Endogenous Desensitization: Changing Host Granulomatous Response to Schistosome Eggs at Different Stages of Infection with Schistosoma Mansoni

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THE PATHOGENESIS of murine hepatosplenic schistosomiasis is related not only to the schistosome eggs [1] but also to the host granulomatous reaction to them, which affects large volumes of liver tissue.[2,3] Andrade and Warren reported, however, that late in the course of infection the size of the granulomas around eggs newly trapped in the liver decreases markedly.[4] This observation suggested that if the host survives the initial severe reaction to the schistosome eggs, the diminished granulomatous response late in the infection might forestall the occurrence of overt disease and result in resistance to the disease. Cheever also observed a diminution in granuloma size in chronic schistosomiasis mansoni, but reported it to be much slighter in degree.[5] Both studies [4,5] suffered from the difficulty of having to differentiate, by microscopic appearance alone, eggs newly trapped in the liver from eggs which had entered the liver in the past.

In an effort to solve this problem, a technique for quantitating granuloma formation devised by von Lichtenberg [6] was utilized. *Schistosoma mansoni* eggs taken from the livers of donor mice were injected via a tail vein into the lungs of recipient mice at different periods in the course of *S. mansoni* infections. The development of granulomas around these eggs in the lungs could thus be quantitated in a situation free of contamination by eggs trapped in the intestines and liver over the course of the infection.

The present study confirmed the previous observation of a marked diminution in the granulomatous reaction late in the course of the infection [4] and noted, in addition, that at the later time periods there appeared to be a more rapid rate of destruction of the schistosome eggs. The results, coupled with the recent finding that the granulomatous reaction around *S. mansoni* eggs is a manifestation of delayed hypersensitivity,[7] suggest

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that the change in granuloma formation may be a form of endogenous desensitization related to the constant high output of egg antigen by the worms.

Materials and Methods

A total of 222 Swiss albino female mice, 18–22 gm. in weight, were each given subcutaneous injections of an average of 16 cercariae of a Puerto Rican strain of *Schistosoma mansoni* obtained from a pool of 30 infected *Australorbis glabratus* snails. The worm burden of a random sample of 8 of the mice was determined 8 weeks after infection by perfusion of their portomesenteric venous systems. The severity of chronic hepatosplenic disease was determined in 5 animals at the seventeenth week of infection by measuring body, liver, and spleen weights as well as portal pressure and ascertaining the presence of portal-systemic collateral circulation according to methods previously described.[1–3]

At 2, 8, 16, 24, and 32 weeks after infection, mice, in groups of at least 20 animals, were each given an injection, via a tail vein, of 1000 *S. mansoni* eggs in 0.5 ml. of 0.9% saline. The eggs were obtained by the method of Coker and von Lichtenberg,[8] at each of the above time intervals, from the livers of groups of 20 donor mice infected 8 weeks previously with large numbers of cercariae. One, 8, 16, and 32 days after the eggs were injected into the lungs of the recipient mice, groups of 5 or 6 mice were sacrificed and the lungs removed for histologic study, as described by von Lichtenberg.[6] Three lung sections from each animal, 5 μ in thickness and at least 250 μ apart, were stained with hematoxylin and eosin. They were searched for schistosome eggs, and the size of each egg, including the reaction around it, was determined by measuring 2 diameters at right angles to each other with a Vickers-A.E.I. Image Splitting Eyepiece. The mean diameter of all egg lesions at each time period was then computed.

In order to eliminate an effect of aging on the granulomatous response of the animals, unsensitized and sensitized (2000 eggs I.P. 1 week previously) Swiss albino female mice in 2 different age groups, 8 and 40 weeks, received 1000 *S. mansoni* eggs each via a tail vein. Within each age group the lungs were removed from 6 unsensitized mice at both 8 and 16 days after the intravenous injection and from similar numbers of sensitized animals at 1 and 8 days. The tissues were prepared and examined as described above.

Although the eggs are usually trapped in the livers of the infected animals, those with severe hepatosplenic disease may develop portal-systemic collateral circulation through which the eggs reach the lungs.[9] At the time of sacrifice, therefore, all animals with visible collaterals were eliminated from the experiment. As the infections were light, only a few mice were thus eliminated. In addition, groups of 5 infected mice which had not received eggs from donor mice were sacrificed at 17 and 24 weeks, and their lungs were sectioned and examined for the presence of eggs.

A group of 32 uninfected mice each received intraperitoneal injections of 6000 *S. mansoni* eggs obtained from 8-week-infected donors every 2 weeks for 32 weeks. At the end of this period 1000 eggs were injected into their lungs via a tail vein and they were sacrificed and examined as described above.

Results

Worm Burden and Hepatosplenic Disease in the Recipient Mice

Schistosome infection in the experimental mice was very light, averaging 3 worms (range 2–6) per mouse. With respect to worm pairs (the 1

male and 1 female necessary for egg production), there was an average of 1.1 (range 1–2) per mouse. Seventeen weeks after exposure, when the disease had reached a chronic state, the mean liver weight was 6.3% ± 0.6% (S.E.) of body weight (normal, 5.0%), the spleen weight was 0.9 ± 0.3% of body weight (normal, 0.6%), the portal pressure was 5.4 ± 0.8 cm. of saline (normal, 4.5), and no gross portal-systemic collateral circulation was seen in any of the animals. Schistosome eggs were not found in the lungs of any of the infected mice examined at 17 and 24 weeks. Hepatosplenic disease was therefore of the very mildest type.

Granulomatous Response to Donor Schistosome Eggs at Various Stages of Infection

The three types of granulomatous responses seen in the 32-week course of the infection were comparable to those seen in: (1) unsensitized, uninfected mice,[7] (2) sensitized mice,[7] and (3) mice desensitized, as described below, by repeated injections of schistosome eggs.

Table 1. Granuloma Formation Around S. mansoni Eggs Injected Intravenously into the Lungs of Mice with Mild S. mansoni Infections at Stages in the Evolution of the Disease from 2 to 32 Weeks

	Duration of infect. (wk.)*	Days after I.V. injection of eggs into lungs			
		1	8	16	32
Total No. of lesions measured (eggs with or without granulomatous reactions)	2	50	100	77	94
	8	60	74	100	70
	16	50	100	58	50
	24	100	100	100	100
	32	54	90	75	78
Diam. (μ) egg alone or with granulomatous reaction (mean ± S.E.)	2	61 ± 2	161 ± 7	203 ± 6	146 ± 4
	8	135 ± 12	242 ± 10	166 ± 4	159 ± 6
	16	92 ± 5	171 ± 5	142 ± 5	127 ± 7
	24	110 ± 4	152 ± 4	105 ± 3	88 ± 2
	32	81 ± 4	136 ± 6	111 ± 4	92 ± 3
Eggs with granulomatous reactions (%)	2	0	86	100	99
	8	63	99	100	100
	16	62	100	100	100
	24	89	100	100	100
	32	59	100	100	100

* At time of egg injection.

Infections of 2 Weeks' Duration. The response of the animals infected with *S. mansoni* for 2 weeks, at which stage the worms are immature and no eggs have been produced (egg output begins at 5 weeks), is essentially that of unsensitized mice (Tables 1 and 2). No reaction was observed

around any of the eggs from the donor animals 24 hr. after their injection into the lungs, the mean diameter of the lesion (61 μ) being that of the egg alone. At 8 days the mean granuloma diameter was 2.6 times that of the egg alone, reaching a peak of 3.3 times the egg diameter at 16 days (Text-fig. 1). Thirty-two days after egg injection the mean granuloma was slightly smaller than that at 8 days. The fully developed granuloma contained the egg in the center surrounded by a collar of epithelioid, giant and mononuclear cells, eosinophils, and fibroblasts (Fig. 1 and 2); necrosis was observed rarely.

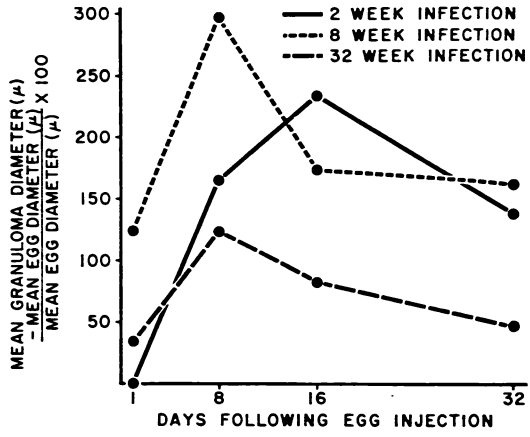
Infections of 8 Weeks' Duration. The granulomatous response to donor schistosome eggs injected into lungs of mice infected for 8 weeks, in which the mature schistosomes had been producing eggs for 3 weeks, was typical of that in uninfected animals injected previously with schistosome eggs (Tables 1 and 2). The inflammatory reaction was both accelerated and augmented. Whereas in the animals infected for 2 weeks there was

Table 2. Granuloma Formation Around S. mansoni Eggs Injected Intravenously into Lungs of Mice Treated with Exogenous Egg Injections as Compared with That in Infected Mice in Which There Is Endogenous Egg Production

Type of response	State of animals	Mean granuloma diam. ($\mu \pm$ S. E.) at time after I.V. injection of eggs into lungs			
		1 day	8 days	16 days	32 days
Unsensit.	Uninfect. & unsensit.	58 ± 3	165 ± 20	206 ± 8	148 ± 8
	2nd wk. of infection (no eggs in tissues yet)	61 ± 2	161 ± 7	203 ± 6	146 ± 4
Sensit.	6000 eggs I.P. 6 wk. previously	97 ± 4	224 ± 5	209 ± 4	165 ± 5
	8th wk. of infection (eggs present in tissues for 3 wk.)	135 ± 12	242 ± 10	166 ± 4	159 ± 6
Desensit.	6000 eggs I.P. every 2 wk. for 32 wk.	79 ± 5	172 ± 6	151 ± 5	118 ± 4
	16th wk. of infection	92 ± 5	171 ± 5	142 ± 5	127 ± 7

no reaction around any of the eggs at 24 hr., in the 8-week-infected animals there were definite reactions around 63% of the eggs, the mean diameter of the lesions being 2.2 times that of the egg alone. This was only a little less than the mean granuloma size seen at 8 days in the mice infected for 2 weeks. The peak reaction not only occurred earlier in the 8-week-infected animals but also, being 4 times greater in diameter than

the egg alone, was significantly larger ($p < 0.0005$) than the peak diameters seen in any of the other experimental groups (Text-fig. 1). The predominant cells in the 24-hr. reaction were eosinophils, but mononuclear cells were also present (Fig. 1 and 2). Eight days after the injection of the eggs into the lungs of the 8-week-infected animals, approxi-



TEXT-FIG. 1. Mean granuloma diameter relative to egg diameter 1, 8, 16, and 32 days after injection of *Schistosoma mansoni* eggs into lungs of mice with *S. mansoni* infections of 2, 8, and 32 weeks' duration.

mately 50% of the granulomas had varying degrees of necrosis, in contrast to only 17% with necrosis in the 2-week-infected animals at that time period. A further contrast was provided in that 11% of the eggs were partially destroyed by 8 days in the animals infected for the longer period of time, while there was no egg destruction in the short-term infections. There was no striking difference between the 2 groups in the cellular composition and fibroblastic activity of the granulomas at this period (Fig. 1 and 2).

Infections of 16–32 Weeks' Duration. By the sixteenth week of infection a marked decrease in the granulomatous reaction observed in the 8-week-infected animals was evident. The diminution continued in the 24- and 32-week-infected animals but at a slower rate (Table 1). The granulomatous response, as in the 8-week-infected animals, was characterized by an earlier onset in that many of the eggs had reactions around them at 24 hr. after their injection into the lungs and the peak granuloma size was reached at 8 rather than at 16 days (Text-fig. 1). The peak granuloma sizes in the 16-, 24-, and 32-week-infected animals, however, were not only much smaller than those in the 8-week-infected animals, but they were all significantly smaller than the peak mean granuloma diameter observed at 16 days in the 2-week-infected animals ($p < 0.0005$) (Table 1). The inflammatory reaction in the desensitized animals 24 hr. after the injection of donor eggs into the lungs consisted predominantly of eosinophils and a few mononuclear cells (Fig. 1 and 2); by 8 days about 40% of

the granulomas had varying amounts of necrosis. In these respects they were similar to the lesions seen in the 8-week-infected mice. The rate of destruction of the schistosome eggs, however, continued to increase with approximately 80% of the eggs being destroyed by 8 days after their injection into the lungs of 32-week-infected animals. The granulomas also appeared to have more epithelioid cells and less eosinophils (Fig. 1 and 2).

The age of the host had little effect on the granulomatous response to schistosome eggs, inasmuch as the reactions of 8- and 40-week-old mice in both the unsensitized and sensitized states were similar both qualitatively and quantitatively.

Uninfected Mice Given Injections of Schistosome Eggs at 2-week Intervals for 32 Weeks

A degree of desensitization equivalent to that observed in the 16-week-infected mice was induced in uninfected mice by the intraperitoneal injection of donor schistosome eggs at 2-week intervals for 32 weeks (Table 2).

Discussion

The granuloma which forms around *S. mansoni* eggs and which is a prime factor in the pathogenesis of hepatosplenic disease in the experimental animal [1-3] has recently been shown to be an immunologic reaction of the delayed hypersensitivity type. In that report, sensitization, an accelerated augmented secondary response, was described around eggs injected intravenously into the lungs of mice which had previously been given a single intraperitoneal injection of eggs.[7] In the present study, desensitization, an accelerated but markedly diminished granulomatous reaction to the eggs in the lungs, was observed following multiple intraperitoneal egg injections. Similar though more exaggerated sensitization and desensitization reactions have now been found during the course of natural, relatively mild *Schistosoma mansoni* infections in mice. When schistosome eggs were injected intravenously into the lungs of mice infected for 2 weeks (the growth and development phase of the worms; egg production begins at 5 weeks), the reaction was virtually identical to that seen in unsensitized animals (Table 2).

At 8 weeks of infection, when the mature worms had been depositing eggs in the intestine and liver of the host for about 3 weeks, the reaction to eggs injected into the lungs was both accelerated and augmented, being similar to that seen in mice which had previously received 1 intraperitoneal injection of 6000 *S. mansoni* eggs (Table 2). The reaction in the 8-week-infected animals was actually somewhat more exaggerated than in

the mice which had received 1 sensitizing egg injection in that it was much larger at 1 and 8 days and had receded more rapidly by 16 days.

Mice infected for 16 weeks had an accelerated reaction to schistosome eggs injected into their lungs which was similar to that in the sensitized 8-week-infected animals, but the granuloma sizes at 8, 16, and 32 days after the egg injection were much smaller. The granulomas were, in fact, quite similar in both rate of development and peak size to those in uninfected mice which had been desensitized by a large number of injections of schistosome eggs (Table 2). The granulomas tended to be even smaller at 24 weeks and by 32 weeks were the smallest such lesions ever observed in untreated animals in this laboratory.[10] At 32 weeks the peak granuloma volume was not only 5.6 times smaller than that in the sensitized 8-week-infected animals, it was even 3.3 times smaller than that of the unsensitized 2-week-infected mice. These results suggest that a partial degree of desensitization develops as a result of the continuous exposure of the host to large amounts of schistosome eggs and their antigens. *S. mansoni* worm pairs have been reported to produce 300 eggs per pair per day, many of which remain in the host tissues.[11] Thus over a 32-week period it may be estimated that each mouse was exposed to at least 134,000 eggs. The greater degree of desensitization in these animals than in the uninfected animals may be explained, therefore, by the fact that the latter received fewer eggs (96,000 per mouse) or perhaps by the periodic nature of the injections as compared to the continuous egg output by the worms.

Despite the smaller granuloma size in the 32-week-infected animals, the eggs appeared to be eliminated more rapidly. This observation is in keeping with that of von Lichtenberg who reported the more efficient sequestration and destruction of egg antigens in sensitized animals.[12] Thus, a series of changes occurs in the later stages of chronic schistosomiasis mansoni which may be beneficial to the host in that less parenchymal tissue is destroyed, less fibrous tissue is formed, the foreign bodies are eliminated more rapidly, and the antigens produced by them are sequestered and destroyed at a faster rate. Since the granulomatous host reaction appears to play a major role in the pathogenesis of hepatosplenic schistosomiasis,[2,4] the smaller, apparently more efficient response of partially desensitized hosts may mediate against the development of overt disease and thus be a factor in the occurrence of resistance to schistosomiasis.

Summary

The granulomatous response to exogenous schistosome eggs injected into the lungs of mice with mild schistosomiasis mansoni infections was studied at different stages in the infection. (The eggs produced by the

worms in the infected animals were all retained in the intestines and liver.) Eggs were isolated from the livers of donor mice infected with *S. mansoni* for 8 weeks and injected via a tail vein into groups of mice with infections of 2, 8, 16, 24, and 32 weeks' duration. The lungs of subgroups of the recipient mice were removed 1, 8, 16, and 32 days after egg injection. After sectioning and staining, the lungs were examined for the presence of eggs and the diameter of each egg, including the host reaction around it, was measured. The animals infected for 2 weeks (egg production begins at 5 weeks) developed granulomas around the injected schistosome eggs in the same manner as uninfected unsensitized animals. Those infected for 8 weeks developed accelerated augmented reactions similar to uninfected animals sensitized by 1 previous intraperitoneal injection of schistosome eggs. The mice infected for 16, 24, and 32 weeks, while forming granulomas at a more rapid rate than the unsensitized animals, developed very small lesions similar to those seen in a group of uninfected mice given intraperitoneal injections of schistosome eggs every 2 weeks over a period of 32 weeks. Aging per se had no effect on granuloma formation since uninfected 40-week-old mice responded in an identical manner to 8 week-old mice. This waning of the granulomatous reaction to schistosome eggs in chronically infected mice was considered to be a form of desensitization, in view of the recent finding that the schistosome granuloma is a form of delayed hypersensitivity. The effect of this endogenous type of desensitization was discussed both in relation to the development of overt hepatosplenic schistosomiasis and to the occurrence of resistance to the disease.

References

1. WARREN, K. S. The etiology of hepatosplenic schistosomiasis mansoni in mice. *Amer J Trop Med Hyg 10*:870–876, 1961.
2. WARREN, K. S. The contribution of worm burden and host response to the development of hepatosplenic schistosomiasis mansoni in mice. *Amer J Trop Med Hyg 12*:34–39, 1963.
3. WARREN, K. S. The pathogenesis of "clay-pipe stem cirrhosis" in mice with chronic schistosomiasis mansoni, with a note on the longevity of the schistosomes. *Amer J Path 49*:477–489, 1966.
4. ANDRADE, Z. A., and WARREN, K. S. Mild prolonged schistosomiasis in mice: Alterations in host response with time and the development of portal fibrosis. *Trans Roy Soc Trop Med Hyg 58*:53–57, 1964.
5. CHEEVER, A. W. A comparative study of *Schistosoma mansoni* infections in mice, gerbils, multimammate rats and hamsters. I. The relation of portal hypertension to the size of hepatic granuloma. *Amer J Trop Med Hyg 14*:211–226, 1965.
6. VON LICHTENBERG, F. Host response to eggs of *S. mansoni*. I. Granuloma formation in the unsensitized laboratory mouse. *Amer J Path 41*:711–731, 1962.

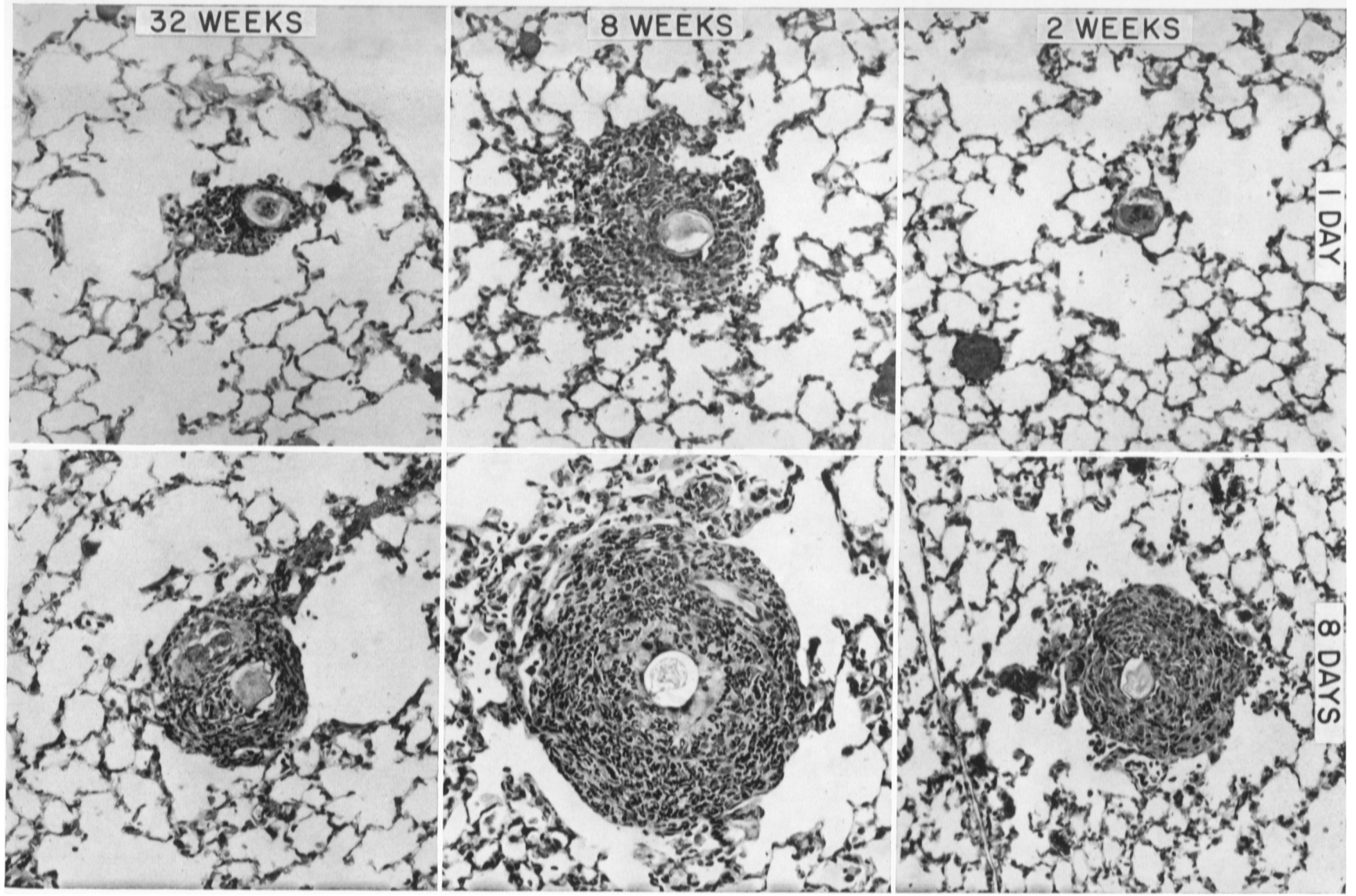
7. WARREN, K. S., DOMINGO, E. O., and COWAN, R. B. T. Granuloma formation around schistosome eggs as a manifestation of delayed hypersensitivity. *Amer J Path 51:*735–756, 1967.
8. COKER, C. M., and LICHTENBERG, F. A revised method for isolation of *Schistosoma mansoni* eggs for biological experimentation. *Proc Soc Exp Biol Med 92:*780–782, 1956.
9. WARREN, K. S. Experimental pulmonary schistosomiasis. *Trans Roy Soc Trop Med Hyg 58:*228–233, 1964.
10. DOMINGO, E. O., COWAN, R. B. T., and WARREN, K. S. The inhibition of granuloma formation around *Schistosoma mansoni* eggs. I. Immunosuppressive drugs. *Amer J Trop Med Hyg 16:*284–292, 1966.
11. MOORE, D. V., and SANDGROUND, J. H. The relative egg producing capacity of *Schistosoma mansoni* and *Schistosoma japonicum*. *Amer J Trop Med Hyg 5:*831–840, 1956.
12. VON LICHTENBERG, F. Studies on granuloma formation. III. Antigen sequestration and destruction in the schistosome pseudotubercle. *Amer J Path 45:*75–93, 1964.

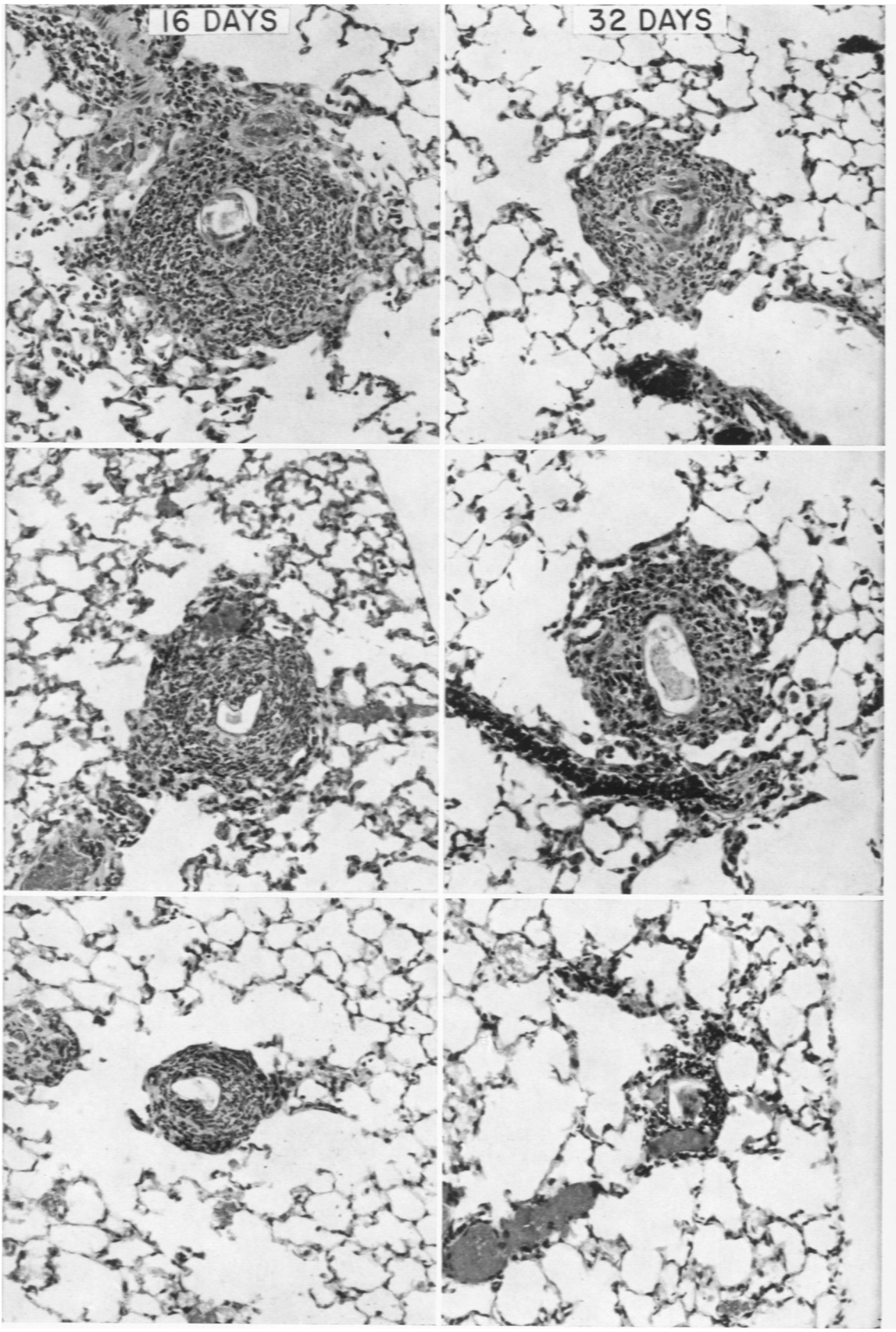
[*Illustrations follow*]

Legends for Figures

Fig. 1. Reaction 1 and 8 days after injection of Schistosoma mansoni eggs into lungs of mice with mild S. mansoni infections of 2, 8, and 32 weeks' duration. Each lesion is representative of mean of between 50 and 100 measured lesions. (After mean granuloma diameter was determined, sections were searched for granulomas representative of mean diameter; these were marked and then photographed.) Hematoxylin and eosin stain. × 150.

Fig. 2. Reaction at 16 and 32 days. See Fig. 1 for details.


32 WEEKS
8 WEEKS
2 WEEKS
1 DAY
8 DAYS


16 DAYS
32 DAYS