Pathology in Mice Resulting from Concurrent Infestations with the Bile Duct Dwellers *Fasciola hepatica* (Trematoda) and *Hymenolepis microstoma* (Cestoda)[1]

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ABSTRACT

Concurrent infestations in white mice were established using infesting doses of 2 *Fasciola hepatica* metacercariae and 10 *Hymenolepis microstoma* cysticercoids. In Sequence I, *F. hepatica* were given on Day 0 and *H. microstoma* on Day 37. *H. microstoma* were given on Day 0 in Sequence II followed by *F. hepatica* on Day 20. In Sequence III, the midacute phases of both infestations coincided, *F. hepatica* being given on Day 0 and *H. microstoma* on Day 15. Pathologies previously described for each infestation were observed in the mice of the concurrent infestation groups and in mice of the single species control groups. In Sequence I, 2 additional pathologies were observed in mice of the concurrent infestation group: (1) the eggs of *F. hepatica* were found in the tissues of the mice and (2) the generalized necrosis in the liver associated with the acute phase of infestation with *F. hepatica* (18–35 days after infestation) was reestablished on Day 60 and continued through Day 120. In Sequence II, the generalized necrosis in the livers of mice of the concurrent infestation group continued through Day 90, 70 days after the infestation with *F. hepatica*. One mouse of the concurrent infestation group in Sequence III was observed to have the eggs of *F. hepatica* in the liver and biliary tissues.

INTRODUCTION

Extensive pathology in the liver is associated with the infestation of mice by 2 bile duct dwellers, the trematode *Fasciola hepatica* and the cestode *Hymenolepis microstoma*. The pathology resulting from the obligatory liver migration by the trematode and its subsequent inhabitation of the common bile duct have been the subject of numerous reports in the literature and have been summarized by Lang (1966, 1967). Lang (1966) divided the course of a primary infestation with *F. hepatica* in mice resulting from a 2-worm infesting dose into 3 phases: (1) the incubation phase (0–17 days after infestation), (2) the acute phase (18–35 days after infestation), and (3) the chronic with repair phase (36–250 days after infestation). During the incubation phase, damage to liver tissue was a direct result of mechanical trauma caused by the migrating worms. Severe liver damage and some mortality were associated with the acute phase of the infestation. Generalized necrosis was present in large areas of the liver and from 30–90 percent of the liver appeared necrotic. The necrosis was associated with lymphocytic infiltrations that were not, generally, located near worm burrows. The chronic phase of the infestation was initiated by the migration of the worms from the liver tissue into the common bile duct. Damaged liver tissue was replaced by regenerated parenchyma and connective tissue. However, the lymphocytic infiltration was maintained.

Although the life cycle of the cestode does not involve a liver migration, the inhabitation of the common bile duct results in extensive liver damage through the release of a toxin by the worms (Simpson and Gleason 1975), and published reports dealing with the pathology associated with the infestation of mice are numerous. These

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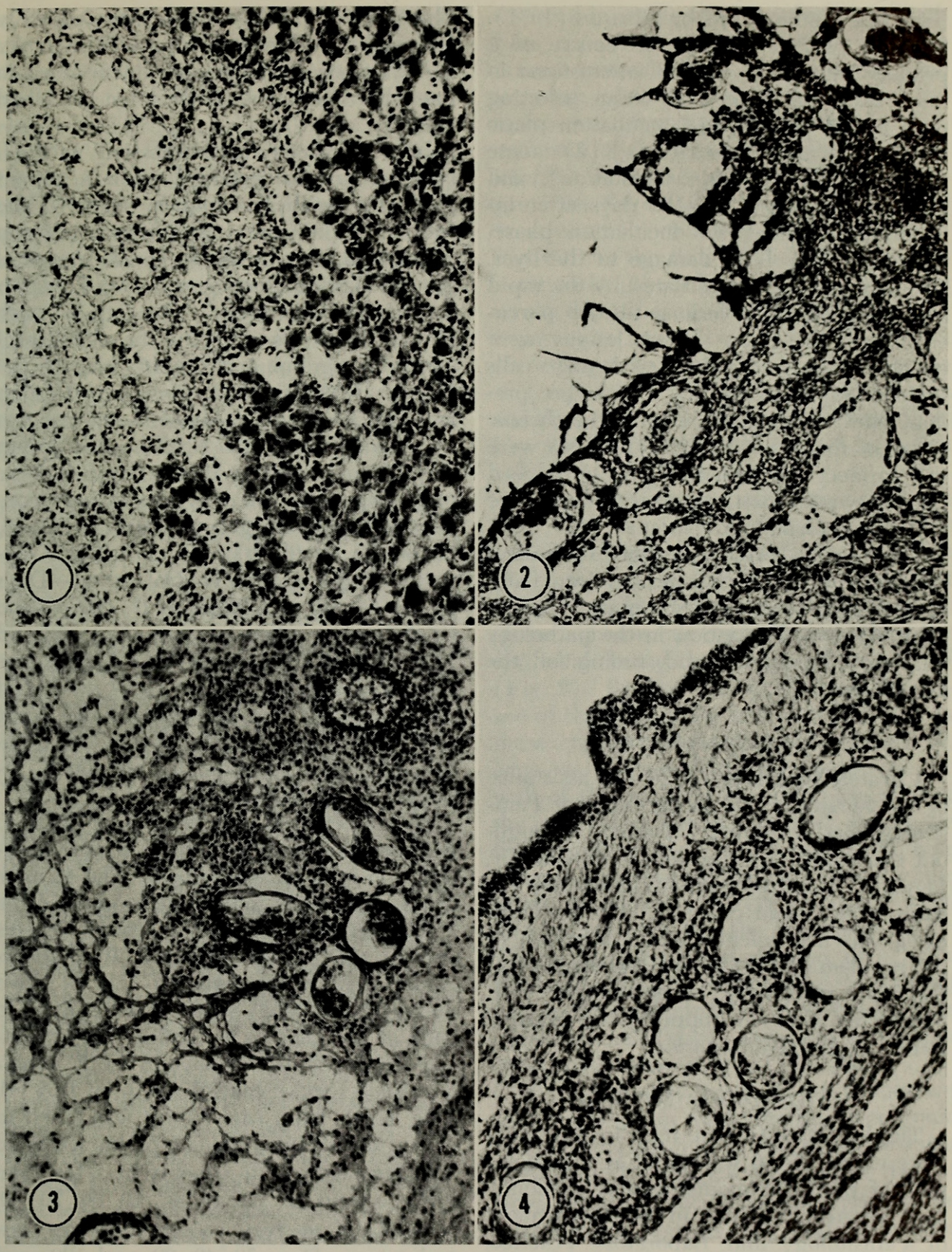

FIGS. 1–4. Pathology in mice resulting from concurrent infestations with *Fasciola hepatica* and *Hymenolepis microstoma*. Fig. 1. Generalized necrosis in the liver on Day 90. Many lymphocytes are present at the periphery of the necrotic area and in the sinusoids. ×140. Fig. 2. Eggs of *F. hepatica* trapped in serous exudate at region where the epithelial lining of the common bile duct has been eroded away (Day 50). ×140. Fig. 3. Eggs of *F. hepatica* in the tissue of the common bile duct on Day 70. ×140. Fig. 4. Eggs of *F. hepatica* in the wall of the cystic duct on Day 80. ×140.

have been summarized by Gleason (1971). Gleason (1971) divided the course of a primary infestation with *H. microstoma* in mice resulting from a 10-worm infesting dose into 3 phases: (1) incubation phase (0–6 days after infestation), (2) acute phase (7–20 days after infestation), and (3) chronic phase (21–150 days after infestation). During the incubation phase, there was very little damage to the liver. The acute phase was initiated by the rapid formation of focal lesions in the parenchyma of the liver. Those lesions were characterized by degenerated hepatic cells surrounded by infiltrating leukocytes, predominantly neutrophils. During the chronic phase of the infestation, focal lesions were still formed, but apparently at a reduced rate, allowing repair processes to keep pace with lesion formation.

This study presents some additional pathology in mice that results from concurrent infestation with *F. hepatica* and *H. microstoma* and variation in the pathology related to sequence and timing of the infestations.

Acknowledgments

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Materials and Methods

The Swiss white mice used in this study were males, 15–16 weeks old at the beginning of the experiments, from a randomly bred strain maintained in the Department of Parasitology and Laboratory Practice, University of North Carolina, for more than 30 years. The stock of *F. hepatica* was isolated from a naturally infested cow in northern California and maintained in the laboratory as described by Lang (1966). The stock of *H. microstoma* was originally obtained from Dr. Arthur Jones, University of Tennessee, Knoxville, and maintained in the laboratory as described by Litchford (1963).

In Sequence I, an infestation with *H. microstoma* was imposed upon a patent infestation with *F. hepatica.* Each of the 64 mice of the concurrent infestation group and the 32 mice of the *F. hepatica* control group received 2 metacercariae of *F. hepatica* by mouth on Day 0. On Day 37, each of the mice remaining in the concurrent infestation group and the 16 mice of the *H. microstoma* single species control group received 10 cysticercoids of *H. microstoma* by mouth. One mouse from each group was killed to obtain tissues for histologic preparations on the days indicated: concurrent infestation group—Days 37, 44, 48, 50, 60, 70, 80, 90, 100, 110, and 120; *F. hepatica* single species control group—Days 20, 30, 50, 80, and 120; and *H. microstoma* single species control group—Days 50 and 120. During this experimental sequence and those reported below, some mice died as a result of the infestations and some mice were killed to determine worm number, size, and location, as previously reported by Gleason (1974).

In Sequence II, an infestation with *F. hepatica* was imposed upon a patent infestation with *H. microstoma.* The 40 mice of the concurrent infestation group and the 24 mice of the *H. microstoma* single species control group each received 10 cysticercoids of *H. microstoma* on Day 0. Two metacercariae of *F. hepatica* were given to each of the mice in the concurrent infestation group and the 32 mice in the *F. hepatica* single species control group on Day 20. One mouse from each group was killed to obtain tissues for histologic preparations on the days indicated: concurrent infestation group—Days 30, 35, 40, 45, 50, 60, 70, 90, and 100; *H. microstoma* single species control group—Days 20, 40, 60, and 100; and *F. hepatica* single species control group—Days 40, 45, 50, 60, and 100.

The timing of the infestations in Sequence III was such that the acute phases

of the infestations coincided. Two metacercariae of *F. hepatica* were given to each of 40 mice of the concurrent infestation group and 28 mice of the *F. hepatica* single species control group on Day 0. Ten cysticercoids of *H. microstoma* were given to each mouse of the concurrent infestation group and 18 mice of the *H. microstoma* single species control group on Day 15. One mouse from each group was killed to obtain tissues for histologic preparations on the days indicated: concurrent infestation group—Days 15, 20, 25, 30, 40, 45, 50, 60, 70, 80, and 100; *F. hepatica* single species control group—Days 20, 25, 35, and 100; and *H. microstoma* single species control group—Days 20, 30, 40, and 100.

The tissue for histologic studies was prepared for microscopic examination using a microtome–cryostat as described by Gleason (1971).

RESULTS

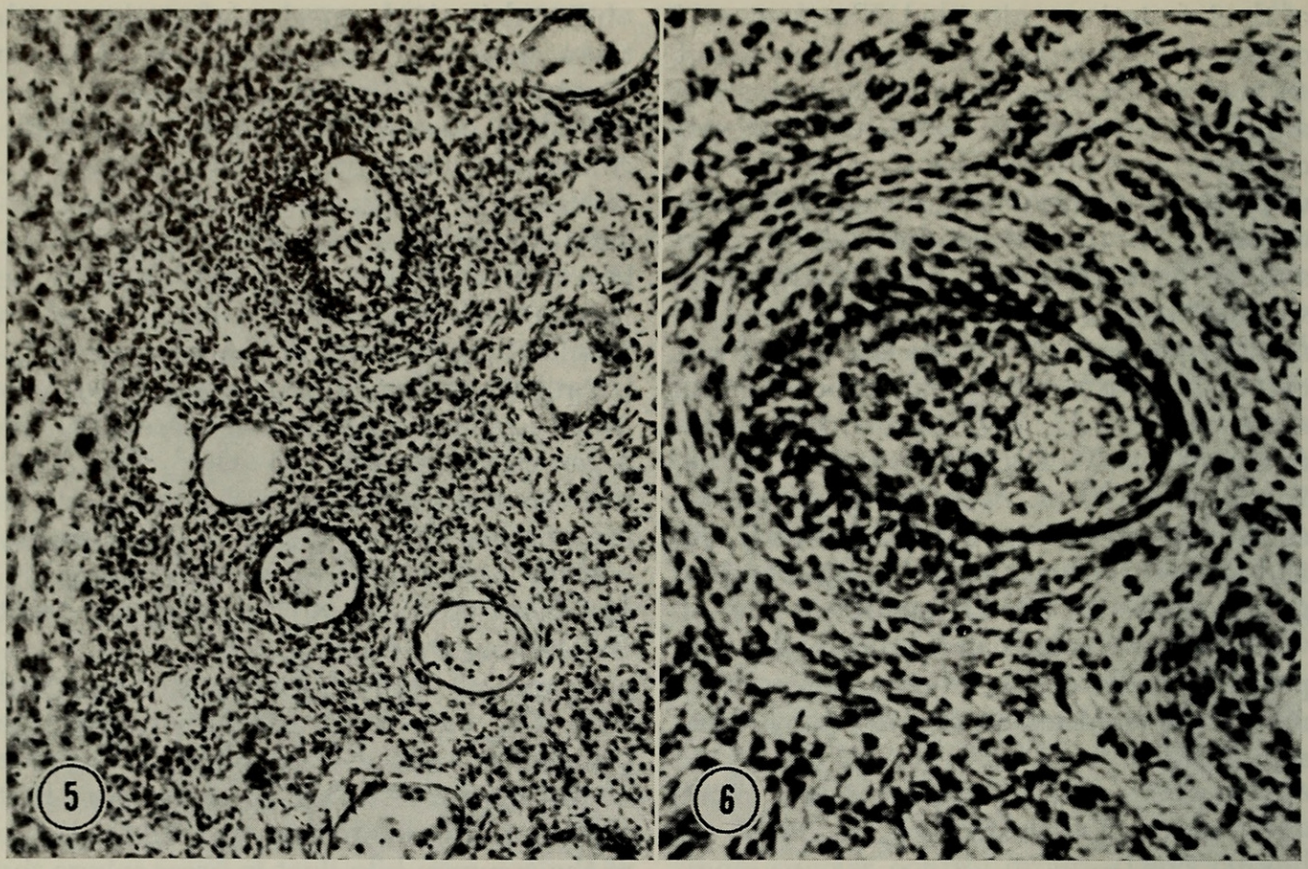
The pathologies in the livers of the *F. hepatica* and *H. microstoma* control mice in the 3 experiments were similar to those described previously for single species infestations resulting from the same infesting doses (Lang 1966, Gleason 1971).

In Sequence I, the mice of the concurrent infestation group were entering the chronic phase of the infestation with *F. hepatica* at the time the *H. microstoma* were given and the pathology in the liver was in the process of healing. The worm burrows were marked by heavy infiltrations of neutrophils and macrophages and the generalized necrosis could be distinguished by the concentration of lymphocytes in addition to the neutrophils and macrophages. The acute phase of the infestation with *H. microstoma*, 6 to 20 days after infestation, occurred 43 to 57 days after the infestation with *F. hepatica*. At that time, focal lesions from the infestation with *H. microstoma* could be clearly distinguished from pathology from the *F. hepatica* infestation by the predominance of neutrophils in the lesions, even though lymphocytes remained prevalent in the sinusoids of the liver.

In addition to the pathology normally observed in single species infestations with these species of worms, 2 types of tissue damage were observed in Sequence I. One pathology observed only in the mice of the concurrent infestation group was the reinstatement of the generalized necrosis characteristic of the acute phase of the infestation with *F. hepatica*. Small areas of the liver were first observed to be affected on Day 60. As the time of the concurrent infestation lengthened, more of the liver was involved, until as much as 50 percent of the organ was necrotic on Day 100. That necrosis was associated with accumulations of lymphocytes in the liver parenchyma and the periphery of the necrotic zone (Fig. 1). Neutrophils were the most prevalent cells within the necrotic areas.

A second type of pathology associated with the concurrent infestation was the presence of eggs of *F. hepatica* in the tissue of the mouse. At Day 50, eggs were first observed in intimate contact with the tissue of the common bile duct in areas where the epithelial lining had been sloughed off (Fig. 2). By Day 70, eggs were in the tissue of the wall of the common bile duct in these exposed areas (Fig. 3). At Day 80, eggs were observed in the tissues of the wall of the common bile duct, the wall of the cystic duct, the gall bladder, and in the liver itself (Figs. 4, 5). Later, eggs were observed in pancreatic ducts, lobular bile ducts, throughout the liver tissue, and some were encapsulated in the peritoneal cavity. The eggs had various degrees of cellular reactions around them. The infiltrating cells were a mixture of neutrophils (the dominant cell type), eosinophils, lymphocytes, and macrophages (Fig. 6).

In Sequence II, mice of the concurrent infestation group were entering the chronic phase of the infestation with *H. microstoma* at the time *F. hepatica* were given. At that time, focal lesions were common in the parenchyma of the liver, and leukocytes (predominantly neutrophils) were abundant in the sinusoids. Those conditions did not affect the liver migration of the *F. hepatica* and there was no reaction around the juvenile flukes. The onset of general-



FIGS. 5, 6. Pathology in mice resulting from concurrent infestations with *Fasciola hepatica* and *Hymenolepis microstoma*. Fig. 5. Eggs of *F. hepatica* in a large pocket in the tissue of the liver, Day 80. ×140. Fig. 6. Egg of *F. hepatica* in the wall of the common bile duct on Day 80. Note the concentric whorls of leukocytes around the egg. ×360.

ized necrosis in the livers of the mice of the concurrent infestation group was similar to that observed in the *F. hepatica* control mice. There was, however, a difference in the longevity of the necrosis. The generalized necrosis was present in the livers of the mice of the concurrent infestation group on Day 90, 90 days after infestation with *F. hepatica*. The extension of generalized necrosis occurred even though the flukes had entered the common bile duct at the normal time for mice, 30 to 35 days after infestation.

In Sequence III, the infestations with *F. hepatica* and *H. microstoma* were administered so that the midacute phase of each infestation occurred on Day 25. At that time, numerous neutrophils and lymphocytes were present in the sinusoids of the liver and generalized necrosis was widespread. The number of leukocytes appeared to be, at a minimum, totally additive in response to the acute phase of each infestation. *F. hepatica* were observed in the common bile duct of the mice of the concurrent infestation group at that time, 5–10 days earlier than in mice of the *F. hepatica* control group.

Eggs of *F. hepatica* were observed in the walls of lobular bile ducts and in the liver tissue of 1 mouse on Day 50. It was the only mouse in Sequence III in which that condition was observed, and the occurrence must have been irregular. The eggs were surrounded with infiltrating leukocytes in the same manner as previously described for Sequence I.

DISCUSSION

As expected, pathologies normally observed in infestations of mice with *F. hepatica* and *H. microstoma* were observed in concurrent infestations with those parasites during the present experiments. Individual pathologies occurred irrespective of the sequence or timing of the infestations as

independent functions of their initiators. Only in Sequence III was there any indication that there might be a synergistic effect of the 2 infestations. In that case, the enormous increase in the number of leukocytes in the sinusoids of the liver in response to the synchronized acute phases may have been more than additive, but quantitative measurement of a response of that nature is difficult.

The pathologies observed in concurrent infestations but not in single species control mice may have been related to a partial or complete blockage of the bile flow resulting from the worm mass of the combined infestations in the common bile duct. Gleason (1974) reported that when an infestation with *H. microstoma* was imposed upon a patent infestation with *F. hepatica*, there was a shift of attachment sites of the cestodes into the proximal region of the common bile duct. That shift brought the scoleces of the cestodes and much of the strobila into intimate contact with the *F. hepatica* present in that region.

The reinstatement or prolongation of generalized necrosis in livers of mice of the concurrent infestation groups of Sequence I and II was not as widespread as during the acute phase of an initial infestation with *F. hepatica*. However, it did affect large areas of the liver. It is probable that this pathology can be attributed to reduction or stoppage of bile flow. Under those conditions, antigenic material present in bile from adult *F. hepatica* in the lumen of the common bile duct would back up into the liver through the intrahepatic ducts. In the liver, the antigenic material would come into contact with sensitized lymphocytes that remain in the liver (Lang 1967). The reaction between the antigenic material and the sensitized lymphocytes would then induce the generalized necrosis.

The second pathology present in livers of mice of the concurrent infestation group of Sequence I and one mouse of Sequence III, but not in the single species control mice, was the presence of eggs of *F. hepatica* in the tissue. That condition could have been caused by the accumulation of eggs in the proximal region of the common bile duct due to a decreased flow of bile. Once trapped in the proximal region, the eggs became entangled in the serous exudate at breaks in the epithelial lining of the common bile duct and were later forced into the tissues through a combination of fluid pressure and mechanical pressure by the worms.

Urquhart (1956) found eggs of *F. hepatica* in the hepatic and biliary tissues of rabbits. He postulated that the eggs entered the tissues through breaks in the epithelial lining of the intrahepatic ducts caused by adult flukes. The eggs were found singly and in clusters, much the same as observed during the present experiments. In the rabbit, however, adult *F. hepatica* were in the intrahepatic bile ducts, while in the mouse the flukes were in the proximal regions of the common bile duct. Thus, in rabbits, more eggs were found in the hepatic tissues. After the eggs entered the tissue, the reaction of the mouse to the eggs was similar to that described by Urquhart (1956) for rabbits. The eggs were invaded by neutrophils, eosinophils, and macrophages. Later, a specific type of granuloma was produced when the eggs were surrounded by concentric whorls of neutrophils, macrophages, and fibroblasts.

The failure to find eggs of *F. hepatica* in tissues of *F. hepatica* control mice, mice of the concurrent infestation in Sequence II, and rarely in mice of the concurrent infestation in Sequence III, would indicate that the timing and sequence of infestation used in Sequence I provided the conditions necessary to force the eggs into the tissues. This probably is correlated with the findings of Gleason (1974) that there was no proximal shift in the attachment sites of *H. microstoma* when an infestation with *F. hepatica* was imposed upon a patent infestation with *H. microstoma* or when the infestations were synchronized so that the midacute phase of each infestation occurred simultaneously.

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