IMMUNOPATHOLOGIC MECHANISMS IN PARASITIC INFECTION WITH EMPHASIS ON SCHISTOSOMIASIS

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Since both the causal agents, and the clinical manifestations of most human parasitic infections have long been described, and effective drugs are now available for many of them, why should research on their immunopathologic mechanisms be worthy of further effort?

First and foremost, because the more deeply we can understand their mechanisms the better will be our ability as physicians to treat and to prevent these endemic diseases in our patients. Second, because the pathogenesis of parasitic diseases has much to teach us that is of general biological value, and can help us analyze other, apparently unrelated disease conditions, and third, because the scholarly pursuit of such knowledge will enlarge the horizons of those who teach and who study parasitic diseases and will make them into better teachers and students, as well as into better physicians.

Moreover, in a recent Editorial in the journal "Science", Minners (1977) has pointed out that scientific opportunities in research on parasitic infections are today greater than ever before. "If we put our minds to it"--he stated--"the next decade may well see research in tropical medicine come to the forefront in the search for new knowledge". Acting out of a similar conviction, Professors T. Lambo and A. Lucas have persuaded the World Health Organization to initiate a special program for research and training in tropical diseases, in which major research emphasis will be placed on disease mechanisms, including immunity and immunopathology, and it is on behalf of this program that I am here.

My task will be to convince you that research on the immunopathology of parasitic infection is today urgent, and eminently worth pursuing. Much progress has already been achieved in clarifying the disease mechanisms of malaria, trypanosomiasis, leishmaniasis, filariasis and schistosomiasis (the target infections chosen by the WHO Special Programme), but many unsolved questions remain. Currently among the most exciting subjects are the prospects for vaccination in both malaria and schistosomiasis, and the cellular and molecular features of these parasites, especially their membrane physiology and their surface antigens and receptors as they relate to immunological host responses. Parasite studies have added new dimensions to basic immunology, such as the diverse mechanisms of evading host immunity discussed by Cohen and Sadun (1976) and have helped to gain a better understanding of delayed hypersensitivity, of immunological modulation, and of immune eosinophil functions.

It should also be stressed that your contributions to this field, in Southeast Asia, can be uniquely valuable because you are in daily contact with the clinical realities of parasitic infections, including some which are as yet quite imperfectly understood, such as gnathostomiasis, angiostrongyloidiasis, or the various endemic liver fluke- and *Brugia* infections. The presence of such endemically infected patients, and your familiarity with their problems, should be your best guides in orienting your research activities and will guard these from remaining sterile or purely academic.

Progress in the immunopathology of parasitic diseases can often be made by the use of relatively simple technology, such as the microscope and test tube, rather than the electron microscope and the scintillation counter. Sophisticated research technology is not to be disparaged, but because of its cost and complex maintenance, it should not be regarded as a primary means for stimulating progress but rather as a tool for pursuing and following up on questions which have already arisen from more direct observations, so that advanced research techniques will come to grow on a broad base of simpler and intermediate technical skills. In building up this kind of research pyramid, the pathology laboratory occupies a very important position, for it is here that clinical observation and basic medical science can best be linked. Historically, the human and/or veterinary autopsy, and the study of surgical and laboratory specimens close to the bedside have been powerful influences on the development of the basic medical sciences. The human autopsy, in particular, provides us with a unique perspective of the disease spectrum which affects a given population, a record of how the causes and courses of disease can change over periods of time, and an identification of problems which will demand concerted research efforts. There is no better way to stimulate rapid scientific progress in many medical disciplines than to facilitate expert and conscientious autopsy pathology observations.

Having emphasized the value of direct observations on spontaneous human and animal disease, we must now turn to experimental studies *in vivo* and *in vitro* which are the primary tools of parasite immunopathology. These model systems strive to imitate nature, while providing the controlled conditions necessary for its understanding, and it is by their use that most of the recent progress in this field has become possible. However, as has so often been said--experiments are rich in pitfalls both of design and of interpretation. One of the most common ones is failure to consider the natural differences in anatomy, physiology and immune reactivity between animal species; another common error is to extrapolate directly from events *in vitro* to what might occur in the intact mammalian host system. We must try to keep these pitfalls in mind as we begin to review some recent research advances.

Because the field of parasite immunopathology is far too vast to be covered succintly, and because protozoal and metazoan diseases are so very different in their disease mechanisms, I shall limit myself here to the immunopathology of the helminthic infection, more specifically to schistosomiasis. This disease is now also of local interest for you, since foci of schistosomiasis have recently been discovered in the Mekong river basin (Sornmani *et al.*, 1971, 1973) in the Lindu Valley, in Sulawesi (Hadidjaja *et al.*, 1972), and possibly elsewhere on the Pacific perimeter where their existence has not yet become widely known.

In addition, Nelson (1972) stated that "Schistosomiasis is the parasitic disease about which more is known than of any other" yet, there are many questions left for us to study, perhaps more than we were even able to ask when we began. A logical division for approaching these subjects is to consider them under two separate headings: Problems related to the disease mechanisms of established infection (i.e. pathogenesis), and those related to protective immunity (i.e. immunogenesis).

SCHISTOSOME PATHOGENESIS

Traditionally, this review would begin with Manson's schistosomiasis, the best studied form of bilharzial infection, but let me depart from this norm by discussing first the pathology of the Mekong Schistosome. Over the

last year and a half Dr. James Byram and I have been able to study its experimental pathology in laboratory rodents in our laboratory in collaboration with Dr. John Bruce and his group (Byram and von Lichtenberg, 1977a).

From our experimental perspective, the Mekong schistosome must be regarded as highly pathogenic for susceptible mammals. This realization first came as a surprise, because the prepatent phase of this organism in mice and hamsters is about 1-2 weeks longer than that of *S. japonicum*, i.e. 5-6 weeks, and its pathology becomes significantly severe only at about 8 weeks. Infection yields as shown by our worm and egg counts are high, 70-75% of cercariae reach full maturity, about 2/3 of these as egg-laying pairs and their survival rates and fertility are comparable to those of any *S. japonicum* strain studied by us thus far. At exposure levels of 5-20 cercariae, all but a few surviving worms reach the portal circulation with egg lesions mainly in the gut, liver and--to a lesser extent--the lung. Virulence is also high since exposure to 15 or more cercariae is uniformly fatal for mice within 2½ months. Mice with 4 pairs of worms succumb within 10 weeks, those with 3 pairs usually by the 15th week. One pair of worms constitutes a heavy, but not a lethal infection for mice. Hamsters survive and tolerate infection somewhat better than mice.

Histologically, as in *S. japonicum* infection, clusters of multiple eggs were frequently grouped together in a single granuloma and these egg foci were the largest, most florid and most destructive ones thus far observed in mice with any schistosome species. More often in mice than in hamsters, we saw granulomas composed of numerous large, vacuolated macrophages of a distinctive appearance (Fig. 1). These "vacuolocytes" did not contain stainable lipids nor appreciable acid phosphatase activity. Electron microscopic studies are in progress.

Other prominent features of Mekong granulomas were central necrosis and prominent peripheral fibroblastic proliferation and collagen deposition. Abscess-like and histiogranulocytic granulomas were also frequently seen, some containing neutrophils, especially in hamsters. Hoeppli phenomena were numerous in hamster livers at 8 weeks, and in mice at 10 weeks, where they are very rarely seen in other schistosome infections. By the 15th week, there was significant calcification of eggs in both the gut and the liver. Plasma cells were numerous, especially in the periphery of granulomas. Eosinophils were prominent, and their behavior will be reviewed in more detail.

Despite clearcut biological differences between the Mekong schistosome and *S. japonicum*, its pathology in rodents was in many respects similar--one might call it an exaggerated version of *S. japonicum* disease. Whether this is also true of canine and of human infection is, unfortunately not known. Both infections, in rodents, contrast sharply from the pathology of *S. mansoni*. Here, as you will recall, single egg deposition is the rule and composite granulomas are rare; egg calcification is uncommon. Typically, the mature granuloma is composed of pale, nonvacuolated epithelioid macrophages and a large complement of eosinophils with few if any neutrophils present (Fig. 2). Hoeppli phenomena are absent in mice and rare in hamsters. Instead of plasma cells, small and immunoblastic lymphocytes surround the granulomas. Fibroblastic proliferation is common, but dense collagen deposition is not, and tends to occur late during chronic infection (von Lichtenberg *et al.*, 1973).

What accounts for these differing histopathologic expressions between schistosome species? In order to answer this question, we must first review the experimental evidence which has been accumulated on the immunopathology of schistosome granulomas.

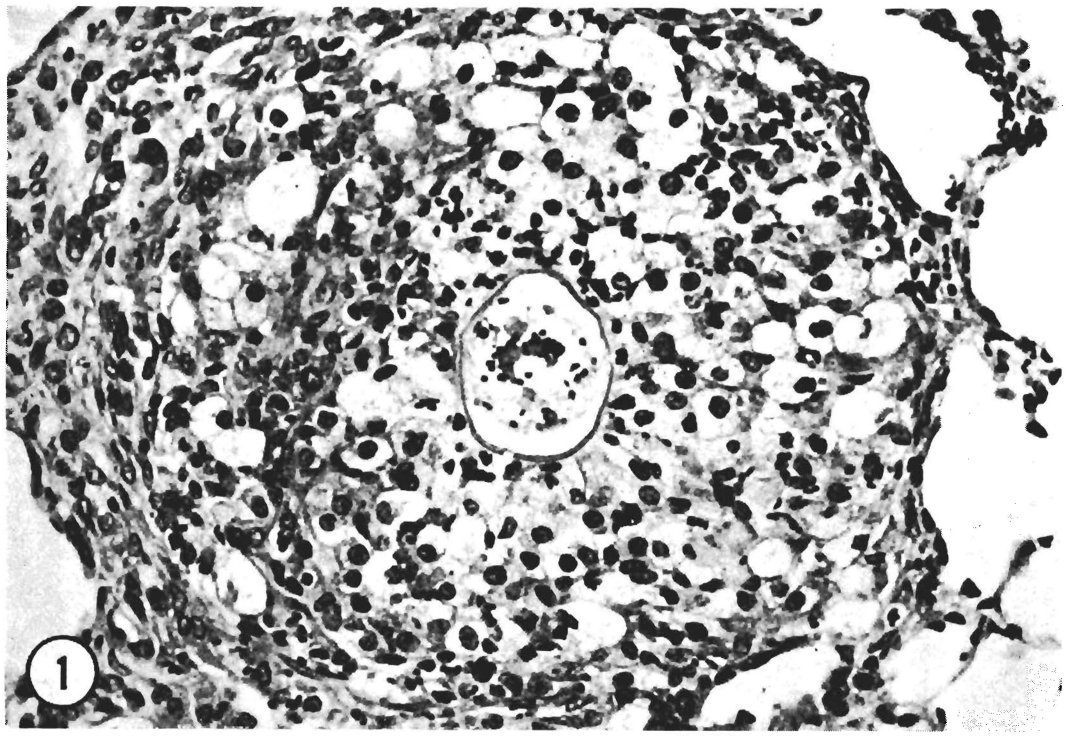


Fig. 1—Hamster infected for $8\frac{1}{2}$ weeks with 100 cercariae of the Mekong schistosome. This lung granuloma, stained by the Dominici technique (x450), shows numerous typical vacuolated histiocytes around an egg with mature miracidium.

The earliest indication that the formation of schistosome egg granulomas was immunologically determined came from experiments in which we injected purified *S. mansoni* eggs by the tail vein into mouse lungs (Coker and von Lichtenberg, 1956). Typical granulomas formed both on primary egg injection, and on challenge, but it was shown that the response in the sensitized host was accelerated and enlarged, i.e. anamnestic. Neither the egg shell alone, nor the nude miracidium were capable of eliciting granulomas, and it was concluded that sustained antigen release from intact eggs was necessary for granuloma formation; this was borne out more directly by the observation that immunofluorescent-stainable egg antigen was sequestered in the central zone of *S. mansoni* egg granulomas where it was eventually katabolized. It was later shown that schistosome egg shells have pores of sufficient size to permit this kind of antigen passage to occur (von Lichtenberg, 1967).

Experiments by Warren and his group at Western Reserve University next demonstrated that accelerated granuloma formation, in the mouse lung model, could be transferred to normal mice by sensitized lymphocytes but not by serum, and that it could be significantly but not entirely suppressed by drugs or by radiation which deplete host lymphocytes or affect cell mediated immunity. This suggested that the *S. mansoni* granuloma is a manifestation of delayed hypersensitivity (Warren *et al.*, 1973); however, today, we would modify this statement, calling the response to *S. mansoni* eggs "T-lymphocyte dependent". This has now been formally proven by Phillips and co-workers and by Byram's studies in our laboratory. Phillips

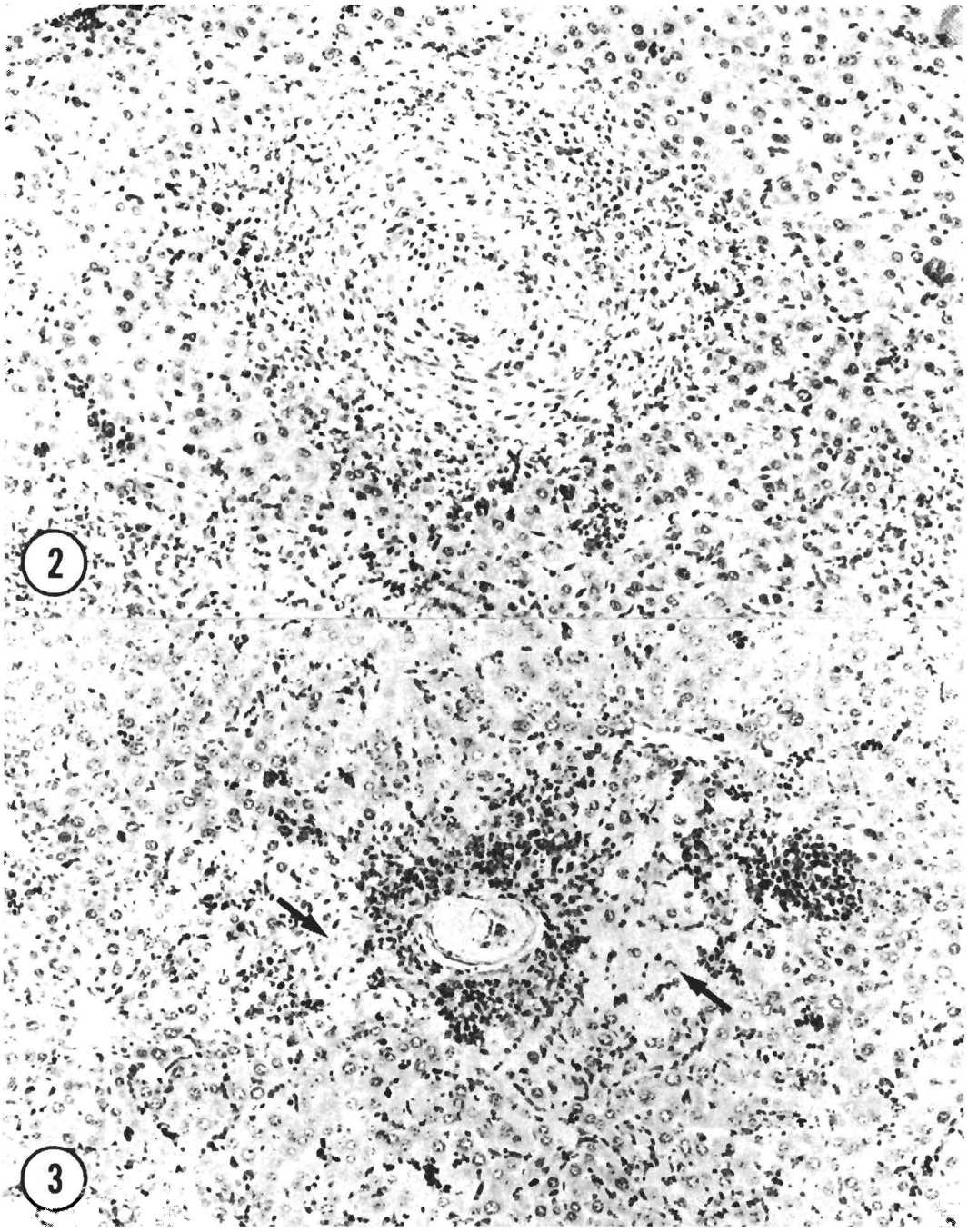


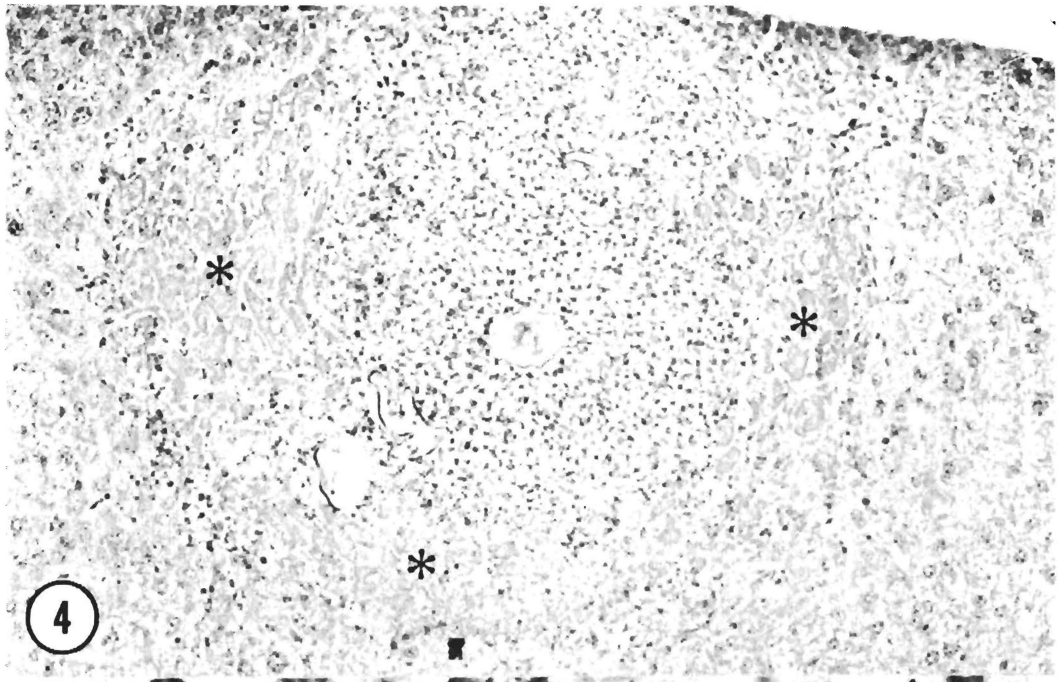
Fig. 2—Nu/+ heterozygote mouse infected for 7 weeks with 200 cercariae of *Schistosoma mansoni*. This liver granuloma (Dominici stain, X185) shows a predominantly histiocytic and fibroblastic composition, with numerous eosinophils, and with lymphoid cells scattered toward its margin. Note intact hepatocytes with ductular proliferation around the granuloma. Compare with Fig 3.

Fig. 3—Nu/nu athymic mouse, conditions identical to those in Fig. 2 The egg focus is much smaller than in the immunocompetent mouse, and contains numerous ordinary macrophages loaded with schtstosome pigment. Adjacent liver cells show typical zonal microvesicular degeneration and acidophilic necrosis (Arrows). Compare with Fig. 2 & Fig. 4.

showed that homozygous nude mice which lack functional T-lymphocytes have much reduced cell responses to *S. mansoni* eggs, but after transplantation of thymus from immunocompetent heterozygote donors of the same mouse strain, these responses were restored to normal size (Phillips *et al.*, 1977). We were further able to demonstrate that the cell responses to eggs in nude mice lack the characteristics of hypersensitivity granulomas in that they do not show epithelioid macrophages, and also fail to render anamnestic responses when tested by the mouse lung granuloma model (Byram and von Lichtenberg, 1977).

Several additional findings of interest emerged from these experiments: In the absence of functional T-cells, there was microvesicular and acidophilic liver parenchymal cell damage around the foci of inflammation induced by mature, but not by immature *S. mansoni* eggs (Fig. 3). At the same time, antigen sequestration was impaired and diffusion of egg materials was evident around the egg foci. Interestingly, when comparing our Philippine *S. japonicum* strain with Puerto Rican *S. mansoni* nude mice, the oriental schistosome eggs caused larger, more abscess-like lesions with a broad fringe of eosinophilic liver cell necrosis around them (Fig. 4), i.e. even in the immunoincompetent host these eggs manifested greater virulence, or destructive power than those of *S. mansoni* (unpublished data). These findings have led us to believe that besides the products elaborated by mature schistosome eggs being antigenic, some are directly cytotoxic for liver cells. Thus far, the only clue to the nature of these toxins has been the extraction from eggs, by appropriate lipid solvents, of lysophospholipids which are known to disrupt plasma membranes, as shown by Smith *et al.* (1971), but it is not clear whether these or other egg products are responsible for the cytotoxicity of schistosome eggs *in vivo*. Heterozygote, immunocompetent mice of the same experimental series which developed normal hypersensitivity granulomas did not show such distinctive hepatocellular damage. Rather, liver cell trabeculae around these granulomas were distorted, and showed some ductular proliferation. Thus the formation of hypersensitivity granulomas, in addition to its harmful effects, seemed to have at least one beneficial effect, namely that of efficiently containing and/or katabolizing potentially cytotoxic egg materials (Byram and von Lichtenberg, 1977b). While it has been suggested that immunosuppression should improve the clinical status of schistosome-infected patients, our findings in nude mice are not encouraging in that respect. Quantitative studies will now be needed to find out whether gain from immunosuppression is sufficient to offset loss from schistosome egg cytotoxicity.

Much research has been devoted to the question how the effect of sensitized T-lymphocytes on *S. mansoni* granuloma formation is mediated. Boros *et al.* (1973) have clearly shown that MIF is elaborated by *Schistosoma mansoni*-sensitized spleen cells, as well as by cells resident in the egg granulomas themselves, and this could account for the aggregation of macrophages around the target egg antigens; in fact, one can now specifically sensitize for-and elicit anamnestic granuloma formation with a soluble schistosome egg extract, named SEA (Boros and Warren, 1971). This egg antigen mixture, or miracidial hatching fluid, will elicit delayed mouse footpad reactions when injected locally, and will incite pulmonary granulomas when adsorbed to appropriate inert particles and injected intravenously into sensitized mice, as confirmed in our laboratory (Dunsford *et al.*, 1974). This leaves little doubt that sensitized T-cells accelerate *S. mansoni* granulomas primarily by cellular immunity mechanisms, such as lymphokines. One of the most interesting among the latter, is a cell product which attracts eosinophils to schistosome



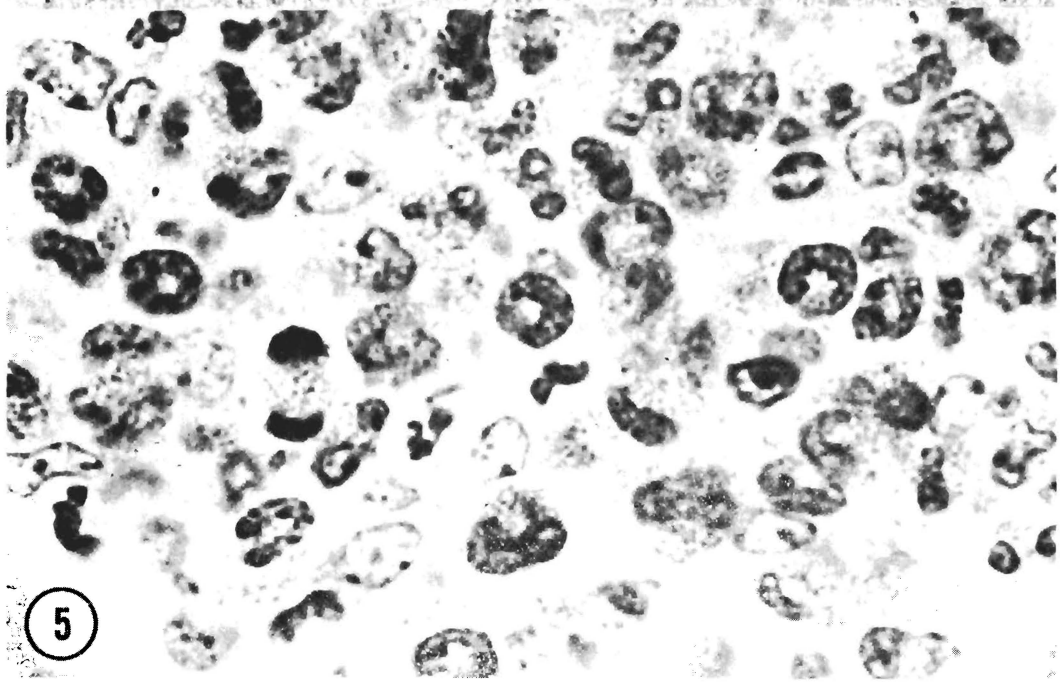


Fig. 4—Nu/nu mouse infected for 7 weeks with 10 cercariae of the Philippine strain of *S. japonicum*. This liver egg focus (Dominici stain, X230) contains numerous neutrophils and resembles a micro-abscess. A zone of eosinophilic coagulation necrosis of liver cells concentrically surrounds the lesion (*). Note its greater severity compared with *S. mansoni* (Fig. 3).

Fig. 5—CF 1 white outbred mouse infected for 10 weeks with 10 cercariae of the Mekong schistosome. A focus of eosinopoiesis is shown in the liver, close to a subcapsular granuloma magnified 1840X (Dominici stain). It is composed principally of myelocytes, showing typical ring-and horse-shoe-shaped nuclei with "open" chromatin. One cell is in metaphase. Note the well developed cytoplasmic granules, which appeared brilliant red in the original slide.

granulomas in a gradient-dependent fashion. Similar to MIF, this material (named ESP) has been obtained by Colley and colleagues from both sensitized spleen cells and from isolated liver *S. mansoni* granulomas (James and Colley, 1975) and, in addition, they find these cells to have a stimulating effect on mouse bone marrow eosinophilopoiesis when co-cultivated in double chambers within the mouse peritoneal cavity (Miller *et al.*, 1976). Significantly, egg reactions in nude and in thymectomized mice are very poor in eosinophils. Conversely, in immunocompetent mice and hamsters, including those infected with the Mekong schistosomes, we have demonstrated proliferation and maturation of immature eosinophils in areas of heavy egg deposition, such as the liver and gut, and in their pathways of lymph drainage, as well as in the spleen. We interpret these findings as extramedullary eosinopoiesis (Fig. 5) and surmise that they could be an expression *in vivo* of the marrow cell eosinopoiesis stimulating lymphokine demonstrated by Colley and colleagues (Byram *et al.*, 1978).

The question remains open nevertheless, whether T-cell stimulation of hypersensitivity granulomas is exclusively mediated by CMI-type mechanisms, or at least in part by their helper activity for B-cells. Thus far, evidence on the latter point has been fragmentary, yet somewhat suggestive: Host IgG_1, IgM and C_3 have all been demonstrated in *S. mansoni* granulomas of mice and baboons by Sogandares Bernal and Brandt (1976) and by Houba (1976). When lymphoid cells of *Schistosoma mansoni* granulomas in cryostat sections were studied for membrane receptor markers such as EAC rosetting, only a few positive cells were found in granulomas elicited by lung egg injection, but liver granulomas in natural *S. mansoni* infection contained significant numbers of presumed B-lymphocytes, which increased to relatively large numbers when the infection was of chronic duration (Boros,1977 unpublished data). This indicates that B-cells and antibody globulins are indeed present in these granulomas, but it does not clarify their functional significance.

It seems quite unlikely that antibody should be required for granulomas to form since anamnestic responses to *S. mansoni* eggs arise concomitant with early MIF production, and before any hemagglutinating antibody becomes detectable in the host serum (Boros and Warren, 1971). Whether antibodies are involved in potentiating the granulomatous response during the acute phase of infection when they attain their highest titers, is unknown; however subsequent to this maximal response, it was shown by Andrade and Warren (1964) that in chronically infected mice granulomas become smaller, resulting in a decrease of liver weight and portal pressure a process now described by the term modulation. Several workers have postulated that antibodies or antigen-antibody complexes may be involved in schistosome granuloma modulation, based on the finding that the serum of chronically infected mice and of chronically infected patients (Colley *et al.*, 1977) contains products capable of depressing the responses of autologous lymphocytes to SEA and to mitogenic lectins such as PHA; however, other possible mechanisms have not yet been ruled out, including an activity of suppressor T-cells.

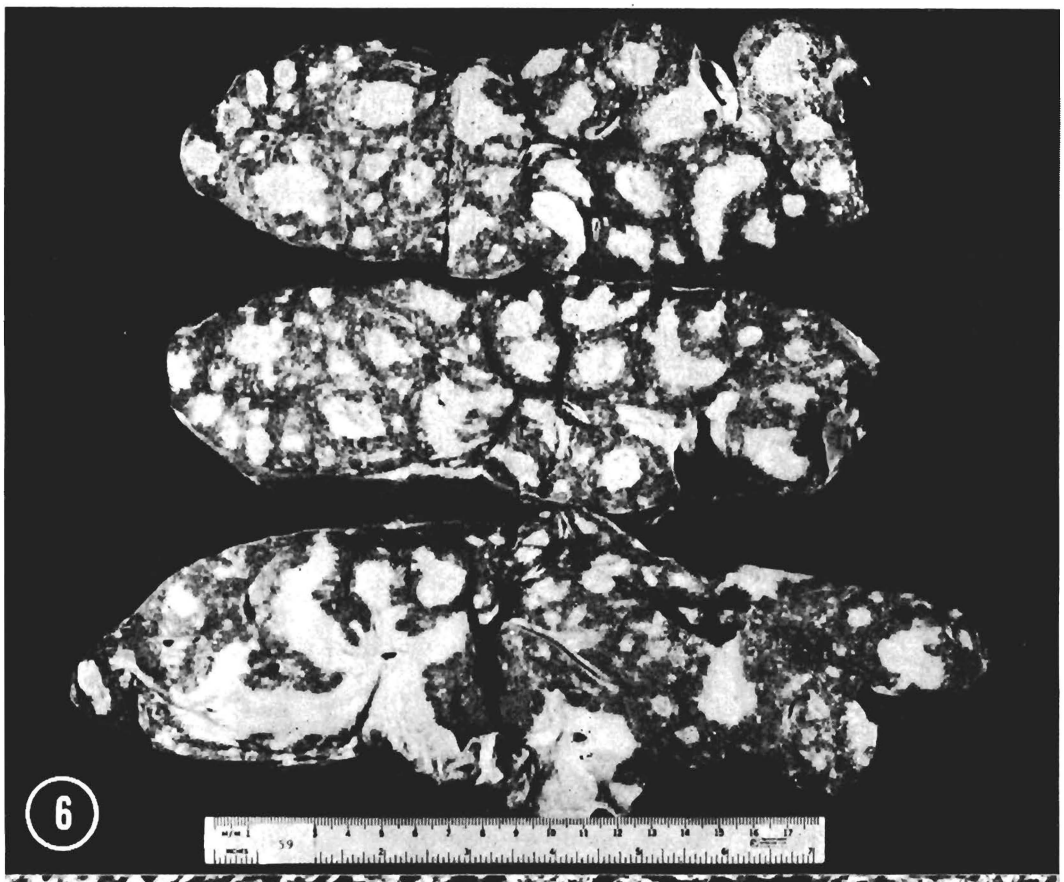
Another interesting possibility which our group has been considering is that antibody, either circulating or locally produced, might be essential for antigen sequestration in *S. mansoni* granulomas. This is based on preliminary evidence that, in T-lymphocyte deprived mice which have undergone thymectomy and sublethal radiation hepatocytotoxic damage can be rendered lesser by passive transfer of immune serum. These unpublished observations from M. Doenhoff's laboratory need to be further confirmed and expanded.

It appears from this brief summary of many studies that our simple view of the *S. mansoni* granuloma as a result of delayed hypersensitivity has changed to a more complex panorama. We now see this reaction as subject to multiple immune regulating mechanisms. The egg granuloma is first elicited by CMI, via T-lymphocytes, but subsequently its size and cellular composition must be under the influence of changing subpopulations of both T- and B-cells. The next years should bring us nearer to a complete understanding of how such immunoregulation works and this understanding will be crucial in any future approach to the immunotherapy of human schistosomiasis, if indeed feasible.

Meanwhile, there has been some progress as well in defining and purifying *S. mansoni* egg antigens. Pelley *et al.*, (1976) of Western Reserve University have obtained a fraction of SEA from *S. mansoni*, named MSA-I,which shows single-protein purity on polyacrylamide gel diffusion and is highly species- and stage specific. Similar research is continuing in the laboratories of Carter and Colley (1978) and of Brown *et al.*, (1978). Unfortunately, analysis of *S. japonicum* antigens is still far behind that of *S. mansoni*, not to mention the Mekong schistosome. From *in vivo* experiments one would surmise that egg products of these latter two schistosome species should substantially differ from those of *S. mansoni*; otherwise, it would be difficult to explain the greater virulence of oriental schistosomes, their peculiarly large and destructive granulomas, and their distinctive cell composition during acute infection. Warren *et al.*, (1975) have suggested basic differences, as well, in immunological mediators. Based on the frequency of plasma cells, and of Hoeppli precipitates (which are presumed antigen-antibody complexes analagous to the COP *in vitro*) it was proposed that *S. japonicum* granulomas might be B-cell rather than T-cell mediated. In further support, *S. japonicum* SEA did not elicit delayed footpad swelling in sensitized mice, but rather marked immediate swelling, and *S. japonicum* eggs did not have a sensitizing effect when given intraperitoneally without Freunds complete adjuvant, as was the case with *S. mansoni* eggs (unpublished data).

It is, however, uncertain whether purified *S. japonicum* eggs still preserve all of their important metabolites or antigenic determinants when they are separated from host tissues. Thus, it has been shown that even in *S. japonicum* infected mice with florid granulomas in their gut and liver, purified eggs injected into the lung do not give rise to granulomas resembling those of natural infection but rather mostly cause only small lesions similar to foreign body reactions. Further research will be needed in this critical area.

There is still a big hiatus from what we know today about the immunopathogenesis of schistosome granuloma formation to what is known about the pathogenesis of severe chronic schistosome lesions in man, such as pipe stem fibrosis, nephropathy, and pulmonary arteritis, but there is now some hope that this gap, as well, will eventually be bridged. Progress in that direction was made by Sadun and colleagues who found that hepatic and renal lesions resembling those in man could be reproduced in the chimpanzee by heavy infections with the Japanese strain of *S. japonicum* (von Lichtenberg *et al.*, 1971). In these apes, pipe stem patterns indistinguishable from those of endemic disease developed quite early, beginning $3\frac{1}{2}$ to 4 months after initial exposure (Figs. 6, 7). These lesions were dose-dependent, and did not require dietary restriction or other manipulations besides schistosome infection itself. Characteristically, pipe stem lesions in the chimpanzee liver began with diffuse inflammation of larger portal fields, and with endophlebitis of portal veins in areas proximal to those of egg deposition, rather than in areas with nume-



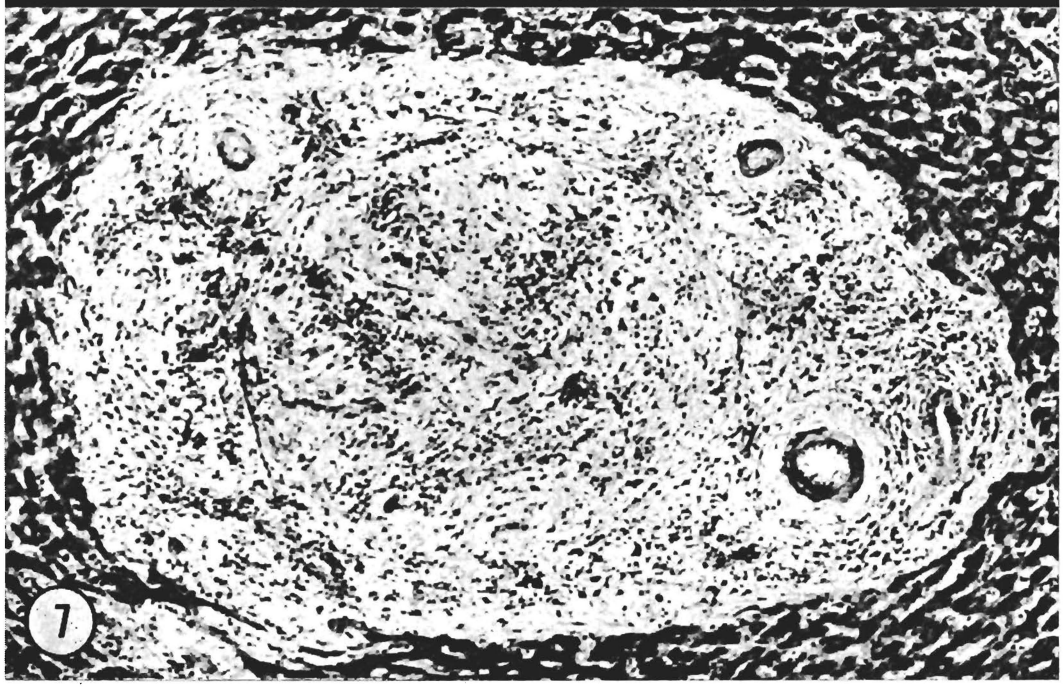


Fig. 6—Established portal liver fibrosis of the classical, broad pipe stem pattern in a chimpanzee heavily infected with *S. japonicum* for 17 months.

Fig. 7—Chimpanzee infected with 2000 cercariae of the Japanese strain of *S. japonicum*, for 2 months. This portal field of the liver (Masson's trichrome, X62) shows the earliest inflammatory precursor lesions of pipe stem fibrosis. Note the diffuse cellular infiltration, edema, plugging and effacement of portal radicle, all seen in a site remote from eggs and granulomas.

rous and confluent granulomas. The nature of this diffuse inflammatory and desmoplastic process which does not occur in small rodent hosts has remained completely unknown. Liver fibrosis in chimpanzees could be prevented by portocaval shunting, whereas this operation did not affect the course of their nephropathy (Sadun *et al.*, 1975).

Experiments in chimpanzees are possible to only few investigators today, and it is therefore fortunate that several workers have reported the development of diffuse large-field portal fibrosis in rabbits heavily infected with *S. japonicum* including glomerular lesions in some of these animals (Tsutsumi, 1971; Jones *et al.*, 1977). Given the relatively good background information available on the rabbit immune system, this host should offer a useful model for studying the pathogenesis of pipe stem fibrosis and for relating it to what is known about granulomatous hypersensitivity. Although little is known as yet about the fate of schistosome antigen, or the deposition of antibody in early, diffuse pipe stem lesions, we have suggested that the conditions under which they appear--high infection intensity and marked host immunological reactivity--suggest a failure of the normal defense mechanisms. Lewert and colleagues have recently postulated that there is activation of clotting mechanisms in the infected rabbit liver by circulating antigen-antibody complexes which might destroy vascular endothelia but clotting could equally well result from the secretion of plasminogen activator by activated macrophages. It is of interest that, in rabbits with pipe stem fibrosis, there has been little evidence of modulation of the granulomatous process, such as is seen in the chronically infected mouse, but this finding is still to be fully evaluated. Because of its potential for analyzing the pathogenesis of pipe stem fibrosis we intend to further develop and study the rabbit model, including the effect of the Mekong schistosome on that host.

The importance of glomerular pathology in endemic schistosomiasis has lately become a matter of dispute. Unlike autopsy data from Egypt, Brazil (Andrade *et al.*, 1971) and the Philippines, epidemiological studies by Lehman *et al.*, (1975) on the incidence of proteinuria in infected populations have shown only limited morbidity attributable to this complication. On the other hand, its research potential for analyzing the immunopathogenesis of schistosomiasis is far from exhausted. There is little doubt that circulating schistosome antigens and/or antigen-antibody complexes play a role in schistosome nephropathy. One of these antigens is a high molecular weight polysaccharide produced in the worm gut but its role in nephropathy is thus far only suggestive (von Lichtenberg *et al.*, 1974). Undoubtedly other antigens of either parasite-or host origin or both are present as well and their precise origins and specificities remain undetermined; moreover, direct demonstration of antigen in the glomerular lesions has not been satisfactorily achieved so as to convince all critical observers.

SCHISTOSOME IMMUNITY

It might seem wasteful to devote much attention to immune protection against the schistosomes in animals, when the existence of human immunity still remains to be convincingly demonstrated. On the other hand, epidemiological data on *S. haematobium* do strongly point in that direction, and there can be little discussion that a human vaccine for the schistosomes, if attainable, would be of great help. The kind of environmental preventive measures that have proven effective in Japan, in parts of China, and in St. Lucia and Puerto Rico are simply not feasible in most of the developing world, and a vaccine would be its best or only hope for speedy progress toward schistosomiasis control.

Inspired by earlier reports of Japanese workers, Vogel and Minning in 1953 pub-

lished a classical series of experiments in macaque monkeys in which they demonstrated self-cure of *S. mansoni* infection and acquisition of resistance to reinfection, but a clear distinction between these two phenomena was first made by Smithers (1962) by showing that even prior to self-cure, these monkeys showed high resistance to cercarial reinfection in the face of persisting, viable adult worms. He later named this phenomenon "concomitant immunity" in analogy with similar findings in host resistance to autologous cancer implants. Smithers and his colleagues next proceeded to show that adult schistosomes can share surface antigens with their hosts, some of which are apparently absorbed from host erythrocytes; thus, they arrived at the postulate that the privileged status of adult worms with respect to host immunity could be the result of their antigenic disguise. By the same token, only the early developmental forms of schistosomula which lack host surface components would be vulnerable to host immune attack.

The hypothesis of host antigen acquisition by schistosomes has stimulated many immunologists to invest a serious scientific effort into the analysis of schistosome immunity and, despite lack of definitive proof, this hypothesis still remains viable today. Excellent reviews of this research by Smithers and Terry (1976), and by Phillips and Colley (1978) are available, as well as the report of a WHO expert committee (1974), and I shall therefore limit myself here to some recent research developments most of which date from 1974 or later, i.e. roughly from the time when work on schistosome immunity in monkeys was largely superseded by work on small animal models and *in vitro* resulting in much accelerated progress in this field.

Our basic model has been the mouse infected with ca. 20-30 cercariae of *S.mansoni* for 10-12 weeks, when its resistance to cercarial challenge reaches a peak. Traditionally, that resistance would be measured by counting adult worms in portal vein perfusates 6 weeks after challenge, and comparing their numbers with the yields of primary infections in normal mice infected at the same time, but Sher has introduced a much more convenient method: that of numbering the schistosomula which emerge from mouse lung snips at 5-6 days after challenge, i.e. at the time when most of the developing parasites have reached the lung, but have not yet migrated on toward the liver. By the use of this method, it could be shown that passive transfer of serum from immune to normal mice did reduce the survival of challenge organisms, albeit not to the levels observed in active immunity. Adoptive transfer of lymphocytes, by contrast, was not successful in mice although Phillips *et al.*, (1977) had shown this to have a small effect in rats when performed at the time when the rats are just beginning to develop their immunity.

The finding that humoral mediators are involved in the immune destruction of schistosomula *in vivo* agrees well with experiments *in vitro* in which it was shown that immune sera of macaques can be lethal for schistosomula even in the absence of cellular elements; however, titers of this lethal antibody failed to correlate with the course of host resistance and it was subsequently shown, in a variety of systems, that more efficient damage to schistosomula could be caused by the cooperation *in vitro* of immune antibodies with a variety of normal host effector cells, including macrophages, neutrophils (W.H.O. 1974) and more recently eosinophils as shown by Butterworth *et, al.*, (1975). Significantly, lymphoid cells were shown to be inactive in all of these systems, some of which are complement-dependent, others independent.

Uncertainty about the relevance of the various *in vitro* systems prompted our group to develop an animal model in which the effector mechanism of schistosome immunity

could be conveniently further analyzed *in vivo*, namely a lung model of immunity in mice somewhat analogous to the egg granuloma model discussed earlier: Cercariae are transformed into schistosomula by penetration of a skin membrane in a test tube, as first described by Clegg and Smithers (1972), and are injected in large numbers into the mouse tail vein, reaching the lung vessels almost immediately. Under these circumstances, cell reaction and attrition of parasites takes place entirely in the lung over the subsequent 5-6 days, and can be quantitatively analyzed by combining histology and lung snip counting of schistosomula (von Lichtenberg *et al.*, 1977).

This model quickly showed us that immune mice differed from normal ones by more extensive and larger cell reactions to challenge parasites, which are enriched with eosinophils and in which schistosomula are killed and converted into typical "residual inflammatory foci". Immune animals also showed much quicker and more efficient killing of schistosomula, especially within the first 24 hours after challenge (Fig. 8). Degranulation of eosinophils against the tegument of somules was seen in the foci of immune mice (Fig. 9), whereas in normal mice neutrophils predominated in these reactions. These findings complemented the results of Mahmoud *et al.*, (1975), who showed that depletion of eosinophils by specific antisera in actively or passively immunized mice substantially impaired their resistance to challenge, and Butterworth's (1975) discovery that, in his ADCC assay, eosinophils are likewise the most efficient killer cells.

In a subsequent analysis Sher (1977) has further defined the cellular and humoral requirements of schistosome immunity in mice briefly, functional T-cells are required for the effector antibodies to be formed, and it thus appears that their helper activity is essential. Transfer of immune immunoglobulin is effective only within 30 hours after challenge indicating that the schistosomula rapidly lose their susceptibility to it. While the antibodies were shown not to be cytophilic, they do require the presence of normal bone marrow elements for their killing activity since their effect is lost in mice depleted by whole body radiation (650 R) and is regained after injection of syngeneic bone marrow cells. In all these respects, the *in vivo* model has behaved similarly to an antibody dependent-cell mediated cytotoxic reaction (ADCC) *in vitro*.

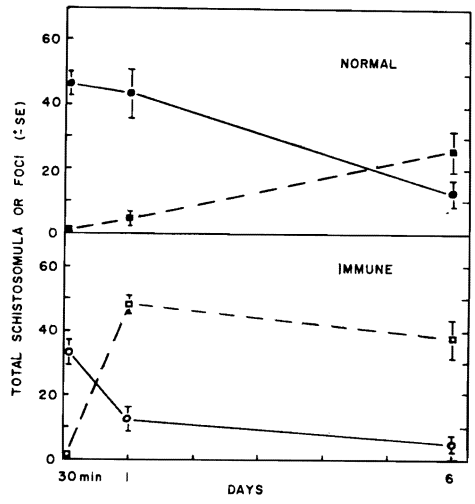


Fig. 8 — Kinetics of schistosomula attrition during intravenous challenge of normal C57BL/6J mice versus mice immunized by a 30-cercaria *S. mansoni* infection. Solid lines = counts of lung schistosomula; broken lines = number of residual inflammatory foci in the lung at each of the intervals shown. Note the reciprocal relationship between these counts, and the marked acceleration of attrition during the first 24 hours seen in the immune mice.

Somewhat surprisingly, it was also shown in these experiments that killing of schistosomula requires the presence of the monoamine 5-hydroxy-tryptamine (Serotonin). Killing was reproducibly inhibited by large doses of reserpine which depletes mast- and other host cells of monoamines, and could be restored by pargyline, a monoamine-oxdase inhibitor. These experiments suggested that more than

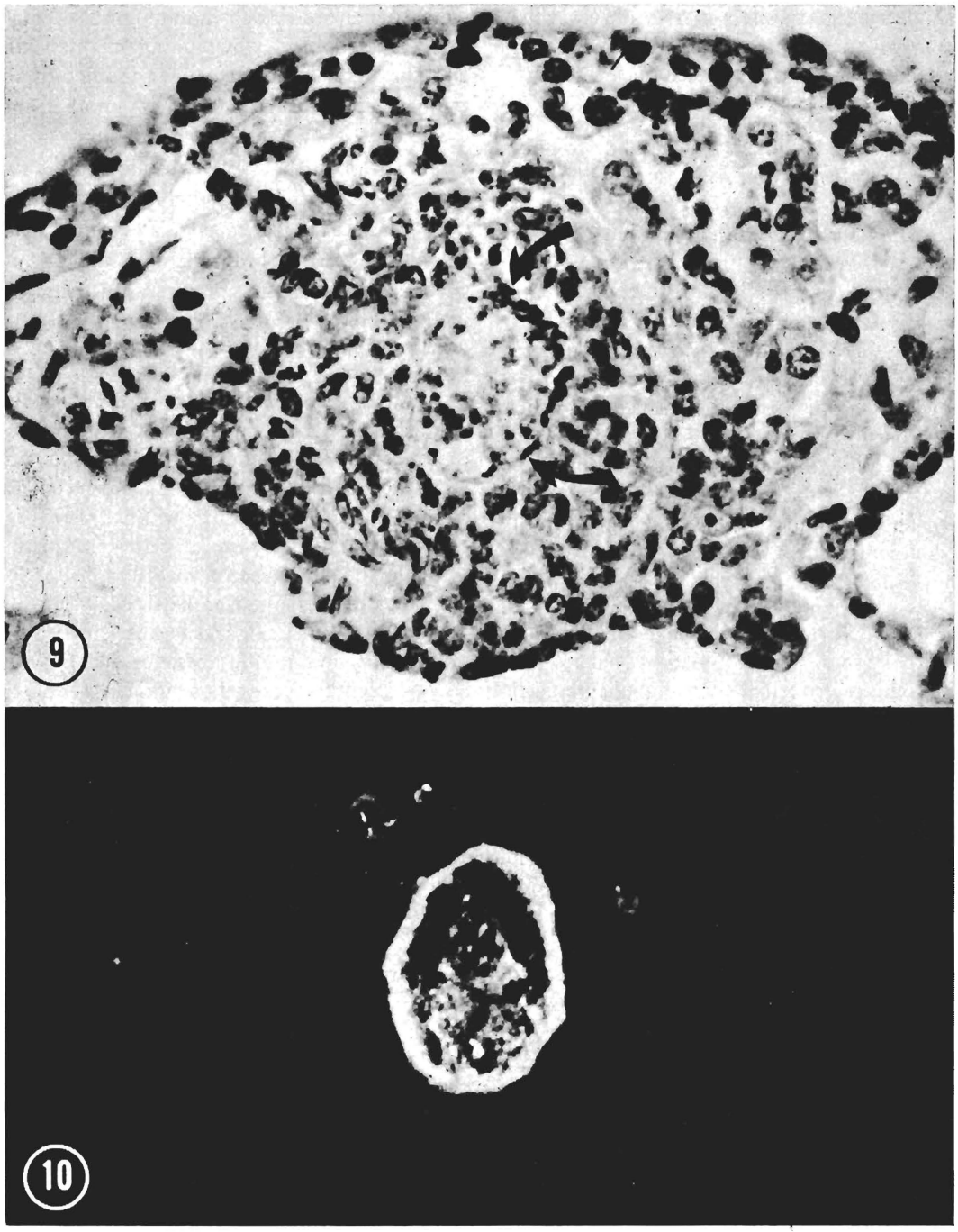


Fig. 9—Typical "killing focus" in the lung of an immune C57BL/6J mouse challenged intravenously with schistosomula of *S. mansoni*, at 24 hours. The tegument of the still intact somule (Arrows) is surrounded by a corona of degranulating and degenerating eosinophils; eosinophils are also mixed with other inflammatory cells in the outer portions of the focus. Dominici stain, X700.

Fig. 10—Cryostat section of lung of an immune C57BL/6J mouse immunized with 30 cercariae of *S. mansoni* and challenged with intravenous somules, at 1 hour. Indirect immunofluorescent stain for IgG_{2A}. Note the marked tegumental fluorescence.

a single mediator system is involved in schistosome immunity in the intact animal, rather than merely the ADCC mechanism effective by itself *in vitro*.

Recently, Drs. Jim Byram and Eyo Imohiosen in our laboratory have shown that schistosomula, one hour after their injection into immune mouse lungs, are covered by immunoglobulins of the subclasses IgG_{2A}, IgG_{2B} and IgA, the first of these being the most prominent (Fig.10). By 24 hours, this antibody coating had largely disappeared (unpublished data). By contrast, C_3 can be shown to be present on the surface of 1-hour lung schistosomula both in normal and in immune mice, thus confirming a similar observation previously made *in vitro* by Sher (1977) that somules possess an activator of C_3 on their surface which acts by the alternate complement pathway.

These latest observations have led us to the hypothesis that the inflammation seen around lung somules in normal mice might be induced by complement activation, whereas the accelerated and augmented reactions in immune mice which are also eosinophil-enriched might be triggered initially by binding or complexing to the parasite surface of antibodies of the subclasses cited, in concert with another, monoamine-dependent mediator system of inflammation. I believe we are now approaching very close to a reasonable hypothesis of the immune effector mechanism against schistosomes *in vivo*, but much more work remains to be done to confirm and expand our observations before we can be absolutely sure.

With respect to the mechanism by which schistosomula--in much reduced numbers--can escape host immune mechanisms and survive to their adult stage, some recent experiments of Imohiosen and Sher (1977) are of interest. They were able to confirm that during the time when schistosomula inhabit the lung of normal mice, they cease expressing their initial surface parasite antigens demonstrable by immunofluorescence and progressively begin to express host antigens (Table 1); the same effect can be demonstrated when schistosomula are cultured *in vitro* for similar

Table 1

On each of the days indicated, schistosomula injected intravenously into normal mice were recovered by the lung snip method, and were stained by the indirect immunofluorescent technique for parasite (upper row) and host (lower row) antigenic determinants. For each sample, 10 random somules were arbitrarily scored for fluorescence intensity (0, 1+, 2+, 3+, 4+,), and the mean score determined.

Staining Reagents	Length of somule residence in mouse lung.								
	0DY*	1DY	2DY	3DY	4DY	5DY	7DY	9DY	12DY
Immune Mouse Serum + Fluorescein-Labelled Rabbit Anti-Mouse IgG	3.7	1.1	0.25	0.2	0.05	0.2	0	0	0
Rabbit Anti-Mouse Red Blood Cell Serum + Fluorescein-Labelled Goat Anti-Rabbit IgG	0	1.1	2.1	2.2	2.4	2.8	3.0	2.6	3.0

* Schistosomula freshly prepared *in vitro*.

periods of time in the presence of host serum and red cells, but not with serum alone. Likewise, the motility of somules which is low immediately after lung injection, progressively increases and reaches its maximum 2 days later; the same increase in motility can be promoted by simply culturing the somules for two days *in vitro;* thus, the described changes in motility and in surface determinants seem to be functions of an adaptational development which could be important for parasite survival. Indeed, when cultured 2-day old schistosomula are injected into the immune mouse lung, their attrition is substantially reduced compared to that of freshly prepared schistosomula, and the cell reactions to them are substantially diminished; both are now comparable to the results observed when fresh schistosomula are injected into normal mice (von Lichtenberg *et al.*, 1977). I should hasten to add that these observations do not constitute any formal proof of the antigen-acquisition hypothesis of Smithers, although they are compatible with it. Other changes which these parasites undergo during their development might be equally important as those of motility, or rate of tegumental turnover (Dean, 1977). We can only hope that further analysis of our model will further define these factors, and that the observations we are now making in the lung model can be validated by comparing them with events during the natural skin route of infection in mice.

Have these studies on the mechanisms of schistosome immunity brought us any nearer to the development of a vaccine? Only the future can tell. Certainly, they have taught us a great deal both about the biology of the parasite and the immunological reactions of the host which was not known before, and I do not regret our involvement in this research. In fact, if this quick and superficial summary of many years of effort in different laboratories has transmitted to you a taste of the intellectual excitement of pursuing the immunopathology of parasitic disease, I shall consider my task as well fulfilled.

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