Twenty yearling sheep were allotted to four groups, each containing five sheep of approximately equal weight. On experimental days 0, 1, 2, 3, and 4, each sheep in each group was given the following inoculations by stomach tube: five sheep in group I (Fasciola hepatica-exposed controls) were given approximately 120 F. hepatica metacercariae each day; five sheep in group II (Haemonchus contortus-exposed controls) were given approximately 10,000 H. contortus infective larvae (L_3) each day; five sheep in group III (F. hepatica and H. contortus-exposed principals) were given approximately 120 F. hepatica metacercariae and 10,000 H. contortus L_3 each day; five sheep in group IV (nonexposed controls) were given sham doses of tap water each day.
Each sheep was weighed at seven-day intervals until experimental day 203 and a fecal sample collected until day 105. Clinicopathologic changes in blood were analyzed in samples collected from each sheep at seven-day intervals. Sheep that died in groups I and III were necropsied; gross pathology was recorded, helminths recovered, and liver tissue taken for histopathologic examination. Two sheep in group I were killed and examined on experimental days 269 and 1,063.

Clinicopathologic and pathologic changes in *F. hepatica*-exposed sheep (group I) were characteristic of chronic fascioliasis. Eosinophilia, leukocytosis, hypoalbuminemia, and progressive macrocytic, normochromic anemia occurred before time of death. One sheep each died on experimental days 162, 168, and 265; 412, 414, and 318 mature *F. hepatica* were recovered in liver bile ducts. Fluke burdens in two sheep that were killed and necropsied on experimental days 269 and 1,063 were 383 and 28, respectively.

Resistance to *H. contortus* exposure was evident in group II sheep; four of five sheep acquired patent infections and two of these were negative for nematode eggs in feces on experimental day 70. Transitory mild eosinophilia and hypoalbuminemia, and normocytic, hypochromic anemia was found, but each sheep recovered naturally within 203 days.
Enhanced pathogenicity of simultaneous exposure to *F. hepatica* and *H. contortus* in sheep was demonstrated by death of three sheep in group III before experimental day 80. The two remaining sheep in group III died two months before two sheep in group I that harbored similar fluke burdens. A macrocytic, slightly hypochromic anemia was found in one sheep that died on experimental day 51; 253 immature *F. hepatica* and 28,000 *H. contortus* were recovered at necropsy. Slightly microcytic, hypochromic anemia, characteristic of *H. contortus* infection, was found in two sheep that died on experimental days 71 and 78. In one sheep, 238 *F. hepatica* and 25,300 *H. contortus* were recovered; the other sheep harbored 332 and 18,100 helminths, respectively. The two remaining sheep died on experimental days 190 and 201; anemia was macrocytic and normochromic, characteristic of chronic fascioliasis. Burdens were 346 and 326 mature *F. hepatica*; 12,600 and 27,600 *H. contortus* were recovered.

Establishment of both helminths and host response to *F. hepatica* infection appeared normal, but development of host resistance to *H. contortus* was inhibited by simultaneous *F. hepatica* infection. Eosinophilic response in sheep with the concurrent infections was less than that of sheep with *F. hepatica* infection alone.
Pathogenicity of Experimentally Induced Concurrent Infections of Fasciola hepatica and Haemonchus contortus in Sheep

by

Paul Joseph Alvin Presidente

A THESIS

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PATHOGENICITY OF EXPERIMENTALLY INDUCED
CONCURRENT INFECTIONS OF FASCIOLA
HEPATICA AND HAEMONCHUS
CONTORTUS IN SHEEP

INTRODUCTION

Pathogenesis and pathology of Fasciola hepatica in experimentally induced chronic infections in sheep have been investigated by several workers (Boray, 1967; Dow, Ross, and Todd, 1968; Furmaga and Gundlach, 1967a, b; Ross, Dow, and Todd, 1967; Rubaj and Furmaga, 1969; Sewell, Hammond, and Dinning, 1968; Sinclair, 1962, 1964; Symons and Boray, 1967, 1968). Naturally occurring epizootics of ovine fascioliasis have also been examined (Hjerpe et al., 1971; Reid et al., 1970; Ross, 1967a, b).

Considerable research on Haemonchus contortus infections in sheep has been done (Andrews, 1942; Charleston, 1965; Fourie, 1931; Malczewski, 1970; Silverman, Mansfield, and Scott, 1970; Stoll, 1943; Veglia, 1915). Clinicopathologic changes in sheep with mixed gastrointestinal nematode burdens have been extensively investigated (Baker and Douglas, 1966; Baker et al., 1952; Campbell and Gardiner, 1960; Holman and Pattison, 1941; Kuttler and Marble, 1960; Shumard, Bolin, and Eveleth, 1957; Whitlock, 1950; Wilson and Turner, 1965). Enhanced pathogenicity was reported when lambs were simultaneously exposed to H. contortus and Nematodirus
spathiger; Nematodirus infection was prolonged in these infections (Kates and Turner, 1960; Turner and Colglazier, 1954). Previous massive exposure to H. contortus larvae caused reduction in numbers recovered and retardation in maturation rate of Nematodirus batus (Mapes and Coop, 1970). Competitive exclusion of H. contortus by simultaneous exposure to Trichostrongylus axei was reported (Turner, Kates, and Wilson, 1962).

Clinicopathology in young calves with concurrent infections of F. hepatica and Ostertagia ostertagi was recently reported (Reid et al., 1967). Pathogenesis of simultaneous F. hepatica and Haemonchus infections in sheep or cattle has not been studied.

The purpose in the present study was to investigate clinicopathologic changes in sheep after simultaneous exposure to F. hepatica and H. contortus.
Since the life cycle of *F. hepatica* was independently described by Leuckart and Thomas in 1883, the common liver fluke has been intensively investigated. Early experiments with *F. hepatica* were reviewed by Gordon (1955) and Dawes and Hughes (1964). Comprehensive literature reviews on the biology of *F. hepatica* were provided by Taylor (1964) and Pantelouris (1965). Pathogenesis of liver flukes in domestic and laboratory animals has been summarized by Sewell (1966) and Sinclair (1967a). Boray (1969) compiled a review of data obtained from experimentally induced infections in sheep and cattle in Australia. Recent literature on fascioliasis in cattle and anemia in ovine infections was discussed by Dawes and Hughes (1970). A comprehensive description of clinical symptoms and pathogenesis of human fascioliasis was recently provided by Náquira-Vildoso and Marcial-Rojas (1971).

Due to considerable variation in host response to *F. hepatica* (Dawes and Hughes, 1964; Dow, Ross, and Todd, 1968; Sinclair, 1969), this review is restricted to a discussion of current knowledge of acute, subacute, and chronic fascioliasis in sheep.

**Acute Ovine Fascioliasis**

Recent experiments by several workers (Boray, 1967; Pullan,
Sewell, and Hammond, 1970; Roberts, 1968; Ross, Dow, and Todd, 1967) have clarified the pathogenesis of this disease in sheep. In the acute disease, death occurred 42 to 62 days after exposure to 4,000 to 12,000 *F. hepatica* metacercariae. Before time of death, symptoms included inappetance, marked weight loss, emaciation, constipation, severe ascites, and anemia.

Clinicopathologic changes observed were normocytic or macrocytic, normochromic anemia beginning five to six weeks after exposure. Increased erythropoiesis was indicated by appearance in peripheral blood of erythrocytes showing anisocytosis, polychromasia, and basophilic stippling; hyperplasia of the red bone marrow occurred. Symons and Boray (1967, 1968) used $^{59}$Fe-labeled plasma to demonstrate rapid movement of iron from bone marrow to circulating erythrocytes in sheep six and eight weeks after exposure. Appearance of poikilocytes in circulating blood before time of death suggested exhaustion of hematopoietic tissues. The major change in leukocyte differential counts was a marked eosinophilia that began two weeks after exposure; Roberts (1968) reported that neutrophilia occurred before death. Hepatic dysfunction was indicated by retarded bromsulphthalien (BSP) clearance (Roberts, 1968), elevated serum glutamic-oxaloacetic transaminase (SGOT) and glutamic dehydrogenase (SGD) levels (Pullan, Sewell, and Hammond, 1970; Sewell, 1967a), icterus of the plasma (Symons and Boray, 1968),
and hypoalbuminemia. Serum total protein levels increased within two weeks after exposure; hypergammaglobulinemia was primarily involved (Pullan, Sewell, and Hammond, 1970; Sewell, 1970).

Postmortem examination revealed severe ascites with moderate to large quantities of blood; the carcass was wet and anemic. Rupture of the liver capsule with hemorrhage into the peritoneal cavity was given as cause of death (Boray, 1967). A generalized, fibrinous peritonitis and perihepatitis were evident. The liver was enlarged, congested, and soft; numerous subcapsular hemorrhagic foci were observed. Histopathologic changes in parenchyma associated with migration pathways of immature flukes were described by Dow, Ross, and Todd (1968). Dissection of liver parenchyma revealed 1,000 to 3,300 immature *F. hepatica*. Growth rate of these flukes was retarded and only a few had entered the bile ducts by time of death.

**Subacute Ovine Fascioliasis**

This disease differs from acute fascioliasis in time that death occurs, number of flukes that become established, and their stage of maturation. Death occurred 56 to 110 days after exposure; a majority of the 500 to 2,000 flukes were recovered in bile ducts (Pullan, Sewell, and Hammond, 1970; Ross, Dow, and Todd, 1967).
Progressive weight loss and anemia began five weeks after exposure. Liver function was improved and eosinophilia less marked than was found during the acute stage.

At necropsy of the sheep, only small amounts of peritoneal fluid were noted and fibrinous peritonitis was less evident. The liver surface was uneven, firm, and biliary cirrhosis had begun (Soulsby, 1965). The reparative tissue reaction prevented fluke entry into bile ducts and extended the migratory period in liver parenchyma (Boray, 1967). Increased size of flukes intensified the mechanical damage causing increased hemorrhage.

In a study of naturally acquired F. hepatica infections in lambs, Reid et al. (1970) reported development of a severe macrocytic, hypochromic anemia. A marked reticulocytosis (8 to 30%) was observed in lambs when packed cell volume (PCV) dropped below 25%. Similar findings were reported by Hjerpe et al. (1971) in an investigation of an epizootic in California among a flock of ewes. Clinicopathologic changes associated with the hypochromic anemia included reductions in serum iron content, bone marrow hemosiderin reserves, and mean corpuscular hemoglobin concentration (MCHC).

**Chronic Ovine Fascioliasis**

This disease has been investigated by workers who examined sheep with experimentally induced infections (Boray, 1967; Furmaga
and Gundlach, 1967a, b; Ibrović and Gall-Palla, 1959; Ross, Dow, and Todd, 1967; Sinclair, 1962, 1964), and those that acquired
*F. hepatica* by natural exposure (Ross, 1967a, b). The chronic
disease resulted from exposure to 100 to 2,000 *F. hepatica*
metacercariae and establishment of 100 to 1,000 flukes in bile ducts.
Time of death correlated with fluke burden, and sheep began dying
16 weeks after exposure. Durbin (1952) reported that in one sheep
a patent infection lasted 11 years; 15 *F. hepatica* were observed
from the bile ducts. Economic losses due to chronic fascioliasis
were discussed by Gordon (1955), and Roseby (1970) demonstrated
a deleterious effect on wool production. Symptoms of chronic
fascioliasis began 8 to 14 weeks after exposure, and included
increased thirst, a progressive anemia, loss of weight and condition;
intermandibular edema and ascites were observed. The anemia
that developed was severe, and PCV was as low as 4% at time of
death (Boray, 1967). This anemia was normocytic or slightly
macrocytic, and normochromic (Furmaga and Gundlach, 1967a;
Compensatory erythropoiesis was indicated by anisocytosis,
basophilic stippling, and poikilocytosis in circulating erythrocytes
(Sewell, Hammond, and Dinning, 1968), red bone marrow hyper-
plasia (Sewell, Hammond, and Dinning, 1968; Sinclair, 1964), and
increased rate of plasma iron clearance and incorporation into
circulating erythrocytes (Sinclair, 1964; Symons and Boray, 1967, 1968). Sinclair (1965) observed a reduction in hemosiderin reserves in bone marrow beginning 87 days postexposure and decreased serum iron concentration at 147 days. A marked decrease in erythrocyte count the following week indicated iron deficiency was the limiting factor in erythropoiesis. Symons and Boray (1967, 1968) used \textsuperscript{59}Fe-labeled plasma to demonstrate that newly produced erythrocytes had a six-day half-life in peripheral circulation. Labeled iron was recovered in bile and feces of infected sheep. Blood loss in feces correlated with degree of anemia observed when \textsuperscript{51}Cr-labeled erythrocytes were injected into infected sheep (Holmes et al., 1967; Sewell, Hammond, and Dinning, 1968). Sewell (1967b) estimated the daily blood loss as 0.5 to 1.0 ml. per fluke. Intestinal re-absorption of iron lost through bile ducts was investigated by simultaneous labeling of erythrocytes with \textsuperscript{51}Cr and \textsuperscript{59}Fe (Holmes and MacLean, 1969). When anemia was hypochromic and host iron reserves were depleted, some reabsorption of labeled iron occurred.

High radioactivity was recorded for expelled ingesta of \textit{F. hepatica} recovered from sheep previously injected with erythrocytes labeled with \textsuperscript{51}Cr or \textsuperscript{59}Fe (Pearson, 1963; Symons and Boray, 1967, 1968). The ingesta contained hematin (Stephenson, 1947; Todd and Ross, 1966), and acetylcholinesterase activity characteristic of host erythrocytes (Frady and Knapp, 1967); the majority
of workers have concluded that mature *F. hepatica* were almost entirely hematophagic. Dawes (1963) and Dawes and Hughes (1964, 1970) criticized some of this work because direct feeding on blood by flukes was physically impossible; the hyperplastic bile duct epithelium separated the fluke from a blood source.

During the chronic stage of fascioliasis, serum total protein decreased because a marked hypoalbuminemia offset the hyperglobulinemia that occurred during the acute stage (Furmaga and Gundzach, 1967b; Ibrović and Gall-Palla, 1959; Sinclair, 1962). Holmes et al. (1968) used $^{131}$I-labeled albumin in infected sheep to demonstrate a hypercatabolism of albumin; the shortened half-life was associated with a loss of albumin into the intestinal tract via the bile. They found similar loss of gammaglobulin when $^{125}$I-labeled globulin-7s was used. Effect of corticosteroid administration on serum protein in infected sheep was examined by Sinclair (1967b). Hyperglobulinemia did not occur and hypoalbuminemia began earlier and was more marked compared to changes in infected, nonmedicated controls.

Serum calcium levels decreased during the chronic stage of infection; nonsignificant changes in serum magnesium, phosphorous and potassium were recorded (Pinkiewitz and Madej, 1967; Sinclair, 1960). Increased serum alkaline phosphatase activity was reported (Hjerpe et al., 1971; Pinkiewitz and Madej, 1967).
Postmortem examination during chronic infection revealed that liver parenchyma was fibrotic and firm, especially the left lobe; main bile ducts were prominent, thickened, and fibrous (Rubaj and Furmaga, 1969); Soulsby, 1965). Histopathologic changes in liver at chronic stage were described by Dow, Ross and Todd (1968) and Rubaj and Furmaga (1969).

Ovine Haemonchosis

Pathogenesis and pathology of *H. contortus* infection in sheep were reviewed recently by Soulsby (1965). Symptoms associated with this infection were principally those caused by anemia. Gordon (1950) reported the following: lack of stamina, pale mucus membranes, intermandibular edema, constipation with hard, dark feces; milk yield was reduced in lactating ewes. Variation in response to exposure was primarily related to condition of the host. Factors that affected outcome of exposure to *H. contortus* larvae included: age of the sheep (Christie, 1970; Lucker, 1952; Poeschel and Todd, 1969; Silverman, Mansfield, and Scott, 1970); host size, weight, and hematologic status (Gordon, 1950); diet of the sheep (Kates, Allen, and Wilson, 1962; Poeschel and Todd, 1969; Shumard et al., 1956; Wier et al., 1948); the hemoglobin type of the sheep (Evans, Blunt, and Southcott, 1963); and number, dosing schedule, and strain of infective larvae used (Conway and Whitlock, 1965; Dineen
et al., 1965; Knapp, 1964a, b). Lambs over eight months of age compensated for blood loss by moderate worm burdens and recovered within 90 days after exposure (Lucker, 1952).

The primary clinicopathologic change in ovine haemonchosis was a marked anemia. Blood was observed in feces beginning 6 to 10 days after exposure; gastric hemorrhage was caused by 4th-stage larvae (Andrews, 1942; Brambell, Charleston, and Tothill, 1964; Clark, Kiesel, and Goby, 1962; Stoll, 1943; Veglia, 1915). A rapid decline in hemoglobin concentration and PCV began six days after exposure; PCV was 6% at time of death 19 to 24 days post-exposure (Lucker, 1952). Blood loss was greater when H. contortus was mature. Brambell, Charleston, and Tothill (1964) used $^{51}$Cr-labeled erythrocytes to demonstrate that 30 ml. of blood was lost in feces eight days after exposure; 500 ml. was lost in feces of one lamb on postexposure day 23. Andrews (1942) measured blood loss in feces of infected lambs; one lamb lost 2,380 ml. in a 10-day period. Blood loss was due to capillary hemorrhage from vacated attachment sites, and to ingestion by the mature worms (Andrews, 1942; Boughton and Hardy, 1935; Fourie, 1931; Veglia, 1915). Martin and Clunies Ross (1934) estimated that for egg production alone, 2,000 female worms required 29 ml. of blood each day. Clark, Kiesel, and Goby (1962) used erythrocytes labeled with $^{51}$Cr alone or in combination with $^{59}$Fe to estimate
mean blood loss as 0.049 ml. per worm per day.

Normocytic or macrocytic, normochromic or microcytic, hypochromic anemia were described in haemonchosis depending on stage of infection (Baker and Douglas, 1966; Fourie, 1931). Increased erythropoiesis was indicated by compensatory myeloid hyperplasia, and anisocytosis, polychromasia, and basophilic stippling in circulating erythrocytes (Baker and Douglas, 1966; Fourie, 1931; Schalm, 1965). Exhaustion of hematopoietic tissues was indicated when poikilocytes and hypochromatic cells appeared in peripheral blood; decreased mean corpuscular volume (MCV) and MCHC suggested from deficiency (Baker and Douglas, 1966; Fourie, 1931; Schalm, 1965; Silverman, Mansfield, and Scott, 1970).

Changes observed in leukocyte total and differential counts during haemonchosis were leukocytosis (Andrews, 1942), a relative lymphocytosis (Silverman, Mansfield, and Scott, 1970), and a relative neutrophilia at death (Andrews, 1942). A marked eosinophilia was recorded 6 to 14 days after exposure, especially if larvae were given in daily doses (Charleston, 1965; Malczewski, 1970). Fourie (1931) and Andrews (1942) reported increased eosinophil counts during the recovery phase of infection.

In sheep with mixed gastrointestinal nematode burdens (including *H. contortus*), hyperglobulinemia and hypoalbuminemia have
been reported (Kuttler and Marble, 1960; Shumard, Bolin, and Eveleth, 1957).

Pathogenesis and pathology of haemonchosis have been studied by several workers (Andrews, 1942; Charleston, 1965; Malczewski, 1970; Stoll, 1943; Veglia, 1915). Migration of larvae to the region of abomasal lamina propria to molt to 4th-stage caused an intense lymphoid infiltration into the area (Charleston, 1965; Malczewski, 1970; Stoll, 1943). Migration of 4th-stage larvae to mucosal surface caused extensive damage to gastric glands and the mucosae; pH increased (Christie, 1970; Christie, Brambell, and Mapes, 1967; Malczewski, 1970), and a marked eosinophilic infiltration into the mucosae occurred (Charleston, 1965; Malczewski, 1970). Lesions were primarily restricted to the mucosae; excessive sloughing and mucus secretion occurred. Hypertrophy of the mucus gland portion occurred and numerous petechial hemorrhages associated with former attachment sites of worms were evident.

**Phlebotomy vs Helminth-Caused Anemias**

Anemia in both haemonchosis and fascioliasis in sheep is apparently posthemorrhagic in nature, and several investigators have attempted to duplicate the condition in infected sheep by repeated phlebotomy. Todd and Ross (1968) reported a macrocytic, normochromic anemia with marginal hypochromasia
occurred in five sheep after removal of 300 ml. of blood for six consecutive days. Significant changes in leukocyte total and differential counts did not occur. Fourie (1931), Andrews (1942), and Richard et al. (1954) found changes in peripheral blood were similar in sheep infected with *H. contortus* to those observed in phlebotomized sheep. Charleston (1964) studied bone marrow changes in *H. contortus*-infected sheep and in phlebotomized sheep. He concluded that resulting anemias were not identical; interference in normoblast maturation and hemoglobinization process in infected sheep was indicated. Also, a slight increase in eosinophil counts in infected sheep was not recorded for sheep that were artificially bled.

Kuttler and Marble (1960) reported significant differences in degree of hypoalbuminemia and hyperalphaglobulinemia observed in sheep with mixed gastrointestinal nematode burdens (including *H. contortus*) when compared to that found in phlebotomized sheep.

Sinclair (1964, 1965) attempted to duplicate the anemia in chronic fascioliasis by repeated phlebotomy. Because of observed differences between infected sheep and phlebotomized sheep, he concluded that a dysfunction of the reticuloendothelial system was the primary cause of anemia. This was characterized by increased erythrocyte destruction and decreased effective erythrocyte production. Other workers (Sewell, Hammond, and Dinning, 1968;
Symons and Boray, 1968, disagreed with this conclusion because they found erythropoiesis was increased, possibly to a maximum. Sinclair investigated the role of the reticuloendothelial system in sheep with experimentally induced *F. hepatica* infections. Effect of corticosteroid administration (Sinclair, 1968) and splenectomy (Sinclair, 1970a) on the course of primary infection, and corticosteroid administration on secondary infection (Sinclair, 1970b) were investigated. He concluded that hyperactivity of the reticuloendothelial system was an integral part of the resistance mechanism to *F. hepatica* infection in sheep. Interference with this system resulted in more severe disease because the normal cellular reaction in the liver was suppressed. Sinclair (1970b) then concluded that the anemia in fascioliasis was primarily hemorrhagic in origin.

Clinicopathologic changes associated with conditions discussed are summarized as follows:
<table>
<thead>
<tr>
<th></th>
<th>Fascioliasis</th>
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<tr>
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<td>+, +</td>
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<tr>
<td>Alkaline phosphatase</td>
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<td>(elevated)</td>
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</table>

NC means no change; + slight change; ++ moderate change; +++ severe change.
MATERIALS AND METHODS

Animals

Twenty sheep, of mixed breeding and 12 to 18 months old, were housed indoors since time of birth in pens with concrete floors. Fecal samples collected from each sheep before the experiment began indicated small numbers of nematode eggs in feces of four sheep; none were found in remaining sheep. The sheep were given a pelleted ration consisting of 62% grass hay, 21% alfalfa, 12% rolled barley, and 5% molasses.

Inoculum

Metacercariae

_Fasciola hepatica_ metacercariae were collected from _Lymnaea columella_, a suitable intermediate host, that was artificially exposed to miracidia hatched from ova. Fluka ova were removed from gall-bladder and liver bile ducts of sheep naturally infected with _F. hepatica_. The number of viable metacercariae was determined by dilution count, and 50 doses containing approximately 120 cysts were drawn from the total collection. The inoculum of 600 metacercariae was given to exposed sheep by stomach tube in five equal doses given on consecutive days.
Nematode Larvae

*Haemonchus contortus* infective larvae (L₃) were recovered from moist, fecal cultures after 27°C. incubation for six days. Nematode eggs were recovered in feces from lambs with experimentally induced *H. contortus* infections. The Kentucky strain of *H. contortus* was used; an isolate was obtained in 1960 and maintained here by serial passage in susceptible lambs. The number of infective larvae was determined by dilution count, and 50 doses containing approximately 10,000 L₃ were drawn from the total collection. The inoculum of 50,000 L₃ was given to exposed sheep by stomach tube in five equal doses given on consecutive days.

**Experimental Design**

On May 1, 1969, the sheep were allotted by body weight to four groups of five sheep each by the method of Gardiner and Wehr (1950). Beginning on May 6 (experimental day 0), and on days 1, 2, 3, and 4, each sheep in the groups was given the following inoculations:

- five *F. hepatica*-exposed controls (group I) were given 120 metacercariae each day;
- five *H. contortus*-exposed controls (group II) were given 10,000 L₃ each day;
- five *F. hepatica* and *H. contortus*-exposed principals (group III) were given 120 metacercariae and then 10,000 L₃ each day;
- five nonexposed controls (group IV) were
given sham doses of water each day. Each group was kept in separate pens with concrete floors, and each sheep was weighed at seven-day intervals until time of death, or until experimental day 203.

**Helminth Egg Counts**

Fecal samples were collected every seven days from each sheep from experimental day 0 until day 105. Presence of *F. hepatica* ova in these samples was demonstrated by sedimentation. Quantitative estimate of nematode egg output in feces was done by the modified McMaster technique; counts were expressed as number of eggs per gram of feces (e.p.g.).

**Hematologic and Serologic Procedures**

Blood samples were collected at seven-day intervals from the jugular vein of each sheep until time of death, or until experimental day 203. Whole blood (five ml.) was collected for serum separation, and five ml. was collected in ethylenediaminetetraacetic acid. Blood smears were prepared and stained with Wright's solution for examination of erythrocytes and making leukocyte differential counts. Packed cell volume (PCV) percentage was determined by microhematocrit. For the first 19 sets of blood samples, total erythrocyte and leukocyte counts were made; hemoglobin concentration was
determined by the biuret method. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were calculated by methods given by Schalm (1965). Serum samples were frozen and albumin concentration was subsequently determined by the biuret method.

Necropsy Procedure

Eight sheep that died and two sheep that were killed were examined for gross pathologic changes; liver tissue was taken for histologic preparation. Sectioned tissues were stained in hematoxylin and eosin for histopathologic examination. Liver from each sheep was collected; gallbladder contents were poured into a beaker and the sediment was examined for fluke ova. Bile ducts were opened and examined for mature flukes. Each liver was then cut in thin strips, placed in a tray, and soaked in warm tap water for approximately two hours. All tissue was examined and washed over a sieve (1.4-mm. openings) before being discarded. Contents in the bottom of the tray were poured over the sieve to recover flukes that emerged from hepatic tissue. Flukes recovered from each liver were placed in petri dishes at 4°C. and allowed to relax overnight. The number of recovered flukes was counted and average length determined by measurement of a 10% random sample. Whole flukes were flattened between glass plates and their lengths measured.
The abomasum was collected from each sheep in group III. Contents and adherent worms were recovered by rubbing the mucosae with the fingertips and washing over a sieve (0.147-mm. openings); material on the sieve was collected and fixed in formalin. Abomasum contents were diluted to a volume of one L. and all worms in a five ml. aliquot were counted. Total *H. contortus* burden was estimated by multiplying the aliquot count by the dilution factor of 200.
RESULTS

**Body Weight Data**

On experimental day 0, mean body weight for group I sheep (*F. hepatica*-exposed controls) was 44.2 kg., for group II sheep (*H. contortus*-exposed controls), 43.1 kg., for group III sheep (*F. hepatica* and *H. contortus*-exposed principals), 42.7 kg., and for group IV sheep (nonexposed controls), 42.7 kg. Weekly mean body weight for sheep in the three control groups are shown (Figure 1); differences between groups were not significant (*P* > 0.05) at any time during the experiment. Each sheep in group IV gained an average 18.3 kg. (minimum and maximum, 15.2 and 23.9) by experimental day 203, and each sheep in group II, 16.3 kg (minimum and maximum, 11.6 and 18.9). Sheep 2 (34.1 kg. on experimental day 0) and sheep 4 (45.0 kg. on day 0) in group I died of chronic fascioliasis on experimental days 162 and 168; both sheep lost approximately 2.5 kg. in the 14-day period before they died (Figure 1). The other three group I sheep averaged 47.5 kg. on day 0, and gained an average 8.9 kg. by day 203. Sheep 1 died on experimental day 265; sheep 3 and 5 were killed and examined on days 269 and 1,063, respectively. These sheep lost or gained little weight in the period between experimental days 140 and 203. Sheep 3 weighed 51.8 kg. on
Figure 1. Mean body weights for 5 Fasciola hepatica-exposed sheep (group I; —), 5 Haemonchus contortus-exposed sheep (group II; ——), and 5 nonexposed sheep (group IV; o—o).
experimental day 203 and 50.0 kg. at necropsy on day 269. Sheep 5 subsequently gained weight and put on considerable fat; it weighed 93.6 kg. at necropsy.

Each sheep in group IV (F. hepatica and H. contortus-exposed principals) died between experimental days 51 and 201; weekly weights are given (Figure 2). Three sheep that died before day 80 showed progressive weight loss for the 28-day period before time of death; two sheep that died on days 190 and 201 lost or gained little weight in the 56-day period before they died.

Helminth Egg Counts

Ova of F. hepatica were recovered in feces from one sheep in each of groups I (F. hepatica-exposed controls) and III (F. hepatica and H. contortus-exposed principals) on experimental day 70. Each of five group I sheep and three of four group III sheep had patent infections on day 77. Each of eight F. hepatica-exposed sheep in groups I and III passed large numbers of fluke ova in feces until time of death or necropsy. Output of fluke ova in sheep 5 (group I) was 16.1 ova per gram of feces on experimental day 1,063. Fluke ova were not recovered in feces from sheep in groups II and IV on any sample day.

Haemonchus contortus egg counts for each sheep in group III (F. hepatica and H. contortus-exposed principals) and mean count
Figure 2. Body weights for each of 5 Fasciola hepatica and Haemonchus contortus-exposed sheep (group III).
for group II sheep (H. contortus-exposed controls) are shown (Figure 3). Four sheep in each of groups II and III had egg counts of 100 to 550 e.p.g. on experimental day 21. Sheep 12 (group III) passed 200 e.p.g. on day 28; none were found in feces from sheep 9 (group II) on any sample day. Mean egg output for group II sheep peaked at 2,790 e.p.g. (minimum and maximum, 150 and 6,300) on experimental day 42 (Figure 3). Three of five group II sheep had no eggs in feces on experimental day 70; output in feces of the other two sheep averaged 425 e.p.g. on day 154. Haemonchus egg counts in feces of three group III sheep peaked at 22,500 e.p.g. on experimental day 56; mean output was 14,000 e.p.g. (minimum and maximum, 1,650 and 30,000) for four sheep on day 70. Two surviving group III sheep averaged 4,850 e.p.g. on day 154.

**Clinicopathologic Findings**

**Erythrocytes**

For sheep in the three control groups, mean weekly values for PCV percentage (Figure 4), erythrocyte counts (Figure 6), and hemoglobin concentrations (Figure 8), are given. For the five principals (group III), mean values for these data for experimental days 0 to 49 are also shown. Individual data for each of five group III sheep are given for PCV (Figure 5), erythrocyte counts (Figure 7), and hemoglobin concentrations (Figure 9).
Figure 3. *Haemonchus contortus* fecal egg counts for each of 5 *Fasciola hepatica* and *H. contortus*-exposed sheep (group III), and mean counts for 5 *H. contortus*-exposed sheep (group II; □--□).
Figure 4. Mean packed cell volumes for 5 *Fasciola hepatica-* exposed sheep (group I; •—•), 5 *Haemonchus contortus*-exposed sheep (group II; ■—■), 5 *F. hepatica* and *H. contortus*-exposed sheep (group III, X—X), and 5 nonexposed sheep (group IV; o—o).
Figure 5. Packed cell volumes for each of 5 *Fasciola hepatica* and *Haemonchus contortus*-exposed sheep (group III).
Figure 6. Mean erythrocyte counts for 5 Fasciola hepatica-exposed sheep (group I; ●●●), 5 Haemonchus contortus-exposed sheep (group II, ■■■), 5 F. hepatica and H. contortus-exposed sheep (group III; X—X), and 5 nonexposed sheep (group IV; ○—○).
Figure 7. Erythrocyte counts for each of 5 Fasciola hepatica and Haemonchus contortus-exposed sheep (group III).
Figure 8. Mean hemoglobin concentrations for 5 Fasciola hepatica-exposed sheep (group I; •-•), 5 Haemonchus contortus-exposed sheep (group II; ■-■), 5 F. hepatica and H. contortus-exposed sheep (group III; X—X), and 5 nonexposed sheep (group IV; o—o).
Figure 9. Hemoglobin concentrations for each of 5 *Fasciola hepatica* and *Haemonchus contortus*-exposed sheep (group III).
Statistical analyses of these data indicated that mean PCV (25.6%) for group I sheep (*F. hepatica*-exposed controls) was significantly lower (P<0.01) than that for group IV sheep (nonexposed controls, 34.3%) on experimental day 70 (Figure 4). Significant reduction in PCV continued for the duration of the experiment; PCV was less than 9% for two sheep at time of death on experimental days 162 and 168. For the two sheep that were killed and examined, PCV was 15.5% on experimental day 169 and 38.0% on day 1,063. Mean erythrocyte count and hemoglobin concentration for group I sheep decreased after exposure; significant reduction (P<0.01) in erythrocyte count (7.06 x 10^6 /cmm.) was found on experimental day 77 (Figure 6), and for hemoglobin concentration (8.42 Gm./100 ml.) on day 70 (Figure 8).

For group II sheep (*H. contortus*-exposed controls), mean PCV (30.1%) was significantly lower (P<0.05) than that for nonexposed controls (36.5%) on experimental day 63 only (Figure 4). Reduction in erythrocyte count was not significant (P>0.05) on any sample day (Figure 6); mean hemoglobin concentration on days 63 (9.68 Gm./100 ml.) and 77 (10.52 Gm./100 ml.) was significantly lower (P<0.05) than that for nonexposed controls (12.20 and 12.58 Gm./100 ml., respectively) (Figure 8).

Significant reduction (P<0.01) in mean PCV for group III sheep (*F. hepatica* and *H. contortus*-exposed principals) began on
experimental day 28 (Figure 4) for erythrocyte count, on day 42 (Figure 6); for hemoglobin concentration, on day 35 (Figure 8). Before death occurred PCV for each group III sheep was 6.5% to 10.0% (Figure 5).

Significant changes in calculated Wintrobe indices are given (Table 1). These data indicate that a progressive macrocytic, normochromic to slight hypochromic anemia occurred in group I sheep. For group II sheep, a transitory normocytic, hypochromic anemia occurred. The following types of anemia were found in group III sheep before time of death: for sheep 13, anemia was macrocytic and hypochromic two days before it died; for sheep 15, normocytic and hypochromic one day before it died; for sheep 11, slightly microcytic and hypochromic eight days before it died; for sheep 12 and 14, macrocytic and normochromic approximately two months before they died (Table 1).

Pathologic changes in circulating erythrocytes for all sheep during the experiment are summarized as follow: Prevalence of anisocytosis, basophilic stippling, diffuse basophilia, Howell-Jolly bodies, and poikilocytosis intensified in three of five group I sheep (F. hepatica-exposed controls) after experimental day 77; for sheep 1 and 5, they appeared on day 126, but were not severe for sheep 5. Macrocytic and hypochromic erythrocytes were observed in blood smears from sheep 3 from experimental day 147 until necropsy on day 269. For group II sheep (H. contortus-exposed controls), these
Table 1. Mean Wintrobe Indices for Sheep in Group I (Fasciola hepatica-exposed Controls), Group II (Haemonchus contortus-exposed Controls), and Group IV (Nonexposed Controls). These Data Before Time of Death are Given for Each Sheep in Group III (F. hepatica and H. contortus-exposed Principals).

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* Difference from nonexposed control value was highly significant (P<0.01).

** Difference from nonexposed control value was significant (P<0.05).
abnormalities were found in four of five sheep on experimental day 35; maximum intensity occurred between days 63 and 77. They were not evident in blood smears from sheep 9 and 10 after day 77, sheep 6 after day 98, and sheep 7 and 8 after day 161. Pathologic changes were severe only in sheep 8. For group III sheep (F. hepatica and H. contortus-exposed principals) these abnormalities were first seen in sheep 13 on day 35, and in all sheep on day 42. Hypochromic erythrocytes were evident in sheep, 11, 13, and 15 before they died. Absence of basophilic stippling, diffuse basophilia, and Howell-Jolly bodies was noted in blood from sheep 12 and 14 on experimental days 84 and 91. Prevalence of all abnormalities intensified on day 98 and continued until time of death. For group IV sheep, erythrocytes appeared normal in all blood smears examined during the experiment.

**Leukocytes**

On experimental day 0, mean total leukocyte count for group I sheep was 8,840/cmm., for group II sheep, 9,840/cmm., for group III sheep, 10,480/cmm., and for group IV sheep, 9,160/cmm. Statistical analysis of leukocyte data indicated significant increase (P<0.05) in total leukocyte counts occurred among group I sheep from experimental day 35 to day 70; mean counts exceeded 11,980/cmm. (Figure 10). On experimental days 21 and 28, total leukocyte
Figure 10. Mean leukocyte total and eosinophil counts for 5 *Fasciola hepatica*-exposed sheep (group I; ——o), 5 *Haemonchus contortus*-exposed sheep (group II; ——■), 5 *F. hepatica* and *H. contortus*-exposed sheep (group III; X—X), and 5 nonexposed sheep (group IV; oo—oo).
counts for sheep 13 (group III) were 14,000 and 14,500/cmm., respectively; on days 42, 49 and 63, counts for sheep 14 exceeded 13,600/cmm. Changes in total leukocyte counts for other sheep in group III and for group II were not significant (P>0.05).

Analyses of leukocyte differential cell count data indicated significant increase (P<0.01) in eosinophil counts occurred in group I sheep (F. hepatica-exposed controls) from experimental day 14 to day 84, and began among group III sheep on day 21. Mean leukocyte total and eosinophil counts for sheep in the three control groups are given (Figure 10). For the five principals (group III), mean eosinophil counts for experimental days 0 to 49 are also shown (Figure 10); data for each of these sheep are given (Figure 11). Peak eosinophilia for sheep in groups I (3,476/cmm.) and III (2,723/cmm.) occurred on experimental day 42. A slight eosinophilic response among group II sheep (H. contortus-exposed controls) peaked on experimental day 21 (884/cmm.) and later, on day 49 (713/cmm.); these counts were not significantly different (P>0.05) from that for nonexposed controls on these days (281 and 191/cmm., respectively) (Figure 10). Decrease in eosinophil counts for sheep 11, 13, and 15 (group III) (Figure 11) was similar to that found among group II sheep (Figure 10). For sheep 12 and 14 (group III) (Figure 11), eosinophil counts paralleled that of group I sheep (Figure 10).
Figure 11. Eosinophil counts for each of 5 Fasciola hepatica and Haemonchus contortus-exposed sheep (Group III).
Among group I sheep, significant increase ($P < 0.05$) in mean neutrophil counts occurred between experimental days 42 and 70; on day 63, maximum count was 3,973/cmm. compared to 2,371/cmm. for nonexposed controls. A significant decrease ($P < 0.05$) in mean neutrophil counts among group I sheep occurred on experimental days 119 and 126 and among group II sheep on day 126. Significant change in mean neutrophil counts for sheep in groups II and III did not occur. Analyses of mean basophil, monocyte, and band counts indicated that significant differences between groups did not occur during the experiment.

**Serum albumin**

On experimental day 0, mean serum albumin concentration for group I sheep was 3.81 Gm./100 ml., for group II sheep, 3.87 Gm./ml., for group III sheep, 3.78 Gm./100 ml., and for group IV sheep, 3.87 Gm./100 ml. For sheep in the three control groups, mean weekly serum albumin concentrations are given (Figure 12). For the five principals, (group III), mean serum albumin values for experimental days 0 to 49 are also shown (Figure 12); data for each of these sheep are given (Figure 13). Statistical analysis of data indicated the following: For group I sheep (*E. hepatica*-exposed controls), significant decrease ($P < 0.01$) in albumin content began on experimental day 21 and continued through day 203, maximum reduction
Figure 12. Mean serum albumin concentrations for 5 Fasciola hepatica-exposed sheep (group I; o--o), 5 Haemonchus contortus-exposed sheep (group II; •--•), 5 F. hepatica and H. contortus-exposed sheep (group III; X--X), and 5 nonexposed sheep (group IV; o--o).
Figure 13. Serum albumin concentrations for each of 5 *Fasciola hepatica* and *Haemonchus contortus*-exposed sheep (group III).
(1.31 Gm./100 ml.) occurred on day 70 (Figure 12); for group II sheep (*H. contortus*-exposed controls), it was found in the period from experimental day 21 to 63, maximum reduction (3.08 Gm./100 ml.) occurred on day 56 and concentration was 4.04 Gm./100 ml. on day 112 (Figure 12); for group III sheep (*F. hepatica* and *H. contortus*-exposed principals), it began on day 14 and values decreased until time of death (Figures 12, and 13). Serum albumin concentration at time of death for two group I sheep was 1.36 Gm./100 ml. (sheep 2, day 154) and 1.6 Gm./100 ml. (sheep 4, day 161) (Figure 12); for five group III sheep, range in concentration was 1.26 to 1.80 Gm./100 ml. (Figure 13).

**Postmortem Findings**

Day of necropsy, and final weight and PCV determined before death are given for eight sheep (three in group I; five in group III) that died, and two sheep (group I) that were killed and necropsied (Table 2).

**Gross Pathology**

Postmortem examination of eight sheep that died revealed intermandibular and subcutaneous edema, and moderate to severe ascites and hydrothorax. Carcasses appeared anemic and blood was thin and watery. Jaundice of skin and mesenteries was noted.
Table 2. Final Weight, Packed Cell Volume, Fecal Sample Analysis, and Helminth Recovery Data for each of 5 *Fasciola hepatica*-exposed sheep (group I), and 5 *F. hepatica* and *Haemonchus contortus*-exposed sheep (group III).

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Group</th>
<th>Day of death</th>
<th>Exposure</th>
<th><strong>Helminth burdens</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Fasciola hepatica</strong></td>
<td><strong>H. contortus</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ova No. in feces*</td>
<td>Flukes (mm.)</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>162</td>
<td>600 none</td>
<td>pos. 412</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>168</td>
<td>600 none</td>
<td>pos. 414</td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>265</td>
<td>600 none</td>
<td>pos. 318</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>269**</td>
<td>600 none</td>
<td>pos. 383</td>
</tr>
<tr>
<td>5</td>
<td>I</td>
<td>1,063**</td>
<td>600 none</td>
<td>pos. 28</td>
</tr>
<tr>
<td>13</td>
<td>III</td>
<td>51</td>
<td>600 50,000</td>
<td>neg. 253</td>
</tr>
<tr>
<td>15</td>
<td>III</td>
<td>71</td>
<td>600 50,000</td>
<td>neg. 238</td>
</tr>
<tr>
<td>11</td>
<td>III</td>
<td>78</td>
<td>600 50,000</td>
<td>pos. 332</td>
</tr>
<tr>
<td>12</td>
<td>III</td>
<td>190</td>
<td>600 50,000</td>
<td>pos. 346</td>
</tr>
<tr>
<td>14</td>
<td>III</td>
<td>201</td>
<td>600 50,000</td>
<td>pos. 326</td>
</tr>
</tbody>
</table>

* The last sample taken before the sheep died.

** The sheep was killed and necropsied. Numbers in parentheses are minimal and maximal values.
for one sheep (13, group III). Perforation of the gallbladder (sheep 12 and 14) or upper duodenum (region of common bile duct, sheep 1) was considered the immediate cause of death for three sheep; numerous mature F. hepatica were recovered in peritoneal fluid from these sheep.

The liver was enlarged in each sheep and gallbladder distension was observed in three sheep (2, 4, and 11). Fluke ova were recovered in bile from eight sheep that were examined after experiment day 71. Numerous subcapsular, hemorrhagic tracts caused by immature F. hepatica were evident on the visceral surface of liver from one sheep (13, experimental day 51). Liver texture changed from soft to firm as the fibrotic reparative process advanced. The "hobnailed appearance" of the visceral surface was observed in sheep (15, 11) that died on experimental days 71 and 78. In sheep that died or were killed after this time, mild fibrosis of liver, especially the left lobe, was evident. Bile ducts on the parietal surface were prominent as duct walls became thickened, distended, and raised. A marked thickening and folding of epithelium of the main bile duct was found in the sheep (5) necropsied on experimental day 1,063.

Helminthologic Data

Final fecal examination results, number of H. contortus recovered in abomasums from each group III sheep, and number and
mean length of *F. hepatica* recovered in liver from each sheep in
groups I and III are given (Table 2). Fluke ova were recovered in
feces of eight sheep that were examined after experimental day 71.
*Haemonchus contortus* e. p. g. counts and worm burdens in group III
sheep at time of death were high, even in two sheep (12, 14) that
died on experimental days 190 and 201.

The first three sheep (13, 15, 11) that died harbored moderate
burdens of *F. hepatica* (238 to 253) and heavy burdens of *H. contortus*
(18, 100 to 28, 000). Order in time that deaths occurred correlates
with the number of *H. contortus* recovered.

Larger *F. hepatica* burdens were recovered in two group I
sheep (2, 412 flukes; 4, 414 flukes) that died before death of the
two remaining group III sheep (12, 346 flukes; 14, 326 flukes). Two
group I sheep (1, 3) lived at least two months longer than two
group III sheep (12, 14) harboring similar fluke burdens. Assuming
that a similar number of *F. hepatica* became established in sheep 5
(group I), significant reduction in its fluke burden occurred in the
2.2-year period after sheep 3 was killed and examined. Total re-
cover recovery was 383 *F. hepatica* in sheep 3 and only 28 in sheep 5.

**Histopathology**

Examination of sectioned liver from four sheep (13, 15, 11, and
5) revealed the following: Fresh migration tracts in parenchyma
were seen only in one sheep (13) that died on experimental day 51. Section through most recently disrupted tissue indicated cellular debris, lysed erythrocytes, and leukocytic infiltration primarily of neutrophils; some eosinophils and macrophages were evident. In older regions of tracts, eosinophils and macrophages containing hemosiderin characterized the infiltrate; early fibroblastic invasion was observed. In sheep (15, 11) that died on experimental days 71 and 78, migratory tracts contained fibroblasts, collagen, macrophages, and plasma cells. By day 1,063, resolution of tissue damage was complete since no evidence of migration tracts were seen in sections from sheep 5.

On experimental day 51, necrotic foci adjacent to fresh tracts were found; these contained hepatocytes showing degenerative nuclear changes of pyknosis, karyorrhexis, and karyolysis. Mononuclear leukocytic infiltration to portal areas adjacent to migratory tracts was evident, and bile ductules showed early proliferative changes within the portal region. By experimental days 71 and 78, plasma cells, macrophages, and some neutrophils characterized the infiltrate in portal areas; bile ductule proliferation and early stages of portal fibrosis were found. Collagen deposition around proliferating bile ductules caused separation of portal triad components. At this time, hyperplastic changes in bile ductule epithelium were evident. Number and height of epithelial cells increased, and in
larger ducts, invagination of the epithelium occurred. By day 1,063, portal fibrosis was extensive and marked adenomatous thickening of the main bile duct epithelium, with a dense underlying collagen layer, were prominent features. Erythrocytes were evident on eroded surfaces of this epithelium, and an intense infiltration of eosinophils, plasma cells, and lymphocytes was found in these areas. In hyperplastic bile ductules and adenomatous structure of the main bile duct, epithelial cells in several areas, had eosinophilic intranuclear inclusion bodies of unknown etiology.

Numerous refractile, crystalline structures in parenchyma were evident in liver sections from sheep examined on experimental days 71 and 78. Zonal fatty degeneration around central lobular veins was found in sheep 11 that died on day 78.
DISCUSSION

Clinicopathologic, gross and histopathologic data for five _F. hepatica_-infected sheep (group I) were characteristic of sheep with chronic fascioliasis. Weight loss, ascites, time of death, and gross liver pathology agree with data reported by Boray (1967, 1969) and Ross, Dow, and Todd (1967). Onset of significant reduction in PCV, erythrocyte count, hemoglobin and serum albumin concentrations, and pathologic erythrocyte condition was as reported by others (Furmaga and Gundlach, 1967a, b; Hjerpe _et al._, 1971; Ibrović and Gall-Palla, 1959; Sewell, Hammond, and Dinning, 1968; Sinclair, 1962, 1964, 1965; Symons and Boray, 1967, 1968). Macrocytic, normochromic anemia in subacute and chronic fascioliasis was reported by Hjerpe _et al._ (1971), Reid _et al._ (1970), Ross (1967a, b). Eosinophilia and leukocytosis observed in the present experiment agree with findings of most workers. Histopathologic changes in liver were similar to descriptions given by others (Dawes, 1963; Dow, Ross, and Todd, 1968; Rubaj and Furmaga, 1969). Presence of eosinophilic intranuclear inclusion bodies in hyperplastic bile duct epithelial cells has not been reported in chronic ovine fascioliasis.

Age resistance to _H. contortus_ infection was demonstrated by sheep in group II. One of five sheep did not acquire a patent
infection, and three of them had no eggs in feces by experimental day 70. Increased erythropoiesis was sufficient to compensate for blood loss, and the normocytic, hypochromic anemia was of short duration. Data similar to these have been reported by others (Baker and Douglas, 1966; Fourie, 1931; Silverman, Mansfield, and Scott, 1970). Eosinophilia and hypoalbuminemia observed in the present experiment agree with data given by Charleston (1965), Kuttler and Marble (1960), Malczewski (1970), and Shumard, Bolin, and Eveleth (1957).

Response of the five *F. hepatica* and *H. contortus*-infected sheep indicated several significant changes when compared to that of the three control groups. Death of three sheep before experimental day 80, and of the two remaining sheep two months before other sheep (group I) with similar *F. hepatica* burdens, indicated enhanced pathogenicity in sheep with concurrent infections. Significant decrease in PCV, erythrocyte count, hemoglobin and serum albumin concentration occurred by experimental day 42, much earlier than was found in group I sheep. Microcytic, hypochromic anemia at time of death of two sheep (15, 11) suggested that the primary causative agent was *H. contortus*. Macrocytic, normochromic anemia at time of death of two sheep (12, 14) indicated that *F. hepatica* infection was responsible.

Establishment of both helminths and host response to *F.*
hepatica appeared normal. Reparative tissue response in sheep with concurrent infections occurred at the time reported for *F. hepatica*-infected sheep by Dawes (1963) and Dow, Ross, and Todd (1968). Inhibition of development of host resistance to *H. contortus* by presence of *F. hepatica* was indicated. Fecal egg counts were higher and the patent period lasted longer in sheep with concurrent infections when compared to that for sheep with *H. contortus* alone. Examination of data for body weights, erythrocyte counts, hemoglobin and serum albumin concentrations, and prevalence of abnormal erythrocytes in blood smears for two sheep in group III (12, 14) indicated improvement in condition, corresponding to time of recovery to *H. contortus* infection by sheep in group II. A marked increase in e.p.g. counts for two sheep (12, 14) from experimental day 154 to time of death suggested that superinfection occurred. This indicates that resistance resulting from primary infection did not occur, and is in marked contrast to data given by Silverman, Mansfield, and Scott (1970). Supportive evidence for impaired response in concurrent infections was found in comparison of mean eosinophil counts for *F. hepatica*-infected sheep and for those with *F. hepatica* and *H. contortus*. Since eosinophilia is characteristic of each helminth infection when studied separately, significant increase over that of sheep with *F. hepatica* alone was anticipated. Results in the present experiment indicate that eosinophilic response in sheep with
simultaneous *F. hepatica* and *H. contortus* infections is less than that for sheep with *F. hepatica* infection alone.

Interference with host response to one nematode species in concurrent infection with two gastrointestinal nematodes was reported by Kates and Turner (1960). They found an extended patent period for *N. spathiger* in lambs when simultaneous *H. contortus* infection was experimentally induced.
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