparative biology and morphology of *Taenia ovis* and *Taenia krabbei* with observations on the development of *T. ovis* in domestic sheep. Can. J. Zool. 40:1287-1311.

WARD, P. A. 1969. Chemotaxis of human eosinophils. J. Exp. Med. 122:121-128.

WEBER, T. B., and R. RUBIN. 1958. The eosinophilic response to infection with the cattle lungworm *Dictyocaulus viviparus*. J. Infect. Dis. 102:214-218.

WEISMAN, J. 1971. New aspects of eosinophilia in *Dictyocaulus viviparus* infections in cattle. *In* S. M. Gaafar (Ed.), *Pathology of Parasitic Diseases*. Purdue University Studies, p. 345-357.

ARTICLE 2

ANTIBODY MEDIATED SECONDARY EOSINOPHILIC RESPONSE TO *TAENIA TAENIAEFORMIS* IN THE RAT

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Running title: Secondary Eosinophilia in Infections with *Taenia taeniaeformis*

Abstract: Normal rats were given intravenous doses of either immune serum or immunoglobulin fractions 24 hr before oral challenge with 1000 eggs of *Taenia taeniaeformis*. Total eosinophil counts per cubic millimeter were performed for 3 days prior to and 6 days after challenge. Sensitized rats showed sharp peaks of eosinophilia 2 to 6 days after this dose. The pattern of the eosinophilic response was similar to that which occurs after challenge of immune infected rats. The differences in peripheral eosinophil levels in passively immunized and normal rats were statistically significant. Immunoglobulin fractions containing protective IgG_{2a} were most effective, but a fraction containing reaginic antibody activity also transferred the secondary eosinophilic response. The findings are discussed in relation to the probable contribution of antigen-antibody reactions to the production of secondary eosinophilic responses in experimental cysticercosis.

INTRODUCTION

Secondary peripheral eosinophilia has been reported to occur in experimental trichostrongylosis in guinea pigs (Rothwell and Dineen, 1972; Rothwell, 1975) and dictyocaulosis in cattle (Weber and Rubin, 1958; Mackenzie and Michel, 1964; Weismann, 1971). Since the challenged animals in these experiments showed a high degree of acquired immunity, an immunologic basis for the secondary eosinophilic response was postulated by several authors. However, the nature of the immune mechanisms which provoke eosinophilia in helminth infections has not been extensively explored.

In recent work Basten and Beeson (1970) have provided evidence that eosinophilic responses to antigens of *Trichinella spiralis* in

rats appear to be lymphocyte dependent. They were unable to show any correlation between the appearance and titer of antibody and the time and degree of eosinophilia, nor were they able to transfer eosinophilic responses with plasma from sensitized animals. Their studies therefore provided no evidence that circulating antibody plays a role in secondary eosinophilia in trichinosis in the rat.

We have previously reported that both primary and secondary eosinophilic responses occur in rats infected with *Taenia taeniaeformis* (Ansari and Williams, 1975). In view of the fact that a remarkable antibody mediated resistance develops in infected rats (Miller, 1932; Campbell, 1936; Leid and Williams, 1974a), we considered the possibility that protective antibodies might be involved in the mechanism of secondary eosinophilia. We report here on the successful stimulation of secondary eosinophilic responses in normal rats passively sensitized with serum immunoglobulin fractions from immune animals.

MATERIALS AND METHODS

Twenty-eight-day-old female Spartan rats [Spb:(SD)BR] (Spartan Research Animals, Haslett, Michigan) were infected with eggs of *T. taeniaeformis* as described previously (Ansari and Williams, 1975). Immune serum was collected either 28 or 35 days later and tested for protective capacity in passive transfer experiments using the procedure described by Leid and Williams (1974a).

Chromatographic fractions of globulins from immune and normal serum were obtained by stepwise elution from DEAE cellulose columns following the method of Musoke and Williams (1975). Pooled fractions under each peak were concentrated to the original serum volume using polyethylene glycol (20,000) and then dialyzed extensively against

phosphate buffered saline pH 7.4 before use in passive transfer experiments. Immunoglobulin concentrations in fractions used for experiments on eosinophilic responses were calculated from the optical density at 280 nm multiplied by a factor derived from the extinction coefficient (Oriol, Binaghi and Boussac-Aron, 1968).

Immunoelectrophoretic analysis of fractions was done in a Gelman apparatus (Gelman Co., Ann Arbor, Michigan) under conditions described by Leid and Williams (1974a). All chromatographic fractions were also tested for activity in the passive cutaneous anaphylaxis test (PCA) (Leid and Williams, 1974b).

Blood for eosinophil counts was obtained by snipping the tail and collecting the sample in a standard white blood cell pipette, after discarding the first 3 drops. The total number of eosinophils/ mm^3 was determined using a direct Speirs-Levy counting chamber (C. A. Hausser & Son Co., Philadelphia, Pennsylvania) in the technique described by Miale (1967).

Eosinophilic responses were followed in groups of six 28-day-old female rats for 2 days prior to and 7 days following passive immunization. Immune serum or immunoglobulin fractions were given intravenously 24 hr before oral challenge with 1000 eggs of *T. taeniaeformis*. One control group received inoculations of fractions of normal serum, but since this was shown to have no effect on the eosinophilic response other control groups were not treated in this way. All rats were killed 1 month after challenge and the livers were examined for developing cysticerci.

RESULTS

Baseline eosinophil counts in normal rats were remarkably consistent and were lower than those obtained in our previous study. The rats in this series of experiments were purchased during late winter months (February-April) whereas those in our previous work on primary infection had been obtained in spring and summer. Whether this difference in eosinophil levels reflects a seasonal pattern is not known, but the low peripheral counts permitted us to measure secondary eosinophil responses in passively immunized rats using small volumes of immune serum (0.5 ml) and relatively low challenge doses (1000 eggs). Basten and Beeson (1970) used 15 ml of plasma per rat in their efforts to passively sensitize recipient animals, and we wished to avoid any stress which might be associated with inoculation of such large volumes.

Samples of immune serum were all tested in passive transfer experiments to determine their protective capacity. Each provided complete protection against a challenge of 1000 eggs. After intravenous administration of 0.5 ml of immune serum challenged animals showed secondary peaks of eosinophilia from 3 to 6 days later. In the first experiment the average value (137 $\pm$ 29.4 S.E.) in sensitized animals on day 4 post infection (p.i.) was significantly different from that in controls (58 $\pm$ 10.1 S.E.) ($P<0.05$, Student's 't' test). Counts for individual rats peaked sharply and then declined (Figure 1), and the daily average values for the group do not illustrate these transient increases. This experiment was repeated, but only 2 of 6 rats showed secondary eosinophilia and the result was not statistically significant ($P>0.05$).

Figure 1. Secondary eosinophilic response in an individual rat (O---O) sensitized with 0.5 ml immune 28-day serum and challenged with 1000 eggs on day 0. The average counts for a group of 6 unsensitized normal rats which also received 1000 eggs on day 0 are indicated by the closed circles (O---O).

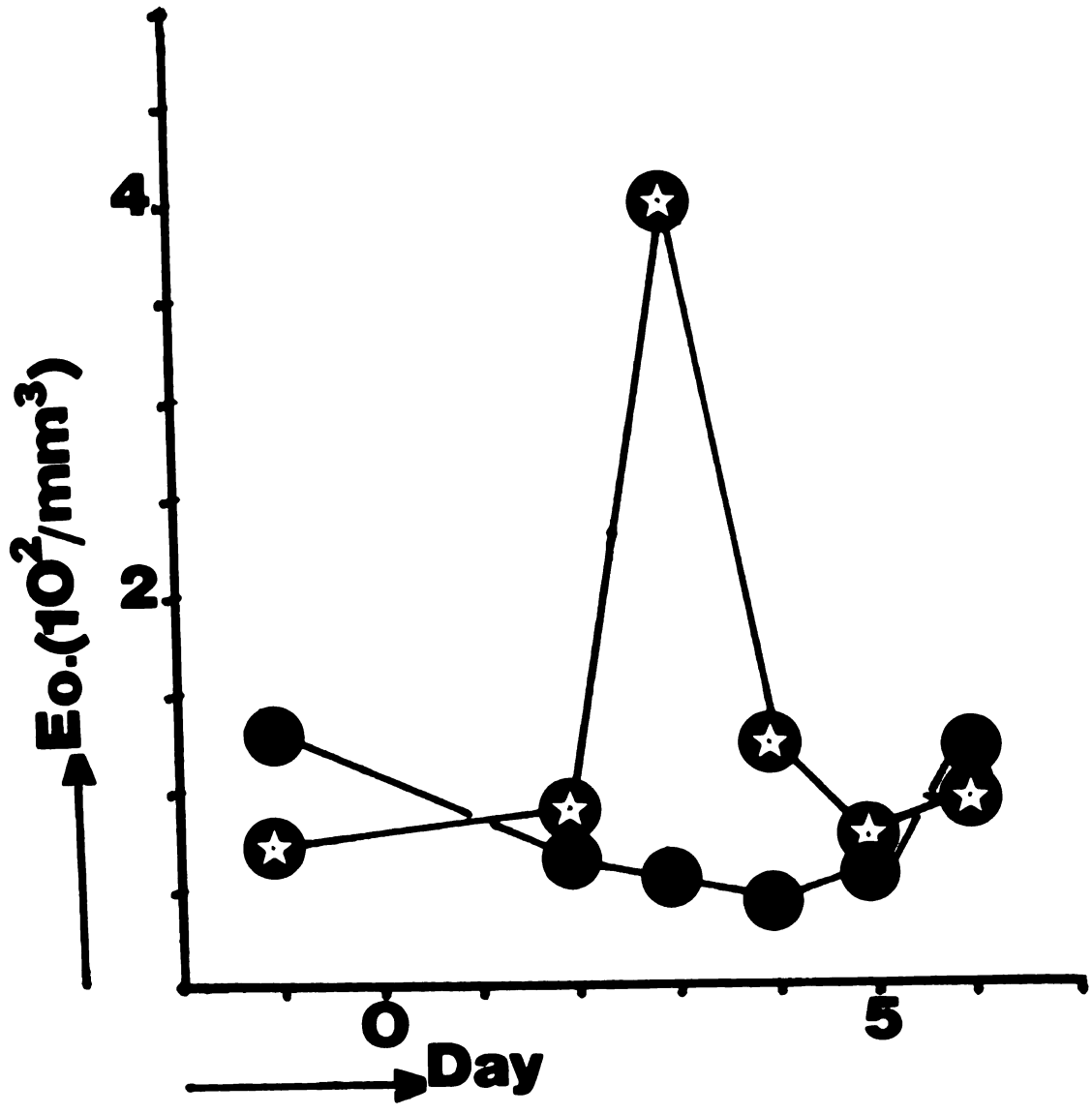


Figure 1

Rats were sensitized with intravenous doses of 300 µg protein of chromatographic fractions of either normal or immune serum globulins. These fractions were eluted from DEAE cellose columns with 0.005 M phosphate buffer (Figure 2) and on immunoelectrophoretic analysis were shown to consist of almost entirely IgG_{2a} immunoglobulins with very slight contamination by other subclasses of IgG_2. The eosinophil counts following challenge in these experiments are shown in Figure 3. The average peripheral eosinophil value for immune IgG_2 sensitized rats (Figure 3B) on day 4 p.i. (218 $\pm$ 35.8 S.E.) was significantly different from that in controls (66 $\pm$ 5.28 S.E., $P<0.005$). No differences were observed in rats given normal IgG_2 preparations prior to challenge (Figure 3A), nor in parallel groups which were given either normal or immune globulins but were not challenged with eggs.

PCA tests were done after a 72-hr latent period and activity was restricted to the immunoglobulin fractions eluted with 0.05 M phosphate buffer. In immunoelectrophoresis this fraction was shown to contain IgG_2 subclasses and IgG_1. Rats sensitized with this fraction showed peaks of eosinophilia 3 to 6 days later and the average on day 4 p.i. (178 $\pm$ 43.5 S.E.) was significantly different from that in controls (66 $\pm$ 5.28 S.E., $P<0.05$). Again individual rats showed sharp peaks as high as 300 eosinophils/mm^3 which immediately fell the following day.

All challenged and control rats were killed 21 days p.i. and those inoculated with immune serum or immunoglobulin fractions were found to have been completely protected against infection. The parasite burden in control rats ranged from 100-145 cysticerci.

Figure 2. Elution profile at 280 nm of DEAE chromatography of globulin fraction of immune 28-day serum. Shaded areas were effective in passive transfer of secondary eosinophilic responses. The fractions eluted with 0.05 M buffer were active in PCA tests.

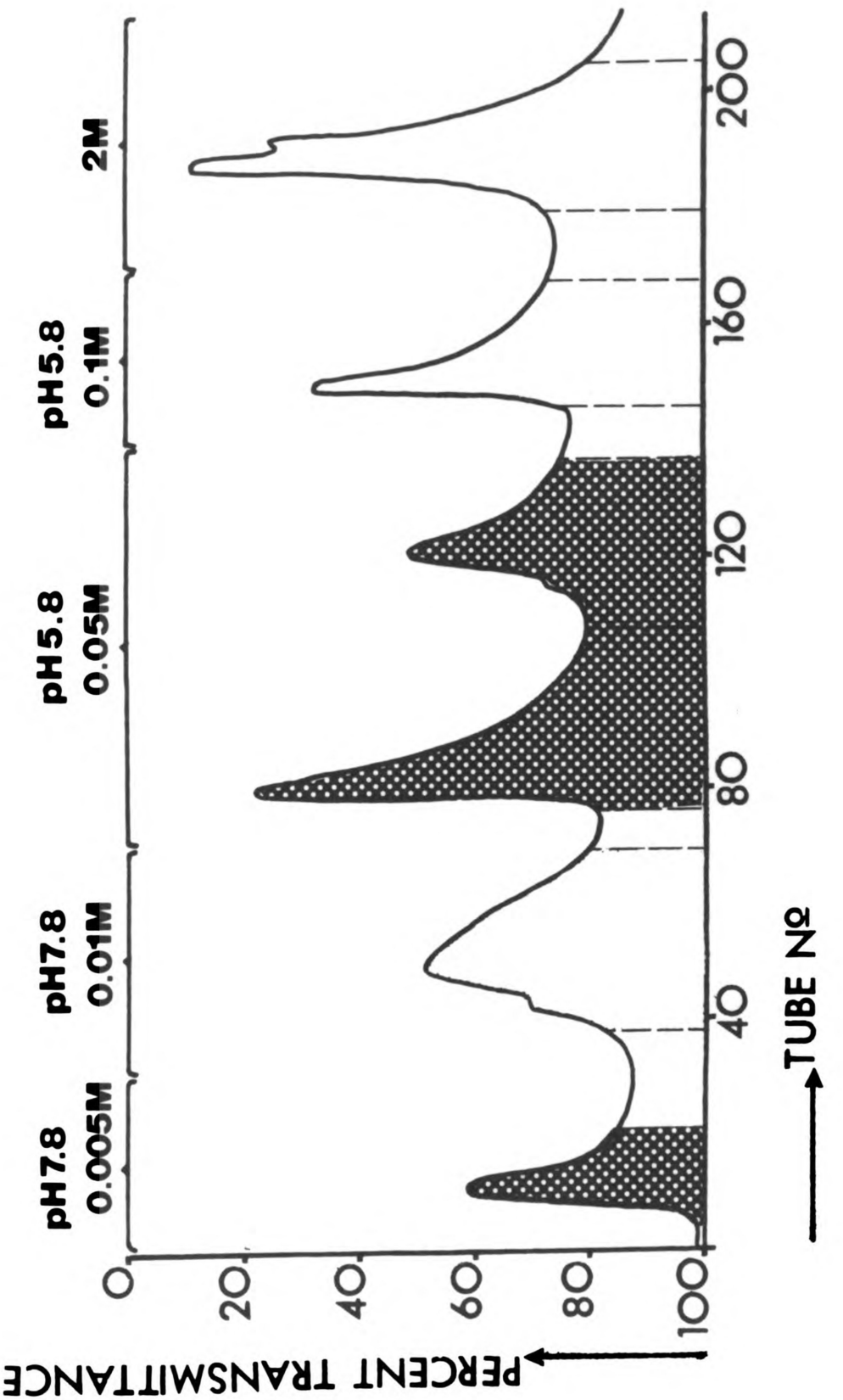

pH7.8
0.005M
pH7.8
0.01M
pH5.8
0.05M
pH5.8
0.1M
2M
PERCENT TRANSMITTANCE
0
20
40
60
80
100
40
80
120
160
200
TUBE Nº

Figure 2

Figure 3. Average peripheral eosinophil values for groups of 6 rats given challenge doses of 1000 eggs on day 0. In 3A rats were sensitized with 0.005 M DEAE fraction of immunoglobulins from normal rat serum (O---O), and normal unsensitized rats served as controls (O--•O). In 3B rats were sensitized with 0.005 M DEAE fraction of immune 28-day serum (O---O) and normal unsensitized rats again served as controls (O---O). Vertical bars represent the ranges for the sensitized groups in each experiment.

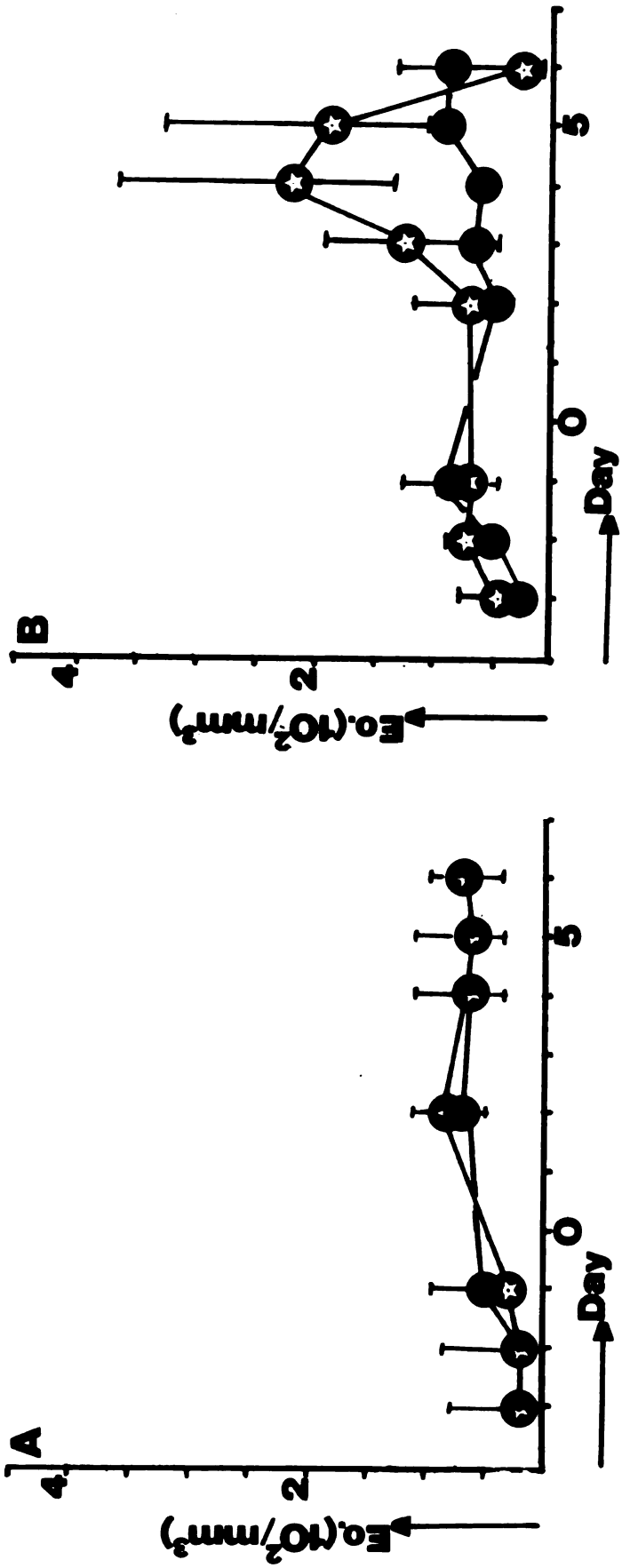


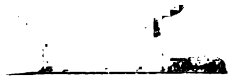
Figure 3

DISCUSSION

Our results indicate that secondary eosinophilic responses to challenge infection with *Taenia taeniaeformis* are at least in part mediated by antibody. Passively transferred IgG_{2a} antibodies, which are known to be highly protective (Leid and Williams, 1974a; Musoke and Williams, 1975), sensitized normal rats for the induction of significant eosinophilic responses after oral challenge. Immunoglobulin fractions containing skin sensitizing reaginic antibodies were also effective, but it is not possible to attribute this to the involvement of reagins since other immunoglobulins were also present. We had anticipated that passive transfer with the IgE-rich fraction might result in more marked eosinophilic reactivity if sensitization of mast cells were important in the mechanism of secondary eosinophilia.

Successful induction of secondary responses with immunoglobulin preparations containing complement-dependent protective IgG_{2a} antibodies suggests that complement-derived factors such as C5A (Kay and Austen, 1972) may be involved in production of eosinophilotactic stimuli. Whether or not locally produced factors are also capable of affecting bone marrow production and release of eosinophils is not known at this time. The single sharp peak of eosinophilia which we observed probably resulted from a transient stimulation of release of cells from the marrow reserve.

Secondary eosinophilic responses in passively immunized rats were not as marked as those which occurred in infected animals challenged with the same dose of eggs (Ansari and Williams, 1975). Individual peaks were generally no higher than 300 to 400/mm^3. This difference was not the result of any deficiency in protective antibody



concentration since none of the passively immunized challenged animals showed any sign of infection at necropsy 3 weeks later. The reduced response may be an indication that mechanisms other than those involving antigen-antibody reactions can contribute to the induction of eosinophilia, and a possible role for specifically sensitized lymphocytes cannot be ruled out. Basten and Beeson (1970) were only able to transfer eosinophilic responses to antigens of *T. spiralis* with lymphocytes. In their system irradiated rats reconstituted with sensitized thoracic duct lymphocytes showed secondary eosinophilic responses after challenge, but recipients given plasma from immune rats did not.

We have recently shown that oncospheres migrate to the liver and survive during the first 24 hr equally well in passively immunized rats and normal rats (Musoke and Williams, 1975). After that time they begin to die in immunized animals although some organisms survive for 48 hr. It seems likely that death of the parasites results from antibody-antigen reactions which stimulate eosinophilic infiltration. Heath and Pavloff (1975) have observed eosinophils infiltrating dead parasites in the livers of passively immunized challenged rats. However, in their experiments challenge doses of 80,000 eggs were used, and the situation is therefore not strictly comparable to our own. In our hands doses of 10,000 eggs are lethal within approximately 1 week and the value of observations made using challenge doses much greater than this is questionable. We are studying the tissue reaction to challenge doses in passively immunized rats in continuing experiments in our laboratory.

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LITERATURE CITED

ANSARI, A., and J. F. WILLIAMS. 1975. The eosinophilic response of the rat to infection with *Taenia taeniaeformis*. J. Parasit. (submitted).

BASTEN, A., and P. B. BEESON. 1970. Mechanism of eosinophilia. II. Role of the lymphocyte. J. Exp. Med. 131:1288-1305.

CAMPBELL, D. H. 1936. Active immunisation of albino rats with protein fractions from *Taenia taeniaeformis* and its larval form *Cysticercus fasciolaris*. Am. J. Hyg. 23:104-113.

HEATH, D. D., and P. PAVLOFF. 1975. The fate of *Taenia taeniaeformis* oncospheres in normal and passively protected rats. Int. J. Parasit. 65:83-88.

KAY, A. B., and K. F. AUSTEN. 1972. Antigen-antibody induced cutaneous eosinophilia in complement deficient guinea-pigs. Clin. Exp. Immunol. 11:37-42.

LEID, R. W., and J. F. WILLIAMS. 1974a. The immunological response of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobulin classes involved in passive transfer of resistance. Immunology 27:195-208.

_____, _____. 1974b. The immunological response of the rat to infection with *Taenia taeniaeformis*. II. Characterization of reaginic antibody and an allergen associated with the larval stage. Immunology 27:209-225.

MACKENZIE, A., and J. F. MICHEL. 1964. Observations on the eosinophil leucocyte response and its specificity after vaccination and infection of cattle with *Dictyocaulus viviparus*. Vet. Rec. 76:1493-1497.

MIALE, J. B. 1967. *Laboratory Medicine: Hematology*, Third Edition. C. V. Mosby Co., St. Louis, p. 834.

MILLER, H. M. 1932. Further studies on immunity to a metazoan parasite, *Cysticercus fasciolaris*. J. Prev. Med. 6:37-46.

MUSOKE, A. J., and J. F. WILLIAMS. 1975. The immunological response of the rat to infection with *Taenia taeniaeformis*. V. Mechanism of action of immunoglobulins involved in passive transfer of resistance. Immunology (in press).

ORIOL, R., R. BINAGHI, and Y. BOUSSAC-ARON. 1968. Preparation d' anticorps monospecifiques anti-immunoglobulines. Annales de l'Institut Pasteur 114:713-726.

ROTHWELL, T. L. W. 1975. Studies of the responses of basophil and eosinophil leucocytes and mast cells to the nematode *Trichostrongylus colubriformis*. I. Observations during the expulsion of first and second infections by guinea pigs. J. Path. 116: 51-60.

_____, and J. K. DINEEN. 1972. Cellular reactions in guinea pigs following primary and challenge infection with *Trichostrongylus colubriformis* with special reference to the roles played by eosinophils and basophils in rejection of the parasite. Immunology 22:733-743.

WEBER, T. B., and R. RUBIN. 1958. The eosinophilic response to infection with cattle lung worm, *Dictyocaulus viviparus*. J. Inf. Dis. 102:214, 218.

WEISMAN, J. 1971. New aspects of eosinophilia in *Dictyocaulus viviparus* infection in cattle. In S. M. Gaafar (Ed.) *Pathology of Parasitic Diseases*. Purdue University Studies, p. 345-357.