THE PATHOGENESIS OF "CLAY-PIPE STEM CIRRHOSIS" IN MICE WITH CHRONIC SCHISTOSOMIASIS MANSONI, WITH A NOTE ON THE LONGEVITY OF THE SCHISTOSOMES

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The hepatic lesion of hepatosplenic schistosomiasis mansoni is characterized by a relatively unique type of fibrosis first described as "clay-pipe stem cirrhosis" by Symmers in 1904.[1,2] A murine model of hepatosplenic schistosomiasis has been described in which many of the characteristic features of the disease such as hepatosplenomegaly, portal hypertension and esophageal varices were present.[3] These animals were heavily infected, however, surviving only 10 to 14 weeks after exposure to the parasite.[4] Gross liver fibrosis was not evident, the pathologic pattern being characterized primarily by granulomas surrounding schistosome eggs in the portal areas.[4] Further studies revealed that mice harboring as few as one pair of worms could develop hepatosplenic disease and survive indefinitely.[5] In such a group of animals infected for 25 weeks, early clay pipestem-like lesions were observed.[6]

The present study was performed in mice infected with *S. mansoni* for as long as 52 weeks. Infections were relatively light and the average worm burdens were uniform over the course of one year. Not only did broad fibrotic bands resembling "clay-pipe stem cirrhosis" develop in the livers of many of the animals but a study of the evolution of these lesions in liver sections has apparently revealed their pathogenesis.

MATERIAL AND METHODS

This study was performed on 100 Swiss albino female mice, 18 to 22 gm in weight, which had been exposed to 35 cercarias of a Puerto Rican strain of *S. mansoni*. The worm burden at 8, 16, 24, 32 and 52 weeks after exposure was determined in 45 of these animals by perfusion of their portal and mesenteric vessels (Table I).[7]

The remainder of the mice (Table II) were weighed and then anesthetized with pentobarbital injected intraperitoneally. A laparotomy was performed and a 22-gauge needle connected to a Sanborn pressure transducer and cathode ray oscilloscope was introduced into the portal vein. After the needle was withdrawn the chest was opened and the thoracic esophagus was examined for the presence of esophageal varices. The

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liver and spleen were removed and weighed. A small portion of the liver (10 to 20 per cent) was preserved in buffered formalin for sectioning and staining with hematoxylin and eosin and Mallory trichrome.

The diameters of the granulomas were measured in stained sections with a Cooke-A.E.I. Image Splitting Measuring Eyepiece. The remainder of the liver was reweighed and preserved at −3°C for egg counts or hydroxyproline determinations. Egg counts

TABLE I

WORM BURDENS OF MICE EXPOSED TO 35 *S. mansoni* CERCARIAS AND STUDIED AT INTERVALS OVER A PERIOD OF ONE YEAR

Intervals (weeks)	Number of animals	Average number of worms	Average number of worm pairs
8	12	8.08 ± .96 *	3.17 ± .49 *
16	9	8.44 ± .88	3.44 ± .56
24	10	8.20 ± .68	3.20 ± .42
32	10	7.30 ± .94	2.90 ± .53
44–49	2	7.00	3.50
52	2	7.50	2.00

* Standard error.

were done by digesting the liver in 200 ml of a 4 per cent solution of potassium hydroxide and counting a 1 ml aliquot in a Sedgewick-Rafter counting chamber.

Collagen measurements were made by hydrolyzing weighed portions of the livers in 6 N HCl at 120° C for 3 hours and analyzing the hydroxyproline content of an aliquot by a modification of the Neuman and Logan procedure.[8] In order to determine the specificity of the analysis, aliquots from all of the control livers and aliquots chosen at random among infected livers at each time interval studied from 8 to 52 weeks, were placed on a 30 cm Dowex 50 ion-exchange column (× 8, 200 to 400 mesh) and proline and hydroxyproline were separated by a modification of the method of Rogers, Kimmel, Hutchin and Harter.[9] It was found that the amino acid levels in the livers of the infected animals were on the average 12.5 per cent lower after column separation than by direct measurement. The final results were all corrected by this factor.

RESULTS

Perfusion 8 weeks after exposure to 35 cercarias of a Puerto Rican strain of *S. mansoni* revealed that the mice harbored an average of 8 worms (3 pairs). Studies performed at 16, 24, 32 and 52 weeks revealed similar worm burdens (Table I). It was impossible to calculate the mortality among these lightly infected animals because groups of mice were sacrificed at various time intervals beginning at 8 weeks. Only a relatively small proportion (14 per cent) of those originally infected for this study died naturally, however, and these deaths occurred from 12 to 49 weeks after exposure with no clustering at any points.

Eight weeks following exposure to *S. mansoni* (Table II) liver weight as per cent of body weight was 42 per cent greater than that of control uninfected animals. The peak liver weight, only slightly higher than that at 8 weeks, was found at 16 weeks, and thereafter there was a gradual

TABLE II

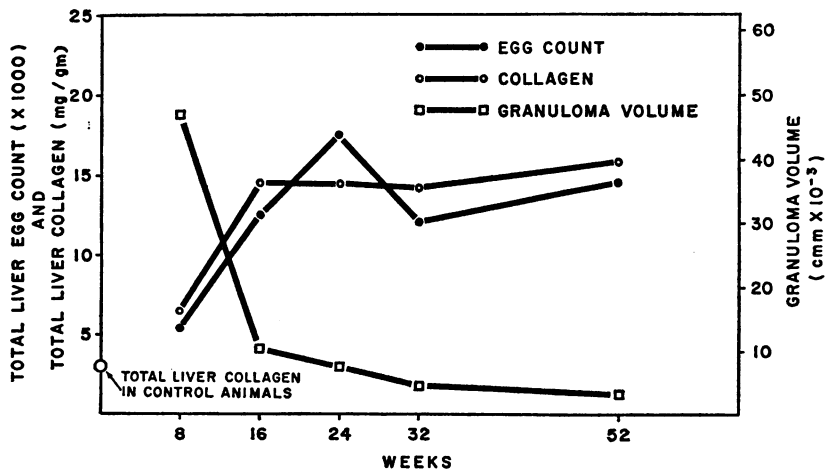
PATHOPHYSIOLOGIC STUDIES IN MICE HARBORING AN AVERAGE OF 3 *S. mansoni* WORM PAIRS OVER A ONE-YEAR PERIOD

	Control	Weeks after exposure 8	16	24	32	52
Number of animals	10	12	12	12	12	7
Body weight (gm)	26.2 ± .79 *	23.2 ± .77	25.5 ± 1.30	29.3 ± .98	28.2 ± .63	30.9 ± 1.36
Liver weight (mg)	1379 ± 55	1734 ± 94	1915 ± 154	2173 ± 119	1983 ± 94	1963 ± 151
Spleen weight (mg)	163 ± 6	358 ± 40	534 ± 50	587 ± 79	383 ± 41	348 ± 81
Portal pressure (cm H_2O)	4.8 ± .33	6.3 ± .39	12.3 ± .96	10.5 ± .76	10.1 ± 1.08	9.1 ± 1.86
Esophageal varices (per cent animals)	0	0	67	90	58	80
Egg count (eggs/whole liver) †	0	5,541 ± 1126	12,721 ± 3363	17,768 ± 3592	12,000 ± 1602	14,394 ± 1784
Granuloma diameter (microns) †	0	446 ± 16	270 ± 6	241 ± 6	215 ± 4	194 ± 6
Liver collagen (mg, wet weight whole liver) †	3.02 ± .16 ‡	6.74 ± .85	14.60 ± 2.10	14.30 ± 1.41	14.10 ± .85	15.90 ± 4.23

* Standard error of the mean.
† Averages of determinations made in 6 livers.
‡ Group of 6 older control mice, average body weight 34.3gm.

decline. By 8 weeks the spleen in the infected mice had increased in weight by 120 per cent, and reached a peak at 24 weeks when there was an overall increase of 260 per cent (Table II). At 52 weeks the spleens averaged the same weight as at 8 weeks. There was only a slight increase of portal pressure by 8 weeks. Its maximum was reached at 16 weeks when it was 156 per cent higher than the control level. Although no esophageal varices were seen at 8 weeks, they were present at 16 weeks in 67 per cent of the infected animals and were observed thereafter in a large proportion of the animals (Table II).

Within 8 weeks after infection the average mouse liver contained over 5,000 schistosome eggs. The peak liver egg content was reached at 24 weeks and fell thereafter (Table II, Text-fig. 1). The maximal average diameter of the granulomas occurred at 8 weeks; by 16 weeks it had decreased markedly, and continued to fall steadily to its lowest level at 52 weeks (Table II). Calculating the average granuloma volume from the measured diameter revealed an even more striking change. The average granuloma volume at 16 weeks was only 22 per cent of that at 8 weeks; by 52 weeks it was 8 per cent of the peak level (Text-fig. 1). When only the period between 16 and 52 weeks was considered, there was a steady and considerable decrease in granuloma volume at each time interval studied. Thus the volume at 52 weeks was only 37 per cent of that found at 16 weeks. The total liver collagen concentration more than doubled by 8 weeks, but it rose to almost 5 times control levels by 16 weeks. Thereafter it remained relatively constant with peak levels occurring at 52 weeks (Table II, Text-fig. 1).



TEXT-FIG. 1. Numbers of schistosome eggs, volume of granulomas surrounding them and collagen content of the livers of mice at various time periods from 8 to 52 weeks after infection with *S. mansoni.*

Liver sections of animals infected for 8 weeks revealed large granulomas surrounding schistosome eggs (Fig. 1). Necrosis was seen in the center of some of the granulomas and the most prominent inflammatory cell was the eosinophil (Fig. 1). When examined at a lower magnification, the granulomas appeared to be scattered essentially within the portal areas (Fig. 4). In animals infected for 16 weeks marked changes were characterized by much smaller granulomas and condensed collagen. The inflammatory response continued to be quite marked at the periphery of the granulomas and eosinophils remained predominant. At this point the granulomas appeared to be occurring in a pattern in many of the animals, seeming to line up into strands (Figs. 2 and 5). By 32 weeks the granulomas were even smaller, the fibrous tissue appeared more dense, inflammation was reduced (macrophages and lymphocytes were now the predominant cells), and many schistosome eggs appeared to be undergoing resorption (Fig. 6). The strands which had been of the thickness of single granulomas were thickened by the addition of more granulomas along the sides and broad clay pipestem-like lesions were beginning to appear in many livers (Figs. 3 and 6). Fifty-two weeks after infection the fibrosis was even more pronounced in some of the livers (Fig. 7). In one of the animals at 52 weeks the various stages in the evolution of the clay pipestems were apparent, one stem being made up almost wholly of individual granulomas in which the schistosome eggs were only partially resorbed and others where eggs were almost completely resorbed and the collagen appeared to have lost its orientation (Fig. 7). The pattern in a section from an operative wedge liver biopsy from a 34-year-old woman with compensated hepatosplenic schistosomiasis proved to be comparable (Fig. 8).

Schistosome pigment first became prominent at 16 weeks. By 52 weeks very large amounts were present, mainly in macrophages at the periphery of fibrotic areas.

Discussion

Symmers described the classic hepatic pathologic lesion in schistosomiasis mansoni before he was even aware that specific species of the parasite existed and prior to any knowledge of a liver disease related infection with schistosomes.[1] At the time he wrote his paper it was believed that all schistosomiasis in Egypt was due to *S. hematobium,* but his observation that all of the schistosome eggs in the liver had lateral rather than terminal spines, indicated that the parasite must have been what was subsequently called *S. mansoni.* In addition, it was not until more than 20 years later that the common disease, Egyptian splenomegaly, was shown

to be of schistosomal origin.[10] Symmers not only described the pathologic features of the liver resulting from schistosomiasis mansoni, he also stated that "this cirrhosis is, I believe, due to the presence of bilharzia ova. . . "[1] Since then, however, the hepatosplenic disease has been attributed to other factors, including malnutrition, dead schistosome worms, and a toxin secreted by living schistosomes.[2]

Studies in the experimental animal have proceeded in a manner different from those in humans.[11] For many years investigators claimed various causations for the hepatic histologic changes in laboratory animals infected with *S. mansoni* in spite of the fact that neither the hepatosplenic syndrome nor the classic pathologic lesion had been observed.[11] In contradistinction to the evolution of knowledge of the human disease, it was first shown that a clinical disorder resembling that of Egyptian splenomegaly occurred in mice infected with *S. mansoni*.[3,4] (A similar disease has recently been shown to occur in mice infected with *S. japonicum*.[12]) The technique used in these experiments produced heavily infested animals all of which died at relatively early stages of the disease.[3,4] The major lesion in these animals was characterized by granulomas surrounding schistosome eggs scattered throughout the liver.[4] The cause of the hepatosplenic disease in this animal model was later shown to be neither dead worms nor toxins, but the schistosome egg without which it would not occur.[13] A later series of experiments revealed that mice infected with as few as 1 pair of worms could develop hepatosplenic schistosomiasis and survive indefinitely.[5] This study continued for a maximum of 25 weeks and early clay pipestem-like lesions were observed in the livers of some of the animals.[6]

The present investigation was concerned with the production of chronic hepatosplenic disease in mice for even longer periods of time. In order to compare the disease state of the animals at various intervals over a projected time span of one year, it was necessary to achieve uniformity of infection. The average worm burden in the mice could be affected by two factors: the death of heavily infected mice or a relatively circumscribed life-span for the worm in the murine host. The former factor was controlled by infecting the animals with a low number of worms and the latter factor was obviated by the demonstration in this study that the worm burden remained constant over the course of one year. The animals in the present study not only had stable schistosome infections from 8 to 52 weeks, but they developed relatively stable hepatosplenic disease. Although there was no alteration in the worm burden, the number of eggs in the livers of these mice did not continue to rise after 16 to 24 weeks. This could be explained if the older worms became sterile, but in view of the presence of viable schistosome eggs in the liver sections at

all of the time periods studied it would appear that a steady state between the schistosome egg output and their resorption by the murine host occurred. While the average egg load thus remained relatively constant, there was a steady and considerable decrease in the average volume of collagen around the individual eggs (Text-fig. 1). Nevertheless, the total liver collagen content, which also reached its peak at 16 weeks, remained constant. Thus, the collagen surrounding the eggs (number of eggs × average volume of collagen around them) decreased markedly and yet the total collagen content of the liver remained constant. This observation was confirmed by examination of the liver sections of mice infected for more than 24 weeks. Broad fibrotic bands were seen in which many of the eggs were partially or completely resorbed.

Andrade and Warren[6] have suggested that the portal hepatitis frequently seen in human and animal schistosomiasis may play a major role in the production of pipestem fibrosis.[2,6] Although portal hepatitis was observed in our animals and a slight fibrotic reaction was seen around some of the portal veins, apparently unrelated to the presence of eggs, it did not seem to be a major factor in the development of the clay pipestem fibrosis. In addition the marked intrahepatic accumulation of schistosome pigment appeared to play no direct role in the pathogenesis of this lesion.

Further studies of the sections of the liver in the present study revealed what may be the evolution of the fibrotic bands. The gradual lining up of individual granulomas into strands apparently following the course of portal tributaries as shown by Cheever in India ink-injected preparations[14] was followed by the addition of more granulomas at the peripheries of the bands. As the eggs were gradually resorbed, the residual collagen appeared to change its orientation from whorls to parallel fibers. In Symmers initial paper he noted large numbers of eggs in the fibrotic areas[1]; this has been confirmed many times since.[2] Nevertheless, many pathologists have seen fibrous bands containing few or no eggs.[2] It is entirely possible that these could have been from cases of prolonged duration in which the infection had died out and the eggs were all resorbed, leaving large amounts of relatively unresorbable collagen. It has been shown that following treatment in the acute stages of experimental schistosomiasis there is a fairly rapid and virtually complete resorption of granulomas.[15,16] Experiments are now in progress on the resorption of collagen in animals treated 40 weeks after infection.[17]

Human schistosomes are known to be capable of living and producing viable ova for more than 30 years.[18] Their average life-span, however, remains unknown. Studies of schistosomal longevity in a poor host such as the rat, indicates a life-span of less than 6 weeks.[19] The present experi-

ments in a highly susceptible host, the first and only one shown to develop a disease resembling the human disorder, have shown that the worms survive for at least one year.

Summary

The pathogenesis of Symmers' "clay-pipe stem cirrhosis," the classic pathologic lesion of human hepatosplenic schistosomiasis, has not been determined. Although schistosome eggs have been considered to be the primary pathogenetic factor, they are relatively rare in fibrotic livers at necropsy. "Clay-pipe stem cirrhosis" has therefore been ascribed to various factors such as coalescence of egg granulomas, diffusible egg or worm toxins, and chronic allergic inflammation in the portal areas.

The present study utilized a murine model hepatosplenic schistosomiasis modified by exposure to fewer cercarias. The degree of infection remained constant with an average of 7 to 8 worms per mouse during the entire period of study. Mortality was low, but hepatosplenomegaly, portal hypertension and esophageal varices occurred within 16 weeks. The development of hepatic fibrosis was studied at intervals for 52 weeks by examining sections stained by the Mallory trichrome method and measuring total liver hydroxyproline content. These observations were correlated with counts of schistosome eggs in the liver and measurements of the average diameter of the collagen around individual eggs.

Sections revealed scattered granulomas at 8 weeks followed by linear strand formation at 16 weeks and coalescence into fibrotic bands at 24 weeks and later. There was apparent resorption of the eggs themselves, as confirmed by peak egg counts at 24 weeks with gradual decline in numbers thereafter. Although the average volume of collagen around individual eggs continued to decrease after reaching its peak at 8 weeks, the highest total liver collagen levels were observed at 52 weeks. It would appear that clay pipestem cirrhosis results from the formation of fibrotic bands derived from residual collagen originating about granulomas. In addition, it was shown that the schistosomes survive for at least one year in a murine host.

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[*Illustrations follow*]

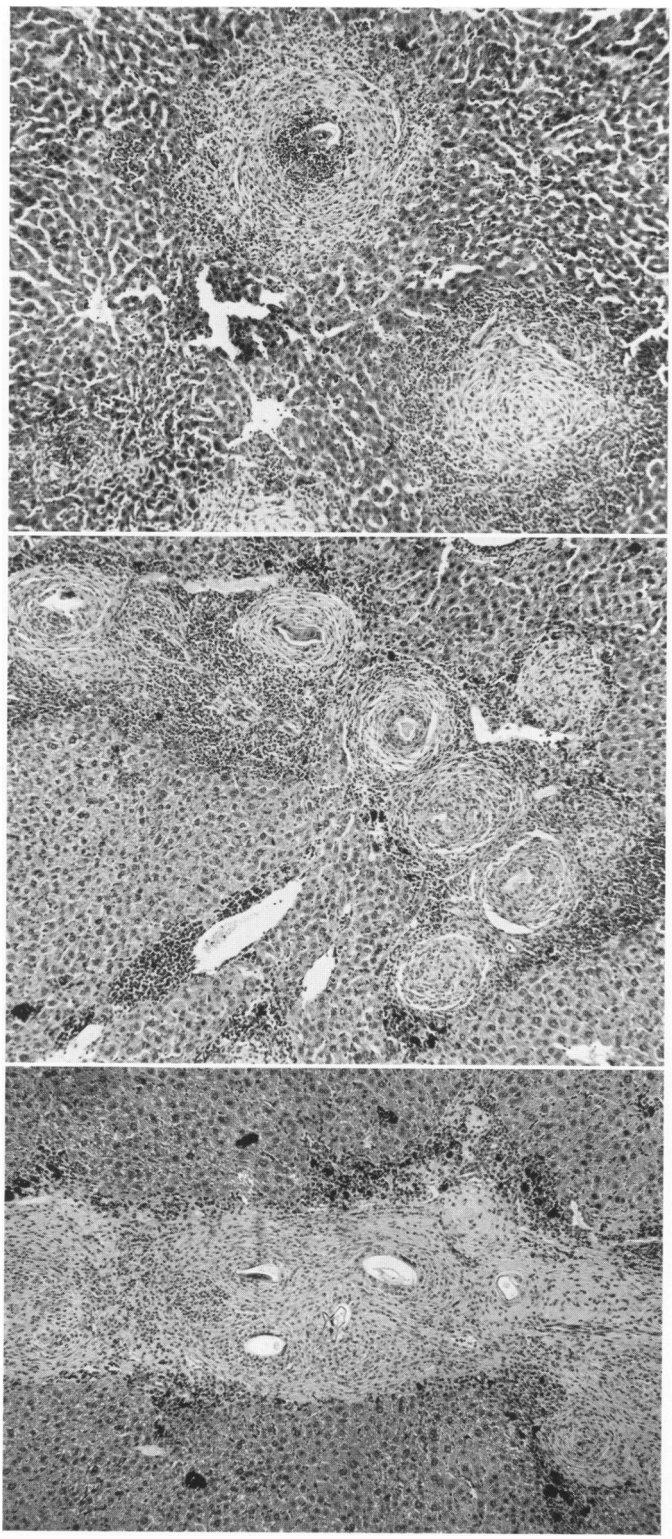
LEGENDS FOR FIGURES

Illustrations were all prepared from sections stained with hematoxylin and eosin.

FIG. 1. Liver. Mouse infected with *S. mansoni* 8 weeks previously. The granulomas are large; the eosinophil is the most prominent inflammatory cell. × 75.

FIG. 2. Mouse infected with *S. mansoni* 16 weeks previously. The granulomas are much smaller and the collagen condensed. The eosinophil continues to be the predominant inflammatory cell. × 75.

FIG. 3. Mouse infected with *S. mansoni* 32 weeks previously. The schistosome eggs are in various stages of resorption. The collagen appears to be changing its orientation from concentric whorls to parallel fibers. Inflammation is less intense and the predominant cells are macrophages and lymphocytes. × 75.



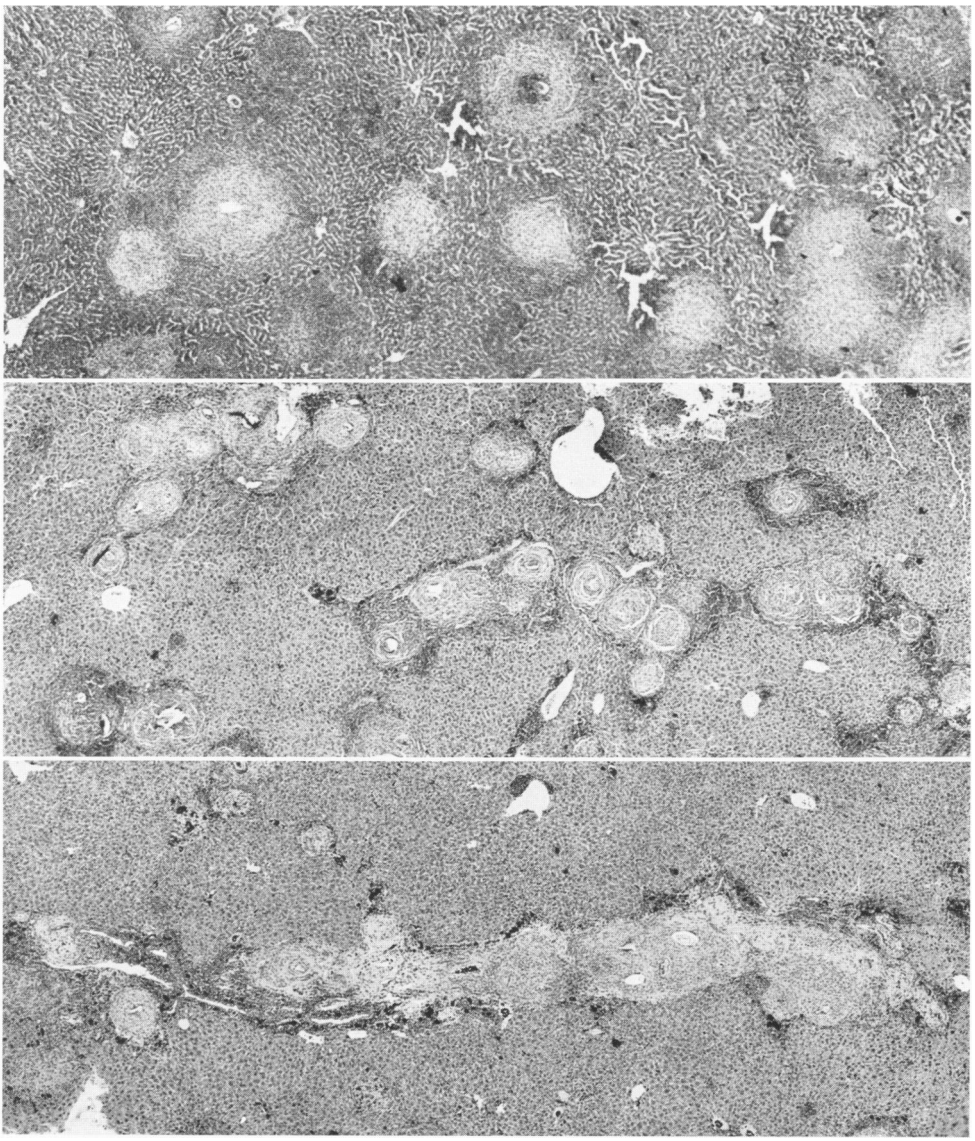


FIG. 4. Mouse infected with *S. mansoni* 8 weeks previously. The large florid granulomas surrounding schistosome eggs are scattered about the portal areas. × 36.

FIG. 5. Mouse infected with *S. mansoni* 16 weeks previously. The much smaller granulomas appear to be lining up into strands following the portal tributaries. × 36.

FIG. 6. Mouse infected with *S. mansoni* 32 weeks previously. Bands more than one granuloma thick have formed. In these many of the eggs have been resorbed and the collagen has been altered from a concentric to a parallel orientation. × 36.

FIG. 7. Mouse infected with *S. mansoni* 52 weeks previously. The apparent evolution of the "clay-pipe stem" pattern is illustrated in this one section. Some of the stems appear to be made up primarily of many small granulomas and others of partially resorbed eggs around which the collagen has changed its orientation. × 36.

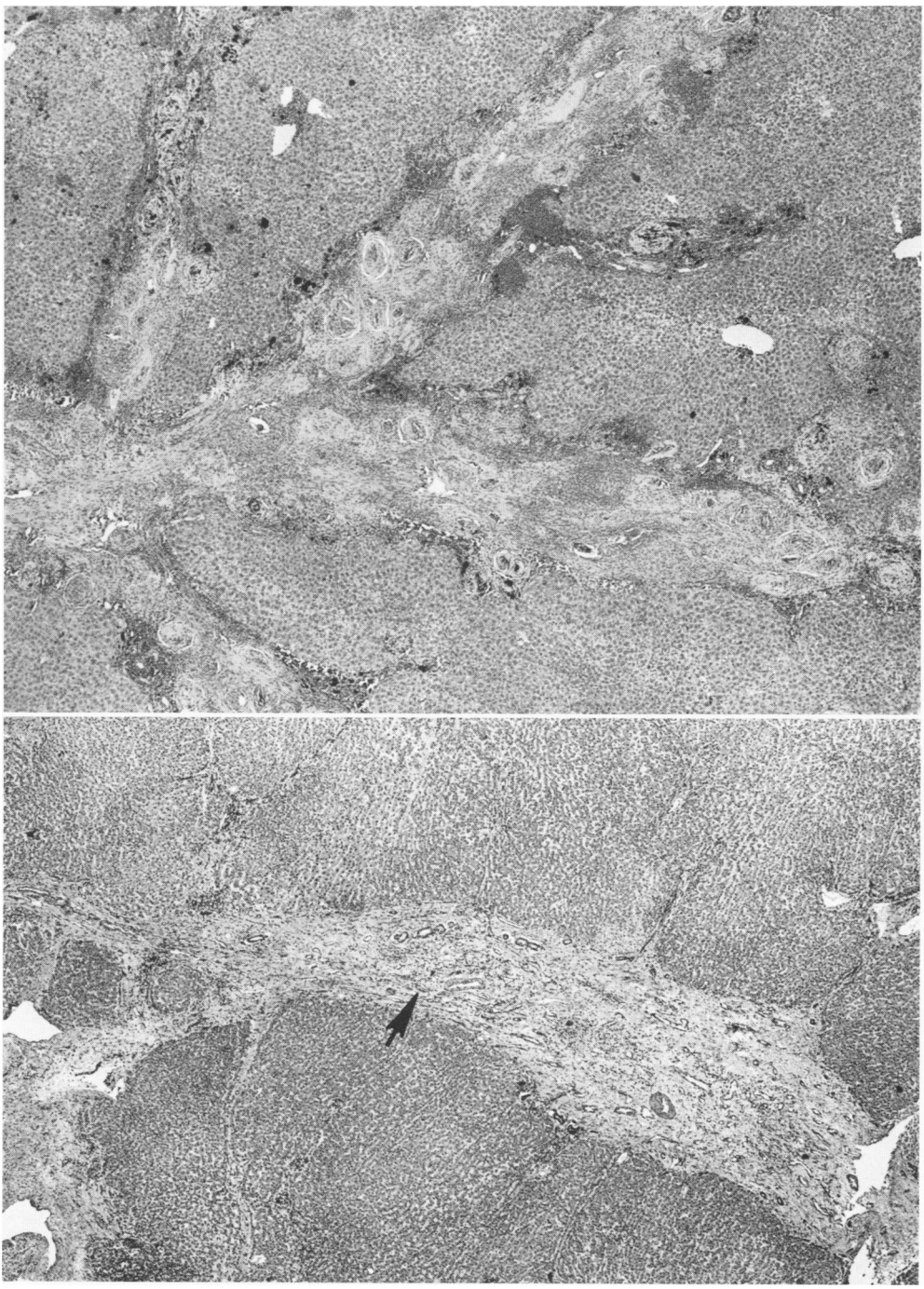


FIG. 8. Operative wedge biopsy in a liver of a 34-year-old white woman with compensated hepatosplenic schistosomiasis. This was presumably an infection of many years duration as schistosomiasis is usually acquired in childhood. One dead, partially resorbed egg (arrow) is observed in the fibrous band and even the collagen surrounding it has a parallel orientation. × 36.