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Characterization of the murine model of schistosomal hepatic periportal fibrosis ('pipestem' fibrosis)

ZILTON A. ANDRADE* AND ALLEN W. CHEEVER†

*Gonçalo Moniz Research Center (FIOCRUZ), 41.945 Salvador, Bahia, Brazil and

†Laboratory of Parasitic Diseases, NIAID, National Institutes of Health, Bethesda, MD 20892, USA

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Summary. During mild (one to two pairs of worms) and prolonged (23 weeks or more) mouse infections with *Schistosoma mansoni*, but not with *S. japonicum*, periovular granulomas and fibrosis were seen to be preferentially located along periportal tissues. This caused fibrotic expansion of the portal spaces on a background of normal-looking hepatic parenchyma, a picture mimicking 'clay pipestem fibrosis' seen in human patients with advanced schistosomiasis. The model was reproduced in outbred and in several strains of inbred mice, and their main characteristics were studied and compared to the human counterpart. A balanced consideration of the similarities and differences between the murine model and human pipestem fibrosis is needed for the adequate utilization of this simple, reproducible and inexpensive experimental model.

Keywords: pipestem fibrosis, experimental schistosomiasis, schistosomal granuloma, *Schistosoma mansoni*, *S. japonicum*

Clay pipestem fibrosis (Symmers 1904) is the characteristic hepatic lesion of advanced schistosomiasis of man. Its experimental reproduction has been achieved in chimpanzees heavily infected with either *Schistosoma mansoni* (Sadun *et al.* 1970) or *Schistosoma japonicum* (Lichtenberg *et al.* 1971). Rabbits infected with *S. japonicum* (Cheever *et al.* 1980), but not with *S. mansoni* (Andrade *et al.* 1988), also develop extensive portal fibrosis resembling pipestem fibrosis. A more practical and workable model was described in the mouse by Warren (1966) and later confirmed by Andrade (1987). However, before the murine model can be more extensively utilized to answer questions concerning advanced hepatic schistosomiasis, details about its reproducibility and main histological features still need to be clarified.

Correspondence: Allen W. Cheever, Laboratory of Parasitic Diseases, Building 4, Room 126, National Institutes of Health, Bethesda, Maryland 20892-USA.

During a mild and prolonged *S. mansoni* mouse infection, the intrahepatic portal vein system is altered in such a way that the parasite eggs are preferentially deposited along the periportal tissues (Andrade 1987). Current laboratory infections, in which 50–120 or even 150 cercariae are used to infect a mouse, usually do not reproduce the model, since a complex distortion of the portal vasculature then occurs, killing the host before a systematized portal fibrosis can develop. Cheever (1969) estimated that the heaviest infections reported in man at autopsy are seldom greater than five worm-pairs per kilogram of body weight, whereas in mice the lightest infections are in the order of 50 worm-pairs per kilogram of body weight. Warren (1966) called attention to two fundamental aspects observed in mice with systematized periportal fibrosis: the animals harboured few worm-pairs and the duration of infection was longer than 16 weeks.

It has been suggested that a model of periportal fibrosis can be obtained at will in the mouse, once a mild (one to three worm-pairs) and chronic (at least 16-week duration) *S. mansoni* infection is produced (Andrade 1987). The present investigation concerns the pathology of prolonged and mild *S. mansoni* and *S. japonicum* infections in mice to define better the murine model of schistosomal pipestem fibrosis.

Material and methods

Outbred albino mice of both sexes, weighing 18–20 g, fed on a commercial balanced diet and water *ad libitum*, were infected at the same time with 15–20 freshly eliminated *S. mansoni* cercariae by the transcutaneous route. The portal system was perfused for the recovery of adult worms by the method of Duvall and DeWitt (1967). An estimation of the number of eggs per gram of liver was made according to Cheever (1970). Fresh pieces of liver and intestines were compressed between glass slides and examined under the microscope for the identification of ovular stages (oogram). Two fragments of each liver were fixed either in buffered 10% formalin, pH 7.4, or in Bouin's fluid, embedded in paraffin and sectioned at 5 μm to give an area of about 2 cm^2. Fragments of the spleen and kidneys were also included. Sections were stained with haematoxylin and eosin, Masson's trichrome, Weigert–Van Gieson and picrosirius red methods. Picrosirius stained slides were examined with and without polarizing light (Junqueira *et al.* 1979).

Histological liver sections were also examined from the following groups of inbred mice: C3H/HeN male mice exposed to 16 *S. mansoni* cercariae (PR-1 Puerto Rican strain) (Richards 1977) subcutaneously and examined 22, 41 and 52 weeks later; BALB/cAnN male mice exposed to 30 *S. mansoni* cercariae subcutaneously (PR-1 strain) and examined 52 or 59 weeks later; 31 C57BL/6N female mice were infected by surgical implantation of one female and one male worm into a mesenteric venule using the NMRI Puerto Rican strain of *S. mansoni* (Stirewalt & Uy 1969) or the Lowell Philippine strain of *S. japonicum* (Cheever & Duvall 1987) and examined 20–54 weeks later.

Results

Parasitological findings

The exposure of outbred mice to a small number of cercariae resulted in failure to identify eggs in the stools of 31 out of 78 animals exposed to cercariae. Of these 31 negative mice, three presented foci of dark pigmentation seen in compression preparations (probably unisexual infections). Another exhibited pigmentation and a few involuting periovular granulomas, but no worms. Twenty-seven animals were not infected, with stool examination, perfusion of the portal system and smashed preparations all yielding negative results.

The numbers of worm-pairs recovered from the portal system of the 43 animals with positive stools generally varied from one to three but five worm-pairs were recovered from one mouse. Infection intensity in C3H and BALB/c mice was one to three worm-pairs and averaged 1.0–1.4 worm-pairs while all C57BL/6 mice were infected with a single pair of worms. Table 1 contains data on number of eggs in the livers. There was no correlation between the degree of portal fibrosis and the number of eggs. Oograms revealed active infection in all animals.

Gross findings

The livers of the infected animals were dark and firm with

Table 1. Summary of findings in *S. mansoni* infected mice

Group	Mouse strain	Weeks infected	Pipestem 0	Pipestem 1+	Pipestem 2+	Pipestem 3+	Healed	Eggs/liver (1000s)	Spleen wt. (mg)	Number of infected mice
1	Outbred	23	20	0	21	6	3	30	NA*	50
2	C57BL/6	20	7	6	0	0	1	24	249	14
3	C57BL/6	52	7	8	8	5	2	62	217	30
4	BALB/c	52	1	4	4	1	0	50	225	10
5	BALB/c	69	3	6	3	1	0	74	265	13
6	C3H/HeN	22	3	4	2	0	0	25	336	9
7	C3H/HeN	41	2	3	3	1	2	27	407	11
8	C3H/HeN	52	0	3	2	0	0	46	420	5

* NA Data not available.

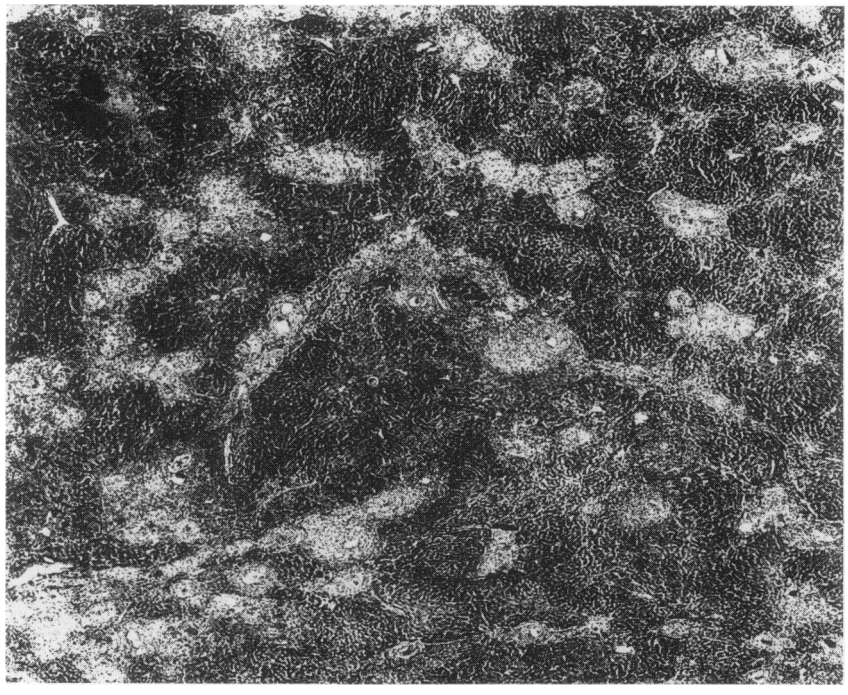

Figure 1. Low power view of murine *pipestem fibrosis*. Periovular granulomas are concentrated along the portal spaces. Liver parenchyma maintains its normal architecture. All photomicrographs are from the livers of C57BL/6 mice infected for 1 year with a single worm-pair. Masson's trichrome stain. × 25.

an uneven external surface showing depressed areas alternating with protruding nodules. The liver capsule was thin, transparent and distended. There were whitish zones of variable sizes and shapes seen on the cut surface, which could be better appreciated after fixation, but the picture did not resemble pipestem fibrosis of humans.

Microscopic findings

Schistosome granulomas tended to be concentrated in portal spaces, enlarging them with fibrosis and chronic inflammation, vascular and ductular proliferation and frequently connecting them with other portal spaces. The expansion of the portal spaces was evident at low magnification (Figure 1), especially when picrosirius stained slides were examined under polarized light (Figure 2). Diffuse periportal fibrosis, not directly related to granulomas or eggs, was minimal or moderate (Figure 3). Fibrosis usually extended beyond the portal area, forming thin, long intraparenchymal septa which sometimes connected with other portal spaces or central veins. Periovular granulomas were usually absent from septal fibrosis. Telangiectatic blood vessels and some dilated lymphatics appeared within and around the portal fibrosis. Focal bile duct proliferation and secretory hyperplasia of biliary epithelium were frequently observed (Figure 4). The main portal vein branch of a given portal space presented irregular subendothelial fibrous thickening and narrowing. Thrombosis with partial organization was seen rarely. Destruction of the muscular coat of the portal vein was also rare. Proliferation and dispersion of smooth muscle cells and increase of elastic tissue were practically absent. There was no correlation between the degree of fibrosis and the number of eggs counted (Table 1). The fibrosis was not systematized, i.e. not all portal branches were affected. In one case, considered as 3+, there was an advanced pipestem appearance in one section, while only isolated granulomas were seen in another section from the same liver. Sometimes massive deposition of eggs and fibrosis formed focal lesions that had no pipestem characteristics. In some cases such focal lesions formed beneath the liver capsule.

The cases not considered to have pipestem fibrosis presented isolated periovular granulomas with a tendency to a periportal distribution, but these granulomas were scattered and did not fuse (Figure 5). In some cases the area of portal fibrosis appeared shrunken and pigmented, the collagen fibres being fragmented, distorted and of variable thickness. In such involuting areas of fibrosis, periovular granulomas were small and rarely contained an egg with well preserved mature miracidium (Figure 6).

In all cases, with or without pipestem fibrosis, the liver parenchyma maintained its normal acinar architecture.

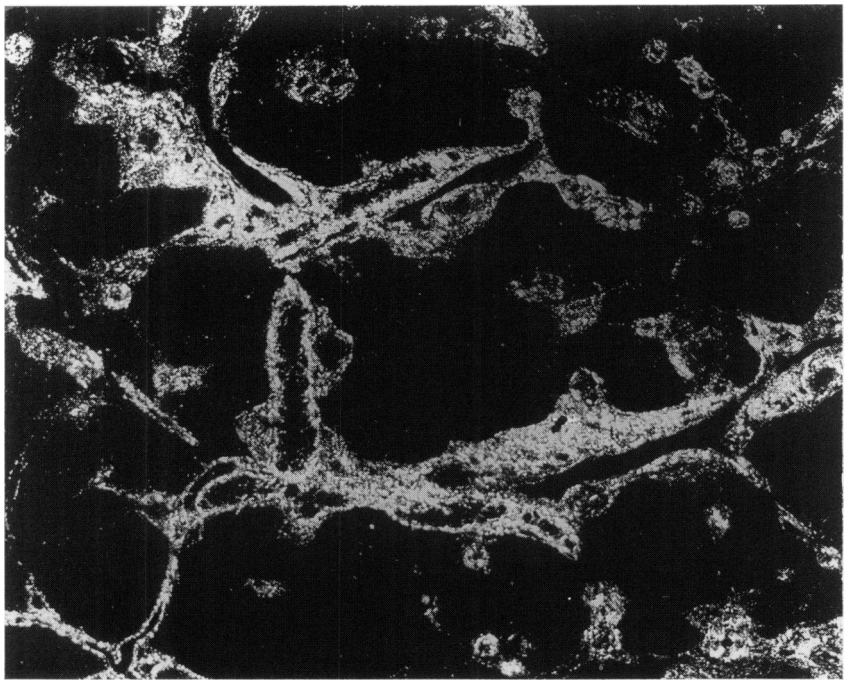

Figure 2. The fibrosis in the enlarged portal spaces exhibits white collagen against a dark background of unstained hepatic parenchyma under polarized light. Picrosirius-red method. × 50.

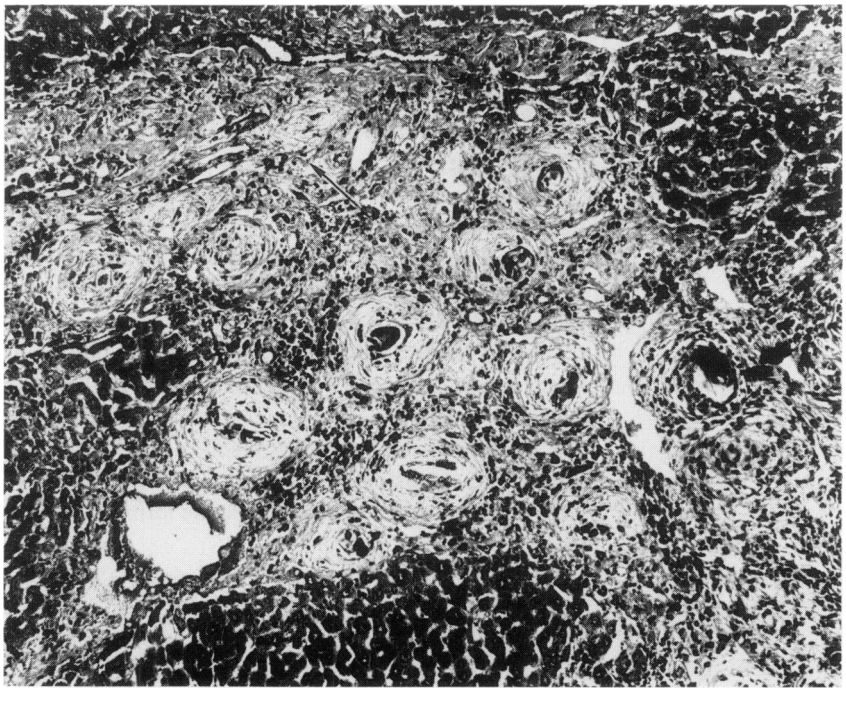

Figure 3. The fibrosis in the enlarged portal spaces is formed mainly by the periovular granulomas, but there is also a component of non-granulomatous fibrosis (arrows). Masson's trichrome stain. × 100.

Only in two cases, both with marked portal fibrosis, were focal areas of nodular hyperplasia noted. Pigment was present in all cases inside sinusoidal cells, within macrophages in granulomas, and free in the interstitial tissue. Increased pigmentation and shrinkage of periportal fibrosis were almost always present and were predominant in some cases. In two 'healed' cases only minimal pigmented scars containing fragments of egg shells were present in an otherwise normal liver. Involuting changes were seen in isolated granulomas also. Fresh and involuting granulomas could be seen side by side (Figure 7).

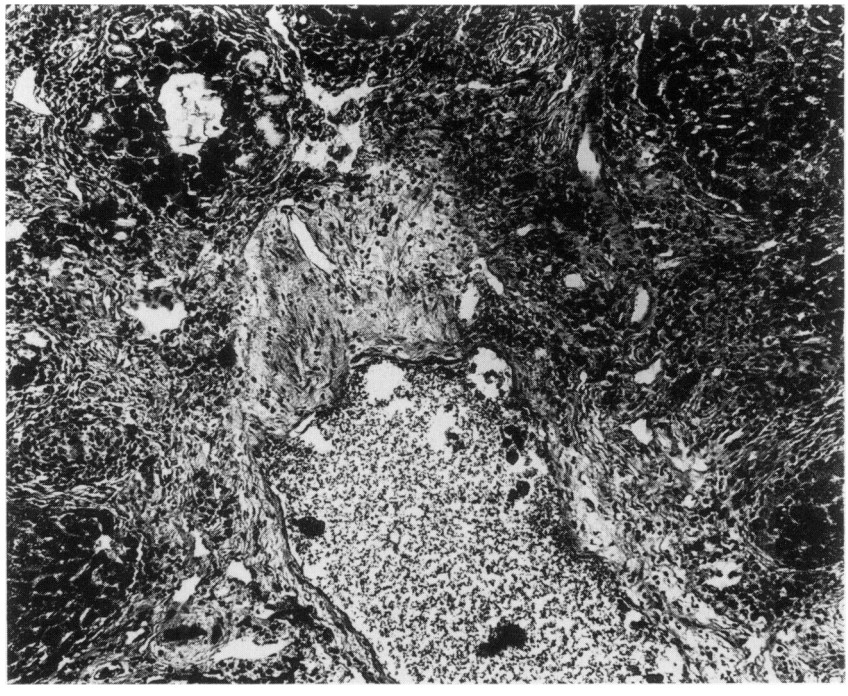

Figure 4. Portal space changes in murine pipestem fibrosis. There are phlebosclerosis of portal vein, small telangiectatic vessels, periovular granulomas, focal mononuclear-cell infiltration and bile duct hyperplasia. Masson's trichrome stain. × 50.

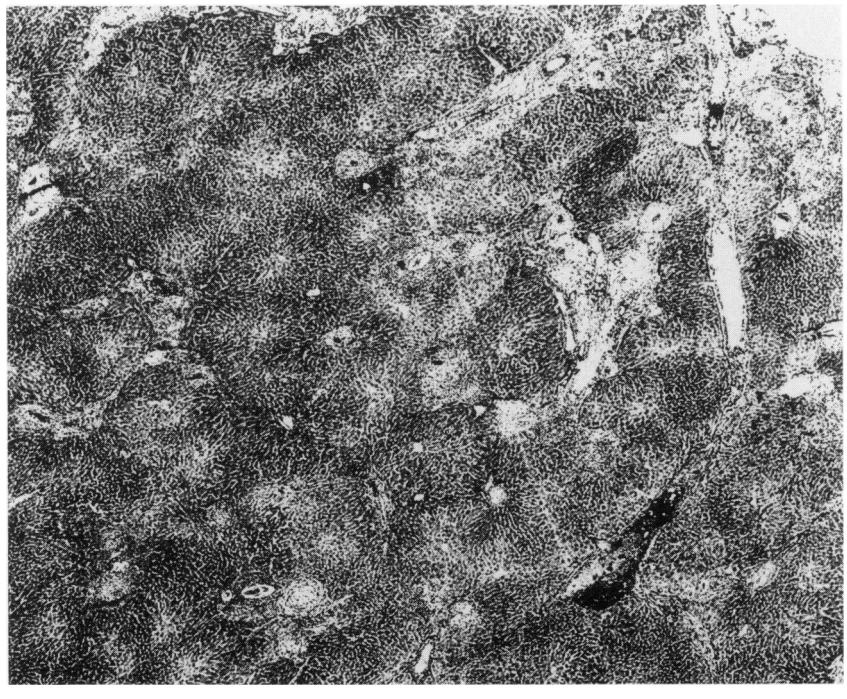

Figure 5. Chronic schistosome infection showing fibrous enlargement of a few portal spaces and isolated periovular granulomas, but failing to reproduce the picture of pipestem fibrosis. Masson's trichrome stain. × 25.

Spleens were moderately enlarged (Table 1) and exhibited mild to moderate enlargement of the red pulp with lymphoid and reticular cell hyperplasia, but chronic congestive changes were minimal or absent. The white pulp was not remarkable, but in some animals it showed variable atrophy. Some kidneys disclosed increased glomerular cellularity and a variable degree of mesangial expansion, but the tubules, vessels and interstitial tissue appeared normal.

Schistosoma japonicum. Mice infected with one *S. japonicum* worm-pair failed to show pipestem fibrosis

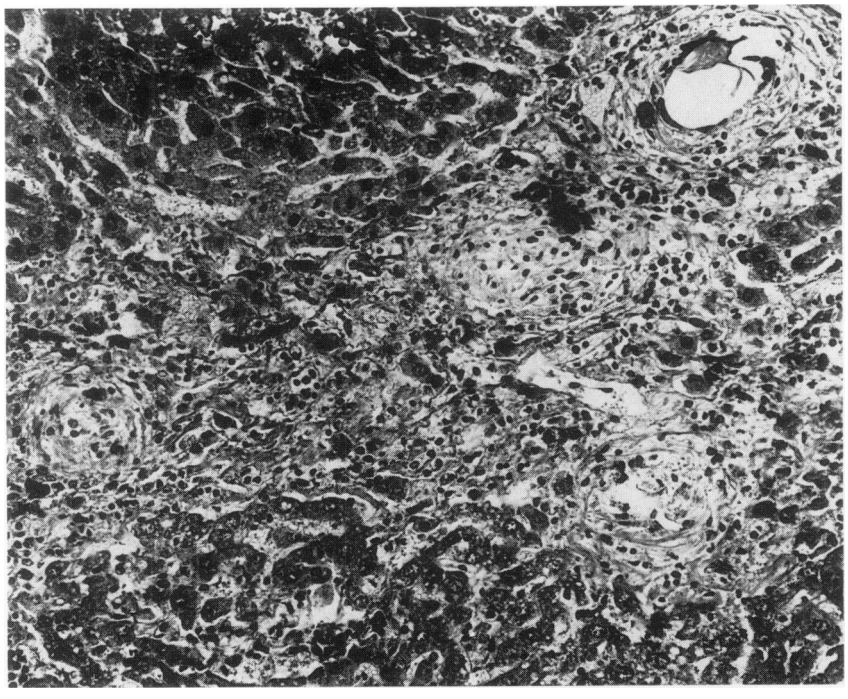

Figure 6. Involuting periportal schistosomal lesions. Connective tissue shows fibres loosely arranged and fragmented both within the granuloma and outside. There is also diffuse mononuclear-cell infiltration. Masson's trichrome stain. × 200.

Figure 7. Prolonged schistosomal infection showing portal fibrosis with both old (long arrows) and fresh (short arrows) periovular granulomas. Masson's trichrome stain. × 50.

although they contained more eggs than did *S. mansoni* infected mice (42 000 eggs/liver at 26 weeks and 87 000 at 54 weeks). The granulomas, which were large and destructive in early infection, soon became smaller. At 20 weeks eggs appeared to concentrate in some portal areas, but were absent from the majority. Fresh granulomas usually contained several eggs, presented central purulent necrosis, vacuolated macrophages and concentric peripheral fibrosis. When a certain amount of portal fibrosis resembling pipestem fibrosis was formed it involved focal areas only and showed a concentration of egg-shells, most of them undergoing calcification.

Sometimes, epithelioid granulomas were formed. Cases of 54 weeks duration revealed involuting fibrosis in some portal areas and absence of fibrosis in other areas. Involuting scars usually contained calcified egg shells and pigment. Spleens in *S. japonicum* infected mice averaged 302 and 218 mg in weight at 26 and 54 weeks respectively.

Discussion

The hepatic histological picture of early schistosomal infection, with granulomas scattered diffusely within the liver parenchyma, clearly differs from mild and late infection where the granulomas are concentrated along periportal areas, leaving the parenchyma undisturbed. This latter picture has been compared to human pipestem fibrosis (Warren 1966; Andrade 1987) and its utility as a model has been already demonstrated in studies on pathogenesis and chemotherapy.

During prolonged infection terminal radicles of the portal venous sytem are progressively obstructed by schistosome egg granulomas. Probably as a consequence of increased intrahepatic portal pressure, fine collaterals become dilated along the entire portal system as demonstrated in injection-corrosion preparations (Andrade 1987). Eggs are then gradually deposited within periportal areas causing chronic inflammation and fibrosis. On the other hand, periportal fibrosis caused by mild and prolonged infection has been shown to be susceptible to degradation after chemotherapy (Andrade & Grimaud 1988), again mimicking the situation with human pipestem fibrosis (Bina & Prata 1983; Homeida *et al.* 1988).

However, the murine model of pipestem fibrosis presents problems of reproducibility and relevance. The present investigation indicates that mild and prolonged *S. mansoni* infection in mice can be expected to cause periportal fibrosis in about 50% of the animals. The proportion was higher in male BALB/c and C3H mice, but even in inbred strains of mice the variability of pipestem formation persisted. This suggests that environmental, parasitological and other factors besides the host genetic background, may interact in the pathogenesis of pipestem fibrosis.

Morphological signs of matrix degradation were seen in all cases examined. In some, involuting changes had probably transformed periportal fibrosis into thin tracts of loose connective tissue containing remnants of egg shells and concentrated dark-brown schistosomal pigment. Longer infections were more likely to show signs of regression. Probably this was the reason for the absence of 1+ grade fibrosis in our outbred mice examined only at 23 weeks. These degradative changes can interfere with interpretation of therapeutic results and may even spontaneously revert to normal in the mouse. Spontaneous regression of clinical hepatosplenic schistosomiasis has been described in human cases followed up for a period of 10 years (Katz & Brener 1966). Histological changes suggestive of focal matrix degradation have also been described in untreated long-standing cases of pipestem fibrosis in humans (Andrade *et al.* 1992).

On the morphological side, pipestem fibrosis in our mice is not as systematized as it is in humans. Periportal fibrosis in the mouse is mainly formed around the granulomas and there is relatively little extra-granulomatous fibrosis. The opposite is seen in man. We have seen diffuse, systematized portal fibrosis in CBA male mice in the material of Henderson and co-workers in which marked portal fibrosis occurs in mice which show striking splenomegaly and lack immunoregulatory idiotypic responses (Henderson *et al.* 1993). These mice also present more striking obstruction of medium sized portal venules than do mice in our experiments. However, vascular changes, including obstruction of large and medium sized portal vein branches, destruction of venous walls, dispersion of smooth muscle fibres and cells into the portal matrix, hallmarks of human pipestem lesion (Andrade *et al.* 1992), are not prominent features of the murine model. Focal bile duct and ductule hyperplasia was a constant finding. Since new biliary canals supposedly induce the formation of new supportive stroma, biliary hyperplasia may contribute to increase portal fibrosis in schistosomiasis. Also, this change has been linked to excessive proline produced by the worms (Bedi & Isseroff 1979), which can by itself stimulate fibrosis. Focal bile duct hyperplasia does occur in humans but is not a prominent feature of Symmers' pipestem fibrosis.

Prolonged *S. japonicum* infection did not result in pipestem fibrosis in the mouse. In *S. japonicum* infected rabbits (Cheever *et al.* 1980) and perhaps in *S. japonicum* infected persons (Tsutsumi & Nakashima 1972) cirrhosis often develops, but it is not present in *S. mansoni* infection in the mouse, the chimpanzee (Sadun *et al.* 1970) or man (Andrade *et al.* 1992) or in *S. japonicum* infection in the chimpanzee (Lichtenberg *et al.* 1971).

Hepatosplenic disease, with portal hypertension and marked hepatosplenomegaly (DeWitt & Warren 1959), is better observed in the mouse during early infection than later when periportal fibrosis develops. Splenomegaly was not remarkable in our material and chronic congestive changes were absent in the spleens examined.

These features are just the opposite of the human condition the model intends to represent.

Like any other experimental model, the murine model of schistosomal pipestem fibrosis presents advantages and limitations. The success of its utilization will depend on the questions to be addressed and on a balanced judgement of the main characteristics of the model, which is what the present investigation has attempted to delineate.

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