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EFFECTS OF THE IMMUNE RESPONSE ON THE HOST IN BILHARZIAL INFECTION

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EFFECTS OF THE IMMUNE RESPONSE ON THE HOST IN BILHARZIAL INFECTION

INTRODUCTION

Host reactions in parasitic infections are ruled by the same immunological principles which regulate all other infectious diseases (6). Therefore, if any of their features appear unique, they must be derived from the specific biological properties of parasites and their products. By the same token, analysis of these variegated responses, so different from classical models, will help to enlarge our perspective of the total range of host defense mechanisms and their interactions in disease.

Schistosome flukes are among the largest tissue dwelling agents and secrete or excrete a variety of enzyme-containing and antigenic products (4,5,17,24,51,55,59). Their mammalian phase culminates with intense and sustained reproductive activity inside host veins. Yet in their natural hosts they rarely produce critical illness and characteristically achieve a stable and long-lasting host parasite balance which may remain entirely subclinical or shift toward eventual host disability or death. In S. mansoni infection, the following key events have been identified through experimental study:

About 28 days after primary exposure those flukes which survive penetration and migration begin reaching sexual maturity; oviposition starts and through about the 10th week of infection the number of eggs deposited in host tissue rapidly escalates. Egg destruction, a slower process, lags behing at first, but sometime around the 4th month of infection, it attains a rate equivalent to deposition so that egg turnover in host tissue stabilizes (7). Even prior to oviposition, antigen is released by maturing worms; thus immunofluorescent antibody can be detected by the 3rd week (21) and, in massive murine infection, circulating antigen is discovered by immunoelectrophoresis on the 26th day (4). Likewise, immediately after deposition of each new schistosome egg, immunofluorescent stainable antigen is diffused for a limited period of

time, probably aggregating a substantial antigen influx in early infection (27). However, during this acute phase, which may be marked by disseminated lymphoreticular activation (21, 37) and by systemic illness (13), multiple antibodies are formed (25, 51) and their titer ascends steeply, as does the degree of cellular sensitization (21). As a result of this, disposal of schistosome antigen becomes accelerated and increasingly efficient, as will be shown in greater detail below. By the time when egg turnover becomes constant, symptomatic remission has ushered in the chronic phase of the infection (13). By this time, too, flukes residing in the mesenteric radicles have completed their differentiation and acquired the capacity to assimilate and transport various metabolites and macromolecules across their integument (54). Whether for this, or for other reasons, their living surface becomes inured to host cells and antibodies by which they are surrounded (and which readily attack any damaged worms). In a similar manner, miracidia protected by intact egg shells, and by release of their secretion maintain an unimpaired 21 to 32 day viability period (38,60) even while surrounded by host cell granulomas. Finally, the host becomes resistant to reinfection, although this varies in degree by species and experimental design (48). In the natural hosts of schistosomes, immunity is feeble, compared to species capable of self-cure (57), but it is likely that, even in man, indefinite summation of worms does not occur, and a relatively stable ceiling or - under favorable conditions - a decline of the infection is eventually reached (41).

Thus, in schistosomiasis, host-parasite balance is the result of mutually counteravailing defense mechanisms of both host and parasite, rather than of low parasite virulence, or suppressed host reactivity. During the chronic phase of the infection, the balance is rarely disturbed except by such events as massive "toxic" superinfection, ectopic lesions in

vital areas, or by intercurrent pathology. In the long run, gradual and repetitive formation and resolution of pseudotubercles leads to structural distortion of organs, and to impaired flow in sensitive vascular territories. This late and sometimes life-threatening pathology is poorly understood but there is evidence that it is related to high egg burdens over long periods of time (9, 50).

The immunopathology of schistosomiasis presents two major problems: The factors which determine acquired resistance, and those which play a role in defense against established infection. While the former have been studied in more detail (28), the latter are of at least equal significance. Part of our research has therefore dealt with the disposal of schistosome egg antigen by the mammalian host. These studies and the pertinent hypotheses will be summarized below.

STUDIES ON THE SCHISTOSOME PSEUDOTUBERCLE

1.- PRODUCTION OF EXPERIMENTAL PSEUDOTUBERCLES: When viable S. mansoni eggs are obtained from mouse livers (11) and are injected intravenously into unsensitized mice, they disperse in the lung forming discrete pseudotubercles which can be sequentially measured and compared with reactions to control particles. Purified egg suspensions are heterogeneous as to age and preservation of individual ova and have lost some antigenicity, but experimental granulomas, while averaging about 150 micra less in mean diameter, are otherwise similar to their natural counterparts in location, and cell composition. After an initial lag of all response, primary experimental pseudotubercles increase to their peak size within 16 to 32 days, then slowly involute and heal, probably prior to the 6th month after onset (26). Ascaris suis granulomas are similar in course, but somewhat faster in onset and healing than schistosome granulomas, and both differ markedly from reactions to insoluble polyvinyl spheres which tend to terminate early by the formation of a thin, fibrous sheath around each plastic

bead (26) (Fig. 1).

2.- STAINABLE SCHISTOSOME EGG ANTIGEN IN PSEUDOTUBERCLES: Using the Coons' immunofluorescent technique with its proper controls (27), it can be shown that in the unsensitized host, an amorphous, specifically stainable product is diffused from eggs for at least 24 hours following injection, after which the material is taken up by phagocytes congregated around the egg. By the 4th day stainable antigen, contrasting vividly with the orange-yellow autofluorescence of the egg shell is deposited on both its inner and outer surfaces and in cytoplasmic particles within granuloma cells. Thus evidence of antigen diffusion is now replaced by a visual image of "antigen-sequestration" (Fig. 2). From the 4th through the 8th day, stainable antigen is rapidly depleted, but fine, powdery particles in the miracidia and host cells remain demonstrable for 60 to 70 days, i.e., past the onset of involution of the pseudotubercle. This sequence originally referred to as "rapid" and "slow" antigen disappearance, is probably the composite result of ending antigen generation in the presence of continuing catabolism (27). The homologous, immunofluorescent circulating antibody does not become detectable until 2 weeks after egg injection (21); thus, as in the case of soluble protein antigen (58), catabolism precedes detectable antibody formation.

When naturally infected mouse liver tissue is stained with the immunofluorescent technique, most granuloma centers fluoresce selectively as if lit by a magic lantern, and the various sequential phases seen after egg injection all appear concurrently. In the best preparations, the miracidial cephalic glands and cortex are stained intensely, together with the glassy antigen deposits along the egg shells (Fig. 2). When specimens are demounted, washed and restained, all these deposits are positive with the Period acid-Schiff stain (27).

3.- REACTIONS TO SEPARATE EGG COMPONENTS: Pure miracidia (31) when injected intravenously, cause a mild leukotactic response (Fig. 6), and disappear without trace within 48 hours; Diffusion of stainable antigen occurs during the first hours after injection similar to the picture seen with whole eggs, but antigen sequestration and granuloma formation do not follow. Pure egg shells, whether obtained by maceration or by sonication-centrifugation (31), cause an inflammatory cell reaction lasting somewhat over two weeks. Some of these egg shells retain traces of Coons'-stainable material, and a few eosinophils and epithelioid cells participate in the early cell response; later, as in the case of the plastic spherules, the shells remain ensheathed by a few stereotactic giant cells or histiocytes (Fig. 4). Reactions to miracidia or egg shells do not qualify as true pseudotubercles and even their aggregate size and duration is lesser than the corresponding reaction to intact eggs, whether viable or heat killed (Fig. 5). Both live and dead intact ova cause the formation of pseudotubercles which are similar in cell composition and reaction profile, but reaction to autoclaved whole eggs is lesser in size and duration than that to viable eggs (Fig. 5)(31). When compared by the immunofluorescent technique, viable eggs are found to generate amorphous stainable antigen during at least the first 4 days of their residence in host tissue, while heat killed eggs show a gradual depletion of this material, together with bluish white autofluorescence suggestive of protein denaturation. Nevertheless, antigen diffusion and its uptake by host cells can be observed in both (27).

4.- HYPOTHESIS OF ANTIGEN SEUQESTRATION: According to this evidence, both miracidial antigen and relatively inert shell material (29) are required for granuloma formation, but the miracidium must be contained in the intact egg shell so that antigen is gradually and continuously released rather than quickly dissipated. The larger size of pseudotubercles caused

by viable, versus heat-killed eggs is explained by their more generous endowment with immunofluorescent diffusible antigen and suggests that this material is a miracidial secretion product. Direct evidence of miracidial secretion and of submicroscopic pores in schistosome egg shells will be supplied below to show that diffusible antigen can indeed be gradually released by schistosome eggs much as drugs are released from so-called "spansules".

A close analogy is evident when the pseudotubercle is compared with the "adjuvant effect", i.e., the enhancement of local cell reaction and of antibody formation resulting when diffusible antigen is injected in the form of oil - or wax-coated particles. In both cases, soluble antigen at first diffuses freely but, as soon as host phagocytes become non-specifically attracted to the particles, newly emerging antigen is taken up by these cells on contact, provided it is macromolecular or attached to a phagocytable carrier. Continued antigen release then results in antigen-sequestration and in primary granuloma formation. This sequence can be triggered in the absence of host sensitization, depending only on the manner in which diffusible antigen becomes available to host cells in situ. Eventually, a gradient of antigen-concentration develops from the center to the periphery of the primary granuloma, systemic antigen diffusion is reduced and, as circulating antibody makes its appearance, the host becomes immunologically responsive.

5.- EFFECT OF SENSITIZATION ON GRANULOGENESIS: When mice are sensitized intraperitoneally with S. mansoni eggs and are then challenged intravenously after suitable intervals, a modified, secondary granulomatous response occurs, which has been analyzed in some detail by our group, including the tragically deceased Dr. Ramón Gómez Mazzei of Asunción, Paraguay (18) and by K. S. Warren and collaborators (63). Some of these studies are still in progress at this writing.

a.- Accelerated and enhanced granuloma formation: This effect was first reported as a twofold or greater enhancement of granuloma size on the 4th day after challenge in 24-hour and 2-week-old mice, sensitized and challenged with Ascaris suis eggs(39). Later, Gómez Mazzei showed in experimental S. mansoni pseudotubercles that both the total cell and the eosinophil response were markedly enhanced by the second day and he was able to demonstrate the specificity of this effect (18). Warren confirmed this further (63), and has shown that sensitization is detectable after 24 hours by quantitating the proportion of eggs showing any cell response whatsoever (Personal Communication). With this method, he and his group are currently exploring the factors in the induction, suppression and passive transmission of the secondary granulomatous response which will be discussed below.

b.- Accelerated antigen disappearance: Whether accelerated secondary cell response results in earlier antigen sequestration, has not yet been explored, but accelerated antigen destruction is well documented: In sensitized hosts, Coons'-stainable antigen is found to be virtually extinguished by the 32nd day after challenge, versus the 70th day in unsensitized subjects (27); in fact, the proportion of granulomas containing stainable antigen is already significantly reduced by the 8th day after challenge (44). Using this experimental endpoint, Peterson showed that the antigen in pseudotubercles is sensitizing in vivo as long as it can be visualized there by immunofluorescence, and irrespective of its coexistence with circulating antibody in the same host (44). While an anamnestic antibody response to repeated egg-challenge has not yet been studied, it has been demonstrated in analogous experimental situations (21).

c.- Reduced total duration of pseudotubercles: Gómez Mazzei noted , although initially larger, that/secondary granulomas actually became smaller than those of unsentitized controls by 70 days after challenge and fewer egg shells were detectable at that time. Since this experiment had not been repeated, he mentioned it without descriptive detail (18). Warren's results also showed a steeper fall in the size of secondary granulomas between the 16th and 32nd days after challenge than occurs in primary granulomas (63).

d.- Enhancement of concomitant alterations: In all these experiments, including the very earliest, systemic alterations were found to accompany granuloma formation. In secondary response, pulmonary alveolitis (26), lymphoid cell mantling of blood vessels (39) and intimal proliferation in pulmonary arterioles were found to be increased (26, 39). When schistosome eggs were injected prior to cercarial infection, splenomegaly was enhanced over that found in the controls (33). When viable schistosome eggs were given by repeated intravenous injection up to 14 times, a pulmonary arteritis was produced in mice which resembled human bilharzial pulmonary arteritis in that hiatuses of the elastica layer and multiple channel formation in arterial lumina (Fig. 6)(2) were present; however, typical angiomatoids and right ventricular hypertrophy did not appear. The design of these unpublished experiments precluded statistical analysis, but granuloma size did not seem to increase beyond the time of the 3rd or 4th successive challenge, i.e., the 2nd to 4th month of observation.

6.- PSEUDOTUBERCLES IN NATURAL INFECTION: Compared with any of the preceding models, the acute phase of cercaria-induced schistosome infection represents a condition of maximal host reactivity. Granuloma size reaches its largest attainable means during the 2nd to 3rd months of infection(7), often giving rise to presinusoidal portal hypertension and to the early

murine hepatosplenic symdrome first described by Warren and deWitt (64). Stainable antigen is calculated to persist for less than 34 days (27), i.e., close to the calculated maximal life span of miracidia. In view of the better preservation of eggs left in situ compared to those in purified suspensions, this may represent at least as effective a host performance as found in the artificially sensitized model. Circulating antibody experiences a steep, anamnestic type of rise with a five-fold or greater increment over the titers attained by a single, purified egg injection (21). Concomitant alterations, including scattered lymphoid cell infiltrates, are likewise maximal at this stage; "lymphoreticular activation" (21, 37, 45) results in hepatosplenomegaly with splenic follicular enlargement and, frequently, hyperglobulinemia (37, 45). As shown by Raslavicius, these manifestations are transmitted to the uninfected parabionts of schistosome-infected mice in the absence of cross-passage of schistosome eggs (45). If the portal vein is ligated to induce collateral formation and egg passage into the mouse lung, a florid pulmonary arteritis replaces the sporadic lesions usually found; this arteritis is more intense than its experimental analogue induced by repeated intravenous egg-challenge (62), but neither of these models fully reproduce human bilharzial cor pulmonale.

Two additional features appear in natural schistosome infection which have not yet been observed in any other experimental model mentioned so far, namely central necrosis of granulomas, and in vivo circumoval eosinophilic precipitate, also called the Hoeppli phenomenon.

a.- Central necrosis of pseudotubercles: This lesion is most frequent in acute sublethal or lethal infection of mice, and in other heavily exposed small laboratory mammals and primates (8, 32, 49). Characteristically, necrosis is circumoval, well limited, and not as extensive as in mycobacterial infection. The necrotic zone may be eosinophilic or may

contain basophilic nuclear fragments or dust. The florid type of pseudotubercle, with numerous centrally aggregated neutrophils is probably a variant or precursor of this lesion. As Cheever has shown, maximal granuloma size in the liver decreases after the transition from the acute to the chronic stage of infection, although mean granuloma size does not clearly diminish (7), but with the waning of large, florid pseudotubercles, central necrosis also becomes rare in chronic infection.

b.- The Hoeppli Phenomenon: Stellate eosinophilic precipitates similar to those of other parasitic and fungal infections (Fig. 7) have been described in schistosomiasis since before 1932 (20), although they have not always been recognized as a lesion distinct from central necrosis. Since 1954 (42), *in vitro* circumoval precipitate has been identified as an antigen-antibody complex, reactive with heterologous antiglobulin (23, 42, 47). Unlike *in vitro* complex, the Hoeppli phenomenon requires an especially intense degree of infection and is correlated with large or rapidly accumulating egg loads in host tissues. It appears during acute schistosomiasis, most frequently between the 9th and 15th week of infection, usually in organs heavily infested with ova. No more than 10% of all granulomas are affected and none containing eggs with immature miracidia show precipitate (34). Although the immunological setting of the Hoeppli phenomenon resembles that of central necrosis, these two features do not often coincide within the same single granuloma. Detailed immunofluorescent studies of *S. mansoni* infected livers of *Mastomys coucha* were undertaken in adjacent serial sections, to show that both antigen and fixed host globulin are present in the Hoeppli precipitate: while antigen predominates in the center, presumed antibody globulin is in excess in the peripheral zone of the complex (Fig.8). Both the Hoeppli phenomenon and the *in vitro* circumoval precipitate are formed on the outer surface of the egg shell and do not appear to affect the vitality of miracidia. Once

formed, the Hoeppli precipitate matures and undergoes degradation parallel to the involution of the entire pseudotubercle (34).

7.- PATHOGENESIS OF THE SECONDARY GRANULOMATOUS RESPONSE: We have shown that in the sensitized host the events which take place in primary granuloma formation are accelerated and their total duration is reduced, thus resulting in more efficient and quicker disposal of schistosome egg antigen; this gain is achieved at the expense of enhanced cellular response both in the pseudotubercles and systemically, and is accompanied by antibody formation. While "infectious allergy" is usually defined by the conversion of a previously negative skin test, the above changes are probably more significant.

Just as intact eggs are required in primary pseudotubercle formation (31), the secondary response also requires sensitization with whole schistosome eggs, either viable or subjected to freezing-thawing; sonicated or mechanically disrupted eggs were found to be ineffective by Warren et al. (Personal Communication) On the other hand, homogenates of primary pseudotubercles remain sensitizing for several weeks after their onset, as shown by Peterson (44). This evidence strongly supports the hypothesis of antigen sequestration and the proposed analogies between schistosome eggs and antigen-adjuvant mixtures which have been discussed earlier.

Procedures which suppress the homograft response also inhibit primary pseudotubercle formation, but have little effect on secondary response. Thus primary A. suis granuloma formation is delayed during the first 8 days post partum in mice (30), but sensitization is not abolished despite their apparent immunological immaturity (39). Warren et al. have successfully inhibited primary S. mansoni pseudotubercle formation by neonatal thymectomy (15) antilymphocytic serum (63) and by a variety of immunosuppressive drugs (14).

How sensitization is mediated, remains unsolved. Warren et al. were

able to transmit the secondary granulomatous response passively by means of sensitized spleen cells, but not by immune serum (63). This experiment clearly proves the systemic nature of sensitization and suggests that it is mediated by delayed hypersensitivity, as also indicated by the relatively slow onset of granuloma formation, and the frequency of concomitant perivenular lymphoid cell infiltrates (61). On the other hand, even a single injection of schistosome eggs induces circulating antibody (21) and the early eosinophilotaxis observed in secondary granuloma formation suggests the formation of antigen-antibody complexes (18, 35). In acutely infected and highly sensitized hosts, clearcut antigen-antibody precipitates occur both in vitro and in vivo (34). I would therefore propose that both delayed hypersensitivity and circulating antibody have closely interrelated roles in mediating secondary pseudotubercle formation, and that antibody formation increases in importance proportionally to the degree of host sensitization. Until methods are found to decode this interplay, cellular and humoral factors in granulogenesis are perhaps best regarded as inseparable. This situation can be allegorically represented by the celebrated Koan riddle attributed to the Zen master, Mokurai as he challenges his pupil, Toyo "Show me the sound of two hands clapping," demands the Master, and Toyo claps his hands. "Good, now show me the sound of one hand clapping" (66).

Perhaps a more tractable enigma is the relationship of in vivo precipitation and of central necrosis in pseudotubercles, both of which tend to occur in highly sensitized hosts. Assuming that circulating antibody reactive with schistosome egg antigen is ordinarily not sufficient to overcome antigen excess in the granuloma center, this relationship might express itself in the customary form of phagocytic antigen-sequestration. Should antibody titer rise to a level sufficient to create a zone of antigen-antibody equivalence adjacent to the mature ovum, in vivo precipitation would occur. In this context, precipitation

can be considered as a form of highly effective antigen-sequestration, a concept applicable to other infections produced by bulky organisms or colonies of organisms, and accompanied by marked host sensitization (19, 36). In the light of this concept, central necrosis might have a critical humoral component, perhaps the formation of soluble antigen-antibody complexes similar to those responsible for other types of immunological cell damage such as arteritis (10, 65), but undoubtedly, cellular sensitivity also plays a role (46). While in many infections central necrosis persists during the entire course of their activity, in schistosomiasis it is largely confined to the acute phase which precedes the stabilization of egg-turnover in host tissues. These clues, and others mentioned earlier, deserve to be followed up by further experimental studies.

The familiar language of immunology has served us well in this discussion, by identifying useful precedents and analogies for most of the phenomena studied. However, if the pseudotubercle is to be fully understood, it must be analyzed on the biochemical and enzymatic level, as well.

8.- ANTIGEN SOURCES IN SCHISTOSOME EGGS: Although the basic structure of miracidia has long been known (16), their physiology and ultrastructure are still poorly understood (22). Likewise, the chemistry and ultrastructure of schistosome egg shell is just beginning to come under scrutiny. Recently Smith has shown that egg shells of S. mansoni, as seen in purified suspensions, consist of an inner, electron-dense layer, a wide middle zone containing submicroscopic pores, and a thin outer layer covered by "microspikes" (Fig. 9). Each of these spikes shows a dense core, a light middle layer, and an outer lining formed by an array of globular subunits (Unpublished). (Fig. 10) Similar structures were seen by Seiti (52) and by Stenger et al. in eggs surrounded by granulomas (56). Histochemically, the miracidial cephalic glands contain protein rich in sulfhydril and tryptophane groups (Fig. 10), together with diastase-resistant PAS-positive material, and with various lipids, but they are neither autofluorescent

nor acid-fast (53); these glands also contain esterase and a variety of other enzymes (1). The egg shell contains a modest admixture of proteins and lipids, but its main structural component is a diastase-resistant PAS-positive, refringent material which shows strong orange-yellow autofluorescence and is relatively resistant to a variety of strong and weak acids, bases and detergents (29). The miracidial envelope consists largely of acid mucopolysaccharide. The Hoeppli phenomenon presents a combination of the histochemical affinities of both egg shells and cephalic glands (Fig. 11), and in this respect differs from in vitro circumoval precipitate, which lacks an identifiable egg shell component. In analogy with the zonation shown by immunofluorescent studies, the outer Hoeppli zone shows a strong affinity for protein rich in indole groups, consistent with the presence of host antibody. These findings suggest that antigenic secretion of the cephalic glands may escape the egg via submicroscopic pores of the egg-shell, and may then form an antigen-antibody complex which results in subsequent decomposition of the delicate outer layer of the egg shell; alternatively, the diffusible product may itself contain an enzyme which catalyzes egg shell decomposition (53). Since the Hoeppli phenomenon forms at the point of miracidial maturity, this product might have the role of a hatching enzyme in the natural reproductive cycle. Further studies of the antigenic components and enzymes of schistosome eggs are urgently needed.

Little is known about the catabolism of egg shells in the granuloma, which extends through its long phase of involution, past the time of disappearance of diffusible antigen. Since lysozyme plays an important non-specific role in defense against mycobacteria (40), this or similar enzymes of monocytic origin (43) may be involved in schistosome egg shell catabolism. Identification of the distinctive egg-shell material, likely to contain highly polymerized glucosamine or glycoprotein (55), could be a first step into an interesting borderland of biochemical immunology, since there is

evidence that granuloma resolution can be accelerated by sensitization although egg shell material is not yet known to be responsive to any physiologically active mammalian enzymes.

9.- IMMUNOLOGICAL SIGNIFICANCE OF PSEUDOTUBERCLES: From the aggregate evidence presented here, pseudotubercles appear to function as auxiliary subunits of the lymphoreticular "establishment" in handling particulate pathogens from which diffusible antigen is gradually released, thus inducing antigen sequestration in situ. These macrophagic cell factories act as immunological receptors and effectors, and potentiate the ability of the sensitized host to catabolize antigen, and to break down residual inert matter during granuloma involution. In this manner, granulomas are uniquely equipped to defend the host against a variety of antigens produced by micro-organisms and haptenic chemical deposits too large or too toxic to be handled by single cell units.

Askonas and Humphrey have suggested that antibody may be locally generated in adjuvant-antigen granulomas (3); since pseudotubercles show a gradient of antigen concentration which decreases toward their periphery, antibody might be a function of lympho-plasmocytic cells which surround the phagocytic core of mature granulomas. This attractive hypothesis would partly account for the enhanced antibody generating potency of antigen-adjuvant mixtures, and for the high antibody levels found in schistosomiasis. However, in order to avoid misunderstanding (12) it should be re-emphasized here that antigen sequestration cannot totally prevent diffusible antigen from reaching and activating the entire lymphoreticular system: therefore, pseudotubercles act by supplementing, rather than supplanting the classical immunological responses to antigenic stimulation.

SUMMARY*

The immunopathology of schistosome pseudotubercles has been reviewed with particular emphasis on antigen sequestration, and on accelerated antigen destruction in sensitized hosts. The sequence of events in primary and secondary pseudotubercle formation has been analyzed and correlated with presently available data on the nature of schistosome egg antigens; the miracidial cephalic glands and the egg shell have been identified as major antigen sources. The interrelated cellular and humoral factors in host sensitization have been explored, with particular attention to precipitate formation and to central necrosis in pseudotubercles, and the Hoeppli phenomenon has been identified as an *in vivo* antigen-antibody complex. The modifications of granulomatous inflammation and its concomitant pathology in the course of natural schistosomi infection have been summarized; finally, a brief evaluation of the immunologic role of granulomas has been presented. The text of the paper should be consulted for details.

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LEGENDS FOR FIGURES

Fig. 1.- Mouse lung, 32 days after intravenous injection of Schistosoma mansoni eggs and of di-vinyl-benzene-copolymer beads. Hematoxylin-Eosin X 240. The schistosome pseudotubercle is at its peak development, whereas the reaction to the plastic bead is reduced to a thin fibrous sheath.

Fig. 2.- Mouse liver, 8 weeks after cercarial infection with S. mansoni. Cryostat section stained with fluorescein-conjugated immune Mastomys globulin by the direct Coons' technique. X 370. The paired miracidial cephalic glands, and the antigen deposits on the inner and outer egg shell surface are intensely positive. Antigen is seen in adjacent granuloma cells, fading toward the periphery. Some granulocytes show non-specific fluorescence.

Fig. 3.- Mouse lung, 24 hours after injection of pure miracidia. Periodic acid-Schiff stain, X 420. The strongly positive miracidium, impacted in a capillary, is disintegrating and has attracted numerous leukocytes to its vicinity. There is no granuloma formation.

Fig. 4.- Mouse lung, 64 days after injection of purified egg shells obtained by sonication-centrifugation. Hematoxylin-Eosin, X 420. The egg appears as a basophilic spiral surrounded by a few histiocytes. This represents the residual stage of the reaction.

Fig. 5.- Graphic representation of cellular reaction diameters around viable and heat killed S. mansoni eggs, and around two types of purified egg shell preparations (see text), at successive intervals after intravenous injection into unsensitized mice.

Fig. 6.- Mouse lung after 4 successive monthly injections of viable S. mansoni eggs. Hematoxylin-Eosin, X 220. The arteriole shows marked intimal proliferation with multiple, cleft-like lumina, appears thickened and surrounded by a dense lymphoid cell infiltrate. An involuting pseudotubercle containing an

LEGENDS FOR FIGURES, (continued)

egg shell is seen in the vessel wall, right of center.

Fig. 7. - Colonic submucosa of baboon, 7 months after exposure to 1000 cercariae. Hematoxylin-Eosin, X 400. Cluster of Hoeppli phenomena in a composite granuloma with central necrosis (an unusual occurrence). Egg on left shows a mature miracidium and fully developed, spectacular Hoeppli corona. Egg on right has a degenerate miracidium and blotchy, ageing Hoeppli phenomenon.

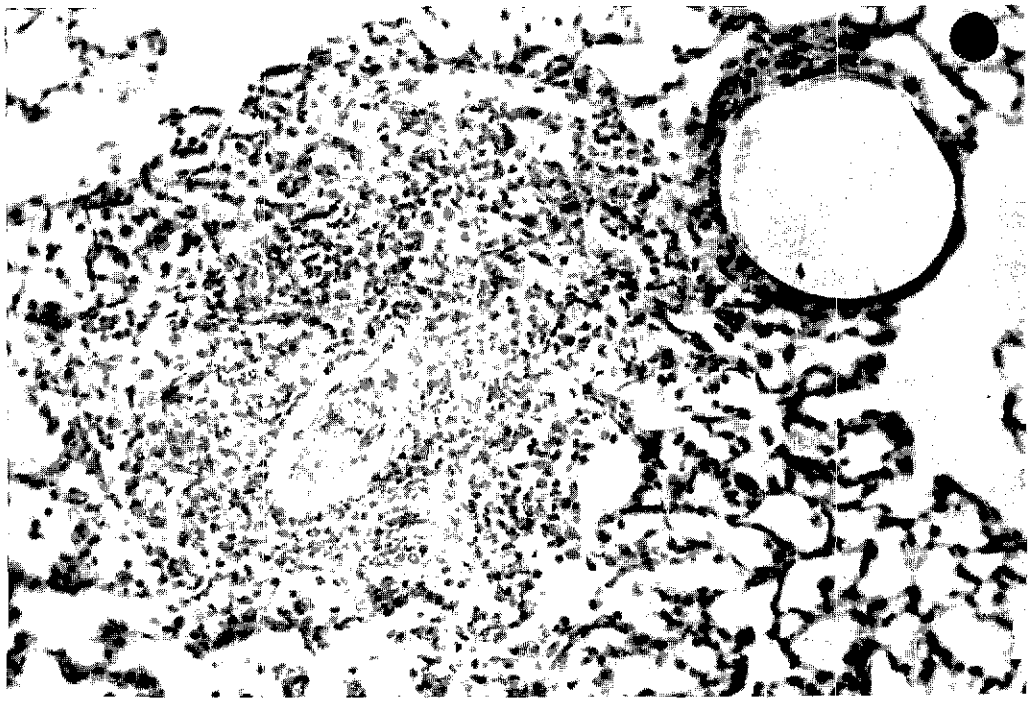
Fig. 8.- Mastomys liver infected for 11 weeks with S. mansoni. Serial cryostat sections stained with rabbit-antimastomys globulin conjugate only, X 370. An apple green halo surrounds the autofluorescent spikes of the Hoeppli phenomenon and blurs their outline. This demonstrates the predominant peripheral location of fixed host globulin in the precipitates. Adjacent sections, stained for antigen, showed the central distribution of the latter.

Fig. 9.- Electron micrograph of schistosome egg shell obtained from a purified suspension; glutaraldehyde-osmium fixed; magnified appr. 43,000 times. Shows the trilaminate egg shell structure described in text, with prominent pores in middle layer, and with closely spaced superficial microspikes. The fine structure of the latter is better seen at higher magnifications.

Fig. 10.- As above, tangential section, X 80,000. Shows the dense cores and the globular arrays of the microspike membrane. A shell pore is seen in the right part of the field.

Fig. 11.- Egg of S. mansoni in mastomys liver, stained by the dimethylamino-benzaldehyde nitrite (Adams, 1960) method for indole groups. X 420. Both the cephalic glands and the early Hoeppli phenomenon seen along the left egg-shell border are strongly positive.

Fig. 1



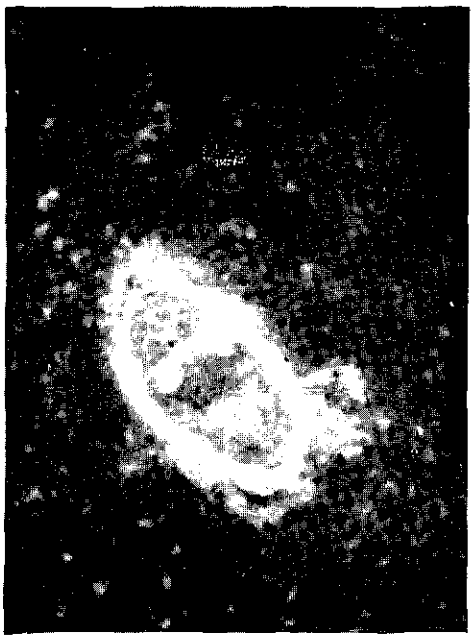
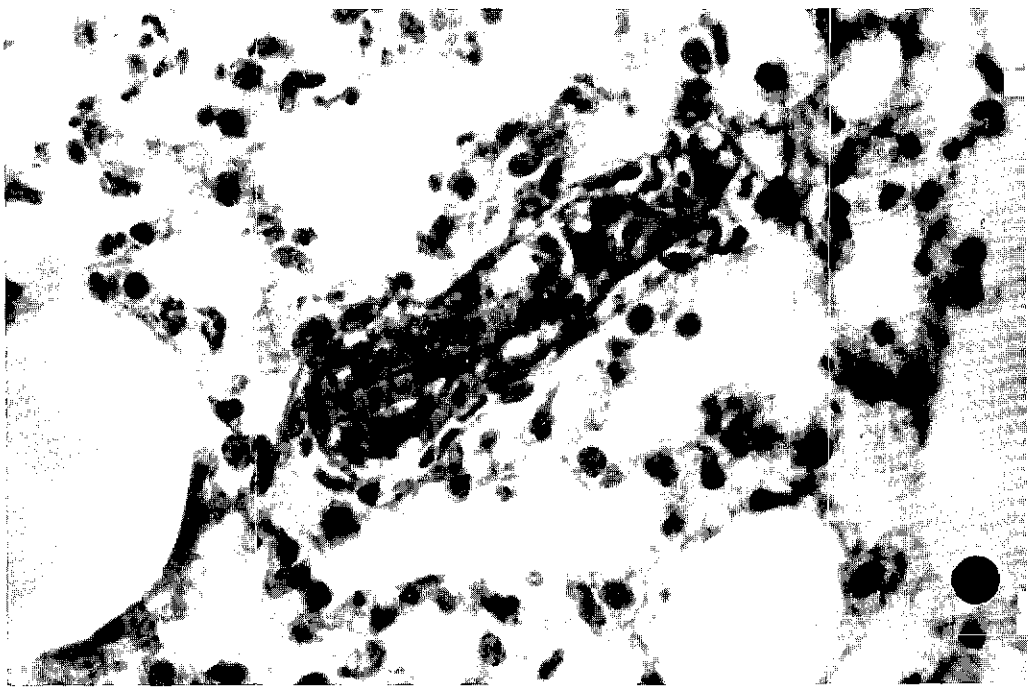


Fig. 2

Fig. 3



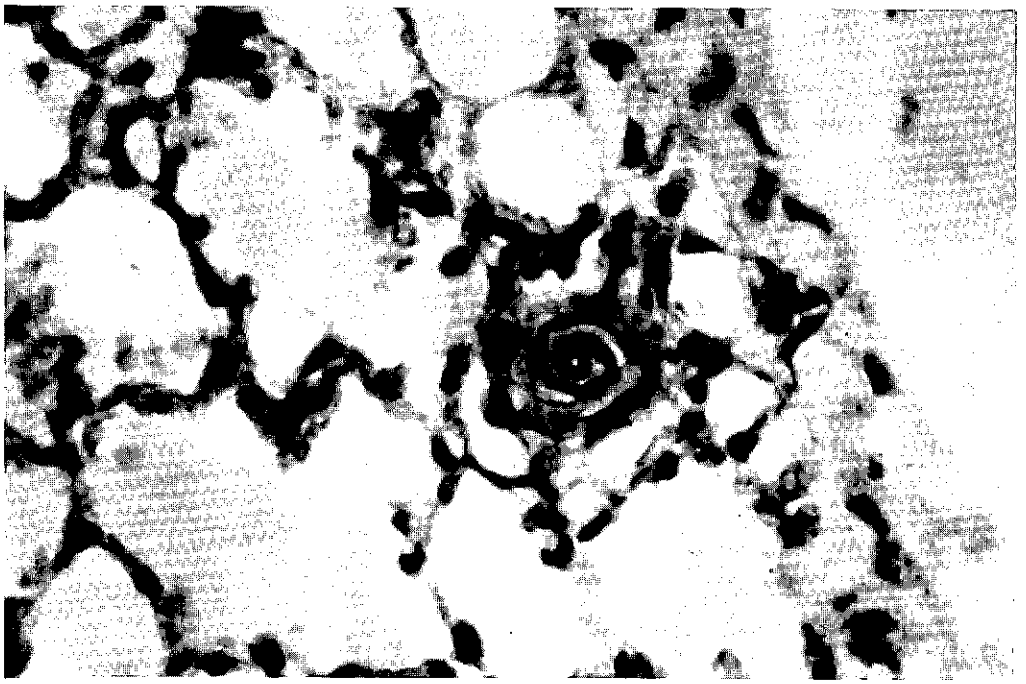
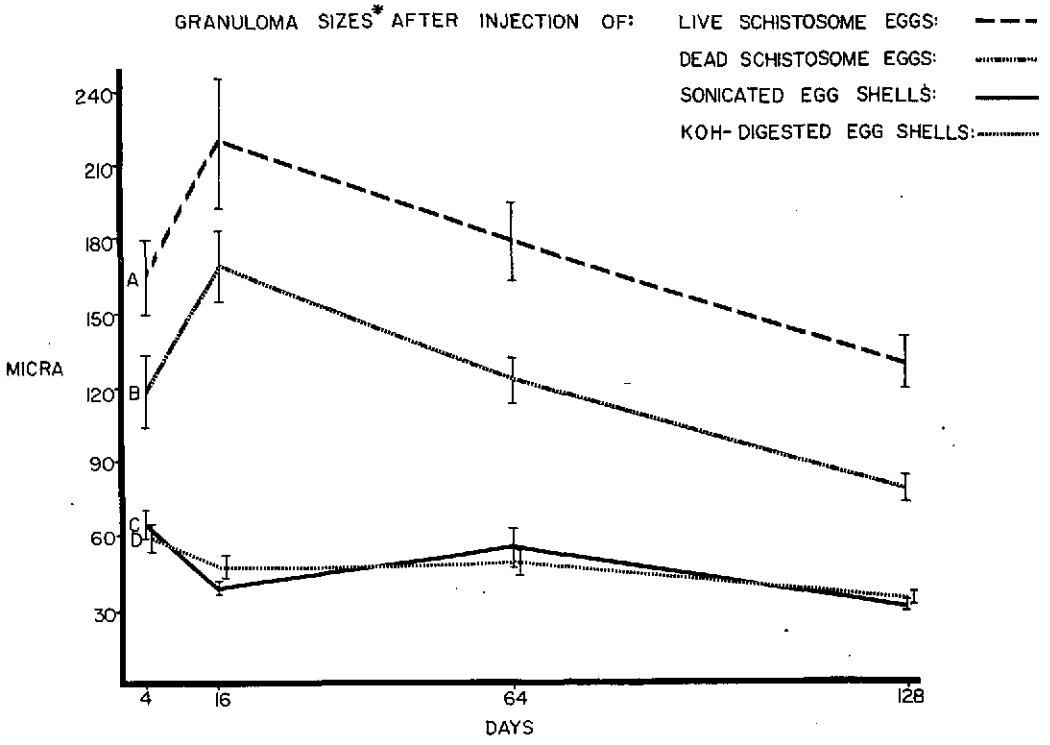


Fig. 4

TABLE III



*VERTICAL BARS SHOW STANDARD ERRORS

Fig. 5

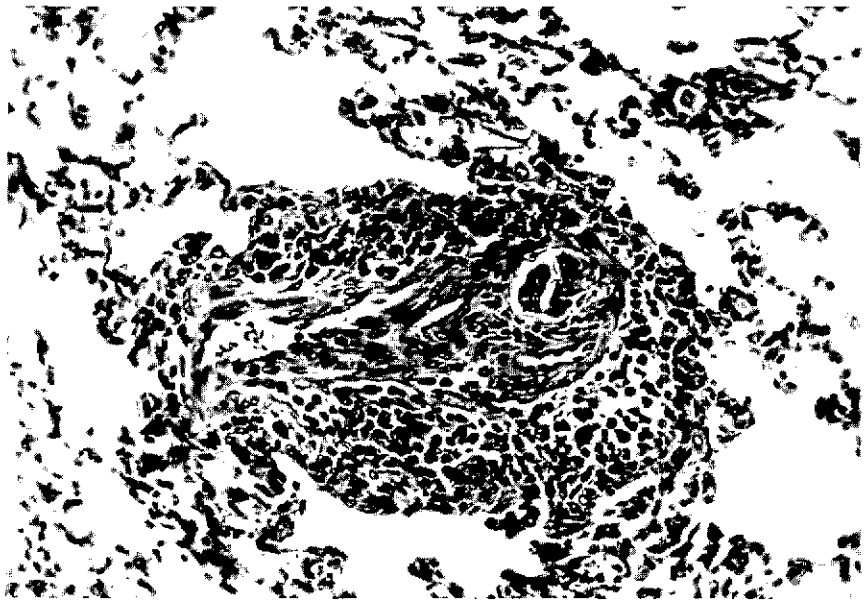
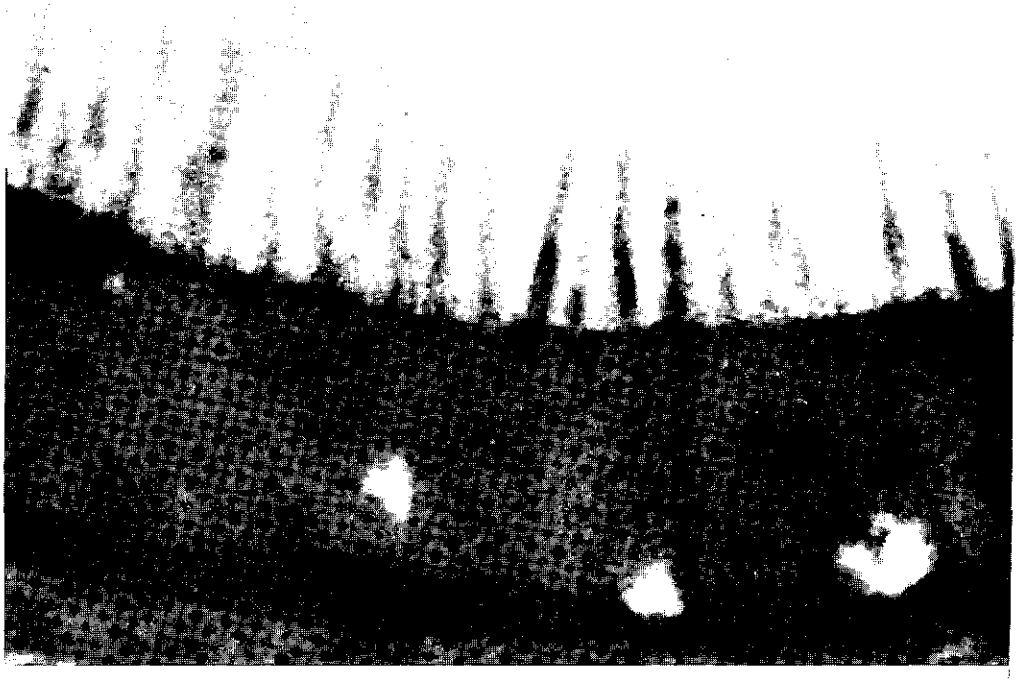


Fig. 6 ←

Fig. 7 ↑

Fig. 8 ↓

Fig. 9 ⟶



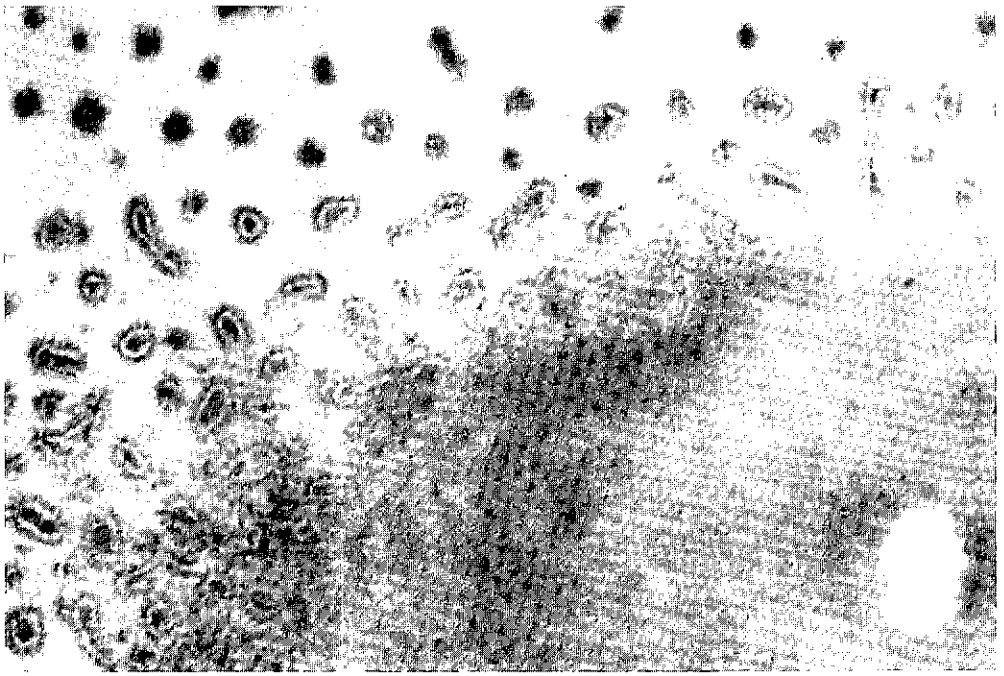


Fig. 10 ↑

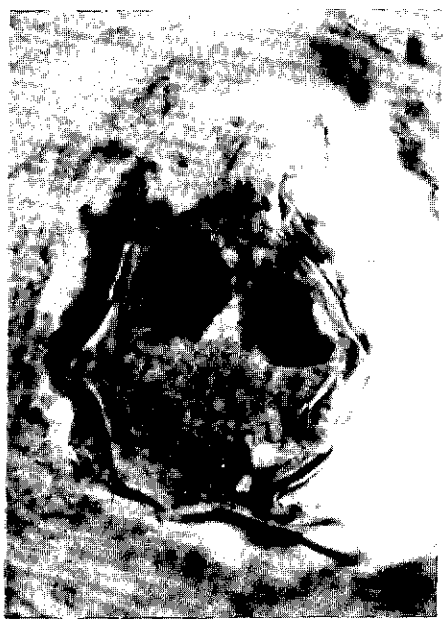


Fig. 11 ↑

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