penetration of fresh cells by merozoites may cause leakage of the plasma proteins required for antigen-antibody complex formation, complement activation and the generation of its chemotactic fragments.

The displacement of infected crypt epithelial cells through crypt basal lamina into the lamina propria was observed during light and electron microscopic examination of infected tissue (Figs. 2-12, 2-13). The mechanism by which these infected cells pass through crypt basal lamina is not well understood. Basal lamina are ubiquitous structures separating epithelial or endothelial cells from underlying connective tissues. They are composed of collagenous as well as non-collagenous glycoproteins (Uitto, Schwartz and Veis, 1980). The collagen unit of the basal lamina has a unique structure which differs from that of interstitial collagens and is resistant to the action of fibroblast collagenase that cleaves other types of collagens (Woolley, Glanville, Roberts and Evanson, 1978; Liotta, Abe, Robey and Martin, 1979). According to Uitto and his coworkers (Uitto et al., 1980) no enzyme has been found that could initiate the degradation of basal lamina collagen in normal tissue under physiological conditions. An enzyme which can degrade some part of basal lamina collagen has been reported in murine tumor cells (Liotta et al., 1979). On the other hand, leucocytes have the ability to penetrate basal lamina in vivo, and the injection of leucocyte extracts into animal tissue has been found to damage their basal lamina (Janoff and Zeligs, 1968). During the development of E. necatrix, heterophil infitration towards the crypt lumen precedes migration of infected cells out to the lamina propria. However, it is not clear whether heterophil penetration breaks crypt basal lamina and facilitates the migration of infected crypt cells or whether infected

infected cells themselves have the ability to break up the basal lamina.

Heterophil numbers increased dramatically during severe infections with E. necatrix and coincided with destruction of tissue and hemorrhage observed during the development of second generation schizonts. The role of heterophils in tissue damage during acute inflammatory reactions in avian species is not well known compared to that of mammalian neutrophils. Anti-leucocyte serum has been used successfully in many species to elucidate their role in tissue damage and their importance in immunological phenomena (Cochrane, 1977, Simpson and Ross, 1971; Mahmoud, Warren and Boros, 1973; Mahmoud, Warren and Peters, 1975; Brochier, Abou-hamed, Gueho and Revillard, 1976; Reuben, Sundaram and Phondke, 1978; Edwards, 1979). Thus it was postulated that anti-heterophil serum may answer some of the questions concerning the role of heterophils in tissue damage seen in E. necatrix infections, including the breakup of the basal lamina of crypt epithelial cells. If the antisera used reduced the numbers of circulating heterophils, further procedures would have been used to obtain a more ptent and monospecific antiserum to evaluate the role of heterophils in E. necatrix infection. However, in this study anti-heterophil serum prepared in rabbits failed to reduce the number of circulating heterophils, regardless of the route of administration. The part played by heterophils in tissue destruction could, therefore, not be studied.

In the study of leucocyte response to E. necatrix infections, an increase in the numbers of circulating eosinophils was the most controversial change observed (Fig. 2-22). The pattern of eosinophilia reported here is similar to that reported with E. tenella infections

(Natt, 1959). On the other hand, Rose et al. (1979a) did not observe such an increase in eosinophils when they studied the peripheral blood leucocyte response to E. maxima of chickens and E. nieschulzi of rats. According to Rose et al. (1979a) eosinophilia in coccidial infection has not been confirmed for E. tenella. In addition, it was not found in a variety of mammals inoculated with Eimeria species (Heil, 1974, cited by Rose et al., 1979a). Nair (1973) observed no local eosinophlic response when he injected live larvae of Ascaris suum and Toxocara canis in chickens. Accordingly he stated that eosinophil numbers are rarely increased in chickens by stimuli which are effective in mammals. In addition, he suggested that the lack of reports regarding the participation of eosinophils in inflammatory conditions of birds may be due to the difficulty of their definite identification in tissue sections with the light microscope. In the study reported in this chapter, there was little difficulty in differentiating eosinophils and heterophils in blood films stained with Wright's stain. The cytoplasmic granules of eosinophils were light pink, round and small and, those of heterophils were deep pink, larger and spindle-shaped. In addition, eosinophils had light grayish-blue cytoplasm and prominent nuclei (Figs. 2-23, 2-24).

The similarity in the eosinophilic response between E. tenella and E. necatrix may reflect the similarity in the pathology and pathogenesis of these two species of chicken Eimeria. The increase in eosinophil numbers in both species occurred after the 9th day post infection and, in the case of E. necatrix, it was dose dependent. The higher the number of oocysts used for inoculation the higher was the eosinophilic response (Fig. 2-22). The eosinophilia coincided with the sexual rather

than the asexual stages of E. necatrix as observed in E. tenella by Natt (1959). It has been noticed that eosinophilia resulted when antigenic material too large to pass through a capillary bed was processed by infiltrating inflammatory cells and not by fixed tissue macrophages (Beeson, 1980). Such material may be provided by sexual or later developing asexual stages of E. necatrix. Stockdale and Fernando (1975) have reported what they called "ghosts" of second generation schizonts of E. necatrix trapped in the lamina propria at day 6 and 7 post infection. Since both E. necatrix and E. tenella are similar in their extreme pathogenicity and development deep in the lamina propria of second generation schizonts, in both cases the mechanism(s) responsible for eosinophilia may be the same. On the other hand, the differences in the eosinophil responses between E. necatrix and E. maxima may reflect the dissimilarity in the pathogenesis of these two Eimeria species.

Changes in peripheral blood leucocytes in chickens infected with Eimeria species have been reported (Natt 1959; Rose et al. 1979a). Rose et al. (1979a) reported a biphasic increase in total leucocyte response to E. maxima. In the case of E. necatrix infection, no such response was observed with the dose of oocysts used in this study. The statement by Natt (1959) that during the first 6 days post infection with E. tenella the total leucocytes remained within normal range was perhaps made because the leucocyte counts of his control chickens were inconsistent during this period. It is believed that total leucocyte response to E. necatrix would also be biphasic if higher inoculation doses were used in this study (see Chapter 3 Fig. 3-6).

The responses of leucocyte other than eosinophils to E. necatrix infection were comparable to those observed after E. maxima (Rose et

al., 1979a) and E. tenella infections (Natt, 1959). Lymphopenia was observed with E. necatrix between day 6 and 8 post infection, similar to the results obtained with E. maxima (Rose et al., 1979a). The lymphopenia in the case of E. tenella infections was more evident between day 5 and 6 post infection. The changes in heterophil numbers reported by Rose et al. (1979a) were biphasic too. This may have been responsible for the biphasic response of the total leucocytes. Such a response was not very obvious with the dose of E. necatrix oocysts used in this study. On the other hand, a higher dose (150,000, Chapter 3, Fig. 3-7) produced a very marked increase of heterophils at day 5 post infection.

A true assessment of the leucocyte response and its role in tissue damage seen in an acute inflammatory response would be complete and more meaningful if accompanied by differential leucocyte counts at the local level, eg. the intestine in the case of E. necatrix. Histological sections usually revealed a prominent heterophil infiltration around early second generation schizonts at day 3 post infection. At the peripheral blood level this was not seen. The opposite was found at day 5 post infection where, at the local level heterophil infiltration was not prominent. It seems that the bone marrow is stimulated throughout the infection but the number of leucocytes in circulation does not reflect the actual changes in the numbers of these leucocytes. The same may be true of other leucocytes, especially the lymphocytes, during the lymphopenia stage.