# Eosinophil-Enriched Inflammatory Response to Schistosomula in the Skin of Mice Immune to *Schistosoma mansoni*

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**Exposure of the mouse skin to *Schistosoma mansoni* cercariae gives rise to acute, exudative inflammation in both normal and immune mice, but the immune response is anamnestically accelerated and is eosinophil-enriched, thereby enhancing opportunities for tegumental contact of schistosomula with host leukocytes, particularly with eosinophils. Many of the inflammatory changes occurring within the first 48 hours after exposure are due to cercarial products, e.g., "penetration tracts," but some remain demonstrable when schistosomula metamorphosed *in vitro* are injected intradermally and are therefore directed against the schistosomula themselves, such as the leukocyte "streaming patterns" seen in their pathways. In contrast to earlier observations in primates, cellular responses to schistosomula in the mouse lung 4 days after penetration are minimal in either normal or immune mice. Thus, immune cellular responses to schistosomula in mice are limited to an early time period after cercarial penetration and are morphologically suggestive of an antibody-mediated response rather than of delayed hypersensitivity. Our observations complement earlier evidence suggesting that antibody-mediated host leukocyte contact with schistosomula initiates the killing of challenge parasites in immune mice, with the eosinophil probably playing a crucial role. (Am J Pathol 84:479–500, 1976)**

SCHISTOSOME IMMUNITY is directed largely against *schistosomula*—the early, migratory worm stages arising from free-swimming cercariae after skin penetration—rather than against adult worms established in portal tributaries.[1,3] Studies of immunized mice have suggested that, in this experimental model, attrition of schistosomula begins prior to their lung transit, which is known to peak 4 to 6 days after exposure.[4] The mechanisms by which schistosomula are killed have not yet been defined but are currently under active study both *in vitro* [5,12] and *in vivo*.[13,21]

The pathogenesis of inflammatory responses to schistosomula in mice likewise remains unclear. Skin inflammation follows cercarial penetration in both nonimmune and immune mice, but is much more intense on reexposure to bird schistosomes ("swimmer's itch").[22] In *Schistosoma*

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*japonicum* infection, a focal exudative dermal response dominated by granulocytes has been described,[23,24] but in *S. mansoni* a diphasic pattern was observed, exudative inflammation changing to a predominantly mononuclear response after the first 24 hours. Divergent histologic patterns could also be reproduced in mice by skin injections of cercarial products according to whether recipients had been passively sensitized by transfer of immune serum or lymphocytes.[25] Lung responses to schistosomula in mice have been described as slight compared to those in the skin,[3] but sporadic retained schistosomula with inflammatory foci have been found in the lungs of mice immunized with *S. japonicum*.[24]

In the present study, we chose the mouse ear pinna as a useful site for quantitative analysis of dermal immune responses [26] and compared the skin and lung cellular responses of immune and nonimmune mice following exposure to cercariae of *S. mansoni*. We also compared cercarial skin reactions with those following intradermal injection of schistosomula which had been transformed from their cercarial stage *in vitro*,[5] with the aim of correlating our findings with what is presently known about the underlying mechanisms of schistosome immunity.

## Materials and Methods

Female mice of the CBA/J and C57/BL6J inbred strains were purchased from the Jackson Laboratories (Bar Harbor, Me.). *S. mansoni* (Puerto Rican strain) cercariae were obtained from infected *Biomphalaria glabrata* snails generously supplied by the Departments of Zoology, University of Michigan, Ann Arbor, Mich. (Dr. Harvey Blankenspoor) and the Department of Tropical Public Health, Harvard School of Public Health, Boston, Mass. (Dr. Edward Michelson). Six- to eight-week-old mice were immunized by intraperitoneal injection of 25 to 35 cercariae each.

### Challenge Infections

After 12 to 16 weeks of infection, immunized animals [4] and age-matched controls were given challenge infections by means of one of the three following procedures.

#### Percutaneous Infection of the Ear Pinna

Mice were anesthetized with Nembutal (Abbott Laboratories), and an ear pinna of each mouse was immersed in small test tube (8 × 30 mm) containing 1.3 ml of a suspension of live cercariae. In different experiments, mice were exposed to infecting doses of either 500, 1000, or 1200 cercariae each. After 15 minutes of exposure, the animals were removed from their position on the test tube rack, and the number of cercariae remaining in the test tubes was enumerated. On the average, 80% of the cercariae in the challenge doses were found to have penetrated the mouse ear tissue.

#### Intradermal Inoculation of the Ear Pinna With Schistosomula

Schistosomula were prepared from cercariae by the skin penetration method [5] and suspended at a concentration of $1.7 \times 10^4$ ml in Earle's lactalbumin (Flow Laboratories) containing 1% heat-inactivated fetal calf serum. Fifty microliters of the suspension was

then injected intradermally into the ear pinna of each mouse using a syringe fitted with a 26-gauge needle. Control injections consisted of fetal calf serum–supplemented media without organisms.

### Percutaneous Abdominal Infection

For examination of inflammatory reactions to schistosomula in the lungs, mice were challenged with 1000 cercariae each at a percutaneous abdominal site by means of the ring method.[27] On the average, 96% of the cercariae were found to have penetrated the infection site in these experiments.

### Histologic Processing

Ear or lung tissue specimens were fixed in either 10% buffered formalin or Bouin-Hollande fixative. Ears were removed at 6, 24, or 48 hours as specified below. Lung tissues were removed from the animals at 4 or 8 days after perfusion with fixative via the intratracheal route. Paraffin-embedded tissues were cut into 4-$\mu$ sections and stained either with hematoxylin and eosin (formalin-fixed tissue) or by means of the Litt modification of the Dominici stain (Hollande-fixed tissue).[28]

### Quantitative Histologic Procedures

Counts of schistosomula in the lung were done by scanning every fifth paraffin section (up to ten per mouse) and determining the mean number of schistosomula per slide per animal. Inflammatory responses to schistosomula in the skin were judged by the following procedures: a) Scanning all schistosomula found in five skin sections and scoring separately all those in which any inflammatory cells were present within a 30-$\mu$ radius of the organism, to yield the percentage of inflammatory responses per total number of schistosomula found per animal. b) Similarly scoring all schistosomula showing direct tegumental contact with host leukocytes. c) Eosinophil percentages were obtained by counting all host leukocytes versus all eosinophils located within a 75-$\mu$ radius of the center of a schistosomulum, as delineated by a standard diaphragmatic field aperture under conditions of Koehler illumination. Again, all schistosomula found in five sections per animal were scored in this manner. All data were statistically analyzed by the Student $t$ test.

## Results

### Histologic Features Shared by Immune and Nonimmune Mice* Pathology of the Ear Pinna in Cercarial Infection

At 6 to 8 hours there was focal and disseminated exudative inflammation prominently involving the dermis, but also extending into the epidermis, and more rarely, the muscle layer. Well over 90% of the inflammatory cells were granulocytes, interspersed with a few monocytes and lymphoid cells, especially in the reticular dermis (Figures 2–10). Plasma cells were extremely rare. Mast cells were numerous and similar in distribution to those of unchallenged controls (Figures 10–12). Occasionally, free metachromatic granules were found interspersed between inflammatory cells in both immune and nonimmune animals.

Most of the denser inflammatory foci appeared to be related to sites of

* Described in chronologic order, but illustrated by feature, i.e., without regard to chronology.

cercarial penetration or to schistomula present either within the same section, or in adjacent step sections (penetration tracts) (Figures 5–7), while others were unrelated or were found in scattered locations, representing nonspecific "scratch marks" (Figure 8). Several of the penetration tracts were marked by disintegrating cercarial tails or bodies (Figures 5 and 7), but most contained only granular debris embedded in granulocytic exudate. Tracts were subcorneal in location and ranging from tiny superficial epidermal slits or burrows to substantial papules lifting the horny layer off the epidermis or eroding the spinous or basal layers, thus denuding the underlying dermis (Figures 5 and 7). Aggregates of granulocytes appeared in the dermis subjacent to these tracts and migrated through the epidermal layers accompanied by focal epidermal cell necrosis and spongiosis, forming microabscess-like superficial collections within the tracts (Figure 7). Occasionally, similar dense granulocytic aggregates were seen at the dermal level as well, some of them underlying apparently unbroken skin (Figure 2). Skin lesions unrelated to schistosomula, or scratch marks, although superficially similar to penetration tracts, were larger in size, more densely infiltrated by neutrophils than cercarial lesions, and largely denuded of a corneal layer (Figure 8). These distinctive features were best observed in animals injected with schistosomula (see below) which lacked cercarial penetration tracts altogether.

Granulocytic reactions to schistosomula varied greatly in intensity as well as distribution. Some parasites were free of significant cell reaction (Figure 1), especially those found embedded within hair follicles, while others had attracted numerous granulocytes (Figure 2). There was similar variation of granulocyte contact with the schistosomulum integument. A common pattern consisted of stream-like granulocytic aggregates in the dermis oriented toward schistosomula either in an upward (Figure 9) or a lateral (Figure 10) direction, depending on whether parasites were intraepidermal or dermal in location. A few other intradermal schistsomula were concentrically surrounded by granulocytes (Figure 2).

Postcapillary venules, especially those near schistosomula or tracts, were markedly dilated and showed leukocyte margination and diapedesis. Red cells inside these vessels appeared clumped, or sometimes crenated or leached; endothelia were swollen or focally detached, especially where schistosomula were entering such vessels (Figure 6). Around some of the venules and at the base of eroded tracts, pink hyaline proteinaceous material was deposited in the dermis and was surrounded by diffuse edema (Figures 6 and 7). Fibroblasts in these sites appeared basophilic and rounded, and connective tissue elements widely separated.

Skin areas close to densely inflamed foci exhibited scattered or clustered

granulocytes in the dermis or epidermis, while sites distant from tracts or schistosomula showed only occasional scratch marks (plus mild congestion and edema) and were otherwise unremarkable. Rarely, invading schistosomula reached the muscle layer, and in one such instance myofibrillary degeneration was seen next to a parasite; elsewhere, muscle foci were marked only by granulocytic aggregates and by plumpness and basophilia of sarcolemmal cells. The ear cartilage was everywhere normal.

*By 24 hours* the overall skin architecture had become increasingly altered. Inflammatory infiltration was greatly intensified, and in some specimens almost confluent, albeit still focally condensed. Its cellular composition remained largely granulocytic with only a mild increase in the proportion of monocytes, macrophages, and lymphoid cells; however, the latter remained scattered and did not form perivascular aggregates or sheets. Mast cells remained largely intact, without discernible change.

Dermal edema was less conspicuous, but focal endothelial, fibroblastic, and sarcolemmal proliferation were augmented. Penetration tracts frequently contained degenerate pus cells, and many had become ulcerated or sloughed or both. While intact granulocytes continued streaming into the eroded sites, the adjacent epidermis showed basal mitotic activity and thickening of its prickle-cell layer up to thrice its normal diameter (Figures 2, 4, and 9). Lesser tracts were beginning to heal by crusting and shedding of superficial squamae and of discolored tissue debris from their thickened epithelium.

While the overall number of dense granulocytic infiltrates at 24 hours was, if anything, greater than at 6 to 8 hours, the number of detectable schistosomula was lesser (see Table 2). Where schistosomula were present, however, they still showed the same range of spatial relationship to inflammatory infiltrates as had been observed earlier, except that concentric foci around schistosomula were somewhat more commonly seen (Figures 2 and 10).

*By 48 hours* the number of schistosomula had diminished further (see Table 2) but the pattern of inflammatory change remained similar to that observed at 24 hours, with some decrease in exudation and vascular damage and with some increase in reparative processes—such as epidermal cell proliferation, superficial tract shedding, and focal dermal fibroblastic activity. Monocytes and lymphocytes remained scattered, and their numbers were modest in proportion to the total infiltrate.

#### Morphology of Schistosomula

Most of the schistosomula observed in the skin of both nonimmune and immune mice appeared morphologically intact. Viewed under oil immer-

sion, some showed a clear linear hiatus between their tegument and the underlying muscle layer ("peeling") without evidence of other structural damage (Figure 4). A few schistosomula (one of every thirty to forty) showed frank tegumental swelling and blebbing associated with vacuolation and nuclear pyknosis of the parenchymal cells (Figure 2); these degenerate parasites were found mostly in the center of microabscess-like granulocyte condensations either in the subcorneal layer or in the dermis; very rarely, leukocytic invasion of degenerating cercarial bodies or schistosomula was noted. No quantitative difference could be established between nonimmune and immune mice with regard to the frequency of damaged schistosomula in the skin, partly because of sampling problems and partly because of difficulty in reliably recognizing subtle damage.

#### Histologic Differences Between Immune and Nonimmune Mice

The single most striking difference found became evident only through the use of the Dominici stain—namely, a dramatic increase in the number and proportion of eosinophils in the inflammatory infiltrate of immune mice, compared with nonimmune mice, as shown in Table 1. The eosinophil percentages in nonimmunized mice were at relatively constant low levels, averaging approximately 2 to 4% of all inflammatory cells surrounding schistosomula, whereas in immunized animals they averaged from 16 to 28% at 6 and at 24 hours, respectively, i.e., an eight- to ninefold increment. Although no eosinophil counts were done at 48 hours, eosinophil enrichment was sufficiently marked in the immune mice to permit two independent observers to correctly determine the immunologic status of individual mice, at all three intervals studied, by qualitative observation only. The greatest density of eosinophils in immune mouse skin was seen in microabscess-like foci with or without schistosomula (Figure 4); in nonimmune mouse skin, such foci were observed more rarely and were largely composed of neutrophils (Figure 3). Contact of eosinophils with the schistosome tegument was only sporadically observed in nonimmune animals (Figure 5) and was somewhat more frequently seen in the immune mice (Figures 2, 4, 9, and 10). Yet, this event occurred in only a small proportion of all schistosomula seen in any one skin section.

Significant quantitative differences in the overall intensity of early cell responses to schistosomula were also established as shown in Table 2.

Six hours after exposure, the overall percentage of cell reactions to schistosomula and the frequency of granulocytic contact with schistosomulum integument were both significantly (up to twofold) greater in the immune (Figures 2, 4, 7, 9, and 10) than in the nonimmune mice

Table 1—Eosinophil Composition of Inflammatory Reactions to Invading Schistosomes in the Skin of Immune vs. Normal CBA/J Mice

| Time | Status | Animals | Total lesions scored | Percent eosinophils (mean ± SD) | |
|---|---|---|---|---|---|
| | | | | Per animal | Per lesion |
| 6 hrs | Normal | 3 | 24 | 1.67 ± 2.89 | 2.08 ± 5.84 |
| | Immune | 3 | 19 | 15.59 ± 8.50 | 17.84 ± 17.78 |
| | | | | $P < 0.05$ | $P < 0.001$ |
| 24 hrs | Normal | 6 | 53 | 3.85 ± 1.46 | 3.68 ± 5.77 |
| | Immune | 6 | 52 | 27.54 ± 8.04 | 28.38 ± 15.58 |
| | | | | $P < 0.001$ | $P < 0.001$ |

(Figures 1, 3, 5, and 6). By 24 hours, the same trend continued, but the differences were no longer statistically significant and remained so at 48 hours.

#### Pathology of the Ear Pinna After Injection of Schistosomula

Lesions at 48 hours were limited to the intradermal bullae marking the injection sites, and to an approximately 1-mm radius around them, except where there had been additional trauma to the tissue on either side of the cartilage. The sham-injected controls showed relatively small bullae and relatively mild neutrophilic infiltration in and around injection tracts with occasional displaced follicles or cartilage fragments. In both normal and immune schistosomula-injected mice, the epidermis overlying or lateral to the bullae was normal except for sporadic scratch marks (Figures 11 and 12). Bullae and surrounding tissues of schistosomula-injected normal mice showed increased granulocytic infiltration compared to sham-injected controls, with adherence of leukocytes to schistosomula inside the bullae and or focal concentration around those situated within the adjacent dermal tissue (Figure 11). Some of the schistosomula showed evidence of

Table 2—Inflammatory Reactions Around Invading Schistosomes in the Ears of Immune vs. Normal Mice

| Time | Status | Total no. of organisms counted | Percent with reaction (±SD) | Percent with cell contact |
|---|---|---|---|---|
| 6 hrs | Immune | 47 | 74.3 ± 16.3 | 49.2 ± 19.1 |
| | Normal | 134 | 32.0 ± 23.6 | 18.0 ± 16.0 |
| | | | $P < 0.05$ | $P < 0.05$ |
| 24 hrs | Immune | 37 | 63.5 ± 14.4 | 32.5 ± 19.3 |
| | Normal | 54 | 33.3 ± 27.6 | 11.0 ± 9.0 |
| | | | NS | NS |
| 48 hrs | Immune | 21 | 44.5 ± 9.9 | 31.3 ± 26.0 |
| | Normal | 99 | 47.5 ± 13.3 | 26.7 ± 16.2 |
| | | | NS | NS |

NS = differences between means which were not significant at the $P = 0.05$ level.

mild or obvious damage, as described earlier, but this did not seem to influence the degree of leukocytic response to them.

Compared to the normal challenged mice, the amount and density of leukocytic infiltration was considerably greater in the immune mice, to the point where their bullous cavities contained abscess-like aggregations of granulocytes around groups of schistosomula (Figure 12). Similarly, the proportion of eosinophils in the exudate as observed in Dominici-stained slides was greatest in the immune mice (Figure 12), lesser in the non-immune mice, and least in the sham-injected controls. These differences were obvious on scanning without need of differential counts.

#### Pathologic Observations in the Lung

Of 281 schistosomula visualized in lung sections of nonimmunized mice (47 per mouse), three were found associated with minimal inflammatory foci and the remainder showed no reaction (Figure 13). However, similar small leukocytic foci were seen sporadically in the absence of schistosomula. Both foci and schistosomula were predominantly in subpleural locations, suggesting a random association between them.

Of 139 schistosomula visualized in immune mouse lungs (28 per mouse), six were associated with small inflammatory foci (Figure 14) and the remainder showed virtually no reaction. However, the lungs of these previously infected mice also contained schistosome eggs and granulomas, as well as desquamative–exudative foci associated with egg deposition in variable numbers. In addition, the lungs showed an increase and focal condensation of leukocytes including eosinophils in and around alveolar capillaries. These latter changes were found to be proportional to the number of eggs seen in individual lungs and were, therefore, not attributable to the challenge schistosomula, although here and there eosinophils were seen associated or in contact with them.

## Discussion

The percentage of schistosomula which normally attain maturity varies with the host species, as does the reduction in their percentage by which immunity is measured. Thus, self-cure and solid immunity are the rule in macaques [1,2,3,20] and rats;[2,21] rhesus monkeys repeatedly vaccinated by exposure to x-irradiated *S. japonicum* cercariae have shown excellent protection.[29] By contrast, mice [2,4,13,14] and, probably, humans [30] show only limited acquired resistance. The *S. mansoni*–mouse model used in our study is characterized by an approximately 60 to 90% maturation reduc-

tion when normal and immune mice are compared either by lung [4] or by portal [2,4] worm recovery methods.

The sites of retention and killing of challenge schistosomula in immune mice still remain to be defined. Normal mice show an approximately 30% skin attrition rate.[31] We were unable to show that this rate was altered in our immune mice since the difference in skin counts was not statistically significant (Table 2). Conversely, the lower numbers observed in lung sections (see page 486) were in keeping with earlier findings indicating that loss of schistosomula begins prior to the fourth to sixth days, when lung migration reaches its peak.[4] If these problems are to be resolved, improved methods are needed for quantitatively sampling schistosomula and for recognizing subtle damage in them.

Regardless of where in the immune host the schistosomula ultimately succumb, the cellular reaction to them clearly has its onset in the skin. In the immune mice, dermal cell responses were accelerated and augmented in anamnestic fashion as early as the sixth hour after penetration, and the chances of surface contact between schistosomula and host granulocytes, including eosinophils, were thereby significantly enhanced. By the time the organisms reached the mouse lung, this focal cell response had abated to a point where it could no longer account for significant worm reductions attributable to host immunity. It is well documented that, during this same interval, schistosomula acquire a number of host antigenic determinants which are thought to protect them from immunologic recognition by the host;[32,33] it is not yet clear, however, whether this process requires contact of schistosomula with any specific host tissue or is merely dependent on time, i.e., on intrinsic parasite development. Thus, it has recently been shown that immunity in mice remains operant even if the challenge entirely bypasses the skin (by injecting schistosomula intravenously).[17] The species of host undoubtedly has an important influence on sequential cell responses to schistosomula,[15] since prominent inflammatory lung foci (tuft-like foci) occur in the highly immune macaque,[34] whereas, in mice, we found only minor lung reactions to schistosomula. Thus, in mice, the cellular effect or mechanisms of immunity appear to act only within a relatively brief time-span following cercarial penetration. Further studies on the time sequence of host reaction to schistosomula are in progress in our laboratory.

Cercarial penetration and metamorphosis, whether in normal or immune host skin, are known to release a multiplicity of enzymatic and antigenic products, including cercarial tails, glycoproteins of the cercarial envelope,[35] acetabular and preacetabular gland secretions,[36-39] and somatic antigens of cercariae which die shortly after host contact. Some of

these products, such as proteases,[38] are direct irritants and can stimulate host mediators of inflammation by degranulating mast cells and liberating vasoactive amines [36] or by activating complement.[40] This probably accounts for the marked similarities between the morphology of primary and immune skin responses to cercarial exposure, described here in some detail. When we eliminated cercarial by-products by injecting schistosomula metamorphosed *in vitro*, primary skin responses were milder and devoid of cercarial penetration tracts and of excematoid epidermal lesions, but in the immune mice an anamnestic inflammatory response to schistosomula nevertheless ensued. Thus, a significant portion of the cellular skin responses to infection is in fact directed against the schistosomula themselves, and this is augmented in the immune host. (It is also well documented that immunity against schistosomes can be elicited by injecting schistosomula alone, without any need of prior cercarial penetration.[41]) Evidently, the skin reaction to cercariae has several morphologic components: some of the lesions (e.g., scratch marks) are nonspecific and others (e.g., the penetration tracts) are best attributed to cercarial activities and products. This leaves the streaming—or concentric dermal migratory patterns of leukocytes in relation to schistosomula—as perhaps the most interesting feature. As in the tuft-like exudative foci reacting to schistosomula in the immune rhesus lung,[34] these migratory patterns suggest the elements of a chase in which either the hunters or the prey may gain advantage, thus ending in capture of the parasite (Figure 2) or in its escape "under protective clothing."[32] That host leukocytes are involved in the immune killing of schistosomula is supported by ample evidence,[6-11,14-16] although the mechanisms are far from understood.

Immunologic analysis of inflammatory responses to schistosome cercariae and schistosomula is obviously a complex task and continues in our laboratory and other laboratories, using the mouse as a prime model. Here, the key problem has been to determine which of the numerous mediator systems invoked in the resistance and/or cellular responses to schistosomula are active *in vivo* and are functionally specific. A symposium summarizing the current status of this research has recently been published.[42] Two findings of the present study may be important: a) the tissue reactions to schistosomula in mice maximally immunized to *S. mansoni* [2,4] were morphologically suggestive of an antibody-mediated process rather than of delayed hypersensitivity; and b) they were eosinophil enriched.

The skin reactions described by us peaked at 6 to 24 hours and were prominently vasoactive and exudative, and overwhelmingly granulocytic. We were unable to find significant mononuclear infiltrates [25,29] within the

48-hour time limit of our study and, therefore, cannot judge whether mice might have developed delayed hypersensitivity responses later, 5 or more days after exposure, as observed in immunized macaques,[29] In any case, skin reactions coincident with the time of maximal lung transit of schistosomula would not be pertinent to the mechanism of immunity.

The striking enrichment in eosinophilic granulocytes observed by us could also be of help in determining the nature of the antibody or antibodies mediating schistosome immunity in mice. Dermal eosinophilia in schistosome-challenged hosts had been mentioned by some early workers [43] and was recently reemphasized in a study involving multiple skin exposure of rhesus monkeys to x-irradiated *S. japonicum* cercariae in which dermal eosinophilia was shown to begin sooner after each successive exposure.[29] In our study in mice, eosinophilia was manifest by the sixth hour after first rechallenge and reached proportions far greater than those of circulating eosinophils seen in mice at 12 to 16 weeks after a primary infection.[44,45] The early eosinophilia and sporadic mast cell degranulation observed here were suggestive of reaginic hypersensitivity,[19,20] but this would not by itself account for specific interactions between eosinophils and schistosomula, as suggested by the tendency of these leukocytes to stream toward and around the parasites. Such interactions were not as frequent and dramatic in immune mice as in Hsü's description of eosinophils destroying schistosomula in immune rhesus monkeys.[29] Whether these eosinophil activities are an integral part of parasite rejection or an epiphenomenon has been a persistent problem in the general immunology of helminth infections.

Studies by Butterworth *et al.* have shown that eosinophils are by far the most efficient of host leukocytes in eliciting $^{51}Cr$ release from schistosomula in the presence of complement-inactivated infected human patient serum *in vitro*;[7] moreover, Mahmoud *et al.* have recently reported that depletion of eosinophils in immune mice by specific antieosinophil serum results in a significant loss of their immunity to *S. mansoni*.[16] Thus, our present report of an eosinophil-enriched dermal inflammatory response in mice immune against schistosomes marks the convergence of several lines of evidence suggesting that antibody-mediated leukocyte-dependent damage to schistosomula could be the first step in the immune killing of challenge parasites, with eosinophils playing a crucial role.

If these assumptions are valid, eosinophil-enriched skin responses to schistosomula in mice should be affected by conditions which either enhance or depress schistosome immunity, and studies of such correlations in our laboratory could furnish clues to the nature of the antibodies mediating both phenomena. At the same time, interest is being focused

on the mechanism of the presumed schistosomicidal action of eosinophils and on their immunologic effector functions [16] which have for so long eluded investigation.

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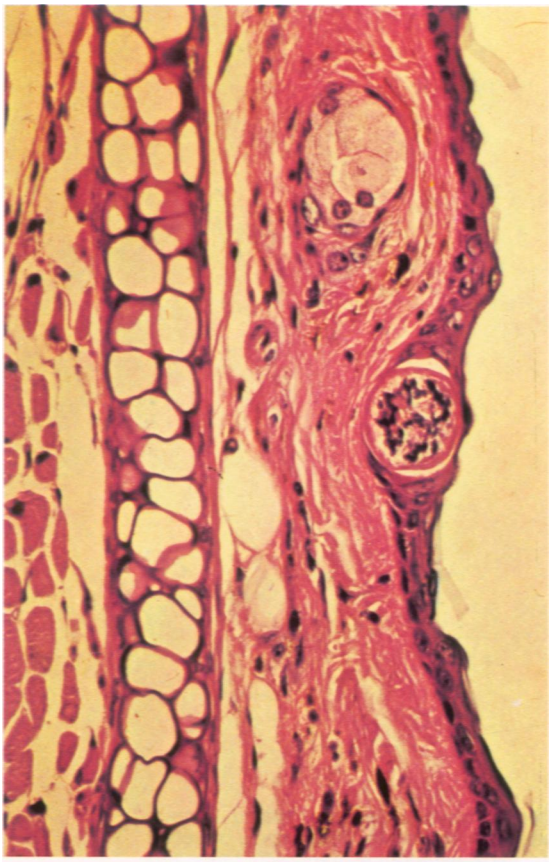
## Legends for Figures

**Figure 1**—Nonimmune mouse pinna at 6 hours showing an intraepidermal schistosomulum without cellular reaction. Note normal skin, adnexae, ear cartilage, and muscle layers. (H&E, × 430)

**Figure 2**—Immune mouse pinna at 24 hours. Damaged intradermal schistosomulum can be seen; note pyknosis, vacuolization, and tegumental blebbing surrounded by a dense granulocytic focus. The epidermis shows proliferation. (H&E, × 700)

**Figure 3**—Nonimmune mouse pinna at 24 hours. Intact intradermal schistosomulum showing pigment in its cecum with streaming neutrophilic reaction is seen. Note a single eosinophil (at 12:30, above parasite), vasodilation, edema, and normally granulated mast cells. (Dominici, × 430)

**Figure 4**—Immune mouse pinna at 24 hours. Subtly damaged intradermal schistosomulum is surrounded by a dense concentric eosinophlic focus in tegumental contact with the parasite. (Dominici, approximately × 1000)



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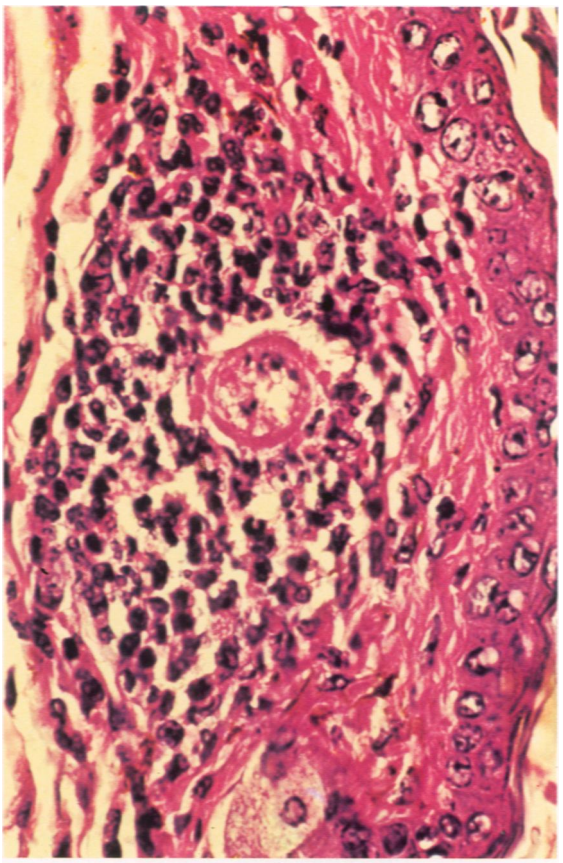

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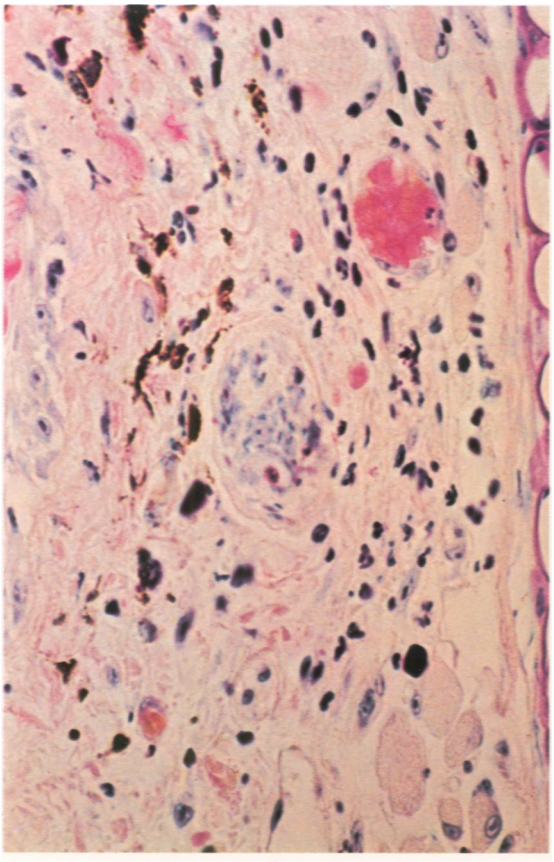

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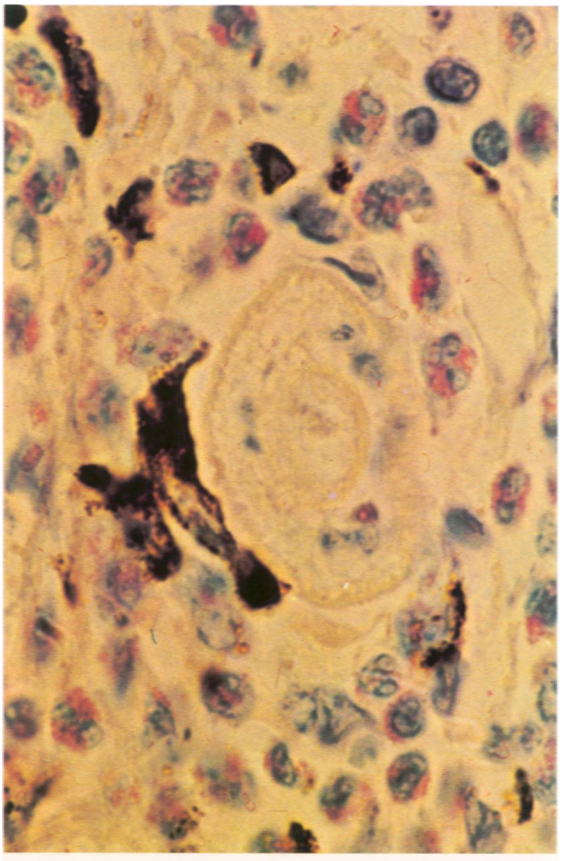

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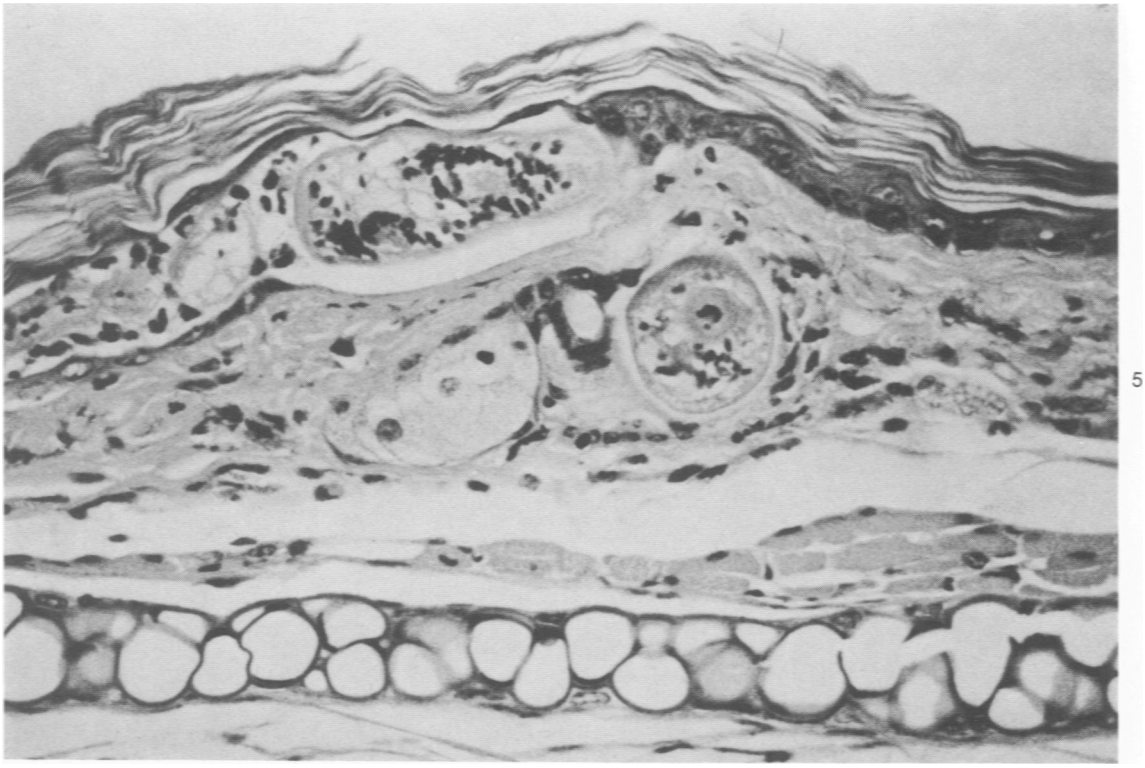
**Figure 5** — Nonimmune mouse pinna after 6 hours shows a metamorphosing intraepidermal cercaria and a dermal schistosomulum, with moderate granulocytic reaction. The intraepithelial organism is contained in an early penetration tract with basal erosion. Sparse granulocytes are contacting the tail and caudal pole of the body. (H&E, × 530)

**Figure 6** — Nonimmune mouse pinna at 6 hours. A normal dermal schistosomulum is caught in the act of penetrating a venule. Note altered endothelium and erythrocytes. There is marked dermal edema. Neutrophils are concentrating toward the base of a penetration tract (cut off from the right margin of this micrograph). (Dominici, × 530)



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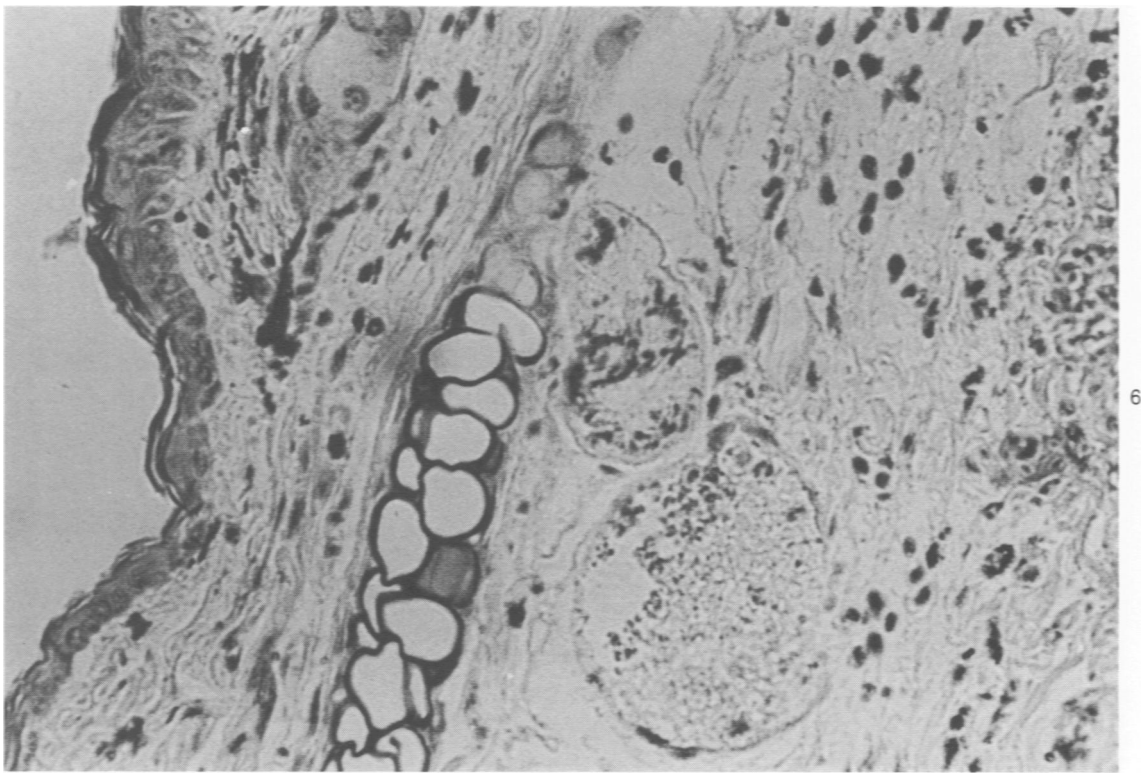

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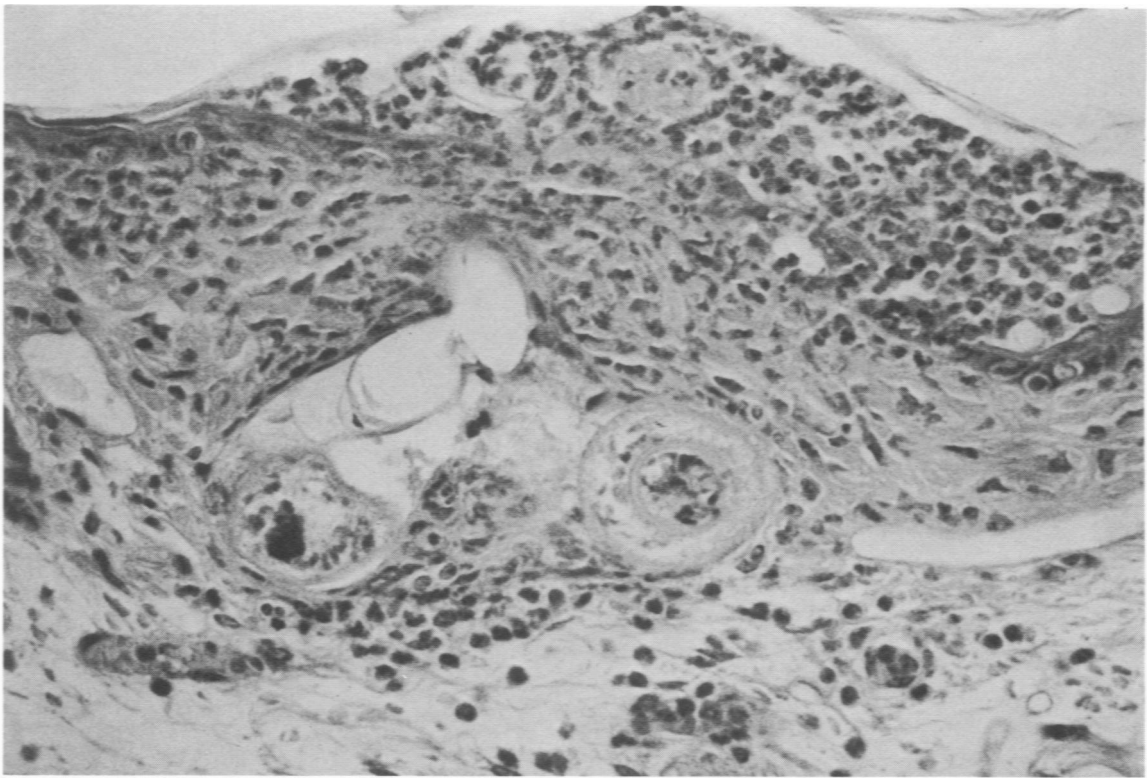


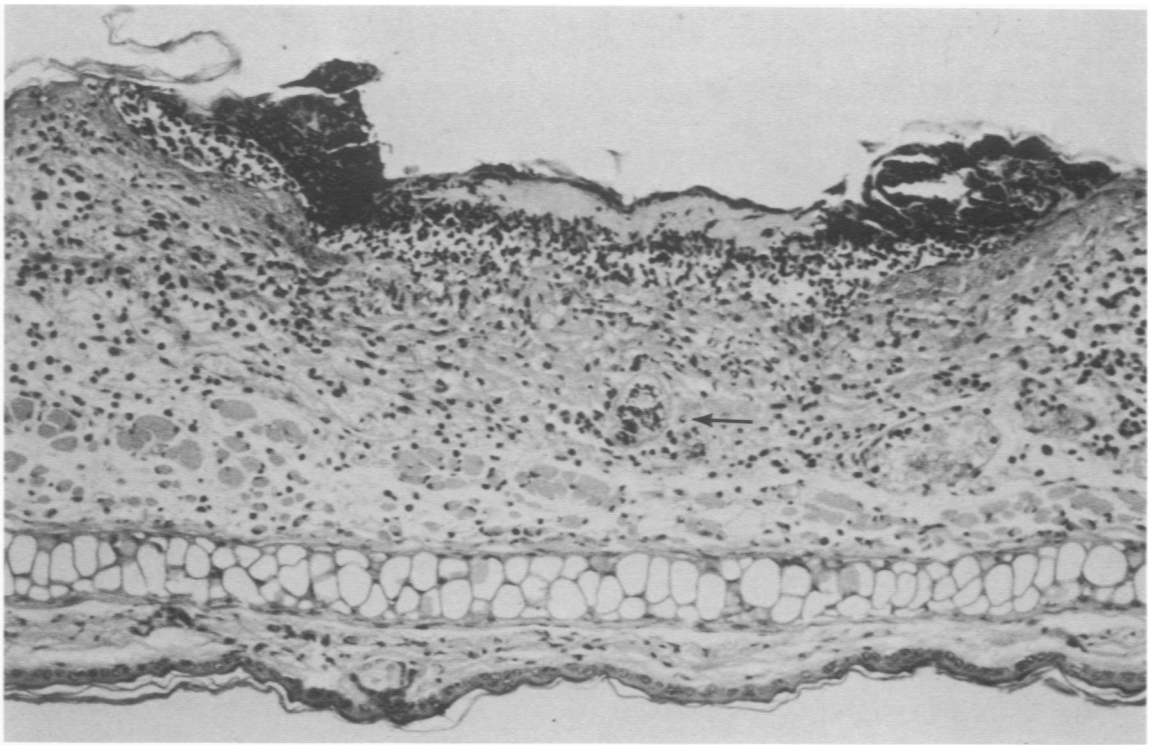
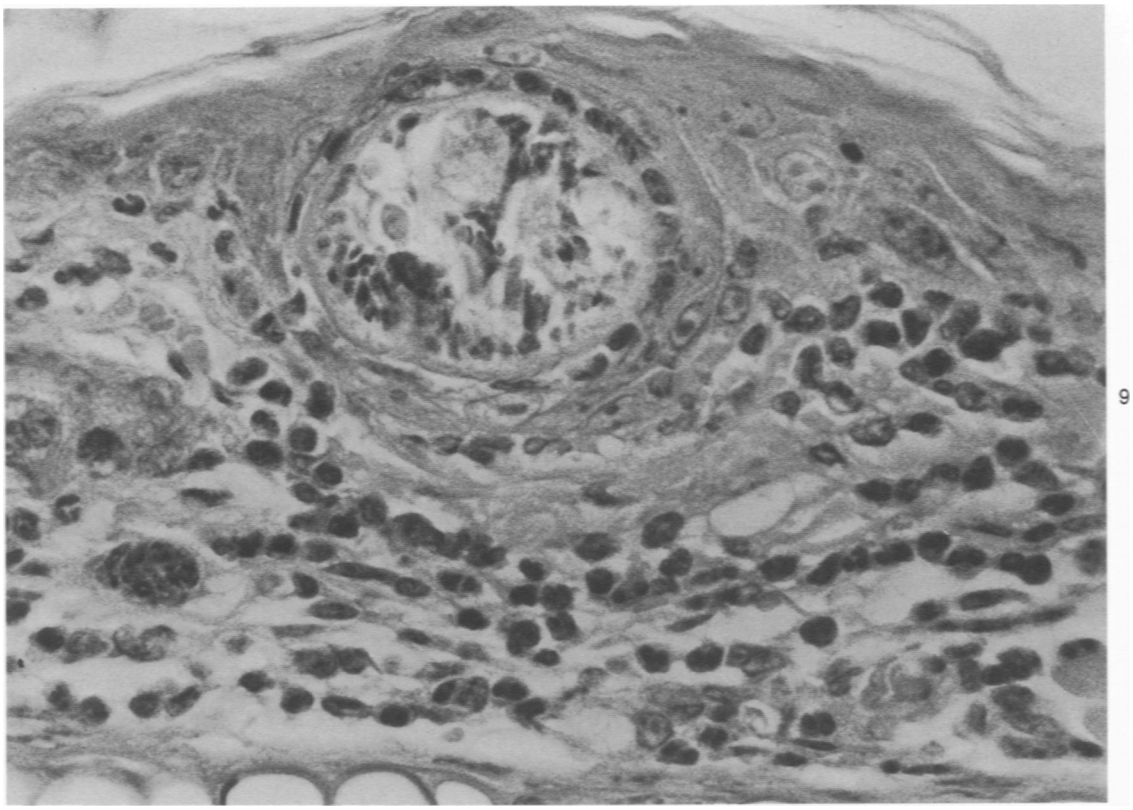


**Figure 7**—Immune mouse pinna at 24 hours showing two normal intradermal schistosomula partly surrounded by a dense, eosinophil-enriched granulocytic reaction with focal tegumental contact, subjacent to a fully developed, basally eroded penetration tract underlying the corneal layer which contains cercarial tail remnants embedded in a microabscess. Note the streaming patterns of leukocytes and the prominent edema in the lower portion of the picture. (H&E, × 530) **Figure 8**—Nonimmune mouse pinna at 24 hours showing a scratch mark situated at the site of a penetrated dermal schistosomulum (*arrow*) with incomplete avulsion of the corneal layer and superficial crusting. Note the extensive erosion of the epidermis with lateral regenerative budding. Most inflammatory cells seen here are neutrophils. (H&E, × 330)



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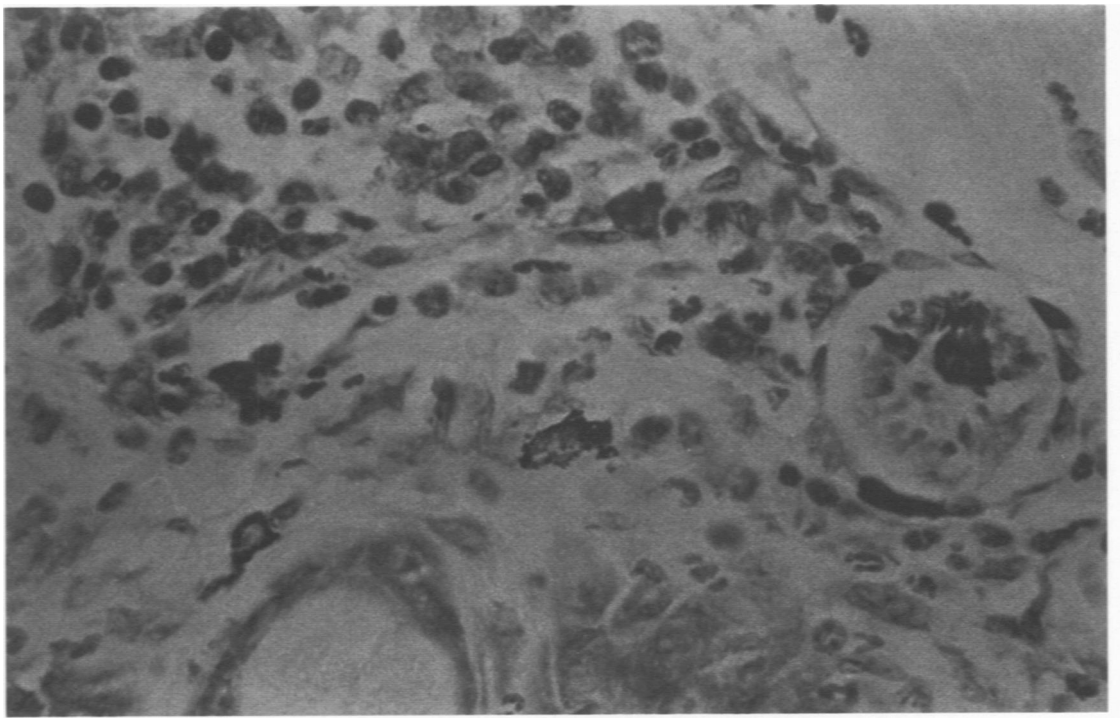

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**Figure 9**—Immune mouse pinna at 24 hours. Normal intraepidermal schistosomula whose tegument is surrounded by a corona of eosinophils which also densely infiltrate the dermis. Note the intercellular edema of the epidermis. (Dominici, × 850) **Figure 10**—Immune mouse pinna at 6 hours. A normal intradermal schistosomulum partially surrounded by a streaming pattern of eosinophils intermingled with some monocytes. Mast cells, including the one overlying the lower contour of the parasite, are not degranulated. (Dominici, × 850)

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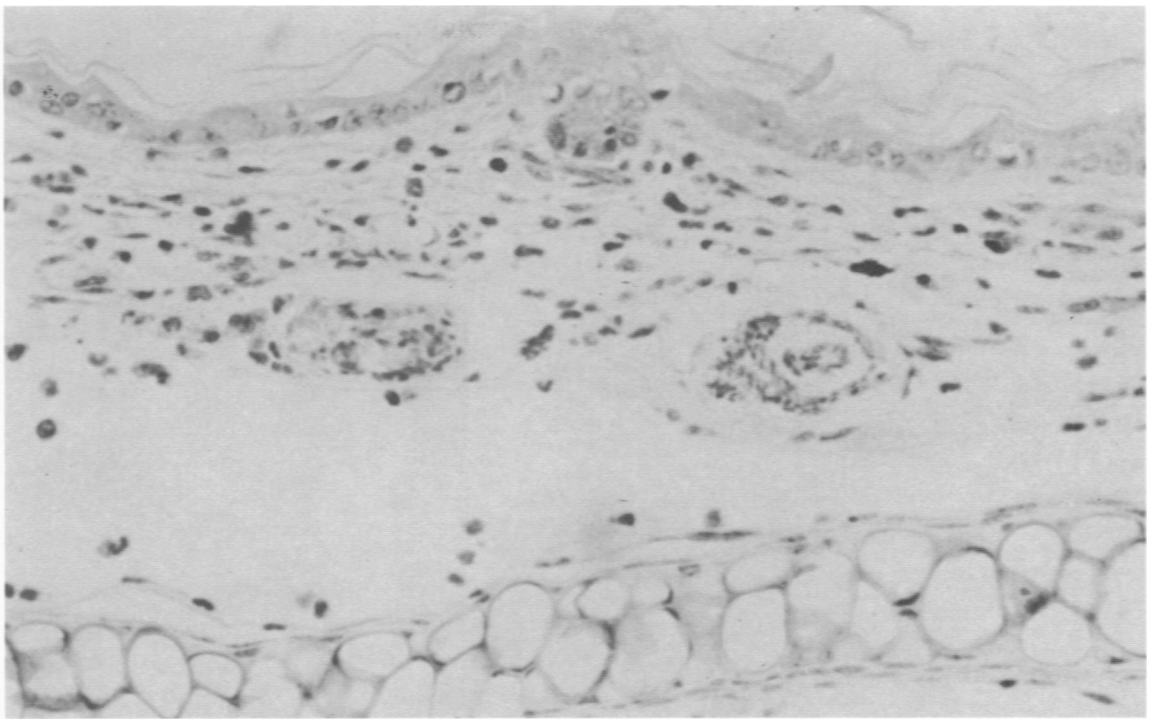

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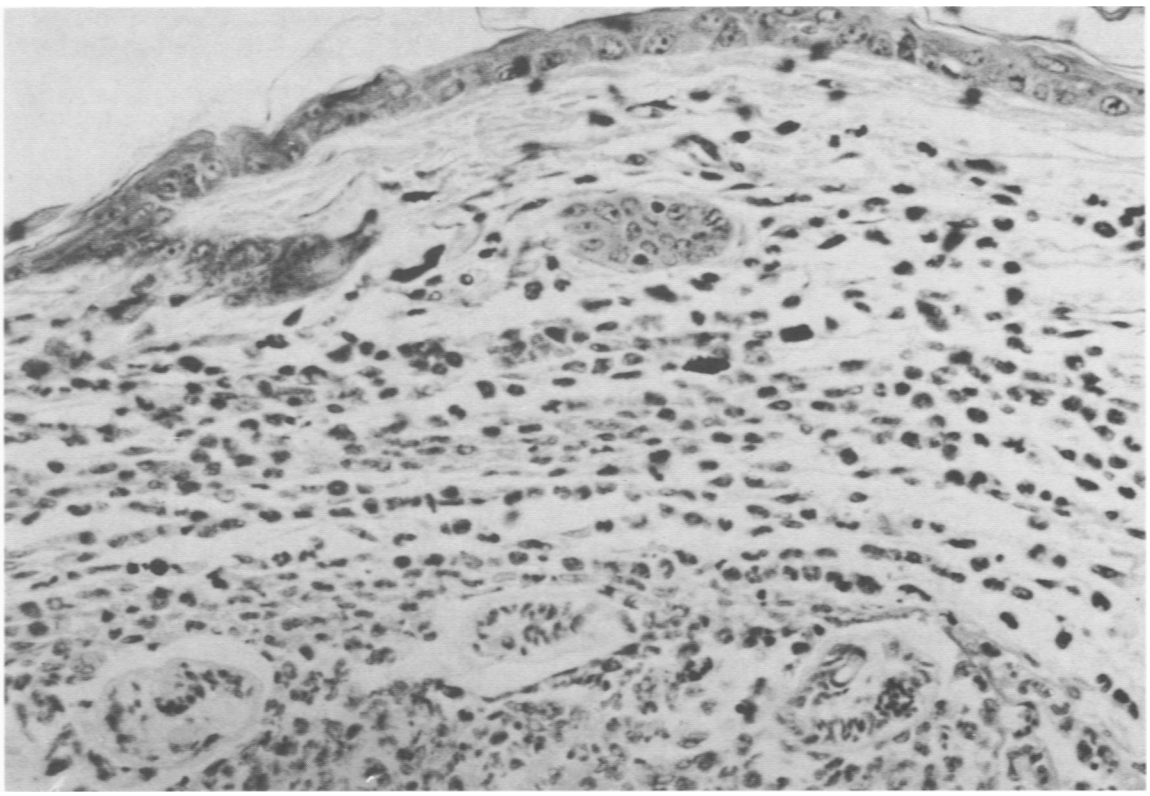
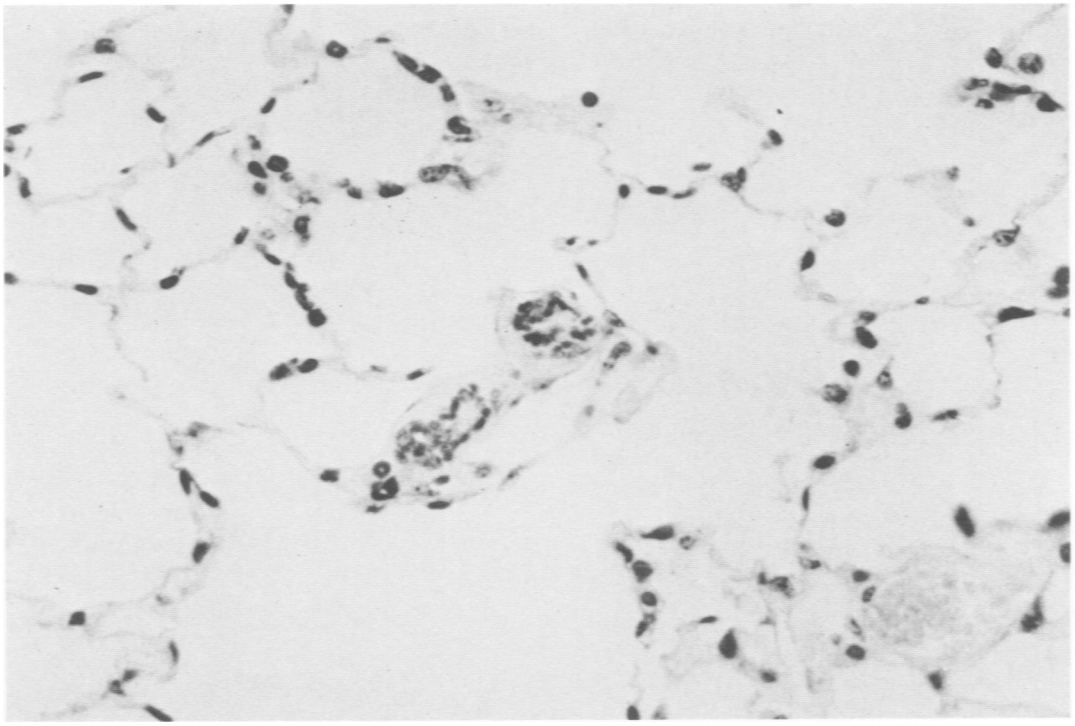


**Figure 11**—Nonimmune mouse pinna injected with schistosomula at 24 hours showing representative granulocytic infiltration of the injection bulla and around the two schistosomula. Note normal epidermis and granulated mast cells. (Dominici, × 530) **Figure 12**—Immune mouse pinna injected with schistosomula at 24 hours; maximal granulocytic reaction fills the injection bulla and surrounds the three schistosomula. Many of these cells are eosinophils. Note, again, normal epidermis and granulated mast cells, similar to those of Figure 11. (Dominici, × 530)



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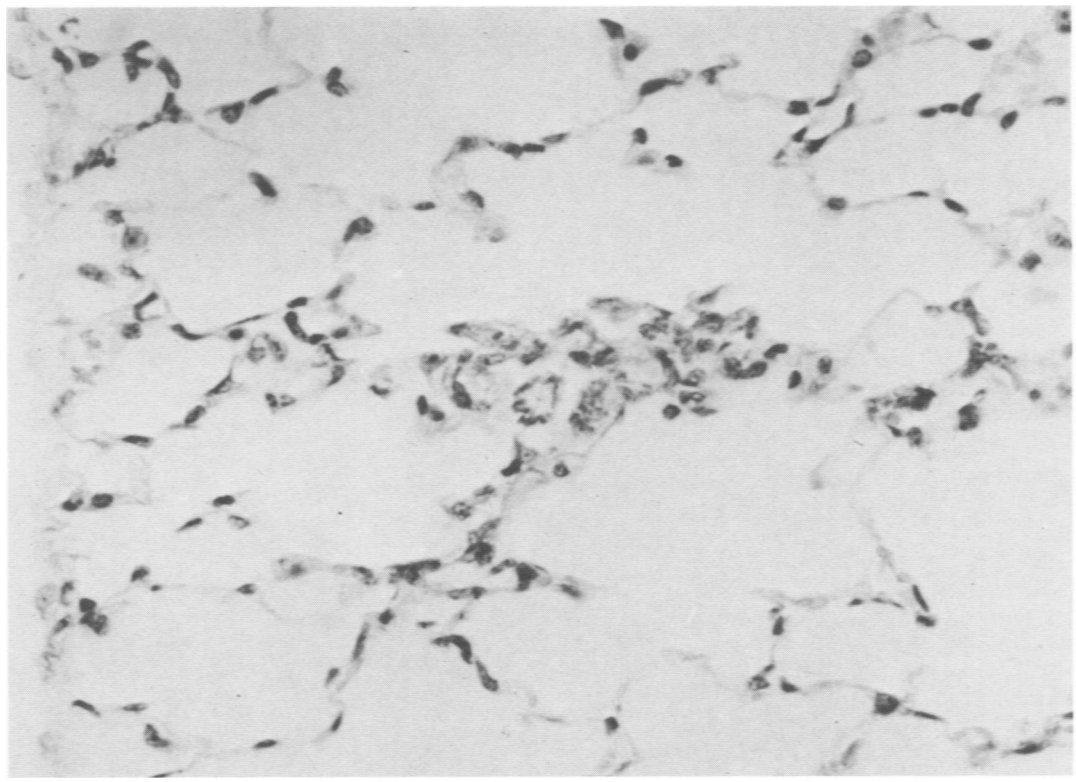

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**Figure 13**—Nonimmune mouse lung showing migrating schistosomulum at 4 days. Two leukocytes are situated in a capillary next to the parasite; otherwise, no cellular reaction is seen. (Dominici, × 530) **Figure 14**—Immune mouse lung showing a schistosomulum at 4 days. A small leukocyte focus has formed next to the parasite which is seen in transverse section, close to the pleura (left). A few of the cells are eosinophils. A few scattered leukocytes are present in the alveolar capillaries. (Dominici, × 530)

[*End of Article*]