Schistosoma mansoni Infection in Mice Depleted of Thymus-Dependent Lymphocytes

II. Pathology and Altered Pathogenesis

Richard D. Buchanan, MD, Douglas P. Fine, MD and
Daniel G. Colley, PhD

Murine schistosomiasis mansoni is a more rapidly fatal disease in hosts deprived of their cell-mediated immune capabilities. Analysis of the histopathology of the disease under these circumstances indicates that rather than the hepatic granulomata characteristic of the normal infection, the host develops zones of liquefactive necrosis in the liver and intestinal mucosa. These lesions are associated with severe parenchymal cell destruction. Such hepatic and mucosal damage, with subsequent toxemia and septicemia, is presumed central to the altered course of the disease (Am J Pathol 71:207–218, 1973).

THE PREVIOUS PAPER demonstrated the pattern of altered immune responses in mice subjected to thymus-dependent (T) lymphocyte depletion and subsequently infected with *Schistosoma mansoni*.[1] Such altered hosts failed to develop cellular hypersensitivity, as manifested by lymphocyte blastogenesis in response to specific antigenic challenge with a soluble schistosomal egg antigen preparation (SEA) and by delayed skin reactivity in response to intracutaneous injection of SEA; furthermore, they failed to develop heat-labile reaginic antibody which mediates passive cutaneous anaphylaxis in the rat. However, heat-stable agglutinating antibodies and antibodies mediating the early dermal response to intracutaneous SEA injection were present in amounts equal to those found in immunologically intact mice with S *mansoni* infection.

Most importantly, T-lymphocyte-depleted mice succumbed earlier during the course of the infection than did normal infected mice. The present study was undertaken to analyze the histopathologic features of schistosomiasis in these altered hosts.

From the Departments of Pathology, Medicine and Microbiology, Vanderbilt University School of Medicine and the Veterans Administration Hospital, Nashville, Tenn.

Supported by Grants HE 08399 and AI 11289 from the National Institutes of Health, US Public Health Service and the Veterans Administration.

Accepted for publication Feb 5, 1973.

Address reprint requests to Dr. Daniel G. Colley, Immunologist, Veterans Administration Hospital, Nashville, TN 37203.

Materials and Methods

The methods of T-lymphocyte depletion and infection were presented in the companion paper [1] and the abbreviations employed below were defined in detail therein. Briefly, these are as follows: (Tx) thymectomized at 5 to 7 weeks of age, (X) irradiated with 850 R total body irradiation, (BM) reconstituted with 5×10^6 nucleated syngeneic bone marrow cells, (Inf) infected with 75 *S mansoni* cercariae and (ATS) rabbit antimouse thymocyte serum. The ATS was administered in two regimens, consisting of a total of 1.2 ml each, injected intraperitoneally. The first regimen was begun immediately preceding the infection (Inf) and the second at 4 weeks postinfection. The two T-lymphocyte-depleted groups are therefore designated Tx-X-BM-Inf and Tx-ATS-Inf-ATS, while the normally infected, immunologically intact group is referred to as Inf or Regular-Inf. Adult worm burdens were determined by perfusion of the mesenteric and portal veins.[2] Three to 6 mice were sacrificed with ether at various times after infection and autopsied. The liver and spleen were removed, weighed and fixed in 10% neutral buffered formalin. Kidneys, lungs, thymus glands (or in previously thymectomized animals that area from which the thymus had been removed) and saline-washed intestines were also removed and fixed in 10% neutral buffered formalin.

Fixed organs were examined at 10 × with a dissecting microscope, and selected tissue blocks were embedded in Paraplast (Sherwood Medical Industries, St. Louis, Mo) for sectioning. Conventional 5-μ hematoxylin and eosin sections were employed for general observations. The Brown-Brenn stain was used to identify microorganisms in tissues.[3] Reticulin preparations [3] were used as necessary to clarify structural details in the reticuloendothelial system. Collagen was identified with a simple polarizing microscope.

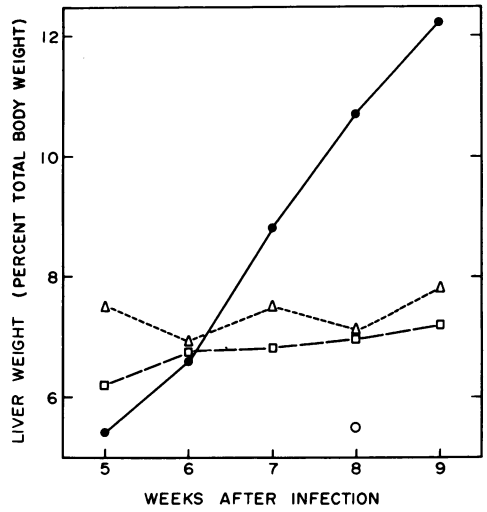
Results

Worm Burdens

The average worm burden in one group of T-lymphocyte-depleted (Tx-X-BM-Inf) mice 5 weeks after infection was 32; this figure remained relatively constant until 9 weeks postinfection, when it fell to 9 worms. For the immunosuppressed group (Tx-ATS-Inf-ATS) the average count at 5 weeks was 40 worms; by 8 weeks postinfection the figure fell to 17; the 1 animal remaining alive at 9 weeks had 11 worms. Normal (Inf) mice had average worm burdens of 36 to 40 which remained stable throughout the 5 to 9 week postinfection period.

Organ Weights

Text-figure 1 shows average serial liver weights expressed as percent of total body weight. In immunosuppressed mice at 5 weeks postinfection (when egg production begins), the liver was 6 to 7.5% of the total body weight, and this did not increase subsequently. In immunologically intact mice (Inf), liver weight began to increase at 6 weeks and rose by 9 weeks to greater than 12%. A similar pattern was seen with the average spleen weights (Text-figure 2), again expressed as percent total body

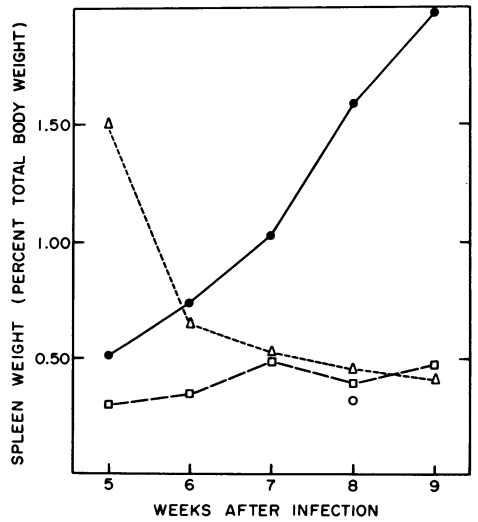


TEXT-FIG 1—Liver weight (expressed as percent total body weight) determined serially in mice Inf only (*solid circles*), Tx-X-BM-Inf (*open squares*) and Tx-ATS-Inf-ATS (*open triangles*). (Liver weight value of age-matched control (uninfected) mice, *open circle*).

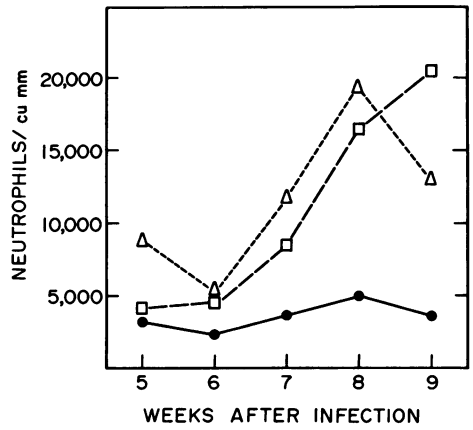
weight. Spleens from normal infected mice increased from 0.50 to almost 2% of total body weight. At 5 weeks (1 week after their second course of ATS), spleens of ATS immunosuppressed mice were enlarged (1.50%), but by 6 weeks they had fallen to 0.65% and remained within the uninfected range thereafter.

Peripheral Leucocytosis

As demonstrated in Text-figure 3, immunologically intact animals (Inf) displayed only slightly variable neutrophil counts from 5 to 9 weeks



TEXT-FIG 2—Spleen weight (expressed as percent total body weight) determined serially in mice Inf only (*solid circles*), Tx-X-BM-Inf (*open squares*) and Tx-ATS-Inf-ATS (*open triangles*). (Spleen weight values of age-matched control (uninfected) mice, *open circle*).



TEXT-FIG 3—Peripheral neutrophil counts (in cells per cu mm) determined serially in mice Inf only (*solid circles*), Tx-X-BM-Inf (*open squares*) and Tx-ATS-Inf-ATS (*open triangles*).

following infection. However, the T-lymphocyte-depleted mice developed marked leukocytosis beginning at 7 weeks after infection.

Histopathologic Features

The lymphoid tissue (spleen, lymph nodes and intestine) of infected immunologically intact animals appeared to be very active, with prominent germinal centers and packed medullary areas. In contrast, the nonfollicular areas of the immunosuppressed animals appeared to be depleted of cells, whereas the germinal centers remained active and normal in appearance. No clear difference was noted between lymphoid tissues in the Tx-X-BM-Inf and the Tx-ATS-Inf-ATS groups, although the degree of lymphoid depletion seemed more constant in the Tx-X-BM-Inf animals. Ova were rarely encountered in the spleen in both intact and immunosuppressed animals.

In intact animals, ovum embolization produced the expected tissue changes in the liver—*ie*, progressive injury of the intrahepatic vascular bed with granuloma formation and periportal reparative fibrosis (Figure 1). Thrombosis of major portal vein branches was infrequent. Small areas of periportal coagulative parenchymal necrosis were frequently seen, usually occuring in proximity to a trapped ovum. Broad areas of parenchymal destruction with collapse were not present. Fibrosis in the granulomas and adjacent periportal tissue was well established by 8 weeks after infection. The liver lesions developed quite differently in the immunosuppressed animals. Granulomas were not observed; the trapped eggs were surrounded by a relatively cell free zone containing fragmented neutrophils and cell debris, with a rim of intact neutrophils at the periphery. Toxic changes often striking in degree were usually

present in the periportal hepatocytes. Little fibroblastic proliferation was observed even in the longest survivors. Most hepatic lesions contained many gram-negative rods and occasional gram-positive organisms. Some animals had widespread bacterial hepatitis with extensive hepatocyte necrosis, but in most the low power microscopic pattern was one of interconnected, largely periportal, poorly contained abscesses containing ova in various stages of preservation. No difference was obvious between the liver lesions of the Tx-X-BM-Inf and the Tx-ATS-Inf-ATS animals (Figures 2 and 3).

The immunosuppressed animals also failed to develop the gut granulomas observed in intact animals (Figure 4). The ova appeared to lie in and near vessels, with only a sparse and nondescript inflammatory reaction (Figure 5). A few of the eggs near the gut surface were surrounded by neutrophils. About half of the immunosuppressed animals had tiny serpiginous hemorrhagic ulcers in the ileum and colon (Figure 6). It was not possible to establish a constant relationship of this lesion to either adult worms or eggs. The presence or absence of this lesion did not appear to correlate with the type of liver lesion present. Extensive small bowel mucosal hemorrhage was noted in 3 of the intact animals.

Granulomatous pneumonitis due to ovum embolization was frequent in the 8 and 9 week intact animals, but was not observed in the immunosuppressed groups. Most of the necropsied immunosuppressed animals had patchy bronchopneumonia; bacteria were demonstrated in the lung tissue only occasionally.

In sections of spleen obtained from the Tx-ATS-Inf-ATS group at 5 weeks, the sinusoids were packed with fragmented cells and neutrophils. This feature was not nearly so striking in later material from this group. Spleen collagenization and/or hemosiderosis was not evident in either intact or immunosuppressed animal groups. No specific renal lesions were present in any of the animals through 9 weeks postinfection.

It should be emphasized that immunosuppressed but uninfected control mice remained healthy, and their peripheral blood neutrophil counts remained stable for more than 6 months.

Discussion

Experimental chronic schistosomiasis in the immunologically intact mouse is characterized by cellular immune reactivity to antigens from schistosomal eggs.[4] The most significant lesion in the disease is hepatic granuloma formation around the egg, with progressive periportal fibrosis ultimately producing portal hypertension.[5] This sequence of events is

accompanied by the development of lymphocyte blastogenic response to SEA at 6 weeks postinfection,[6] by the development of delayed intradermal hyperactivity to SEA at about the same time,[7] by the development of humoral immune response to SEA as measured by ear swelling,[7] PCA[1] and Bentonite agglutination,[1] and by the development of a marked peripheral blood eosinophilia.[8,9] The liver enlarges, becoming by 9 weeks approximately 12% of the entire body weight, as opposed to a normal of about 6% (Text-figure 1). Simultaneously, the spleen weight increases from 0.5 to 2.0% body weight. These weight changes appear to reflect increased reticuloendothelial activity and developing portal hypertension.

It seemed logical to ask whether chronic schistosomiasis, robbed of the host's delayed hypersensitivity granulomatous reaction to egg antigens, might not be a much more benign illness.[5,10] Attempts to answer this question[11,12] produced no definite evidence that any of the immunosuppressive modalities used might affect the hepatosplenic disease.

It has recently been reported[13] that the use of an antiinflammatory drug (propiomazine) in murine schistosomiasis produced partial inhibition of hepatic granuloma formation and increased the mean survival time by approximately 1 week. The mechanism by which the drug exerts this influence is at present unclear.

The present study indicates that rigorous depression of the cellular immune response can dramatically alter the course of relatively severe (worm burdens of 32 to 40) chronic murine schistosomiasis, but far from having a salutary effect, it produces a rapidly fatal systemic disease. Egg-induced granulomas are not seen, but rather the ova are surrounded by a zone of liquefactive necrosis containing cell debris and fragmented neutrophils. This hepatic lesion is often contaminated by bacteria (probably of gut origin), providing a locus from which fatal toxemia or septicemia may develop. Many of the immunosuppressed animals had tiny hemorrhagic ulcers in the ileum. The pathogenesis of this lesion was not clear from our study. It may, of course, have provided a portal of entry for invading gut flora, but identical foci of hepatic bacterial invasion occurred in animals without this gross enteric lesion. Hemorrhagic ileal lesions were noted in a few of the immunologically intact animals as well.

We are unable to clearly delineate the pathogenic mechanism which results in the egg-induced liquefactive necrotic lesions observed. It is possible that the host, incapable of mounting a cell-mediated immune response which normally circumscribes the eggs, is defenseless against the enzymatic activities and phospholipids which have been described

for schistosomal eggs and the embryos they contain.[14–18] It has been shown that sequestration and destruction of diffusible egg material is one role of the schistosomal granuloma.[19] Another explanation which might result in lesions similar to those observed involves the possible formation of immune complexes (in the absence of cell-mediated reactivity), and their subsequent interaction with complement and polymorphonuclear leucocytes, to produce tissue damage.

The decreased worm burdens observed in our T-lymphocyte-depleted mice just prior to death may be related to the developing sepsis mentioned. It has been recently shown that bacteremia with certain gram-negative microorganisms exerts a marked schistosomicidal effect on adult *S mansoni* worms.[20]

References

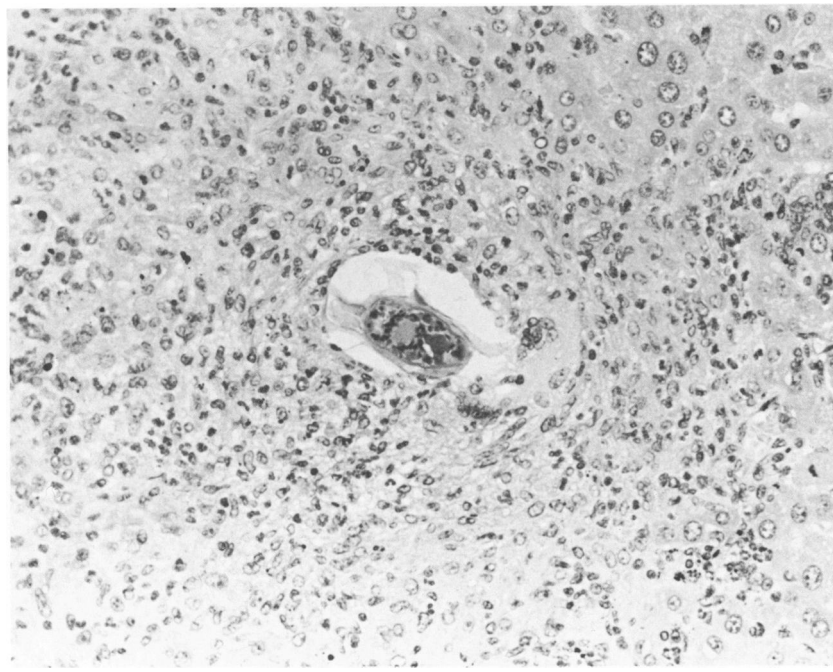
1. Fine DP, Buchanan RD, Colley DG: *Schistosoma mansoni* infection in mice depleted of thymus-dependent lymphocytes. I. Eosinophilia and immunologic responses to a schistosomal egg preparation. Am J Pathol 71:193–206, 1973
2. DuVall RH, DeWitt WB: An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am J Trop Med Hyg 16:483–486, 1967
3. Luna LG, Ed: Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, Third edition. New York, McGraw-Hill Book Company, 1968
4. Warren KS, Domingo EO, Cowan RBT: Granuloma formation around schistosome eggs as a manifestation of delayed hypersensitivity. Am J Pathol 51:735–756, 1967
5. Warren KS: Worms, Immunological Diseases. Edited by M Samter. Boston, Little, Brown and Company, 1971, pp 668–686
6. Colley DG: Schistosomal egg antigen-induced lymphocyte blastogenesis in experimental murine *Schistosoma mansoni* infection. J Immunol 107:1477–1480, 1971
7. Colley DG: Intradermal immune responses to a schistosomal egg antigen during experimental murine *Schistosoma mansoni* infection. Proc Soc Exp Biol Med 140:772–775, 1972
8. Colley DG: Development and correlation of antigen-induced lymphocyte blastogenesis and eosinophilia during murine schistosomiasis. Fed Proc 31:664, 1972 (Abstr)
9. Colley DG: *Schistosoma mansoni:* Eosinophilia and the development of lymphocyte blastogenesis in response to soluble egg antigen in inbred mice. Exp Parasitol 32:520–526, 1972.
10. von Lichtenberg F: Discussion of Warren KS, 1968, Pathophysiology and pathogenesis of hepatosplenic schistosomiasis mansoni. Bull NY Acad Med 44:280–294, 295–300, 1968
11. Domingo EO, Warren KS: The inhibition of granuloma formation around *Schistosoma mansoni* eggs. II. Thymectomy. Am J Pathol 51:757–767, 1967
12. Domingo EO, Warren KS: The inhibition of granuloma formation around

Schistosoma mansoni eggs. III. Heterologous anti-lymphocyte serum. Am J Pathol 52:613–631, 1968

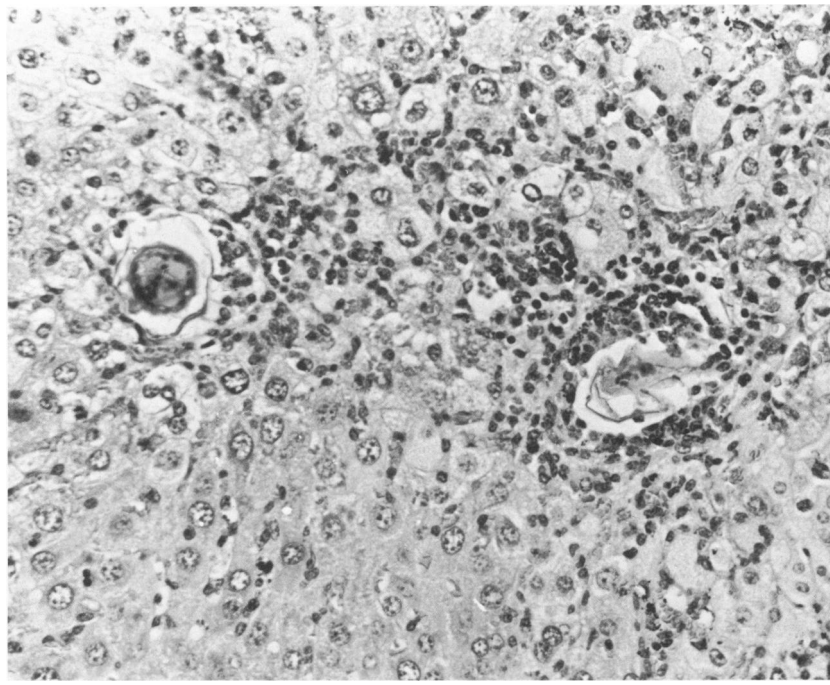
13. Henson E, Tremaine W, Lockard V, Smith T, Arhelger R, Brunson J: Inhibition of granuloma formation and prolonged survival in experimental schistosomiasis. J Reticuloendothel Soc 11:313–324, 1972
14. Lewert RM, Lee CL: Studies on the passage of helminth larvae through host tissues. I. Histochemical studies on the extracellular changes caused by penetrating larvae. II. Enzymatic activity of larvae *in vitro* and *in vivo*. J Infect Dis 95:13–51, 1954
11. Pepler WJ: Histochemical demonstration of an acetylcholinesterase in the ova of *Schistosoma mansoni*. J Histochem Cytochem 6:139–141, 1958
16. Dusanic DG: Histochemical observations of alkaline phosphatase in *Schistosoma mansoni*. J Infect Dis 105:1–8, 1959
17. Kloetzel K: A collagenase like substance produced by eggs of *Schistosoma mansoni*. *J Parasitol* 54:177–178, 1968
18. Smith TM, Lucia HL, Doughty BL, von Lichtenberg FC: The role of phospholipids in schistosome granulomas. J Infect Dis 123:629–639, 1971
19. von Lichtenberg F: Studies on granuloma formation. III. Antigen sequestration and destruction in the schistosome pseudotubercle. Am J Pathol 45:75–93, 1964
20. Ottens H, Dickerson G: Studies on the effects of bacteria on experimental schistosome infections in animals. Trans R Soc Trop Med Hyg 66:85–107, 1972

Acknowledgments

The authors thank Miss Carol Grigg for excellent technical assistance and Dr. Roger M. Des Prez for generous encouragement and discussion.



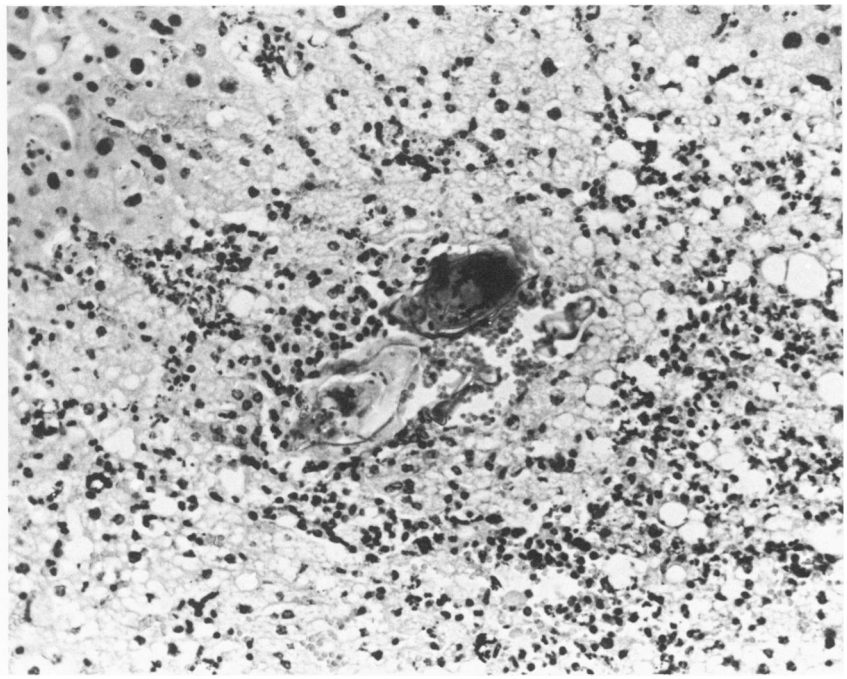
1



2

Fig 1—Normal-Inf mouse; 7 weeks postinfection. Liver with well formed portal granulomata. There is considerable collagenization. The adjacent liver cells appear normal (H & E, × 640).
Fig 2—Sacrificed Tx-ATS-Inf-ATS mouse; 7 weeks postinfection. Depressed reticuloendothelial response around eggs in portal area. Note vacuolar degeneration in hepatocytes (H & E, × 640).

3



4

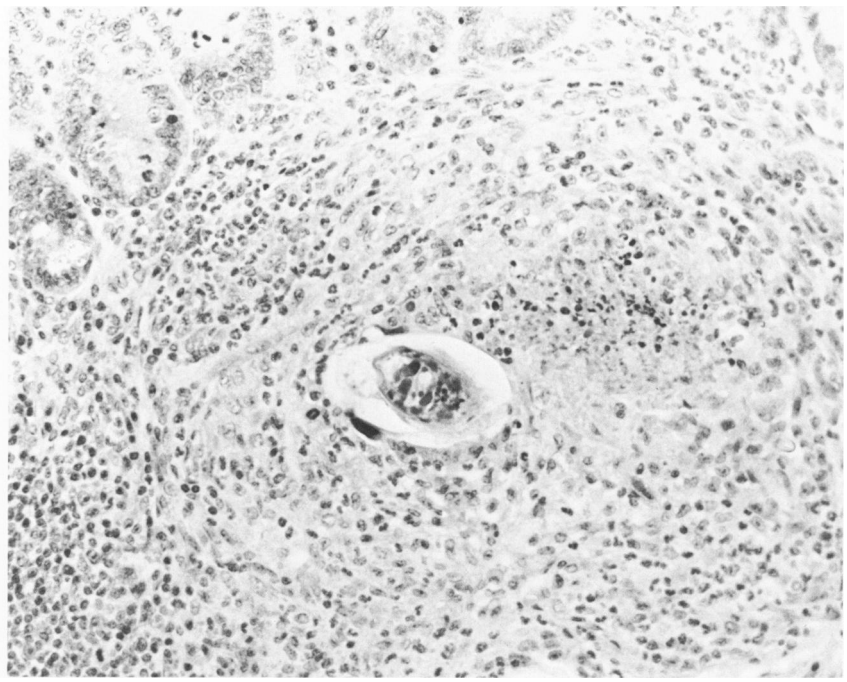
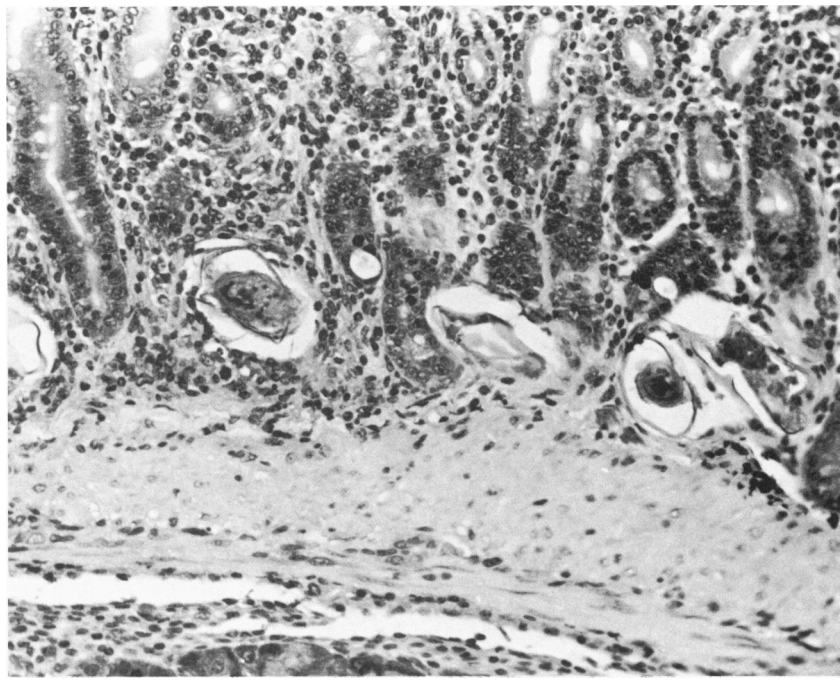
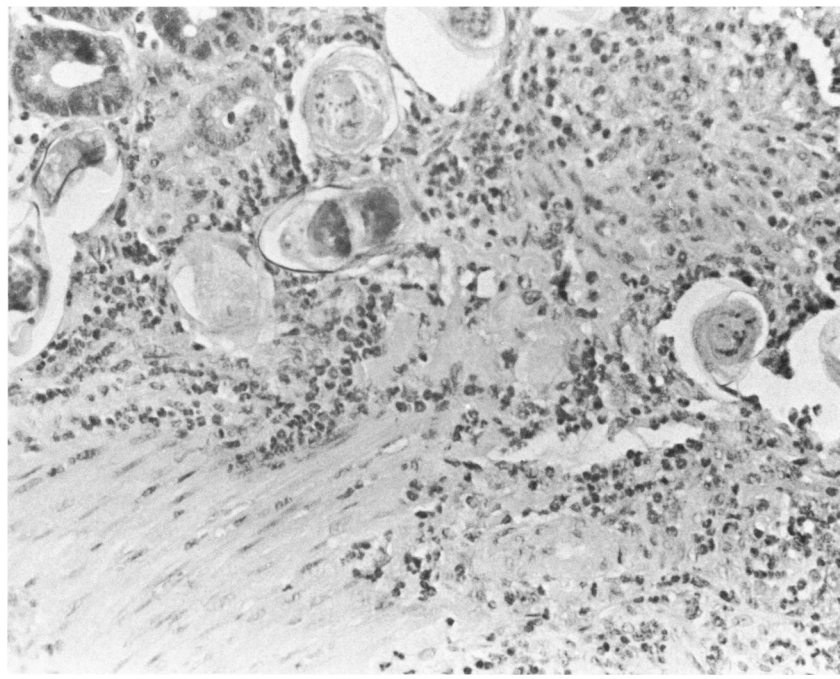


Fig 3—Autopsied Tx-ATS-Inf-ATS mouse; 7½ weeks postinfection. Extensive hepatic necrosis surrounding eggs in portal area. Lesions contain fragmented polymorphonuclear leukocytes, numerous gram-negative organisms (H & E, × 640). **Fig 4**—Normal-Inf mouse; 7 weeks postinfection. Granuloma around eggs in submucosa of ileum (H & E, × 640).



5



6

Fig 5—Sacrificed Tx-ATS-Inf-ATS mouse; 7 weeks postinfection. Several eggs in venules of the ileal mucosa. Very little inflammatory response is present (H & E, × 640). **Fig 6**—Sacrificed Tx-ATS-Inf-ATS mouse; 8 weeks postinfection. Tissue block taken from ulcerative lesion, ileum. High power micrograph of an area within a characteristic serpiginous lesion. Extensive necrosis in mucosa, muscle, surrounding group of eggs. Numerous gram-negative organisms present in lesion (H & E, × 640).

[*End of Article*]