THE INHIBITION OF GRANULOMA FORMATION AROUND *Schistosoma mansoni* EGGS

II. THYMECTOMY

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The pathogenesis of murine hepatosplenic schistosomiasis may be related not only to the parasite eggs [1–7] but also to the granulomatous response of the host to them.[8,9] When the schistosomes do not produce eggs the disease does not occur,[1,5] and when egg production is stopped in the early stages the disease rapidly improves.[3] It has been suggested, however, that in spite of continuing egg production hepatosplenic disease might be prevented or ameliorated if the host reaction to the eggs could be suppressed.[10] Partial suppression of granuloma formation around schistosome eggs with 5 different immunosuppressive drugs has been reported recently.[10] Other methods of inhibiting granuloma formation have been suggested by the finding that the granulomatous response to *S. mansoni* eggs is a form of delayed hypersensitivity.[11]

Neonatal thymectomy, while having an effect on the production of only some of the circulating antibodies, is a particularly effective suppressant of all types of delayed hypersensitivity reactions in which it has been tried.[12–15] Accordingly, the effect of this procedure on granuloma formation around *S. mansoni* eggs was studied. Ablation of the thymus within the first 3 days following birth resulted in greatly diminished granuloma formation around schistosome eggs, but did not affect the different type of granuloma which formed around inert plastic beads. Sensitization of thymectomized mice by prior injection of schistosome eggs resulted in the formation of relatively large granulomas, although they were significantly smaller than those of sensitized control animals.

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Materials and Methods

Thymectomies were performed on Swiss albino mice within 3 days of birth using the method of Kaplan[16] with the following modifications: hypothermia was used instead of ether anesthesia, and the thymus was aspirated through a Pasteur pipette instead of being removed by forceps. On return of the neonates to their mothers, the mothers were anesthetized with intraperitoneal injections of low doses of pentobarbital (0.082 mg./gm.) in order to reduce cannibalism.

Of the 246 neonates which were thymectomized by this procedure, 113 (46%) were available for study. The remainder were either excluded because thymic remnants were found when they were sacrificed or they were lost to study due to surgical mortality, wasting disease, or cannibalism. Of the 50 mice (⅕ of each litter) which were operated upon as described above but without removal of the thymus (sham-operated), 10 were lost mainly because of cannibalism. Total white cell counts using Neubauer chambers and differential white cell counts made on Wright-stained smears were performed on 12 thymectomized and 12 sham-operated mice. The absolute lymphocyte counts averaged 4822/cu. mm. ± 944 in the sham-operated mice as com-

Table I

EXPERIMENTAL PROTOCOL

Experiment	*Procedure*	*Animals*		*Period of observation*
		Condition	*No.*	
Granuloma formation around *S. mansoni* eggs in lungs	1000 eggs in 0.5 ml. saline per mouse injected via tail vein	Thymectomized Sham-operated	30 17	16 days post injection
Effect of egg load on granuloma size	2800 & 6000 eggs in 0.5 ml. saline per mouse injected via tail vein	Thymectomized	8	16 days post injection
Granuloma formation around inert foreign body	6000 plastic beads in 0.5 ml. saline per mouse via tail vein	Thymectomized Sham-operated	7 10	2, 4, and 8 days post injection
Effect of previous egg injection (sensitization) on subsequent granuloma formation	7000 eggs in 0.5 ml. saline per mouse by I.P. injection; 4 weeks later, 1000 eggs in 0.5 ml. saline per mouse via tail vein	Sensitized, thymectomized Sensitized, sham-operated	4 7	16 days post injection
Penetration of cercariae & subsequent maturation to adult worms	As described[19]	Thymectomized Unthymectomized, unoperated control	8 10	8 wk. post infection
Development of hepatosplenic schistosomiasis mansoni	Shaved abdomen of each mouse exposed to 15 cercariae. Determination 8 weeks later of body, liver, & spleen weights; portal pressure; presence of esophageal varices; hematocrit	Thymectomized Unthymectomized, unoperated control	8 8	8 wk. post infection

pared to 2644/cu. mm. ± 286 in the thymectomized mice. This difference was significant at the 0.005 level.

Both thymectomized and sham-operated mice were used in the experiments summarized in Table I when they reached 5–7 weeks of age. To perform these studies, schistosome eggs were isolated by the method of Coker and von Lichtenberg [17] from the livers of young Swiss albino female mice in the eighth week of infection with a Puerto Rican strain of *Schistosoma mansoni.* A large proportion of these eggs were shown to be viable by hatching them in fresh water. Divinyl-benzene-copolymer beads (obtained from Bio-Rad Laboratories) were screened through a 70-mesh filter, triple washed. and suspended in normal saline. Their average diameter as determined in our studies was 51 ± 1.5 μ (range 28–66).

Varying amounts of schistosome eggs or beads (Table I) suspended in 0.5 ml. of 0.9% saline were injected via the tail vein into the lungs of Swiss albino female mice 18–22 gm. in weight.[18] At the time intervals noted in Table I, groups of mice were anesthetized with pentobarbital; after 0.5 ml. of buffered formalin was injected intratracheally, their lungs were removed and fixed in the same solution. At this time the thymectomized animals were examined carefully for thymic remnants; any suspicious material was preserved in buffered formalin. Three sections 5 μ in thickness were taken from each lung, each section being at a distance of at least 250 μ from the preceding one. They were stained with hematoxylin and eosin and then examined for schistosome eggs or beads. The size of each egg or bead, 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 the lesion for each time period was calculated.

To determine the effect of thymectomy on infection with schistosomes, 8 mice, thymectomized 6 weeks previously, and 10 unoperated controls were exposed for 60 min. to exactly 40 cercariae each of a Puerto Rican strain of *S. mansoni,* using a method recently described.[19] The number of cercariae which had penetrated these animals was determined; 8 weeks later the animals were sacrificed and the number of adult worms they harbored was determined by the method of Yolles *et al.*[20] The effect of thymectomy on the development of hepatosplenic disease was studied by exposing 8 thymectomized and 8 unoperated control mice to 15 cercariae by the method of Olivier and Stirewalt.[21] Eight weeks after exposure the animals were anesthetized and the portal pressure, hematocrit, and body, liver, and spleen weights were determined as previously described. Portions of the livers were preserved in buffered formalin and treated as described above (for the lungs) including the measurement of mean granuloma size.

Serums obtained from 2 groups of thymectomized and control animals, one infected with *S. mansoni,* the other injected intravenously with schistosome eggs were sent to Dr. Elvio Sadun, Director of the Department of Medical Zoology, Walter Reed Army Institute of Research, Washington, D.C., for determination of the presence of circulating antibodies by the fluorescent antibody test with fixed cercariae as antigen.[22]

Results

Granuloma Formation

Around Schistosome Eggs, Sham-operated and Thymectomized Mice. The mean granuloma diameter at 16 days in the sham-operated mice was 192 ± 94 μ, which was similar to that of unoperated mice previously reported (206 ± 8 μ).[11] This represents a lesion with a diameter 225% larger than the egg diameter alone (Table II). The average granuloma diameter for the thymectomized animals was 92 ± 1 μ, an increase of only 56% over the egg diameter alone (Table II). The difference be-

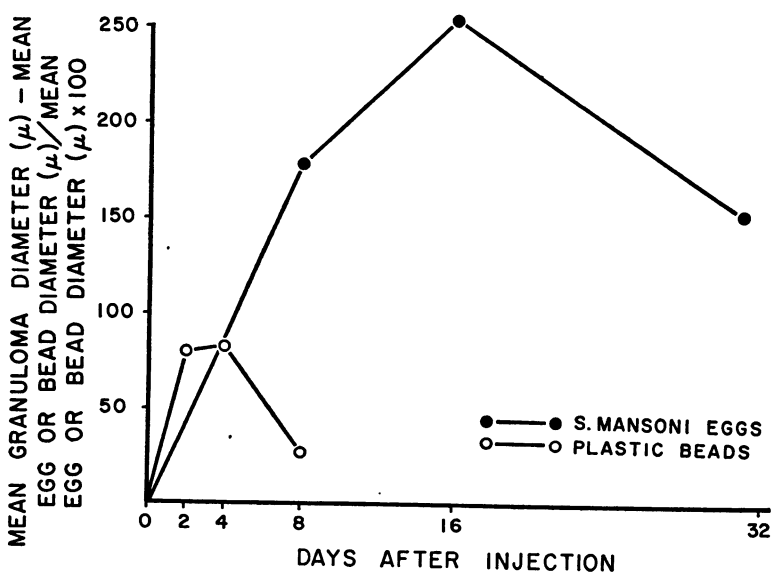
TABLE II

GRANULOMA SIZE IN MICE 16 DAYS AFTER I.V. INJECTION INTO THEIR LUNGS OF S. MANSONI EGGS

Measurements	*Mice*	
	Thymectomized	*Sham-operated*
Mice studied (total no.)*	30	17
Eggs measured, with or without granulomatous reaction (total no.)	526	386
Mean diameter of lesion ($\mu \pm$ S.E.)	92 ± 1	192 ± 4
Av. increase in granuloma diameter relative to egg diameter (%)	56	225
Calculated granuloma volume (cu. mm. × 10^{-4})	4	37
Eggs in lungs without reaction (%)	56	8

* Studied: 2–3 lung sections per mouse.

tween the sham-operated and thymectomized animals was highly significant ($p < .0005$). In terms of volume, the mean size of the presumably sperhical granulomas in the sham-operated mice was approximately 9 times that in the thymectomized mice. Furthermore, only 8% of the eggs in the sham-operated animals were completely free of cellular reaction, as compared with 56% in the thymectomized animals (Table II). While the eosinophil was the predominant cell in granulomas of the sham-operated mice 16 days after egg injection, mononuclear cells were more numerous in the granulomas of the thymectomized mice (Fig. 1).



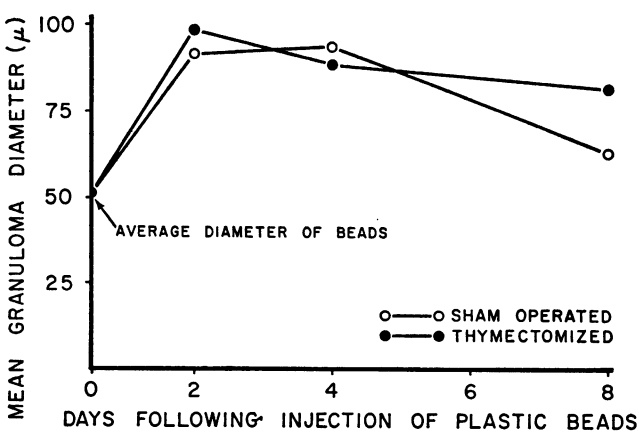
TEXT-FIG. 1. Evolution of granulomas induced by viable biologic products (*S. mansoni* eggs) and presumably inert particles (divinyl-benzene-copolymer beads) injected I.V. into lungs of mice.

In addition, whereas central necrosis and macrophage-like alveolar exudation were seen frequently in the sham-operated mice, these abnormalities were observed only rarely in the thymectomizd animals.

In relation to the effect of egg load on granuloma size, there was no significant difference in the average granuloma diameter among the lungs of the thymectomized animals which contained 1–10, 11–20, or more than 21 eggs per section ($p > 0.4$).

Around Plastic Beads, Sham-operated and Thymectomized Mice. Following the intravenous (I.V.) injection of plastic beads into the lungs of the sham-operated animals, a mononuclear type of inflammation around the particles was maximal at 2–4 days and subsided almost completely by 8 days. In contrast, the schistosome egg-induced inflammation reached its peak at 16 days, and subsided by 32 days (Text-Fig. 1). At its peak, the bead-induced inflammation represented an 80% increase in size over that of the bead size alone, in contrast to an increase of 225% in the case of the schistosome egg (Text-Fig. 1). The reaction of the thymectomized animals to plastic beads was similar in every way to that seen in the sham-operated mice (Text-Fig. 2), in contrast to the marked difference in the response of the 2 groups to schistosome eggs.

Around Schistosome Eggs, Sensitized Sham-operated and Sensitized Thymectomized Mice. Following sensitization by the injection of schistosome eggs intraperitoneally (I.P.) prior to their I.V. injection into the lungs, the mean granuloma diameter at 16 days in the sham-operated group was $180 \pm 5\ \mu$ as compared to $144 \pm 5\ \mu$ for the thymectomized group, a difference which was statistically significant ($p < .0005$) and can be seen in Table III. Nevertheless, the mean granuloma size in the sensitized thymectomized animals was much larger than that in the unsensitized thymectomized mice ($p < .0005$), indicating that sensitization



TEXT-FIG. 2. Size of divinyl-benzene-copolymer bead-induced granulomas in lungs of thymectomized and sham-operated mice.

TABLE III

GRANULOMA SIZE IN SENSITIZED * MICE 11 DAYS AFTER I.V. INJECTION INTO LUNGS OF S. MANSONI EGGS

	Sensitized mice	
	Thymectomized	*Sham-operated*
Mice studied (total no.)†	4	7
Eggs measured (total no.)	88	139
Mean diameter of lesion ($\mu \pm$ S.E.)	144 ± 5	180 ± 6
Av. increase in granuloma diameter relative to egg diameter (%)	144	205
Calculated granuloma volume (cu. mm. × 10^{-4})	16	30
Eggs in lungs without reaction (%)	6	9

* Sensitized by I.P. injection of 6000 *S. mansoni* eggs 1 month prior to their I.V. injection.
† Studied: 4–5 lung sections per mouse.

occurred (Fig. 1). Furthermore, the percentage of eggs around which granulomas formed in the sensitized thymectomized animals (94%) was similar to that in the sensitized sham-operated animals (91%).

Worm Development, Hepatosplenic Disease, Granuloma Formation

Unoperated Control and Thymectomized Mice Infected with S. mansoni. The penetration of cercariae into the thymectomized animals, which averaged 90 ± 0.9% for a 60-min. exposure period, was similar to that of 92 ± 0.9% in the unoperated control animals. Of those cercariae that penetrated, 40 ± 6.0% developed into mature worms in the thymectomized animals as compared to 43 ± 3.9% in the control animals.

The development of hepatosplenic schistosomiasis was not prevented by neonatal thymectomy, since both unoperated and thymectomized animals showed comparable changes in liver and spleen weights, portal pres-

TABLE IV

HEPATOSPLENIC DISEASE IN MICE 8 WEEKS FOLLOWING EXPOSURE TO 15 CERCARIAE OF S. MANSONI

	Mice	
Parameters	*Unoperated controls* *	*Thymectomized* *
Body wt. (gm. ± S.E.)	27 ± 4	26 ± 2
Liver weight to body weight (%)	7.6	8.9
Liver granuloma diameter ($\mu \pm$ S.E.)	360 ± 2	373 ± 10
Spleen weight to body weight (%)	1.7	1.2
Portal pressure (cm. of saline ± S.E.)	8.8 ± 1	7.6 ± 0.4
Hematocrit (% ± S.E.)	38.0 ± 1.5	40.6 ± 2.6

* Average of 8 mice.

sure, and hematocrit following exposure to the same numbers of cercariae (Table IV). Granuloma formation in the livers of both groups was similar, with mean diameters of 360 ± 2 μ in the control mice and 373 ± 10 μ in the thymectomized mice.

Circulating Antibody

Thymectomized and Control Mice Injected I.V. with Schistosome Eggs or Infected with S. mansoni. Both thymectomized and unoperated control animals with mild *S. mansoni* infections developed circulating antibodies. Following the injection of schistosome eggs alone, 88% of the sham-operated mice and 43% of the thymectomized animals developed detectable antibodies (Table V).

Discussion

The granuloma which forms around schistosome eggs appears to be a manifestation of delayed hypersensitivity as demonstrated by the occurrence of a sensitization reaction which is specific and can be transferred with cells but not with serum.[11] Previous studies in which schistosome granuloma formation was inhibited by 5 different immunosuppressive drugs supported this assumption, as the results were similar to those found with other delayed hypersensitivity systems.[10,12] The effect of neonatal thymectomy was examined in the present study because this procedure is associated with an impairment of immunologic capacity in which delayed hypersensitivity, in particular, is depressed.[12-15] While thymectomy reduced the granulomatous response to schistosome eggs, it did not alter the ability of the mice to react to a presumably inert foreign body. These results add to the increasing volume of evidence that the reaction to the *S. mansoni* eggs is an immunologic response.

Although granuloma formation was greatly diminished in unsensitized

Table V

CIRCULATING ANTIBODIES IN MICE AS DETERMINED BY IMMUNOFLOURESCENT TECHNIQUE[23]

Procedure	*Animals*	*Mice (No.)*			
		Total	*Reactive*	*Weakly reactive*	*Non-reactive*
Exposed to 15 cercariae of *S. mansoni* 8 weeks previously	Unoperated	7	6	1	0
	Thymectomized	10	9	1	0
Injected I.V. with 6000 *S. mansoni* eggs 16 days previously	Sham-operated	8	7	0	1
	Thymectomized	7	3	0	4

mice by neonatal thymectomy, the suppressant effect was largely overcome by sensitization. This phenomenon—the ability of previously unreactive thymectomized mice to react following booster antigen injections—has previously been described for both immediate and delayed hypersensitivity reactions.[23] These observations may explain why neither granuloma formation nor hepatosplenic disease in animals infected with *S. mansoni* was inhibited by thymectomy, the continuous output of eggs in the infected animals having a similar effect to repeated doses of antigen.

The attempt to inhibit granuloma formation induced by schistosome eggs is of more than academic importance. In the mouse—the only experimental animal in which a syndrome of hepatosplenic schistosomiasis closely resembling that seen in humans has been produced [6,24]—the granulomas resulting from the deposition of eggs in the tissues appear to be a key pathogenetic factor. Unlike the granulomatous diseases in which the granuloma may play a role in limiting the multiplication and dissemination of the etiologic agent, the granuloma-inducing factor in schistosomiasis neither multiplies nor disseminates. The only postulate advanced as to the protective role of the granuloma in schistosomiasis is that it sequesters potentially harmful antigen produced by the schistosome eggs.[25] It has been suggested, however, that this sequestration may result in antigen concentration in the granulomas high enough to promote central necrosis, thereby leading to scarring in the healing phase.[25] These multiple small granuloma scars have been shown to coalesce into the fibrous bands of typical "clay-pipe stem cirrhosis." [6] A method, either immunologic or pharmacologic, that can prevent granuloma formation will not only test this hypothesis but might provide a means of preventing the development of schistosomal disease in the experimental animal.

Summary

The effect of neonatal thymectomy on granuloma formation around schistosome eggs (which has recently been shown to be a manifestation of delayed hypersensitivity) has been investigated. Thymectomized animals developed granulomatous reactions around schistosome eggs one-ninth the volume of those found in sham-operated controls. Many of these animals, however, continued to form circulating antibodies as demonstrated by an immunofluorescent procedure. In contrast, the different type of granuloma formation induced by inert plastic beads was unaffected by thymectomy. Thymectomized mice sensitized by the prior injection of schistosome eggs developed relatively large granulomas, which nevertheless remained significantly smaller than the granulomas in the sham-operated sensitized animals. In thymectomized animals in-

fected with *S. mansoni,* however, the hepatic granulomas were as large as those in the sham-operated mice and the animals in both groups developed hepatosplenic disease of comparable severity.

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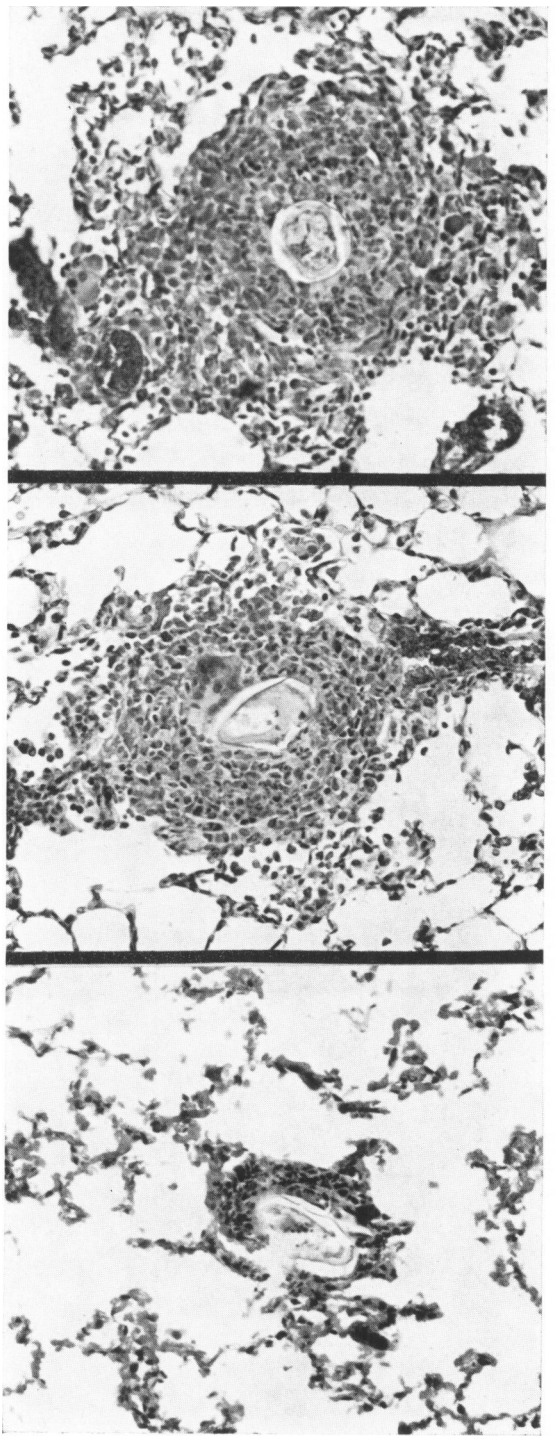
LEGEND FOR FIGURE

FIG. 1. Representative granulomas (after mean granuloma diameters for each experimental group was determined, sections were searched for a granuloma representative of mean diameter for group; this granuloma was marked and then photographed) in unsensitized sham-operated (*top*), sensitized thymectomized (*middle*), and unsensitized thymectomized (*bottom*) mice 16 days following injection of *S. mansoni* eggs. Hematoxylin and eosin stain. × 150.



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