Inhibition of Granuloma Formation Around *Schistosoma mansoni* Eggs

IV. X-irradiation

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THE GRANULOMATOUS REACTION around schistosome eggs in the lungs of mice appears to be a form of delayed hypersensitivity. Direct evidence for this hypothesis has been provided by cell transfer studies,[1] and indirect evidence, by the inhibition of granuloma formation by neonatal thymectomy [2] and rabbit anti-mouse lymphocyte serum,[3] immunosuppressive measures which primarily affect cell-mediated hypersensitivity phenomena. X-irradiation, another method of immunosuppression, was examined in the present study because this measure primarily affects the formation of circulating antibody and has relatively little effect on delayed hypersensitivity except when administered in near-lethal doses. In addition, both repeated low-dose and single high-dose X-irradiation induce a marked lymphopenia. The use of X-irradiation, therefore, made it possible to investigate the effects of suppression of antibody formation and depression of circulating lymphocyte levels on granuloma formation.

Materials and Methods

Technique for Studying Granuloma Formation

Schistosome eggs were isolated by the method of Coker and von Lichtenberg [4] from the livers of Swiss albino mice which had been infected 8 weeks previously with a Puerto Rican strain of *Schistosoma mansoni*. Divinyl-benzene-copolymer beads (BioRad Labs), similar in size to the schistosome eggs, were screened through a 240-mesh steel seive, trapped on a 360-mesh seive, and triple-washed with normal saline. A total of 1000 eggs or 6000 beads suspended in 0.5 ml of normal saline were injected into the lungs of mice via a tail vein. Other mice were sensitized by

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an intraperitoneal injection of 5000 eggs in 0.5 ml of normal saline 1 week prior to their intravenous injection.

As noted in Table 1, at different periods after intravenous egg injection mice were anesthetized with pentobarbital, 1 ml of 10% buffered formalin was injected intratracheally, and the lungs were removed and placed in containers filled with formalin. Three sections 5 μ thick and 250 μ apart were cut from each lung, stained with hematoxylin and eosin, and examined for eggs or beads. The size of each egg or bead and the reaction around it was determined by measuring two diameters at right angles to each other with a Vickers A. E. I. image-splitting eyepiece. The mean diameter of approximately 50–100 lesions was determined for each time period, and the standard error calculated. For each experimental group a granuloma of the mean size was then located and photographed. At the time the animal was sacrified, white blood cell and differential counts were done, and blood was collected for antibody determinations.

X-irradiation

A General Electric Maxitron X-ray machine (250 kv, 15 ma, with a filter of 1 mm of Al and 0.5 mm of Cu) was used for all treatment schedules. The distance from the beam to the target was 76 cm, and the dose rate was 25 R per minute, as measured by a Victoreen dosimeter. Ten mice at a time were X-irradiated in a dorsal-to-ventral direction while enclosed in a circular plastic container. It was determined that the $LD_{50/30\ days}$ (50% mortality in 30 days) for the Swiss albino mice used in these studies is 600 R.

Groups of mice were exposed to 7 R or 30 R whole-body X-irradiation 5 days per week for a period of 8 weeks. At the end of this period the mice were given intravenous injections of schistosome eggs or beads, X-irradiation continuing until the animals were sacrificed as shown in Table 1. Another group of mice was subjected to a single dose of 450 R 24 hr prior to intravenous egg injection (Table 1).

Table 1. Protocol for Study of Effect of Repeated 7 R or 30 R X-irradiation or a Single Dose of 450 R X-irradiation on Granuloma Formation Around *Schistosoma mansoni* Eggs or Divinyl-Benzene-Copolymer Beads Injected Via a Tail Vein into the Lungs of Mice

Treatment group	X-irradiation (time prior to IV injection)*	Eggs injected (No.)		After egg injection (days)‡
		IP†	IV	
Eggs: unsensitized mice				
Control	0	0	1000	1, 8, 16, 32
7 R	8 wk	0	1000	1, 8, 16, 32
30 R	8 wk	0	1000	1, 8, 16§, 32
450 R	24 hr	0	1000	1, 8, 16, 32
Eggs: sensitized mice				
Control	0	5000	1000	1§, 8
30 R	8 wk	5000	1000	1, 8
Beads				
Control	0	—	6000	2
30 R	8 wk	—	6000	2

* The 7-R and 30-R doses were given 5 days/week and were continued after eggs were injected into lungs; 450 R was in a single dose.

† Given 1 week prior to IV injection.

‡ After IV injection of eggs, when lungs were removed for examination; 6 mice at each time interval except where day is marked § at which time there were 5 mice.

Antibody Determination

A passive hemagglutination method for the study of antibodies to *S. mansoni* eggs was devised in our laboratory by Dr. D. L. Boros. Sheep red blood cells were fixed with 1% glutaraldehyde, tanned with a 1:2000 dilution of tannic acid, and sensitized at pH 6.4 with a 1:2000 dilution of *S. mansoni* egg antigen prepared by the method of Anderson.[5] Antiserums were obtained from mice having had a bisexual worm infection for 8–11 weeks. The highest titer obtained with this system was 1:160. Preincubation for 2 hr of 0.1 ml of antiserum with a similar volume of a 1:10 dilution of antigen rendered the test negative.

Results

Mortality

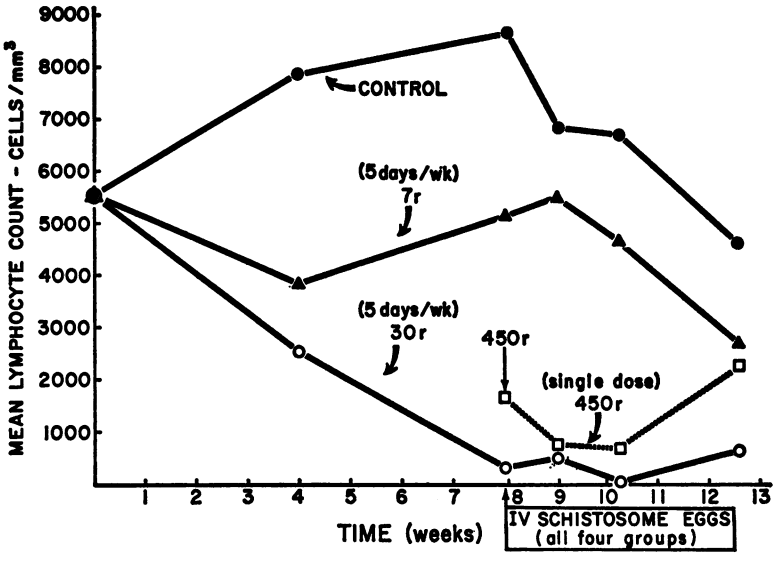
No deaths occurred during the entire period of experimentation among either the 48 animals in the control group or the 30 animals exposed to repeated 7 R X-irradiation. Of the 50 mice in the 30-R (repeated) X-irradiation group, 2 died prior to the time of egg or bead injection, and 2 of the 30 mice in the 450-R (single-dose) treated animals died within the first week following X-irradiation.

Lymphocyte Counts

Prior to the onset of X-irradiation, the lymphocyte count of a group of 10 mice averaged 5533 ± 532 cells per cubic millimeter. After 4 weeks of X-irradiation the mean counts in 6 animals exposed to 7 R and 6 animals exposed to 30 R were respectively, 3850 and 2738, while the mean count in 6 control mice was 7940/cu mm (Text-fig 1). After 8 weeks of X-irradiation, schistosome eggs were injected intravenously, and lymphocyte counts were made each time groups of animals were sacrificed to study granuloma formation. During the 32-day period after egg injection the lymphocyte level in the animals exposed to 7 R was moderately but consistently low, but in those animals exposed to 30 R it was markedly decreased, at one time period to less than 2% of the control level (Text-fig 1, Tables 2–4). Single-dose X-irradiation at 450 R led to a rapid and marked diminution in lymphocyte count. By 8 days it was at its lowest level where it remained at 16 days; by 32 days, however, it had begun to rise (Text-fig 1, Table 2).

Circulating Antibody in Unsensitized Mice

It was noted in the unsensitized, untreated mice that 16 days after intravenous egg injection 3 of 6 mice had antibody titers of 1:80, and at 32 days 4 of 5 mice had titers of 1:40. Mice treated with repeated 7 R X-irradiation had no measurable circulating antibody at any time period studied nor did those treated with 450 R (single-dose) X-irradiation. In



TEXT-FIG 1. Effect on the circulating lymphocyte concentration of repeated 7R and 30R X-irradiation (5 days/week) for 8 weeks prior to and 32 days after the injection of schistosome eggs, as well as the effect on the lymphocyte count of a single dose of 450 R given 1 day prior to injection of schistosome eggs.

Table 2. Effect of Repeated 7 R or 30 R X-irradiation or a Single Dose of 450 R on Lymphocyte Counts and Granuloma Formation at Different Time Periods after the IV Injection of Schistosome Eggs into the Lungs of Mice

	Day 1	Day 8	Day 16	Day 32
	No. Mice/No. Eggs Measured			
Control	6/88	6/54	6/100	6/100
7 R	6/100	6/99	6/100	6/100
30 R	6/66	6/56	5/69	6/73
450 R	6/100	6/100	6/90	6/100
	Mean Granuloma Diameter (μ) ± SE (% Eggs with Reaction)			
Control	54.27 ± 1.17 (0)	150.78 ± 12.95 (57)	209.00 ± 8.75 (93)	162.40 ± 7.30 (92)
7 R	57.84 ± 1.39 (0)	147.11 ± 6.81 (65)	200.76 ± 8.46 (95)	163.45 ± 6.35 (96)
30 R	59.36 ± 1.72 (6)	106.00 ± 7.36 (63)	229.82 ± 10.64 (100)	182.62 ± 6.01 (100)
450 R	52.18 ± 1.30 (0)	86.20 ± 5.28 (38)	100.67 ± 6.64 (34)	244.03 ± 9.63 (100)
	Mean Lymphocyte Count (Cells/cu mm) ± SE			
Control	7943 ± 960	6875 ± 542	6657 ± 699	4623 ± 969
7 R	5066 ± 716	5571 ± 764	4704 ± 701	2785 ± 435
30 R	320 ± 65	565 ± 78	73 ± 37	647 ± 119
450 R	1622 ± 287	743 ± 50	743 ± 107	2240 ± 224

the 30-R group, however, 1 of 4 mice had an antibody titer of 1:40 at 16 days, and 1 of 5 mice a titer of 1:80 at 32 days (Table 5).

Granuloma Formation Around Schistosome Eggs in Unsensitized Mice

The injection of eggs intravenously into unsensitized mice produces a predictable pattern of granulomatous reaction in the lungs: 1 days after their injection eggs are noted in the pulmonary arterioles without any reaction around them; at 8 days, 57% of the eggs have a reaction around

Table 3. Effect of Repeated 30 R X-irradiation on Granuloma Diameters and Lymphocyte Counts after IV Schistosome Egg Injection into Lungs of Mice Which Had Been Previously Sensitized by IP Injection of Eggs

	Day 1	Day 8
No. mice/No. eggs measured		
Control	5/71	6/100
30 R	6/51	6/56
Mean granuloma diameter (μ) ± SE (% eggs with reaction)		
Control	74.31 ± 4.60 (47)	235.84 ± 8.64 (100)
30 R	61.21 ± 3.50 (24)	215.48 ± 12.42 (96)
Mean lymphocyte count (cells/cu mm) ± SE		
Control	7696 ± 611	7435 ± 812
30 R	515 ± 131	1192 ± 164

Table 4. Effect of Repeated 30 R X-irradiation on Granuloma Diameters and Lymphocyte Counts 2 Days after IV Injection of Plastic Beads into the Lungs of Mice

	Day 2
No. mice/No. eggs measured	
Control	6/100
30 R	6/100
Mean granuloma diameter ± SE (% eggs with reaction)	
Control	129.94 ± 4.23 (93)
30 R	127.38 ± 4.53 (94)
Mean lymphocyte count (cells/cu mm) ± SE	
Control	11635 ± 1795
30 R	202 ± 66

Table 5. Effect of Repeated 7 R or 30 R X-irradiation or a Single Dose of 450 R X-irradiation on Circulating Antibody after Intravenous Injection of Schistosome Eggs into Unsensitized Mice

	Day 1	Day 8	Day 16	Day 32
Control	0/6	0/6	3/6*	4/5†
7 R	0/6	0/6	0/6	0/6
30 R	0/6	0/6	1/4†	1/5*
450 R	0/6	0/6	0/5	0/6

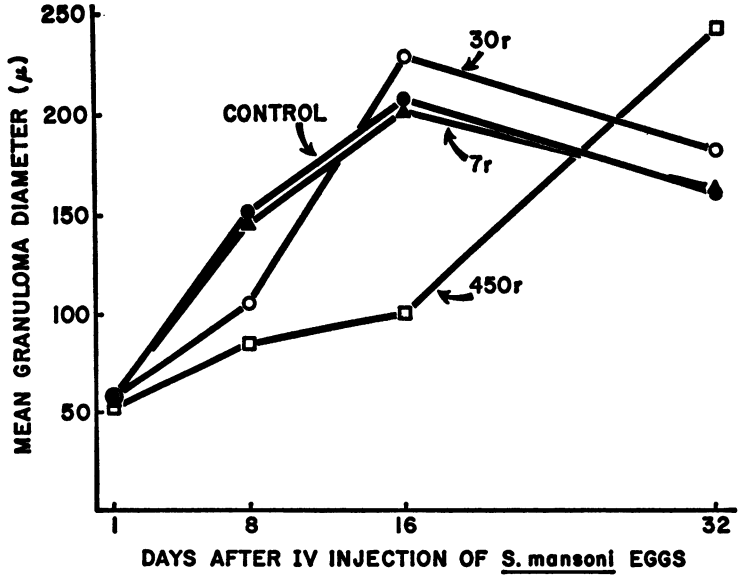
* Titer positive at 1:80.
† Titer positive at 1:40.

them which consists of epithelioid cells, macrophages, eosinophils, lymphocytes, and an occasional giant cell. Peak granuloma size occurs at 16 days, the diameter of the lesion being three to four times that of the egg alone. After this the granuloma size decreases, and at 32 days the diameter is approximately the same as it was at 8 days (Text-fig 2, Table 2).

Repeated 7 R X-irradiation had no effect on granuloma diameter, nor did it alter the number of eggs showing reactions (Text-fig 2, Table 2, Fig 1). Repeated 30 R X-irradiation also had little effect, except at 8 days when the mean granuloma diameter was significantly smaller than that of controls: $p < 0.05$ (Text-fig 2, Table 2, Fig 1). Mice exposed to a single dose of 450 R had significantly smaller granulomas at 8 and 16 days than controls after egg injection ($p < 0.05$). At 32 days after egg injection, however, the mean granuloma diameter in the 450-R group was greater than that of the peak reaction in the controls (Text-fig 2, Table 2, Fig 1). This marked increase in granuloma size was coincident with a rise in the lymphocyte count (Text-fig 1).

Granuloma Formation Around Schistosome Eggs in Sensitized Mice

In sensitized mice there is an accelerated and markedly enhanced reaction to schistosome eggs. At 24 hr after egg challenge, almost 50% of



TEXT-FIG 2. Effect of repeated 7R and 30R X-irradiation and a single dose of 450 R on granuloma formation around schistosome eggs injected intravenously into the lungs of mice.

the eggs have reactions around them. Peak size occurs at 8 days and the mean granuloma diameter is larger than that seen in unsensitized mice at 16 days. Sensitized mice exposed to repeated 30 R X-irradiation had both significantly smaller granulomas ($p < 0.05$) and fewer eggs with reactions around them 1 day after egg injection. However, at 8 days—the time of the peak reaction in sensitized mice—the mean granuloma size in the X-irradiated group was just as large as that in the controls (Table 3, Fig 2).

Granuloma Formation Around Plastic Beads

Plastic beads injected via the tail vein of unsensitized mice elicit a foreign-body reaction which characteristically evolves rapidly, peak reaction size occurring at 2–4 days, and declines by 8 days. The mean diameter of the peak cellular response is much less with beads than with schistosome eggs.

Mice exposed to repeated 30 R X-irradiation showed a marked lymphopenia at 2 days. The mean granuloma diameter at this time, however, was similar to the control value (Table 4, Fig 3).

Discussion

Much evidence has been gathered suggesting that the schistosome egg granuloma is a form of delayed hypersensitivity. A series of studies has been performed utilizing measures such as neonatal thymectomy [2] and antilymphocyte serum,[3] which tend to suppress delayed cell-mediated reactions to a greater degree than immediate humoral antibody-mediated reactions. Since immediate hypersensitivity phenomena have been shown to be radiosensitive when X-irradiation is given prior to antigenic stimulation,[6-9] and cell-mediated reactions have been demonstrated to be relatively radio-resistant,[10-14] it was important to determine the effect of X-irradiation on the schistosome granuloma. Both 7 and 30 R X-irradiation given 5 days per week over a 2-month period (cumulatively, 280 and 1200 R, respectively) inhibited circulating antibody, but essentially did not suppress granuloma formation. Mice exposed to 450 R single-dose X-irradiation 24 hr prior to egg challenge exhibited both inhibition of circulating antibody and partial suppression of granulomatous reaction at 8 and 16 days.

Granuloma inhibition by 450 R is consistent with the data of Uhr and Scharrf:[10] antibody formation in guinea pigs was inhibited by exposure to 200 R X-irradiation, but delayed skin reactivity to diphtheria toxoid was only slightly affected; 400 R resulted in a greater degree of inhibition, and 800 R caused virtually complete suppression of delayed

skin reactivity. Dixon, Talmage, and Manrer [13] demonstrated that most rabbits treated with 400 and 600 R showed inhibition of circulating antibody to BCG as well as suppression of delayed skin reactivity to this antigen. Porter, Moseley, and Murray [14] demonstrated that 800 R X-irradiation to rabbits ($LD_{50/30\ days}$ = 800 R) resulted in acceptance of bone marrow transplants in 15% of the animals, while 900 and 1000 R resulted, respectively, in 38% and 62% acceptance. Recently, Volkman and Collins [15] demonstrated that 400 R whole-body X-irradiation of mice sensitized with purified protein derivative or *Salmonella gallinarum* antigen suppressed footpad swelling following challenge with the appropriate antigen. These data indicate that cellular hypersensitivity phenomena are relatively radioresistant and that a single high dose of X-irradiation is required to affect such a system. The granulomatous reaction to schistosome eggs in mice behaves in this manner, lending further credence to the idea that the schistosome granuloma is a manifestation of delayed hypersensitivity. Moreover, the inhibition of circulating antibody in chronically X-irradiated mice without concomitant inhibition of the granulomatous reaction suggests that circulating antibody plays little role in granuloma formation.

Our data also contribute further evidence suggesting that lymphopenia per se does not result in the suppression of cell-mediated phenomena. Although antilymphocyte serum produces a marked prolongation of skin homograft acceptance time,[16,17] it is not clear that inhibition of cell-mediated reactions is secondary to the lymphopenia produced by this agent. Other postulates on the mode of action of antilymphocyte serum include transformation of small lymphocytes into blast cells which are immunologically incompetent,[18] and selective destruction of the particular lymphocytes which play a role in delayed hypersensitivity reactions.[19] X-irradiation also results in severe lymphopenia but, in contrast to the effect of antilymphocyte serum, prolongs homograft acceptance to only a minor degree.[20] The effects of both antilymphocyte serum and X-irradiation on the schistosome egg granuloma are similar to their effects on homograft rejection. Both immunosuppressive measures result in marked lymphopenia, but only the antilymphocyte serum suppresses granuloma formation. These data, therefore, bolster the concept that peripheral lymphopenia per se is not essential for the inhibition of cell-mediated phenomena.

While repeated 7 R and 30 R X-irradiation caused lymphopenia and inhibited the formation of circulating antibody, these doses had essentially no effect on the granulomatous reaction in unsensitized or sensitized mice. A single dose of 450 R had a similar effect on circulating

lymphocyte and antibody levels but did suppress granuloma formation for a period of several weeks. It is concluded that the effect of X-irradiation on the schistosome egg granuloma is consistent with that reported in other delayed hypersensitivity models. Moreover, it is noted that neither a decrease in circulating antibody nor lymphopenia per se significantly affects granuloma formation.

Summary

Mice given intravenous injections of schistosome eggs have a reproducible granulomatous reaction in the lungs which peaks at 16 days. Treatment with repeated 7 R and 30 R whole-body X-irradiation for 8 weeks prior to egg injection resulted in moderate to marked lymphopenia and suppressed circulating antibody, but had no appreciable effect on granuloma formation. Single-dose 450 R X-irradiation, which resulted in lymphopenia and inhibited circulating antbody, did suppress granuloma formation for a period of about 3 weeks after egg injection. Mice sensitized with an intraperitoneal dose of eggs 1 week prior to egg challenge and exposed to repeated 30 R X-irradiation showed a marked lymphopenia, but no change in granuloma size at the peak granuloma response time of 8 days. Also, mice treated with repeated 30 R X-irradiation and given intravenous injections of plastic beads demonstrated a marked peripheral lymphopenia without any change in the peak foreign body granulomatous response at 2 days. These findings indicate that the schistosome granuloma is relatively radioresistant, and that its behavior is similar to that of other cellular hypersensitivity phenomena. In addition, because granuloma formation was unimpaired in spite of the suppression of circulating antibody and the occurrence of marked lymphopenia, it is concluded that neither of these factors in and of itself plays a major role in the granulomatous reaction to schistosome eggs.

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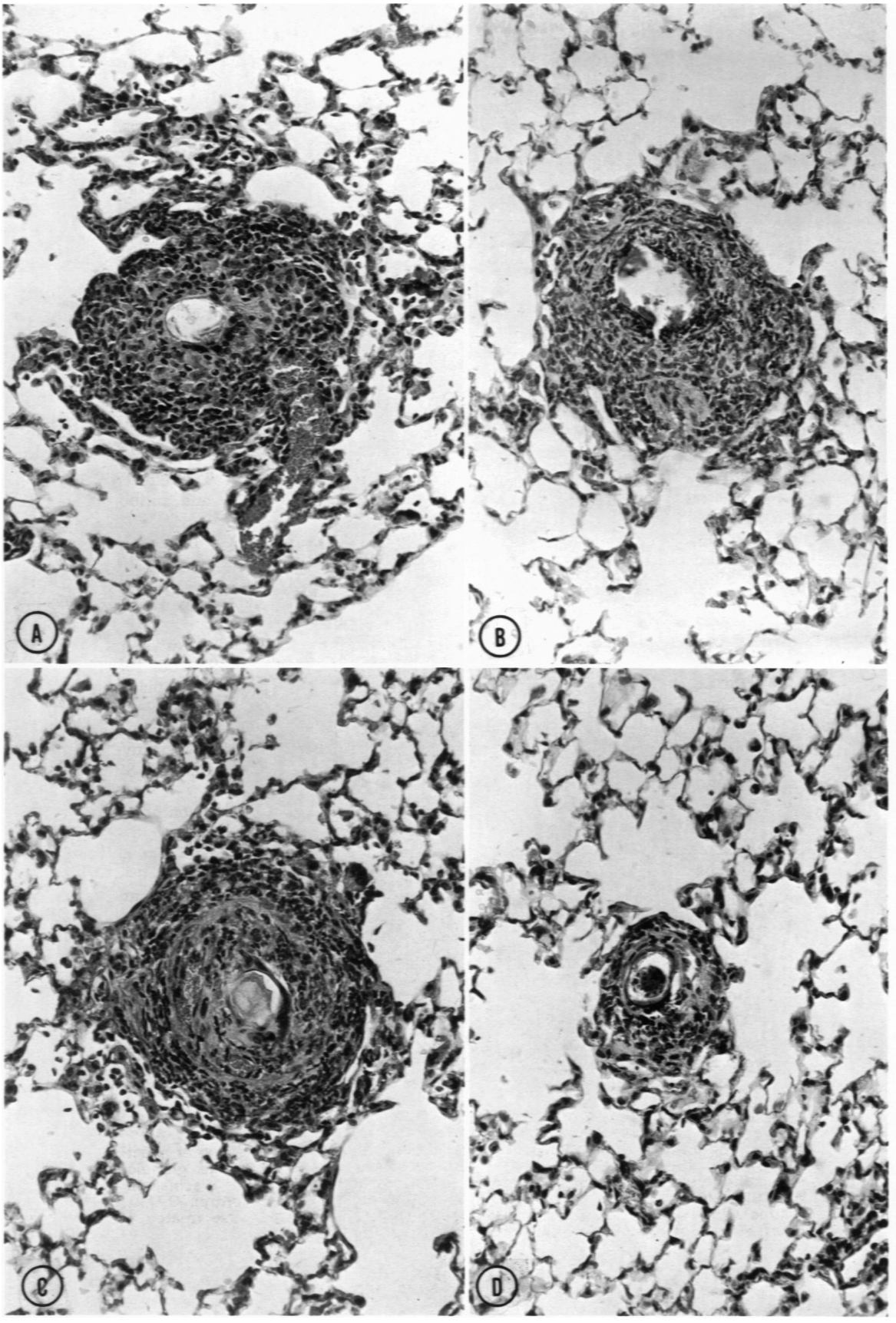
Legends for Figures

Fig 1. Photomicrographs of representative granulomas in mice 16 days after injection of eggs into the lungs. Hematoxylin and eosin. × 225. **A.** Control mouse: granuloma consisting of eosinophils, lymphocytes, and epithelioid cells surrounding a schistosome egg. **B.** Mouse treated with repeated 7 R X-irradiation: similar to control. **C.** Mouse treated with repeated 30 R X-irradiation: similar to control. **D.** Mouse treated with 450 R single dose: marked diminution in granuloma size.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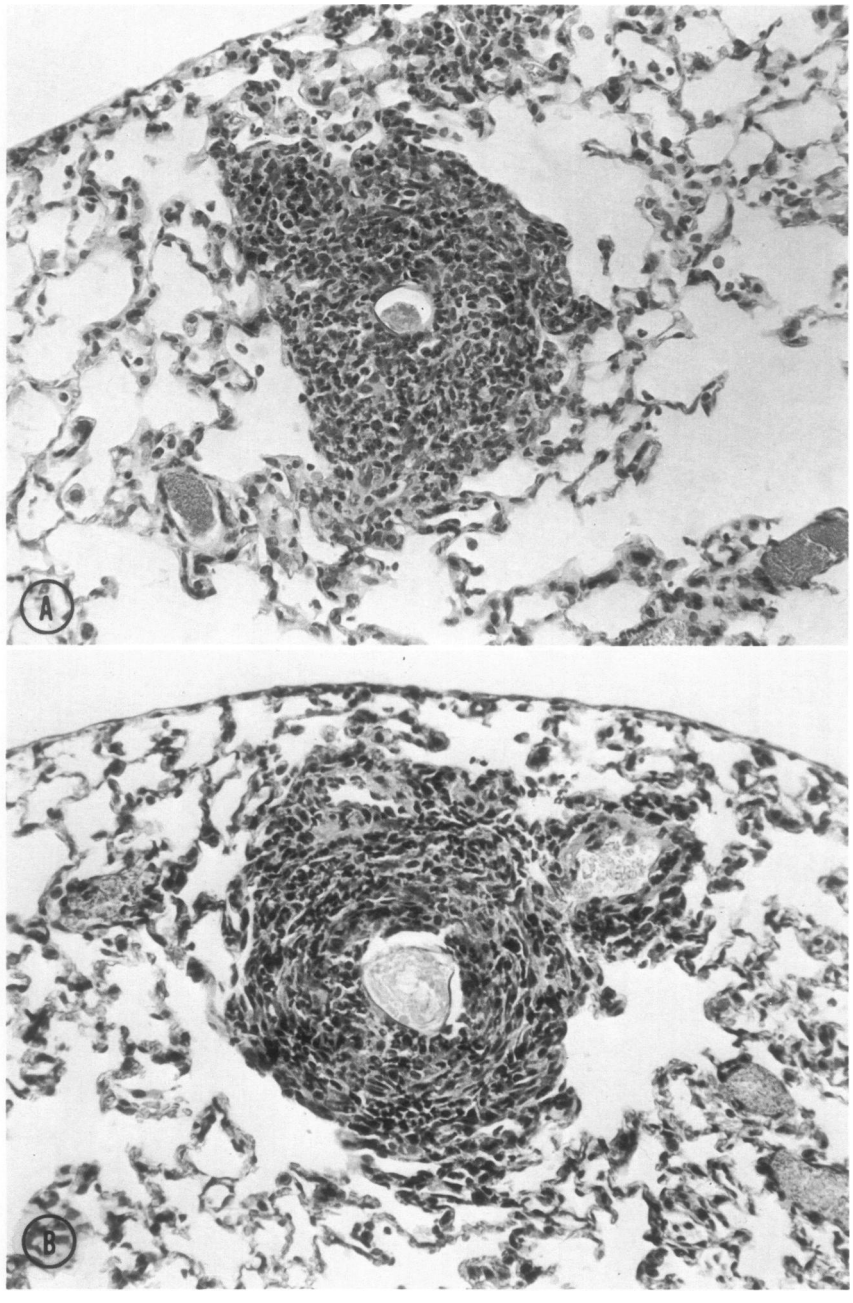

Fig 2. Photomicrographs of representative granulomas 8 days after an intravenous injection of schistosome eggs into the lungs of mice sensitized by a prior intraperitoneal injection of eggs. Hematoxylin and eosin. × 225. **A.** Control mouse: reaction consisting of lymphocytes, eosinophils, and epithelioid cells surrounding a schistosome egg. **B.** Mouse treated with repeated 30 R X-irradiation: similar to control.

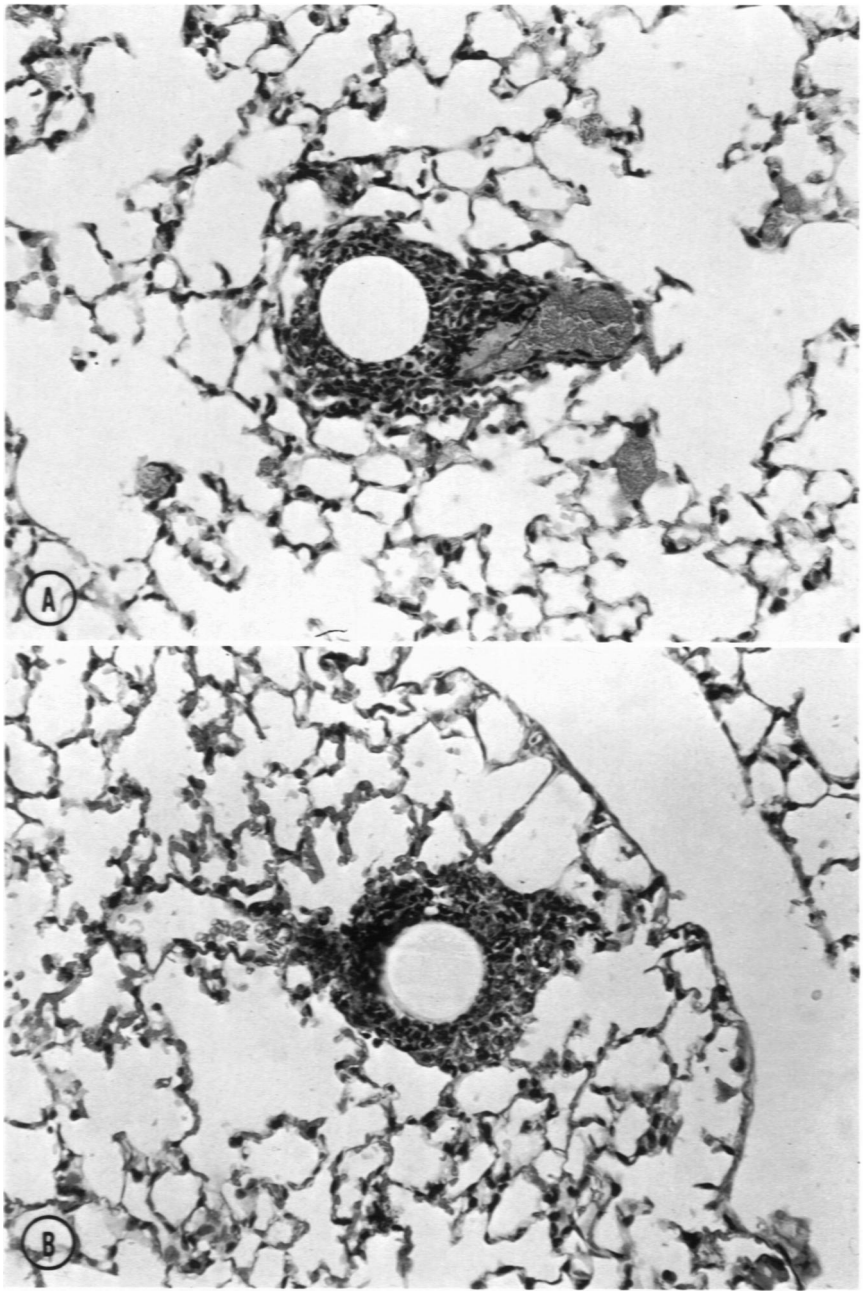

Fig 3. Photomicrographs of representative granulomas 2 days after injection of divinyl-benzene-copolymer beads into the lungs of mice. Hematoxylin and eosin. × 225. A. Control: granuloma consisting of large numbers of neutrophils with some mononuclear cells. B. Mouse treated with repeated 30 R X-irradiation: similar to control.