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Title: PATHOGENICITY OF EXPERIMENTALLY INDUCED

    CONCURRENT INFECTIONS OF FASCIOLA HEPATICA

    AND HAEMONCHUS CONTORTUS IN SHEEP

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Twenty yearling sheep were allotted to four groups, each con-

taining five sheep of approximately equal weight. On experimental

days 0, 1, 2, 3, and 4, each sheep in each group was given the fol-

lowing 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 in-

fective 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 $\underline{F}$. hepatica and $\underline{H}$. 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 $\underline{F}$. hepatica and 28,000 H. contortus were recovered at necropsy. Slightly microcytic, hypochromic anemia, characteristic of $\underline{H}$. contortus infection, was found in two sheep that died on experimental days 71 and 78. In one sheep, 238 $\underline{F}$. 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 $\underline{F}$. hepatica; 12,600 and 27,600 $\underline{H}$. contortus were recovered.

Establishment of both helminths and host response to $\underline{F}$. hepatica infection appeared normal, but development of host resistance to $\underline{H}$. contortus was inhibited by simultaneous $\underline{F}$. hepatica infection. Eosinophilic response in sheep with the concurrent infections was less than that of sheep with $\underline{F}$. hepatica infection alone.

Pathogenicity of Experimentally Induced Concurrent
Infections of <u>Fasciola</u> <u>hepatica</u> and <u>Haemonchus</u>
<u>contortus</u> in Sheep

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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 Gundłach, 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 battus (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 Hae-monchus infections in sheep or cattle has not been studied.

The purpose in the present study was to investigate clinico-pathologic changes in sheep after simultaneous exposure to F. hepatica and H. contortus.

REVIEW OF LITERATURE

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 sur-
face 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, hypo-
chromic 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;

Sewell, Hammond, and Dinning, 1968; Sinclair, 1962, 1964, 1965).

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

^{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 ^{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 ^{51}Cr and ^{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 F.

hepatica recovered from sheep previously injected with erythrocytes

labeled with ^{51}Cr or ^{59}Fe (Pearson, 1963; Symons and Boray,

1967, 1968). The ingesta contained hematin (Stephenson, 1947;

Todd and Ross, 1966), and acetylcholinesterase activity character-

istic of host erythrocytes (Frady and Knapp, 1967); the majority

of workers have concluded that mature $\underline{F}$. 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 hyper-globulinemia that occurred during the acute stage (Furmaga and Gundłach, 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 hypo-albuminemia 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), hyperglobulinema 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 associ-
ated 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 macro-
cytic, normochromic anemia with marginal hypochromasia

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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 hemoglobination 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 produc-

tion. 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 cortico-

steroid administration on secondary infection (Sinclair, 1970b)

were investigated. He concluded that hyperactivity of the reticu-

loendothelial system was an integral part of the resistance mech-

anism to F. hepatica infection in sheep. Interference with this

system resulted in more severe disease because the normal cellu-

lar 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:

| | Fascioliasis | | | Nematodes | | |
	acute	sub-acute	chronic	haemon-chosis	mixed infec-tion	Phle-botomy
Weight loss	+++	++	++	+, ++	++	++
Edema	+	+	++	++	NC	NC
Ascites	+++	++	+, ++	NC	NC	NC
Anemia						
normocytic	++	+	++	+	++	
macrocytic	+	++	+, ++	+		++
microcytic				++, +++	+	
normochromic	++	++	++	+	++	++
hypochromic		+		++, +++	+	+
Abnormal red cells	+++	+++	+++	+++	++	++
Leukocytosis	NC	++	++	+	+	NC
Eosinophilia	+++	+	+	+, ++	+	NC
Neutrophilia	++	+	+	++	+	NC
Hypoalbuminemia	+	+++	+++	++	++	+
Hyperglobulinemia	+++	++	++	++	++	+
Bromsulphthalein retention	+++	++	+	NC	NC	NC
SGOT, SGD levels (elevated)	+++	++	+	NC	NC	NC
Alkaline phosphatase (elevated)	+++	++	+	NC	NC	NC

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 (L_3) 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 L_3 were drawn from the total collection. The inoculum of 50, 000 L_3 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 L_3 each day; five F. hepatica and H. contortus-exposed principals (group III) were given 120 metacercariae and then 10, 000 L_3 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 micro-hematocrit. 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 ex-

amined 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 approxi-

mately 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 $4^{o}C$. 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 bur-
den 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 experi-

mental 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 ex-

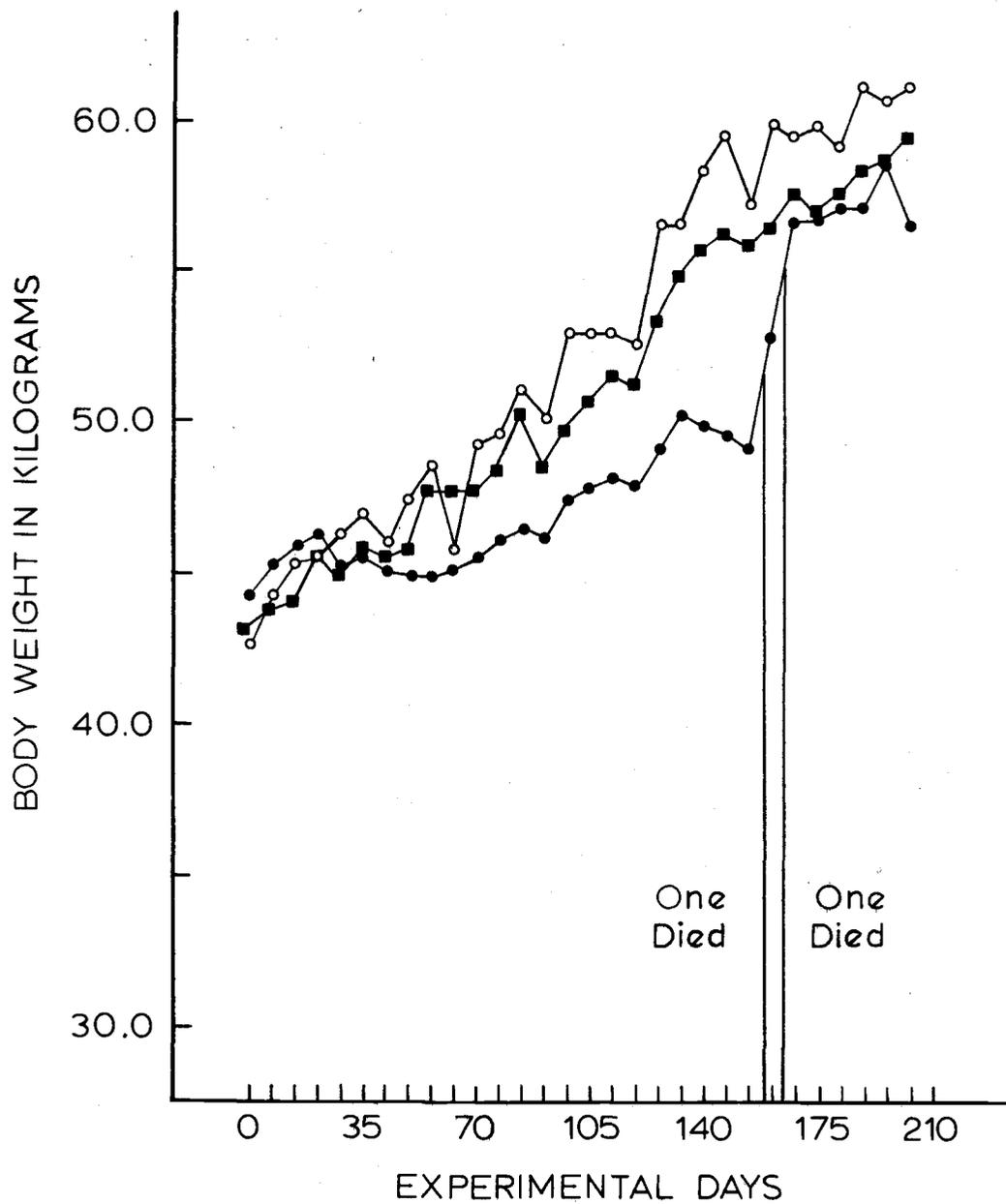perimental days 140 and 203. Sheep 3 weighed 51.8 kg. on

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

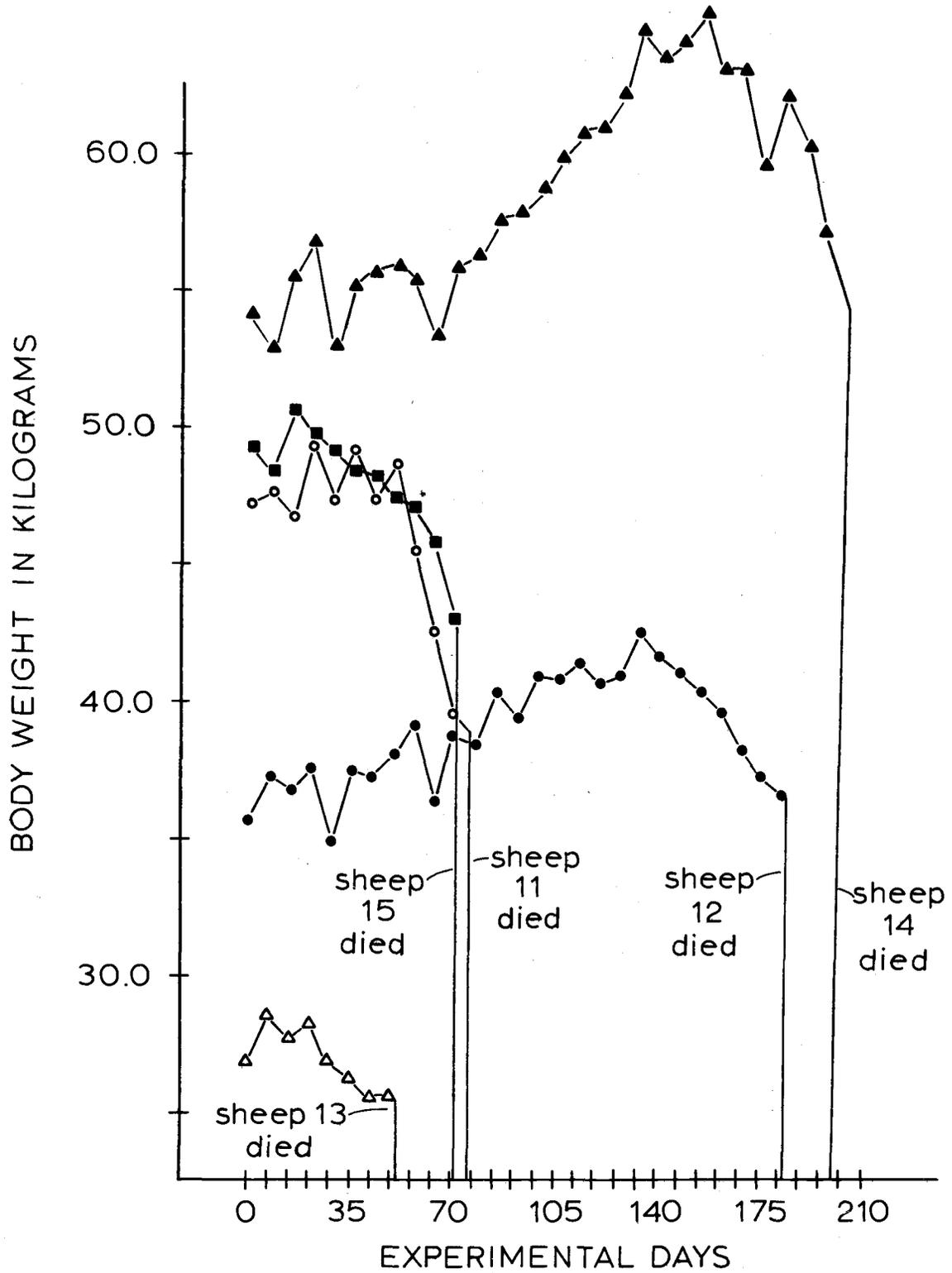(F. hepatica and H. contortus-exposed principals) and mean count

Figure 2. Body weights for each of 5 Fasciola hepatica and Haemonchus contortus-exposed sheep (group III).

for group II sheep (H̱. 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 experi-

mental day 56; mean output was 14,000 e.p.g. (minimum and maxi-

mum, 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).
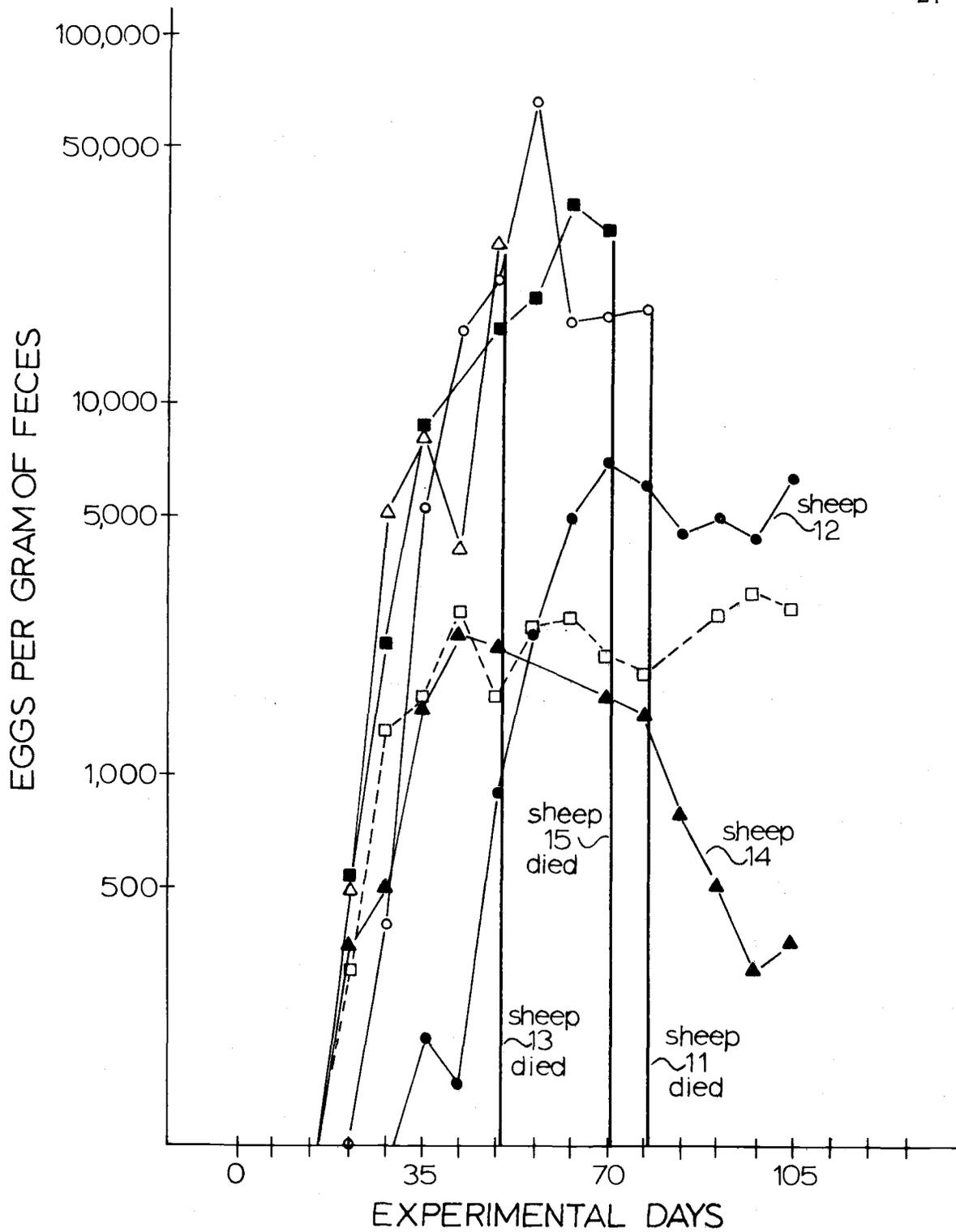
Figure 3. Haemonchus contortus fecal egg counts for each of
5 Fasciola hepatica and H. contortus-exposed sheep
(group III), and mean counts for 5 H. contortus-
exposed sheep (group II; □--□).

Figure 4. Mean packed cell volumes for 5 <u>Fasciola hepatica</u>-
exposed sheep (group I; ●──●), 5 <u>Haemonchus</u>
<u>contortus</u>-exposed sheep (group II; ■──■), 5 <u>F</u>.
<u>hepatica</u> and <u>H</u>. <u>contortus</u>-exposed sheep (group III,
X──X), and 5 nonexposed sheep (group IV; o──o).

Figure 5. Packed cell volumes for each of 5 <u>Fasciola hepatica</u>
and <u>Haemonchus contortus</u>-exposed sheep (group III).

Figure 6. Mean erythrocyte counts for 5 <u>Fasciola hepatica</u>-
exposed sheep (group I; ●——●), 5 <u>Haemonchus</u>
<u>contortus</u>-exposed sheep (group II, ■——■), 5 <u>F.</u>
<u>hepatica</u> and <u>H</u>. <u>contortus</u>-exposed sheep (group III;
X——X), and 5 nonexposed sheep (group IV; o——o).

Figure 7. Erythrocyte counts for each of 5 <u>Fasciola</u> <u>hepatica</u> and <u>Haemonchus</u> <u>contortus</u>-exposed sheep (group III).
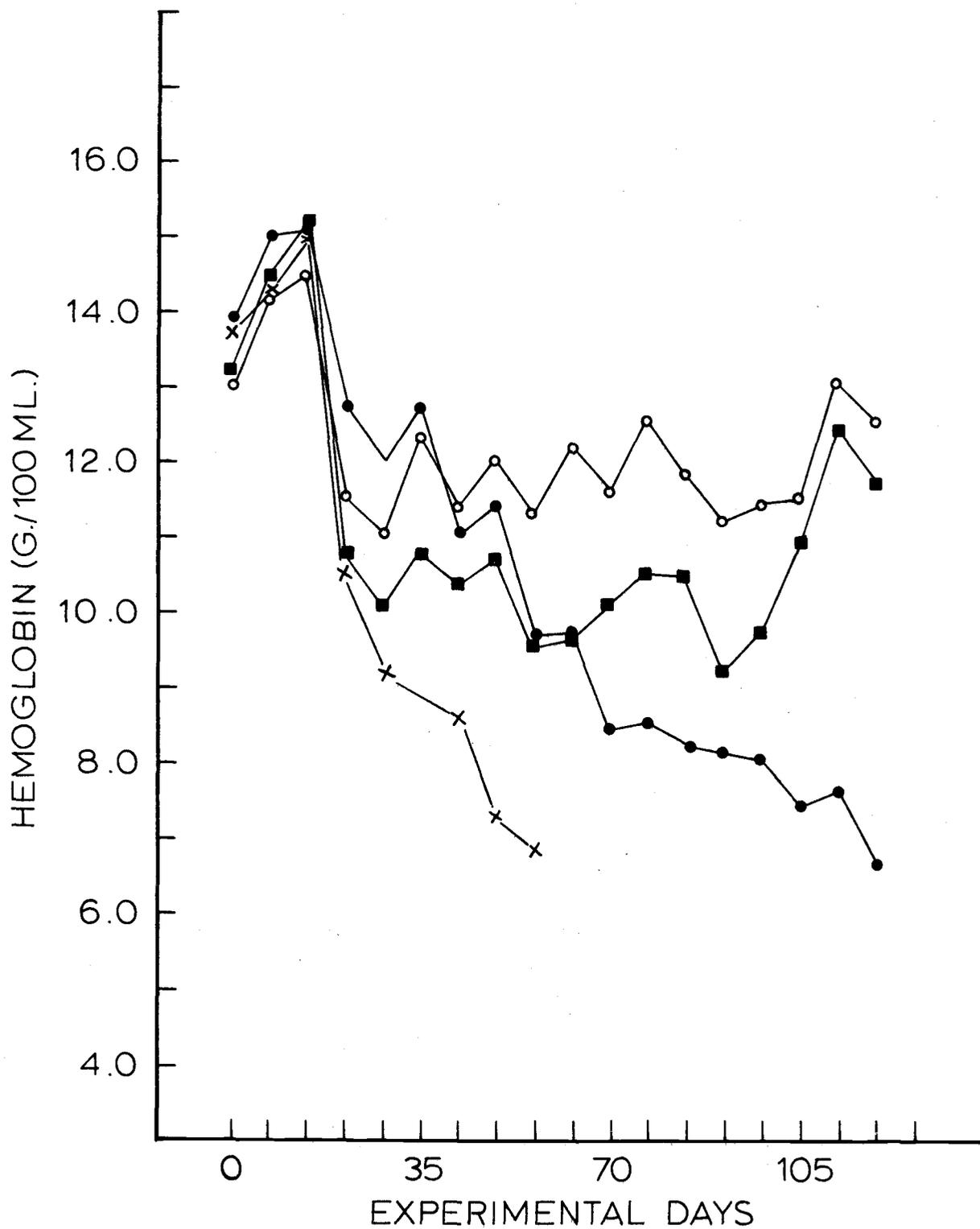
Figure 8. Mean hemoglobin concentrations for 5 Fasciola
hepatica-exposed sheep (group I; ●——●), 5 Haemonchus
contortus-exposed sheep (group II; ■——■), 5 F.
hepatica and H. contortus-exposed sheep (group III;
X——X), and 5 nonexposed sheep (group IV; o——o).
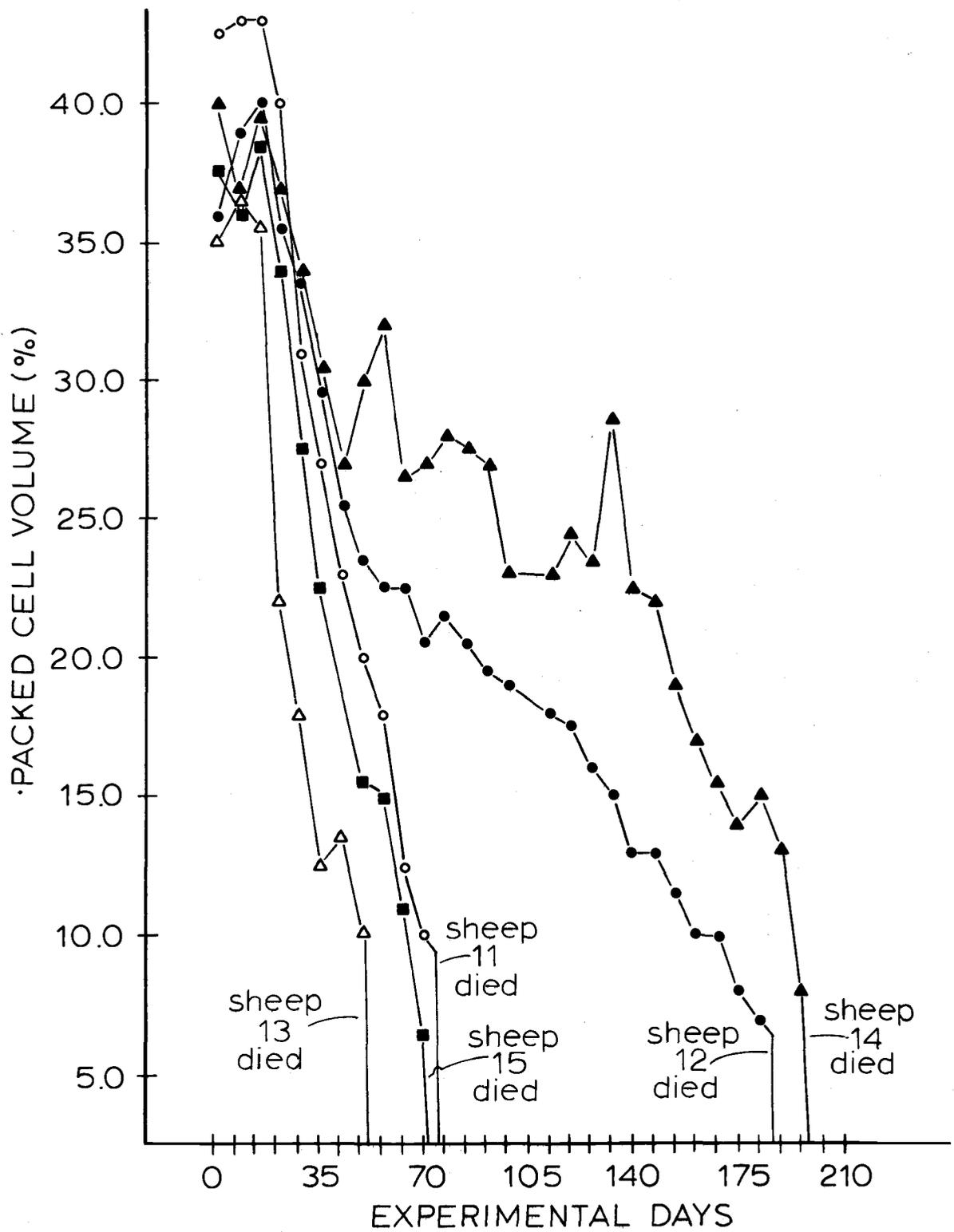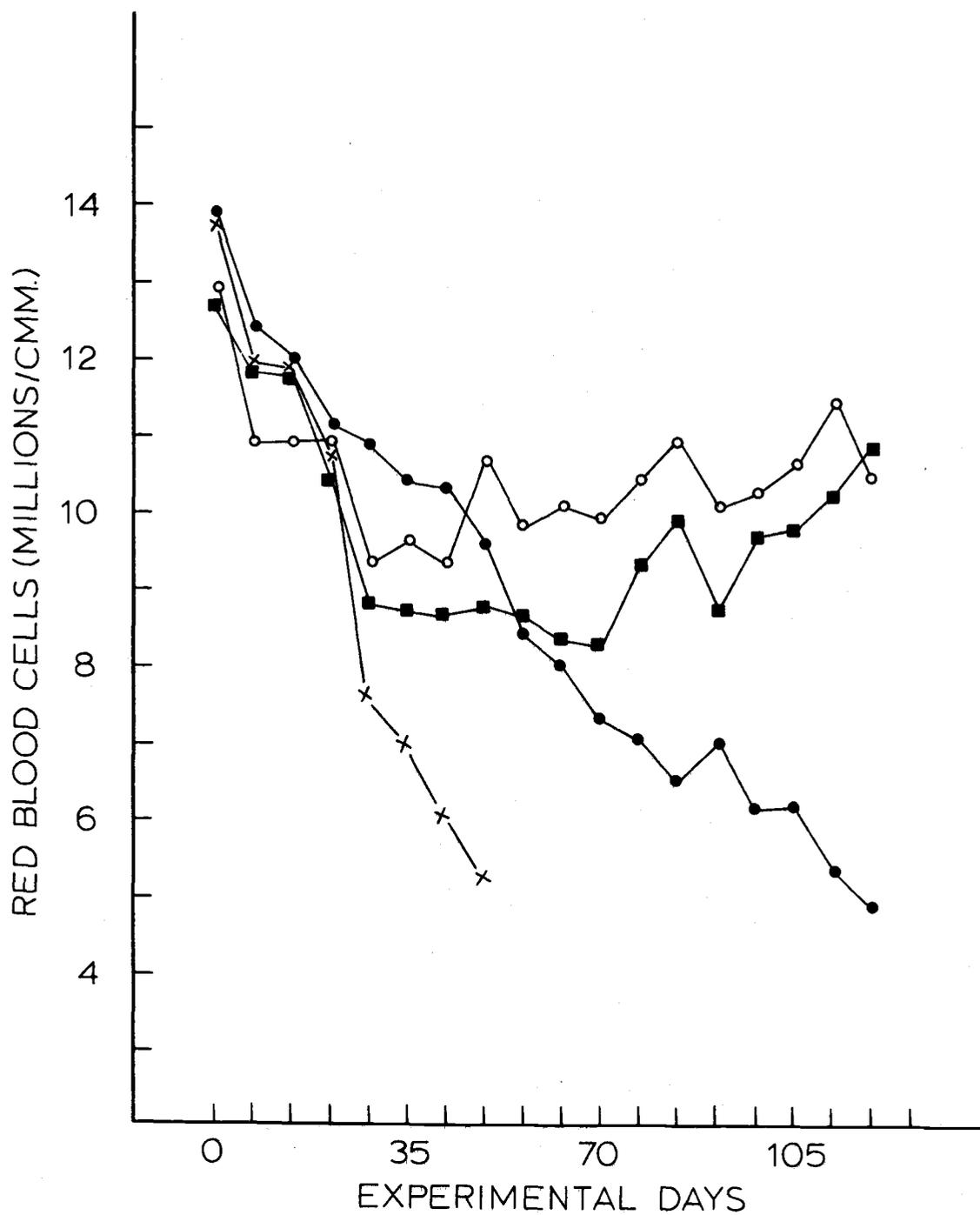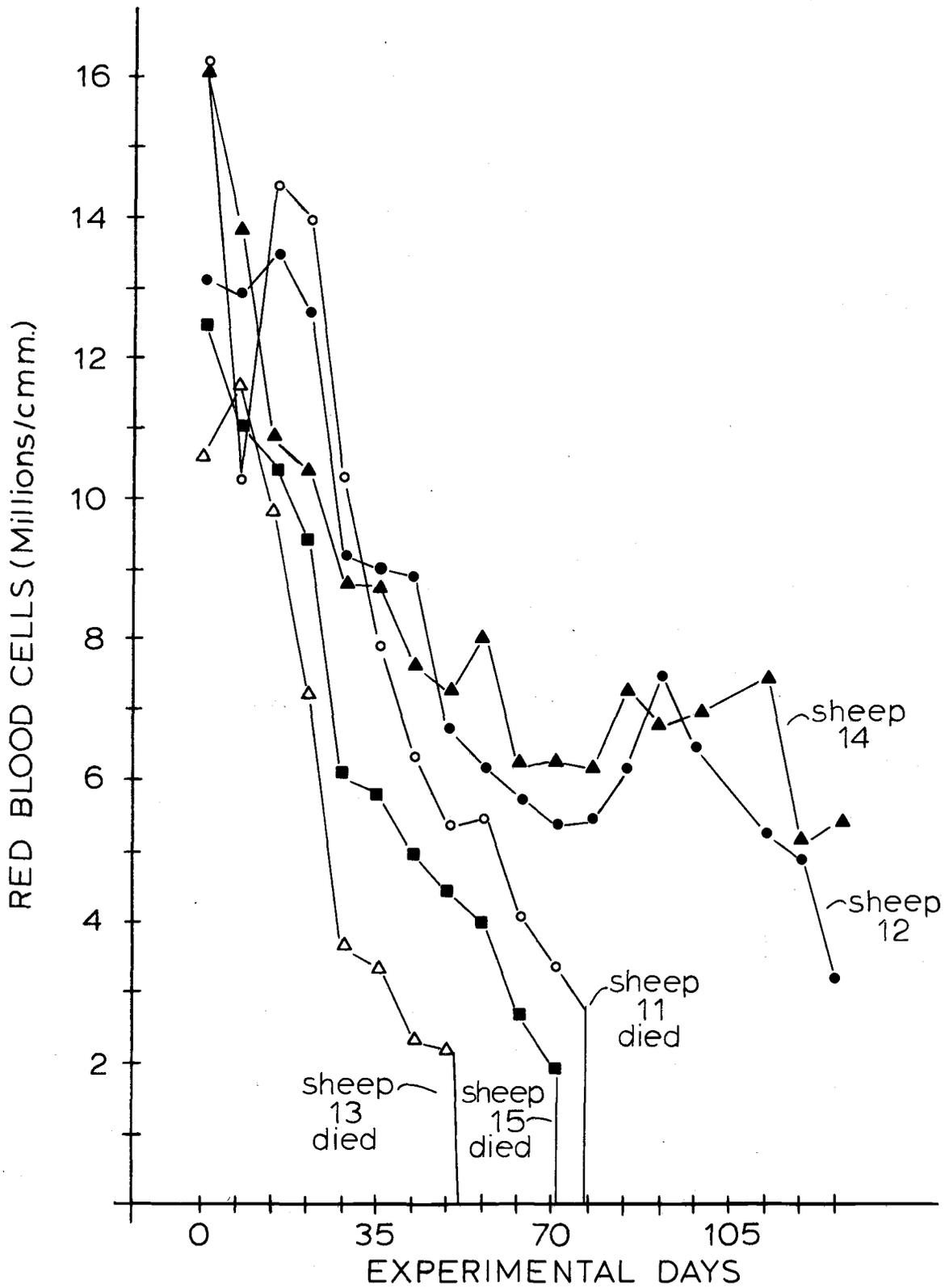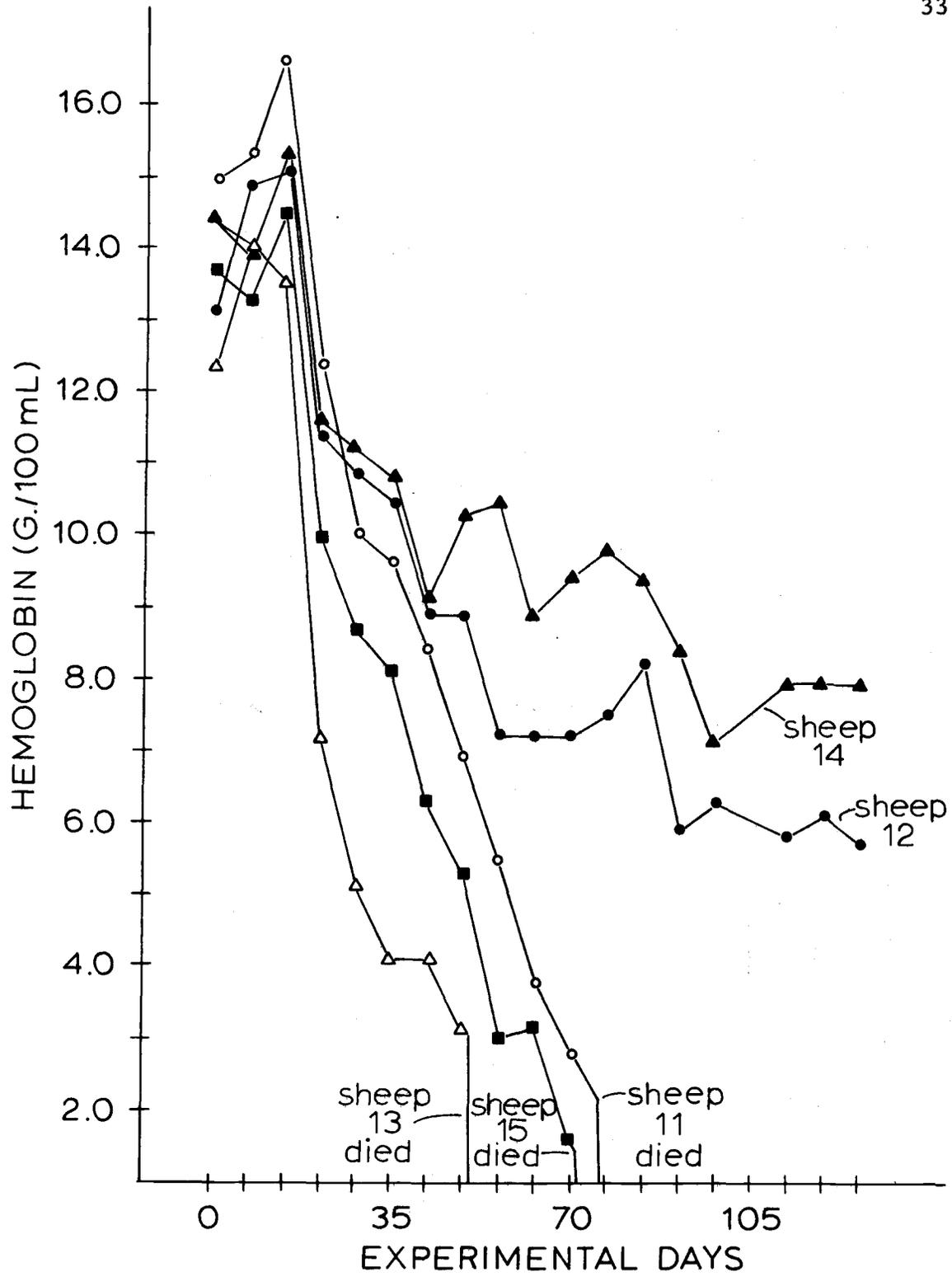
Figure 9. Hemoglobin concentrations for each of 5 <u>Fasciola</u>
<u>hepatica</u> and <u>Haemonchus</u> <u>contortus</u>-exposed sheep
(group III).

Statistical analyses of these data indicated that mean PCV

(25. 6%) for group I sheep (F. hepatica-exposed controls) was sig-

nificantly 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, hyprochromic 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).

Group	Sheep No.	Experimental day	Mean corpuscular volume (cμ)	Mean corpuscular hemoglobin (μμg.)	Mean corpuscular hemoglobin concentration (%)
I		49	35.1	12.0	31.4
II		49	36.4	12.4	34.0
III	13	49	44.2*	13.7	31.0*
IV		49	33.0	11.4	34.2
I		70	35.2	11.6	32.9
II		70	36.6	12.2	33.6
III	15	70	32.8	8.1	24.6*
	11	70	29.2	8.2	28.0*
IV		70	34.7	11.7	33.8
I		91	39.1**	11.8	30.3*
II		91	37.5	11.1	29.7*
IV		91	33.7	11.2	33.1
I		126	46.6*	14.5	31.3
II		126	33.9	10.9	32.2
III	12	126	49.2*	17.5	35.6
	14	126	42.7*	14.4	33.6
IV		126	35.0	12.0	34.1

* 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
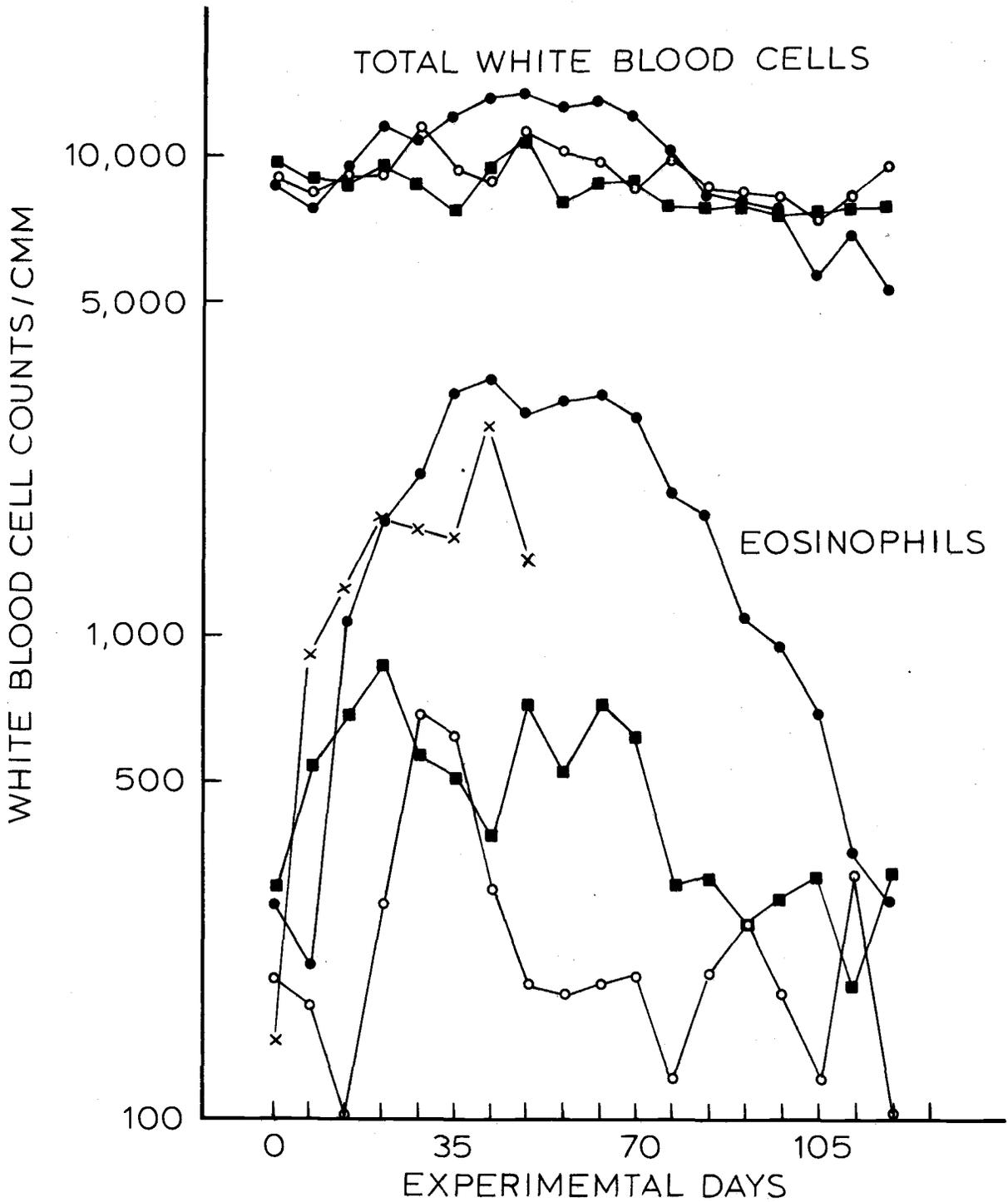
Figure 10. Mean leukocyte total and eosinophil counts for
5 Fasciola hepatica-exposed sheep (group I; ●——●).
5 Haemonchus contortus-exposed sheep (group II;
■——■), 5 F. hepatica and H. contortus-exposed
sheep (group III; X——X), and 5 nonexposed
sheep (group IV; o——o).

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).
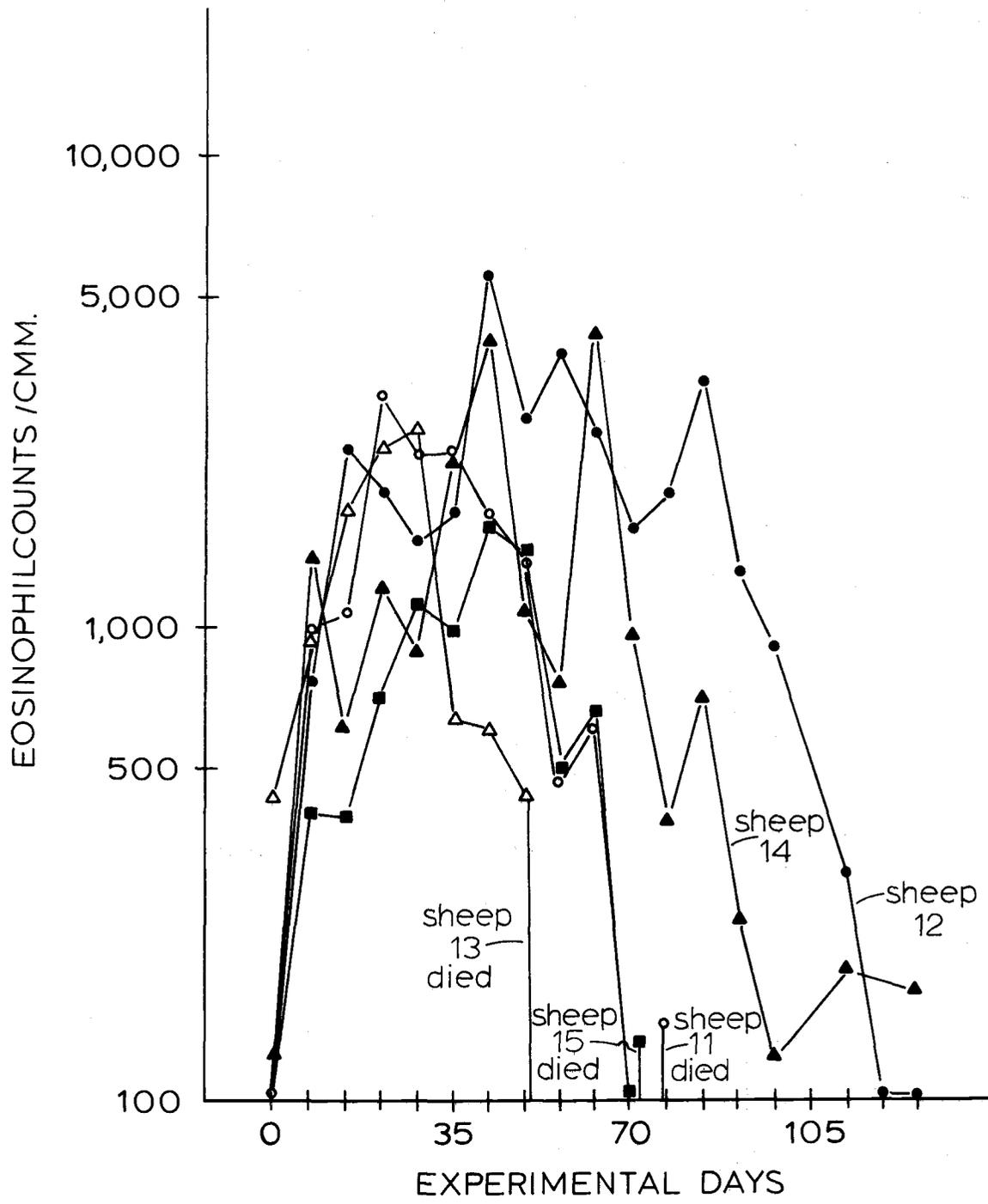
Figure 11. Eosinophil counts for each of 5 <u>Fasciola</u> <u>hepatica</u>
and <u>Haemonchus</u> <u>contortus</u>-exposed sheep (Group III).

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 ex-

perimental days 0 to 49 are also shown (Figure 12); data for each of

these sheep are given (Figure 13). Statistical analysis of data in-

dicated the following: For group I sheep (F. hepatica-exposed con-

trols), significant decrease (P< 0. 01) in albumin content began on ex-

perimental day 21 and continued through day 203, maximum reduction
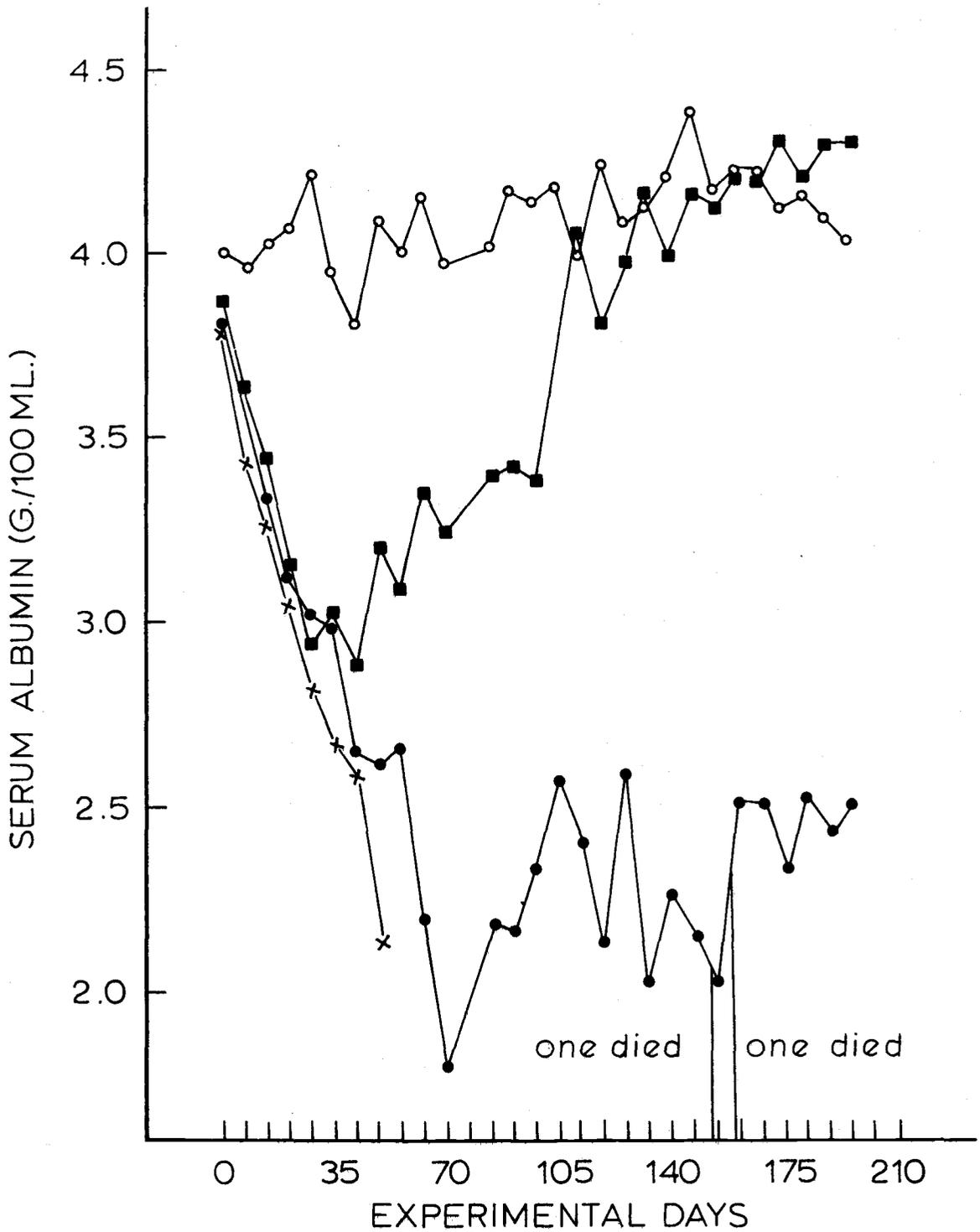
Figure 12. Mean serum albumin concentrations for 5 Fasciola
hepatica-exposed sheep (group I; ●——●), 5 Haemonchus
contortus-exposed sheep (group II; ■——■), 5 F.
hepatica and H. contortus-exposed sheep (group III;
X——X), and 5 nonexposed sheep (group IV; o——o).
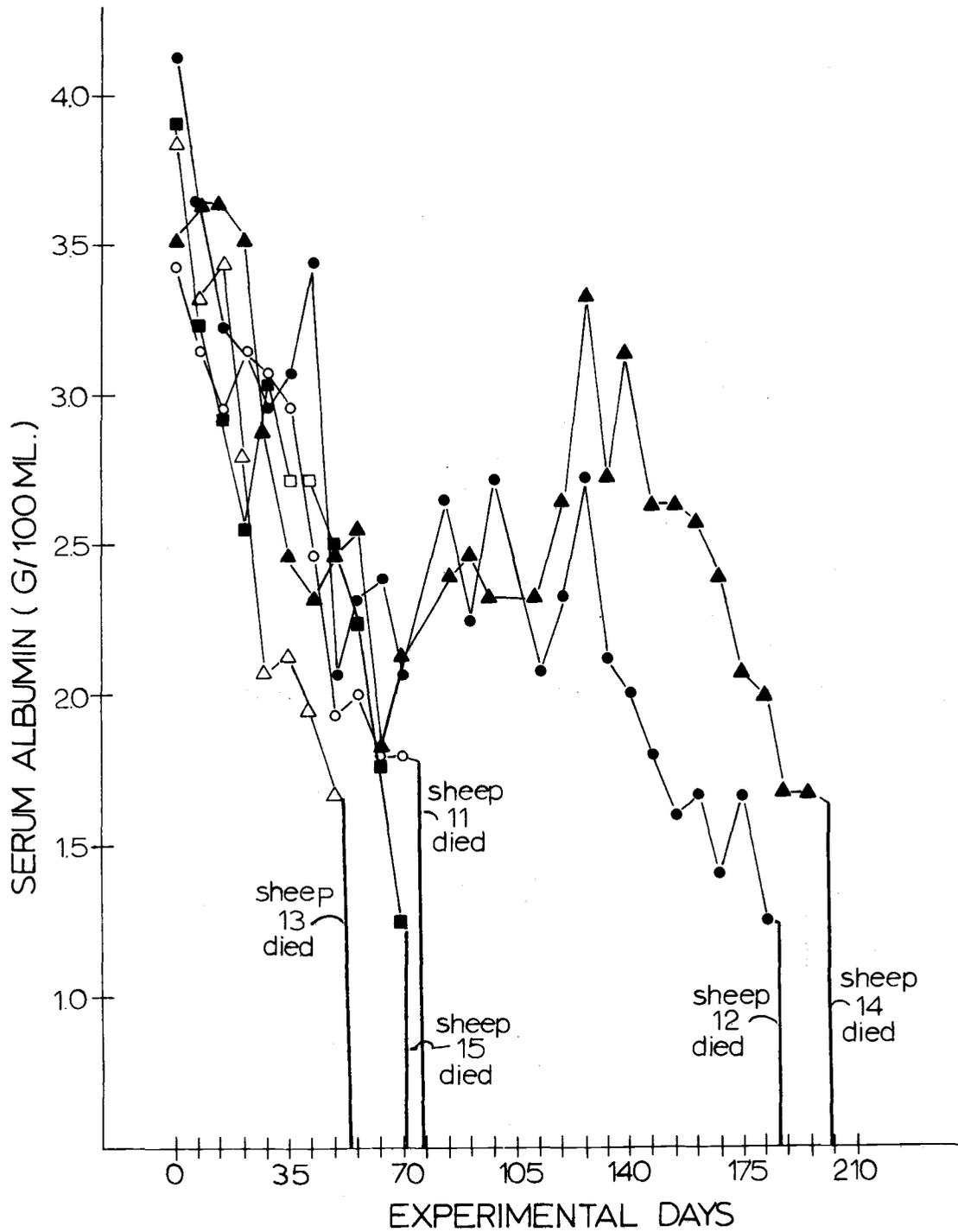
Figure 13. Serum albumin concentrations for each of 5 Fasciola hepatica and Haemonchus contortus-exposed sheep (group III).

(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 de-

creased 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).

Sheep No.	Group	Day of death	No. of F. hepatica	No. of H. contortus	Ova in feces*	No. of flukes	Mean length (mm.)	No. measured	Eggs per gram of feces*	No. of worms	Final weight (kg.)*	Final PCV (%)*
			Exposure		Fasciola hepatica				H. contortus			
2	I	162	600	none	pos.	412	29.6 (23-35)	50	---	---	35.2	7.5
4	I	168	600	none	pos.	414	31.0 (25-36)	50	---	---	42.3	8.0
1	I	265	600	none	pos.	318	27.0 (22-32)	32	---	---	---	---
3	I	269**	600	none	pos.	383	33.0 (27-41)	38	---	---	50.0	15.5
5	I	1,063**	600	none	pos.	28	28.5 (24-33)	26	---	---	93.6	38.0
13	III	51	600	50,000	neg.	253	6.2 (4-9)	25	27,900	28,000	25.7	10.0
15	III	71	600	50,000	neg.	238	15.4 (9-22)	24	30,000	25,300	43.2	6.5
11	III	78	600	50,000	pos.	332	18.9 (14-25)	33	18,200	18,100	38.6	7.0
12	III	190	600	50,000	pos.	346	30.9 (25-35)	35	15,500	12,600	36.8	7.0
14	III	201	600	50,000	pos.	326	27.8 (22-35)	33	13,800	27,600	57.3	8.0

* 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 $\underline{F}$. 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 $\underline{F}$. hepatica (238 to 253) and heavy burdens of $\underline{H}$. contortus (18, 100 to 28, 000). Order in time that deaths occurred correlates with the number of $\underline{H}$. contortus recovered.

Larger $\underline{F}$. 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 $\underline{F}$. 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 recovery was 383 $\underline{F}$. 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, macro-
phages, 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. Mono-
nuclear 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 prolifer-
ating 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 reduc-

tion in PCV, erythrocyte count, hemoglobin and serum albumin

concentrations, and pathologic erythrocyte condition was as reported

by others (Furmaga and Gundłach, 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. Histo-

pathologic 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. Examina-

tion of data for body weights, erythrocyte counts, hemoglobin and

serum albumin concentrations, and prevalence of abnormal erythro-

cytes 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 concur-

rent 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 re-

ported 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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