[Jap. J. Parasit., Vol. 20, No. 1, 40-44, 1971]

Schistosomiasis Japonica: Models for the Pathogenesis of Hepatosplenic, Intestinal and Cerebral Disease

KENNETH S. WARREN*

Departments of Preventive Medicine and Medicine, Case Western Reserve University and University Hospitals, Cleveland, Ohio, U.S.A.

(Received for publication; December 5, 1970)

Animal models have played an important role in the elucidation of the pathogenesis of the various disease syndromes associated with *Schistosoma mansoni* infection (Warren, 1964a; Warren, 1968). Studies of a murine model of hepatosplenic disease have demonstrated that the schistosome egg, rather than toxic worm products or dead adult worms, is the major parasite factor responsible for the occurrence of this syndrome (Warren, 1964a; Warren, 1968). The host inflammatory and fibrotic reaction to the eggs (granuloma or pseudotubercle) has been shown to be a necessary concomitant (Warren, 1968), and this host response has been found to be an immunologic reaction of the delayed hypersensitivity type (Warren *et al.*, 1967). Models of intestinal (Domingo and Warren, 1969) and pulmonary schistosomiasis (Warren, 1946) have been developed and the same factors—the schistosome egg and the host granulomatous reaction to it—have been implicated as the major pathogenetic mechanisms.

Although similar investigations with schistosomiasis japonica might appear to be redundant, there are major differences between *S. mansoni* and *S. japonicum* with respect to both the eggs and the host responses to them (Warren and Domingo, 1970). The *S. japonicum* eggs are smaller, spherical rather than ellipsoidal in shape, and essentially have no spine; their daily rate of production is approximately 10 times that of *S. mansoni*. In addition, while the latter eggs are produced individually, *S. japonicum* eggs are laid in large aggregates. These eggs frequently calcify while those of *S. mansoni* do so only rarely. The "Hoeppli phenomenon", an eosinophilic material found around the eggs embedded in host tissues, which appears to be an antigen-antibody complex, tends to occur in experimental animals more frequently around *S. japonicum* than *S. mansoni* eggs.

With respect to the host granulomatous reaction, it was believed that "the eggs of *S. japonicum* usually produce more severe lesions than those of *S. mansoni* and *S. haematobium*, partly because each fertilized egg seems to exert a more acute toxic effect on the surrounding tissue" (Meleney *et al.*, 1953). The fact that the *S. japonicum* eggs are laid in bunches not only in mice but also in dogs, monkeys and man, has made it relatively impossible to compare the host reaction to these eggs with that to the other species of eggs (Warren and Domingo, 1970). Using the von Lichtenberg method in which eggs were isolated from the livers of infected mice and injected into the pulmonary microvasculature of uninfected animals, we have demonstrated recently that the granulomatous response to individual *S. japonicum* eggs is far less than that to *S. mansoni*

* At present on sabbatical leave as Visiting Professor at the London School of Hygiene and Tropical Medicine and the Royal Free Hospital, London, England.

The investigations reported herein were supported by grants from the United States-Japan Co-operative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases of the Department of Health, Education and Welfare, The Rockefeller Foundation and the Commission on Parasitic Diseases, Armed Forces Epidemiological Board.

or *S. haematobium* eggs (Warren and Domingo, 1970). In addition, secondary exposure to *S. japonicum* eggs did not lead to an anamnestic reaction as it did with those of the other species (Warren and Domingo, 1970). These factors, plus the time scale of the development of the lesions, tend to indicate that the reaction around single *S. japonicum* eggs is more a foreign body than an immunologic response (Kellermeyer and Warren, 1970). It is possible, however, that an immunologic reaction does occur when the eggs are deposited in bunches. Another factor which may play a role in the pathogenesis of schistosomiasis is the length of time in which the eggs remain in the tissues. Those of *S. japonicum* appear to be destroyed more rapidly than *S. mansoni* eggs despite the fact that sensitization, which accelerates egg destruction, does not occur with the former (Warren and Domingo, 1970). Since the inflammatory and fibrotic host reaction to the schistosome eggs is such an important factor in the pathogenesis of the various disease states associated with schistosomiasis, fundamental differences in the etiology of the reactions may result in disease processes that are basically different.

Initial experiments with models of hepatosplenic schistosomiasis japonica appeared to confirm the prevalent view, based primarily on the egg output, that schistosomiasis japonica was far more severe than schistosomiasis mansoni (Warren and Moore, 1966; Moore and Warren, 1967). At first, mice with fairly heavy *S. japonicum* infections were studied in the acute stages of infection, revealing that the severity of hepatosplenic disease was comparable to that induced by six times as many pairs of *S. mansoni* worms (Warren and Moore, 1966). Subsequently, a direct comparison was made between schistosomiasis japonica and mansoni (again in the acute stage of infection—until the 12th week) in that all of the animals harbored only one worm pair (Moore and Warren, 1967). Seven to ten times as many *S. japonicum* eggs were found in the livers at each time period studied; concomitantly the livers and spleens were larger, portal pressures were higher, there was a greater percentage of esophageal varices, and hematocrits were lower than in the *S. mansoni* infected mice.

An unexpected opportunity was then afforded to examine long term *S. japonicum* infections in the course of a study of the pathogenic capacities of four different geographic strains (Philippine, Formosan, Japanese and Chinese) (Warren and Berry, in preparation), similar to an investigation previously performed in our laboratories with *S. mansoni* (Warren, 1967). Although Hsu and Hsu (1960) have previously studied the pathogenicity of *S. japonicum* strains they exposed mice to large numbers of cercariae and recorded only mortality. In our investigations each mouse harbored only 1 worm pair, and the following parameters were measured: body, liver and spleen weights, portal pressure, liver histology and egg counts, esophageal varices and hematocrit (Warren and Berry, in preparation). On the assumption that mortality would be high, it was originally planned to study the animals at 8, 10 and 12 weeks. Two factors, however, enabled us to study the disease for much longer periods (20 and 40 weeks): a high infection rate, and an exceedingly low mortality.

With respect to the severity of hepatosplenic disease, the Philippine strain was most pathogenic, followed by the Formosan, Japanese and Chinese strains, the latter of which caused only mild disease (Warren and Berry, in preparation). For instance, peak portal pressure with the Philippine strain was 135% above normal as compared to 35% with the Chinese strain. At one point 50% of the mice infected with the former strain had esophageal varices, while no mice infected with the latter strain ever showed this abnormality. The animals infected with the more pathogenic species tended to have more eggs in their livers. Although vast numbers of eggs were found in the livers of the animals infected for 40 weeks (the Formosan strain averaged 103,000 per liver) inflammatory reaction was relatively slight.

Periportal inflammation accompanied by some fibrosis was seen only in animals infected with the Japanese strain.

During the course of the above strain study, intestinal obstruction was observed in 80% of mice infected for 40 weeks with the Formosan strain and in 60% of those infected with the Philippine strain (Warren, 1969). When examined at 36 weeks, none of the animals infected with the less virulent Chinese strain showed gross signs of obstruction (Warren and Berry, in preparation). A single site of obstruction was found in 7 of 8 mice infected with the Formosan strain while 2 sites were seen in 4 of 6 mice infected with the Philippine strain. Of the 19 sites of obstruction, 13 were in the upper half of the small intestine, 2 at about the midpoint, three in the terminal ileum and one in the rectum. The cause of the obstruction was a localized thickening of the intestinal wall due to masses of parasite eggs and the host inflammatory reaction to them.

Gross intestinal obstruction has not been observed in long term *S. mansoni* infections in experimental models (Warren, 1966). While a facile explanation for this phenomenon would be the far greater egg output of *S. japonicum* females, another factor appears to have been operative : *S. japonicum* worms seem to remain in one or only a few locations, depositing large numbers of eggs in circumscribed areas. In contrast, studies of intestinal schistosomiasis mansoni have revealed a relatively even distribution of eggs throughout the small bowel (Domingo and Warren, 1969).

This tendency of *S. japonicum* worms to remain in one location provides a clue to the cause of the relatively high incidence (2–4%) of cerebral schistosomiasis japonica. In most cases, at surgery or autopsy a large localized mass of eggs is found in the cerebral veins, although worm pairs have never been observed in the brain. Recently, we have attempted to develop an animal model of this syndrome using a Japanese strain of *S. japonicum* and African green monkeys (*Cercopithecus aethiops*) (Jane *et al.*, 1970). In order to examine the different theories of the etiology of cerebral schistosomiasis japonica—entry of schistosomulae directly into the brain through emissary veins, worm or egg emboli, or egg-laying worm pairs in the cerebral veins—20 monkeys were treated in the following manner : 1) cercariae were placed on the shaved heads of 2 monkeys ; 2) viable worm pairs were injected into the carotid artery of 2 monkeys ; 3) eggs or plastic beads similar in size were injected into the carotid artery of 10 monkeys ; and 4) viable worm pairs were placed proximal to a ligated jugular vein or within the sagittal sinus of 6 monkeys. No neurological symptomatology was noted with most of these methods with the exception of the group which was given a second injection of massive numbers of schistosome eggs ; this led to right hemiparesis or decerebrate rigidity in 4 animals.

With respect to the lack of results with worm pairs in the venous circulation of the brain, it was not surprising that no effect was observed after cranial exposure to cercariae as the schistosomulae seemed to go through their usual cycle, passing through the lungs and terminating in the mesenteric vessels. In the studies in which worms were transplanted into the cerebral veins not only were there no neurological changes, but no worm pairs were found in the brain at postmortem even though the animals were throttled. Recent studies by Smithers' group have shown, however, that transplantation of worms from different host species (in this case, mouse to monkey) results either in prolonged debility or in the death of the parasite (Smithers and Terry, 1969). Future such studies should involve transplantation within the same primate species. With respect to injections of eggs or beads, no neurological change followed a single burst of as many as 50,000 eggs or 85,000 beads. It seems, therefore, that the brain can tolerate vast numbers of microemboli. Although the above investigation did not solve the question of the pathogenesis of cerebral schistosomiasis japonica, it is the first such study published and provides techniques and suggests path-

ways which might elucidate the problem.

Most English language textbooks of clinical parasitology (Faust and Russell, 1964; Belding, 1965), tropical medicine (Manson-Bahr, 1960), medicine (Brown, 1967) and liver disease (Sherlock, 1968) make little or no distinction between schistosomiasis japonica and mansoni with regard to pathogenesis and clinical disease, the only exception being a greater severity ascribed to *S. japonicum* infection because of higher egg output. In fact one major textbook states, "The lesions produced in infection with *Schistosoma japonicum*, as well as their sequence of development are practically the same as those in *S. mansoni* and *S. haematobium* infection" (Faust and Russell, 1964). For the last 5 years we have been developing animal models of schistosomiasis japonica to complement our 15 years of experience with schistosomiasis mansoni, and we have been struck with great potential differences in the disease processes.

Patients exposed to *S. japonicum* cercariae are more likely to develop heavy infections, despite the low output of cercariae by *Oncomelania* spp., for the following reasons: layering of the cercariae on a 2-dimensional surface rather than throughout a 3-dimensional volume of water, very rapid penetration of the skin, and higher percentage of adult worm maturation. The greater efficiency of this organism in infecting its definitive host may account for the rarity of swimmer's itch and relative frequency of Katayama fever.

With respect to the development of chronic disease, the Asian parasite was considered to cause more severe reactions for two reasons: one, it produced more eggs, and two, the host reacted to the eggs more violently. The first reason may be a gross over-simplification, the second one may be untrue. Although large numbers of eggs are produced by *S. japonicum*, they are smaller in size, are laid in large aggregates and individually cause less inflammation than *S. mansoni* eggs. Thus, with respect to their effect on circulation through an organ, each group of *S. japonicum* eggs may have little more effect than a single *S. mansoni* egg. Furthermore, the different types and degrees of host granulomatous reactivity to the *S. japonicum* eggs may result in less residual fibrosis.

It may be suggested, therefore, that it is the patients with very heavy *S. japonicum* burdens, which probably occur more frequently than in schistosomiasis mansoni, which tend to develop obstruction to hepatic blood flow. Under these circumstances vast numbers of eggs may in themselves contribute significantly to blockage of the hepatic circulation and even produce a significant degree of parenchymal destruction. Another major difference between *S. mansoni* and *S. japonicum* of pathogenetic importance is the recent observation that while *S. mansoni* worms appear to migrate about as they deposit their eggs, those of *S. japonicum* have a tendency to remain localized. This may explain the greater prevalence of intestinal obstruction and central nervous system disease in patients infected with the Asian organism.

Thus the comparative study of schistosomiasis mansoni and japonica in animal models has revealed differences between the organisms which indicate that the pathogenesis of these diseases may be quite different. In the past the belief that the disease processes were virtually identical may have obscured some important clinical differences between schistosomiasis japonica and mansoni.

References

1) Belding, D. L. (1965): Textbook of Parasitology, 3rd Edition, Appleton Century Crofts, New York.
2) Brown, H. W. (1967): Schistosomiasis, in Cecil-Loeb Textbook of Medicine, Edited by Beeson, P. B. and McDermott, W., 12th Edition, W. B. Saunders Co., Philadelphia and London.
3) Domingo, E. O. and Warren, K. S. (1969): Pathology and pathophysiology of the small intestine in murine schistosomiasis mansoni, including a review of the literature. Gastroenterology, 56, 231-240.
4) Faust, E. C. and Russell, P. F. (1964): Craig

and Faust's Clinical Parasitology, 7th Edition, Lea & Febiger, Philadelphia.
5) Hsu, S. Y. L. and Hsu, H. F. (1960): On the virulence of the geographic strains of *Schistosoma japonicum*. Am. J. Trop. Med. Hyg., 9, 195-198.
6) Jane, J. A., Warren, K. S. and van der Noort, S. (1970): Experimental cerebral schistosomiasis japonica in primates: J. Neur. Neurosurg. Psych., 33, 426-430.
7) Kellermeyer, R. W. and Warren, K. S. (1970): The role of chemical mediators in the inflammatory response induced by foreign bodies: Comparison with the schistosome egg granuloma. J. Exp. Med., 131, 21-39.
8) Manson-Bahr, P. H. (1960): Manson's Tropical Diseases, 15th Edition, Cassell, London.
9) Meleney, H. E., Sandground, J. H., Moore, D. V., Most, H. and Carney, B. H. (1953): The histopathology of experimental schistosomiasis. II. Bisexual infections with *S. mansoni*, *S. japonicum* and *S. haematobium*. Am. J. Trop. Med. Hyg., 2, 883-913.
10) Moore, D. E. and Warren, K. S. (1967): Hepatosplenic schistosomiasis mansoni and japonica compared in mice each infected with one pair of worms. Trans. Roy. Soc. Trop. Med. Hyg., 61, 104-109.
11) Sherlock, S. (1968): Diseases of the Liver, 4th Edition, Blackwell Scientific Publications.
12) Smithers, S. R. and Terry, R. J. (1969): Immunity in schistosomiasis. Ann. N. Y. Acad. Sci., 160, 826-840.
13) Warren, K. S. (1964): The correlation between experimental and human infection with *Schistosoma mansoni*. Nature, 201, 899-901.
14) Warren, K. S. (1964): Experimental pulmonary schistosomiasis. Trans. Roy. Soc. Trop. Med. Hyg., 58, 228-233.
15) Warren, K. S. (1966): The pathogenesis of "clay-pipe stem cirrhosis" in mice with chronic schistosomiasis mansoni with a note on the longevity of the schistosomes. Am. J. Path., 49, 477-489.
16) Warren, K. S. (1967): A comparison of Puerto Rican, Brazilian, Egyptian and Tanzanian strains of *Schistosoma mansoni* in mice: Penetration of cercariae, maturation of schistosomes and production of liver disease. Trans. Roy. Soc. Trop. Med. Hyg., 61, 795-802.
17) Warren, K. S. (1968): Pathophysiology and pathogenesis of hepatosplenic schistosomiasis mansoni. Bull. N. Y. Acad. Med., 44, 280-294.
18) Warren, K. S. (1969): Intestinal obstruction in murine schistosomiasis japonica. Gastroenterology, 57, 697-702.
19) Warren, K. S. and Moore, D. E. (1966): Murine hepatosplenic schistosomiasis japonica. Am. J. Trop. Med. Hyg., 15, 22-27.
20) Warren, K. S. and Domingo, E. O. (1970): Granuloma formation around *Schistosoma mansoni*, *S. haematobium* and *S. japonicum* eggs in unsensitized and sensitized mice: Size and rate of development, cellular composition, cross-reactivity and rate of egg destruction. Am. J. Trop. Med. Hyg., 19, 292-304.
21) Warren, K. S., Domingo, E. O. and Cowan, R. B. T. (1967): Granuloma formation around schistosome eggs as a manifestation of delayed hypersensitivity. Am. J. Path., 51, 735-756.
22) Warren, K. S. and Berry, E. G., A comparison of hepatosplenic schistosomiasis in mice each infected with one pair of the Philippine, Formosan, Japanese or Chinese strain of *Schistosoma japonicum*, in preparation.