STUDIES ON GRANULOMA FORMATION

III. Antigen Sequestration and Destruction in the Schistosome Pseudotubercle

F. v. Lichtenberg, M.D.

From the Department of Pathology, The Peter Bent Brigham Hospital and Harvard Medical School, Boston, Mass.

Antigenic sites of *Schistosoma mansoni* (S.M.) and *Schistosoma japonicum* (S.J.) can be selectively stained either by the "direct" or the "indirect" Coons technique. Stainable schistosome antigen (SSA) abounds in the cuticle of worms and cercariae.[1] Anderson and Sadun, therefore, used cercariae prestained with rhodamine-bovine-albumin (RBA) for their modified indirect Coons test and found it to be a sensitive diagnostic screening method.[2] SSA in schistosome eggs and granulomas in mammalian tissues was first demonstrated by Andrade, Paronetto and Popper[3] by means of the direct and indirect Coons staining with human and mouse serums. Magalhaes Filho[4] obtained similar results with serum from a naturally resistant host. Studies on the immunofluorescence of schistosomula (immature migratory worms, intermediate between the cercarial and adult stages) and adult worms in mammalian tissues have not yet appeared. These would be essential for an understanding of the mechanism of acquired resistance in *Macaca mulatta*. In this subject resistance can be produced by long-term S.J. or S.M. infection,[5,6] by a zoophilic strain of S.J.,[7,8] or by irradiated cercariae of S.M. and S.J.[9-11]

The antigenic structure and chemical features of the schistosome egg are largely unexplored. The eggshell is said to be chitinous,[12] and an acid-fast material is present in eggshells and in miracidia.[13] Andrade and Barka[14] have suggested that the major antigenic component in eggs is a polysaccharide or glycoprotein. Smithers and Williamson[15] found relatively large amounts of a glucosan-type compound in purified egg fractions. Lewert and Lee[16] have suggested that a ground substance depolymerase is secreted by miracidial penetration glands, the normal substrates of which are the host-snail mucoids; the enzyme is contained in a periodic-acid-Schiff (PAS) positive secretion product.

The experimental schistosome pseudotubercle or granuloma has been

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shown to follow a reproducible time sequence with respect to onset, duration and healing time. Its final healing stage, which is attained about 6 months after injection in the unsensitized host, coincides with the disappearance of residual egg material.[17] Granulomatous tissue reaction around parasite eggs is delayed in the newborn [18] but enhanced and accelerated in the sensitized adult mouse.[19] It has, therefore, been postulated that the pseudotubercle is a response to antigens which remain accessible to nearby phagocytes following their deposition in host tissue, perhaps because of low diffusibility. After an initial period of diffusion, these antigens would be walled off or "sequestered" by phagocytes and would eventually be ingested and metabolized mostly within the granuloma. Depending on the nature of the antigen, circulating antibody might or might not be induced, but, in either case, the host would be sensitized and become capable of accelerated antigen-sequestration and destruction.

A standard technique has been established for the immunofluorescent staining of schistosome antigens in mammalian tissue sections, and the above hypothesis was tested in two successive stages: (a) study of the SSA of eggs under conditions of natural, i.e., cercaria-induced, infection both in host tissues and *in vitro;* and (b) study of the fate of SSA in experimental granulomas produced by purified schistosome eggs [20] in nonsensitized and in sensitized mice.

Material and Methods

The immunofluorescent technique was evolved and tested in schistosomula and worms at different stages of development, obtained as follows: 25 adult and 25 infant (14 hours) Swiss white mice were exposed to 400 cercariae each of the Puerto Rican strain of S.M., the adults by a glass well fixed to the abdominal skin under Nembutal® anesthesia, the babies by permitting them to crawl in shallow glass dishes. Both adults and newborns were sacrificed in groups of 4 to 5 after 1, 24 and 72 hours (for study of the skin), after 7 days (lung), and after 14 days (lung and liver). Ten additional adult mice were infected by tail exposure [21] with 125 to 150 cercariae each, and killed in groups of 5 on the 14th and 20th days for study of the liver.

Similarly, 29 adult and 27 infant (14 hours) white rats were exposed to 500 cercariae each, the newborns by crawling, the adults by intraperitoneal injection. Groups of 4 to 5 newborn rats were sacrificed after 1, 24 and 72 hours for study of the skin, and on the seventh and 14th days (lung). Groups of 4 to 5 adults were killed after 7, 14 and 21 days (lung), and on the 21st and 28th days (liver).

All fresh tissue samples were quick-frozen with dry CO_2 (the mouse and rat lungs after intratracheal injection of buffered normal saline) and were stored at $-22°$ C. in sealed polyethylene bags. They were serially sectioned at 4 to 8 μ on a cryostat-microtome ($-22°$ C.) and stored up to 4 weeks at 6° C. without drying. Every fifth section was stained with hematoxylin and eosin for identification of schistosomula or worms. The 4 to 6 sections adjacent to those containing a parasite were saved for immunofluorescent study, and the remainder were discarded.

The following immunofluorescent reagents were prepared:

Immune Mouse Serums. Mice infected with 100 S.M. cercariae 8 weeks previously

(supplied by Dr. H. van der Schalie, Department of Zoology, University of Michigan, Ann Arbor) were killed by exsanguination, and their serums pooled after liver inspection insured the presence of pseudotubercles. The serums from mice with minimal liver involvement were discarded. Serums were frozen in 1 ml. aliquots and tested for cercarial fluorescence *in vitro* by the method of Anderson, Sadun and Williams.[2] Positivity at dilutions of at least 1:32 was required for use.

Absorbed Immune Mouse Serums. Serums were prepared as indicated above, but were incubated at 8° C. for 12 hours with 1 gm. per ml. acetone-extracted schistosome-infected liver powder, prepared according to Coons.[22] Immune serums and serums absorbed with whole intact *Syphacia obvelata* worms were used for comparative evaluation of the absorption effect.

Normal Mouse Serum. This was pooled from pathogen-free mice (Charles River Laboratories, Cambridge, Mass.), frozen and tested as indicated above, to be negative at dilutions above 1:4 (weak positivity at low dilutions varied in successive "normal" mouse batches). All of the above serums were diluted 8-fold prior to use.

Conjugated Rabbit Antiglobulin. Fluorescein-isothiocyanate-conjugated rabbit-anti-mouse globulin (Sylvana, Lot 114632) was double-absorbed with acetone-extracted normal mouse liver powder (Baltimore Biological Laboratories) according to Coons,[22] stored frozen in 1 ml. aliquots and diluted before use in a mixture of freshly prepared RBA and buffered normal saline to yield a 4-fold dilution of antiglobulin in a 2-fold dilution of RBA.

Unconjugated Rabbit Antiglobulin. Same source, batch and dilution as given above.

Staining proceeded as follows:

(1) The slide was removed from the refrigerator and immediately fixed in acetone (3 minutes). (2) It was dipped in saline, partly evaporated and the edges were blotted. (3) Immune mouse serum was layered on the section (16 minutes). (4) Sections were washed in 2 changes of buffered saline (1 hour), partly evaporated and the edges blotted. (5) Conjugated rabbit antiglobulin-RBA mixture was layered on the sections (40 minutes). (6) Specimens were washed in 2 changes of saline (1 hour) and mounted in buffered glycerol.

For purposes of controlling staining specificity, as many as 6 adjacent slides were processed as indicated above, but with the following individual modifications: Control 1: Step 3 omitted. Control 2: Normal serum used in step 3. Control 3: Absorbed immune serum used in step 3. Control 4: Step 5 omitted. Control 5: Unconjugated rabbit antiglobulin used in step 5. Control 6: Unconjugated, followed by conjugated antiglobulin used in step 5. As an additional control, normal homologous tissue was stained by the standard technique.

Studies of Egg Antigen in Cercaria-Induced Infection

Antigen in cryostat sections was studied in 5 adult mouse livers excised 8 weeks after exposure to 125 S.M. cercariae and in 5 similar livers 10 weeks after exposure. Immunofluorescent staining proceeded as above, except that prescanning of slides with hematoxylin and eosin was unnecessary since random sections showed numerous eggs. In addition, serial cryostat sections of 2 of the 8-week-infected livers were glycerin-mounted, sequentially in the following order: (a) unstained, (b) treated with immune mouse serum only, and (c) fully processed, so that the effect of each type of treatment on the same egg could be studied by ultraviolet and phase microscopy. After careful removal of the cover glasses, slides mapped in this manner were washed in 3 changes of buffered saline, refixed in acetone (5 minutes) and restained with the Mowry-MacManus variant of the alcian blue-PAS stain [23] and the Millon and the Sakaguchi reactions according to Baker (cited by Pearce [24]). Controls stained with nonimmune serums and with unconjugated antiglobulin were treated similarly.

For *in vitro* study, suspensions of S.M. eggs were obtained from 5 mouse livers infected 8 weeks previously.[20] These were incubated for 2 hours in buffered isotonic

NaCl under normal conditions of room lighting and temperature, so that they contained viable eggs, broken eggshells and free miracidia. Aliquots of 4,000 eggs were stained in 1 ml. centrifuge tubes, washed by triple centrifugation in buffered normal saline, mounted in saline under sealed cover glasses and inspected immediately. The immunofluorescent method and controls were essentially as above, except that immersion in mouse serum was prolonged to 4 hours and antiglobulin was diluted 1/6 without the addition of RBA.

Studies of the Fate of SSA in Experimental Granulomas

Immunofluorescent staining was carried out in the lungs of 98 adult mice which had received intravenous injections of approximately 1,200 purified eggs of S.M. each,[17] as follows:

Series 1. Viable eggs were injected into 50 nonsensitized mice, which were sacrificed in groups of 2 to 6 animals at 1, 6, 24 hours and 1, 2, 4, 8, 16, 24, 32, 43 and 70 days.

Series 2. Eggs autoclaved for 10 minutes at 100° C. were injected into 21 nonsensitized mice which were sacrificed in groups of 2 to 4 animals at the same intervals as above minus the last 3.

Series 3. Viable eggs were injected into 27 mice which had been sensitized with approximately 800 such eggs intraperitoneally, 30 days prior to challenge.[19] They were sacrificed in groups of 3 to 5 animals at 1 hour and 1, 2, 4, 8, 12 and 32 days.

All tissues to be compared were processed simultaneously with strict adherence to the standardized immunofluorescent technique.

In order to minimize variations in SSA due to differences in individual development of eggs prior to their injection, 50 eggs in each group were scanned under ultraviolet light, and the 10 brightest eggs were selected for observation. In addition, eggs with and without SSA were differentially counted in 5 lungs of sensitized and in 5 lungs of nonsensitized mice, both challenged simultaneously with 1,200 eggs and excised 8 days after injection; the differences were statistically analyzed. Initially, all slides were observed with a Reichert UV microscope, and later with a Zeiss microscope equipped with an Osram 200 HBO burner, 0.8 mm. dry darkfield condenser, exciter filters BG-12 (4 mm.), UG-2 and BG-38, neutral filter 0.7 and barrier filter 50. Color photographs were taken with a Leitz Micro-Ibso attachment on Super-Ansco Daylight film (SA 100/50°). Color and black and white prints were prepared from the color transparencies (by Mr. Leo Goodman, Boston City Hospital).

Results

All stages of worm development showed specific fluorescence of striking intensity and definition (Figs. 3 and 4). The bright, apple-green color and particulate appearance helped to distinguish it from the fainter background and nonspecific fluorescence, sometimes seen in vascular lumens, endothelium and basement membrane. Host and parasite nuclei were negative. Schistosomula and worms showed diffuse fluorescence, with the strongest staining (Fig. 3) seen in the cuticles, intestinal linings and intestinal content. In the skin and lung stages, bright staining occasionally occurred in apical glands (Fig. 4). One hour after penetration, free SSA droplets were seen in host tissues close to schistosomula (Fig. 4), but were absent 24 hours later. All controls, except control 3 (absorbed immune serum) resulted in total abolition of specific staining. The latter showed clearly perceptible weakening.

SSA in schistosome eggs in tissues was seen in two forms: Aggregates

of glassy, homogeneous precipitate appeared on the penetration glands of miracidia and coated the eggshells with irregular bands and masses of brightly fluorescent material; halos of tiny, dust-like granules and flecks were found for the most part near the eggs in the centers of granulomas (Fig. 1). On critical focusing, some granules appeared inside miracidia or in host cell cytoplasm, while many others were superimposed on cytoplasm where it had been in contact with specific serums. After overstaining, the glassy and dusty aggregates merged around the egg, with a sunburst-like effect. Fainter diffuse staining was seen in miracidial cytoplasm and cuticles and occasionally in the necrotic centers of granulomas. SSA varied individually, and was most intense in well-developed, cellular granulomas. Late, fibrotic nodules showed little or no SSA, whereas the miracidium exhibited whitish autofluorescence. This was rare in the 8-week livers, but occurred in about 20 per cent of granulomas in the 12-week livers. All slides containing eggs, regardless of staining, showed strong orange autofluorescence of eggshells. Controls (1, 2, 3 and 6) revealed mild, nonspecific fluorescence of the miracidial penetration glands, but none in the rest of the miracidial cytoplasm.

Intact eggs *in vitro* were largely negative, a few exhibiting faint internal fluorescence. Circumoval precipitates were brightly stained and easily spotted (Fig. 5). Free miracidia and those in broken eggs showed intense staining, particularly of the penetration glands, cilia and cuticles. Droplets of stained precipitate adhered to the cuticle and tangled with the cilia.

Prior to processing, and in stained controls, occasional eggs showed a slight fuzziness of their outline with phase contrast; this was also stainable with PAS. After treatment with immune mouse serum, circumoval precipitates were seen *in vitro* where eggs were incubated in immune serum for 4 hours but not in cryostat sections (incubated for 15 minutes). After adding either conjugated or unconjugated rabbit antiglobulin, the precipitates which were demonstrable by immunofluorescence were evident with phase contrast, except that the smaller SSA granules were difficult to visualize. When restained with PAS-alcian blue, these precipitates showed a well-contrasted purple-pink color (Fig. 2). The Millon and Sakaguchi reactions were negative in all instances.

Viable eggs injected into nonsensitized mice (series 1) initially appeared surrounded by halos of SSA, which flooded the adjacent alveoli and tissue spaces concentrically within 20 to 50 μ, and emitted fading streamers into vascular lumens. During the first 6 hours eggshells were uncoated or scantily coated with SSA (Fig. 6). One to 4 days after injection, a new, brightly stained glassy coating appeared in many eggs, and, simultaneously, staining re-appeared in some of the penetration

glands, so that the eggs resembled those in the liver (Fig. 7). The glassy precipitates reached their greatest staining intensity on the second day, and remained well defined until the fourth day; by the eighth day glassy deposits were reduced in width, frayed in outline and faded in color (Fig. 8). From then on, they disappeared in progressive numbers and were entirely abolished by the 32nd day. Fading of the powdery halos occurred at a much slower pace, but was evident by the 32nd day. However, even on the 70th day, after which observations were discontinued, 3 of 50 eggs still showed small powdery halos (Fig. 9). The remainder of granulomas on that date contained degenerated eggs and eggshells, lacking SSA. Miracidial cytoplasm also was slow to fade. As miracidia degenerated, many of them began to show a brilliant, whitish autofluorescence; spotty specific staining, however, remained superimposed on this white background until about the time the powdery halos disappeared (Figs. 8 and 9).

In series 2 (autoclaved eggs, nonsensitized mice) miracidial cytoplasm showed diffuse whitish autofluorescence throughout the period of observation (24 days) and specific fluorescence of miracidia was abolished. A thin, stringy or fuzzy SSA-coating of the eggshell was seen in early specimens. This coat did not regenerate subsequently, but underwent progressive disintegration and fading. A lesser amount of SSA was seen at all stages. Otherwise, results were similar to those in series 1.

In series 3 (viable eggs, sensitized mice) the sequence from the first hour through the second day was similar to that in series 1, except that the fluorescent deposits appeared brighter and more plentiful (Fig. 10). Fading and fraying of glassy deposits was noted from the fourth day onward, and most halos were dramatically dimmed by the eighth day, so that their appearance was comparable to that on the 32nd day in series 1 (Fig. 11). On the latter date, SSA had totally disappeared from the granulomas in series 3. Degeneration and disappearance of miracidia were likewise accelerated. The cellularity and size of granulomas was greater in series 3 beginning on the second day, and eosinophils, made conspicuous by rhodamine stain, were present earlier and in larger numbers (Fig. 11).

Differential counting of granulomas in sensitized and nonsensitized mice on the eighth day after challenge revealed persisting SSA in 43 per cent of the former and in 76 per cent of the latter, with a significance of $P < 0.01$.

Discussion

Although the antigens of worms and eggs differ in their response to diagnostic tests[25,26] and in their relative protective effect against

cercarial reinfection,[27] they share reactivity with the immunofluorescent antibody. Other techniques are needed to define the multiple antigen-antibody systems in schistosome infection.[26,28,29] Immunofluorescent staining of eggs is probably related to the precipitin antibodies responsible for the circumoval test response,[30,31] the spontaneous "Hoeppli phenomenon,"[32] and the miracidial immobilization test.[33] The indirect immunofluorescent technique appears to label larger (glassy) and smaller (powdery) egg-antigen deposits differently, but does not reveal their precise cellular localization or chemical nature. It improves the detection of circumoval precipitates *in vitro* and might therefore be diagnostically useful when antibody titers are low. Egg-antigen required "uncovering" by seepage through the capsule (circumoval test), breakage of eggshells or histologic sectioning before reacting. The labeled egg-antigen has a polysaccharide moiety[14-16] which remains PAS-stainable after reacting with antibody. This material may well be secreted by miracidial glands as a vehicle for the snail-penetrating enzyme. However, its chemical structure and biologic role in the mammalian host are uncertain.

Polysaccharide antigens generally elicit primary tissue eosinophilia, and eosinophilia is prominent in the schistosome pseudotubercle.[32,34] However, SSA is not the sole determinant of pseudotubercle formation. Unpublished studies shave shown that live, SSA-containing miracidia do not cause typical pseudotubercles, whereas intact live or autoclaved whole eggs do. This raises the question whether eggshells contribute adjuvant-like materials (chitin, lipoprotein, acid-fast material) or act by prolonging the antigen-releasing capacity of miracidia or both.

In natural infection, the miracidia of schistosome eggs remain viable in host tissues for 3 weeks or longer.[35,36] SSA production continues during part of this time, since it was shown to increase in staining intensity between the first hour and the second day after injection of live purified eggs; this was not the case with autoclaved eggs. Furthermore both SSA content and granuloma size are less in purified eggs than in eggs stained directly in infected mouse livers, and still less after autoclaving eggs when hosts of similar sensitivity are infected.[17,37] This suggests a relation between the amount of SSA and the degree of host cell response. Most likely, schistosome eggs keep releasing SSA into host tissues during part of their life span as with sustained-release drug capsules (spansules).

Experimental pseudotubercle development in the nonsensitized mouse exhibited 4 successive stages: (1) A phase of diffusion lasted at least 24 hours and coincided with the early lag period of host cell response. Halos of spreading SSA suggested that antigen was disseminated sys-

temically and that there was an outpouring of edema from sites of vascular injury near the egg. (2) A phase of antigen sequestration coincided with the formation of a host cell halo between the fourth and eighth days. SSA became limited largely to the centers of granulomas. "Glassy precipitates" reappeared in miracidial penetration glands and along the eggshells, suggesting continued antigen production. (3) A phase of rapid antigen destruction accompanied the growth of the granuloma to its maximum size, attained between the 16th and 32nd days. This phase was marked by the disappearance of the "glassy" antigen deposits, and was followed by the fourth stage. (4) A phase of slow antigen destruction continued for more than 70 days, during the declining and healing phase of the granulomatous reaction. Gradually, all residua of SSA in miracidia and host cells were extinguished, and only eggshells and degenerated eggs remained in the healing granulomas.

This sequence was accelerated and enhanced in sensitized mice: The abundance and brightness of SSA at early stages of granuloma formation suggested enhanced antigen sequestration (Fig. 11). Antigen destruction *in situ* on the other hand was sufficiently accelerated to qualify it as an immune response, since the time span was shortened more than twofold; this observation proved to have statistical significance. The host cell response in the sensitized mouse also began earlier and reached larger size, a phenomenon shown previously in experimental ascaris granuloma.[19]

In cercaria-induced schistosome infection, host reactivity should at least have equaled that of the mouse sensitized by a single intraperitoneal egg injection. Here it was calculated from available data related to the onset of oviposition and maximum egg survival[35,36] that SSA was removed from individual pseudotubercles within a maximum of 34 days, compared with a time span of less than 32 days in the experimentally sensitized mouse. Both these time spans require more accurate definition.

Most of the phenomena observed can be explained as the result of two mutually antagonistic processes, the sustained elaboration and release of SSA by surviving eggs, and the phagocytic-catabolic activity of host cells. Prior to host cell response, diffusion of SSA would be a function of its viscosity and solubility; later, antigen would be locally detained by phagocytes. Continued SSA production during early phagocytosis would lead to "sequestration." Increased phagocytic and catabolic activity and decrease in the rate of SSA production would be evidenced by rapid antigen disappearance. Conversion to slower fading remains unexplained, but implies the presence of an antigenic fraction difficult to metabolize, or a change in the rate of phagocyte metabolism.

In essence, then, the pseudotubercle may be considered to be a specialized phagocytic host response by means of which schistosome egg antigen is sequestered and metabolized *in situ;* this is followed by host sensitization, as stated in the working hypothesis.

The accelerated antigen destruction in schistosome pseudotubercles of sensitized mice is analogous to that reported by Talmage, Dixon, Bukantz and Dammin[38] in a study on the disappearance of isotope-labeled protein antigen from the serums of immune animals. However, in the present study the time spans were more prolonged, and antigen destruction correlated with phagocytic activity, suggesting that in granulomas antigen catabolism was ultimately a cellular function. Whether accelerated phagocytosis and antigen catabolism are mediated by circulating antibody[39] or by delayed hypersensitivity or both remains unresolved.

It is also uncertain to what extent findings in schistosome pseudotubercles are applicable to other granulomatous reactions where both the chemical nature and the rate of release of antigens differ. This is the case with depot antigens formed from haptenic metals such as zirconium[40] or with bacteria capable of prolonged reproduction.[41] Studies of specific antigens with analogous methods are required, and preliminary experiments employing methylcellulose filter particles loaded with various antigens have recently been begun. The central problem in such studies will be enhanced capacity of the sensitized phagocyte to deal with specific antigens.

There are analogies between the experimental model and natural schistosome infection, but there are also major differences. These are the greater life span and SSA content of eggs in natural infection, the presence of adult worms and their metabolic antigens, and the sustained egg-laying of the female worm, which results in an escalatory challenge of the host with a constantly mounting egg load. Nevertheless, it seems likely that the change in reactivity responsible for accelerated granuloma formation also takes place in natural infection. The acute phase of schistosomiasis marked by hepatosplenomegaly and eosinophilia usually begins before a large number of eggs have been shed and subsides while increasing numbers of eggs continue to be deposited in host tissue. During the chronic phase of schistosomiasis, clinical symptoms may be absent or mild at a time when there is severe abnormality in the human intestine, liver or lung[42]; the same is true in laboratory mice[43] and rhesus monkeys.[44] Cortisone administration has a damaging effect, and tends to add degenerative parenchymal changes to the granulomatous lesions.[45] Some of these features can be explained by increased antigen sequestration during the chronic phase of schistosomiasis. The lessening of general symptoms could be due to curtailment of egg-antigen

dissemination. On the other hand, larger antigen concentration *in situ* might promote central necrosis of pseudotubercles, and thereby lead to scarring during the healing phase of multiple granulomas. In addition, the larger size of granulomas in the sensitized or chronically infected host might favor obstruction of portal radicles[46] and lung arterioles, so that the net effect of sensitization on the host would be a mosaic of "protective" and of "harmful" effects. Further work will be needed to characterize the polysaccharide-containing egg-antigen chemically.

Summary

Eggs of *S. mansoni* were studied by the indirect immunofluorescent technique in order to ascertain the distribution and relative amount of stainable schistosome antigen *in vitro* at various stages of host reaction. The results were correlated with previous data on the histologic features of experimental pseudotubercle production.

Both worms and eggs in mammalian tissue showed selective immunofluorescent staining. A major egg-antigen produced by miracidia contained polysaccharide and could represent the vehicle of [redacted] snail-penetrating hyaluronidase. Deposits were labeled in penetration glands, along the eggshell and in small granules in phagocytes of the pseudotubercle. The antibody responsible for immunofluorescence was related to *in vitro* circumoval precipitate.

After intravenous injection of eggs into nonsensitized mice, 4 successive stages of antigen disposal could be distinguished: prior to host cell reaction (1 to 24 hours) antigen was disseminated. Coincident with accretion of a cell halo (24 hours to 4 days), antigen production continued, and there was uptake by host phagocytes. This phase of "antigen sequestration" was followed by rapid antigen destruction ending with the disappearance of capsular deposits as the granuloma reached its peak size (16th to 32nd day). Finally, residual antigen in miracidia and host cells was extinguished, coincident with the healing phase of the granuloma (over 70 days). In mice presensitized by intraperitoneal egg injection, both antigen disposal and host cell reaction were accelerated. Antigen sequestration appeared to be enhanced and the antigen disappeared prior to the 32nd day. In the naturally infected mouse liver, antigen destruction was completed within 34 days of oviposition.

The findings were considered to be the result of a balance between antigen release by eggs and phagocytic-catabolic activity in host cells. Secondary pseudotubercle formation was considered to be an enhanced response in which phagocytosis and antigen disposal were accelerated. The pathogenetic significance of these concepts is discussed.

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[*Illustrations follow*]

Legends for Figures

All figures except 2 and 5 represent cryostat sections treated with immune mouse serum followed by rabbit-anti-mouse-globulin conjugated with fluorescein isothiocyanate and counterstained with rhodamine-bovine-albumin. In Figure 2, the cover glass was removed after Coons's staining, the tissue was re-fixed and re-stained with Mowry's periodic acid-Schiff-alcian blue technique. Figure 5 represents an *in vitro* modification of the immunofluorescent stain, involving "circumoval precipitin reaction." Figure 3 was photographed with Reichert equipment, the remainder with Zeiss equipment.

Fig. 1. Egg of *S. mansoni* in a mouse liver granuloma 8 weeks after cercarial exposure. The orange-yellow autofluorescence of the shell outlines the miracidium. The nuclei are negative, but the cytoplasm shows powdery fluorescence. The penetration gland is not shown. The eggshell is embedded in a brightly fluorescing glassy precipitate which fades outwardly into a corona of powdery granules occupying and overlying cells in the center of the granuloma. Occasional granules appear in peripheral host cells. Rhodamine-counterstained eosinophils are scattered through the granuloma which extends beyond the margins of the field. × 520.

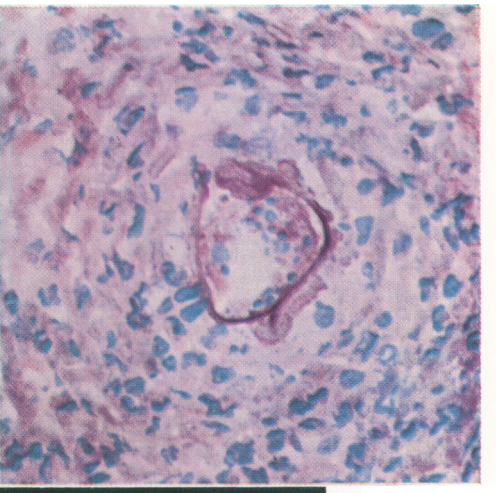
Fig. 2. Similar egg, after PAS-alcian blue staining. The glassy precipitate stains pinkish red, and the smaller powdery granules are barely discernible to the right of the egg. The nuclei of miracidial and host cells are counterstained blue. × 520.

Fig 3. Adult male schistosome occupying a portal radicle 21 days after cercarial exposure. The tuberculate cuticle shows intense staining with a sharp external and somewhat blurred internal outline. Bright staining of the cecal lining is evident. An unidentified cluster of cells stains brightly in the left upper portion; the remainder of the worm is somewhat less bright. × 160.

Fig. 4. Schistosomulum of *S. mansoni* in the mouse skin, 1 hour after cercarial penetration. The cuticle stains brightly, the intensely stained center corresponds to eosinophilic apical gland content. Parasite nuclei are small and unstained. Stained material in granular form is scattered along the trajectory of migration, diffusing into a lymphatic toward the left. There is no host cell reaction. The horny epidermal layer is counterstained orange-yellow. × 540.

Fig. 5. Egg of *S. mansoni in vitro* showing a circumoval reaction stained with fluorescent antiglobulin. The miracidium, outlined by the autofluorescent eggshell, is essentially unstained. The circumoval precipitates outside the shell are brightly stained. × 600.

1



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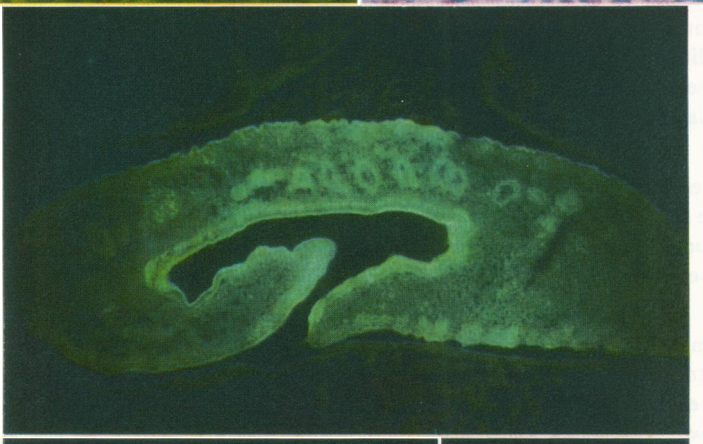

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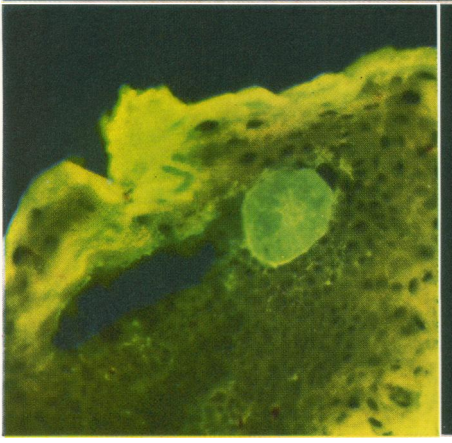

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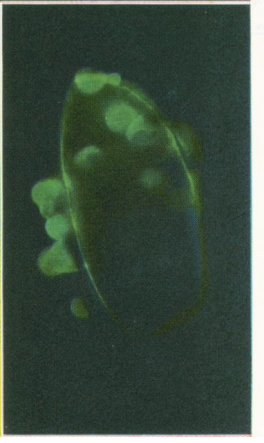

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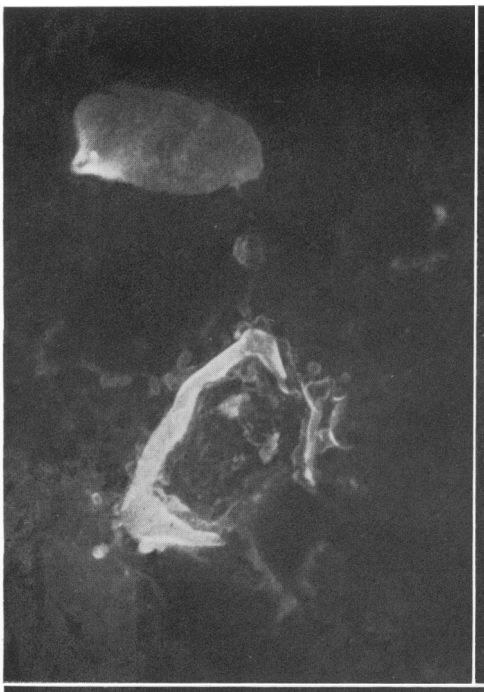
FIG. 6. Below, section of an egg; above, tangential view of an empty eggshell of *S. mansoni* in the lung, 1 hour after injection of purified eggs into a nonsensitized mouse. The eggshell lacks miracidial structure and shows autofluorescence. The miracidium of the egg, outlined by its strongly autofluorescent, broken shell, shows a partly evacuated pair of penetration glands, a faintly positive miracidium and is surrounded by somewhat dimly staining antigen. The latter diffuses into the surrounding alveolar and vascular spaces. A host cell reaction is lacking. × 540.

FIG. 7. Similar egg, 4 days after intravenous injection. The penetration gland is filled with brightly stained antigen. There is abundant glassy precipitate around the eggshell, and powdery granules appear in the halo surrounding the egg. A few rhodamine-stained eosinophils are included. This stage of "antigen sequestration" resembles that shown in infected mouse liver (Fig. 1). × 600.

FIG. 8. Similar egg, 8 days after intravenous injection. The phase of "rapid antigen removal" is illustrated. The miracidium, inside the eggshell, exhibits whitish autofluorescence. The glassy coating has faded extensively; residua of it, moth-eaten and faintly stained, can be seen at the upper margin of the shell. The powdery granules show little modification and are limited to the granuloma site. The host cell halo, containing several eosinophils, is of moderate size. × 540.



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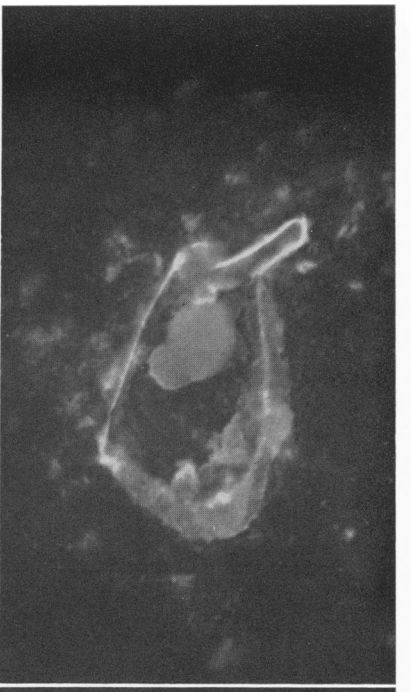

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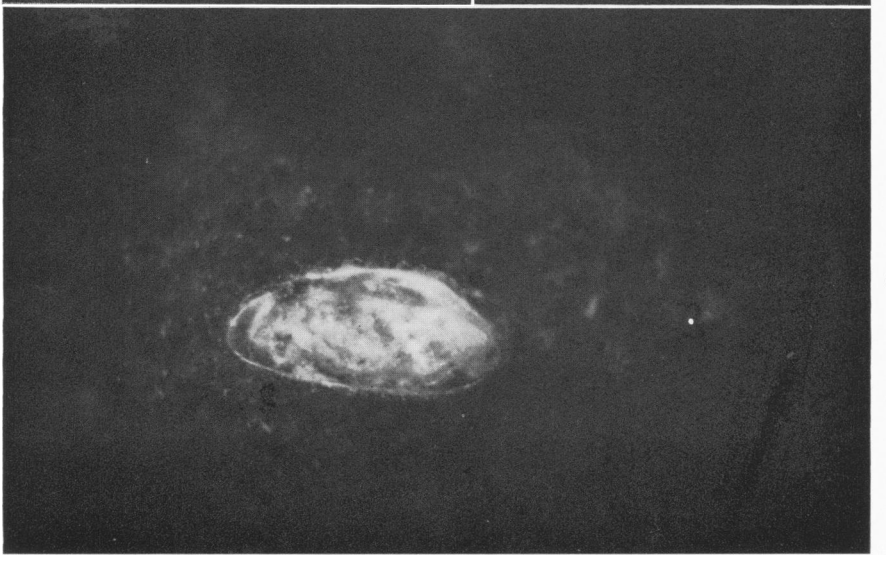

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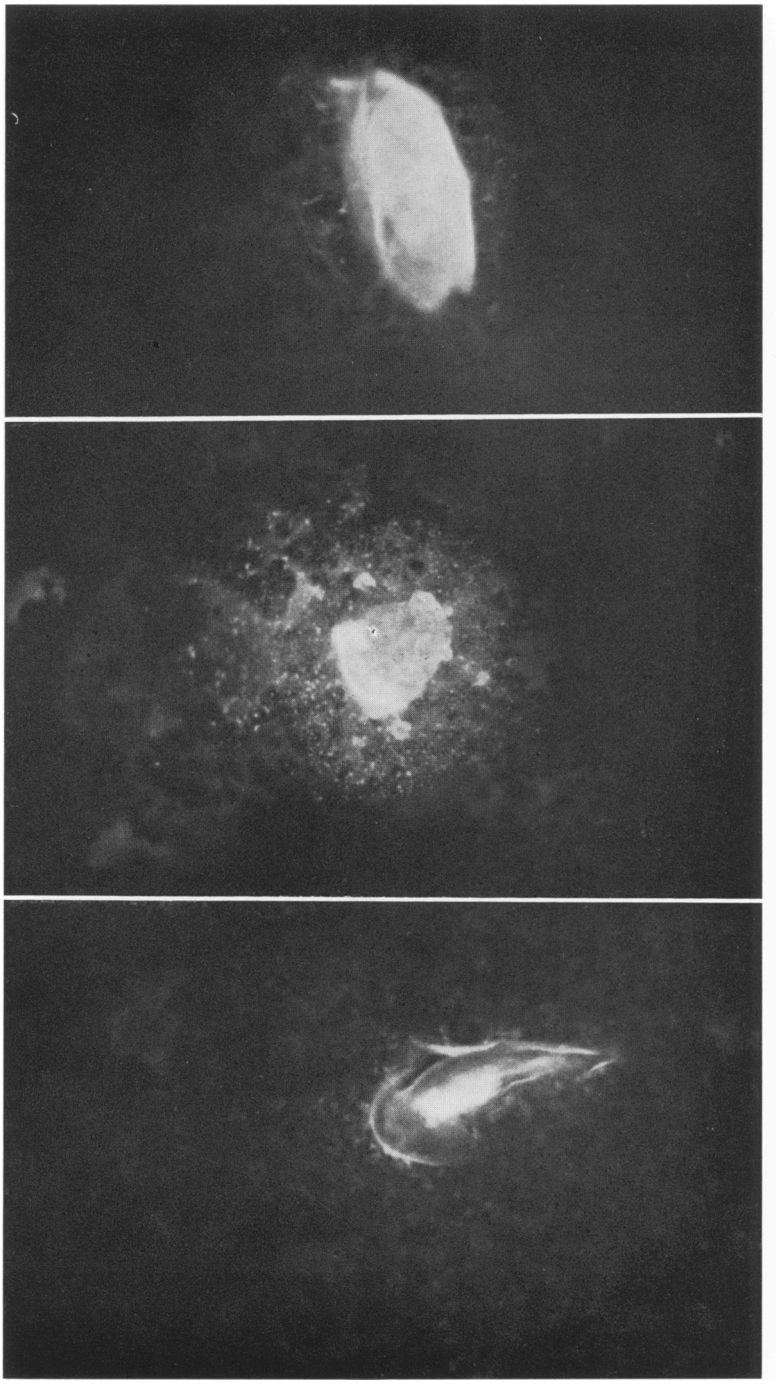
FIG. 9. Similar egg (series 1), 70 days after intravenous injection. The degenerated egg, surrounded by a small, residual host cell halo, exhibits minute powdery granules limited to the area of cell reaction. A single eosinophil is present. Most eggs lacked specifically stainable antigen at this stage. × 540.

FIG. 10. Viable egg of *S. mansoni,* 1 day after intravenous injection into a mouse pre-sensitized by intraperitoneal egg-injection. The autofluorescent eggshell is sectioned tangentially, and the miracidial nuclei are faintly visible through it. An extremely bright halo of densely packed, specifically stained granules is seen around the egg. Host cell reaction has begun and includes a few eosinophils. Compare with Figures 6 and 7. × 540.

FIG. 11. Similar egg (series 3), 8 days after intravenous injection. The eggshell is fragmented and the miracidium degenerated. Partial digestion of the miracidium is seen at its lower margin. There is extensive host cell reaction mainly of neutrophils and eosinophils, extending to the left border of the field. Both the glassy and the powdery fluorescent material are markedly reduced in amount compared with Figure 8. × 540.



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