cross-infection with any of the species studied. However, they found that lesions produced by E. tenella in chickens which had been immunized with E. necatrix were not as severe as they expected. Although very severe lesions were produced in chickens which had been immunized with E. tenella when challenged with E. necatrix. Rose (1967a,b) found partial cross-protection between E. maxima and E. brunetti and also between similar stages of E. necatrix and E. tenella when occupying the same site. The possible immunological relationship between E. maxima and E. brunetti was questioned and reexamined again by Hein (1971a). She showed conclusively that chickens completely immune to E. maxima were fully susceptible to low or high doses of E. brunetti.

It has been shown that immunological specificity of some Eimeria species extends to strains of the same species (Rose, 1978; Jeffers, 1978; Long, 1974; Long and Millard, 1978). Joyner (1969) found that two strains of E. acervulina were not immunologically identical and that chickens which were sqlidly immune to one strain would support a degree of infection with the other. Norton and Hein (1976) compared two laboratory strains (Houghton and Weybridge) to a fresh isolate of E. maxima. Complete immunity towards the homologous strain was obtained but was not sufficient to protect against heterologous challenge.

iii. Immunogenicity of endogenous stages

The complexity of the endogenous stages of Eimeria led to many speculations concerning the role of each stage in the induction of protective immunity. Functional antigens which induce immunity to infection are not present uniformly throughout the various stages of the coccidian life cycle (Rose, 1978). Much of the experimental evidence suggests that asexual stages, particularly the second schizogonic stages

of E. tenella, E. necatrix and E. maxima, are more functionally immunogenic than the sexual ones (Rose, 1976, 1978; Rose and Hesketh, 1976; Horton-Simth, 1949; Kendall and McCullough, 1952). That sexual stages are inefficient inducers of protective immunity has been shown after infection with the second or third generation merozoites of E. tenella, E. necatrix and E. maxima. In the case of E. tenella and E. necatrix, the sexual stages were poorly immunogenic even against their homologous stages (Horton-Smith, Long, Pierce and Rose, 1963; Rose, 1967a; Rose and Hesketh, 1976). The sporozoites do not seem to be very immunogenic either. Long and Millard (1968) found that even after meticlorpindol or methyl benzoquate exerted an inhibitory action on the sporozoites of E. tenella, E. mivati and E. acervulina, for as long as 60 days, infections relapsed on withdrawal of the drug. Even this prolonged contact with large numbers of sporozoites did not induce immunity.

iv. Antigens

Soluble and particulate antigens have been used widely as a tool for detecting serum antibodies and cell-mediated responses (Rose, 1973, 1978). Sporozoites and merozoites have been used as a particulate antigen for simple agglutination tests, lysis, neutralization of invasiveness or as antigen for fluorescent antibody tests (Burns and Challey, 1965; Herlich, 1965; Cerna, 1966, 1967, 1970; Cerna and Zalmanova, 1972; Rose, 1973; Kouwenhoven and Kuil, 1976; Kuil, and Kouwenhoven, Dankert-Brands and Kol, 1977). Soluble antigens have been prepared by extraction of free parasite stages or infected tissue and used for precipitation in liquid or agar gel and in complement fixation tests (Pierce, Long, Horton-Smith, 1962; Rose and Long, 1962; Rose,

1959, 1973). According to Rose (1973) no systematic analysis of the antigens has been carried out but antigens are numerous and probably differ at different endogenous and exogenous stages of the life cycle. Working with liver coccidiosis of rabbits, Rose (1959) demonstrated that antigens obtained from bile on different days after infection showed different precipitation patterns in agar immunodiffusion tests. Many precipitation bands which represent antigens were detected and the greatest number coincided with both schizogony and gametogony.

It has been demonstrated that some antigens were shared by different stages of the same species (Cerna and Zalmanova, 1972; Kouwenhoven and Kuil, 1976), and by different species (Cerna, 1970) and that some even shared determinants with host antigens (Asherson and Rose, 1963; Augustine and Ridges, 1963). Cerna (1966, 1967, 1970) using the fluorescent antibody test demonstrated circulating antibodies to coccidial antigens in rabbits, mice and chickens infected with various Eimeria species. Using the indirect fluorescent antibody test, Cerna (1970) studied the cross-reaction of 4 species of coccidia; Eimeria tenella of chickens, E. stiedae and E. magna of rabbits and E. pragensis of mice. Common antigens were detected between merozoites of the two rabbit species but either no reaction or a reaction, only with low dilutions of the antisera was found between the other species studied. Kouwenhoven and Kuil (1976) tested antisera prepared in chickens by oral immunization and in rabbits by subcutaneous inoculation of sporozoites in Freund's Complete Adjuvant. In both antisera, fluorescence was never associated with the first generation schizonts and gametocytes but sporozoites and second generation schizonts gave positive results. Rose (1978) attributed the failure of chicken immune

serum to react with gametocytes to the poor immunogenicity or the lability of their antigens. The results obtained when rabbit antisera were used in the test showed that sporozoites do not have antigens in common with gametocytes.

Crude soluble antigens from different stages and from infected tissue have all failed to induce protective immunity (Rose, 1978).

2. Factors affecting host immune response

A certain degree of resistance to reinfection of the host is manifested as a reduction in parasite multiplication and pathogenic effects (Rose, 1978). She postulated that the outcome of reinfection is determined by a number of factors which influence the immunological response of the host. These factors are:

i) number of oocysts

ii) mode of inoculation

iii) immunosuppression by other infections

iv) immunogenicity

The immunogenicity of the species involved was discussed earlier (page 38).

i. Number of oocysts

Rose (1973) found that infection with small numbers of oocysts (subclinical infections) resulted in immunity to a massive challenge dose. However, Mukkur and Bradley (1969) found that the greater the number of E. tenella oocysts inoculated, the greater the immunity to challenge. On the contrary, Hein (1968) reported that a high level of resistance against reinfection was obtained by two low doses ($8x10^4$ and $1.6x10^5$) of E. acervulina oocysts while chickens given 3.2×10^5

and 6.4×10^5 oocysts at the same time intervals did not appear as effectively immunized.

According to Rose (1978) factors which may reduce parasite multiplication during primary infection such as anticoccidial drugs or enteric infections which disturb the intestinal epithelium, will lower the stimulus and therefore the immune response.

ii. Mode of oocyst inoculation

The mode of oocyst administration has a marked effect on immune response and protection against subsequent infection (Babcock and Dickinson, 1954; Joyner and Norton, 1973, 1976; Long and Millard, 1977; Rose, 1978). Babcock and Dickinson (1954) reported that immunity to E. tenella developed after the administration of 1.6×10^3 oocysts whether the dose was given as a single inoculum or given over a period of 2 to 5 days. On the contrary, Joyner and Norton (1973) showed that the immunity developed by the daily administration of a very low number of oocysts over a period of time (5/day for 28 days) was significantly greater than that developed in chickens receiving the same total number of oocysts in a single immunizing dose. Similar results were obtained by Joyner and Norton (1976) using daily inoculations of 1 or 5 oocysts of E. maxima and 5 or 20 oocysts of E. acervulina for 20 days and 25 days respectively. According to Rose (1978), comparison between the low "trickle" and single inoculations is difficult because the intervals between immunization and challenge inocula is different. Continuous exposure to low grade infection is regarded to be more comparable with the mode of immunization under field conditions (Joyner and Norton, 1976; Rose, 1978). The natural mode of immunization under field conditions was studied more closely by keeping chickens in litter pens

(Long and Millard, 1977). In some pens all chickens received single immunizing inocula while in other pens uninoculated chickens were placed with seeder chickens to determine whether they could become immunized by exposure to natural infection. These results showed that good protective immunity developed even with the poorly immunogenic E. necatrix. Long and Millard (1977) suggested that the effective immunization obtained in the chickens kept in litter pens was achieved by reinfection with small numbers of oocysts which enhanced the immune response.

iii. Immunosuppression by other infections

Infections which interfere with the immune system of the chicken will affect the immune response to coccidial infection (Rose, 1978). Under field conditions two viral diseases, Marek's disease and infectious bursal disease, are known to interfere with the immune system of the fowl and consequently impair their immunity to coccidial infection (Biggs, Long, Kenzy and Rootes, 1968; Anderson, Reid, Lukert and Fletcher, 1977; Rose, 1976, 1978; McDougald, Karlsson and Reid, 1979). Chickens infected with Marek's disease virus have been shown to be more susceptible to primary and subsequent infection as judged by oocyst production with E. mivati and E. maxima (Biggs et al, 1968). This probably explains the well documented association between infection with Marek's disease virus and field outbreaks of coccidiosis (Randall, Grant and Sutherland, 1971). Rice and Reid (1973) compared the development of immunity to E. tenella and E. maxima in chickens exposed to Marek's disease virus at 1 day and 4 weeks of age. Chickens were challenged at 35 days of age with 1×10^5 oocysts of E. tenella or 2×10^5 oocysts of E. maxima. Their results showed that immunity to

coccidia was increased by delaying exposure time to Marek's disease virus. Similarly, chickens infected with infectious bursal disease virus prior to immunization with E. tenella showed significantly less protection than chickens given the virus after 14 days of coccidial immunization (Anderson et al., 1977).

3. Effect of Immune Response on the Parasite

According to Rose (1978) the effect of the host immune response on parasite development is most easily examined when immunity is complete, indicated by the termination of the life cycle before oocyst production and by the absence of pathogenic effects. When immune rabbits were challenged with E. stiedae normal excystation occurs and sporozoites penetrate the epithelial cells but further development was not found (Horton-Smith, Long, Pierce and Rose, 1963a). Similarly, in chickens completely immune to E. tenella, excystation and penetration of sporozoites occurred normally and some were found in the crypts 24 hours later but no nuclear division or first generation schizonts were found (Horton-Smith, Long and Pierce, 1963b). Leathem and Burns (1967) have shown that E. tenella sporozoites recovered from the host cells of immune chickens within 24 hours after infection were capable of infecting a susceptible host. Rose and Hesketh (1976) have shown that the sporozoites of E. maxima were also inhibited in immune hosts and that this inhibition was reversible within 38-48 hours if the sporozoites were transferred to a non-immune environment. On the other hand, total and irreversible inhibition of sporozoites in the immune host was found later than 72-88 hours post-infection (Rose and Hesketh, 1976).

The inhibition of the parasite in the immune host is not confined to the sporozoites. Second generation merozoites inoculated through the cloaca or directly into the ceca invaded the cecal epithelial cells but failed to grow and were not detectable 30 hours after infection (Horton-Smith, et al., 1963b).

In the case of the very immunogenic species, E. maxima, a solid immunity terminates the life cycle before oocyst production (Rose, 1978). In the less immunogenic species like E. mivati, a successive reduction in the numbers of oocysts follows sequential challenges although oocyst production shows a similar pattern to that seen in a primary infection (Rose, 1978).

4. Effector mechanisms

i. Specific antibodies

McDermott and Stauber (1954) were the first to demonstrate agglutination of second generation merozoites of E. tenella using serum from recovered, orally infected chickens or chickens immunized with merozoite suspensions. Later, specific antibody response to coccidial infection was detected by many investigators using different serological methods which included agglutination, dye exclusion, precipitation, complement fixation test, immunofluorescence and lysis, immobilization, and neutralization of the invasive stages (Heist and Moore, 1959; Rose, 1959, 1961; Pierce, Long and Horton-Smith, 1962; Rose and Long, 1962; Augustin and Ridges, 1963; Long, Rose and Pierce, 1963; Andersen, Lowder, Hammond and Carter, 1965; Burns and Challey, 1965; Herlich, 1965; Cerna, 1966, 1967, 1970; Cerna and Zalmanora, 1972; Morita, Tsutsumi and Sockawa, 1972; Abu Ali, Movseijan, Sokolic and Tanielian,

1976; Kouwenhoven and Kuil, 1976; Kuil et al., 1977; Davis, Parry and Portor, 1978).

According to Rose (1978) antibodies are usually detected in the serum within a week after infection, peaking shortly thereafter and then are no longer detectable, although the chickens remain resistant to subsequent infections. Using the double immunodiffusion test, Rose and Long (1962) have shown that resistance to reinfection with E. maxima, E. necatrix, E. tenella and E. acervulina was accompanied by the development of circulating antibodies in chickens. Although the double immunodiffusion test is neither sensitive nor quantitative, it was possible to demonstrate that there were marked differences between the four species studied. The highly immunogenic species E. maxima induced an earlier and higher concentration of antibodies, while with E. necatrix, which is the least immunogenic, the response was delayed and slight (Rose and Long, 1962; Rose, 1978). Rose and Long (1962) also observed that challenge infection did not usually enhance antibody production in chickens which were completely immune and had no detectable serum antibodies. Similar results were obtained with E. stiedae infections in rabbits (Rose, 1959, 1961). It was suggested that, where resistance is complete, the parasite fails to establish itself. This lack of an antigenic stimulus may be responsible for the absence of a secondary antibody response (Rose, 1978; Rose and Long, 1962). Although circulating antibodies did not show a secondary response, Davis et al. (1978) demonstrated that the immunoglobulin profile of cecal contents, for primary and secondary infection with E. tenella, resulted in elevated levels of IgA, although IgG and IgM generally remained very low or were undetectable.

Abu Ali *et al*. (1976) found that the antibody response in chickens to *E. tenella* oocysts given orally was better than that of subcutaneously inoculated birds. They also reported that serum antibody titres from orally infected chickens tended to decrease 14 days post infection irrespective of a second infection but that subcutaneous inoculation followed by subsequent oral infection resulted in a dramatic increase in serum antibody titres.

According to Rose (1974b) the mode of action of protective circulating antibodies against coccidial infection is unknown. As stated earlier, circulating antibodies which lyse and agglutinate the invasive stages have been demonstrated but their presence does not necessarily correlate with nor is a prerequisite for immunity (Rose, 1973, 1978). These antibodies are an indication of developing or recently acquired immunity; once this is established they are usually undetected (Rose, 1978). Despite the similarities of antisera produced by oral infection or by parenteral injection of non-viable parasite antigens, the latter did not protect animals from oral infection (Rose, 1961; Horton-Smith *et al*, 1963b). They were, however, effective in protecting against an intravenous challenge infection with sporozoites of *E. tenella* (Long and Rose, 1965). Rose (1974b) showed that the *in vitro* test which correlates best with protective activity of circulating antibodies to *E. maxima* is the neutralization of the invasive stages.

Rose and Long (1969) and Rose, Long and Bradley (1975) showed an increase in gut permeability to macromolecules in chickens immune to *E. acervulina*, *E. maxima* and *E. praecox*. The increased permeability in immune chickens early after challenge represents a mechanism by which