CHAPTER ONE

GENERAL INTRODUCTION

Coccidiosis is one of the most commonly diagnosed diseases of chickens despite the use of anticoccidial drugs for its control (Long, 1978, Lee and Onderka, 1978). Nine species of Eimeria are known to parasitize chickens. These species have a characteristic specificity for particular regions of the intestinal tract (Long, 1978; Ryley, 1975).

Chickens infected with Eimeria may show listlessness, a hunched appearance, anorexia, weight loss, decreased weight gain, diarrhea, dysentry and death (Ryley, 1975). The outcome of infection depends on the species of Eimeria, intensity of infection and host resistance. Some species of Eimeria develop superficially in villous epithelial cells, while others develop deep in the intestinal lamina propria. Pathogenicity is generally correlated with such differences in the site of development (Ryley, 1975).

Eimeria necatrix is one of the most pathogenic species infecting chickens and usually produce severe hemorrhagic enteritis in association with the development of second generation schizonts. Lesions are most pronounced in the middle third of the small intestine, close to Meckel's diverticulum but, in heavy infections, they may be present along the entire intestine. Inflammation and lesions usually begin after the migration of crypt epithelial cells harboring second generation schizonts into the lamina propria. Uninfected tissue in the vicinity of cells harboring these parasites seems to be particularly affected. The development of second generation schizonts is accompanied initially by

massive infiltration of heterophils and later by mononuclear leucocytes.

The process of inflammation involves a variety of complex humoral and cellular components as yet incompletely understood. Although inflammation plays a vital role in recovery from infection, pathological changes also occur as a result of these inflammatory responses (Mims, 1977). The proportional contribution of microbial or host factors in inflammation may not be clearly identified. In most infections, direct and indirect types of damage contribute to injury and in a given disease one or the other may be more important (Mims, 1977).

Mast cells, basophils, platelets, neutrophils and to a lesser extent macrophages and lymphocytes contain potent mediators of inflammation (Bainton, Nichols and Farguhar, 1976; Camuss, Mencid-Huerta and Benveniste, 1977; Bainton, 1980, Austen, 1980). An inflamatory stimulus may release, activate or induce synthesis of the mediators, the damaging effects of which, for example lysosomal hydrolases or terminal components of the complement system, can be irreversible. In contrast, certain other mediators such as histamine and prostaglandin may produce earlier and potentially reversible aspects of inflammation (Weissmann, 1975).

The mechanism of tissue damage by avian coccidial parasites has not been investigated in terms of the role of parasite products or infiltrating leucocytes. The purpose of this study was therefore to study the possible pathogenetic role of products of second generation schizonts of E. necatrix. Hypothetically, the leakiness of the plasma membranes of cells harboring second generation schizonts facilitate the diffusion of their own metabolic products and those of the developing

parasite. These products may induce direct or indirect tissue damage. Indirectly, injury may occur by chemotaxis of leucocytes with subsequent release of their lysosomal enzymes which may induce tissue damage. Attempts were made to identify such parasite products using serological as well as chromatographic techniques. The pathological changes associated with the development of E. necatrix, in particular of second generation schizonts, as well as blood leucocyte changes in response to the various stages of its life cycle were also studied.

Since the early development of second generation schizonts was accompanied by massive heterophil infiltration, studies were undertaken to relate this to lesion severity. Since intraperitoneal injection of an irritant induced massive peritoneal heterophilia and leucophilia, this was used in attempts to alter development of enteritis possibly by changing priority of leucocyte migration.

LITERATURE REVIEW

Coccidia belong to the class Sporozoa of the phylum Apicomplexa. The most widely studied genera are, Eimeria, Isospora, Tyzzeria and Wenyonella, many species of which are parasites of veterinary and medical importance (Long, 1978). They are common intracellular parasites of animals and man, develop mainly in the alimentary tract and, when present in large numbers, cause a serious disease called coccidiosis.

Coccidiosis in domestic birds is of major economic importance, usually where intensive methods are used for poultry production (Ruff and Reid, 1977; Long, 1978).

Losses in chickens were reduced after introduction of preventive anticoccidial drugs in the late forties but reduced weight gain, egg production and feed conversion remain problems in chickens with low level infections. In broiler production the losses from morbidity with coccidia have been estimated at 1/2 - 1 cent/lb. According to Ruff and Reid (1977) losses would total between 60-120 million dollars based on an annual production of 3,000,000,000 broilers in the United States alone. In addition the cost of anticoccidial drugs is estimated to be between 35 and 40 million dollars annually (Ruff and Reid, 1977). In the United Kingdom the cost of anticoccidial drugs is estimated to be about 2.9 million Pounds Sterling annually and an estimate of their cost worldwide is 40 million Pounds Sterling for broilers alone (Long, 1978).

Avian Coccidia

Nine genera of coccidia are known to infect birds with the genus Eimeria containing the species of major economic importance (Reid, 1975; Ruff and Reid, 1977).

The main features of members of the genus Eimeria are: (1) the oocysts always contain four sporocysts and within each are two sporozoites; (2) prominent host specificity; (3) no cross protection between different species; (4) marked locational specificity for development within the host (Long, 1978).

The life cycle of typical Eimeria sp. may be divided into three stages: sporogony, schizogony and gamogony. Schizogony and gamogony occur within the host while sporogony usually takes place outside the host, leading to the formation of the infective stages, the sporozoites. All stages of the life cycle are haploid except for the zygote which is diploid.

Eimeria Species Infecting Chickens

Most or all of the nine species of Eimeria known to infect chickens are found worldwide, wherever intensive search has been made (Reid, 1975). They vary greatly in pathogenicity but all are considered pathogenic to some degree. Species determination depends mainly on site of development, morphology, pathological changes and absence of cross protection when one species is tested against another in live birds. Eimeria necatrix will be described in detail as this was the species studied.

Eimeria necatrix, Johnson, 1930

i. Oocyst morphology

The oocysts of E. necatrix are ovoid and measure 13 - 22.7 by 11.3 - 18.3 µm (mean 16.7 by 14.2 µm) (Tyzzer et al., 1932; Pellerdy, 1974). Larger measurements have been reported by Becker (1952), 19.3 by 16.5 µm and even larger by Davies (1956), 20.5 by 16.8 µm and by Edger (1955), 20.4 by 17.2 µm.

The oocyst wall is thin, smooth, and has no micropyle. Sporulation time ranges from 21 - 48 hours at room temperature (Pellerdy 1974; Davies, 1956). Sporocysts are long, ovoid and fill the entire space within the oocyst and, according to Tacla (1967, cited by Pellerdy, 1974), they measure 9 - 15 by 4.5 - 7.4 µm (average 12 by 6 µm). The banana-shaped sporozoites measure 7.9 - 11.3 by 1.3 - 2.1 µm, have large refractile granules and a nucleus close to the pointed end (Mohamed, 1969 cited by Pellerdy, 1974).

ii. Life cycle

The life cycle of E. necatrix will be used to illustrate the life cycle and development of avian Eimeria. Variations in the life cycles between Eimeria species occur in the prepatent period, number of schizogonic generations, and location of the parasites within the digestive tract and other organs eg. the kidneys in E. truncata of geese.

a) Endogenous development

In E. necatrix, endogenous stages were first described by Tyzzer, Theiler and Jones (1932), and later by Davies (1956) and Mohamed (1969, cited by Pellerdy, 1974). The mechanical action of the gizzard breaks

up the oocyst releasing the sporocytes. The action of trypsin as well as bile and high levels of carbon dioxide (Doran, 1962, 1966; Farr and Doran, 1962; Nyberg, Baver and Perdue, 1958) in the small intestine activates the sporozoites which escape from the sporocysts (Long, 1978). Sporozoites penetrate crypt epithelial cells and start to round up by 12 hours (Mohamed, 1969 cited by Pellerdy). It has been reported by Van Doorninck and Becker (1957) that the sporozoites of E. necatrix first invade the lamina propria of the small intestine where most of the sporozoites are engulfed by macrophages and carried to the crypt epithelial cells where the macrophages appear to disintegrate.

The nucleus divides by a process of asexual multiple fission known as schizogony. As the parasite grows, the host cell and its nucleus enlarge and bulge into the crypt lumen (Stockdale and Fernando, 1975). Mature first generation schizonts are seen as early as 60 hours post infection.

At the end of the 3rd day post infection mature first generation schizonts rupture releasing merozoites which penetrate the surrounding crypt epithelial cells and start the second cycle of schizogony. Early second generation schizonts appear in clusters as intracellular inclusions in crypt epithelial cells (Stockdale and Fernando, 1975). Stockdale and Fernando (1975) observed that infected crypt epithelial cells separate from non-infected cells and appear to migrate into the lamina propria, forming nests of second generation schizonts. These schizonts are conspicuously large and usually cause drastic changes in the parasitized cell and its nucleus. At 102 hours post infection, schizonts have many nuclei but they are not mature until the 109th hour at the earliest, at which time second generation merozoites are released

These released merozoites invade epithelial cell in the ceca and, to a lesser extent in the rectum to develop into either third generation schizonts or to gammonts (Pellerdy, 1974).

There is controversy concerning the cell in which second generation merozoites develop. Lee and Long (1972) and Long (1973) suggested that those of E. necatrix and E. tenella develop in fibrocytes of the lamina propria. Stockdale and Fernando (1975) stated that cells infected by the second generation schizonts resemble macrophages functionally and morphologically, but added that the evidence supporting the epithelial nature of these cells was demonstrated by the presence of desmosomes between clumps of infected cells (Bergman, 1970, cited by Stockdale and Fernando, 1975; Fernando, Pasternak, Barrell and Stockdale, 1974). It seems therefore that these infected epithelial cells, through the activity of the parasite are altered almost beyond recognition (Ryley, 1975).

As stated earlier, most of the sexual stages of E. necatrix localize in the ceca; a few of them may be present in the large intestine (Pellerdy, 1974). The microgametocytes (male gametocyte) produce large numbers of biflagellate microgametes which fertilize the macrogametes (female gametes) producing a zygote which form two walls and becomes an oocyst. The oocyst, which is a highly resistant transmission stage, is excreted in feces. The prepatent period of E. necatrix, which is the minimum time required to complete endogenous development, is at least 6 days (Pellerdy, 1974).

b) Exogenous development

The oocysts, once formed, do not develop further until excreted.

Specific environmental conditions such as optimum temperature, moisture and oxygen are required to permit sporulation. The oocysts undergo both nuclear (diploid to haploid) and cellular division resulting in the typical formation of four sporocysts, each containing two sporozoites. For E. necatrix oocysts, the minimum time required for sporulation is 18 hours (Reid, 1975; Pellerdy, 1974). Of the nine species infecting chickens E. necatrix produces the fewest oocysts, per oocyst ingested (Reid, 1975).

iii. Clinical signs

E. necatrix infects chickens mainly at 5-7 weeks of age even though there is experimental evidence of infection in one day old chickens (Pellerdy, 1974). Infected chickens show listlessness, drooping wings, inappetence and dehydration (Mohamed, 1969 cited by Pellerdy, 1974); Reid, 1975). E. necatrix infection causes a higher mortality than any other species except E. tenella. Deaths usually occur on the 5th day, peak on the 7th, and conclude on the 12th day post infection (Reid, 1975). Davies (1956) reported 100% mortality in chickens experimentally infected with 1×10^4 oocysts. Mortality generally starts early in the life cycle (during maturation of second generation schizonts), but signs of morbidity may last for a week longer than with most other species. Chickens that recover from a severe infection appear weak, anemic and emaciated (Tyzzer et al., 1932).

iv. Macroscopic lesions

Macroscopic lesions can not be detected during the maturation of first generation schizonts but appear 2.5 - 3 days post infection after first generation merozoites invade crypt epithelial cells. The peak of lesion severity appears between the 4th and 7th day post infection,

especially in the middle portion of the small intestine close to Meckle's diverticulum (Reid, 1975; Pellerdy, 1974). Early changes which can be seen from the serosal surface include petechial hemorrhages and whitish opacities representing nests of second generation schizonts. Petechial hemorrhages are regularly present and seem to commence within the schizont nests (Tyzzer et al., 1932) which are located deep in the lamina propria accounting for their easy visiblity through the serosa. According to Pellerdy (1974) the bleeding seen in E. necatrix infection always starts from the centre of a schizont nest.

The severity of infection, and of lesion development, depends upon the number of oocysts infecting the chicken. Twenty thousand oocysts or more cause a severe hemorrhagic enteritis during the acute phase, and as the infection dose increases, the lesions are detected earlier (Hein, 1971b). Chickens receiving 8×10^4, 3.2×10^5 or 1.1×10^6 oocysts developed severe lesions on day 3 and in those inoculated with 2×10^4 oocysts lesions appeared on day 4. On day 3 lesions were more severe in the two groups receiving the two higher doses than in the group receiving 8×10^4 oocysts (Hein, 1971b).

Massive dilatation or "ballooning" of the middle part of the intestine is typical of severe E. necatrix infections. In such cases the entire intestinal lumen is filled with clotted blood and fibrin. These changes usually are accompanied by thickened mucosa and very fragile intestinal walls (Pellerdy, 1974).

The changes discussed so far accompany the maturation of the second generation schizonts, while only mild catarrhal inflammation accompanies the development of sexual stages in the ceca.

v. Microscopic changes

No obvious histopathological changes appear during the development of first generation schizonts of E. necatrix. This stage is found in enlarged crypt epithelial cells, and is hard to detect especially in low dose infections. The lesions seen in E. necatrix infection develop in association with second generation schizonts (Tyzzer et al., 1932). Accordingly, the initial changes are localized in and around infected crypt epithelial cells which contain second generation schizonts.

Early second generation schizonts can be found in crypt epithelial cells by 3 days post infection (Stockdale and Fernando, 1975). Inflammatory cells, predominantly heterophils, are seen to infiltrate the area, and can be seen around, between and in the lumen of infected crypts. Later in the infection two layers of epithelial cells can be detected; an outer circle of cells which are parasitized and an inner circle of unparasitized cells lining the crypt lumen (Stockdale and Fernando, 1975). The lumina of many infected crypts are distended by inflammatory exudate and are usually lined by flattened epithelial cells (Stockdale and Fernando, 1975).

Tyzzer et al (1932) recognized that parasitized cells are hypertrophic and have large nuclei with excess chromatin. Stockdale and Fernando (1975) described large gaps in the lamina propria close to the schizont nests and in some cases these gaps were continuous with the lumen of an adjacent crypt. The nature of these gaps is not well understood. The nests of second generation schizonts often develop deeply enough to appose and separate the muscularis mucosa and in many cases rupture the inner layers of the external muscle of the small intestinal wall (Stockdale and Fernando, 1975).

Long (1973) reported that massive infiltration of heterophils, lymphocytes and pyroninophilic cells in the lamina propria and muscularis mucosa accompanies the development of second generation schizonts. Hemorrhages into the lumen of infected crypts are frequent although their origin is not obvious. Stockdale and Fernando (1975) attributed hemorrhage to the rupture of distended capillaries in the lamina propria. According to Tyzzer et al. (1932) hemorrhage is not restricted to the crypt lumen but also occurs in the mucosa, and the villi.

The terminal stages of second generation schizont maturation correspond with the peak of enteritis. At this stage, second generation merozoites are released into the lumen in large numbers together with desquamated epithelial cells, red blood cells and leukocytes (Long, 1973; Pellerdy, 1974).

Following the release of second generation merozoites healing and regeneration occur. A fibrin network intermingled with mononuclear cells replace the destroyed crypts (Tyzzer et al, 1932). According to Stockdale and Fernando (1975) the muscularis mucosa regenerates by the formation of smooth muscle cells. However, Tyzzer et al., (1932) believe that ruptured muscle is replaced by fibroblasts.

The development of sexual stages of E. necatrix in the cecal epithelial cells is accompanied by negligible histopathological changes except for the crowding of gametogenic stages or third generation schizonts within cecal epithelial cells and moderate leukocytic infiltration (Tyzzer et al., 1932; Pellerdy, 1974).

Factors Influencing Pathogenicity

Unlike bacteria or viruses, the coccidia have a predetermined,

self-limiting life cycle, with a more or less fixed number of asexual stages and sexual stages terminating the parasites existence in the host. Despite many studies, the mechanisms by which disease is induced by coccidia are relatively unknown (Ruff and Reid, 1977).

The pathogenicity of coccidia, even within a given species, is variable. Many poorly defined factors, such as the number of oocysts injested, the virulence of particular isolates or strains, environmental factors acting on the parasite, the genotype and immune status of the host, may influence the severity of infection and the outcome of the disease (Fernando, 1981).

1. Oocyst dose

Since the coccidia have a self-limiting life cycle, it is generally accepted that severity of the disease and the oocyst output depend on the numbers of infecting oocysts (Long, 1973; Long and Horton-Smith, 1968).

This is not true of all species, for example Krassner (1963) showed that with E. acervulina, fewer oocysts were produced as the dose of oocysts was increased. The same was observed by Williams (1973) in E. tenella in chickens and by Lotz and Leek (1970) with E. intricata in heavily inoculated sheep. This phenomenon was attributed to the so called "crowding factor" in which insufficient numbers of epithelial cells were available when exceptionally large doses of oocysts were given (Long, 1973). Long (1973) attributed the "crowding factor" observed in heavy infections to the production of interferon or interferon-like substances. Chickens given an artificial interferon inducer were protected against E. maxima infection (Long and Milne,

1971). Fayer and Baron (1971) observed that interferon had no effect on sporozoite penetration but inhibited the intracellular development of E. tenella in cultured cells.

Another explanation for the "crowding effect" offered by Long (1973) is that immunity may commence as a result of the exposure to a massive number of early stages of the parasite and that this immunity is effective against later stages of the life cycle. According to Long (1973) such effects may be operative in hosts infected with Eimeria which have long prepatent periods. In support of this kind of effect, Lotz and Leek (1970) have found degeneration of second generation schizonts of E. intricata of sheep 17 days after heavy infection.

Although many workers have shown that the severity of the disease increases with an increase in the number of oocysts ingested, Leathen and Burns (1968) found that mortality as a result of E. tenella infection was higher in chickens infected with 5×10^4 or 1×10^5 oocysts than in chickens given 10 million oocysts. Fernando (1981) suggested that the establishment of very large numbers of sporozoites and the subsequent development of the early stages produce a host response resulting in loss of some invasive stages, later in the life cycle.

2. Size and site of endogenous stages

Most coccidial species develop within the intestinal epithelial cells of their specific host. Some Eimeria species like E. praecox develop only in the epithelial cells along the side of the villi, while others like E. acervulina develop first in the crypts of Lieberkuhn and later in the superficial epithelial cells of the villi (Long, 1973). In

E. tenella and E. necatrix first generation schizonts develop in the crypts, while second generation schizonts develop deep in the lamina propria. According to Fernando (1981) the species that develop superficially within villous epithelial cells, are less pathogenic than those that develop deeper within the lamina propria. Those species (E. tenella, E. necatrix and E. bovis) which develop deep in the lamina propria tend to have very large schizonts (50 to 80 µm in diameter) and cause disruption of blood vessels leading to hemorrhage into the intestinal lumen (Long, 1978).

The size of endogenous stages is another important factor affecting pathogenicity of Eimeria sp. Stages which are restricted to epithelial cells of the villi, above the host cell nucleus in position and do not exceed 15 µm in diameter may cause little damage because only cells at the tip of the villi are destroyed. E. praecox is a good example of such an infection where only a slight to moderate cellular response accompanies the infection (Long, 1967, 1973). Species like E. maxima and E. brunetti in which the endogenous stages are large (gametocytes) and develop below the nucleus of parasitized villous epithelial cells close to the basement membrane, tend to cause inflammatory responses and greater effects on the host (Long, 1973, 1978).

Those Eimeria species that develop in other organs, such as E. truncata in geese renal epithelial cells and E. stiedae in rabbit biliary epithelium, tend to cause more damage (Long, 1973, Fernando, 1981). In rabbits infected with E. stiedae there appears to be a proliferation of the capillary bile duct epithelium disproportional to the number of coccidia present (Kotlan and Pellerdy, 1936, cited by Pellerdy, 1974). Bachman and Menendez (1930) reported that jaundice is

a feature of liver coccidiosis of rabbits. In E. truncata infection of the goose, the kidneys are infiltrated with numerous inflammatory cells and there is necrosis of the tubular epithelial cells (Long, 1973).

3. Virulence and viability of oocysts

In most species of coccidia the oocysts are discharged from the host in an undeveloped state and must sporulate before they are infective to their host. According to Long (1978) sporulation does not occur below 12°C or above 39°C; the optimum is 28-31°C. Within this range of temperature the rate of sporogony and the number of oocysts which develop normally, depends on the temperature (Kheysin, 1972). In winter or in hot climates sporulation does not occur readily. Low temperatures may cause temporary inhibition of sporulation which is usually resumed when temperatures increase above 12°C but long exposure to cold temperatures (eg. 26 weeks at 4°C) are effective in destroying viability (Long, 1978). Edgar (1954) reported that oocysts exposed to a temperature of 45°C were killed in 24 hours, as were those stored at -12°C for seven days. The effect of temperature has an epizootiological significance for birds kept outside but not for those inside, where temperatures are consistently more than 15°C (Horton-Smith, 1957; Long, 1978). In many species of coccidia the sporozoites form within 2 to 4 days under optimum conditions but a few more days are required for the oocysts to become infective (Kheysin, 1972). For example the oocysts of E. intestinalis and E. magna of rabbit complete sporulation within 3 days, but the same oocysts require 5 days to become infective (Kheysin, 1947b, 1948, cited by Kheysin, 1972). The oocysts of E. maxima complete

sporulation with 27 hours, but require a few more hours to become infective (Edgar, 1954).

The sporulation process cannot occur in an environment lacking oxygen or moisture and oocysts die rapidly under such conditions (Kheysin, 1972; Long, 1973). Horton-Smith and Long (1954) showed that oocysts of chicken Eimeria survive only about three weeks in deep litter and that the number of oocysts in litter are kept more or less constant by the discharge of new oocysts.

It was noted that sporozoites within oocysts lost their energy source, amylopectin (a homogeneous polymer of glucose) completely (Kheysin, 1972) or significantly (Vetterling and Doran, 1969) after long periods of storage at 4°C. Vetterling and Doran (1969) also reported that only a few oocysts that had been stored for 2 years were infective and a dose of 2×10^6 was necessary to produce a patent infection. Oocysts stored for 6 years were not infective at all.

It is not valid to assume that batches of oocyst cultures of the same species, obtained from field cases of coccidiosis are similarly pathogenic when used to infect susceptible animals (Long, 1973). There is experimental evidence that some strains of the same species differ in their pathogenicity (Joyner, 1969; Joyner and Norton, 1969; Shumard and Callender, 1970; Long, 1970). Long (1970) demonstrated that two laboratory strains of E. tenella produced different mortality levels when inoculated to chick embryos. Joyner (1969) reported that of two strains of E. acervulina, the more recently isolated strain had a higher reproductive potential and that the two strains were not immunologically identical. Chickens which were solidly immune to one strain would support a degree of infection with the other.

A wide difference in the relative pathogenicity, as measured by mortality and weight loss, have been found when chickens were inoculated with equal numbers of 14 isolates of E. tenella (Shumard and Callender, 1970). Doran, Vetterling and Augustine (1974) compared infectivity and oocyst production in vitro, and pathogenicity and oocyst production in vivo in three strains of E. tenella (Wisconsin, Weybridge and Beltsville strains). They found that the Wisconsin isolate caused the greatest reduction in weight gain, while the Weybridge strain caused the highest mortality. Most oocysts were produced by the Wisconsin strain and the fewest by the Beltsville strain. In chicken kidney cell cultures, the Wisconsin strain showed the highest percent infection but the Beltsville strain produced the most oocysts. The cecal lesions produced by the three strains were similar (Doran et al, 1974).

4. Host factors

i. Age:

Most farm animals, raised in an environment where exposure to coccidia takes place at an early age, develop immunity to infection which is maintained by reinfection with low doses of oocysts (Long, 1978). This led several early workers to conclude that younger animals are more susceptible to infection with coccidia than their older counterparts (Long, 1973; Fernando, 1981). However, it is well known now that both old chickens and mammals, when raised coccidia-free, are as susceptible or more susceptible than very young animals to a similar dose of oocysts (Long, 1973, 1978; Fernando, 1981).

Hein (1968, 1971b, 1974) reported that young chickens were highly susceptible to the pathogenic effects of E. acervulina, E. necatrix and

E. brunetti. Oocyst production in 6-week old chickens infected with E. acervulina was almost 10 times greater than in 2-week old chickens given a similar number of oocysts but there was earlier growth retardation, longer duration of growth depression and slower recovery in the 2-week old chickens. Using the Packed Cell Volume (PCV) and mean weight as an indicator for the severity of infection, she found that with certain doses of E. necatrix (eg. 5×10^3) the effect was greater in 2-week old than in 6-week old chickens. Mortality tended to occur earlier in younger chickens when the oocyst dose was increased, though all chickens in both groups died from the infection (Hein, 1971b). Similar differences in growth retardation was found in 2 and 6-week old chickens infected with E. brunetti. Mortality in 2-week old chickens increased as the dose of oocysts increased, but was sporadic in the 6-week old chickens (Hein, 1974).

Long (1978) considered the increase in parasite replication in infected chickens as a sign of increased susceptibility. If this were true, then Hein's (1974) results which showed that the reproductive potential (oocysts produced per oocysts inoculated) of E. necatrix was higher in 2-week old than in 6-week old chickens given 0.12×10^4, 5×10^3 and 2×10^4 oocysts, should mean that young chickens are more susceptible at lower doses of infection. On the other hand, Kouwenhoven (1972), working with E. acervulina, showed that oocyst output did not coincide with the increasing pathogenic effect and that there was no correlation between individual body weight, weight loss and oocyst production within groups of chickens of the same age infected with the same number of oocysts. Kouwenhoven (1972) also observed that the older chickens suffered from a longer and more serious weight loss than the

younger birds. These observations contradict those of Hein (1968). Long (1973) concluded that it is preferable to differentiate between age susceptibility as judged by oocyst production and that judged by susceptibility to the pathogenic effect of the parasite. It is, therefore, likely that with the hemorrhagic forms of coccidiosis in chickens (E. tenella and E. necatrix) young birds are more susceptible, but that with other infections older birds are more susceptible (Long, 1973).

Davis, Boughton and Bowman (1955) found that under farm conditions, E. alabamensis was relatively rare in calves 3 to 9 weeks of age and common in those 3 to 9 months of age, while the reverse was true with E. ellipsoidalis. Experimental infection with E. alabamensis resulted in less pathogenic effects in calves under 3 weeks of age than in calves over this age. The resistance of very young calves may be caused by a variety of age-associated physiological differences in the host (Long, 1973).

ii. Breed and sex

Most of the work done on genetic resistance and susceptibility has been with chickens and appears to extend to several species of Eimeria (Long, 1978; Fernando, 1981). Johnson (1927; cited by Jeffers, 1978) was probably the first to suggest that there is a genetic variation in resistance to avian coccidiosis. Later, many investigators found that some breeds of chickens are more susceptible or resistant to one or more species of coccidia (Rosenberg, 1941; Edgar, King and Johnson, 1951; Long, 1968a).

Genetic differences in embryonic susceptibility to E. tenella exist but do not point to the response of chickens from the same mating. This