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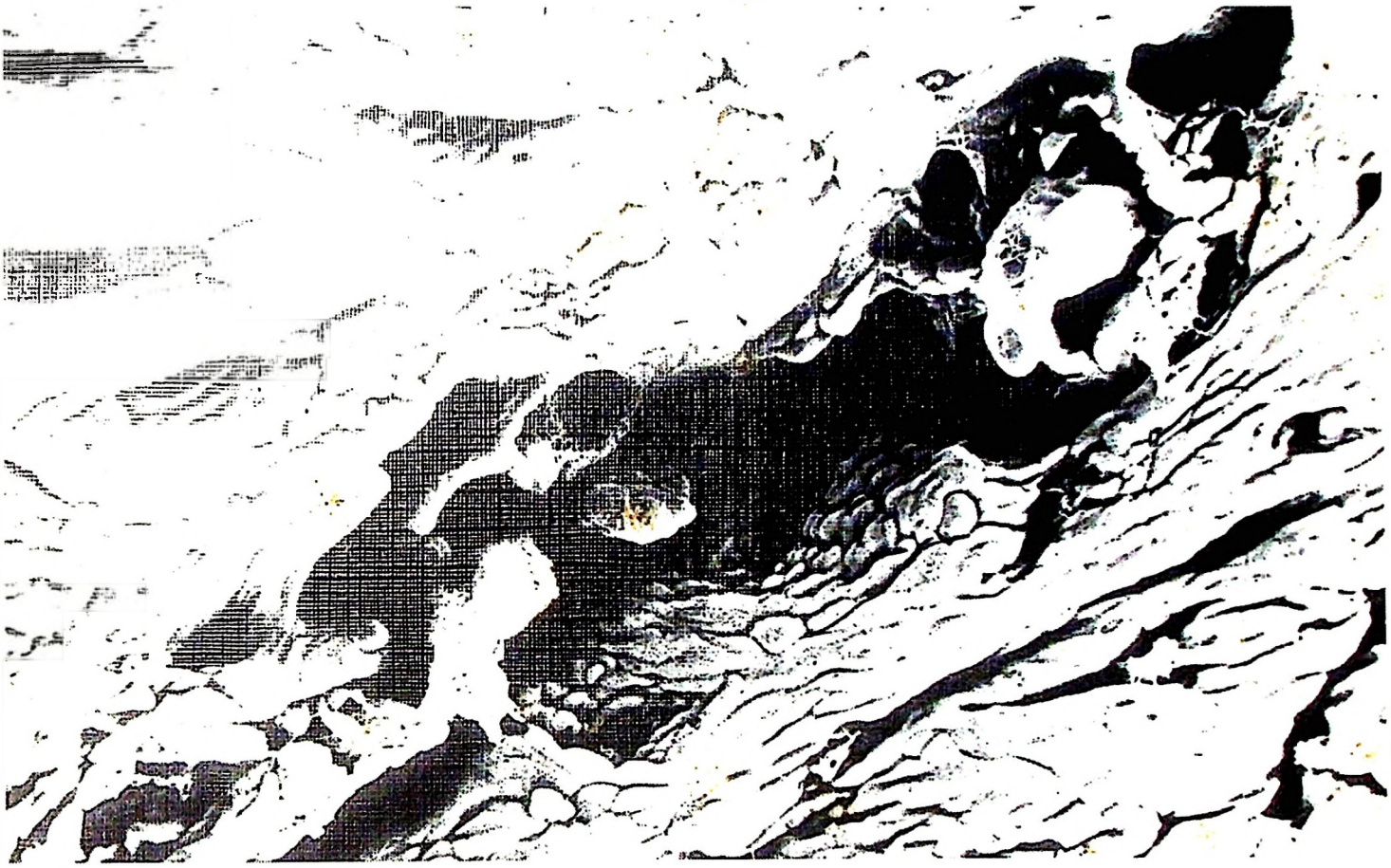
A light and electron microscopic study



Ph.D. Thesis

WILLIAM D. SEMUGURUKA

Department of Pharmacology and Pathobiology
Royal Veterinary and Agricultural University
Copenhagen, Denmark, 1992

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Illustration on cover: Schistosome eggs emerging through and near glandular pits of colonic mucosa in a hamster.

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CONTENTS

1. INTRODUCTION

Schistosomiasis in livestock is recorded in many parts of Africa and appears to be widespread and endemic in northern, eastern and southern Africa (Hussein, 1968, Majid, 1980, Malek, 1969, Dinnik and Dinnik, 1965, Lawrence, 1978c). Although the disease is usually of a chronic nature in most livestock, there have been reports of acute infection, sometimes in outbreak proportions, among cattle in Sudan (Eisa,1966; Hussein, 1968), Zimbabwe (Lawrence, 1976, 1977d) and South Africa (Reinecke, 1970; Van Wyk *et al* , 1974). In a mainly slaughterhouse survey in Tanzania, Dinnik and Dinnik (1965) recorded infections in cattle of between 31-50% in northern, central and southern parts of the country. In a more recent epidemiological study in one region in Tanzania, Kassuku *et al* (1986) recorded a 30.8% prevalence rate of infection in cattle.

The veterinary importance of livestock schistosomiasis, in particular that in cattle and to some extent also in sheep and goats, is beginning to be realized. This is not only because it can cause deaths under conditions favorable for heavy infections but also because of lowered growth rates and productivity in its often chronic form (Dargie, 1980; Lawrence, 1977d; McCauley *et al.* 1983, Saad *et al,* 1980). In some countries like Tanzania, the importance of the disease is probably overshadowed by diseases such as East Coast Fever, trypanosomiasis and fascioliasis which are endemic in the same areas where schistosomiasis occurs. Furthermore, owing to the similarity of some of the clinical signs characteristic of these diseases and schistosomiasis, diagnosis in favor of these other diseases is likely thus lowering the recorded incidence of schistosomiasis. However, with increasing need for cattle productivity and the changing pattern in livestock management that make greater exposure to challenge by schistosomiasis possible, the disease is bound to gain more veterinary attention in Tanzania as it has in a country like Sudan.

The most important species responsible for bovine schistosomiasis in Africa are *Schistosoma bovis (S. bovis)* (northern and eastern Africa, north of 10°S) and *Schistosoma mattheei (S.mattheei)* (southern Africa, south of 10°S) (Dinnik and Dinnik,1965, Christensen *et al*,1983). In Tanzania, particularly in the southwestern part of the country, *S. bovis* and *S. mattheei* coexist although *S. bovis* is reported to be the dominant species.

The life cycle of *S. bovis* and *S. mattheei* is probably similar. That of *S. bovis* has been closely studied by Lengy (1962). The adult worms live in the mesenteric and portal veins in the definitive host (cattle, probably sheep, goats and other ruminants). *S. bovis*, like other schistosomes, is a digenetic trematode and to complete the life cycle the mature miracidium in the egg must reach the intermediate host, a fresh water snail (in Tanzania either *Bulinus africanus* or *Bulinus truncatus*). To reach the intermediate host the eggs must cross a venule/vein wall, the intestinal lamina propria and the intestinal epithelium into the intestinal lumen and be excreted with the feces into the snail habitat.

The pathogenesis, pathology and pathophysiology of the disease in the final host has been much studied in human schistosomiasis mansoni (reviewed by Warren, 1973). It is well established that the egg stage of the life cycle is responsible for the major clinical and pathological changes in human and animal schistosomiasis (Warren, 1973).

Although research in animal schistosomiasis has tended to lag behind that in the human disease, some studies have now been done on the clinical manifestations, pathogenesis, pathology, and pathophysiology of *S. bovis* infection in cattle and other domestic animals.(Dargie, 1980; Hussein, 1971; Hussein *et al*, 1975, 1976; McCauley *et al*,1983; Saad *et al* 1980, 1984). Similar work has been done with *S. mattheei* in cattle and sheep (Lawrence, 1978c). In all these studies in cattle the involvement of the egg in the development of disease in the major target organs, the liver and the intestines, has been established. As for the adult worms, it is the dead ones that cause significant pathological changes.

Pathological studies in schistosomiasis have paid great attention to the liver. However, changes in the alimentary tract, particularly the intestines, have been studied to explain the clinical signs such as diarrhoea, which is sometimes bloody, anemia, and loss of body condition that develop especially on primary exposure to infection (Dargie, 1980; Hussein,1971; Hussein *et al*, 1975; Saad *et al*, 1980; Lawrence, 1976, 1977b, 1977d, 1978a). Biochemical, nutritional and radioistope studies have been undertaken to establish the clinicopathology and pathophysiology of *S. bovis* primary infections (Saad *et al*, 1980, 1984). Similar studies with *S. mattheei* have been undertaken in cattle and sheep (Dargie, *et al*,

1973, 1977; Lawrence, 1977b; Taylor *et al*, 1977). Dargie (1980) has used data from some of these studies to summarize the pathogenetic mechanisms believed to be responsible for the diarrhoea, anemia and loss of body condition and productivity.

As already mentioned, the egg in the intestinal wall plays the major role in the development of bovine intestinal schistosomiasis. The hemorrhage and loss of body fluids are considered to occur across the intestinal wall. The intestines probably inefficiently allow nutrients to be absorbed. However, the precise location and nature of some of these changes have not been directly established. Pathomorphological studies have also been undertaken at the light microscopic level by many investigators. Although informative, the studies have not fully explained the basis of some of the lesions and clinical observations. Supportive studies at the electron microscopy level that might have shed light on the pathogenesis of intestinal schistosomiasis due to *S. bovis*, are lacking.

Intestinal lesions occur as the eggs emigrate through the intestinal wall to the exterior and because of eggs which are trapped in the intestinal wall. Some of the pathological changes observed are also probably the mechanisms by which the eggs are excreted in the feces. Studies on the mechanisms of egg excretion have been going on for many years. Doenhoff *et al* (1986) have recently partly reviewed the studies done so far but comprehensive reviews of earlier works are given by Kuba (1963), Bruijning (1968) and Warren (1973).

Many hypotheses have been put forward by various investigators. However, many of the proposals do not appear convincing as explanations of the precise way(s) the eggs migrate from the intravascular position to the exterior. Doenhoff *et al* (1978, 1986) and Dunne *et al* (1983) have demonstrated that egg excretion is an immunologically dependent mechanism which involves both cell-mediated, and, later during the cause of infection, humoral responses. Doenhoff *et al* (1986) have also claimed that the liver granuloma which is T-cell dependent for its development, plays a central role in the excretion of the egg although it is not clear how this is accomplished. The authors further note that inflammatory thrombi can form around eggs intravascularly and that these may help rupture the endothelium allowing the eggs to penetrate into the adjacent tissues from which the eggs reach the intestinal lumen by help of peristalsis.

Ngaiza *et al* (1990) support the concept of immune dependency of egg excretion. They have demonstrated that blood platelets assist in the extrusion of the egg from the intravascular location and indicate that in early egg excretion there is reversible thrombocytopenia, that substantial fibrin formation is not induced and that thrombosis is avoided through various modulation mechanisms. Doenhoff *et al* (1986) and Ngaiza *et al* (1990), both observed an increase in tissue load and reduced fecal egg excretion in mice deprived of T-cells or platelets, but did not state whether the eggs are retained entirely or mostly within the blood vessels. The possible interaction of the effector cells of the vascular walls with the eggs is not known, and the mechanisms by which the eggs penetrate to the extravascular space are not yet clarified.

From previous studies on mechanisms of egg excretion (Kuba, 1963; Bruijning, 1968; Warren, 1973) it is apparent that investigators have observed different cells and noncellular material around the intravascular eggs as well as different reactions to their presence. The different observations may be related to different hosts studied and different species of parasites used in the investigations.

Excretions/secretions by eggs and their role in the emigration of the eggs from the host body are strongly advocated (Kuba, 1963; Warren, 1973). Kloetzel (1967) has shown a collagenase-like substance secreted by eggs. Smith *et al* (1971) have found and localised in the miracidium compounds which disrupt plasma membranes in similar manner to phosphatides. However, it is still unclear whether egg products participate in the *in vivo* migration of the eggs and what specific lesions they may actually produce.

Egg secretions and peristalsis are said to facilitate egg migration from the extravascular position to the intestinal lumen (Warren, 1973). However, in some definitive hosts many eggs remain in the intestinal wall or are washed to the liver by the blood (Warren, 1973; Lengy, 1962b). Lenzi *et al* (1987) have shown that the periovular inflammatory cells, particularly the eosinophils which produce a collagenase enzyme, favor the passage of the eggs through the intestinal wall. Sher *et al* (1990), while studying interleukin-5 (IL-5) and its eosinophilopoietic activities and influence in the development of granulomas in murine schistosomiasis, noted decreases in tissue eosinophils in the intestines, among other tissues,

but there was no reduction in egg output as judged by fecal egg counts.

The foregoing shows how unclear are the mechanisms involved in egg excretions. Some of these mechanisms are also involved in the development of some of the lesions in the intestine.

The aim of the present study, therefore, was to investigate the morphological changes during the transmucosal migration of *S. bovis* eggs from the intravascular lumen to the extravascular and subenterocytic space and further through the epithelium into the intestinal lumen. The pathological changes during early and late patency will be studied in field and experimental infections in the definitive host (cattle) and laboratory animals (hamster). Both light and electron microscopy will be used for recording of the changes.

2. LITERATURE REVIEW

EPIDEMIOLOGY

Schistosomiasis in cattle is caused by *S. bovis* and *S. mattheei*. *S. bovis* is distributed in northern and eastern African countries north of latitude 10°S while *S. mattheei* occurs in eastern and southern African countries south of 10°S (Dinnik and Dinnik, 1965; Christensen, *et al*, 1983).

In Sudan about 90% of 2 years old cattle are infected with *S. bovis* although the prevalence of infection is lower in older cattle (Majid *et al*, 1980). In a survey in Tanzania, Dinnik and Dinnik, (1965) estimated that over 50% of cattle on some farms harbored *S. bovis*. In a more recent survey, some regions of Tanzania cattle were found with about 30% prevalence of infection by *S. bovis* (Kassuku *et al*, 1986). The trend is similar for *S. mattheei* infections in cattle in southern African countries (Lawrence, 1978e).

Although animal schistosomisis is generally a chronic infection, severe acute cases of the disease have been reported. In Sudan, Eisa (1966) reported an outbreak in which over 8000 cattle were lost. In another outbreak in Sudan there was 100% morbidity and up to 50% mortality in calves due to *S. bovis* infection (Hussein, 1968). Van Wyk *et al*, (1974) have reported a severe outbreak in cattle due to *S. mattheei* infection at one farm in South Africa. In this outbreak infection occurred from a drinking trough the water of which had massive concentrations of cercariae. Outbreaks of schistosomiasis probably occur if conditions suitable for intensive transmission of infection exist. Such conditions include presence of limited water resource which is suitable for supporting the snail host species, intramolluscan larval schistosome development and existance of a nonresistant cattle population (Christensen, *et al*, 1983). The conditions prevailing during the outbreak described by Van Wyk, *et al* (1974) illustrate these points well.

Transmission of infection in schistosomiasis is usually through the skin. The manner by which the cercariae penetrate the skin and transform into schistosomules and adult worms has been studied by many workers. The mechanism of penetration of *S. bovis* has been recorded by Lengy (1962b). Transmission of the infection *per os* has been experimentally demonstra-

ted in goats (Kassuku *et al*, 1985). This route is a less efficient way compared to the percutaneous route (Lengy, 1962b). Even then serious disease outbreaks have occurred in cattle in which the oral route was considered the major method of infection (Van Wyk,*et al*, 1974).

LIFE CYCLE OF *S. BOVIS*

Detailed study of all the stages of the life cycle of *S. bovis* have been undertaken by Lengy (1962a, 1962b). However, only the adult and the egg stages of the cycle are of interest for the present study.

Mature worms *in copulo* live in the mesenteric veins of the definitive host. The female leaves the male to lay eggs in the venules deep in the wall of the intestine. The depth to which the female lays the eggs depends on a number of factors. These include the stage of infection in the host and the severity of infection. In late stages of infection the mesenteric and intestinal veins develop phlebitis. The phlebitis is thought to be partly due to immunological reactions mostly to dying worms but also to live, adult worms (Lawrence, 1978b). Lawrence (1978b) has also observed that in heavily infected animals phlebitis develops in early stages and is of high prevalence. This is not the case in light infections. Phlebitis in the large mesenteric and intestinal veins prevents the female worms from reaching venules in the deeper part of the intestine. This results in eggs being laid in the submucosa and even serosa. Such eggs are unlikely to reach the intestinal lumen.

The number of eggs present in the uterus of the worm at any one time is in the range of 3-65 (Lengy, 1962b). The eggs are in small aggregates of 3-8 at irregular intervals. However, the eggs may be more concentrated or in tandem arrangement more or less longidutinally, with the pointed end always directed posteriorly (Lengy, 1962b). The uterine egg arrangement is more or less maintained after they are laid. After the female has withdrawn to the larger vessels, the arrangement may change as the eggs exit to the extravascular position.

The eggs are immature when laid. It takes between 7-8 days for the embryo to reach the mature miracidial stage in the case of *S. bovis* (Lengy, 1962a, Malek, 1969). Whether maturity is reached while intravascularly or extravascularly, is not directly stated. However,

some investigators have observed immature eggs in the extravascular space and in feces (Lawrence, 1978a). Mature and immature eggs within blood vessels have also been observed (Kuba, 1963).

Eggs depend on their excretions/secretions to exit from the vessel lumen and other mucosal tissues (reviewed by Warren, 1973). However, the excretions/secretions are produced better by mature miracidia. This means that miracidia in eggs need to mature intravascularly, as put forward by Ngaiza *et al* (1990).

The life span of the miracidium in the egg is about 21 days for *S. mansoni* (Warren,1973). Eggs that are excreted with a mature miracidium have a better chance of perpetuating the life cyle. Such eggs would be at least 6 days old from the time of being laid. Even if the egg was delayed in the intestinal tissues during emigration, it would continue to mature and would still have 2-3 weeks to reach an intermediate host (Bruijning, 1968).

MECHANISMS OF SCHISTOSOME EGG EXCRETION

Many suggestions have been made on how the eggs leave the intravascular and extravascular areas to reach the intestinal lumen. Detailed reviews on the subject thave been written by Kuba (1963) and Bruijning (1968). The subject has been partly reviewed by Warren (1973) and Doenhoff (1986). However, it is necessary to put into perspective the mechanisms of egg excretion in the present study.

Each mechanism suggested for the excretion of eggs from the host seems to have some credibility. However, the mechanisms suggested so far are not entirely satisfactory and remain mostly as hypotheses.

The mechanisms suggested include rupture of the blood vessels with or without hemorrhage (reviewed by Kuba, 1963). The rupture is thought to be caused by the female during egg laying or by the eggs themselves. Probably the female lays the eggs directly into the perivascular tissues. Also proposed was migration of the eggs through tissues due to movements of the contained embryo. Lengy (1962a) has observed that mature miracidia made very occasional movements within the egg. It has also been noted that eggs with immature

miracidia are often found in extravascular positions and in feces (Lawrence, 1978a). Another suggestion is that of necrosis and rupture of the blood vessel. The necrosis is said to be a sequel to inflammation or to result from egg secretions. *In vivo* microscopy studies have revealed that clusters of eggs can compromise blood flow with resultant necrosis of the vessel wall and subsequent liberation of the eggs to extravascular tissues (Bloch,1980). In the same study, however, single eggs in venules were seen to emigrate through the vessel wall using the nonspined end. The eggs did so without deformation of the wall or hemorrhage.

It has been suggested that the spine plays an important role in the extravasation of the egg. In an elaborate and thorough study, Bruijning (1968) demonstrated that the lateral spine of *S. mansoni*, by catching on to the vessel wall, prevented the egg from being carried by the blood. He further observed that peristalsis tended to move the egg against the direction of the blood flow. In this process the egg assumed an oblique position which enabled it to penetrate the vessel to reach the extravascular space. However, some schistosomes, e.g. *S. japonicum*, lay eggs that have no spines.

Other suggested mechanisms of egg excretion involve enzymatic or toxic substances secreted by the unhatched eggs. The suggestion is made by many investigators (reviewed by Kuba, 1963 and Warren, 1973). The method is probably in co-operation with other mechanisms. Egg secretions of antigenic substances and their role in the pathogenesis of schistosomiasis has been established by *in vivo* and *in vitro* investigations of *S. mansoni* infection in mice (reviewed by Smithers and Doenhoff, 1982). However, few substances in egg excretion/-secretions have shown properties that might play a role in the excretion of the eggs. Mention of these was made under 'Introduction'. The unequivocal identification of the secreted substances and their possible effects on the tissues require further elucidation.

The secreted antigenic and enzymatic substances reach the extraovular space through micropores in the eggshell (Stenger *et al*, 1967; Race *et al*, 1969). In *S. mansoni* eggs, the micropores are cribriform sieve-like structures comprised of minute anastomosing channels (Neill *et al.*1988). Neill *et al*,(1988) have demonstrated the existence of a cellular envelope (von Leichtenberg's envelope) just under the eggshell. They claim that the envelope effects a barrier against passive diffusion of macromolecules. This means there would be a selective

passage of lytic substances necessary for the egg excretion. Such a process would need a strong external (to the egg) attractant for the lytic egg macromolecules. The cellular envelope is distinct in mature but not in immature miracidia (Neill *et al*, 1988). Selective diffusion of macromolecules would thus be unlikely from eggs with immature miracidia. Probably eggs with immature miracidia exit by other means. There appear to be no ultrastructural studies on *S. bovis* eggs to compare with those on *S. mansoni* eggs.

It has also been suggested that endothelial cells, leukocytes and fibroblasts participate in eggs excretion. The involvement of these cells has been reported from light microscopic studies. The degree of cellular attraction to the intravascular egg is thought to depend on the stage of differentiation of the contained embryo. Thus, mature eggs would have greater attraction of cells and therefore better facilitation of their exit to the extravascular space (reviewed by Kuba, 1963).

The phase of emigration from the extravascular space to the intestinal lumen is said to be facilitated by actions of egg excretions/secretions and peristalsis (reviewed by Kuba, 1963 and Warren, 1973). Peristalsis probably has a role in intestinal schistosomiasis especially with mature eggs which are capable of secretions. However, often both mature and immature eggs are found in feces (Lawrence, 1978b). There are other digenetic schistosomes, e.g. *S. nasalis*, (Rao, 1933), whose predilection site, the nasal mucosa, lacks peristaltic movements.

The passage of the eggs through the intestinal wall is also said to be aided by actions of periovular inflammatory cells, particularly the eosinophils (Lenzi *et al*, 1987). This is probably what was referred to by other investigators as ulcer or abscess-like lesions in the intestinal mucosa (Kuba, 1963; Warren, 1973). It is now well known that neutrophils and eosinophils contain enzymes that degrade various components of extracellular matrix (Slauson and Cooper, 1990; reviewed by McEwen, 1992). It is conceivable that these cellular accumulations about the egg or its environs facilitate the exit of the egg to the intestinal lumen. However, many eggs with mature or immature miracidia are seen in the lamina propria or lumina of intestinal glands with or without periovular cellular exudate (Hussein, 1971; Lawrence, 1978a). It has been shown that suppression of factors that particularly stimulate eosinophilopoiesis and maturation, e.g. interleukin-5 (IL-5), does not reduce fecal

egg load (Sher *et al*,1990). Probably therefore, other mechanisms, in addition to some already suggested, operate in egg emigration to the intestinal lumen.

Dependency on the host's immunological responses for the excretion of the eggs has recently been investigated. This was indicated in the 'Introduction' above. The immunological mechanisms of egg excretion are also strongly advocated by Damian (1987). He sees it as one way by which the schistosome parasite has adapted to the host immune responsiveness by directing this to the propagation of its life cycle.

Many of the studies on mechanisms of egg excretion have been on *S. mansoni*. *S. bovis* has been comparatively less studied. However, the mechanisms for egg excretion in both schistosomes are probably similar since their definitive habitats in the final host are similar.

CLINICAL SIGNS

Following percutaneous infection of cattle with *S. bovis*, clinical signs may be observed as early as five weeks p.i. and they are usually evident from 7-8 weeks p.i. (Hussein, 1971). A similar period is recorded for *S. mattheei* infection in cattle (Lawrence, 1977d). The clinical signs are related to the onset of oviposition and excretion of eggs through the intestine (Hussein, 1971; Lawrence,1976). The severity of the clinical signs depend on the intensity of infection, susceptibility of the host, duration of the infection and, to some extent, the nutritional status of the animals (Lawrence, 1976, 1977d; Saad *et al*,1980).

In naturally occurring *S. bovis* infection of cattle, the disease is always severe in calves and in other age groups undergoing a primary heavy infection. Clinical signs include diarrhoea, which is sometimes bloody, sunken eyes, loss of body condition and anemia (McCauley *et al*, 1983). Similar clinical signs have been observed in cattle experimentally infected with *S. bovis* (Hussein, 1971; Massoud, 1973; Saad *et al*, 1980). Both experimental and natural *S. mattheei* infections in cattle show a clinical picture similar to *S. bovis* infections (Lawrence, 1976, 1977d; van Wyk *et al*, 1974). In cattle exposed to heavy primary *S. mattheei* infection an acute intestinal schistosomiasis develops which is followed by a subacute form especially in cattle on a low level of nutrition (Lawrence, 1977d). The acute form is characterized by dysenteric diarrhoea and animals may die in a week or two. Diarrhoea and persistent

unthriftiness characterize the subacute intestinal disease. The subacute form is probably the more common in the natural condition. A chronic hepatic syndrome has also been described in cattle infected with *S. mattheei* (Lawrence, 1976). The clinical signs of this form are referable to hepatic insufficiency. This form is said to be fatal.

Some of the clinical signs observed in bovine schistosomiasis are similar to those in, e.g., trypanosomiasis, East Coast Fever, and fascioliasis. Obviously this complicates the field diagnosis of schistosomiasis. This may, in part, be responsible for the lack of records on clinical schistosomiasis in those countries in which these other diseases are endemic.

CLINICAL PATHOLOGY AND PATHOGENESIS

Cattle infected with *S. bovis* develop anemia, hypoalbuminemia, hyperglobulinemia and eosinophilia (Saad *et al*, 1980). Similar changes are also encountered in cattle with *S. mattheei* infection (Lawrence,1977b). Also observed in *S, mattheei* infection in cattle is neutrophilia in lighter infections but a neutropenia in heavy ones (Lawrence, 1977b). The severity of the clinicopathological changes depend on the intensity and stage of the infection. The changes are severe during the early patency in heavy infections. During this period there are also high fecal egg counts. Thus the development of these features as well as the clinical signs of the disease are related to the excretion of the egg through the intestine (Bushara, *et al*, 1980; Lawrence, 1977b; Saad *et al*, 1980).

The eosinophilia that is observed starts before patency of the disease. It has been observed as early as 3-4 weeks p.i., with maximum values being recorded at about 8 weeks p.i. (Saad *et al*, 1980). The eosinophilia is considered to be a reaction of the host to live and dead worms and to eggs trapped in tissues.

There are few records on changes in the blood neutrophils in bovine schistosomiasis. The neutropenia observed by Lawrence (1977b) was thought to be from hemodilution or some specific sequestration in areas of inflammation. On the other hand he attributes the neutrophilia he observed to stimulation by adrenal cortical hormones.

The anemia that develops is thought to arise from hemorrhage and/or hemodilution caused

by expansion of plasma volume (Bushara *et al*, 1980; Dargie, 1980; Lawrence, 1977b; Saad *et al*, 1980, 1984). As is the case with other clinical signs, anemia is prominent in early patency when fecal egg counts are high. These investigators consider the excretion of the egg through the intestinal wall a major cause of the hemorrhage and therefore the anemia. It has also been observed that whereas there was loss of blood into the intestinal tissues, there was also poor erythropoietic response and reduction in the lifespan of the red blood cells (Saad, *et al*, (1980). The causes of theses changes are not clearly explained. It is also not clear whether the hemorrhage was entirely from mechanical damage of blood vessels by the emigrating eggs or whether it was due to inflammatory factors. Equivalent results were obtained with *S. mattheei* infection in cattle (Lawrence, 1977b) but anemia began to develop during the prepatent period. The author thought it was due to hemoconcentration caused by diarrhoea. In a study of schistosomiasis in sheep using *S. mattheei* attenuated in hamsters, Dargie *et al*, (1973) observed large numbers of eggs in the intestinal wall. However, fecal egg counts were low, there was no serious clinical disease and there were no hemorrhagic lesions in the gut. It is not clear in this study whether the large numbers of eggs were mainly intravascular or extravascular. If they were extravascular, then the mechanical damage by eggs as a cause of hemorrhage becomes doubtful.

The hypoalbuminemia that is observed is also explained on the basis of intestinal hemorrhage and plasma volume expansion (Bushara *et al*, 1980; Saad *et al*, 1984). The hyperglobulinemia is presumed to result from antigenic stimulation (Smithers and Terry, 1969). It begins before patency but reaches peak levels during the period of maximum egg production and adult worm elimination (Saad *et al*, 1980).

As already mentioned above, diarrhoea is a common manifestation of schistosomiasis in cattle. In many of the investigations that have been done, diarrhoea has been considered to be due to the excretion of the eggs through the intestinal wall (Dargie, 1980; Hussein, 1971; Lawrence, 1977b, 1978a; Saad *et al*, 1980). As with many of the changes observed in this disease, diarrhoea is also severe in heavy primary infections during maximum egg excretion which is in early patency. Clinically, however, diarrhoea has been recorded before patency (Hussein, 1971). It has also been observed to begin to diminish while fecal egg counts are still high (Lawrence, 1977b). It has therefore been suggested that factors in addition to the

effects of egg excretion probably operate in the pathogenesis of diarrhoea.

Damage is done to the mucosa of the small and large intestines by the exiting eggs. In *S. bovis* infections in cattle, major damage is in the small intestines, especially in the first three months of the disease. In later stages the colon also shows severe damage (Saad *et al*, 1980). In *S. matheei* infections damage is in both the small and large intestine. However, the diarrhoea seen soon after patency in *S. matheei* infection is associated mainly with damage to the colon mucosa (Lawrence, 1978a). With damage to the mucosa there are high plasma and red blood cell loss to the intestinal tissues (Saad *et al* 1980), hence the hemorrhagic diarrhoea. It is probable that physical damage to the venules and mucosal epithelium by the exiting eggs is contributory to the development of diarrhoea. However, inflammatory reactions to the emigrating and trapped eggs, as well as other factors, probably also contribute to the development of diarrhoea. The precise nature of these factors needs more clarification.

GROSS PATHOLOGY

Gross lesions are most evident in calves exposed to primary heavy infection. There are variable degrees of hydroperitoneum, hydrothorax and sometimes hydropericardium (Hussein, 1971, 1975; Massoud, 1973). The small intestines are most affected although lesions may also be observed in the cecum, colon and rectum (Hussein, 1971, 1975).There are diffuse or punctate hemorrhages in the mucosa of the small intestine. The mucosa may sometimes be opaque or thickened and there may be ulcers. In heavy infections the mucosa may be intensely congested and coated with mucous exudate rich in schistosome eggs (Hussein, 1975). Schistosome eggs, however, are not grossly visible. "Sandy patches" of tiny granulomas are seen in the mucosa and serosa.

There are hemorrhagic foci in the cecal, colonic and sometimes rectal mucosa (Massoud,1973). Although gross pathological changes are usually prominent in the small intestine prominent congestion and hemorrhage have been observed in the colon at 3-6 months p.i. (Saad, *et al*, 1980). This is attributed to the shifting of the worms from the small intestine to the colon. Also observed in *S. bovis* in cattle are prominent engorgement, occlusion by thrombi, thickening and tortuousness of intestinal veins (Hussein, 1975; Massoud, 1973).

In bovine schistosomiasis, due to *S. mattheei* gross lesions are closely similar to those in *S. bovis* infections. However, severe colonic mucosal congestion and hemorrhage are seen in early patency. As in *S. bovis*, the colon lesions are said to be due to a shift of worms from the small intestine but the shift occurs earlier (Lawrence, 1977d, 1978a). Also observed in *S. mattheei* infection, are enlargement and hyperemia of the ileocecal valve (Lawrence, 1977d, 1978a).

In *S. bovis* infection of cattle, gross lesions are also observed in the liver. The lesions vary depending on the severity and duration of illness. Tiny whitish or yellow foci may be scattered under the capsule and on the cut surfaces (Hussein, 1971, 1975; Massoud, 1973). In heavily infected animals there may be fibrosis and thickening of portal tracts. In such cases the liver is hardened. There also may be elevated grayish nodules consisting of dilated portal veins occupied by thrombi in which masses of living or degenerated worms may be embedded (Hussein, 1975)

LIGHT MICROSCOPIC LESIONS

Histopathological changes in natural and experimental *S. bovis* infection in cattle have been described by Bushara *et al*, (1980); Hussein (1971,1975) and Massoud (1973). In the intestines there were different degrees of catarrhal to hemorrhagic inflammation of the mucosa. There were also superficial epithelial cell desquamation, increased number of goblet cells and multiple superficial hemorrhages. Early in the disease the mucosa was generally infiltrated with eosinophils, macrophages, lymphocytes and plasma cells. Many eggs with intact miracidia were seen in rows and without cellular reactions. However, degenerate eggs were also found. These had provoked variable degrees of granulomatous reaction. Some granulomas were formed by epithelioid and giant cells with variable numbers of eosinophils and mononuclear cells. Central necrosis and Hoeppli phenomenon were common in some of the granulomas (Hussein, 1971). A proportion of the eggs was surrounded by large numbers of eosinophils and sometimes neutrophils to form what looked like microabscesses. Some of the microabscesses opened into intestinal lumen. The granulomas were prominent in the submucosa and lower tunics as the infection became older. Granulomatous lesions and other inflammatory changes were noted to cause thickening of the intestinal tunics. Although

similar changes occurred in the colon, they were less severe and extensive than in the small intestine.

Vascular lesions were also described in the submucosa and the subserosa (Hussein, 1975; Saad *et al*, 1980). They included intimal and medial proliferative changes and/or eosinophil and mononuclear cell infiltrations. Adventitial and perivascular eosinophil and mononuclear cell infiltrations were also described as were granulomas in different layers of the veins in the submucosa.

The intestinal lesions observed in *S. bovis* infection were similar to those in *S. mattheei* infections. The sequential development of the intestinal lesions in experimental *S. mattheei* infection in cattle have been described in detail by Lawrence (1978a). Early hemorrhagic lesions in the colon in *S. mattheei* infection are a feature of the disease.

Apart from the microscopic changes in the intestine there were lesions in the liver. As in the intestines the major lesion in the liver was the granuloma. Granulomas were seen in the portal areas and sometimes intraparenchymally (Hussein, 1975). Some granulomas were composed of a core of one to five eggs surrounded or engulfed by giant cells.They had a fringe of eosinophils, lymphocytes, plasma cells, epithelioid cells and fibroblasts. Other granulomas were densely packed with lymphocytes and eosinophils while others were concentrically fibrosed and presented an onion-peel appearance (Hussein, 1975). Some granulomas showed the Hoeppli phenomenon. Portal and periportal fibrosis were also observed.

Hussein (1971) has described extensive medial hypertrophy and hyperplasia of intrahepatic portal veins in *S. bovis* infection in cattle. He considered these lesions to be peculiar to bovine schistosomiasis only.

Granulomatous lesions have also been described in the pancreas, mesenteric lymph nodes and lungs but no lesions were observed in the spleen in primary infections (Bushara, *et al*, 1980; Hussein, 1975). However, intense focal eosinophilic infiltrations were observed in the spleen in cattle after heavy reinfection with *S. bovis* (Bushara, *et al*, 1980).

The kidney is not often affected in bovine schistosomiasis. However, diffuse interstitial inflammation,fibrous thickening, perivascular cuffing of renal arterioles and focal glomerulonephritis, have occasionally been observed in cattle following heavy reinfection with *S. bovis* (Bushara, *et al*, 1980). Proliferative glomerulonephritis, however, is a common lesion in *S. mansoni* infections in man. It is experimentally reproducible in animals (reviewed by Andrade and van Marck, 1984). The schistosomal nephropathy is an immune complex disease whose important prerequisite is prolonged heavy infection.

In cattle infected with *S. bovis*, the main lesion in the target organs is the granuloma. The pathogenesis of the lesion is assumed to be similar to that in *S. mansoni* infection in man. The pathogenetic mechanisms of this lesion have been reviewed by Warren (1982). They involve delayed hypersensitivity responses to the schistosome eggs.

ELECTRON MICROSCOPIC LESIONS

Ultrastructural studies of intestinal schistosomiasis are limited. None on *S. bovis* infection in cattle have been found. In their study on murine schistosomiasis mansoni, Bogitsh and Wikel (1974) observed vesiculation of nuclei, hypertrophy of membranous elements and depletion of glycogen in intestinal epithelial cells in contact with active eggs. They also observed that mitochondria in the epithelial cells near viable eggs displayed intracristal granules whose number per mitochondrion varied from one to four. These investigators speculated that the mitochondrial changes were brought about by the soluble egg antigens circulating in the blood or lymph.

Bogitsh and Wikel (1974) also found that in early infections the serosal muscularis granulomas in the small intestine had little collagen. However, the granulomas contained a large number of eosinophils, some neutrophils, fibroblasts and, occasionally, plasma cells, lymphocytes and epithelioid cells. On the other hand, in late infections the serosa-muscularis granulomas were composed largely of fibrous tissue elements.

Although Bogitsh and Wikel (1974) observed that the intravascular eggs were in intimate contact with the endothelium no changes were reported in the cells or the egg. They also did not speculate on how the egg exited to the extravascular space.

THE HAMSTER IN EXPERIMENTAL SCHISTOSOMIASIS

The hamster has been described as an excellent model for the study of *S. mansoni* infections. The animal is highly susceptible to *S. mansoni* and in it there develops a stable and long term infection (Warren, *et al*, 1967). The hamster is also susceptible to *S. bovis* infection (Lengy, 1962b). Differing severities of disease can be produced in the hamster by exposure to different intensities of infection with *S. bovis*. Although the hamster is an excellent model for schistosomiasis research, there appears to be no descriptions of the systematic pathology of the disease in this animal.

3. MATERIALS AND METHODS

ANIMALS

A. EXPERIMENTAL ANIMALS

1. Eleven, male, 5-6 months old Jersey calves weighing between 96-106 kg.
2. Six male, 10 months old Jersey calves weighing between 168-236 kg.
3. Fifteen female, 3 months old Golden hamsters weighing between 92-124 gm.

B. FIELD CASES

Cases of naturally occurring bovine schistosomiasis in the Iringa Region, Tanzania. The animals were from 1-2 years old to more than 7 years old of either sex or castrates brought for slaughter at the abattoirs in Iringa town. The cattle were mostly Shorthorn Zebu or crosses of these with Boran.

All experimental infections were carried out at the Royal Veterinary and Agricultural University, Denmark.

The experimental calves were born and kept indoors up to and throughout the experiment. They were fed hay/straw (barley) and supplemented with barley grain and minerals. They also had free access to fresh water.

Some animals developed pneumonia and others ringworm. They were given appropriate medication before, on and after exposure to *Schistosoma bovis* (*S.bovis*) cercariae.

Hamsters were purchased from Bartin and Kingman Ltd, England. They were caged separately and fed a special guinea pig commercial feed *ad libitum*. They had free access to fresh water.

The field cattle, from which specimens were collected, were indigenous and grazed on natural pastures. They were brought for slaughter from cattle auctions. Cattle brought for auction came from both within and outside Iringa region. It was therefore difficult to establish the precise origin of the animals. However, specimens were also collected from

some cattle coming from places known to have many swampy areas. Fecal examinations on various farms in these areas had shown evidence of *S.bovis* infection.

EXPERIMENTAL INFECTIONS

A. INFECTIVE MATERIAL

Cercariae of *S.bovis* were the infective material for both the experimental calves and hamsters. The *S.bovis* cercariae were originally from infected *Bulinus africanus* snails collected from Iringa Tanzania. At the Danish Bilharziasis Laboratory (DBL), two thousand of these cercariae were given to each of two calves. Following peak appearance of eggs in feces, the calves were used as a source of miracidia for infecting snails (*Bulinus wrightii*) at the DBL. Cercariae from these snails were then used for infecting the experimental calves and later on the hamsters. Usually cattle in the field in the Iringa Region most probably become infected with *S.bovis* only, whereas the most southern and southwestern parts of Tanzania may have both *S.bovis* and *S.mattheei* (Dinnik & Dinnik, 1965).

B. PROCEDURES

1. Calves

All calves were exposed to infection percutaneously. Because the intended infective dose (5,000 cercariae) per calf could not be achieved at one harvest, the calves were infected twice at seven days intervals. The first dose was 2,717 cercariae and the second 3,000 cercariae.

In the initial experiment, seven calves were infected. The tails were closely shaved, thoroughly cleaned and then rinsed with distilled water. Plastic bags containing the infective material were placed on the tail with the shaved part of the tail submerged in the infective water. The tail was kept submerged for 30 minutes (duration of exposure to cercariae) (Fig. 1). During the time of exposure the plastic bag with its contents was kept close to the body of the calf for warmth so as to keep the cercariae actively moving.

After the 30 minutes of exposure to cercariae, the bags and their contents were removed from the tails. The tails were held for a while by the assistants until they were reasonably dry. This was done to avoid shaking of the tail and thus losing some of the infective material

still on the skin and also to prevent the control calves, that were being kept in the same room, from licking the wet tails and thereby becoming infected via the oral mucosa. For this initial experiment, four calves were kept as control animals.

In the second experiment two calves, initially infected when 5-6 months old, were reinfected with a total of 4,555 cercariae, at the age of 10-11 months. At the same time, two other calves of approximately the same age (10-11 months) were infected for the first time with the same number of cercariae using a procedure similar to that used in the initial experiment. During this second experiment, two calves were kept as control animals.

2. Hamsters

Ten hamsters were infected and five hamsters kept as control animals. The hamsters were each infected with 300 cercaria percutaneously using the Ring Method as follows: The hamster was anaesthetized intraperitoneally with a solution consisting of one part Nembutal (pentobarbitone sodium) (50 mg/ml), one part ethyl alcohol (96%) and nine parts distilled water (or physiological saline), at the dose rate of 1 ml of the solution per 100 gm of body weight. The hairs on the lower abdomen were then thoroughly clipped with scissors and the area rubbed with gauze soaked in deionised water after which the animal was placed on a clean table top and properly fixed to it by adhesive tape. A heavy nickel ring (2.7 cm high and 2.4 cm inside diameter) was placed on the clipped abdominal area, the ring firmly pressed down and attached properly to the abdomen and the table top by adhesive tape (Fig. 2). The 300 cercariae were added to the ring using a 1 ml sterile disposable syringe. The cercariae were left in contact with the skin in this manner for 30 minutes. Following this exposure, the water with whatever remained in it was removed from the ring using a 1 ml sterile disposable syringe. The exposed hamster was freed of the other materials used during the procedure, placed in a cage, transferred to a warm room (20°C-25°C) and constantly observed during recovery from anaesthesia.

NATURAL INFECTIONS

The cattle from which specimens were collected for histopathology were naturally infected, probably by the percutaneous route while standing in infected water. Infection through the oral mucosa when drinking infected water may have occurred. The number of cercariae to which

these cattle had been exposed is impossible to determine. However, they were likely to have been repeatedly exposed to infection whenever grazing at infected swamps or at infected waters.

CLINICAL EXAMINATIONS

A. CALVES

All experimental calves, infected and control animals, were clinically examined weekly starting by the third week p.i.. Starting week five p.i. fecal and blood samples were taken. The fecal samples were examined for the presence of schistosome eggs, and the blood samples were subjected to a general hematological examination including total count of erythrocytes, white blood cells and differential white cell counts. Sampling blood and feces were continued until week fourteen p.i..

B. HAMSTERS

The hamsters were routinely observed at the time of feeding and watering. Fecal samples were collected for schistosome egg counts on week seven p.i..

NECROPSY AND COLLECTION OF SPECIMENS

A. EXPERIMENTAL CALVES

Necropsy was performed from the seventh week post infection, when eggs appeared in the feces for the first time. Hence forward, necropsy was done at 2 weeks intervals and at each necropsy one infected and one control calf were examined.

The calves were killed with a captive bolt pistol. Through the cranial lesion, a plastic rod of about 2 metres was introduced so as to pass down the vertebral column destroying the spinal cord to effect complete paralysis of the animal.

Immediately after removing the plastic rod, the abdomen was opened to expose the small and large intestines for the collection of specimens. All the procedures were carried out within about five minutes from the time the animal was stunned. Specimens for light microscopy (LM) were collected from three different places from each of the proximal, middle and distal, small intestines and proximal, middle and distal large intestines. Specimens for LM

were also collected from the abomasum, mesenteric lymph nodes, liver, lung, heart, kidney, spleen and urinary bladder. Specimens for electron microscopy (EM) were taken only from the small and large intestines at the same sites as for LM.

Tissues for LM were fixed in 10% neutral, buffered formalin and kept for later processing.

For EM, the specimens were immediately immersed in a cold (4°C) mixture of paraformaldehyde and glutaraldehyde (Appendix 2) in a large Petri dish, gently and lightly stretched and pinned to a soft board in the Petri dish with the mucosal surface facing up. This procedure prevented the rolling up of the wall of the intestine due to muscle contractions. After initial fixation for 15-20 minutes, thin slices about 1 mm wide, of the entire intestinal wall, were cut and diced in the fixative under a stereomicroscope. About 10 small blocks containing all layers of the intestine were transferred to vials with fresh fixative and kept at 4°C for processing for transmission electron microscopy (TEM).

Specimens for scanning electron microscopy (SEM) were obtained from the same specimens as used for TEM. The pieces with sides not exceeding 3-7 mm were trimmed and put in vials with fresh fixative as was the case for TEM. The specimens were kept at 4°C for later processing.

B. HAMSTERS

The necropsy of hamsters was started at week seven p.i. when the schistosome eggs appeared in the feces for the first time. Thereafter necropsies were performed with two weeks intervals or whenever an animal appeared too ill or died. At each necropsy one or two infected and one control hamster were examined.

The hamsters were deeply anaesthetized by giving 1 ml of Nembutal (50 mg/ml pentobarbitone sodium) intraperitoneally. The abdominal organs were then exposed by removing the skin and abdominal muscles and specimens for EM and LM were collected. Specimens were taken from the small and large intestines at sites similar to those from calves.
The sampling and fixation procedure for LM and EM (TEM and SEM) was the same as that for calves.

C. FIELD CASES

Before the cattle brought for slaughter at the slaughterhouse were killed, fecal samples were collected at the time of ante mortem examination and checked for the presence of miracidia by the miracidia hatching technique (Appendix 4). For later identification at slaughter each animal, from which a fecal sample was taken, was given a number that was tied around its neck. The animals that showed evidence of schistosomiasis before slaughter were requested to be slaughtered as quickly as possible. This, unfortunately, did not always happen. However, even the animals whose feces were negative for miracidia were examined after slaughter as well as others randomly selected and not previously examined for miracidia. Thus, a total of seven to ten intestines were examined each time.

Cattle brought to the slaughter house were killed by cutting the spinal cord at about the first and second cervical vertebra and then bled. This was often done by the time I arrived at the slaughter house and it took time to get the intestines for examination. The slaughterers had their own procedure to follow that could not be altered to collect fairly fresh specimens of the intestines. Specimens for LM were collected from the section of the intestine opposite the mesentery in whose veins or venules there were schistosomes.

The intestinal and mesenteric lymph node specimens were fixed in 10% neutral, buffered formalin and kept at room temperature until processing at the Sokoine University of Agriculture (SUA), Morogoro.

LIGHT MICROSCOPY

Tissue specimens that were fixed in 10% neutral, buffered formalin were trimmed to appropriate size, dehydrated by passing through increasing concentrations of alcohol up to absolute alcohol, then cleared in xylol and embedded in paraffin wax. Sections were cut at 5 μm using a Reichert-Jung microtome. All sections were initially stained with hematoxylin and eosin (HE). Selected sections were stained by the picric acid-acid fuchsin (van Gieson) method, Luna's method for eosinophils and by the periodic acid-Schiff (PAS) Method.

SCANNING ELECTRON MICROSCOPY

The intestinal specimens that had been trimmed to the size of 3 mm^2 to 7 mm^2, (whole thickness), fixed in a mixture of paraformaldehyde and glutaraldehyde (Appendix 1) and kept at 4°C were processed (dehydrated) as shown in Appendix 3. The specimens were left in the 100% water free acetone at 4°C until ready for critical point drying.

Critical point drying was carried out in CDP 020 Critical Point Drier (Balzers Union). The specimens were placed in the pressure chamber where the acetone was replaced by liquid carbon dioxide (CO_2): The specimens were rinsed five times in liquid CO_2 to remove all acetone. The chamber was then heated to above 31°C (corresponding to a pressure of 73 bar) which is the critical temperature of CO_2. By passing the critical temperature of CO_2 at the corresponding pressure the change from liquid to gas occurs without boiling. The temperature was kept above 31°C and pressure lowered without liquid being reformed and thus the tissue specimens dried with a minimum of disruption and distortion of structure.

After critical point drying, the dried specimens were mounted on brass cylinders, 10 mm high and 10 mm in diameter, by means of double-adhesive tape and " Silver Paint" (the silver paint thus providing conductive properties between specimen and brass holder).

After the 'Silver Paint' was dry, gold was applied in a thin layer by sputter coating in a Polaron High Resolution Sputter Coater E 5400.

Scanning electron microscopy of the mounted specimens was done in a JSM 840A (JEOL) microscope at an accelerating voltage of 15 Kv and a beam current of 3×10^{-10} or 3×10^{-11} mA. The results of the study were recorded at appropriate magnifications on AGFA APX 100 film (professional) using an Olympus or a Mamiya camera.

TRANSMISSION ELECTRON MICROSCOPY

The diced slices of whole intestinal wall thickness were processed to embedding level as shown in Appendix 1.

After embedding, sections for tissue orientation and selection of suitable areas for the study at EM, were cut at 1 μm on an ULTRACUT E (Reichert Jung) microtome, stained with toluidine blue and examined light microscopically. Ultrathin sections were cut at 75nm and mounted, some on naked copper grids (300 mesh), others (2/5 per section) on coated grids(150 mesh). The sections on grids were stained with lead citrate (Reynolds, 1963) and uranyl acetate (Watson, 1958) but with reduced staining time owing to earlier block staining before the process of dehydration . Study of the mounted sections was done in a JEM 1200E (JEOL) electron microscope at an acceleration voltage of 60 KV. The results of the study were recorded on a 9 x 6.5 cm AGFA SCIENTIA film.

4. RESULTS

CLINICAL FINDINGS

A. Calves

1. Experimental Infections

No clinical signs ascribable to schistosomiasis were observed during the experimental period.

2. Natural Infections

Only animals from which fecal samples were collected had antemortem examination. Nearly all these animals were thin looking and some had loose feces. In a few cases there was diarrhoea and the feces had reddish mucoid material.

B. HAMSTERS

During the period of investigation, some hamsters had blood stained or dark-brown semisolid feces. In some cases the dark-brown feces were watery and caused matting of the perineal hairs. The affected hamsters were dull and had staring coat. Some animals had labored breathing. Others became recumbent until death or when taken for necropsy.

MACROSCOPIC FINDINGS

A. CALVES

1. Experimental Infections

a. Gastrointestinal tract

In both experiments there were very few gross lesions in the gastrointestinal tract at all stages of the disease. A few punctate foci of congestion/hemorrhage were seen in the mucosa of the anterior and middle small intestine at 11 weeks p.i. In both experiments at 7-18 weeks p.i. there were worms of either sex in the mesenteric veins in a few specimens. Worms were also observed in the subserosal veins at 11 weeks p.i.

b. Liver

Few gross lesions were observed in the liver in both experiments. Few grey-yellow foci, sometimes in aggregates, were observed under the capsule of the liver from 7 to 18 weeks p.i. In some specimens there were a few linear to irregular whitish-grey subcapsular areas.

2. Natural Infections

a. Gastrointestinal tract

In a few animals there were patchy areas of congestion of the intestinal mucosa while in other cases the mucosa had few scattered areas of punctate congestion/hemorrhage. Occasionally there was reddish-grey nodular thickening in the duodenal mucosa or yellow-brown thickening of the mucosa of the jejunum and ileum. Eleven of the thirty five cattle examined had yellow-whitish nodules, 1-3 mm in diameter, under the serosa near the attachment of the mesentery to the duodenum, jejunum and ileum. Similar nodules, occasionally congested, were seen along the veins in the mesentery attachment to the small intestines. There was also some thickening of the mesenteric veins for short distances from the mesentery attachment to the small intestine.

All the thirty five cattle from which specimens were collected had worms in various parts of the mesenteric veins.

b. Liver

There was no opportunity to examine these.

B. HAMSTERS

a. Gastrointestinal tract

No gross lesions were seen in the intestines at 7 weeks p.i. At 8-10 weeks p.i. the following changes were observed.

There was variable diffuse hyperemia of the mucosa of the small intestine. There were also occasional foci of congestion/hemorrhage visible through the serosa of the jejunum. In other cases the foci were widely distributed. The contents of the small intestine were either small amounts of reddish mucus, or small amounts of dark-red, semisolid to fluid material. In some cases the contents were yellow-brown, slimy, tenacious, adherent material.

There were numerous very tiny granular areas visible through the serosa of the jejunum. The cecum had dark-grey or dark- brown fluid or semisolid contents and was often distended by gas. The cecal mucosa had focal or linear areas of congestion/hemorrhage in few or many areas. In the anterior portions of the colon the contents were fluid and variably dark-brown

or black. They were semisolid, yellow-brown tenacious, adherent material in the distal colon. The colonic mucosa had few or widely distributed foci of hemorrhage/congestion visible through the serosa. The rectum was similarly affected, but had semipelleted feces.

From 11 to 14 weeks p.i., there were dark-red, slimy fluid contents in some small intestines. In others the contents were tenacious adherent materials. The mucosa was diffusely congested with raised dark-grey studs here and there. The duodenum and jejunum had many tiny granular spots visible through the serosa. At 14 weeks p.i. there were yellow-brown nodules, approximately 1-1.5 mm in diameter, under the serosa of the jejunum and along the mesentery attachment to it (Fig 3). Similar nodules were seen along the mesenteric veins draining the affected areas of the jejunum. The same structures, but smaller in size, were seen at similar areas at other parts of the small intestines. In the colon and cecum, the nodules were under the serosa only. The cecal and colonic mucosa were diffusely congested with dark red spots, 0.5-1 mm in diameter, in some cases. They had tiny raised brown spots under the serosa at 11 weeks p.i. The walls of the small and large intestines were thickened. One animal, at 11 weeks p.i. had no gross intestinal lesions.

In the whole period of experimental infections worms (males and females) were seen in mesenteric veins at 8-9 weeks p.i. A few worms were also found free in the peritoneal cavity.

b. Liver

The liver was of about normal size or slightly enlarged. At 7 weeks p.i. some liver lobes had yellow-brown spots of about 0.5-1 mm in diameter. At 8-10 weeks p.i. the liver was dark-brown with yellow-brown spots of variable size under the capsule and on cut surfaces. The organ had a variably rough surface throughout the experimental period. In some cases the surface had an almost morocco leather appearance. At 10 weeks p.i. worms were seen in veins at cut surfaces of the liver.

At 11-14 weeks p.i. the liver was grey brown and had uneven and rough surfaces with yellow-brown spots, 1-1.5 mm in diameter, under the capsule. Sometimes there were yellow-brown areas on the cut surfaces. At 14 weeks p.i., there were tiny, nodular structures as

those seen in the intestines. The liver appeared smaller than normal.

c. Other organs

At 8-10 weeks p.i. some hamsters had variable degrees of hydrothorax either with clear fluid, or with whitish, flocculent material which sometimes coagulated on exposure to air. There was prominent hydroperitoneum, the fluid being cloudy or blood stained in some cases. In the **stomach** there were edema and congestion of the mucosa which had also dark-red spots in the glandular region. The oesophageal and glandular parts of the stomach had often light to dark-brown contents. Some **lungs** had a few areas of congestion. At 11 weeks p.i. the lungs had many dark red spots, 1-2 mm in diameter, under the capsule. In the period of 8-10 weeks p.i. the **heart** musculature was flabby. At 11 weeks p.i. the atria of the heart were markedly distended with a very dark coagulum. In this same animal the **pancreas** had numerous pin-head areas of congestion/hemorrhage. The **spleen** was smaller than normal in the 8-10 weeks p.i. period of study. At 11 weeks p.i. the spleens were enlarged and dark-red though in late stages of the infection they were actually smaller. The **mesenteric lymph nodes** were variably enlarged.

LIGHT MICROSCOPY

For ease of description, histopathological changes have been broadly grouped as follows:

i. Those in which the predominant cell was the eosinophil. Additionally, these lesions had diffuse appearance and boundary.

ii. Granulomatous lesions in which the mononuclear cell was dominant. Mononuclear cells here refers to macrophages, lymphocytes and plasma cells. The term large mononuclear cells will be used to refer to all other mononuclear cells with a large, lightly staining nucleus and the epithelioid cells. In the mononuclear cell lesion, eosinophils in variable proportions were present. The lesions had a cellular composition and morphology generally associated with most granulomas.

iii. Other inflammatory changes.

iv. Intravascular schistosome eggs and related changes other than those in (i), (ii), (vi).

v. Extravascular eggs and related reactions other than those in (i), (ii).

vi. Lesions involving veins. Intravascular adult worms and their related changes will also be described here.

A. CALVES

1. Experiment I

a. Gastrointestinal tract

i. Lesions in which the eosinophil was predominant.

There was a general diffuse increase in eosinophils and mononuclear cells in the lamina propria at the anterior and middle small intestine, However, the prominent and frequently seen lesion at 7 weeks and 11 weeks p.i. was eosinophil cells accumulation around one or several schistosome eggs. A few mononuclear cells were scattered among the eosinophils. The lesions had indefinite morphology and border and although they were seen in all layers of the mucosa of the small intestine, the superficial third was more affected. The pathological changes were more widespread in the middle than in the distal small intestinal mucosa. Occasionally they were also observed in the submucosa of the anterior small intestine.

Several modifications in the lesions were observed. Some of the eggs were immediately surrounded by a zone of strongly acidophilic amorphous material, the so-called Hoeppli phenomenon. The Hoeppli phenomenon was sometimes more prominent at the pointed ends of longitudinally sectioned eggs (Fig. 4). The intense accumulations of eosinophils were observed extending from the basal lamina propria to the subepithelium. Often in these tracts of eosinophils there were no eggs. Where the eosinophil accumulations were just below the epithelium, sometimes eosinophils were observed among the epithelial cells and in the intestinal lumen. In some lumina of crypts or intervillous spaces, eggs in an eosinophil exudate were observed.

The intense eosinophil accumulations about eggs were less observed in the mucosa of the intestines at 18 weeks p.i.

ii. Granulomatous lesions.

The prominence of the granulomatous lesions varied with the duration of the disease. A wide range of granulomas was observed, particularly in the small intestine and sometimes in the anterior colon at 11 and 18 weeks p.i. The cellular composition of the central and peripheral parts of the various granulomas seen is given in Table 1.

It is evident that granulomas characterized by a core of mononuclear cells (macrophages and lymphocytes), or eosinophils or a mixture of these were occasionally observed at 7 weeks p.i., in the mucosa of the small intestine. They were, however, more prevalent in the mucosa of the small intestine at 11 weeks p.i. At 18 weeks they were somewhat less prevalent than at 11 weeks p.i. In the mucosa of the small intestine these granulomas were seen more in the basal lamina propria. This type of granuloma was also observed in the submucosa, particularly at the middle and distal small intestine. They were occasionally observed in the mucosa and submucosa of the anterior colon.

The granulomas with epithelioid cells and/or giant cells were more prevalent at 18 weeks p.i. than at 11 weeks p.i. These granulomas were also more clearly delineated by a fringe of small darkly staining mononuclear cells and fibroblasts, or fibroblasts and collagen.

Although some were observed in the mucosal basal lamina propria, they were more frequent in the submucosa. A few of the granulomas in the mucosa of the small intestine were observed to occupy the entire thickness of the mucosa at 11 weeks p.i. In such areas the mucosa was obviously thickened. This thickening was associated with variable morphological changes in the villi and crypts. At both 11 and 18 weeks p.i. a few eggs with their granulomatous reactions were observed in the lumina of crypts or in the intervillous spaces.

Table 1: Types of Granulomas in the Intestine in Experiment I

Core of granuloma*	Cells around core	Duration p.i. 7wk	11wk	18wk	Level in intestine SI M	SI SM	LI M	LI SM
egg+eosi	mono+eosi	-	+	+-	+-	-	-	+
egg+mono/ eosi	mono/eosi	+-	++	+-	+	+-	+-	-
egg+mono	mono+eosi	+	++	+	++	+	+	+
egg+Hoep#	eosi	+	+-	-	+	+	+-	+-
egg+Hoep#	mono+eosi	-	+	+	+	+	-	+
egg+Hoep# +-eosi	giant+mono +-eosi	-	+	+	+	+	-	-
egg+Hoep#	eosi+giant +mono+fibr	-	+	+	+	+-	-	-
egg+epit	mono+-eosi +fibr	-	+	++	+-	++	-	+-
egg+giant	mono+eosi+ fibr	-	+	++	+-	++	-	+-

Explanation to Abbreviations:

eosi = eosinophils; epit = epithelioid cells; giant = giant cells;
Hoep = Hoeppli phenomenon; mono = mononuclear cells; fibr = fibroblasts;
mono/eosi = either of the two

SI = Small Intestine; LI = Large Intestine; M = Mucosa; SM = Submucosa
wk = week; p.i. = post infection

* The different types of cores of granulomas sometimes had no evidence of eggs.
\# The Hoeppli phenomenon was present in some lesions and absent in others.
\+ The lesion was observed
+- The lesion was sometimes observed.
++ The lesion was predominant

The cores of most of the granulomas had more eggs with intact miracidia. Some granulomas, however, had dead miracidia. Granulomas with intact or dead miracidia were sometimes found in the same area of the intestine. The Hoeppli phenomenon was observed in some granulomas. This reaction was seen at all the stages of the disease. At 7 weeks p.i., however, the reaction was seen in association with the predominant accumulation of eosinophils around eggs. The granulomatous lesions, like other pathological changes observed, were prevalent in the small intestines. Similar lesions were observed in the anterior colon. However, they were fewer and low in intensity.

iii. Other inflammatory changes.

At 11 and 18 weeks p.i. the mucosa of the anterior and middle sections of the small intestines had increased fibrous connective tissues and mononuclear cells at the same location. In some of the affected areas remnants of eggs could be identified.

iv. Intravascular eggs and related changes.

In venules and capillaries at different levels of the mucosa at all sections of the small intestine, eggs were found containing mature or immature intact miracidia. Other eggs had early embryos or were empty. In this intravascular location eggs had a cellular mantle of variable layers. Mostly it was a single layer of markedly attenuated mononuclear cells often unrecognizable from the endothelial cells in close contact with the egg (Fig. 5a). The blood vessels with eggs had little or no cell reaction around them. At a few sites in the mucosa of the middle small intestine, several eggs were found in tandem fashion inside or outside a blood vessel up to the subepithelium (Fig. 6a). Many intravascular eggs were encountered at 11 weeks p.i. compared to 7 weeks and 18 weeks p.i. These eggs were more located in the mucosa of the middle small intestine, but also to a lesser degree in the distal and the anterior sections. Intravascular eggs were much less observed in the submucosa of the middle and distal small intestine and none were encountered in the colon.

v. Extravascular eggs and related changes.

Extravascular eggs with or without viable miracidia and little or no cellular reaction around them were also found in the mucosa, sometimes in the same location as the intravascular eggs. This type of eggs was much less observed in the submucosa of the middle and distal small intestine and none were encountered in the colon.

Eggs with intact or degenerate miracidia lying inside the lumen of the crypts or intervillous space were seen covered by eosinophils and other cells. At some sites in the superficial part of mucosa, eggs so covered were seen to be partly emerged to the lumen of the intestine. In the lamina propria at such places were eosinophils or a mixture of eosinophils and mononuclear cells (Fig. 7). Some of the partly emerged eggs were in close proximity to granulomas in the subepithelium, and occasionally emerged eggs with intact miracidia were found in the intervillous spaces or crypt lumina with a mantle of granuloma cells. The

emerged eggs in the intervillous spaces or crypt lumina and other partly emerged eggs were seen more often at 7 and 11 weeks p.i. than at 18 weeks p.i. There seemed to be more of these in the middle sections of the small intestine than at other sections. Some emerged and partly emerged eggs were also observed in the mucosa of the anterior colon.

vi. Lesions involving veins.

Changes involving the walls of submucosal veins and their surroundings were encountered mainly in the middle and distal sections of the small intestine at 11 weeks and 18 weeks p.i. The changes consisted of infiltrations in the adventitia and outer media by a great number of eosinophils alone or mononuclear cells and eosinophils or just mononuclear cells. In a few veins there were medial hypertrophy and narrowing of the lumen. At 18 weeks p.i. granulomas were observed in one side of the wall of some veins. In this position they caused narrowing of the vessel lumen. The granuloma had variable cellular composition as observed elsewhere in the intestine.

Another observation involving the veins was the presence of pairs of male and female worms inside the veins in the submucosa, the mesentery and, occasionally, the tunica muscularis. In some of these occupied veins, the wall was markedly stretched with great attenuation of endothelial cells to very thin lines. No adult worms were seen in the mucosa. This observation was made at 18 weeks p.i. only in all sections of the small intestine. The frequency of lesions in the veins was much less compared to other lesions described.

b. Liver

In a few portal triads there were a few small granulomas. In the core of some of them there were large mononuclear cells with or without eggs. In others there were giant cells around the eggs in the core. The cores of these granulomas were surrounded by mixture of eosinophils and small mononuclear cells.

c. Other organs

At 11 weeks p.i. a few granulomas were found in the **mesenteric lymph nodes**. Some of granulomas had a core of an egg with Hoeppli phenomenon surrounded by eosinophils or epithelioid cells. The cores were enclosed by a mixture of mononuclear cells and eosinophils

with a thin surrounding zone of fibroblasts. At 7 and 11 weeks p.i. the Malpighian corpuscles of the **spleen** were surrounded by many neutrophils and eosinophils. These cells were also increased in numbers in the pulp but not to the same extent as around the corpuscles.

2. Experiment II

a. Gastrointestinal tract

i. Lesions in which the eosinophil was predominant.

The intense eosinophil reaction seen in Experiment I at 7 weeks p.i. was less prominent and less frequent than in Experiment II. Few such areas were observed in the superficial part of the mucosa of the small intestine. Occasionally they were encountered in the submucosa of the distal small intestine and in the mucosa of the anterior colon. Modifications in the lesion similar to those in Experiment I were observed.

ii. Granulomatous lesions.

Granulomatous lesions were also the main pathological changes in this group of animals. The various types of granulomas and their distribution in the different layers of the intestines are shown in Table 2. The remarks given under Experiment I about the occurrence and distribution of granulomas with a core of mainly large mononuclear cells, with or without eosinophils and a core of epithelioid cells or giant cells, apply also to Experiment II. A few granulomas were observed in the superficial part of the mucosa, partly projecting into the lumen of a crypt. A few were just under the epithelium.

iii. Other inflammatory changes.

The mucosa of the anterior and middle sections of the small intestine had areas with an increased amount of connective tissue and number of mononuclear cells at the same location. In some of the affected areas there were remnants of eggs.

iv. Intravascular eggs and related changes.

Few intravascular eggs were seen in the mucosa of the middle and distal sections of the small intestine. The eggs were observed more often in calves with primary infections. The eggs had many features in common with intravascular eggs in Experiment I.

Table 2: Types of Granulomas in the Intestine in Experiment II

Core of granuloma	Cells around core	Duration p.i. 11 wk		Level in intestine**			
				SI		LI	
		1ry	2ry	M	SM	M	SM
egg+eosi	mono+-eosi	+	+-	+	+-	-	+
egg+mono	mono+-eosi	+	+	+	+-	+	+
egg+mono/ eosi	mono/eosi	+	+-	+	+-	+-	-
egg+Hoep#	eosi	+-	+-	+	+-	+-	+-
egg+Hoep# +-eosi	giant+mono +-eosi	+	+	+	+	-	-
egg+Hoep#	mono+eosi	+	++	+	++	-	+-
egg+epit	mono+eosi fibr	+	++	+-	++	+-	+
egg+giant	epid+eosi +mono+fibr	+	++	+	++	-	+-
egg+Hoep#	eosi+giant	+-	+-	+	+	-	-

Explanation to Abbreviations: See under Table 1.
1ry = Primary infection
2ry = Calves given a second infection
** = No difference between primary and secondary infections

v. Extravascular eggs and related changes.

Eggs in intervillous spaces or crypt lumina were observed in the superficial part of the mucosa of the middle and distal small intestine. The emerged eggs were few compared to those seen in Experiment I at 11 weeks p.i. Some of the eggs were in an exudate of mainly eosinophils. Very few were observed with a mantle of granuloma cells.Eggs in the lamina propria were also observed in the small intestine. They had little or no cellular reactions around them. Some of the eggs had intact immature or mature miracidia. In others, however, there were no miracidia or these were dead. All in all, the calves with primary infection had more eggs in the lamina propria than those exposed to a second infection.

vi. Lesions involving veins.

Venous changes were occasionally observed in Experiment II in calves that were exposed to a second infection. There was mononuclear cell infiltration in the adventitia and perivascular tissues around the affected veins. However, no schistosome eggs or their remains were seen in the lesions. The lesions were located in the submucosa of the anterior small intestine.

b. Liver

Few granulomas, some containing eggs, were observed in the portal triads. Most granulomas were composed of mainly small mononuclear cells. In other granulomas the eggs were surrounded by giant cells, epithelioid cells and a distinct capsule of connective tissue.

c. Other organs

No lesions were detected in the **spleen, pancreas** and **mesenteric lymph nodes**

3. Natural Infections

Specimens were collected from thirty five cattle. The majority of the specimens were from different sections of the small intestine; others were taken from the colon. At postmortem specimens were taken from sections of the intestine opposite the mesenteric veins which contained schistosome worms. Specimens were also taken from cattle which had shown miracidia in fecal samples antemortem.

i. Lesions in which the eosinophil was predominant.

Lesions in which the eosinophils were the predominant cells were not as prevalent as the mainly granulomatous lesions. In a few specimens, however, these lesion were the most dominant and occurred mostly in the superficial part of the mucosa. The lesions had features similar to those seen in the experimental infections.

ii. Granulomatous lesions.

The granulomas were the most frequent type of lesions observed in all the specimens. A variety of cellular combinations in the granulomas were observed as in the experimental disease. However, cores with mainly large mononuclear cells in the presence or absence of an egg/egg material with or without Hoeppli phenomenon and with or without giant cells, were the most common. The periphery had a mixture of eosinophils and small mononuclear cells. Some granulomas were surrounded by fibroblasts and collagen. Most of the granulomas were small although a few large ones were observed, some occupying the entire thickness of the mucosa.

As in experimental infections, the granulomas with cores of mainly epithelioid and/or giant

cells were prevalent in the submucosa. They did, however, occur in other layers. The granulomas with cores of mainly macrophages, lymphocytes with or without eosinophils, were seen more often in the mucosa. In the mucosa, however, granulomas were seen more in the basal lamina propria. In a number of specimens granulomas of different cellular compositions were observed in the same location. Also observed was the occurrence of granulomas only in the submucosa or mesentery in some specimens.

iii. Intravascular eggs and related changes.

Intravascular eggs, some with a mantle of one or more layers of mononuclear cells, were found in 9 of 35 specimens. They were mostly in mucosa and the eggs had little or no cellular reaction around them. At a few sites, groups of up to five eggs were seen in tandem arrangement close to the epithelium. Around these and others in batches, the blood vessel wall was markedly distended. The eggs had intact immature or embryonic miracidia. Occasionally intravascular eggs, singly or in batches, were found in the submucosa.

iv. Extravascular eggs and related changes.

Extravascular eggs with very little or no cellular reaction were encountered in the middle and superficial part of the mucosa of the small intestine. Some of the eggs had mature while others had immature miracidia. Eggs with intact or degenerated miracidia were observed in the intervillous spaces or crypt lumina. Some of the eggs had the Hoeppli phenomenon around them.

v. Lesions involving veins.

Pathological changes involving blood vessels, mainly the venules and the veins, were substantial. The changes were similar to those found in the experimental infections. These, however, were more prevalent and severe, particularly in the serosa and mesentery. They were also more prevalent in the small intestines than elsewhere. The greatest number was in the mesentery and serosa (23 of 35 specimens) followed by the submucosa (18 of 35 specimens). None were found in the mucosa. Mild lesions in the submucosa were similar to those found in the experimental infections. In severe cases there were proliferative changes in the media. There was also intimal and endothelial hyperplasia with partial or complete occlusion of the vessel lumen. In some veins some of the enlarged smooth muscles of the

inner layers of the media were vacuolated. Among the proliferative smooth muscles in the media were few elastin fibers. Additionally, there were intimal/endothelial papillary projections into lumina of some of the veins, thus dividing the lumen and almost occluding it (Fig. 10). In a few such affected mesenteric veins there were intact worms.

In the affected veins thickening of the walls was mainly due to changes in the smooth muscles. However, marked thickening of the media and intima was also partly due to the increase in collagen in the outer intima and inner media. Collagen was also found among the hyperplastic endothelial cells that were more or less occluding the vessel lumina.

The inflammatory changes in blood vessels were similar to those in experimental infections. In a number of veins, particularly in the serosa and mesentery, eosinophils and/or mononuclear cells not only affected the perivascular tissues but often involved all layers of the vessel wall. Whereas granulomas observed in the experimental infections were localized in the paravascular tissues, granulomas, observed in field cases were found in all layers of the vessel wall, sometimes causing complete closure of the vessel lumen. As in the experimental infections, the majority of the granulomas had a core of large mononuclear cells with or without giant cells and eggs. Additionally, the Hoeppli phenomenon was not uncommon in the field infections. The cores of the granulomas consisted largely of eosinophils which also formed a halo around the cores together with mononuclear cells and some fibroblasts.

It was not unusual to come across veins with marked proliferative changes and infiltration of eosinophils and mononuclear cells in the media and adventitia as well as the perivascular tissues. Some veins had only perivascular aggregations of small mononuclear cells. In all these lesions the accompanying arterial vessels were unaffected.

An occasional lesion encountered in the mesentery was early thrombus formation at a site occupied by a pair of intact male and female worms.

vi. Summary of lesions.

Involvement of the different layers of the intestines with the schistosome eggs was variable

in natural infections. No significant lesions were seen in the mucosa of the 6 of 35 specimens but granulomas were always present in the submucosa. Lesions with prominence of eosinophils were mostly in the mucosa. In only a few cases were these lesions, as well as intravascular and extravascular eggs without cellular reactions, the major changes indicative of schistosomiasis. Usually there were additional lesions particularly in the submucosa, serosa and mesentery. Lesions involving blood vessels, especially in the serosa and mesentery were present in 23 of 35 specimens. In 4 of 35 specimens the vascular lesions were the only evidence for the presence of schistosomiasis. The vein lesions were second in importance in 9 of 35 (25%) of the natural infections investigated. Vascular lesions, more or less similar to those in the small intestine were encountered in the few specimens of the large intestine.

B. HAMSTERS

a. Gastrointestinal tract

Lesions were more extensive and severe compared to those in calves. Lesions were found at all levels in all sections of the intestines and mesentery. The hamster has no obvious muscularis mucosa in the small intestine. There is therefore no clear distinction between mucosa and submucosa. Thus, lesions in the small intestine were mucosal, muscular, serosal or mesenteric in distribution.

i. Lesions in which the eosinophil was predominant.

Prominent and frequent eosinophil reactions in the presence or absence of eggs were the main changes at 7-8 weeks p.i. The eggs in these changes had intact immature or mature miracidia. However, the marked aggregations of eosinophils in calves at 7-11 weeks p.i. were not so intense in hamsters. Also in these the Hoeppli phenomenon was not seen at any of the stages of the disease studied. As in calves, tracts of eosiniphils extending to the subepithelium from the lower parts of the lamina propria were observed in the hamsters. These lesions were mostly in the mucosa of the small intestines at 7-8 weeks p.i. However, a few lesions were observed in the mucosa of the colon. From 8 weeks p.i. the lesions were infrequently observed compared to other lesions. Mild diffuse accumulations of eosinophils were, however, observed in the small and large intestine up to 14 weeks p.i..

ii. Granulomatous lesions.

From 7 weeks p.i. onwards areas of diffuse mononuclear cell infiltrations (macrophages, plasma cells and lymphocytes) were present in all layers of the intestines and in the mesentery. The mononuclear cell infiltration was mild and discrete at 7 weeks p.i. At 9 weeks p.i. the infiltration was intense and widespread. This lesion along with the obviously granulomatous ones (see below), caused marked thickening of the wall of the intestines. 11).

Lesions with the unequivocal morphology of a granuloma were seen as early as 7 weeks p.i. although other inflammatory changes were more prominent at this stage. The granulomas were seen as small aggregates of eosinophils and mononuclear cells in varying numbers around schistosome eggs. Some of the granulomas had large, pale staining mononuclear cells among which were scattered a few eosinophils. At 7-8 weeks p.i. the lesions were at all levels of the mucosa. They were, however, seen more frequently in the small intestine. Finally, granulomas in low numbers were present in the submucosa of the colon. The granulomatous lesions were the main changes from 9 weeks p.i. They were at all levels in all sections of the intestines including the rectum and mesentery. This was unlike the experimental infection in calves where involvement of the colon was occasional and only in the anterior part.

The granulomas had variable cellular compositions. However, the variation was not as diverse as that observed in the calf infections (Table 1). The most common type of granuloma had large, pale staining nuclei. Occasionally, giant cells were seen in these. The margins of such granulomas were delineated by small, darkly staining nuclei or fibroblasts and collagen. Some granulomas had only one egg but others had many eggs scattered all over (Fig. 9). In the same granuloma some eggs had intact mature or immature miracidia while in others the miracidia were degenerated. Other types of granuloma had a core of eosinophils. From this core eosinophils extended to the subepithelium. In other types with a core of large or small mononuclear cells, there was a fringe of eosinophils. From this fringe eosinophils also extended to the subepithelium. In some of the granulomas there was necrosis in the center of the lesions. In none of the stages of the disease investigated, was a Hoeppli phenomenon observed in association with the granulomas. In later stages of the

infection (12-14 weeks p.i.) the granulomas with large mononuclear cells were the most predominant in all parts of the intestine.

Certain features were associated with the presence of granulomas in the intestines. Some of the granulomas, particularly those composed of the large mononuclear cells, occupied the entire thickness of the mucosa. Other granulomas extended from the subserosa to the subepithelium (Fig. 9). Owing to the lengthwise coalescence of several granulomas, large areas of the mucosa and submucosa were occupied. These features resulted in marked thickening of the intestinal wall. At the thickened area villi and intestinal glands were shortened or had disappeared all together. Where the granulomas extended to the subepithelium, some of them breached the epithelium. Segments of such granulomas around eggs were located in the intervillous spaces or intestinal gland lumina. Fibroplasia near some of the granulomas were seen to extend to other areas. This was observed at all levels of the intestine. Granulomas in the subserosa and serosa formed nodular growths with variable degrees of fibrosis.

iii. Intravascular eggs and related changes.

Intravascular eggs were observed in hamsters as in calves but they were in larger numbers and more widespread. In the early stages of the disease, the intravascular eggs were particularly numerous in the mucosa of the small intestine. However, between 9-11 weeks p.i. several eggs in venules and veins were in both the mucosa and submucosa of the intestines. Intravascular eggs were also seen in the tunica muscularis and subserosa of the intestines but in smaller numbers.

Many of the features of the intravascular eggs in calves were also observed in hamsters but more prominently. Many of the intravascular eggs observed had intact intermediate mature or immature miracidia while others were empty. The eggs occurred singly or in batches of three or more. Eggs observed in batches caused marked distention of the vessels (Fig. 10), some of which were close to the epithelium. One to several layers of mononuclear cells formed a mantle around the eggs. In some vessels the mantle anchored the egg to the endothelium (Fig. 5b). Eggs in tandem arrangement were more common in the hamsters than in calves. Such eggs were in all sections of the intestines including the rectum. Some eggs

in tandem fashion seemed to have passed through the vessel wall into the subepithelial area or even to the intestinal lumen (Fig. 6b). In some cases, batches of eggs were in a blood vessel in an of bleeding. There was also an increased number of leukocytes in the neighborhood of intravascular eggs. However, leukocytosis was also observed in vessels without eggs.

There were partly emerged eggs in some venules/capillaries. Some eggs were seen in the subendothelial area. Some of these eggs had a single layer of cells on the luminal side. This layer was continuous with cells at the point where the egg entered the vessel wall. At the point of emergence, the egg with an intact miracidium attracted little perivascular cellular reaction. However, the egg with a dead/degenerate miracidium attracted eosinophils and mononuclear cells. In a few specimens at 11 weeks p.i. eggs or their remains were observed in thrombi in mesenteric veins of the large intestines.

iv. Extravascular eggs and related changes.

More extravascular eggs were observed in hamsters than in calves. They were in similar or different locations as the intravascular eggs. For ease of description, extravascular eggs have been put into two groups.

1. This group consisted of eggs with little or no cellular reaction around them in the mucosa, submucosa, tunica muscularis and the subserosa. In the lamina propria the eggs were observed near blood vessels or very close to the intestinal epithelium. Many of the eggs were single, some were in batches and a few were in tandem arrangement. Some eggs were in an area of hemorrhage. Many of the eggs had intact mature or intermediate mature or immature miracidia. When a cellular reaction was present around the eggs it consisted mostly of a single cell layer closely apposed to the egg as if it were the intravascular mantle still present. As in the case of intravascular eggs, extravascular eggs were numerous in the period of 9-11 weeks p.i. At 14 weeks p.i. few eggs could be seen in the mucosa of the small intestine although appreciable numbers were seen in the mucosa and submucosa of the colon.

2. The second group of extravascular eggs were those in emerging or emerged positions with respect to the epithelium and lumen of the intestine. This group was observed in all

sections of the intestines, including the rectum. They were located mainly in the superficial part of the mucosa. In earlier stages of the disease (7-8 weeks p.i.) they were more numerous in the middle small intestine than elsewhere. Later, however, (9-11 weeks p.i.), they were many in all sections of the intestines in many specimens. The following observations were made: (i) Some eggs were observed in the intervillous spaces or in the intestinal gland lumina. Here the eggs, often with intact mature miracidium, were surrounded by mainly eosinophils (7-8 weeks p.i.) or eosinophils and some mononuclear cells in later stages. (ii) Partly emerged eggs were observed in the intervillous spaces or intestinal gland lumina, as seen in calves (Fig. 8). Some of the eggs had their pointed ends facing the intestinal lumen. (iii) Some eggs in the intervillous or intestinal gland lumina had no cell cover. Some of these eggs had mature and others immature intact miracidia. (iv) Eggs were observed with the pointed ends through the epithelium unaccompanied by any cellular reaction (Fig. 11). At this site in some specimens the epithelial arrangement was partly distorted. As in calves some of the eggs near the epithelium had the spined end pointing away from the intestinal lumen while others had it the other way round. (v) Eggs were observed in the subepithelial area preceded by a few layers of eosinophils at the basal lamina of the epithelium. (vi) Extravascular eggs in tandem arrangement were seen with the egg at the top already through to the intestinal lumen. In the colon some eggs in the situations described above were seen just to the side of the glandular pit opening or just below it. Also many such eggs were seen next to the basal lamina of the colonic epithelium without cellular reaction.

v. Lesions involving veins.

Lesions involving walls of veins and their perivascular tissues were less common than in calves. There were paravascular and subintimal granulomas which were composed of mainly large mononuclear cells. The vascular lesions were located mainly in the submucosa and subserosa, and occasionally in the mesentery of the colon.

Worms, singly or in pairs, were observed in veins only in the mesentery of the intestines, especially that of the colon, in all stages of the disease studied. Some of the mesenteric veins with worms had perivascular mononuclear cell accumulations and the nearby tissues were infiltrated with similar cells and eosinophils. This change was observed now and then from

8 weeks p.i. Apart from the perivascular cellular reaction that was observed, no other pathological changes were seen.

Thrombosis was another lesion that involved blood vessels. The mesenteric veins of the colon were particularly affected. In some thrombi there were trapped eggs or their remnants. The thrombosis was prominent at 11 weeks p.i.

vi. Other microscopic changes.

There were variable degrees of congestion and hemorrhage at various levels in all sections of the intestines from 8 weeks p.i. In some specimens there were erythrocytes among epithelial cells in villi with widely distended capillaries. At some stages of the disease, e.g. 11 weeks p.i., there was generalized congestion of the intestines. Obvious rhexis of blood vessels in areas of hemorrhage could not be established. Some tissue sections of the small intestines, however, had eggs in tandem arrangement intravascularly in an area of hemorrhage. The blood vessel appeared to have been damaged since, in some places, some eggs in the chain were outside the vessel.

In some specimens the intestines had a hemorrhagic exudate overlying the epithelium without evidence of hemorrhage in the underlying lamina propria. At 8 weeks p.i. there was separation at the lateral sides of villous epithelial cells. In the small intestine and to a lesser extent in the large intestine, there was, in early patency, leukocytosis in venules and capillaries, whether they contained eggs or not.

b. Liver

There were prominent and extensive granuloma formations from the seventh week p.i.. The granulomas were mostly of the large mononuclear cell type with or without eggs in the core which was sometimes necrotic. The granulomas were most frequent in the portal areas. Other granulomas were subintimal, causing partial or complete occlusion of the lumen of the vessel. Many eggs were present in the granulomas. Some granulomas had eggs with intact miracidia while in others the eggs were degenerated.

In addition to granulomas, there were hepatocyte necrosis and fibrosis at different stages.

Intravascular eggs, singly or in groups, adhered together by mononuclear cells, were observed in portal veins and their tributaries. Some eggs were partly emigrated or subendothelial as was observed in the intestines. Thrombosis was prominent and extensive in the portal veins. In some veins eggs were trapped in the thrombi. In other veins thrombi were formed near intact worms (Fig. 12). Some of the thrombi were very large and occlusive; some were undergoing organization. Thrombosis was observed from 9 weeks p.i. onwards. At 10 weeks p.i. there was prominent hemorrhage especially under the capsule of the liver. These hemorrhagic areas also contained many eosinophils. However, rupture of blood vessels was not encountered.

c. Other organs

In the **lungs** there were small granulomas with either a core of large mononuclear cells around an egg or a core of only eosinophils and mononuclear cells. Intravascular and extravascular eggs with little cellular reaction were also observed. Leukocytosis, largely from neutrophils, was quite prominent in the pulmonary vessels of the lungs more or less in all the stages of the disease. Thrombosis was also occasionally observed in some pulmonary vessels. Mature and immature worms were seen in the lungs at different stages of the disease. The **mesenteric lymph nodes** had granulomas in the cortex and medulla. In the subcapsular sinuses and medulla there were numerous eggs with or without cellular reaction. Many giant cells unassociated with eggs were also present in these areas. Adult worms were seen in mesenteric veins draining the lymph node. The **pancreas** had few granulomas of the large mononuclear cell type and several areas containing eggs without reaction. There were eggs inside venules in earlier stage of the infection. However, eggs were less observed from the 11th week p.i. There were many intravascular eggs in the **spleen** in the early stages of the disease but less so in the later stages. Adult worms were also observed in the large veins. Granulomas of the large mononuclear cell type were seen. However, the prominence of the lesion did not match the many eggs that were present. The **kidneys** had hyaline casts in the lumina of tubules. This was extensive in some specimens.In epithelial cells of some tubules there were hyaline droplets. Lesions directly related to the presence of eggs or worms were not seen.

SCANNING ELECTRON MICROSCOPY

A. CONTROL ANIMALS

1. Calves

The appearance of villi of the small intestine varied from finger-like to leaf-like or tongue-like, even in the same animal and sometimes in the same area. In the early stages in the proximal small intestine there were ridge-like, interconnecting villi (Fig. 13). In later stages there were very few interconnections.

2. Hamsters

There were also differences in the appearance and disposition of villi in the intestines of hamsters at different stages of the experiment. In the early stages they had a finger- or tongue-like morphology on and between ridges. Later on they were thicker and with blunt tips which had the appearance of the side of a closed boxer's glove. The colonic mucosa also had a variable appearance during the experiment. Early on, glandular pits were easily identified on the mucosal folds. Later, each fold had numerous shorter and smaller folds among which the glandular pits were slit-like and difficult to distinguish from the folds (Fig. 14).

B. EXPERIMENTAL ANIMALS

1. Calves

Few changes were detected in these animals. In one calf, at 7 weeks p.i., some villi of the anterior small intestine had excessive loss of epithelium with the cells rounding up and shriveling to small pale granule-like structures and subsequent exposure of the subepithelial tissues.

2. Hamsters

At 7 weeks p.i. the intervillous spaces of the distal small intestine contained irregular masses the surface of which were formed by globular elements with many projections or with discoid morphology and what appeared to be mucus threads (Fig. 15). The villi showed no obvious changes.

In the anterior colon, also at 7 weeks p.i., the surface of the mucosa had ovoid to elliptical patches of rough granular appearance, equal in size to areas of intact epithelial cells, among the mucosal epithelial cells. A similar change, though more pronounced and affecting larger areas of the colonic mucosal surface, was seen at 8 weeks p.i. This seemed to represent areas of epithelial loss. Also evident at 7 weeks p.i. was separation of individual or groups of cells giving the surface of the mucosa a fissured appearance. There were also cracks in the mucosal surface. Along some of the cracks were discoid and globular elements. Some of the mucosal surface cracks linked several glandular pits.

At weeks 8, and 10 p.i. the extrusion zones and their surrounding areas at the tips of many villi of the distal small intestine had increased numbers of globular cellular elements. Some of these cells had microvilli on their surfaces, others appeared swollen and had irregular surfaces with swollen microvilli and yet others had uneven but smooth surfaces, (Fig. 16).

In the colon of some animals at 8 weeks p.i. the mucosal surface had a swollen appearance which was easily appreciated about the glandular pits openings (swollen lip appearance).

In the colon at 9 and 10 weeks p.i., several mucosal areas showed disruption in the normal appearance. There were 50-100μm, roughly circular or 50-300μm by 50-100μm roughly linear areas of disruption. In such areas there were cracks in the mucosa or the mucosal surface cells were loose, separated, and appeared round and shrunken or were shrunken but held together in groups. In some other areas of disruption the surface cells of the mucosa were lost, leaving an opening at which were round cells with microprojections on their surfaces and discoid cells (Figs. 17a, 17b).

In some disrupted areas, just to the side of an opening into the glandular pit, were apparently spindle shaped structures with the free ends tapering to a point. The edges of the hole through which these structures emerged had variably loosened cells and fibrillar material some of which was stuck to the surfaces of the structures. These structures conformed to the morphology of schistosome eggs (Figs. 17a, 18a, 18b). The extent of the exposed part of the egg was variable so that in some cases only the tip or the whole pointed part of this and a fair part of the body of the egg were visible. In one area up to seven eggs were seen

emerging and the area of emergence had cracks and obvious loosening of the cells around it comparable to a mushroom emerging through the soil (Fig. 19a). All the eggs that were seen emerged presented the nonspined end of the spindle-shaped egg. In some of the areas of the emerging eggs there was only loosening of the cells forming the edges of the hole of emergence, in other areas the mucosal surface cells were disrupted, loose, shrunken and/or glued together, (Figs. 18a, 18b, 19a, 19b) or were absent. The parts of the eggs visible above the mucosal surface were observed to have folds or ridges on them (Figs. 17a, 19b). On some eggs these folds were in a spiral arrangement. Other emerged eggs had damaged external cover at the pointed part of the egg.

TRANSMISSION ELECTRON MICROSCOPY

Specimens from the calves had fewer areas that were suitable for electron microscopic study than those from the hamsters. It was also observed that schistosome egg contents fell out leaving empty spaces in specimens mounted on naked grids. However, the contents remained in place on coated grids although contrast in the EM was somewhat reduced. In the TEM studies, therefore, both types of grid were used.

Changes related to the presence of the eggs were studied at the following stages:

a. Intravascular eggs (hamsters only)
b. Subendothelial eggs (hamsters only)
c. Extravascular eggs at a distance from blood vessels but within the the intestinal wall. This involved study of eggs with (i) very little or no cellular reaction about the egg and (ii) conspicuous cellular reaction (nongranulomatous and granulomatous lesions) (hamsters and calves)

No specimens were found with eggs at the stage of actually entering and crossing the endothelium and subendothelium or the intestinal epithelium.

A. HAMSTERS

a. Intravascular eggs

Some of the vessels containing eggs had a single, continuous or interrupted layer of smooth muscle cells just outside the endothelium while others had none. The vessels were therefore either small veins, venules or capillaries. Blood vessels with eggs inside were easily found

in the hamsters at 7 week p.i. but not at later stages of infection (11 weeks p.i.).

The intravascular eggs examined had viable embryos at different stages of development (Fig. 20). The egg shell consisted of very dense, homogeneous, amorphous material and was of almost even thickness. There were micropores scattered in the shell in sections taken from the middle of the spindle- shaped egg. These micropores were not seen to open directly on the outer surface of the egg shell. The outer surface of the egg shell was covered by numerous cone-shaped microspines of variable heights. The egg shell had variable types of cells covering it. In some blood vessels the egg was partly covered by monocytes in close proximity to the egg or partly by these and thin extensions of endothelial cells. The endothelial cell extensions also covered the luminal side of the monocyte egg cover (Fig. 20) forming junctions over the monocytes. On other parts of the same egg there was only an endothelial cover without intervening monocytes. On other eggs in different blood vessels, the cell mantle was mainly composed of monocytes. These, in some places, formed two layers of cells. However, even here the luminal side of the monocytes was partly covered by thin endothelial cells extensions (Fig. 21). A composite sketch of the area under description is shown in Fig. 22.

The interface between the egg shell surface and the host cells had dense flocculent or finely particulate material in aggregate or diffuse masses. In some parts of the interface microspines were interspersed with membrane-bound small vesicles with medium dense contents, giving the area an irregular reticulate appearance (Fig. 23). Some of the eggs and their cell cover in the lumen were adherent to one side of the vessel wall. Here the endothelial cover on the luminal surface of the egg and that next to the area of attachment were continuous (Figs. 21, 22b).

The cells covering the egg and the endothelium at the site of attachment had prominent Golgi complexes, with more than one in some of the cells. Numerous small vesicles were seen throughout the cytoplasm. Endothelial cells appeared to contain unusual amounts of organelles while monocytes had many secretory granules. Some endothelial cells had vesicles with heterogenous contents including myelinoid figures. Where the endothelium from the unoccupied side was continuous with that of the part covering the egg, some areas of basal

laminae of the endothelium and smooth muscles were poorly evident. Also in this area between adjacent smooth muscle cells there were dense myelinoid figures. In the immediate perivascular tissue of such an area were emigrated eosinophils (Fig. 21). Between the endothelium and the subjacent smooth muscle cells as well as their subjacent areas were medium dense to lucent droplets or vesicles. However, this was not in all areas of egg attachment.

There was increased margination and emigration of leukocytes, particularly neutrophils and monocytes, sometimes in areas close to the intravascular egg. In these areas endothelial cells had prominent Golgi complexes and other organelles as seen in association with the attached egg. Obvious margination and emigration were seen at most stages of the disease.

There was a variety of cells immediately outside the vessel wall on the side with or without an intravascular egg. Neutrophils, eosinophils and monocytes, similar to those covering the intravascular egg, were numerous. However, neutrophils outnumbered eosinophils. From their closeness to the outside of the vessel they appeared to have just emigrated. These extravascular leukocytes, particularly the monocytes, like their intravascular counterparts, had a morphology consistent with increased synthetic activity. Similar cells and others in the lamina propria formed the rest of the perivascular tissue elements. In some of the eosinophils the granules had low density. Some other cells had vacuoles whose contents were homogenous material mixed with myelinoid figures. Other had vacuoles with lucent particulate material that was mixed with cytomembranes. In some areas, the spaces between cellular extensions and the extracellular space around some eosinophils had small globular or vesicular structures with homogeneous dense contents.

b. Subendothelial eggs

The position of the egg, emigrating leukocytes, the vessel walls, the abluminal side of the egg and the perivascular tissues, is shown in the composite sketch in Fig. 27. Eggs in the subendothelium were studied at 8, 9, and 11 weeks p.i.

In some of the specimens the egg had an intermediate mature miracidium, i.e., with some recognizable organization of organs. In the other specimens the state of the miracidium could

not be determined because of the shelling out of the egg contents during tissue processing. The egg shell structure was similar to that of the intravascular egg. The blood vessels involved were venules and capillaries.

In the lumen of the vessel with the subendothelial egg, leucocyte margination was frequently observed. Some of the marginating monocytes and endothelial cells had increased synthetic activity as was observed in the venules with intravascular eggs. There were more neutrophils marginating and emigrating close to the subendothelial egg areas than other leukocytes.

In some specimens the egg was just under the endothelium. In some areas neutrophils were interposed between them. In such areas the luminal surfaces of the neutrophils were covered by thin extensions from adjacent endothelial cells which formed tight junctions over the neutrophils. At the abluminal side of these emigrating neutrophils, they were in contact with the egg shell surface except for the peripheral parts where thin extensions of endothelial cells were between the eggs and the neutrophils (Fig. 25). In other segments of the vessel the same egg was directly under the endothelium. The endothelium was markedly stretched over the egg. Gap and tight junctions as well as nonjunctional associations between endothelial cells were observed over the egg. At the points where the endothelium over the egg continued with that of the unoccupied side, subendothelial structures such as smooth muscle cells were deflected from the endothelium.

Apart from neutrophils emigrating with the egg, already emigrated neutrophils and activated monocytes (macrophages) were evident just outside the vessel wall near subendothelial eggs. In cytoplasmic extensions of the macrophages covering the egg and in cells nearby, there were areas of cytoplasmic rarefaction with clumping of the cytoplasmic matrix.

The interface area at the subendothelium was delineated by intact endothelial or neutrophil cell membrane and the egg shell surface.The area was very narrow in some cases. It had medium dense, very finely particulate flocculent material. Additionally, a few small globular or vesicular structures were occasionally seen. The interface between the egg shell and the host cell cover on the side not under an endothelium had slightly more dense but finely

particulate material. The globular or vesicular structures here were slightly larger and obviously membrane-bound (Fig. 26).

At the side of the egg not directly under the endothelium the cells around the egg were macrophages sometimes accompanied by eosinophils. Some of the eosinophils closest to the egg surface had some secretory granules with medium to dense contents and no crystalloid bar while in others the crystalloid bar was faded and in a flocculent matrix. Other granules had vague membrane outlines. Some macrophages had a variety of large vacuoles with heterogenous contents. Fibroblasts had markedly dilated cisternae. At these sites no cellular elements immediately around the egg could be identified as those displaced from the subendothelium now occupied by the egg.

Most eggs examined in or just outside a venule/vein had their long axis parallel to the vessel wall. However, one egg had its pointed end pushing at the endothelium (Fig. 10). The tip of the pointed end was almost at the basal lamina. The endothelial cell being pushed was in close contact with the egg shell except for an interface of medium dense finely particulate material. The endothelial cell in contact with egg showed no changes. However, some cells close to the endothelium and just outside the area where the egg was pushing had many myelinoid whorls. In the intercellular spaces in this perivascular area there were many membrane-bound vacuoles with lucent contents. The pointed end of the egg had a thicker shell compared to that at the middle parts of the egg. Also at this part of the egg there were comparatively more microspores.

c. Extravascular eggs

i. Eggs with little or no cellular reaction.

The eggs examined were from specimens obtained at 7-11 weeks p.i. They were in the small or large intestine at some distance from blood vessels. Some were very close to the basal part of a crypt or villus epithelium. Others were in the middle or basal area of the lamina propria. The eggs had miracidia at different stages of development. Except for an egg whose contents had been shelled out during tissue processing, all the eggs examined had viable miracidia. The egg shell composition was similar to that of the intravascular and subendothelial eggs (Fig.27).

The interface between the egg shell and the host cells contained variable materials. In some cases the interface had medium dense to dense contents of finely particulate material. At other eggs the material was dense to very dense and homogenous with microspines projecting into it. The material was of variable thickness and density in different areas in some specimens. There was a highly dense line separating the material from the host cells in some places but not in others. At some eggs near the base of a villus or crypt epithelium, the dense homogeneous material extended between or about fibrillar structures and cellular elements of the lamina propria to the basal lamina of the overlying epithelium (Fig. 28). At other eggs, in addition to medium dense finely particulate material in the interface, there were small membrane- or nonmembrane-bound vesicles or globules. These were of variable size and number and had medium dense to dense contents. The cell cover limiting the interface was of variable composition at different eggs. Immediately forming the inner layers of the cover of some eggs were cytoplasmic extensions, probably of macrophages, fibroblasts or smooth muscle cells. The rest of the cell cover was composed of obvious macrophages, fibroblasts, some eosinophils, plasma cells and an occasional mast cell. These cells were in proportions ordinarily observed, or slightly increased, in the lamina propria. Mingled with these cells were cytoplasmic extensions of cells whose origin could not be identified.

A variety of changes were observed in the cells and cellular extensions. In some cytoplasmic extensions immediately around the egg there were vesicles with lucent to dense flocculent materials. In other cellular extensions there were myelinoid figures. In some extensions mitochondria had lost cristae to form medium dense particulate material However, other mitochondria showed little change. Other cellular extensions and areas between adjacent cells had denser areas which in some places alternated with less dense portions. Some of the affected areas involved collagen.

In the cytoplasm of some fibroblasts and plasma cells close to the egg there were lipid droplets similar to those in miracidial cells inside the egg. In some eosinophils the secretory granules had light contents, no crystalloid bar and indistinct delineating membranes. At other sites there were small membrane-bound vesicles or globules in intercellular spaces near places of indistinct cell membranes, some sites of collagen and other fibrillar material. The vesicles were similar to those seen in the interface between the egg shell and surrounding

host cells (Fig. 29). At these sites, however, they appeared more dense and larger. Some smooth muscle cells here had rarefaction and clumping of cytoplasmic matrix materials (Fig. 29). At sites where the egg was close to the basal part of the mucosal epithelium, cells between the egg and the epithelium had vacuolar figures with lucent, homogeneous or flocculent material in the cytoplasm. The peripheral cytoplasm of smooth muscles cells was dense, a change also seen in the basal lamina of the adjacent epithelium (Fig. 13).

One egg in the immediate subepithelium was found. This egg was very close to the basal lamina and was parallel to it (Fig. 30). The immediate cover of the egg was composed of very fine cytoplasmic extensions, probably belonging to macrophages, fibroblasts or smooth muscles cells. The rest of the egg cover were obvious plasma cells, macrophages and fibroblasts. Apart from a few phagosomes in the macrophages, there were no changes in the cellular cover of this egg.

ii. Eggs with conspicuous cellular reaction.

Two types of cellular reaction to the eggs were encountered: A *nongranulomatous* reaction that was common in the earlier stages of the disease and a *granulomatous* type that was common in later stages. These changes were in both the small and large intestines.

Most eggs examined had viable intermediate mature or mature miracidia. Some were encountered that were degenerate and in one case of a granuloma there were both an egg with a mature viable miracidium and pieces of intact egg shell from an egg whose contents had been phagocytosed.

The structure and composition of eggs examined were as described under eggs outside blood vessels above. However, eggs sectioned at the narrow end showed the shell to be somewhat thicker and with more micropores compared to the middle wide part of the egg.

Nongranulomatous lesions. These were located at different levels of the mucosa. Some were close to the base of the epithelium of a villus or crypt and others were in the lower third of the lamina propria. In earlier stages of the disease the cells closest to the egg were eosinophils and an occasional macrophage or other mononuclear cell. The peripheral part of

the lesion, however, had a more heterogenous mixture of cells including neutrophils and a few mast cells. The eosinophils were closely apposed to each other and to the few other cells that were mixed with them but they did not interdigitate.

In other nongranulomatous lesions, however, macrophages and eosinophils were equally present close to the egg with the occasional neutrophil and mast cell in the periphery. The cells with their cytoplasmic extensions formed the immediate cover of the egg shell surface. The interface was of variable thickness, being almost nonexistent in some cases. The host cell membrane delineating the interface was intact in some places and indistinct in others. The interface areas had materials similar to that observed in previously described cases.

In some lesions a number of eosinophils closest to the egg had secretory granules with lucent to almost empty contents, vague membrane outlines and the entire cytoplasmic matrix was less dense (Fig. 31). However, such affected cells were comparatively few. At other lesions, however, eosinophils had only few, lucent granules. Macrophages and fibroblasts in the peripheral zones of some of the lesions had large lipid drops and phagosomes. Also, in some of these nongranulomatous lesions, a few of the closely approximated, interboundary areas were more dense while others, not so approximated, had medium dense, finely particulate material and small globular or vesicular structures as seen in other specimens.

Granulomatous lesions. The lesions studied were located in the lamina propria of the small and large intestines. Some were near the base of the mucosa epithelium, in the basal third of the lamina propria or the subserosa. A few were near blood vessels or paravascular in position in the lamina propria.

Early granulomas at 7 weeks p.i. and mature ones at 11 weeks p.i. were examined. The main cells in the early granuloma were a mixture of macrophages and eosinophils.Some were close to the egg shell surface but others were not. These cellular elements were closely apposed to each other without collagen among them except at the periphery where it probably belonged to the lamina propria. In other granulomas, in addition to the macrophages and eosinophils, there were occasional neutrophils and mast cells in the middle or periphery of the lesion. Also plasma cells were observed here. In the more mature granuloma, the major

cells were epithelioid and other mononuclear cells. However, a few eosinophils were present although not close to the egg. Plasma cells were also observed along with fibroblasts. In mature lesions collagen was found between epithelioid cells. The individual fibers were thin and not as easily appreciated toward the core as at the periphery of the lesion. Although giant cells had been observed light microscopically in some of the granulomas, none were encountered at TEM.

At the interface between the egg shell surface and the host cells there was a membrane that was conspicuous in some places but not in others. The interface was of variable thickness around the egg in the same or in different granulomas. In places where membrane separation of the egg surface was not obvious, there was highly dense, homogeneous material into which microspines of the egg shell projected. At some points the material extended into the intercellular areas of the surrounding cells whose cytoplasm adjacent to this material exhibited similar high density. In the material there were vesicles or vacuoles whose contents varied from very dense, dense to medium or lucent. There were membranous structures in some of them. Where the interface had membrane separation, the host cells were very closely applied to the egg shell surface. In other lesions the interface separation from the host cells had dense homogeneous, finely particulate material in which were interspersed globular or vesicles as observed elsewhere.

In the rest of the cells surrounding the egg, eosinophils had changes similar to those seen in nongranulomatuos lesions (Fig. 31). In mononuclear cells, particularly the macrophages, a variety of changes were observed. The frequency and intensity of these changes varied from granuloma to granuloma. In the cytoplasm there were vacuoles and vesicles of different sizes. Their vacuolar contents varied from medium dense to dense homogenous, flocculent or amorphous material. In other vacuoles contents were heterogeneous and consisted of smaller vesicles with medium dense to dense material among membranous stacks or myelinoid whorls or figures of variable density. Other vacuoles had highly dense contents of aggregates of what might have been smaller vesicles. Additionally, some cells close to the egg shell surface and some toward the periphery had foci of rarefaction and clumping of the cytoplasmic matrix material. This change was also observed in von Lichtenberg's envelope, a viable egg's syncytial cytoplasm.

In other cells and in different granulomas, some cells close to the egg shell surface had free ribosomes of lower density. Mitochondria in cells at different levels of the granuloma had variable loss of cristae and their contents were dense particulate material with or without tiny vesicles. Areas between cells close to the egg in several other places in the lesion and perigranuloma tissues had higher density. These areas included cytoplasmic extensions of other cells, collagen fibers which additionally looked frayed and subplasmalemmal cytoplasm of adjacent cells. Some of the above cytoplasmic changes were seen in lamina propria macrophages, fibroblasts and smooth muscle cells surrounding the granuloma. The changes were also observed in cells between the lesion and the basal part of the epithelium. Much distended cisternae of the rough endoplasmic reticulum (RER) and other large vacuolar structures with lucent contents or just empty, were observed in some of these cells or in the intercellular area.

In the granuloma with both a viable and a destroyed egg the egg shell compositions and structures were similar. The egg shell segments of the destroyed egg were surrounded on both sides by epithelioid cells. On the inner side the shell and the epithelioid cells were in very intimate contact. The outer side, however, had an interface with medium dense finely particulate material (Fig. 32). No egg shell material was identified in the surrounding phagocytes.

Changes were observed in the mucosal epithelial cells overlying nearby granulomatous reactions. Some epithelial cells had dilated cisternae of RER and vacuoles with medium dense to dense, finely particulate or amorphous material.In others, vacuoles had contents of either whorls of membranous structures or myelinoid figures. In many cases some mitochondria had cristae dissolved into flocculent or finely particulate material.

Eggs close to the base of the epithelium in an area of congestion and bleeding were also examined. The specimen was from a later stage of the disease (11 weeks p.i.). At necropsy there was generalized congestion of the intestines. The egg shell structure was similar to that seen in other specimens. The egg contents had fallen out during tissue processing. The immediate cellular cover of the egg and changes in the host cells were not unlike those seen in the earlier examined lesions. However, in the periphery of the lesion macrophages,

fibroblasts, few eosinophils and neutrophils were mixed with erythrocytes. An egg was observed in the lamina propria in close contact with both erythrocytes and macrophages as well as smooth muscle cells and fibroblasts. In these nucleated cells and in similar cells elsewhere in that area there were vacuoles with heterogeneous contents and some with greatly distended RER. Tissues nearby and elsewhere had disorganized collagen and other fibrillar material. A few venules examined in the area of congestion and hemorrhage had sites with markedly attenuated and discontinuous endothelial cells. The cytoplasmic matrix of the endothelial cells had medium to lucent density.

The overlying intestinal epithelial cells had swollen mitochondria with dissolved cristae, forming tiny vacuoles. The cells had also vacuoles with highly heterogeneous contents and dilated endoplasmic reticulum. The cytoplasmic matrix of many of the cells was lucent to medium dense. In between some epithelial cells there were erythrocytes. In some areas epithelial damage, through which bleeding took place was observed (Fig. 33). Here, some epithelial cells had lost microvilli. The cytoplasmic matrix of these cells had medium dense particulate material, RER was dilated and there were large vacuoles and phagosomes. Also seen in the cytoplasm were erythrocytes in different stages of degradation. The erythrocytes were in transcellular migration to the intestinal lumen (Fig. 33). In some of these cells some mitochondria were well preserved while others were damaged as described earlier. However, in many of the epithelial cells examined, most of the damaged mitochondria were concentrated near the nucleus (Fig. 33). A few specimens were examined in which there were adult worms inside mesenteric veins. The endothelium was thin and attenuated and had numerous tiny vesicles and many multivesicular bodies. In some endothelial cells there were more than one Golgi complex. Extending from the perinuclear region to the plasmalemma in some endothelial cells were wavy bundles of very fine filaments which in some places were in transverse section. The wavy filaments formed about a third of the cytoplasm. In some parts of the vein the worm cuticle was in contact with the endothelial surfaces. In others places there were erythrocytes and thrombocytes in between.

B. CALVES

Eggs encountered in this group were *extravascular* in the lamina propria of the mucosa of the small intestine and were associated with both *nongranulomatous and granulomatous lesions*. Specimens were taken at 7, 11, and 18 weeks p.i. Some of the lesions were close

to the crypt or villus epithelium. One lesion examined was in the submucosa and this was from a calf that was infected twice and the specimen was collected at 32 and 11 weeks p.i. after first and second exposures, respectively.

Eggs that were sectioned at the middle, widest part had lost their contents during tissue processing. However, those sectioned at or toward the narrow end had medium dense to dense irregularly reticular material. The egg shell structure and composition were similar to those of eggs in the hamster study.

Nongranulomatous lesions. These were seen in the early stages of the disease (7 and 11 weeks p.i.). Unlike in the hamsters, identification of eosinophils in the calves was not straightforward as nearly none of the secretory granules had a crystalloid bar. However, on the basis of the large size and numbers of the secretory granules, variable size and shape, heterogenous contents, multiple nuclear lobes and the light microscopic appearance of the lesions, it was possible to distinguish the eosinophils from neutrophils, macrophages and mast cells.

As in the hamsters, the cells closest to some eggs with nongranulomatous reaction, were eosinophils. The cells were sometimes an almost pure population (Fig. 31b). At other eggs there was a mixture of macrophages and eosinophils. Whether or not eosinophils or macrophages were the closest to the egg surface, the host cells were intimately applied to the egg surface sometimes with and sometimes without obvious membrane separation. At the sites of close contact some eosinophil secretory granules were almost empty although they had intact limiting membranes. At these sites there were also cytoplasmic vacuoles containing laminated structures. Also seen in the cytoplasm of cells near the eggs were areas of higher density with apparent structural disorganization of organelles therein. There was increase in density at cellular boundaries as described for the equivalent lesion in the hamsters. In areas where an interface existed the contents were as those seen in similar lesions in the hamsters.

Other changes seen in cells around the egg were in the cytoplasm. They were similar to those in the nongranulomatous lesions in the hamsters. However, in calves the rarefaction and clumping of cytoplasmic materials were in many cells and affected larger areas of the

cytoplasm. In some cells the cytoplasm had irregular nonmembrane-bound, very dense areas of variable size. In other cells there were aggregates of vesicular structures with medium dense as well as lucent areas or barely identifiable membranous fragments, all these in a low density matrix. This type of lesion was sometimes near the base of an epithelium. Some fibroblasts close to such a lesion had nuclei with several ovoid or elliptical areas of high electron density and cytoplasmic changes as seen in other cells. Some cells and cytoplasmic extensions near the basal lamina had irregular, dense to medium dense foci in the cytoplasm in which were ribosome clumping and mitochondrial swelling. Cell boundaries here showed swollen enlarged cytoplasmic projections with dense contents, a change also seen in interboundary areas of some cells elsewhere in the area. The epithelium overlying this area did not show much change.

Granulomatous lesions. Most eggs in this type of lesion had lost their contents during tissue processing. Thus, the state of the miracidium could be ascertained only in few eggs.

Two types of granulomatous lesions were seen in Epon sections. One had a dark fringe immediately around the egg and the other did not.

The lesion without a dark fringe around the egg had many features similar to the equivalent lesion in the hamster but the cytopathological changes were more pronounced in the calves. The early granuloma had a mixture of cells, i.e., eosinophils and macrophages, closest to the egg shell surface and nearby areas but at the periphery a few neutrophils and mast cells were seen. Cytoplasmic changes were similar to those observed in the nongranulomatous lesions. In later granulomas the macrophage and epithelioid cells were dominant but eosinophils were still present. In addition, fibroblasts were seen in the middle and peripheral parts of the granuloma. Cytopathological changes were generally similar to those in the nongranulomatous lesion. Collagen was obvious in between cells, especially in the peripheral areas.

In the granuloma where the egg had a dark fringe, the material immediately surrounding the egg had a highly irregular circular outline. In the specimens examined at 11 and 18 weeks p.i. the eggs had been sectioned near or at their narrow end. The egg contents here were dense irregularly reticular material. The material immediately around the egg was somewhat

similar to that seen in hamsters (Fig. 28) but was thicker and more complex. The layer of the material was dense to highly dense, finely particulate material. In the material were numerous highly polymorphic areas which varied from linear to round to mostly bizarre shapes, that contained either highly dense, medium dense or lucent material. Evident in this material, and partly responsible for the diverse and heterogenous composition, were partly degraded, but still recognizable, cytoplasmic organelles as well as some unidentifiable structures. The organelles were more difficult to identify the closer they were to the egg shell surface. Myelinoid whorls or figures, membrane- and nonmembrane-bound vesicles within vesicles and vesicles with highly dense contents, lamellar structures some of which were dilated or condensed into dark lines, areas of honeycomb appearance, and others with bizarre shapes and composition, were all observed in the material. At 11 weeks p.i. the material formed a finely granular background while at 18 weeks p.i. it was amorphous.

The material described above was in intimate contact with the egg shell without any membrane separation, i.e., it appeared to be continuous with the shell surface, the egg microspines projecting directly into it. Adjacent to this material were host cells with membrane separation in some areas though not in others. In some lesions this interface area with or without membrane separation had one or several layers of small vesicular or globular structures whose density and contents were similar to that of the cytoplasmic matrix of the immediately surrounding cells (Fig. 34). These vesicular or globular structures were almost identical to those seen in hamsters (Fig. 29).

The highly dense material around the egg was surrounded by cells with a variety of changes. Cells nearer to the material and therefore also to the egg, had medium dense, finely granular cytoplasmic matrix. In this there were irregular areas of rarefaction and clumping of the cytoplasmic matrix. In some cells a distance from the egg but still within the lesion, the cytoplasm contained areas of highly dense, nonmenbrane-bound material or areas with myelinoid structures. In other lesions cells close to the material had similar changes but with more pronounced organelle degradation (Fig. 35). Rarefaction and clumping of the cytoplasmic matrix, myelinoid figure formation, vesicles of heterogenous contents and mitochondrial changes as well as increased density of cytoplasmic extensions between cells and interboundary areas of cells were also observed. This was in cells toward the periphery

of the lesion and in some cells between the lesion and the base of nearby epithelium. Most of the cytopathological changes were marked in cells close to the egg and the highly dense material surrounding it. The changes were less so in the periphery where affected cells included fibroblasts and smooth muscle cells of the lamina propria.

A small granuloma in the submucosa was examined in a specimen taken at 32 and 11 weeks after first and second exposures, respectively. The egg shell structure and composition were similar to that in others lesions. The egg contained a mature, dead miracidium. The surface of the egg shell was surrounded in most parts by a multinucleated giant cell. Its boundary with mononuclear cells, mostly macrophages and fibroblasts, that surrounded it exhibited multitudes of cytoplasmic foldings and infoldings with great a diversity of patterns. The cytoplasmic foldings of the giant cell were of variable thickness and greater density compared to those of the macrophage whose few foldings interdigitated with those of the giant cell (Fig. 36). Collagen was present in these areas of interdigitation. The egg shell surface was clearly separated from the giant cell in some areas but not in others. The areas contained small vesicular or globular structures comparable to the ones seen other lesions. The globular structures were in several layers. The giant cell cytoplasm had numerous mitochondria but the RER was poorly developed. Peripheral to the giant cell, there were, in addition to the mononuclear cells, a few eosinophils and neutrophils. In the mononuclear cells there were cytoplasmic changes similar to those seen in the outer zones of equivalent lesions in the hamsters and calves.

5. DISCUSSION

The clinical and gross pathological manifestations of *S. bovis* infection in calves were minor in both experiments in the present study. Calves were exposed to 4,000-5,000 cercariae per animal, percutaneously. This infective dose is probably toward the minimum when compared to other studies. In one such study, each calf received between 9,000-10,000 carcariae (Saad et al, 1980). In another study, calves were given 70,000 cercariae (Bushara, *et al*, 1980). In cattle with schistosomiasis, egg output by the worms is rapidly reduced (Lawrence, 1973). Therefore, it is in heavy infections that serious clinical disease can develop. In nature, however, cattle are usually exposed to low or moderate infections; hence the high prevalence of infection but the low incidence of serious disease (Majid, *et al*, 1980).

Early in the present experimental study, it became obvious that the low response of the calves to infection by *S. bovis* would not provide sufficient and relevant lesions, particularly for ultrastructural study of the transmucosal migration of eggs. In order to ensure fair availability of lesions for EM, hamsters were infected with a high dose of 300 *S. bovis* carcariae per animal. The hamster is known to be highly susceptible to *S. bovis* (Lengy, 1962; Warren 1973). The hamsters in the present study developed serious clinical and pathological changes almost comparable to those in sheep exposed to heavy infection with *S. bovis* (Hussein, *et al*, 1976)

In the interpretation of scanning electron microscopical changes, variations in the morphology of the intestinal mucosa with the duration of the experiment had to be taken into consideration. In normal animals the morphology of villi varies with species, age, intestinal microflora and immune status of the individual (Jubb, *et al*, 1985). In the present study, there were variations in the appearance of the villi of the small intestine in both control hamsters and calves in early and late stages of the experimental period. In earlier stages the calves exhibited ridge-like, interconnecting villi in the small intestine. In later stages, there were fewer interconnections between the ridge-like villi. Such villi are rarely observed in weaned ruminants (Jubb, *et al*, 1985). In the present study the calves were 8-10 months old. The factors behind these morphological differences could not be established in the present study.

The structure and composition of the eggshell were similar regardless of the stage of development of the miracidium or whether the egg was intravascular or extravascular. The structure and composition of *S. bovis* egg shell resemble those of *S. mansoni* (Stenger *et al.* 1967; Neill *et al.* 1988). In the present study, however, no micropores were observed opening directly to the outside. Thus the cribriform morphology of the micropores in *S. mansoni* eggs cannot be confirmed to exist in *S. bovis* eggs. There are also no published reports on the egg shell structure of *S. bovis* to compare with the present study. The egg shell of *S. bovis* appears to have comparatively more microspores in the narrow than the wide part of the spindle-shaped egg. Although *S . mansoni* eggs are not as spindle-shaped as those of *S. bovis*, Race *et al.* (1969) found the micropores located mainly in the anterior portion of the egg. Unlike these investigators, however, the micropores observed in the present study had no lining by trilaminar unit membranes. The microspores are thought to be routes for passage of egg excretions/secretions (Stenger *et at*, 1967; Race, *et at.* 1969). The more microspores there are at one part of the egg shell the greater the likelihood of higher concentration of excretions/secretions there. This might partly explain the light microscopic observations of the emphasized Hoeppli phenomenon at the narrow parts of the eggs (Fig. 4). It might also be related to the observed cellular aggregations, mostly of platelets, at the spine and poles of *S. mansoni* eggs when added to citrated whole blood from uninfected mice (Ngaiza, *et al*, 1990). Also in the present study the narrow part of the egg had comparatively thicker egg shell than the wide central part. In the SEM study, egg observed emerged did so with the pointed end (Fig. 19). The comparatively thicker shell of the narrow end might be related to use of this part of the egg for tissue penetration. However, these observations need further study in comparison with the structure of eggs of *S mattheei* and human schistosomes other than *S.mansoni*.

The intravascular schistosome egg is separated from the extravascular space by approximately 0.1 μm to 1.0 μm of endothelial and subendothelial tissues. The eggs of *S. bovis* are about 132 - 247 μm long and 38 - 60 μm wide. Therefore, the barrier to the extravascular space is very thin. As some of the venules have an interrupted single layer of smooth muscle cells, the egg has therefore only the endothelium and its basal lamina to cross to reach the extravascular space. And where such a venule is close to the base of a mucosal epithelium, the distance to the intestinal lumen is comparatively short for the egg.

Past investigations on the intravascular egg and the tissue reactions to it have given different results. Some investigators observed the eggs to be surrounded by leukocyte inflammatory cells, endothelial cells and fibroblasts (Kuba, 1963). Others have reported that the eggs were surrounded by inflammatory thrombi or aggregates of platelets (Doenhoff, *et al*, 1986; Ngaiza, *et al*, 1990). These authors also claim that these cellular/tissue reactions about the eggs aid the eggs in reaching the exterior. Other investigators have claimed that necrosis of the vessel wall by the egg helps it to get to the extravascular space (Bloch, 1980).

In the present study, leukocytes identified as monocytes formed one or several layers around the intravascular egg. Additionally, the luminal side of this cellular mantle had an endothelial cover that was continuous with the rest of the vascular endothelium. However, this was the case only for the eggs already adhered to the vessel endothelium. Thus, unlike in past investigations no inflammatory thrombi, aggregates of platelets or fibroblasts were found. The observation of leukocytes around the eggs is similar to the results of some earlier studies, but in the present study clear identification of the leukocytes and delineation of endothelial cover over the rest of the cellular mantle was possible with TEM.

For schistosome eggs to emigrate to the extravascular space, they must first adhere to the vessel endothelium. In the present study, eggs were observed to do so in two ways. Some eggs became impacted in venules/capillaries of smaller calibre than their diameter. They then became covered by a cellular mantle as described already. The other way of adhesion to the vessel wall was by way of a cover of monocytes. These facilitated the eggs' adherence.

Monocytes, like all other leukocytes, are able to adhere to the endothelial surfaces because of the latter's specific receptor molecules for the cells (Slauson and Cooper, 1990). In addition, endothelial cells secrete various factors some of which aid leukocyte adherence to and emigration through the endothelial layer (Harlan, 1985). On the other hand, leukocytes, in particular monocytes and neutrophils, secrete factors which alter endothelial functions thus promoting their adherence and emigration through the endothelium. Some of the leukocyte secretory factors have been shown to promote endothelial cell growth in culture (Harlan, 1985). Endothelial cell proliferation *in vitro* has also been observed to be stimulated by some soluble factor secreted by intact *S. mansoni* eggs (Freedman and Ottesen, 1988). Thus the

cellular mantle of monocytes around the intravascular eggs and the endothelial cell growth over the cellular mantle are probably involved in the eventual emigration of the egg to the exterior through their various secretions. Also in the present study neutrophils were observed emigrating close to the intravascular egg or in contact with the subendothelial egg. The neutrophils were also prominently present just outside vessels with the subendothelial or the just exited eggs. That these neutrophils, monocytes and endothelial cells, closely associated with the schistosome eggs, were involved in the egg emigration was supported by ultrastructural observation indicating heightened synthetic activities in these cells. Some of the cells, however, exhibited organelle changes usually associated with increased membrane turnover.

The cytoplasmic changes observed in the cells associated with the eggs might have been induced by secretions from the eggs. This is a method of egg emigration advocated by several earlier investigators (Kuba, 1963; Warren, 1973). The changes may also have been due to immunological responses to the egg. Immunological dependency of excretion of the egg has been propounded by a number of investigators (Doenhoff, *et al,* 1986; Damian, 1987). However, the effector cells have not been clearly identified, although Ngaiza, *et al*, 1990) have shown that thrombocytes might be some such cells. In the present study the monocytes and neutrophils which were morphologically associated with the eggs appear to effect the emigration to the extravascular space. In animals with heavy infections, and therefore numerous eggs exiting, there would probably be reduction in monocyte and neutrophil blood counts. Although no blood cell counts were done in the present study, there is reported neutropenia in early patency in cattle heavily challenged with *S. mattheei* (Lawrence, 1977). It seems, however, that neutrophil and monocyte counts are parameters seldom investigated in schistosomiasis.

Apart from cytoplasmic changes that were observed in cells associated with the intravascular and subendothelial eggs, there were materials in the interface between the egg shell and the cellular mantle, that might have been related to the egg emigration. The finely particulate or flocculent material was more obvious with the subendothelial eggs. So were the membrane- and nonmembrane-bound structures particularly in the interface opposite that under the endothelium.These materials probably represent breakdown products of the basal lamina type

IV collagen, laminins or other extracellular matrix components in the subendothelium space occupied by the egg and its cellular mantle. Neutrophils and probably other leukocytes possess a variety of enzymes capable of digesting these compounds (Harlan,1985, Slauson and Cooper, 1990). The material could also stem from the action of excretory/secretory products of the schistosome eggs. The abundancy and obvious presence of these materials, especially in that part where the egg was in contact with perivascular tissue where emigrated neutrophils, monocytes (now macrophages) and eosinophils were present in large numbers, is a further indication of an interaction between these cells and the egg in its emigration to the exterior.

Although clear inflammatory changes were not observed in relation to emigration to the extravascular space the following were seen. There was prominent leukocytosis in venules and adherence of neutrophils and monocytes to the endothelium. These and the ultrastructural changes indicative of an activation of endothelial cells and monocytes of the egg cell cover are probably signs of inflammation. Marked adherence of neutrophils to the endothelium is said to be an early sign of inflammation (Harlan, 1985). However, these changes may be the influence of excretions/secretions of eggs *per se*.

Necrosis of the vessel wall from inflammatory reactions or other causes associated with the intravascular egg is thought to be one way the egg emigrates to the extravascular space (Kuba, 1963; Bloch, 1980). Whereas necrosis of the vascular tissue apposed to the egg and its cellular cover was not observed in the present study, it is conceivable that the marked stretching of the vessel wall and the pressure on the endothelium and subendothelial tissues caused by eggs laid in batches (Fig. 10) could lead to necrosis, vessel wall breakdown and passive release of eggs into the extravascular space. However, eggs were laid singly, in batchess or tandem arrangement. Pressure necrosis may therefore, be one of the ways of egg emigration to the extravascular space, especially in early patency at the time of high egg output.

The laying of eggs in tandem fashion (Fig. 6a) might cause mechanical break of the vessel wall thereby leading to deposition of eggs extravascularly. If the laying was close to the mucosal epithelium, as observed in some specimens in the present study, eggs may be laid

directly into the intestinal lumen (Fig. 6b). This, however, is probably occasional when the worm has laid large numbers of egg, at a go, in tandem fashion. This would be at the height of oviposition.

Some extravascular eggs, seen with little or no cellular reaction, had immature or mature miracidia. Such eggs were probably in transit to the exterior or had just arrived in the area. Those with some degree of cellular reaction might have been at the site earlier or were capable of some excretion/secretion which had induced some reaction. At light microscopy the cellular composition did not differ much from that of the rest of the mucosa or there were slightly more eosinophils, plasma cells, mast cells and macrophages. At TEM, however, the cytoplasm of the cells or their extensions immediately surrounding some of the eggs showed various cytopathological changes ranging from dilation of endoplasmic reticulum, degradation of mitochondrial cristae, to formation of myelinoid whorls. These changes may have been due to materials from the eggs. However, they can be induced by hypoxia, chemical compounds, biological toxins or physical factors (Slauson & Cooper, 1990). Their prominent occurrence in cells near the egg shell surface rather than in those not so near, probably indicates some damaging influence from the schistosome eggs.

As in the case of the subendothelial egg, evidence for probable extracellular matrix degradation was seen in the form of membrane- or nonmembrane-bound globules or vesicular structures. The quantity and density of these structures were more prominent around some of the extravascular eggs than others. They were also observed between fibroblasts and collagen around some of these eggs.

Also observed in the interface was flocculent or homogenous, finely particulate material, as seen around the subendothelial egg. The material, however, was dense to highly dense and thicker around some extravascular eggs. At some eggs, the material extended for a distance between cells or their extensions. The TEM appearance of the material when dense and thick around the eggs had some similarity to that of antigen-antibody complexes, e.g., that seen in the immune-mediated glomerulopathy of schistosomiasis mansoni (Andrade *et al*, 1984) or the glomerulopathy in the golden hamster infected with *S. haematobium* (Sobh *et al*, 1991). However, the material could also be antigen or antibody in high concentrations or

some other products of extracellular matrix breakdown. Antigen-antibody complexes form the major part of the Hoeppli phenomenon in schistosomiasis (von Lichtenberg *et al*, 1966; Smith and von Lichtenberg, 1967). The Hoeppli phenomenon in relation to findings in the present study will be discussed in detail later.

In the present study, the material thought to be antigen-antibody complexes was seen only at TEM in hamsters. It was seen around eggs with little or moderate cellular reaction. Material of similar TEM appearance was seen in the interface around eggs in granulomas in hamsters and calves.

Other extravascular eggs in the mucosa were observed in lesions in which the predominant cells were eosinophils and in the granulomas. Eggs in these lesions had immature or mature miracidia. Some of the miracidia were intact while others were degenerated or dead.

The lesions in which the eosinophils predominated were prominent and much more intense in calves than in hamsters. Thus, in calves they appeared like microabscesses. Microabscesses in connection with schistosome eggs have been reported by several investigators (Hussein, 1971; Lawrence, 1978a). Some investigators have reported these to have a large proportion of polymorphs which were attributed to bacterial presence (Lawrence, 1978a). In the present study, the lesions were observed in both cattle and hamsters but no bacteria were observed. As the observed tracts of eosinophils extended from the basal layers of the mucosa to the epithelium and as many eosinophils were seen among the epithelial cells, the tracts are probably routes of emigrating eggs. Evidence for that is further provided by the observation of eggs surrounded by cells, mainly eosinophils, in lumina of crypts and intervillous spaces adjacent to the point where tracts of eosinophils joined an epithelium or entered the intestinal lumen. Eggs were also observed close to the epithelium preceded by a zone of eosinophils. With SEM intervillous bodies covered by cells with the morphology of leukocytes, were observed in the early rather than in the later stages of the disease.

In lesions in which eggs were surrounded by mainly eosinophils, the presence of cytopathological changes in these cells, increased density in the intercellular areas and the apposed cytoplasmic matrix, vesicular structures between collagen fibers some of which were close

to the basal lamina of the mucosal epthelium, were probable indication of the effects of toxic or lytic factors. Such factors might be from the schistosome eggs or from the eosinophils. Eosinophils contain a variety of substances which include proteolytic factors (McEwen 1992). These probably play the role of lysing extracellular matrix, basal lamina and probably also damaging other cells. In the process, eggs find their way to the intestinal lumen. These findings in the present study support the idea expressed by Lenzi *et al*, (1987) that eosinophils favour emigration of the schistosome eggs to the exterior. The occurrence of intense eosinophil accumulations around eggs, mostly in the superficial part of the mucosa in the early phase of the disease when egg excretion in feces is at its peak (Hussein 1971, Saad *et al*, 1980, Bushara *et al* 1980) and the other observations already stated above, lend further support to this manner of egg emigration through the mucosa of the intestines. However, many eggs were observed in the present study and have been observed by other investigators (Kuba, 1963, Lawrence, 1978a) not to have eosinophils around them in the mucosa and in the intestinal glandular lumina. This, and the observation that suppression of factors, e.g., interleukin-5 which stimulate eosinophilopoeiesis and maturation does not reduce fecal egg load (Sher *et al*, 1990), indicate the possible existance of more than one way of excretion of eggs.

The egg is also thought to be excreted through the intestine by actions of its own excretions/secretions. Several workers have investigated this possibility mostly through *in vitro* studies as well as through some light microscopical investigations (Kuba, 1963; Kloetzel, 1967, 1969; Smith *et al*, 1971; Asch and Dresden, 1974). In the light microscopical studies, the investigators tried to demonstrate absence of certain connective tissue fibers around the schistosome eggs in the intestines as evidence for lysis by egg excretions/-secretions (Kuba, 1963; Kloetzel, 1969). In the present study, lysis of tissues immediately surrounding the eggs as reported by some of the authors was not observed. In the TEM study the material observed immediately around some of the eggs cannot be ascribed to digestion of particular connective tissue fibers. As pointed out earlier, part of the material seen may be antigen-antibody complexes.

Eosinophils and macrophages are major effector cells in the body defence against schistosomes (Capron and Dessaint, 1987). In lesions in which eosinophils were the

predominant cells and in the early granulomas, eosinophils were closely apposed to the egg shell surface. It is reported that eosinophils effect destruction of parasites by degranulation and release of cytotoxic substances (Capron and Dessaint, 1987; Kephart, *et al*, 1988; McEwen, 1992). In the present study some eosinophils closely apposed to the egg had very few granules. The membrane binding of some granules was poorly visible. Also some of the eosinophils had lucent to medium dense matrix. However, the number of eosinophils thus affected was low. Other eosinophils had highly dense areas of cytoplasmic matrix in which there were degenerated organelles. The egg shell in apposition with the eosinophils seemed intact as was the miracidium inside the egg. These observations were made in specimens taken at 7 - 11 weeks p.i.. The presence of degenerating eosinophils indicates probable damage by toxic excretions/secretions from the egg or from disintegrating eosinophils. It seems therefore that the intact egg shell is an effective barrier to cytotoxic substances in eosinophils. Even after the egg shell is broken and egg contents phagocytosed at the end of the three weeks life span (Warren, 1973), the egg shell may persist in granulomas for many weeks. In the present study egg shell remnants with structure and composition of that of intact eggs were observed in granulomas at 3 months p.i.. They were surrounded by epithelioid cells.

It is possible, therefore, that the intense cellular reaction seen around some eggs may be related to the integrity of the egg shell. Damage to the egg shell may occur at the time of egg laying, as the egg moves in and through blood vessel walls and from peristaltic contractions of the intestines.

Some eggs detained in the extravascular tissues of the mucosa were in early or late granulomatous lesions. In early granulomas the eosinophils were predominant near the egg shell. The eosinophils were mature. There were no collagen fibers observed in these early granulomas. In late granulomas, in both calves and hamsters, fine fibers were observed among macrophages and epithelioid cells closer to the egg, but thicker and more prominent fibers were in the periphery of the granuloma. Even though the macrophage type of cell was predominant in late granulomas, eosinophils were still present as were occasional neutrophils and mast cells. These finding are similar to those reported by Bogitsh *et al,* (1974) in murine intestinal schistosomiasis mansoni. In the mice, however, granulomas were mainly

in the muscularis-serosal areas. In hamsters and calves in the present study, granulomas were found in various layers of the intestine. However, they were predominant in the basal mucosa and submucosa in late stages of the disease, a feature also reported by Hussein (1971) and Lawrence (1978a) in cattle.

Also observed in the present study in both hamsters and calves was material of variable appearance in the interface between the egg shell and cells immediately surrounding the egg. The material was observed in both early and late granulomas. It was more prominent in calves and in late granulomas. The material in the interface was dense or highly dense, homogenous and amorphous in some early granulomas, particularly in hamsters. In some granulomas the material was highly dense and contained a variety of organelle debris. The organelle debris was more easily identifiable the further it was from the egg shell surface. This was particularly so in the calves in granulomas at 11 and 18 weeks p.i.. In cells surrounding the interface material, again more so in calves than in hamsters, there was evidence of organelle degeneration. The organelle degeneration was more advanced in cells immediately surrounding the interface material than those further away. These features resulted in several, variable layers of ultrastructurally observable damage, with the most severe damage being close to the egg shell and the least damage at the most peripheral cells around the egg.

Some of the above observations are similar to those by Williams *et al*, (1969) in their TEM study of the Splendore-Hoeppli phenomenon in phycomycosis. In the present study, there was apparent gradation of damage suggestive of continuing excretion and spreading of toxic material in a concentration gradient from the miracidium in the egg. Also in the late granuloma, the macrophages which were close to the egg shell, were the targets of damage whereas in phycomycosis Williams *et al*, (1969) observed mainly involvement of eosinophils.

Also observed in the present study, and not reported by Williams *et al*, (1969), was the presence of numerous dense to highly dense vesicles or globules in the boundary between the interface material composed of organelle debris and cells immediately surrounding the material (Fig. 34). These structures resemble spherical microparticles found in extracellular locations under certain circumstances (Ghadially, 1988). Although they may be found in

small amounts and with different densities in normal tissue, abundant spherical microparticles are indicative of cell injury and are debris cast off by injured cells. In the present study, vesicles or globules with TEM appearance of spherical microparticles were observed at the periphery of irregularly radiating columns of the highly dense interface material (Fig. 34). Some host cells apposed to these structures had cytoplasmic matrix rarefaction and clumping. These features and the organelle degeneration already referred to above are highly indicative of injury to host cells by some material from the surrounding schistosome eggs. The cytoplasmic clumping and rarefaction are probable early indications of cytotoxic damage (Slauson and Cooper, 1990).

The structures resembling spherical microparticles were also observed in connective tissues around the granulomas in the lamina propria. They were observed between collagen fibers and fibroblast and other cells surrounding some eggs that had little or moderate cellular reaction. They were also observed close to the basal lamina of mucosal epithelial cells where the egg was close to it. These changes in close association with the eggs, both in the granulomas and nongranulomatous reactions, are a morphological indication that eggs emigrate through excretion/secretion of lytic factors. Secreted factors from cellular reactions to the egg may partly contribute to the changes observed.

The highly dense material with variably degenerating cell organelles in the granulomas was observed in Epon sections from calves, at light microscopy, as a thin, dark blue fringe. It was not demonstrable in hamsters. The material was probably equivalent to the Hoeppli phenomenon that was observed in hematoxylin and eosin sections in calves from 7 weeks p.i. onwards. As already commented upon, the Hoeppli phenomenon is thought to be antigen-antibody complexes (von Lichtenberg *et al*, 1966; Smith and von Lichtenberg, 1967). The Hoeppli phenomenon occurs in highly sensitized animals and is associated with high egg burdens (von Lichtenberg *et al*, 1966). In the present study, the infective dose of *S. bovis* carcariae given to the calves was low to moderate in amount. The Hoeppli phenomenon was observed in the calves early in the infection, and throughout the study period. It was common in both experimental and natural infections. It is also reported as a common observation in cattle by several investigators (Bushara *et al*, 1980, Hussein, 1971, Lawrence 1978). However, in these reports some cattle received heavy infections. Although the Hoeppli

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phenomenon is not reported in hamsters by light microscopy, there was TEM evidence of the phenomenon in these, however, it was much less intense than it was in calves. The intensity and much organelle debris in the material immediately around the egg and the continuing cytopathological changes in cells surrounding the material, probably indicates more vigorous reactions between the host cells and egg material in calves. In cattle, therefore, the Hoeppli phenomenon is probably indicative of an active and successful defense against schistosomiasis bovis.

The defense against schistosomiasis is largely effected by eosinophils and macrophages (Capron and Dessaint, 1987). The Hoeppli phenomenon has been shown to contain large amounts of extracellular major basic protein (MBP), a component of eosinophil granules that is specifically toxic to helminths (Kephart, *et al*, 1988). Other eosinophil granule materials/factors are probably also present in the Hoeppli phenomenon. In the cattle investigated in the present study, some eggs with Hoeppli phenomenon seen in early stages of the infection had intact miracidia and were in the superficial layers of the mucosa. In other cases the eggs had dead miracidia. The successful destruction of the egg miracidium probably depends on the integrity of the egg shell which has been commented on already.

In some granulomas with or without the Hoeppli phenomenon, cells at different distances from the egg showed variable organelle degeneration. This was seen in early or late granulomas in hamsters and calves. Miracidia in eggs and the egg shells surrounded by these cells were intact in some of the granulomas. These observations indicate the protective role of the granulomas for the rest of the host tissues since the granuloma cells are damaged in the process. These observations further support the view expressed by Reis and Andrade (1987) that the granuloma around the egg protects the host from miracidial secretions. However, this protection by the cell-mediated granuloma is for a limited period before humoral antibodies, which are more effective, take over (Doenhoff *et al*, 1986). Doenhoff *et al* (1986) believe that in addition to protection of the host, the granuloma and other immunological responses of the host also ensure survival of the schistosome parasites. Some of these surviving eggs in the granulomas eventually reach the intestinal lumen. Such eggs, with their mantle of granuloma cells, were seen in the present study in intervillous spaces or crypt lumina. Similar observations are reported by Damian (1987). This author and others

(Doenhoff *et al*, 1986) have observed a correlation between fecal egg loads and the size of the granulomas in the liver. No correlation between intestinal granulomas and fecal egg loads has been reported so far. The authors' suggestions of the dependency of egg excretion on host immune responses probably includes specific and nonspecific responses since egg antigen secretions occur after laying and maturation of the miracidium. The marked leukocytosis in association with intravascular eggs that was observed at the beginning of investigations in hamsters in the present study may or may not have been due to specific immune response to infection. As already discussed for the present study, neutrophils and monocytes appear to play a major role in the exit of the intravascular egg to the extravascular space.

Eggs surrounded by different combinations of cells in granulomatous reactions were observed at LM in the present study. As reported by earlier investigators, granulomas become more prevalent in the submucosa as the infection gets older (Hussein, 1971; Lawrence, 1978a). A similar observation was made in the present study in cattle in both experimental primary infections, experimental reinfections and natural infections. In the present study, in primary infections, lesions with predominance of eosinophils were observed largely in the superficial layers of the mucosa soon after patency. However, only few and poorly delineated granulomas were observed soon after patency in cattle. These were mostly in the different layers of the mucosa. In the reinfection experiment some granulomas were observed both in the mucosa and submucosa early in the disease. The lesions with mainly eosinophils was also observed in the reinfected calves but was not prevalent as in primary infections. In the natural infections that were investigated, a proportion of the specimens had the lesions of mainly eosinophils in the superficial layers of the mucosa. In other specimens from natural infections, granulomas were observed mostly in the submucosa only or in different parts of the mucosa and submucosa.

Although one cannot make conclusive statements on the basis of the two experiments and the few field observations above, it is possible to have an idea on whether one is dealing with a recent or late infection and whether the infection is a primary one or a reinfection. A somewhat similar suggestion was made by Lawrence (1978a). However, he based his suggestion on histological estimation of egg counts in the different layers of the intestine.

Granulomatous lesions and other pathological changes were observed in the veins of the intestines and their corresponding mesentery. They were present together with proliferative changes which, however, occurred earlier than inflammatory reactions. The inflammatory and proliferative changes observed in the blood vessels are similar to those reported by Hussein (1971), Bushara *et al*, (1980) and Lawrence (1978b). In the present study, however, the changes were less severe and less extensive in the experimental infections, probably due to light the infections that were used. Also a few animals in the present study were kept for up to 31 weeks. Cattle with heavy schistosome infections develop vascular lesions earlier than those with light infections (Lawrence, 1978b). This is probably not so in hamsters, although they were exposed to heavy infection. Also vascular lesions were less common in the hamsters than in the experimental calves which received light infections. This may be a reflection of species differences between hamsters and calves in response to schistosome infections.

The vascular inflammatory lesions are claimed to be partly caused by living worms and partly by dead ones (Lawrence, 1978b). Eggs also contribute to the development of vascular lesions. In both experimental and natural infections with *S. bovis* in the present study no dead worms were observed associated with the vascular lesions. In *S. mattheei* infections of cattle the host steadily eliminates the adult worms (Lawrence, 1973, 1978). The intravascular dead worms give rise to lesions in the intestinal walls. In the present study of *S. bovis* infection in calves, infective doses were comparatively lower than in the studies with *S. mattheei* (Lawrence, 1978b). However, in cattle, there may be differences in elimination of adult worms of the two schistosome species. There appears to be no previous study of this aspect in *S. bovis* infections.

Histologically intact worms were observed in veins in the submucosa and mesentery but no inflammatory lesions were seen directly associated with them. TEM did not reveal inflammatory changes in the veins occupied by the worms. The apparent increase in and prominence of cytoplasmic fine filaments in endothelial cells are probably related to the physical overstretching of the venule wall by worms. Some venules in hamsters were distended to over five times their normal calibre by the worms.

Vascular changes were commonly observed in the natural infections in the present study. In some specimens the vascular lesions were the only indication of schistosomiasis. This point is useful for diagnosis, particularly when tissue specimens are submitted without parasitological results. Since the vascular lesions develop in late stages their presence indicates an old schistosome infection.

As already pointed out by some investigators (Lawrence, 1978a), vascular lesions prevent the female worm from reaching deeper layers of the intestines for oviposition. Hence eggs and egg-associated lesions appear in the deeper layers and mesentery in later stages of infections. The females, however, rarely go beyond the venules in the submucosa. In the experimental infection in hamsters and calves and in the natural infections in the present study, no worm sections were seen beyond the lower parts of the submucosa, relative to intestinal lumen. The worm diameter was often much larger than the venules and capillaries.

In the present study, extravascular eggs were also observed retained in the lamina propria in areas of hemorrhage or congestion. Some venules near the eggs had gaps in the endothelium whose cells were prominently attenuated and their cytoplasmic matrix was lucent or medium dense. The hamster, from which specimens were found with these lesions, had macroscopically widespread congestion of the intestines. Although venule lesions are suggestive of damage which would lead to hemorrhage, it was not possible to establish whether the endothelial changes were due to the direct effect of the eggs in the intestine. The endothelial changes observed were probably related to hypoxia resulting from the congestion. The congestion , however, may be related to other systemic effects of the schistosome infection that could not be established in the present study. Hypoxia of congestion may have been responsible for the cytopathological changes in the mucosal epithelial cells between which erythroytes were seen to escape to the intestinal lumen. Mitochondrial changes reported by Bogitsh *et al*, (1974) in the epithelial cells close to what the authors call active granulomas were not observed in the present study. The authors attributed the mitochondrial changes to circulating schistosome antigen. Mitochondrial changes seen in the present study in the mucosal epithelial cells probably came about in a similar manner to changes in other organelles as discussed above.

Diarrhoea, which is sometimes haemorrhagic, is a feature of heavy schistosome infection in cattle (Hussein 1971, Lawrence 1977d, 1978a). From previous clinicopathological studies of the pathogenesis of the diarrhoea, it seems that the basic cause is the damage done by the eggs emigrating through the intestinal wall to the intestinal lumen (Bushara, *et al*, 1980, Saad, *et al*, 1980). Hemorrhagic diarrhoea was observed in hamsters but not in calves in the present study. In the LM studies it was difficult to establish exactly the damage done by the emigrating egg other than inflammatory changes. The observed increase in connective tissue and the thickening of the mucosa would probably lead to diarrhoea when extensive. However, although some hamsters had some areas of the mucosa and submucosa occupied by granulomas and fibrosis, intestinal thickening was not uniform. At TEM, extensive cytopathological changes were observed in the enterocytes in some specimens from hamsters but they could not be clearly attributed to the direct effect of the eggs. Nevertheless, such changes would probably also lead to diarrhoea. Mucosal surface damage was best assessed by SEM in the present study. The emigrating eggs leave minute to large, though still microscopical, areas of displaced surface mucosal cells. Some of the displaced cells appeared shrunken or shrivelled and glued together or dispersed. This appearance of the cells is suggestive of degeneration or necrosis. The degenerative or necrotic appearance might have resulted from the effects of the excretion/secretion of the emigrating egg or lytic action of eosinophils surrounding the exiting egg. Emigrating eggs surrounded by eosinophils were observed histologically as discussed earlier.

The other type of damage by the eggs was seen as loosened epithelial cells around the eggs. Although somewhat dislodged, the cells were not shrunken and were in their original areas or slightly displaced (Figs. 18, 19). Such could occur from the forceful piercing of the epithelium by the pointed end of the egg. In the present study, all the eggs observed emerging through the epithelium did so with the pointed nonspined end. Some emerging eggs did so through crypts or glandular pits or close to these. In some of these sites there were also leukocytes and erythrocytes. These were mostly likely coming from the subepithelial tissue. At some damaged and cracked mucosal surfaces there were five or more eggs emerging in only a small area (200μmx200μm) (Fig. 19a). It is conceivable that eggs may emerge through the same spot several times. Thus, the greater the number of eggs emerging per unit area and repeated emergence in the same area, the greater the likelihood

of severe diarrhoea. This view based on actual observations of the types of mucosal surface lesions, reinforces observations by earlier investigators (Hussein 1971, Bushara *et al*, 1980, Dargie 1980, Saad *et al*, 1980) based on pathophysiological and clinical studies. Therefore, eggs, whether they are without cellular reaction and quickly exit through the mucosa (Lawrence, 1978) or are temporarily or permanently trapped, may induce lesions with various pathophysiological effects including diarrhoea.

Apart from areas directly damaged by emerging eggs, there were foci of necrotic or degenerating epithelial cells or cracks in the mucosal surface. Also observed by SEM was increased epithelial cell loss, particularly in the mucosa of the colon of hamsters. This loss was not in direct association with emerging eggs. These lesions were not appreciated with LM. The lesions were probably related to inflammatory reactions, edema or congestion in the intestinal wall. These lesions might interfere with mucosal epithelial functions and lead to diarrhoea.

At SEM, the small intestine of some hamsters had increased epithelial cell accumulations at the extrusion zones of villi. This feature was again not easily observed at LM or TEM. Increased accumulation of cells at the extrusion zone of villi may result from accelerated cell division in the proliferative compartment of the crypts or reduced shedding at the extrusion zone. Which of these was in operation in the present study was not determined. Excessive accumulation of cells at the tips of villi is a common feature that can be caused by a number of infectious agents (Landsverk, 1992). Schistosomiasis is probably one other infection with this feature.

Hemorrhagic diarrhoea was observed in hamsters in the present study. TEM study of venules showed gaps in the endothelium in some specimens but not in association with eggs. As opinioned earlier in the discussion, intravascular eggs in the process of emigration have an endothelial cover on the luminal side. This effectively seals off the lumen and prevents bleeding. However, it is possible that when there are numerous eggs exiting, the endothelial cover may be inadequate and hemorrhage might occur as it does when massive numbers of neutrophils emigrate creating many gaps between endothelial cells (Harlan, 1985). In many hamsters, in the present study, there were severe liver lesions including phlebitis, granuloma-

tous lesions, necrosis of hepatocytes, fibrosis in vessels and in the parenchyma. There was also thrombosis associated with worm and/or eggs. However, there were fibrinous thrombi unassociated with any parasitic materials. Thrombosis was also observed in the lungs, intestines, pancreas, mesentery and spleen. In some of these tissue they were mainly fibrin thrombi. Therefore, the hemorrhages seen in the intestines and the thrombosis may be partly related to liver failure and other systemic effects of the heavy schistosome infection in the hamsters. The hypothesis earlier advanced on necrosis and egg emigration can also be applied to the pathogenesis of hemorrhage. Eggs in tandem arrangement may be forced through the thin venule or capillary walls leading to hemorrhage. The hemorrhage from these sources would be apparent in heavy infections at the height of oviposition. Morphological changes as evidence for some of these physical causes of hemorrhage was present in the current study. These support some of the hypotheses propounded in earlier studies on the excretion of the schistosome eggs (Kuba, 1963).

Minute foci of hemorrhage on the surface of the intestinal mucosa were seen by SEM. These were at points of egg emergence or cracks in the mucosal surface. Such sites of hemorrhage are not observable with LM and the hemorrhagic exudates may be wrongly assumed to have come from another part of the intestines.

6. CONCLUSIONS

Most of the structure of *S. bovis* is similar to that of *S. mansoni*. However, there may be differences in the composition and structure of the micropores.

Intravascular eggs probably reach the extravascular space with the aid of monocytes and neutrophils. In heavy infections, however, the eggs may be forced through the vessel wall if they are laid in batches or tandem arrangement. This manner of emigration may lead to bleeding.

Extravascular eggs probably reach the intestinal lumen through the action of lytic factors from the miracidia and leukocytes, particularly the eosinophils. The presence of spherical microparticles and other debris around the eggs and between cellular and fibrous elements attests to this method of emigration.

Nongranulomatous and granulomatous lesions are induced by eggs temporarily or permanently trapped in the wall of intestines. Cytopathological changes in cells in these lesions probably stem from the effects of miracidial excretions/secretions. The cellular reactions around the eggs are partly protective to the rest of the host cells. However, the observed miracidial survival may also be partly due to the protection of these cellular reactions.

Schistosome eggs emerge through the mucosal surface probably by the actions of lytic factors from the eggs and by the forceful piercing by the pointed ends of the eggs. Diarrhoea probably develops from such damages.

It is possible to identify recent and old, natural infections by the occurrence and distribution of accumulations of eosinophils and granulomas in the intestines. Vascular lesions in the intestines may also be useful in the differential diagnosis of schistosomiasis.

7. SUMMARY

Chapters 1 and 2

Clinical signs and pathological changes in animal schistosomiasis are often attributed to the effect of the eggs trapped in or migrating through the intestinal wall. Research on the emigration of the egg to the exterior from the intravascular space and the related pathological changes have given inconclusive findings. In the present study these aspects of schistosomiasis were investigated by light and electron microscopy.

Chapter 3

Eleven calves and ten hamsters and appropriate controls were used in this study. Each calf was given about 5,000 cercariae. Hamsters received 300 cercariae each. Cercariae were of *S. bovis* that originated from Iringa, Tanzania. Infection was given percutaneously. The study included specimens from thirty five naturally infected cattle. The specimens were collected from a slaughterhouse in Iringa, Tanzania.

Chapter 4

Clinical signs: The disease was mild in the experimental calves. Although loss of body condition and diarrhoea were observed in natural infections of cattle this could not be attributed to schistosomiasis alone. Hamsters had diarrhoea which was sometimes bloody. They became recumbent until death or when taken for necropsy.

Gross Pathology: Few punctate foci of congestion/hemorrhage were seen on the intestinal mucosa of the experimental calves. In the natural infections some cattle had yellow-white subserosa nodules near the mesenteric attachment. Similar nodules along the mesenteric veins which were also thickened for a short distance from the intestines, were also observed. In hamsters the intestines were variably congested. Intestinal contents were fluid or semifluid and dark red or brown. In later stages the mucosa had raised dark-grey studs. In the subserosa there were yellow-brown, tiny nodules along the jejunal mesenteric attachment. The liver was of normal or reduced size. Livers had rough almost morocco leather-like surfaces. Congestion and edema of the stomach, focal congestion in lungs and pancreas and hydrothorax and hydroperitoneum were seen in some hamsters.

Light microscopy: Intense accumulations of eosinophils, locally or in the form of tracts in which eggs were present or absent, characterized the early lesion in the intestinal mucosa. The Hoeppli phenomenon was observed in some of the lesions. Granulomas were few and mostly noncapsulated in the early stages, especially in the calves. Granulomas became predominant in later stages in both calves and hamsters. Eggs with intact or dead miracidia were present in the granulomas. Many eggs with miracidia in different stages of development were present in some individual granulomas in hamsters. In calves the granulomas were mostly submucosal. They were in all layers in the hamsters resulting in variable intestinal thickening. Calves showed marked variation in granuloma cellular composition. The Hoeppli phenomenon was common in the granulomas in experimental and natural infections in calves.

Many intravascular and extravascular eggs were observed especially in the hamsters in early stages. They were mostly in the superficial and middle layers of the mucosa. Intravascular eggs had a single or more layers of mononuclear cells around them. Eggs were laid singly, in batches or in tandem arrangement. Whether intravascular or extravascular, some eggs had intact immature or mature miracidia. Some extravascular eggs had intact or degenerated/dead miracidia. Eggs in batches caused great distention of the venule while some in tandem arrangement seemed to have been pushed through the vessel wall or, in some cases, right through the mucosal epithelium. Extravascular eggs without cellular reaction were seen close to the mucosal epithelium. Some of the eggs had the pointed end piercing the epithelium. Eggs surrounded by eosinophils or granuloma cells were in the subepithelial areas or in crypt lumina, glandular pits or intervillous spaces.

Proliferative and inflammatory changes in veins in the submucosa and mesentery were prominent in natural infections of calves. Subintimal, medial and perivascular egg granulomas were encountered particularly in calves with natural infection. Intact worm sections without tissue reactions were found in the submucosal and mesenteric veins. There was prominent parasitic thrombosis in mesenteric veins and liver vasculature in hamsters. However, nonparasitic thrombosis was also encountered in these.

Granulomatous lesions, parenchymatous necrosis and fibrosis, phlebitis and hemorrhage were prominent in livers of hamsters. Changes in the liver of calves were few. Granuloma formation was evident in the spleen, pancreas and mesenteric lymph nodes of hamsters only.

Scanning electron microscopy: Interconnecting ridges of villi were common in the proximal small intestines of control and experimental calves. Few changes were seen on the mucosa of the intestines of calves. In hamsters the following were observed. Irregularly shaped structures considered to be eggs covered in cellular exudate were seen in the intervillous spaces in hamster intestines. There was marked accumulation of cells at the extrusion zones of villi. On the surface of the mucosa irregular areas of disruption were common in the colon at 9-10 weeks p.i. At some of these sites there were loose, round and shrunken cells. At other sites the cells had been loosened but were intact. Erythrocytes and globular spiked leukocytes were at some of these sites too. At some of these areas of mucosal disruption were emerging eggs. All emerging eggs did so with the nonspined pointed end. Eggs were also seen emerging through glandular pits or just to the sides of these.

Transmission electron microscopy: The egg shell had very dense homogenous material. It was comparatively thicker at the narrow than the wide parts of the spindle-shaped eggs. There were also more micropores in the narrow than the wide parts of the eggs. Numerous microspines formed the outer surface of the egg shell.

Intravascular eggs were surrounded by a single or several layers of monocytes which attached some eggs to the endothelium. The luminal side of this cellular mantle had an endothelial layer which was continuous with the rest of the vascular endothelium.

The luminal side of subendothelial eggs was in contact with endothelium and neutrophils at some points. There was increased margination and emigration of neutrophils and monocytes at these sites. These cells and eosinophils were increased in numbers in the perivascular tissues surrounding the abluminal side of the subendothelial eggs. Some cells associated with the intravascular and subendothelial eggs showed increased synthetic activity while others had organelle degradation.

Extravascular eggs induced none, little, nongranulomatous and granulomatous reactions. However, even where there was no obvious cellular reaction, the host cells immediately around the eggs had degenerative changes. These changes were marked in nongranulomatous and granulomatous lesion. Cytopathological changes were also seen in the perigranuloma cells. Increase density of intercellular areas and the apposed cytoplasmic matrix was evident in the lamina propria tissues and basal lamina of the epithelium adjacent to the egg lesions.

Mature eosinophils formed the major cellular component of some nongranulomatous and early granulomatous reactions. Eosinophils were in contact with the eggs. Some were degranulated and had foci of highly dense areas in the cytoplasmic matrix. Other eosinophils has granules of lucent density. Some eggs in these lesions had intact egg shell and miracidia. Early granulomas lacked collagen or other fiber formations.

Late granulomas contained mainly macrophages and epithelioid cells. Eosinophils, neutrophils and mast cells were few. Giant cells were prominent in a few granulomas. There were cytopathological changes as seen in other cells. Fine fibers were seen in the inner part of the granulomas. Thicker fibers that were collagen were seen toward the periphery of the granulomas. Pieces of egg shell seen in some granulomas had a structure similar to that of intact eggs.

Intravascular eggs, subendothelial and extravascular eggs were associated with flocculent or finely particulate material in the interface between the egg shell and the surrounding cells. Also in the interface were globular or vesicular structures. These interface materials were small in amount and intensity around the intravascular eggs and the luminal side of the subendothelial eggs. They were prominent around some extravascular eggs in both calves and hamsters. In calves, in which the materials were most prominent, they are the equivalent of the Hoeppli phenomenon seen at light microscopy. Although the Hoeppli phenomenon is not described in hamsters in light microscopy, the interface materials seen at TEM are probably also the Hoeppli phenomenon equivalent. The vesicular or globular structures were also observed among connective tissue cells and fibers between the egg lesions and the mucosal epithelium.

Venules near some extravascular eggs in an area of bleeding showed gaps in the endothelium whose cells had lucent cytoplasmic matrix. Macrophages, eosinophils and erythrocytes surrounding these venules and the eggs and the epithelial cells overlying such areas had variable degrees of degeneration. The cytoplasm of some overlying epithelial cells contained degenerating erythrocytes which were also present between the epithelial cells.

Chapter 5

The egg shell of *S. bovis* is comparatively thicker and with more micropores in the narrow than the wide parts of the spindle-shaped egg. These features may have pathogenetic consequences.

Intravascular eggs appear to reach the extravascular space in association with monocytes and neutrophils. An endothelial cell cover over the egg and its cellular mantle seals off the vascular lumen and prevents bleeding. Intravascular eggs probably also reach the extravascular space by overdistention and necrosis of the vascular wall by eggs in batches. Eggs in tandem arrangement may be pushed right through the vessel wall and nearby mucosal epithelium into the intestinal lumen. Eggs laid in large batches and in tandem arrangement may cause hemorrhage. Hemorrhage is also likely to occur from inadequate endothelial cover of the emigrating eggs when these are numerous. These ways of hemorrhage are likely in heavy infections in early stages of the disease.

Extravascular eggs probably reach the intestinal lumen by actions of lytic factors from eggs and leukocytes, especially eosinophils. Evidence for this was seen in the form of particulate or flocculent material and spherical microparticles in association with the eggs and between cellular and fibrous elements around the eggs and the rest of the lamina propria. Early nongranulomatous and granulomatous reactions and late granulomas are induced by temporarily or permanently trapped eggs in the intestinal wall. Cellular elements in these lesions surrounding the eggs show cytopathological changes probably due to the effects of excretions/secretions from the miracidia. To some extent, therefore, the granulomas protect the rest of the host cells.

Schistosome eggs appear to emerge through the mucosal surface by lytical factors from eggs

and leukocytes as well as by forceful piercing by the pointed ends of the eggs. Some eggs emerge through crypt lumina and glandular pits. It is through these damages that loss of fluids and therefore diarrhoea occur.

The distribution of the early, intense accumulations of eosinophils and the late granulomas in the different layers of the intestine may be of use in identifying recent and old infections in the field. Pathological changes in veins in the intestines interfere with egg laying close to the mucosal surface. The lesions occur late in the disease. They may be useful for differential diagnosis in the field.

8. SAMMENDRAG

Kapitel 1 og 2

De kliniske tegn og de patologiske forandringer ved schistosomiasis hos dyr sættes ofte i forbindelse med æggenes passage af tarmvæggen. Undersøgelser af æggenes vandring fra karlumen og til tarmlysningen har givet modstridende resultater. Nærværende studium omhandler undersøgelser af disse forhold belyst ved lys- og elektronmikroskopi.

Kapitel 3

Elleve kalve og 10 hamstere og kontroller blev brugt i undersøgelserne. Hver kalv blev inficeret med 5.000 cercarier og hver hamster med 300 cercarier af *Schistosoma bovis* erhvervet i Iringa, Tanzania. Infektionen fandt sted perkutant. Undersøgelserne inkluderede desuden materiale fra 34 naturligt inficerede stykker kvæg fra Tanzania, slagtet på slagterier i Iringa.

Kapitel 4

Kliniske symptomer: Sygdommen forløb mildt hos de eksperimentelt inficerede kalve. Skønt vægttab og diarré blev observeret i det naturligt inficerede kvæg, kunne dette ikke alene tilskrives schistosominfektionen. Diarré, der kunne være blodtilblandet, blev undertiden set hos hamsterne, som blev afkræftede og uvillige til at rejse sig.

Makroskopiske forandringer: Hos de eksperimentelt inficerede kalve sås punktformede blødninger og/eller hyperæmi i tarmslimhinden. I det naturligt inficerede kvæg sås gullige subserøse knuder nær krøstilhæftningen. Lignende knuder kunne ses langs krøsvenerne, som var fortykkede i et kort forløb fra krøstilhæftningen. I hamsterne var tarmene i varierende grad hyperæmiske, og tarmindholdet var rødbrunt og flydende eller halvflydende. I de senere stadier i infektionen var der mørkegrå "tappe" ud fra slimhinden i tarmen, og i subserosa sås gulbrune knuder langs fasthæftningen af mesojejunum. Leveren fra inficerede hamstere var af normal størrelse eller noget mindre, og overfladen var som "Marokko-læder". I nogle hamstere sås endvidere hyperæmi og ødem i ventriklen, lunge- og pankreasstase samt hydrothorax og hydroperitoneum.

Lysmikroskopi: De tidlige tarmforandringer var kendetegnede ved udtalte ansamlinger af eosinofile granulocytter med eller uden relation til tilstedeværende schistosomæg. Hoeppli-fænomenet kunne iagttages i nogle af disse ansamlinger. Til at begynde med var granulomer kun fåtallige og især i kalvene uden indkapsling, men granulomer blev mere iøjnefaldende senere i infektionsforløbet både i kalve og hamstere. Æggene kunne indeholde intakte eller døde miracidier hos begge dyrearter. I kalvene så granulomerne mest i submukosa, hvorimod de i hamsterne kunne ses i alle tarmvæggens lag, hvorved de kunne forårsage varierende grader af fortykkelse af tarmvæggen. Kalvene udviste stor variation i sammensætningen af granulomerne, hvad angik de cellulære bestanddele. Hoeppli-fænomenet var hyppigt iagttaget i både eksperimentelt og naturligt inficeret kvæg.

Specielt hos hamstere kunne der ses mange intra- og ekstravaskulære æg tidligt i infektionen. De var især beliggende i de superficielle og midterste lag af slimhinden. De intravaskulære æg var omgivet af et eller flere lag af mononukleære celler. Æggene kunne ses enkeltvis, i grupper eller på række, og de kunne ligesom de ekstravaskulære æg rumme intakte immature eller mature miracidier. I de ekstravaskulære æg kunne endvidere lejlighedsvis ses degenererede/døde miracidier. Når æggene lå i klumper i karrene (venulerne), kunne de forårsage kraftig dilatation af karrene, og i tilfælde, hvor æggene lå på række i karrene, syntes det, som om de blev skubbet igennem karvæggen eller i nogle tilfælde direkte gennem slimhinden. Ekstravaskulære æg uden cellereaktion omkring blev set tæt ved tarmepitelet. Nogle af æggene kunne ses med den spidse ende igennem epitelet. Æg omgivet af eosinofile granulocytter eller granulomceller sås subepitelialt og mellem villi i tarmlumen.

Proliferative forandringer og betændelsesforandringer var udtalte i venerne og i submukosa og krøset i naturligt inficerede kalve. Æggranulomer beliggende subintimalt samt i tunica media og perivaskulært blev specielt iagttaget i kalve med naturlig infektion. Tværsnit af schistosomer uden vævsreaktion blev observeret i krøsvener og i vener i submukosa. Der var udtalte parasittromber i krøsvenerne og i levervenerne hos hamsterne, men også ikke-parasitære tromber blev fundet. Granulomer, nekrose og fibrose samt flebitis og blødning var iøjnefaldende i leveren fra hamsterne, hvorimod der kun var ubetydelige patologiske forandringer i leveren fra kalvene. Granulomer i milt, pankreas og krøslymfeknuder sås kun i hamsterne.

Scanning-elektronmikroskopi: Få forandringer blev observeret i slimhinden i tarmen fra kalve. Indbyrdes forbundne "kamme" af villi sås såvel i kontroller som i eksperimentelt inficerede kalve. Hos hamsterne sås mellem villi uregelmæssige strukturer, der blev tolket som værende schistosomæg dækket af et cellulært ekssudat. På villis ekstrusionszoner sås ansamlinger af celler, og i colon sås 9-19 uger efter infektionen uregelmæssige områder med kontinuitetsadskillelse i epitelet. På nogle af disse steder lå løsrevne, runde eller skrumpne celler, men også røde blodlegemer og runde, takkede leukocytter kunne observeres disse steder. På flere af disse steder med kontinuitetsadskillelse i epitelet og i krypterne sås æg stikke frem med deres spidse ende forrest.

Transmissions-elektronmikroskopi: Skallen på æggene bestod af et meget tæt, homogent materiale og var forholdsvis tykkere i den smalle ende af æggene end i den bredere ende. I den smalle ende var der ligeledes flere mikroporer. På skallens overflade kunne ses talrige "mikrokamme".

Intravaskulære æg var omgivet af et eller flere lag af monocytter, som kunne fasthæfte nogle æg til endotelet. Den luminale del af cellelaget omkring æggene var beklædt med endotel, der dannede en direkte overgang til det øvrige vaskulære endotel.

Den luminale del af de subendoteliale schistosomæg var visse steder i kontakt med endotelet og neutrofile granulocytter, og disse steder var der øget margination og emigration af neutrofile granulocytter og monocytter. Disse celler og eosinofile granulocytter var øget i antal i det perivaskulære væv omkring den abluminale side af de subendoteliale æg. Nogle af cellerne, der sås i kontakt med intravaskulære og subendoteliale æg, udviste øget synteseaktivitet, mens andre havde disintegrerede organeller.

Ekstravaskulære æg inducerede ingen eller kun ringe granulomatøs eller non-granulomatøs reaktion, men selv, hvor der der ikke var nogen cellulær reaktion, udviste værtscellerne umiddelbart rundt om æggene degenerative forandringer. Disse forandringer var til stede både i non-granulomatøse og granulomatøse forandringer. Cytopatologiske forandringer kunne også iagttages i værtsceller omkring granulomerne. Øget densitet af de intercellulære områder og den tilgrænsende cytoplasmatiske matrix var iøjnefaldende i lamina propria og

i basallamina til tarmepitelet op til de læsioner, som schistosomæggene havde forårsaget i tarmvæggen.

Mature eosinofile granulocytter udgjorde hovedbestanddelen af celleinfiltrationerne i en del non-granulomatøse og tidlige granulomatøse forandringer. De eosinofile granulocytter var i tæt kontakt med schistosomæggene, og nogle af granulocytterne var degranulerede med områder med øget densitet af cytoplasmaet. I andre eosinofile granulocytter var granula transparente. En del æg i disse områder havde intakt skal og rummede miracidier. De tidlige granulomatøse forandringer var uden kollagendannelse og elastiske tråde.

De ældre granulomer indeholdt især makrofager og epiteloidceller, hvorimod eosinofile og neutrofile granulocytter og mastceller kun optrådte sparsomt. Kæmpeceller kunne ses i enkelte granulomer. Cytopatologiske forandringer sås i forskellige celler. Tynde fibre kunne ses i det indre af granulomerne. Tykkere kollagene tråde sås i periferien af granulomerne. Stykker af skaller fra schistosomæg, der kunne ses i nogle granulomer, havde samme struktur som skaller fra intakte æg.

I området mellem schistosomæggene og de omgivende celler kunne ses et fnugget eller vesikulært materiale, hvad enten det drejede sig om intravaskulære eller ekstravaskulære æg eller æg beliggende subendotelialt. Dette materiale var sparsomt til stede omkring intravaskulære æg og på den luminale side af subendoteliale æg, men var i udtalt grad til stede omkring ekstravaskulære æg både hos kalve og hamstere. Hos kalve, hvor materialet var mest udtalt, var det identisk med Hoeppli-fænomenet, som det kunne erkendes ved lysmikroskopi. Skønt Hoeppli-fænomenet ikke er beskrevet hos hamstere, er det sandsynligt, at det intercellulære materiale, der er fundet omkring schistosomæg i hamstere ved TEM, ækvivalerer med Hoeppli-fænomenet. De vesikulære strukturer blev også observeret i bindevævet mellem tarmepitelet og de af schistosomæggene inducerede skader i tarmslimhinden.

I et område med blødning i slimhinden kunne der i venuler i nærheden af ekstracellulære æg ses åbninger i endotelet, hvis celler havde et klart cytoplasma. Makrofager, eosinofile granulocytter og røde blodlegemer i nærheden af disse venuler samt de tilgrænsende

tarmepitelceller udviste varierende grader af degenerative forandringer. Degenererede røde blodlegemer kunne endvidere ses interepitelialt og intracellulært i enterocytterne.

Kapitel 5

Skallen af *S. bovis*-æg er tykkere og har flere mikroporer i den smalle ende end i den brede ende, hvilket kan have patogenetiske implikationer.

Intravaskulære æg synes at nå til det ekstravaskulære område sammen med monocytter og neutrofile granulocytter. Et endotelcellelag over æggene og cellerne omkring dem adskiller æggene fra karrets lumen og forhindrer blødning. Intravaskulære æg kommer sandsynligvis også ekstravaskulært via en overudstramning og nekrose af karvæggene, når det drejer sig om klumper af intravaskulære æg. Æg, der ligger på række i karrene, kan måske direkte blive "skubbet" gennem karvæggen og tarmepitelet og ud i tarmlumen.

Blødning kan sandsynligvis også opstå på grund af mangelfuldt endoteldække omkring de migrerende æg, når disse er talrige. Sidstnævnte mulighed er særlig sandsynlig ved kraftige infektioner tidligt i infektionsforløbet.

Ekstravaskulære æg når sandsynligvis frem til tarmlumen ved hjælp af lytisk aktivitet af stoffer fra æggene og fra granulocytter og da især fra de eosinofile granulocytter. Evidens for dette var tilstedeværelsen af det fnuggede materiale og de sværiske mikropartikler i tæt relation til æggene og mellem bindevævet omkring æggene og resten af lamina propria. De iagttagne former for granulomer menes alle at være induceret af schistosomæg, der midlertidigt eller permanent er blevet fastholdt i tarmvæggen. De cytopatologiske forandringer, som cellerne omkring schistosomæggene udviser, skyldes sandsynligvis påvirkninger fra ekskretoriske og/eller sekretoriske stoffer fra miracidierne. Man kan derfor sige, at granulomerne i nogen grad beskytter resten af værtscellerne.

Schistosomæg synes at trænge gennem tarmepitelet ved hjælp af lytiske faktorer fra æggene og leukocytter såvel som ved aktiv passage formidlet af æggenes spidse ende. Nogle æg trænger gennem epitelet i krypterne. Det er på grund af disse migrationinducerede skader, at der kan tabes væske til tarmlumen, hvorved diarré kan opstå.

Fordelingen af de tidlige, omfattende ansamlinger af eosinofile granulocytter og af de sene granulomer i de forskellige lag af tarmvæggen kan måske være nyttige til identifikation af nye og gamle infektioner under naturlige forhold. Patologiske forandringer i tarmens vener kan interferere med deponeringen af æg tæt ved slimhindeoverfladen. Sådanne forandringer ses sent i infektionsforløbet og kan måske have differentialdiagnostisk betydning ved naturlige infektioner.

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10. APPENDICES

Appendix 1

Procedure for Dehydration, Clearing and Embedding of Tissues for Transmission Electron Microscopy

1. Rinse in 0.2 M Na cacodylate buffer three times for 15 minutes each time
2. Post-fix in 1% osmium tetroxide buffered in o.2 M Na cacodylate and keep in darkness for 1 hour 30 minutes
3. Repeat (1) above
4. Stain the blocks in 0.5% uranyl acetate* for 2 hours at 20°C
5. Transfer to 75% alcohol for 10 minutes
6. Transfer to 90% alcohol for 10 minutes
7. Transfer to 96% alcohol for 10 minutes
8. Transfer to 100% alcohol three times each, time for 20 minutes
9. Transfer to 1:1 mixture, 100% alcohol: propylene oxide, for 10 minutes
10. Transfer to propylene oxide for 15 minutes
11. Transfer to propylene oxide for 15 minutes
12. Transfer to 2:1 propylene oxide:Epon**, for 30 minutes
13. Transfer to 1:1, propylene oxide:Epon**, for 30 minutes
14. Transfer to 1:2, propylene oxide:Epon**, for 30 minutes
15. Transfer to Epon** and leave overnight
16. Transfer to Epon** [the following day] after adding DMP to the Epon**
17. Polymerize at 60°C for 48 hours [two days]

Key:

***Uranyl acetate (0.5%)**

0.4 g uranyl acetate in 20 ml of distilled water; filter; keep in darkness

****Epon**

Epon (Glycide ether 100)	45.5 ml
DDSA (Dodecenyl amberacid anhydride)	31.0 ml
MNA (Methyl nadic anhydride)	23.5 ml
	100.0 ml

Appendix 2.

Paraformaldehyde - Glutaraldehyde Fixative

Paraformaldehyde	4 g
Distilled water, 60°C	100 ml
Dissolve in fume cupboard	30 min.
1N NaOH (Titrisol ampulla)	A few drops until solution is clear

Cool

Add:

Glutaraldehyde, 25%	40 ml

0.2 M cacodylate buffer, pH 7.4 to make 200 ml

Final pH: 7.2 - 7.4

Final paraformaldehyde concentration: 2%

Final glutaraldehyde concentration: 2.5%

Appendix 3.

Process for Dehydration for Scanning Electron Microscopy

1. Rinse in 0.2 M Na cacodylate buffer three times for 5 minutes each time
2. Transfer to 25% acetone for 15 minutes
3. Transfer to 40% acetone for 15 minutes
4. Transfer to 60% acetone for 15 minutes
5. Transfer to 70% acetone for 15 minutes
6. Transfer to 90% acetone for 15 minutes
9. Transfer to 100% acetone (new bottle) for 20 minutes
10. Transfer to 100% acetone for 20 minutes
11. Transfer to 100% acetone for 20 minutes
12. Transfer to 100% water free acetone at 4^{oc} until the next processing stage, Critical Point Drying.

Appendix 4.

Procedure for Harvesting Miracidia After Obtaining Fecal Specimens (Miracidium Hatching)(Christensen, N.Ø., et al, 1984)

1. Transfer a 5 g fecal sample to the 100 ml container using the spoon and add 50 ml of saline
2. Apply the screw cap and shake thoroughly by hand to obtain a thin suspension of saline and fecal material
3. After shaking, add another 50 ml of saline
4. Pour the sample through the sieve into the specimen glass and using the wash bottle, spray the sieve with an additional 100 ml of the saline to ensure that no eggs remain on the mesh
5. Fill up the specimen glass with saline
6. Leave the specimen glass for 30 minutes in the dark
7. Pour off the supernatant without disturbing the deposit
8. Refill the specimen glass with saline, resuspend the deposit in the saline using the applicator stick and leave the specimen glass for another 30 minutes in the dark
9. Repeat steps 7 and 8 until the supernatant becomes clear
10. Pour off the supernatant without disturbing the deposit
11. Refill the specimen glass with fresh water, resuspend the deposit in the fresh water using the applicator stick and leave the specimen glass for 30 minutes in the dark
12. Pour off the supernatant without disturbing the deposit
13. Pour the deposit into the glass Petri dishes and using the wash bottle, spray the specimen glass thoroughly with fresh water to ensure transfer of all the deposits to the Petri dishes (transfer only small amounts of deposit to each Petri dish)
14. Leave the Petri dishes under strong artificial illumination at a temperature of 20-25°C for 30 minutes
15. Check for emergence of miracidia using the microscope with x10-40 magnification

INTESTINAL LESIONS ASSOCIATED WITH TRANSMUCOSAL MIGRATION OF EGGS IN CALVES AND HAMSTERS INFECTED WITH *SCHISTOSOMA BOVIS*

A light and electron microscopic study

11. FIGURES

Ph.D. Thesis

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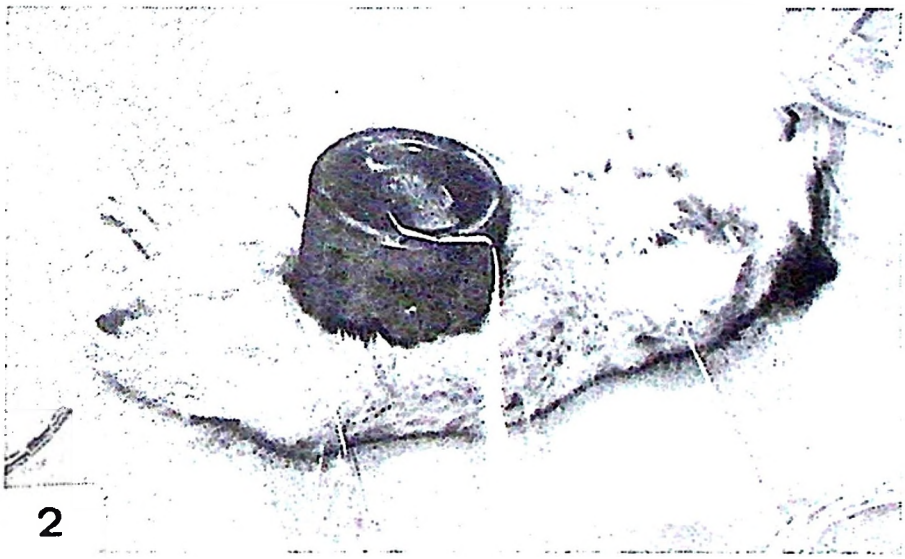
Fig. 1. Procedure for infection of calves. The shaved tail is submerged in infective water contained in a plastic bag.

Fig. 2. Procedure for infection of hamsters. The infective material is put into the metal ring placed on the clipped abdominal area.

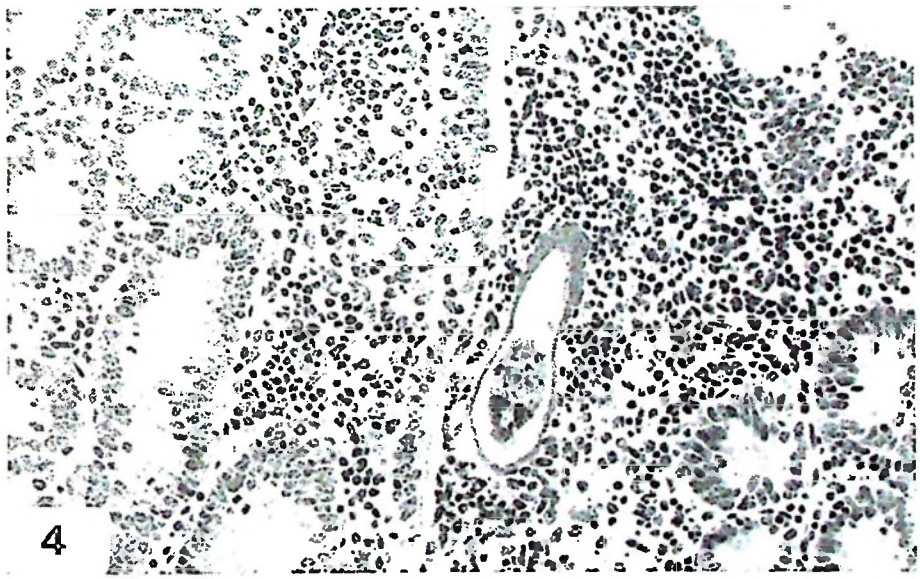


2

Fig. 3. Small nodules (arrows) under the serosa at the mesenteric attachment to the jejunum. The nodules were yellow-brown. Hamster.

Fig. 4. An egg in an accumulation of eosinophils in the lamina propria and surrounded by homogenous material (Hoeppli phenomenon) most pronounced at the pointed end. Calf. H.E. 25x objective magnification.

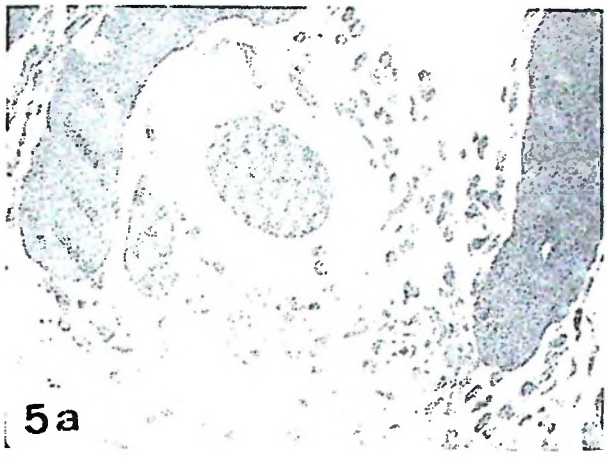
3



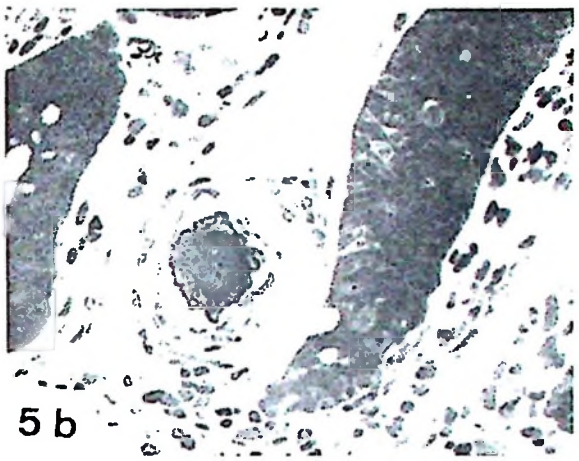
4

Fig. 5. Intravascular eggs. Small intestine. (a) The egg is surrounded by a thin single layer of attenuated mononuclear cells. (b) The egg is surrounded by several layers of cells which anchor the egg to the endothelium. Epon section. Toluidine Blue. 40x objective magnification.

Fig. 6. (a) Eggs in tandem arrangement immediately below the epithelium. Small intestine. Calf. (b) Eggs in tandem arrangment with the topmost egg through the epithelium into the intestinal lumen (star). Small intestine. Hamster. EG = Egg. H.E. 25x objective magnification.



5a



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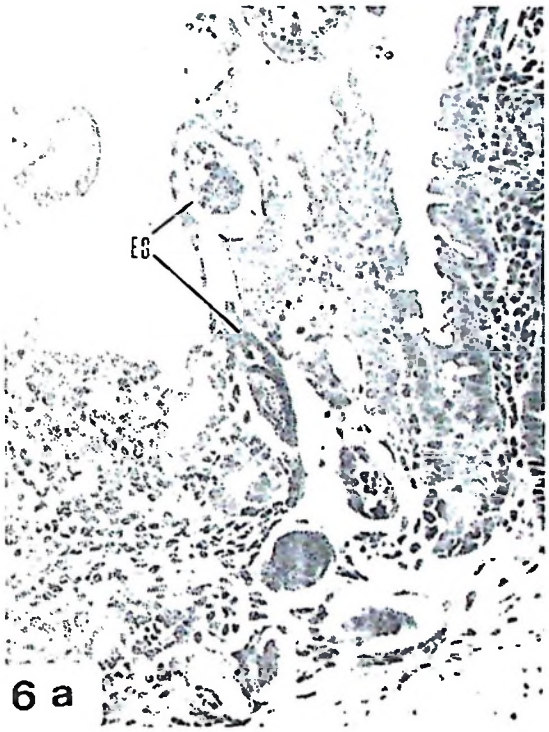
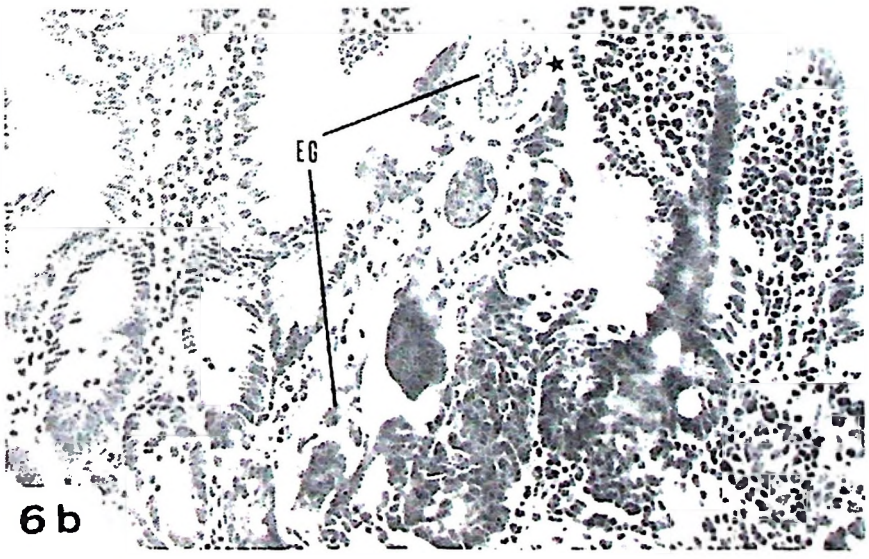


6 a

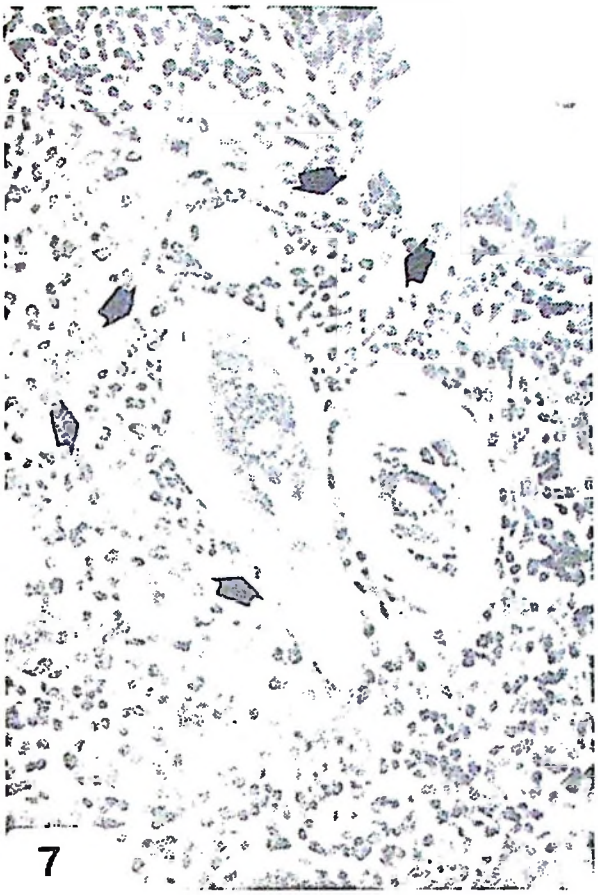


6 b

Fig. 7. A partly emerged egg in the intervillous space (arrows) has the luminal side covered by eosinophils and the abluminal side still in the lamina propria surrounded by a mixture of eosinophils and mononuclear cells. Small intestine. Calf. H.E. 40x objective magnification.

Fig. 8. A vein with proliferative changes leading to occlusion of the lumen. The adventitia and perivascular area are infiltrated with eosinophils and mononuclear cells. Small intestine. Calf. H.E. 25x objective magnification.

Fig. 9. A composite granuloma with eggs in which miracidia are at different stages of development. Immature miracidia (thin arrow). Mature miracidia (thick arrow). The granuloma occupied the entire wall of the intestine. Small intestine. Hamster. H.E. 10x objective magnification.



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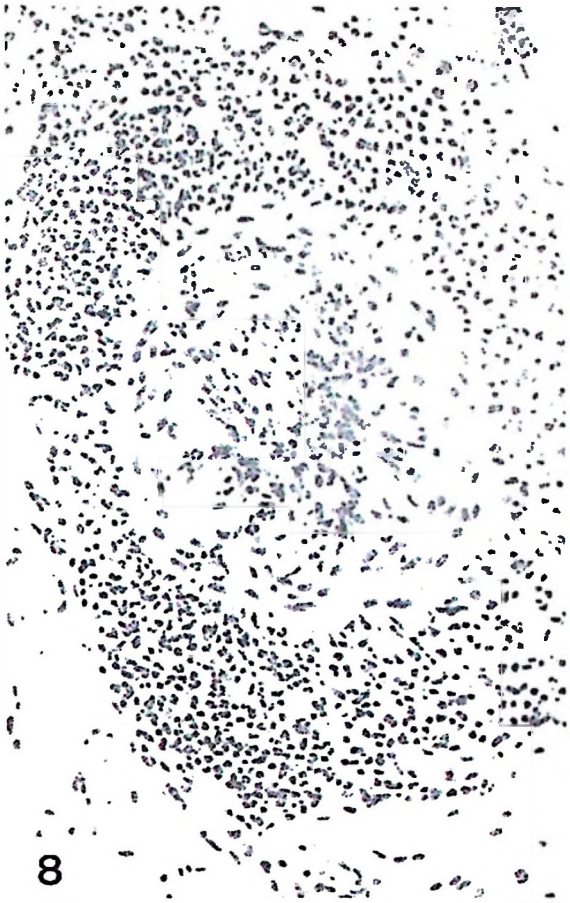

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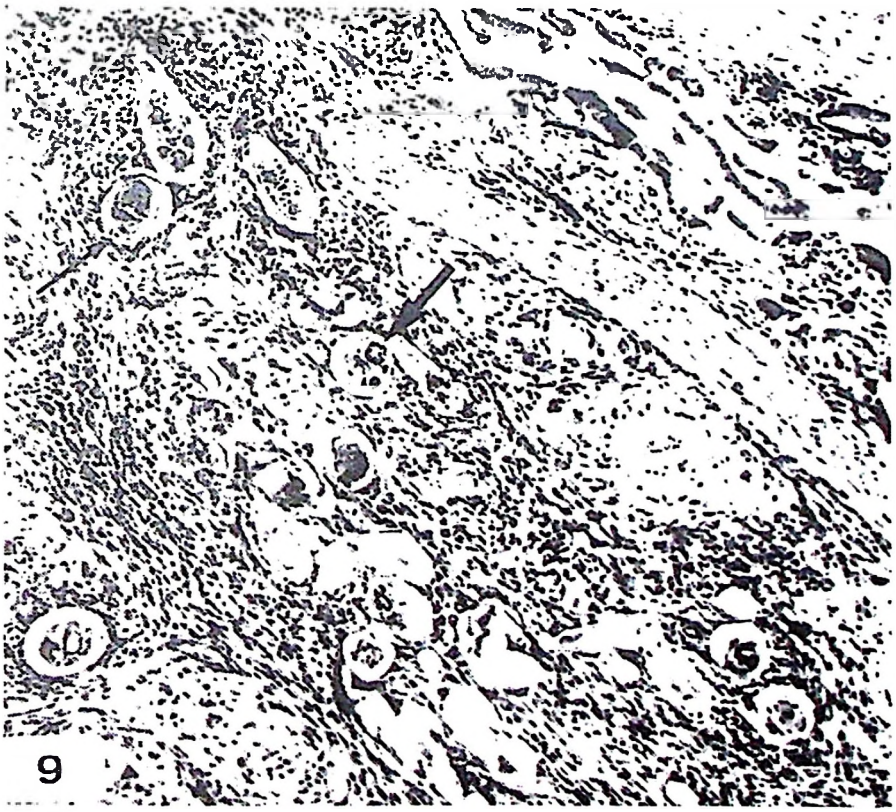
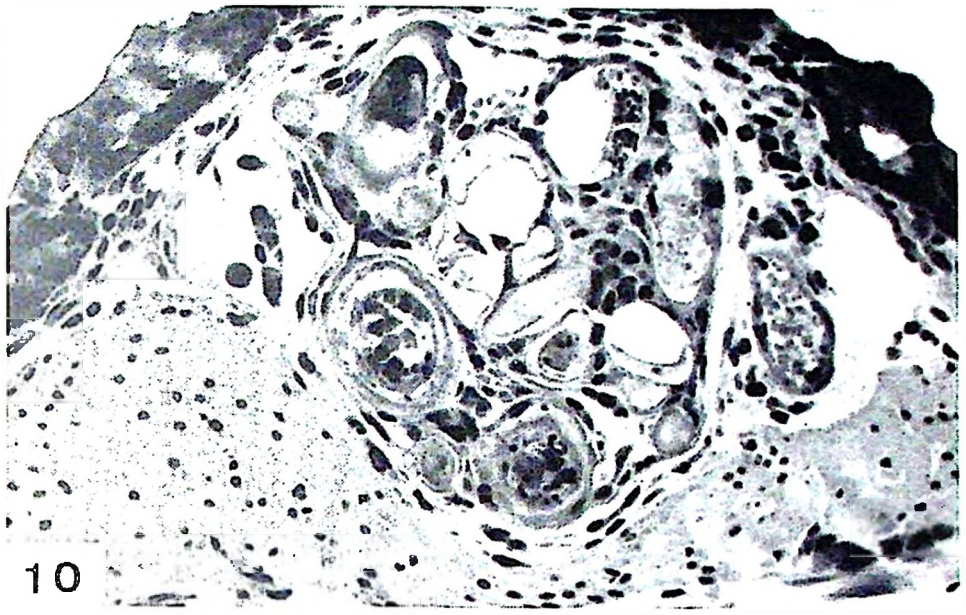


9

Fig. 10. Conglomerate of eggs inside a submucosal venule. The venule is markedly distended. The eggs are more or less surrounded by mononuclear cells. Hamster. H.E. 25x objective magnification.

Fig. 11. The pointed end of an egg pushing through the basal lamina and epithelium of a villus. There is some physical distortion of the epithelium. Small intestine. Hamster. H.E. 100x objective magnification.



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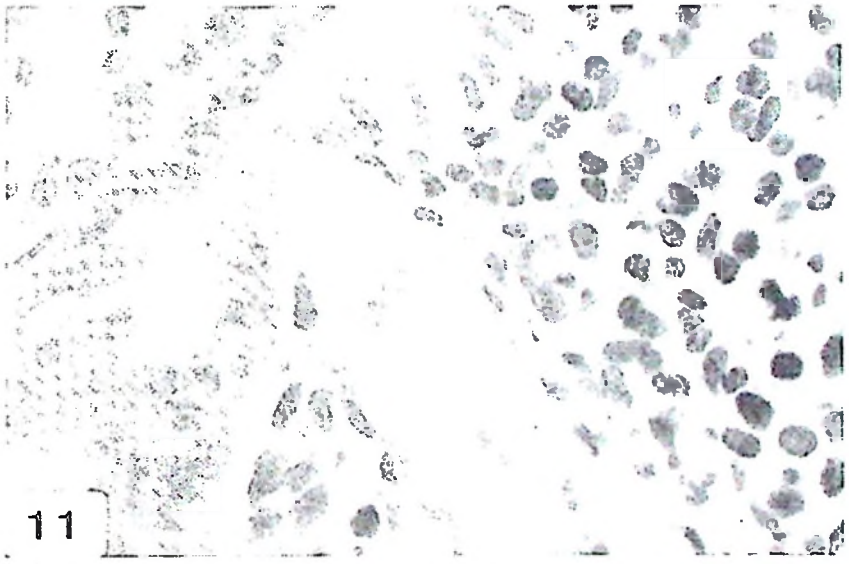
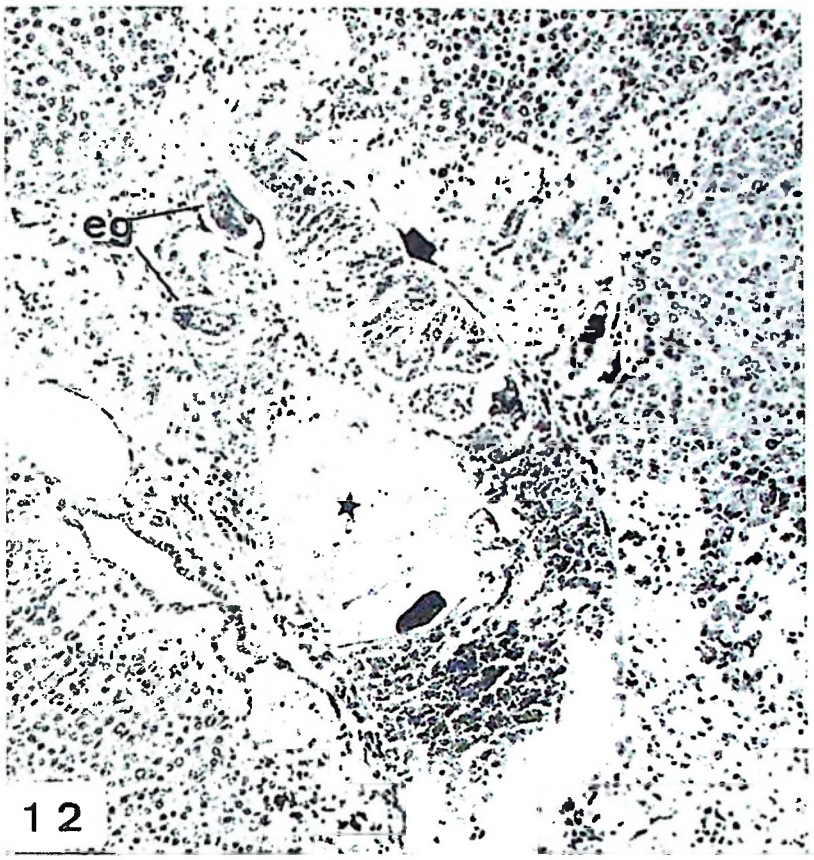


11

Fig. 12. A thrombus (star) in a portal vein in the presence of eggs (EG) and an intact worm (arrow). Liver. Hamster. H.E. 25x objective magnification.

Fig. 13. Interconnecting ridge-like villi. Proximal small intestine. Control calf. Bar = 100μm



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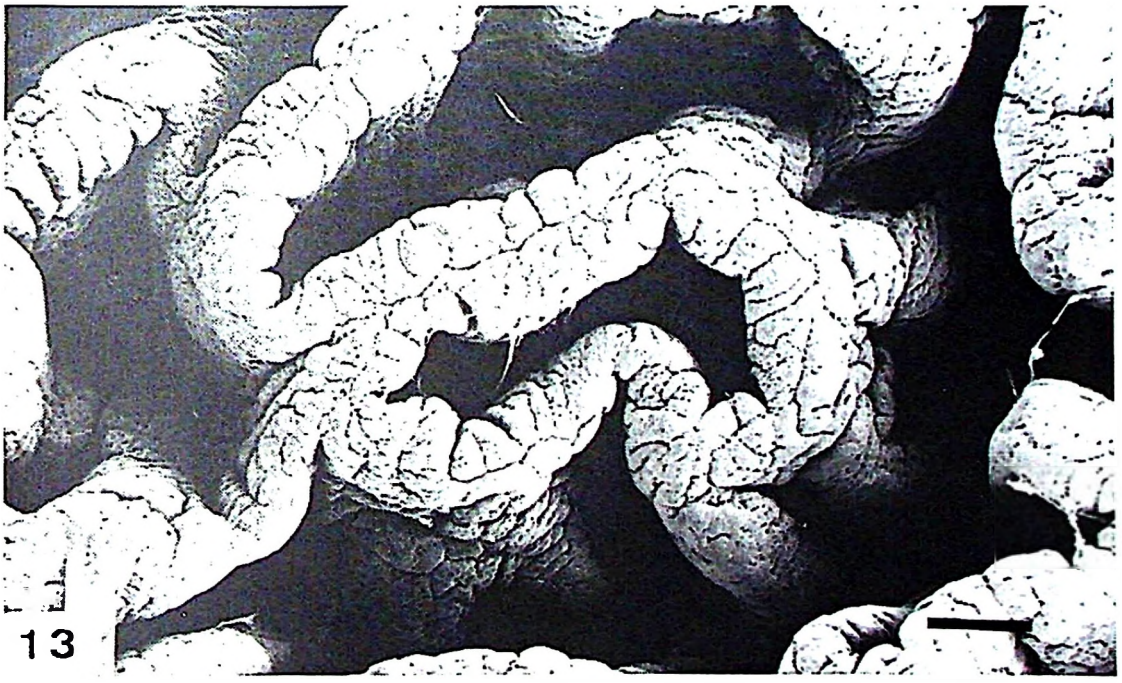


13

Fig. 14. Mucosal fold with numerous smaller and shorter folds among which are slit-like glandular pits (arrows). Colon. Control hamster. Bar = 100μm

Fig. 15. Irregular masses (star) between villi. They are probably schistosome eggs covered by cellular exudate. Small intestine. Hamster. Bar = 100μm.


14


15

Fig. 16. Markedly increased number of cells at extrusion zones of villi. Some of the cells have swollen microvilli. Small intestine. Hamster. Bar = 100μm.

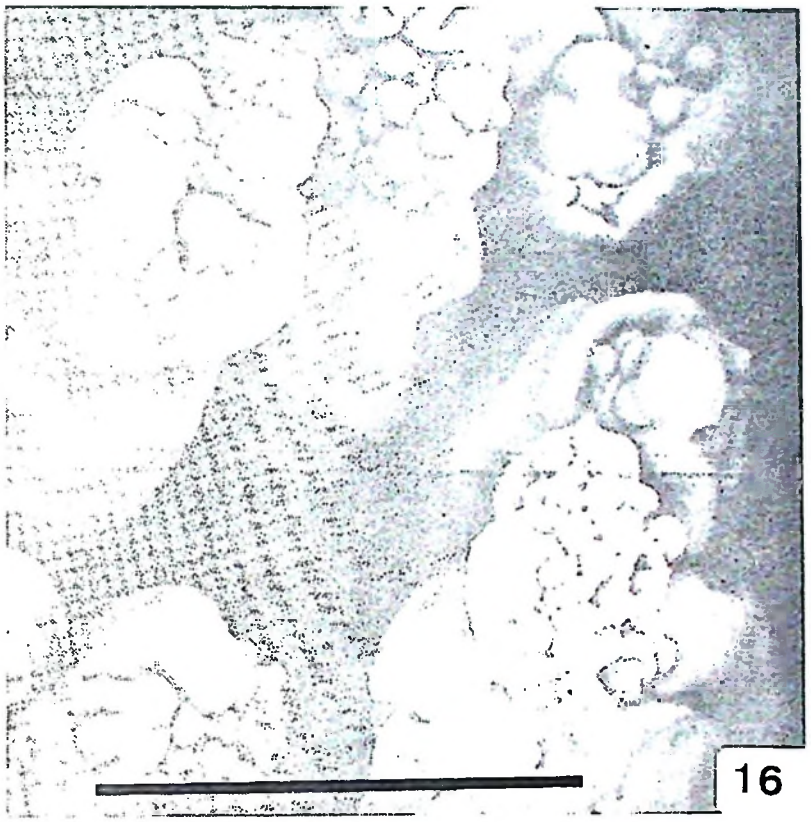
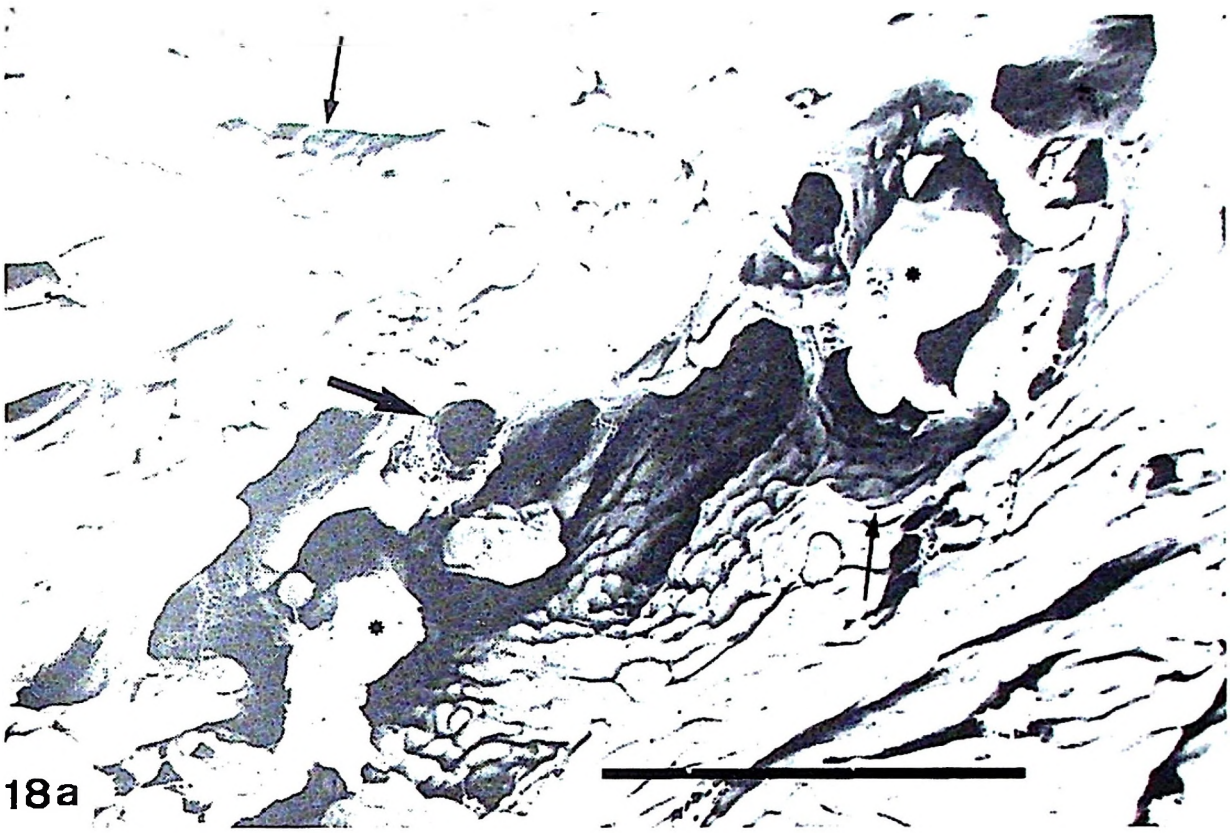

16

Fig. 17. (a) Area of disruption by emerging eggs (EG), leaving an opening at which are (b) globular cells with microprojection (L) (probably leukocytes) and discoid cells (probably erythrocytes). Colon. Hamster. Bar (a) = 100μm, (b) = 10μm.

17a

17b

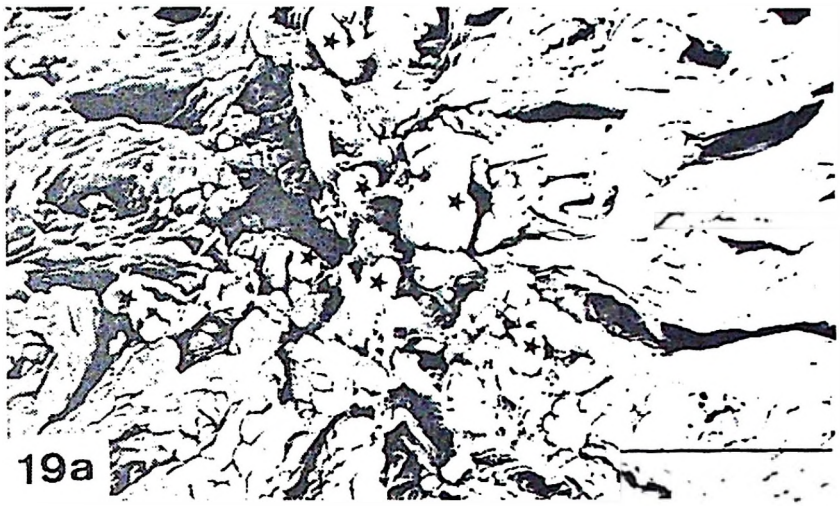
Fig. 18. (a) Schistosome eggs (asterisk) emerging through and near glandular pit openings. Hole through which an egg emerged (thick arrow). Glandular pits (thin arrow). (b) Higher magnification of one of the eggs in (a). Note the loosening of mucosal surface cells around hole of emergence. Colon. Hamster. Bar (a) = 100μm, (b) = 10μm.



18a

18b

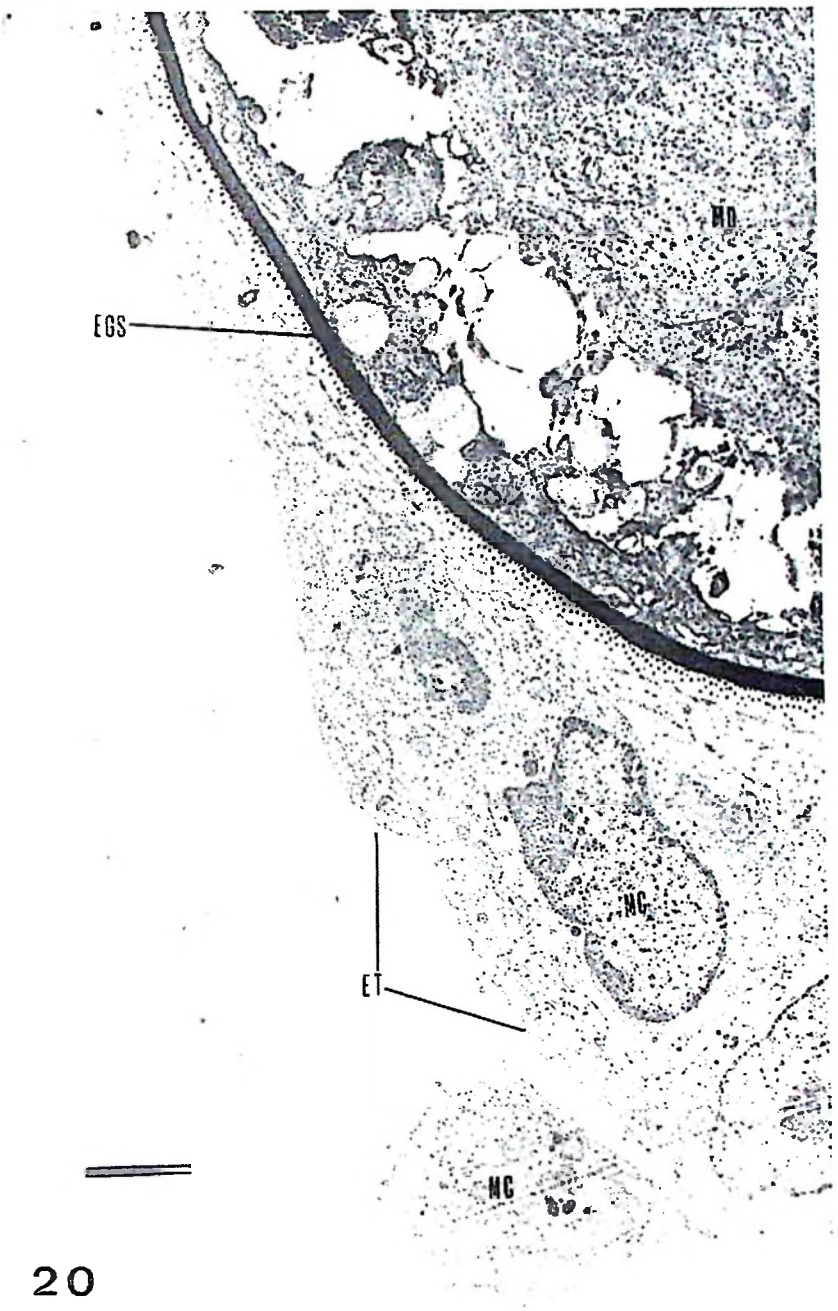
Fig. 19. (a) Many eggs (stars) emerging with the nonspined end and causing loosening of mucosal surface cells. Some of the loosened cells are round and shrunken. (b) Closeup on one of the emerging eggs. Note folds in its surface (arrows). Colon. Hamster. Bar (a) = 100μm, (b) = 10μm.



19a

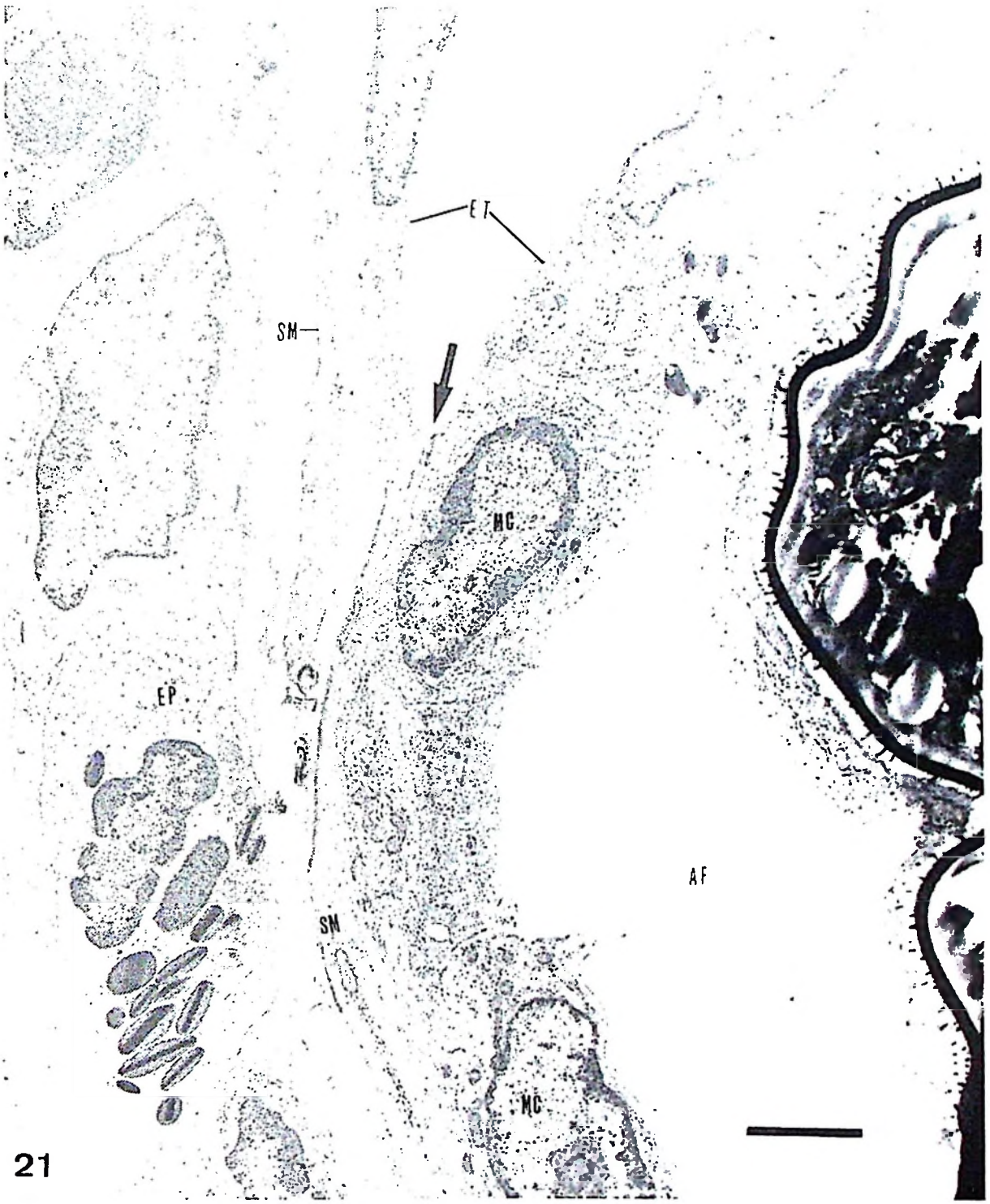
19b

Fig. 20. Monocytes (MC) form the immediate cell cover of an intravascular egg. The monocytes are covered by endothelial cells (ET) or their extensions. EGS = Egg Shell. MD = Miracidium. Small intestine. Hamster. Bar = 2μm.



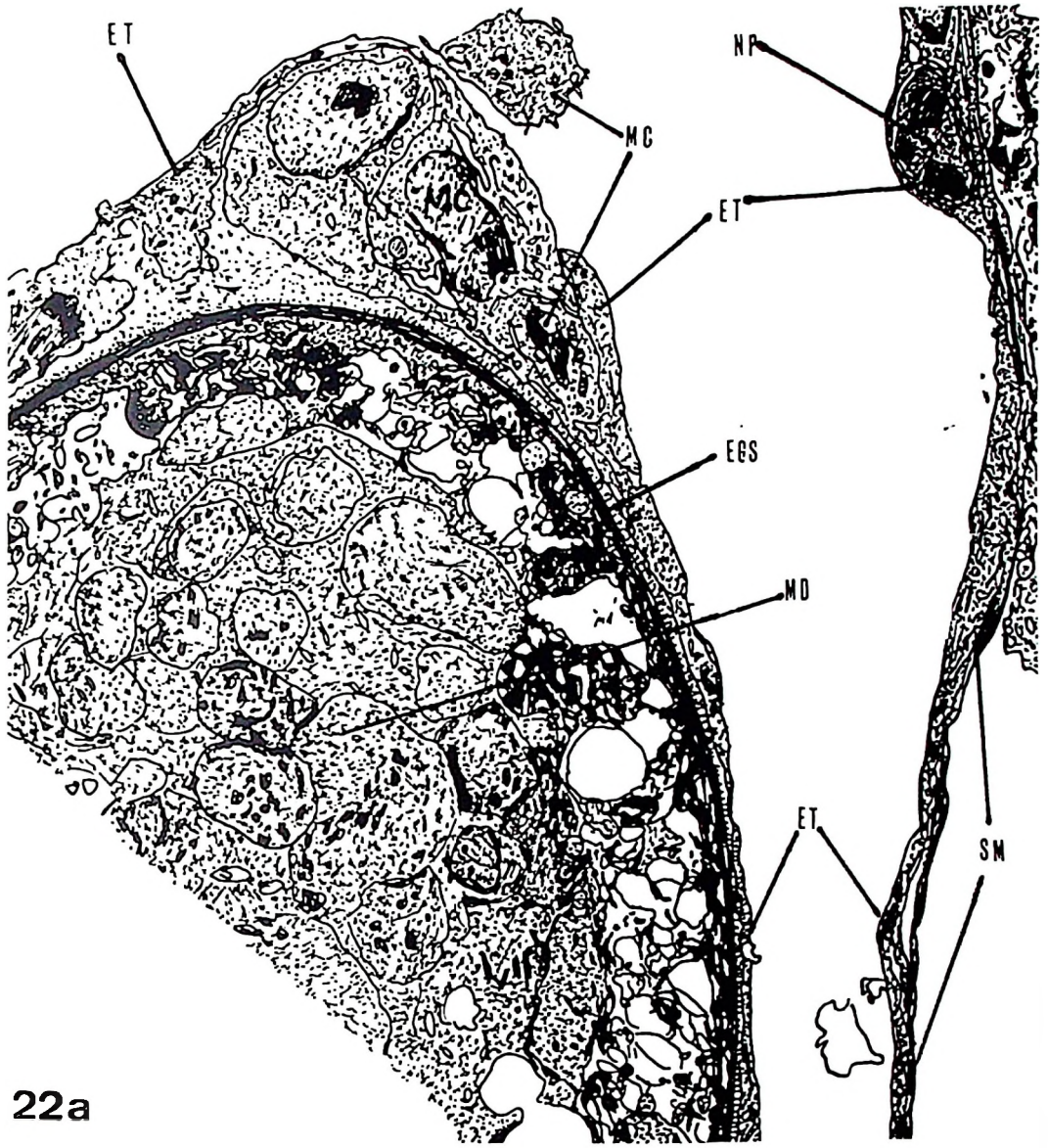
20

Fig. 21. Monocytes forming several layers around an intravascular egg. The luminal side of the cellular mantle is covered by endothelial cells (ET) or their extensions. Endothelium over the egg and vessel is continuous (arrow). AF = Artefactual separation of mantle cells. EP=Eosinophil. ET = Endothelial cells. MC = Monocyte. SM = Smooth muscle. Small intestine. Hamster. Bar = 2μm.



21

Fig. 22 (a) and (b). Composite sketch incorporating parts of Figs. 20 and 21. Eggs described in TEM under "Intravascular eggs". AF = Artefactual space. EGS = Egg Shell. EP = Eosinophil. ET = Endothelial Cell. MC = Monocyte. MD = Miracidium, MØ = Macrophage.


ET
MC
NP
ET
EGS
MD
ET
SM
22a

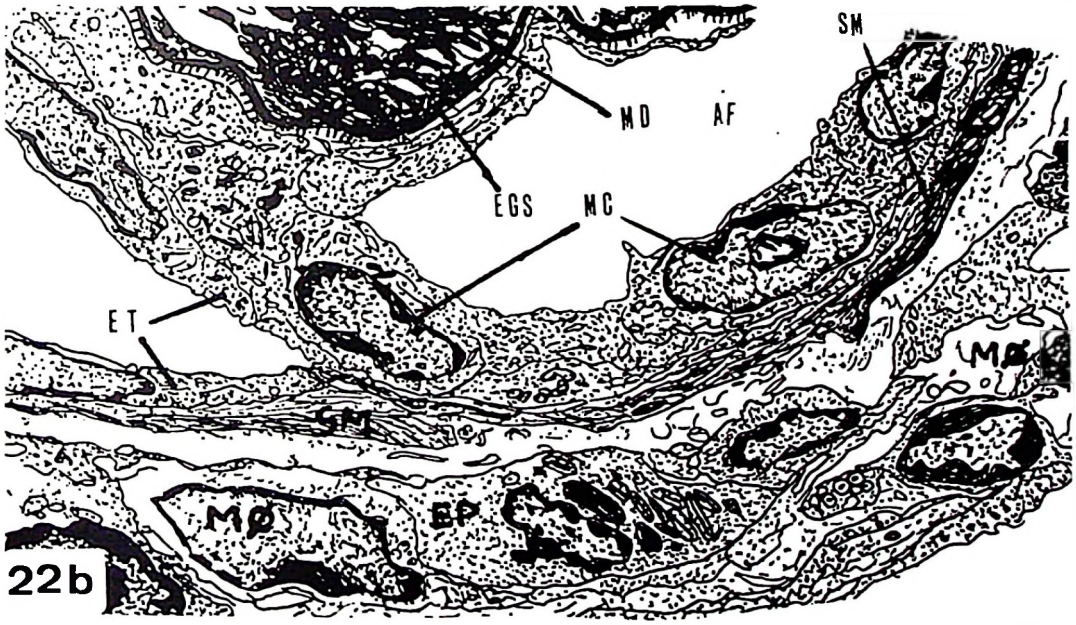

SM
MD
AF
EGS
MC
ET
MØ
MØ
EP
22b

Fig. 23. Inferface between egg shell and host cell has flocculent material (small short arrow), small membrane bound vesicles (long arrow) and microspines in transverse sections, all mixed to give an irregular reticular appearance. Small intestine. Hamster. Bar = 500 nm.

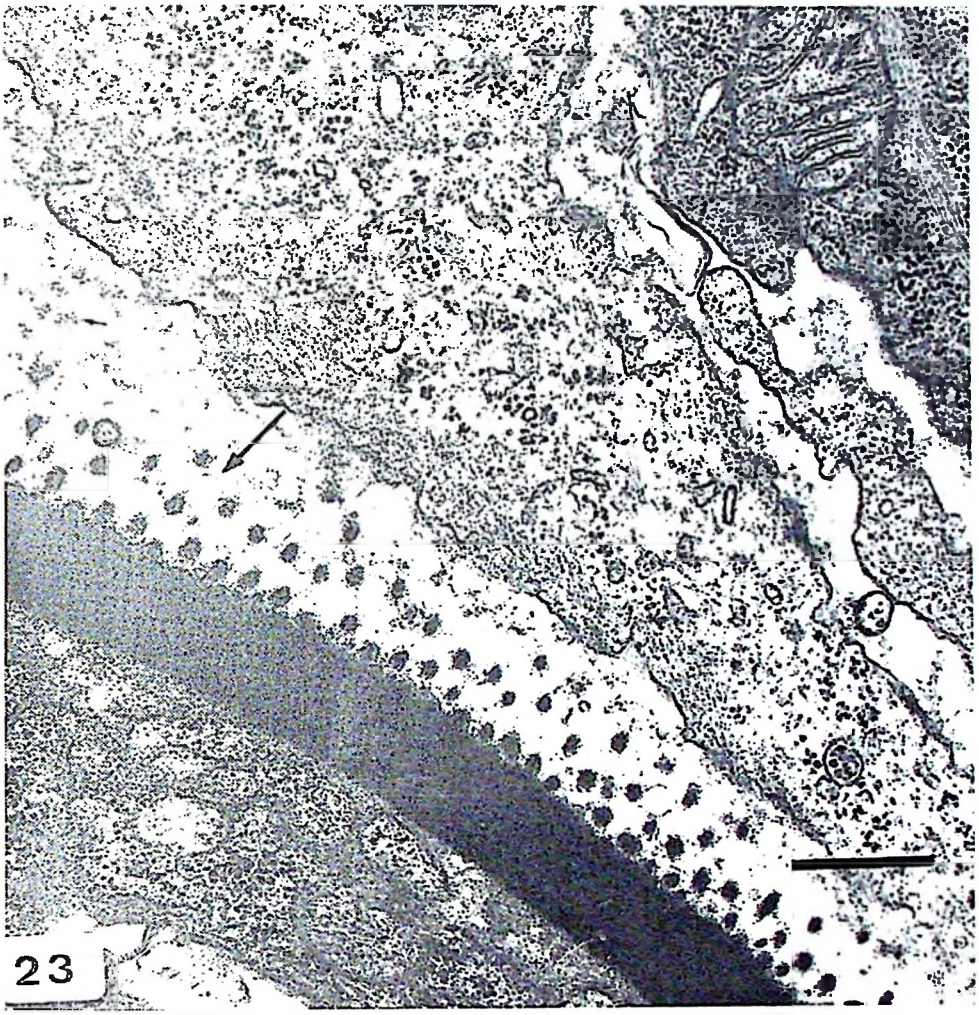

23

Fig. 24. Composite sketch incorporating parts of Figs. 25 and 26, Eggs described in TEM under "Subendothelial eggs". EGS = Egg Shell. ET = Endothelium. ABL = Egg side away from subendothelial space. LM = Egg side directly under endothelium. MC = Monocyte. MD = Miracidium. MS=Microspines. NP = Neutrophil. PVT = Perivascular tissue.

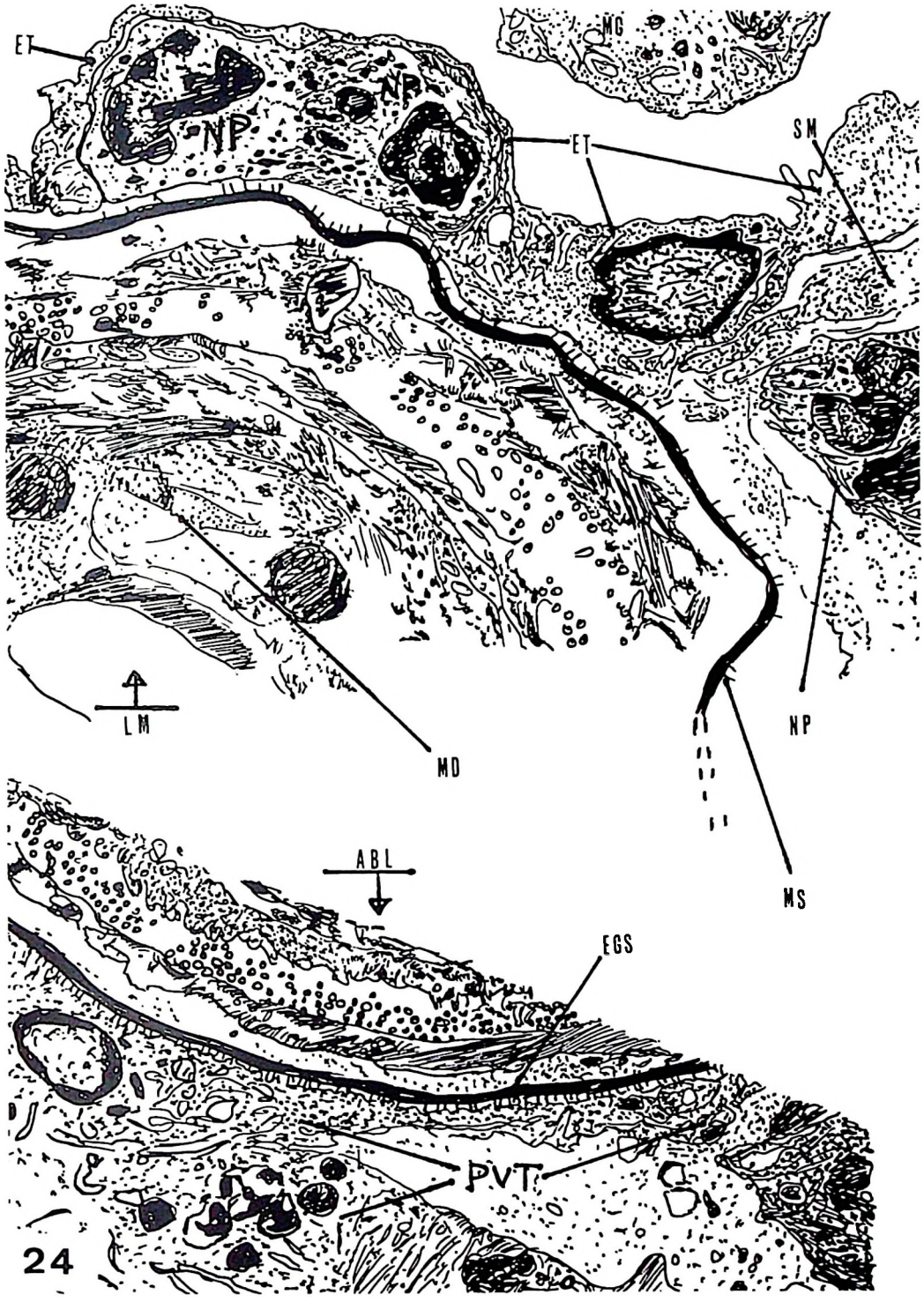

ET
NP
NP
MG
ET
SM
LM
MD
NP
MS
ABL
EGS
PVT

Fig. 25. An egg with a mature miracidium in the subendothelial space together with an emigrating neutrophil (np). The luminal surface of the neutrophil is covered by thin extensions of endothelial cells (ET). The abluminal side of the neutrophil is partly in contact with the egg shell and endothelial cell extensions (ET). Small intestine. Hamster. Bar = 1μm.

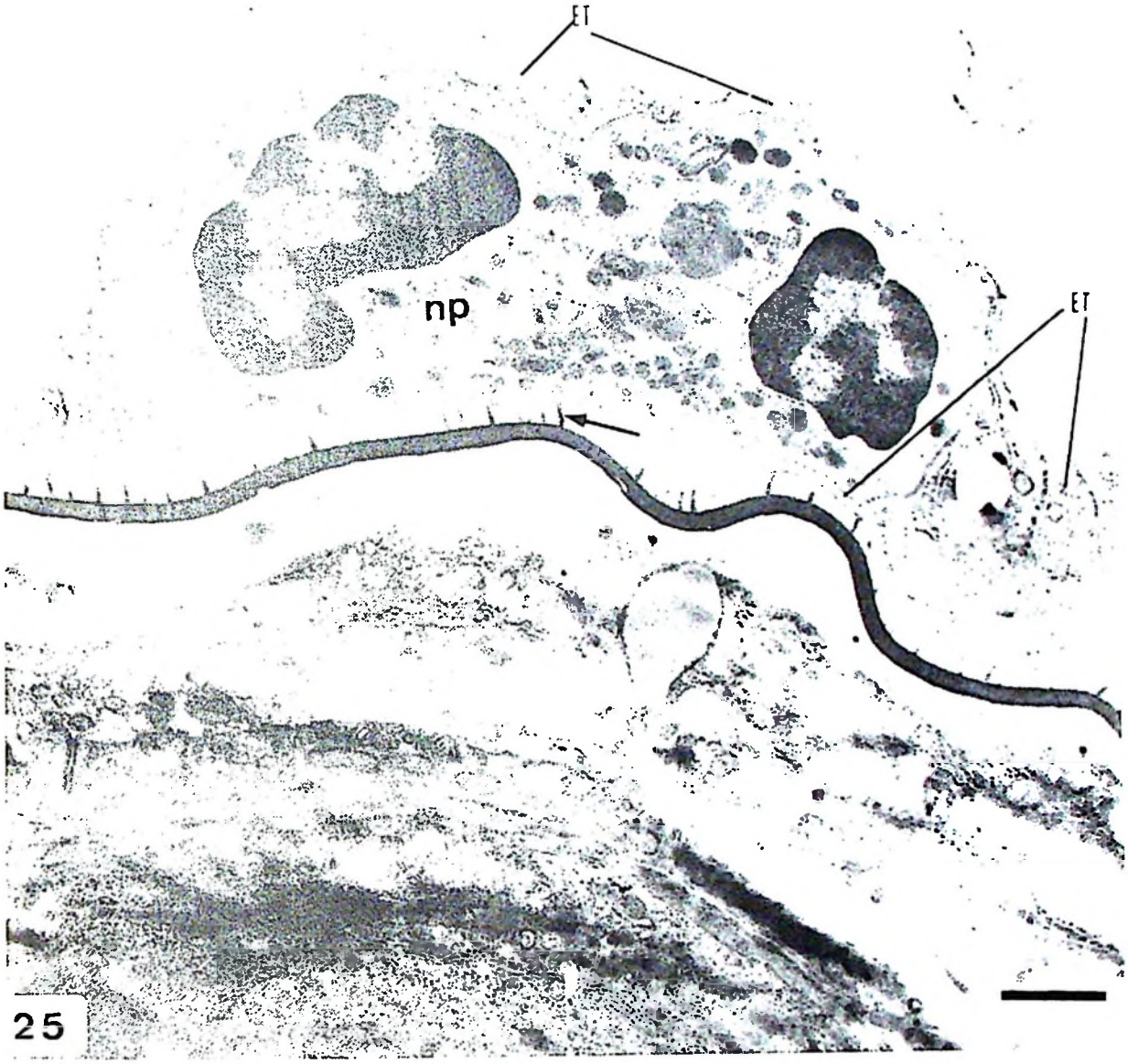
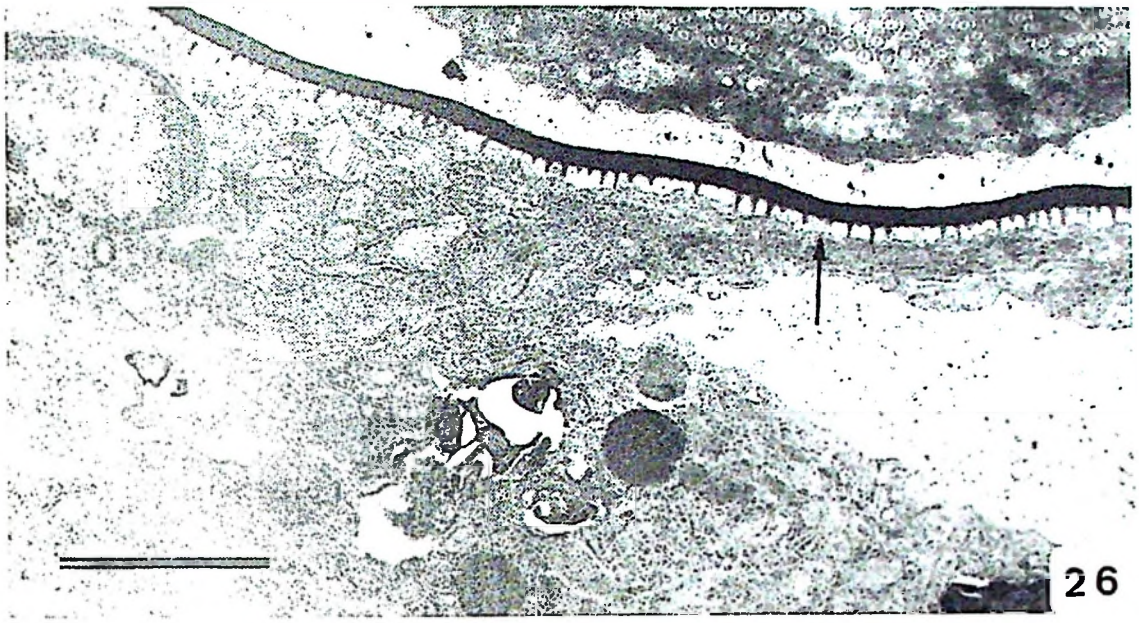

ET
np
ET
25

Fig. 26. Interface between the host cells and egg shell at the side not under the endothelium. There are vesicular structure (arrow) which are dense, large and membrane-bound. Small intestine. Hamster. Bar = 2μm.

Fig. 27. An extravascular egg with an intact immature miracidium (md). The egg shell (egs) is highly dense and uniformly thick. No micropores are evident in this section. Microspines are cone shaped and vary in height. Small intestine. Hamster. Bar = 2μm.



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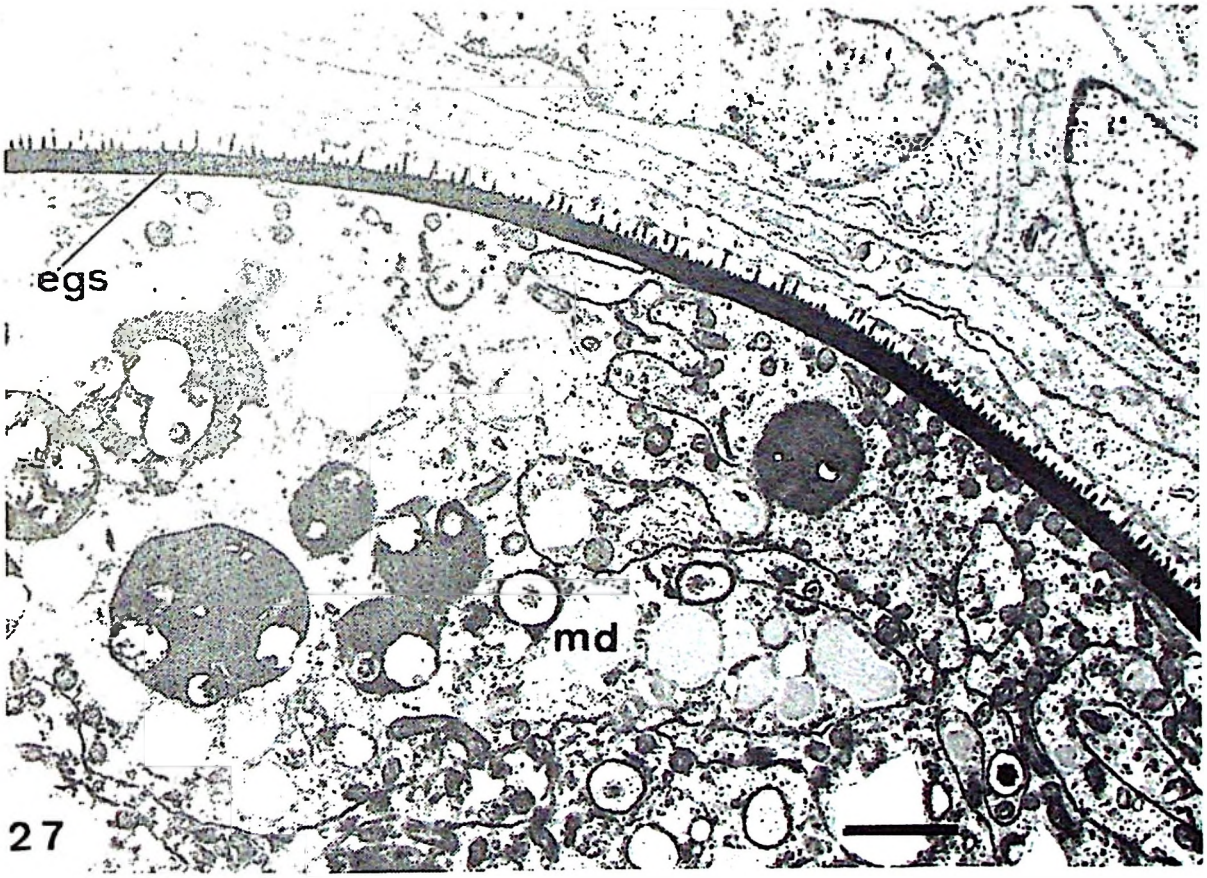
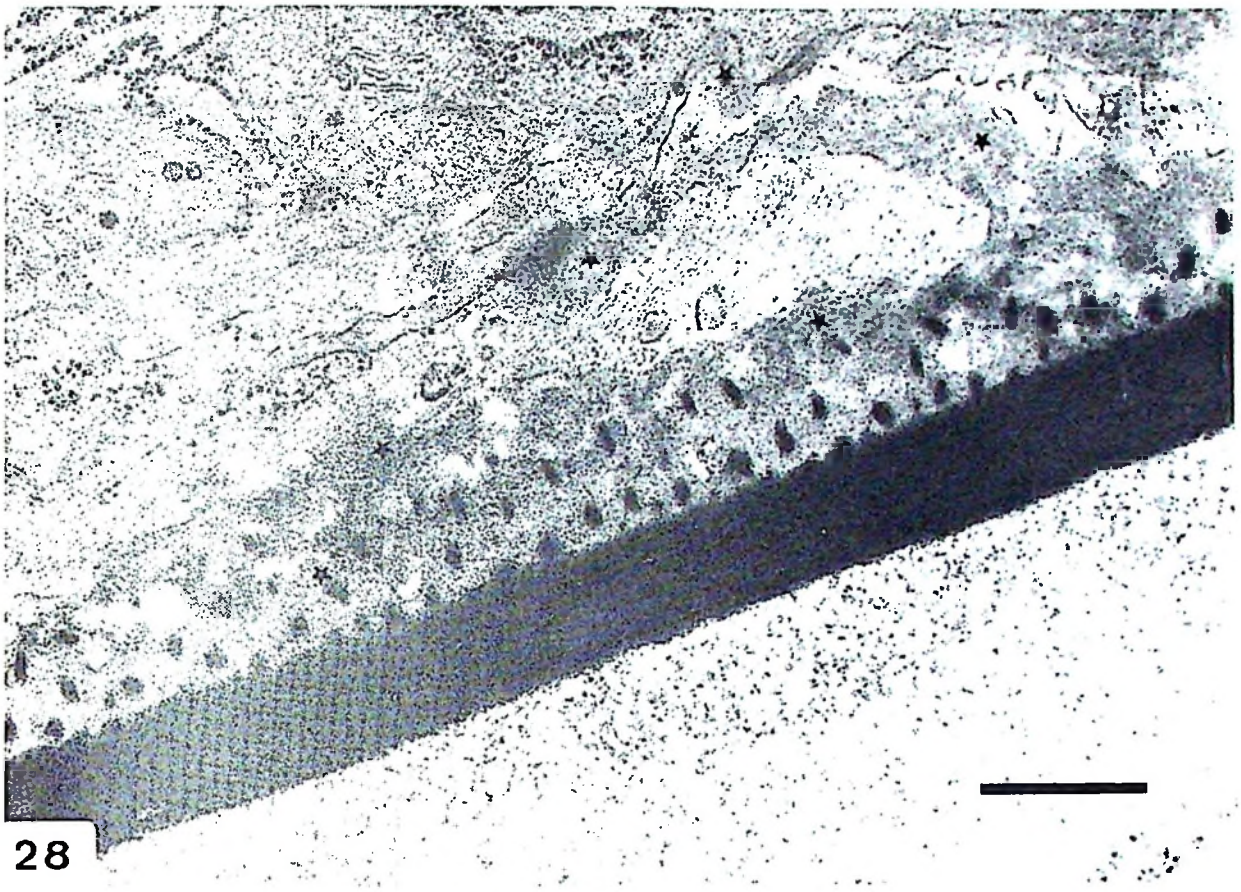


27

Fig. 28. Dense homogenous material (star) in the interface between egg shell and host cells.It extends between fibrillar structures and cellular elements. Small intestine. Hamster. Bar = 500nm.

Fig. 29. Many membrane-bound vesicles or globules (arrows) in the interface between egg shell and surrounding host cells. Extravascular egg. Colon. Hamster. Bar = 1μm.



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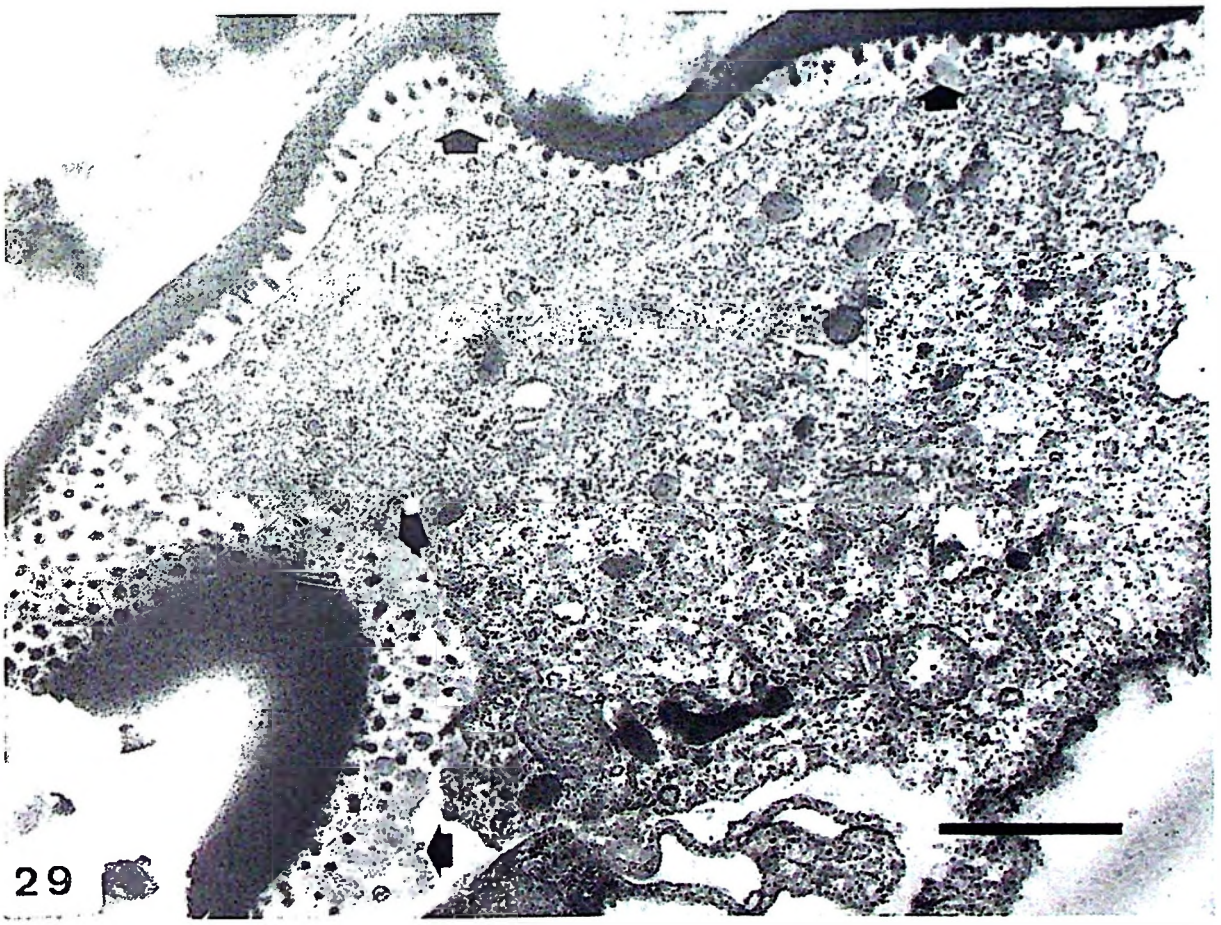
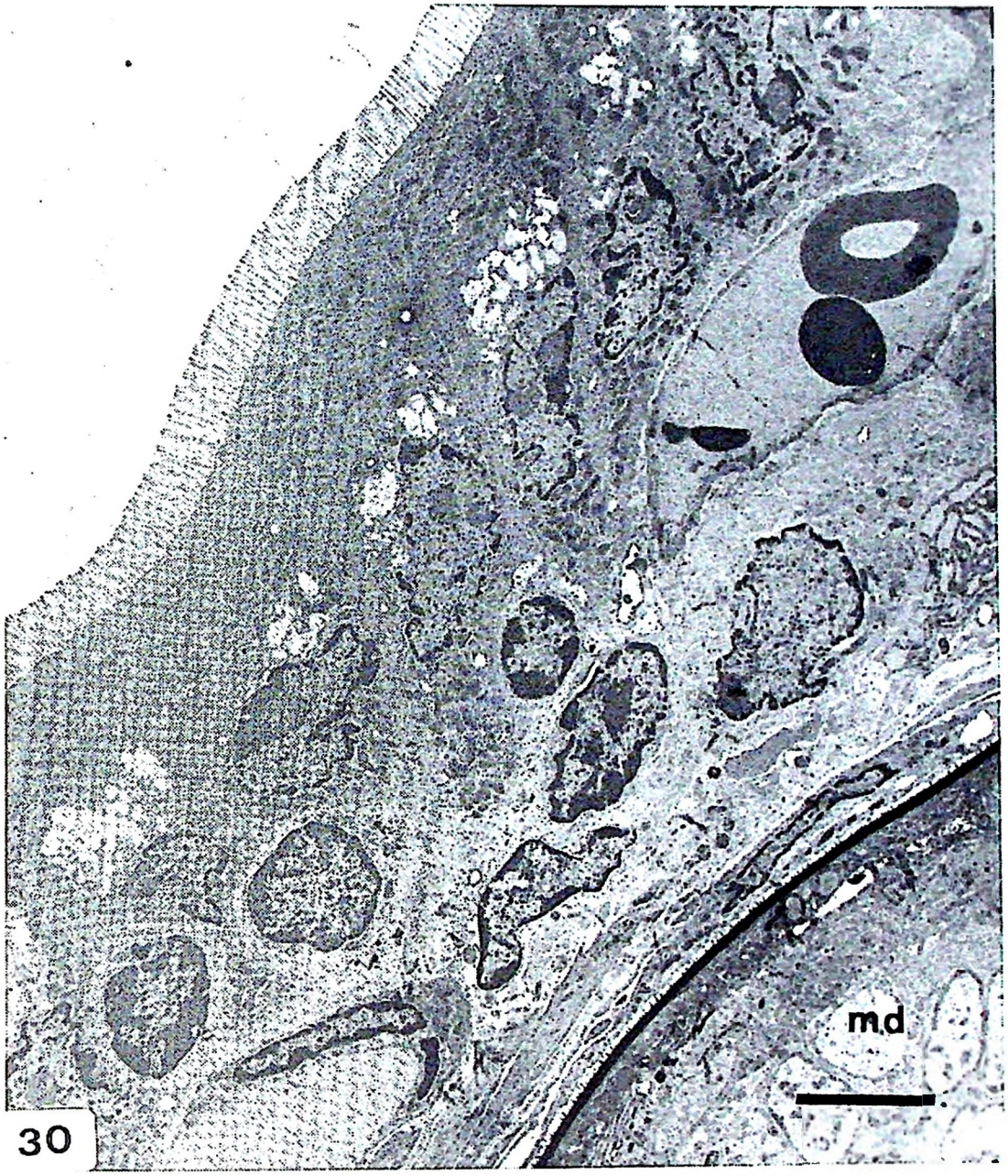


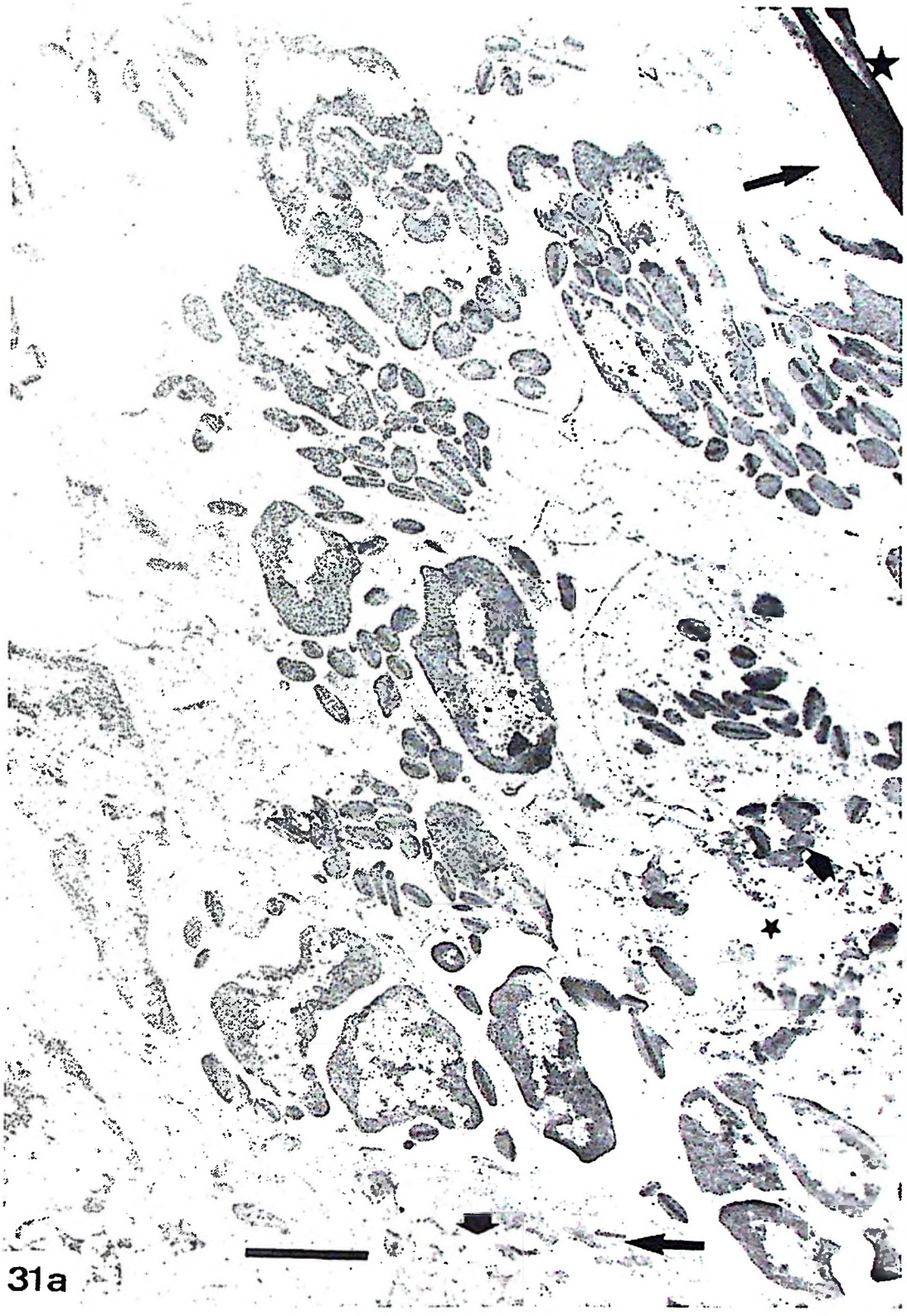
29

Fig. 30. A subepithelial egg which is roughly parallel to the epithelium. The egg has an intermediate mature miracidium (md). There is minimal cellular reaction and cytopathological changes. Small intestine. Hamster. Bar = 5μm.



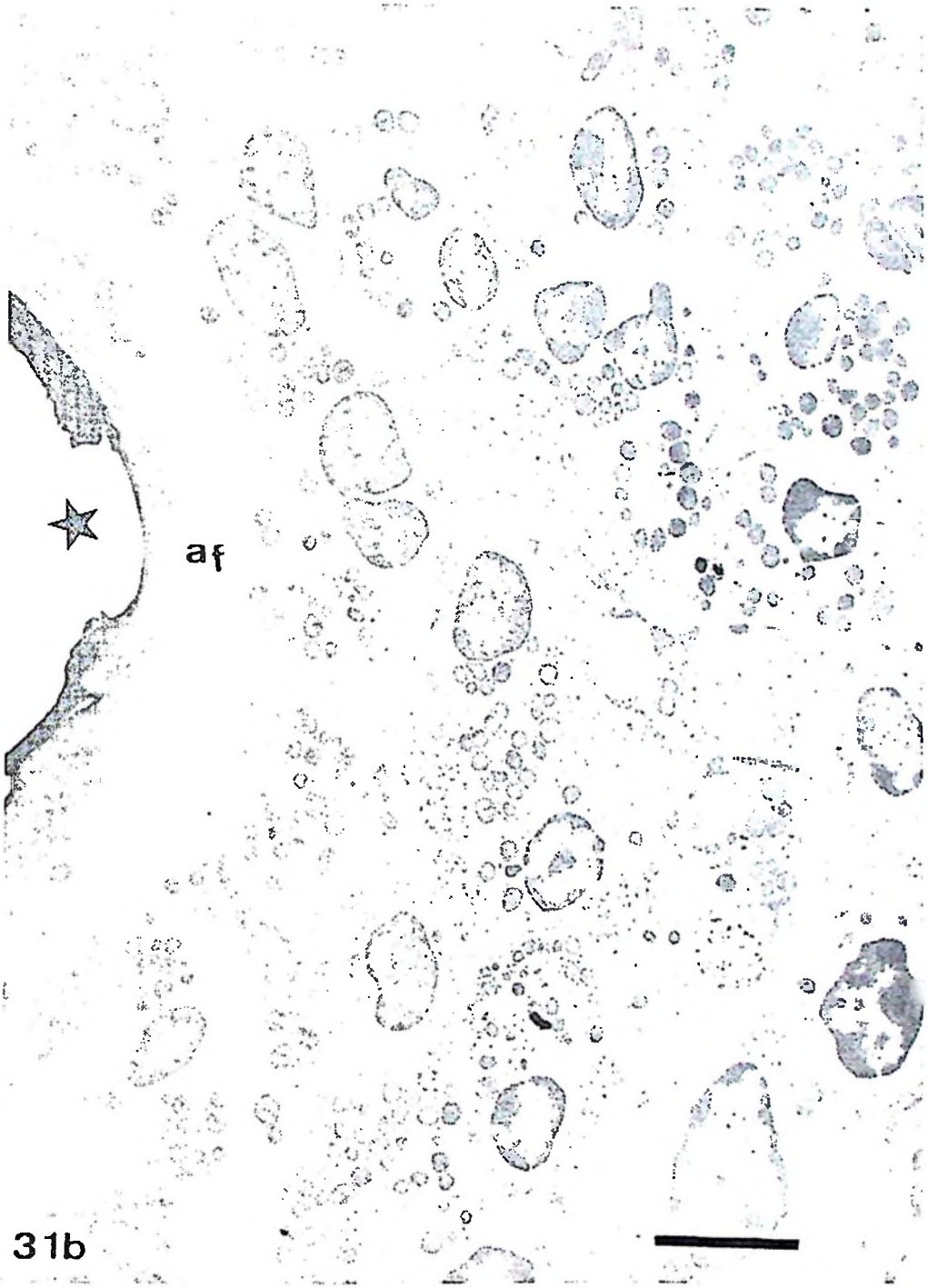
30

Fig. 31. (a) Accumulation of mostly mature eosinophils. Some of the eosinophils around the egg (large star) have granules with low density (long arrow), or are empty (thick short arrow). Some eosinophils have rarefied cytoplasmic matrix (small star). Nongranulomatous/early granulomatous lesion. Small intestine. Hamster. Bar = 2μm.



31a

Fig.. 31. (b) An almost pure population of mature eosinophils around an egg. Unlike those in hamsters, calf eosinophils have no crytalloid bar. All cells are closely approximated but there are no interdigitations. Nongranulomatous/Early granulomatous lesion. af = Artefactual separation. Large star = Egg area. Small Intestine. Calf. Bar = 5μm.



31b

Fig. 32. Egg shell remains in a granuloma. The structure and composition is similar to those of viable eggs. The shell is surrounded by epithelioid cells on the inner (in) and outer aspects (ot). Colon. Hamster. Bar = 1μm.

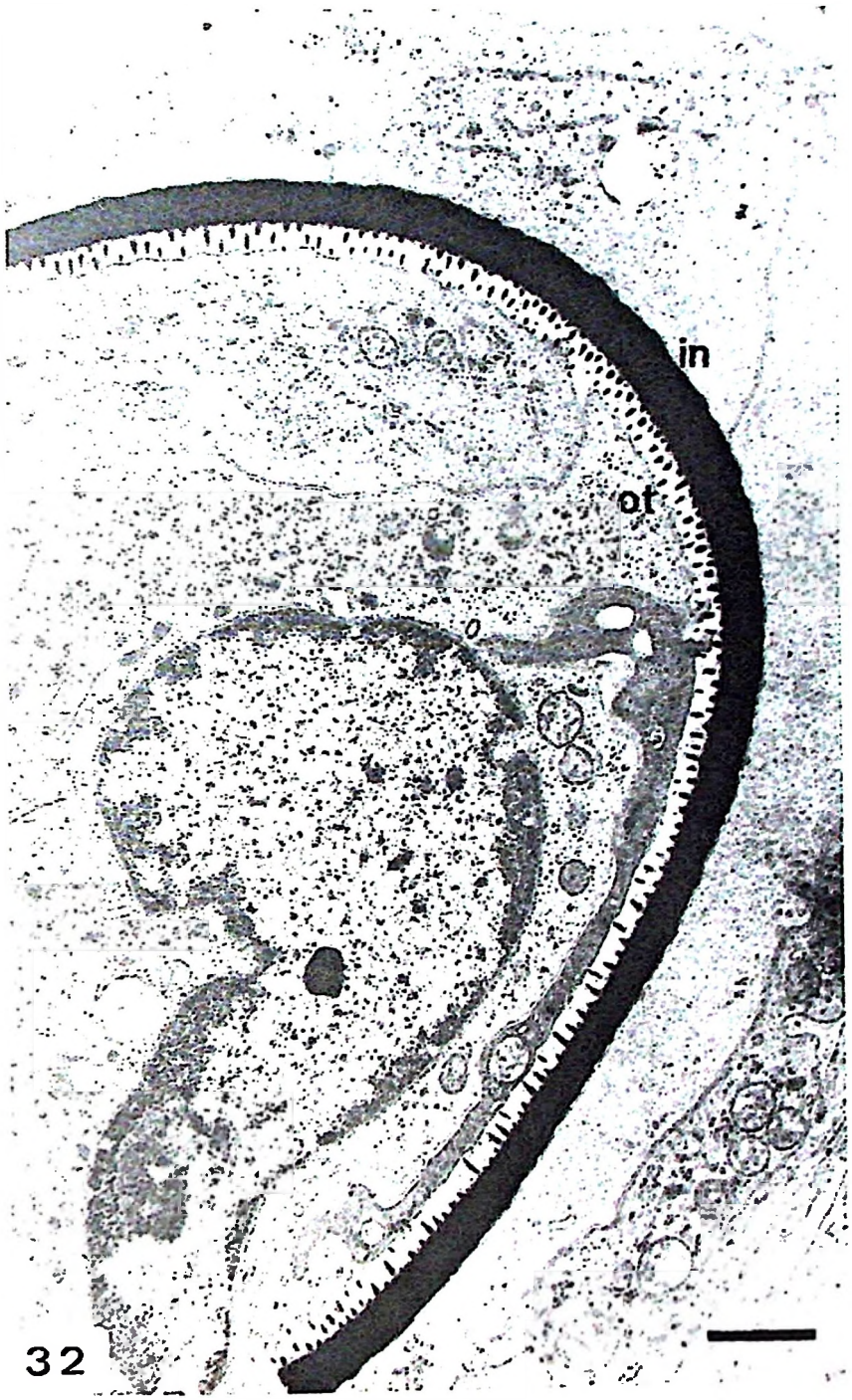

in
of
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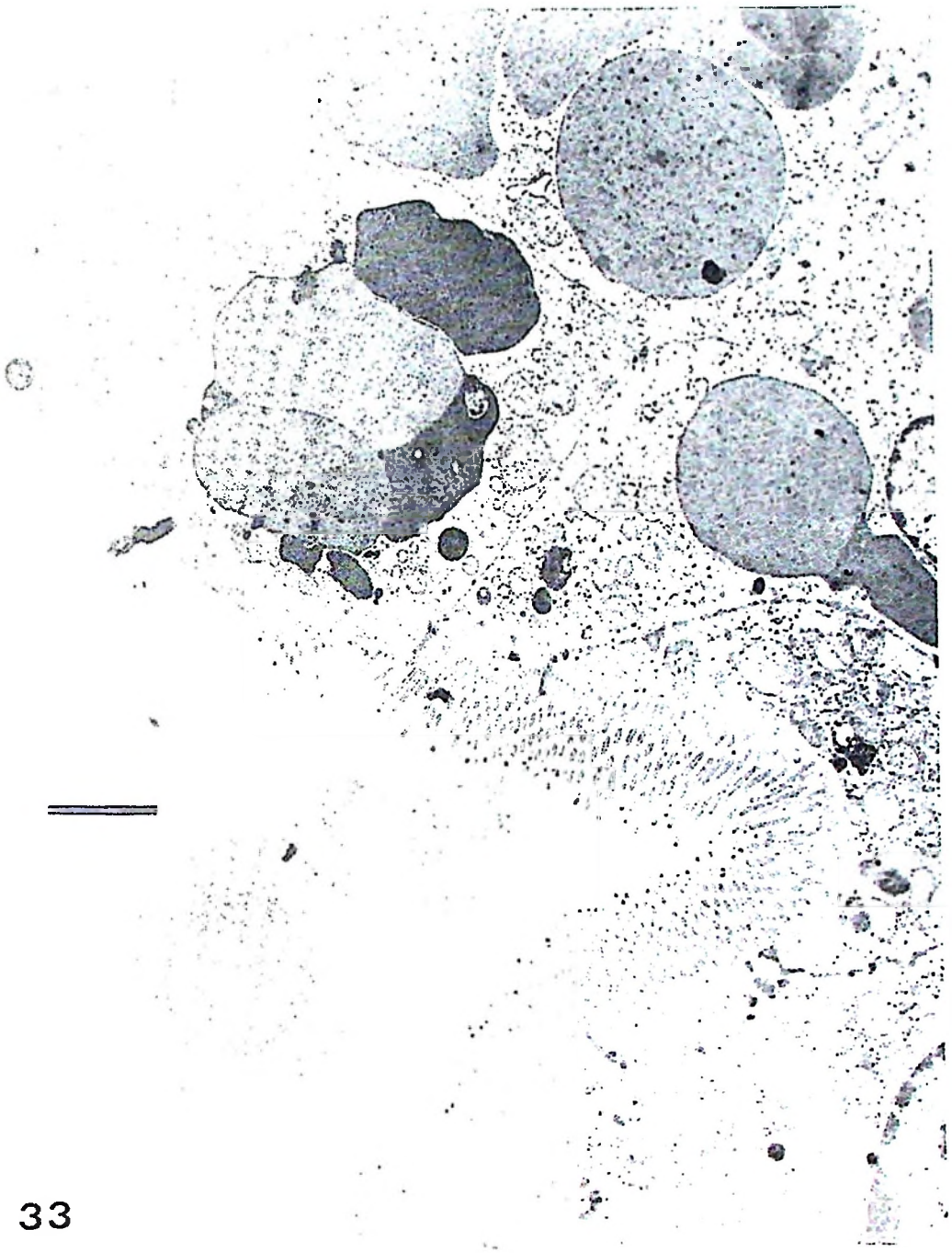
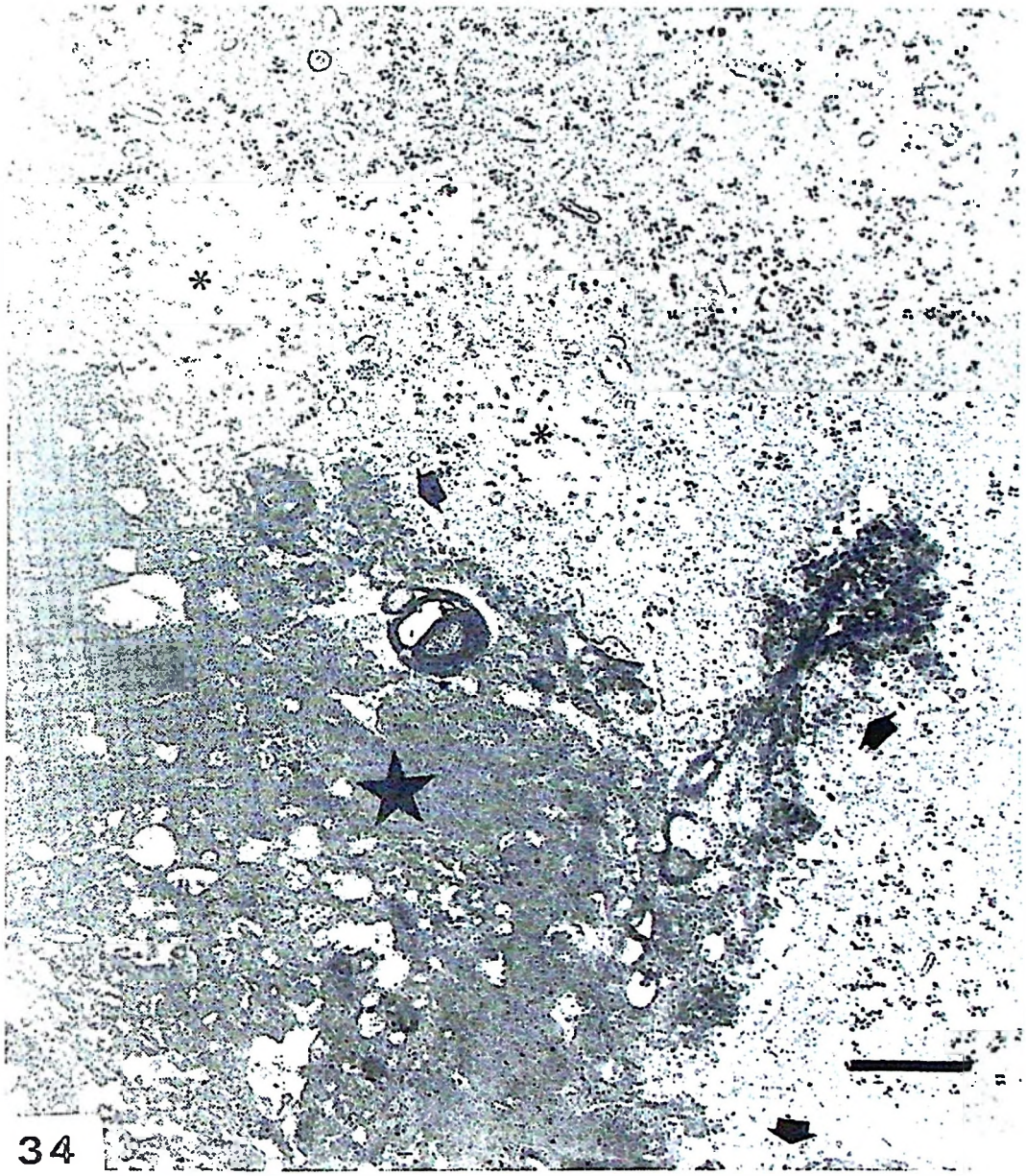
Fig. 33. Damaged enterocytes. Note transcellular migration of erythrocytes and their degeneration. There is enterocyte disintegration with loss of microvilli. Small intestine. Hamster. Bar = 2μm.



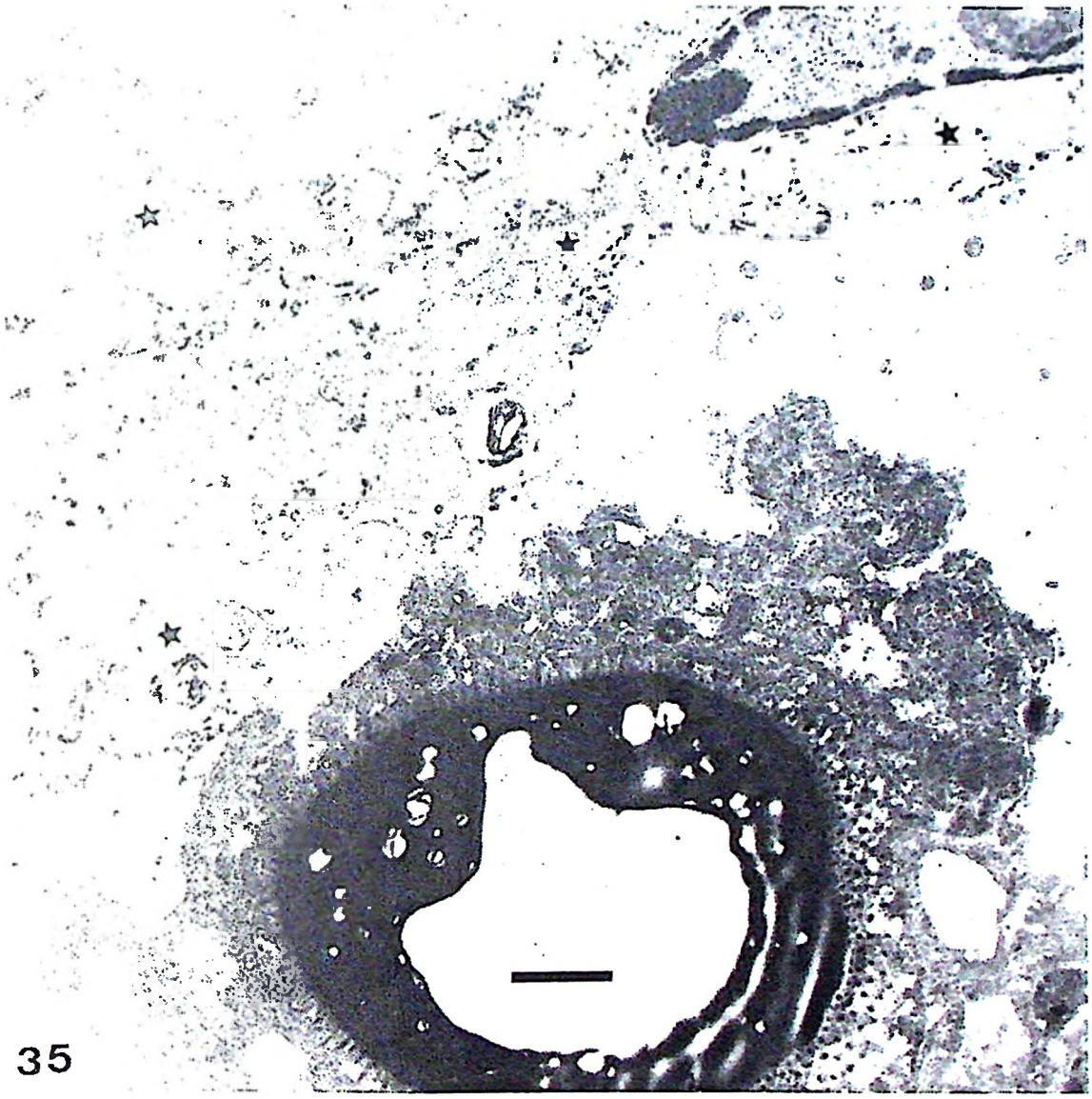
33

Fig. 34. The interface between the highly dense heterogenous material around the egg and the surrounding host cells has vesicles or globules (arrows) whose density is almost similar to that of the cytoplasm of host cells. There is cytoplasmic matrix rarefaction and clumping (asterisk). Small intestine. Calf. Bar = 500nm.



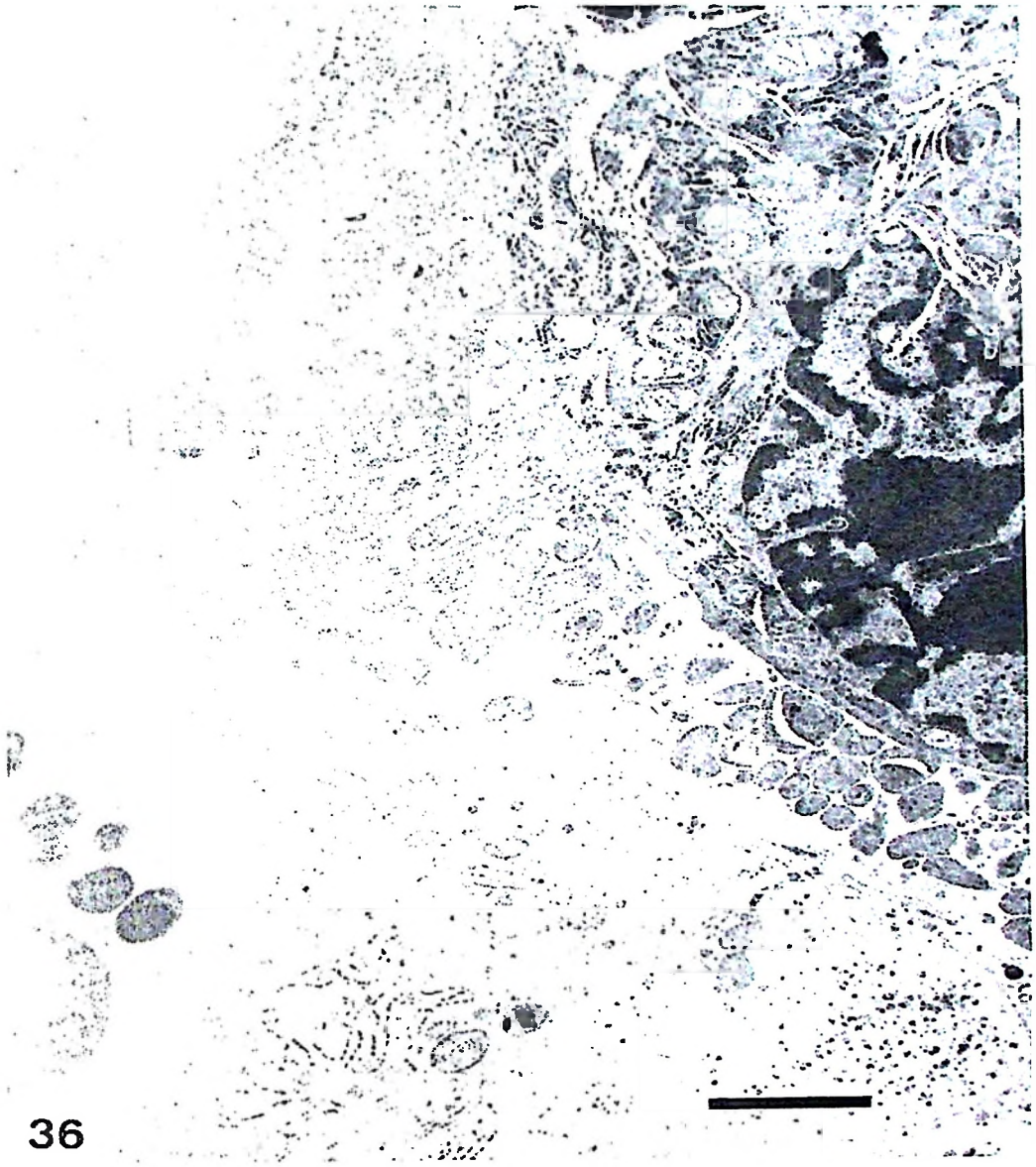
34

Fig. 35. An egg with highly dense heterogenous material around it. The egg shell is particularly thick at this level (narrow end). Cells surrounding the highly dense material have pronounced organelle degradation (star). Small Intestine. Calf. Bar = 1μm.



35

Fig. 36. A giant cell adjacent to an egg in a granuloma. The cytoplasmic foldings of the giant cell are denser than those of other cells with which it interdigitates. Colon. Calf. Bar = 1μm.



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