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GRANULOMA FORMATION AROUND EXOGENOUS EGGS OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA JAPONICUM* IN MICE

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Summary

Hepatic granulomata which were qualitatively and quantitatively similar to those seen in infections established with cercariae were induced by surgical injection of exogenous eggs of *Schistosoma mansoni* and *Schistosoma japonicum* via the mesenteric veins of previously unexposed albino mice. Thereafter, their comparative histopathologic studies were made. The maximum mean sizes of granulomata were attained on Day 32 with viable eggs of these parasite species. Although *S. mansoni* eggs produced significantly larger lesions (368·4 ± 21·5 μm) than eggs of *S. japonicum* (205·8 ± 18·6 μm) at the peak period, the difference in the mean granulomal size showed no correlation with either the time of onset or the severity of pathologic changes produced. Thus, eggs of *S. japonicum* with smaller granulomata, evoked pathologic changes which were earlier in onset and more severe than those produced by the same quantity of *S. mansoni* eggs. Since the most obvious variables (the quantity of eggs, the sequence of their arrival in the liver, the timing of observation, and the strain of experimental animals) were controlled, it was concluded that the most severe and fatal effects produced by eggs of *S. japonicum*, as previously suggested, are largely due to differences in the cytotoxic and antigenic peculiarities of this species.

Résumé

Les granulomes hépatiques qui étaient qualitative et quantitativement semblables à ceux observés dans des cas d'infection èstablies grâce au cercariae furent induits par l'injection chirurgicale d'oeufs exogènes de *Schistosoma mansoni* et de *Schistosoma japonicum* via les veins mésentériques de souris albino non précedemment exposées. Ensuite des ètudes comparatives histopathologiques furent faites sur elles. Les tailles moyennes maximum des granulomes furent atteintes au 32e jour avec des oeufs viables deces especès de parasites. Bien que les oeufs *S. mansoni* aient produit des lésions considérablement plus importantes (368·4 ± 21·5 μm) que les oeufs du *S. japonicum* (205·8 ± 18·6 μm) à la périod de pointe, la différence au niveau de la taille moyenne du granulome n'a révélé aucune correlation avec le commencement ou la gravité des changements pathologique produits. Ainsi, les oeufs du *S. japonicum* à plus petits granulomes provoquent les changements pathologiques qui furent précédement constatés et qui ètaient plus graves que ceux produits par la même quantité d'oeufs *S. mansoni*. Puisque les variables les plus èvidentes (la quantité des oeufs, la séquence de leur introduction dans le foie, la durée de l'observation, et la tension subie par les animaux expérimentaux) étaient in changées il a été conclu que les effets les plus graves et les plus fatals produits par les oeufs du *S. japonicum* tels que précédemment annonces, sont dûs en grande partie aux différences constatées au niveau des particularités cytotoxiques et antiquéniques de cette espèce.

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Introduction

Several investigations have incriminated the eggs as the major parasite component in the

pathogenesis of human schistosomiasis and in the development of overt disease (Dewitt & Warren, 1959; Cheever, 1961; Hsu & Hsu, 1961; Lichtenberg, 1962; Moore, Groove & Warren, 1977; Edungbola & Schiller, 1979). Likewise, various studies (Meleney *et al.*, 1953; Warren & Domingo, 1970) have shown that the pathogenesis of schistosomiasis varied in that the onset and severity of pathologic changes produced depend on the species of the parasite and of the mammalian host being studied. Meleney *et al.* (1953) reported that the pathologic changes induced in the laboratory hosts by *S. japonicum* were more severe than those produced by *S. mansoni* or *S. haematobium* partly because eggs of *S. japonicum* are deposited in bunches, whereas those of *S. mansoni* are produced and reached mammalian tissues singly. For this reason, Warren & Domingo (1970) indicated that this difference has made it relatively impossible to compare the response induced in the host by eggs of *S. japonicum* with that of *S. mansoni* or *S. haematobium*.

Furthermore, using the tail vein egg injection technique, Warren and co-workers (Warren *et al.*, 1975; Warren, Grove & Pelley, 1978) could not induce pulmonary granulomata with eggs of *S. japonicum* in the unsensitized mice whereas they succeeded with eggs of *S. mansoni* and *S. haematobium*. Thus, Warren and Boros (1970) concluded that this failure has rendered the use of the lung model unsuitable for investigating the aetiology of *S. japonicum* lesions. This important limitation has led to the attempt made in this current study to induce and compare granulomal formation around viable eggs of *S. mansoni* with that of *S. japonicum* using the alternative surgical injection technique described recently by Edungbola and Schiller (1979).

Materials and methods

Albino mice of Swiss-Webster CD strains, about 5-weeks-old, were infected with eighty cercariae of the Puerto Rican strain of *S. mansoni* by tail immersion. The same strain of mice, infected with *S. japonicum* were obtained from Dr John I. Bruce of the Research Foundation, University of Lowell, Massachusetts, U.S.A. Both groups were killed 10 weeks post infection and viable eggs isolated from their livers and intestines as described by Moore *et al.* (1977).

Groups of uninfected female mice (Swiss-Webster CD Strains) were given 3000 viable eggs of either *S. mansoni* or *S. japonicum* via their mesenteric veins. Five recipient hosts in each of the two groups were sacrificed at intervals of 1, 8, 16, 32, 64 and 100 days following the egg injection. The details of the surgical and histological procedures employed are as described by Edungbola and Schiller (1979).

Results

The formation of hepatic granulomata around viable exogenous eggs of *S. mansoni* and *S. japonicum* commenced on Day 8. Thereafter, the lesions increased in size until they reached the peak on Day 32.

Table 1 shows that although the maximum mean sizes of hepatic granulomata induced with eggs of both schistosome species were attained on Day 32, eggs of *S. mansoni* elicited significantly larger granulomatous lesions (368·4 ±

TABLE 1. Mean sizes of hepatic granulomata around *S. mansoni* and *S. japonicum* eggs in mice (mean ± s.e. μm).

	Days after inoculation of eggs					
	1	8	16	32	64	100
Schistosoma mansoni granuloma (mean diam.)	NR	241·1(1±13·4)	256·3(±11·3)	368·4(±21·5)	360·3(±17·7)	220·6(±11·8)
Schistosoma japonicum granuloma (mean diam.)	NR	155·6(±10·7)	198·9(±16·2)	205·8(±18·6)	162·1(±12·0)	102·4(±9·1)
Eggs with or without reactions measured (av. no.)	100	100	100	100	100	100
Eggs with histologic (%) reactions	* 67·4	83·9	91·7	91·8	97·6	94·4
	† 84·1	96·5	98·6	99·2	93·5	89·0

* *Schistosoma mansoni*
† *Schistosoma japonicum*
NR = no histologic reaction; egg only ($x = 60·3 \pm 5·2$ μm)

FIG. 1. *Schistosoma japonicum* eggs located at the periphery of a granuloma on Day 16. Note the accumulation of neutrophils, plasma cells and mononuclears around a necrotic centre (× 84).

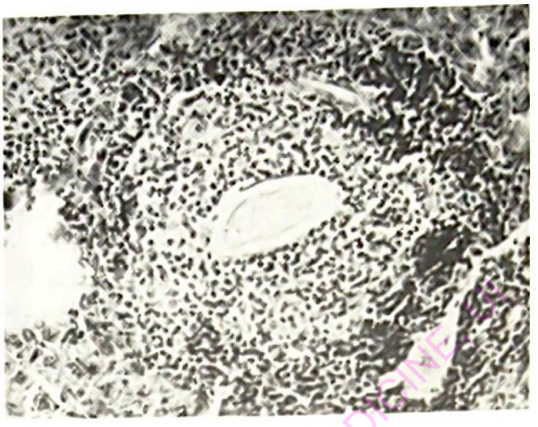

FIG. 2. A preponderance of eosinophils around *S. mansoni* egg with a miracidium on Day 16 (× 57·4).

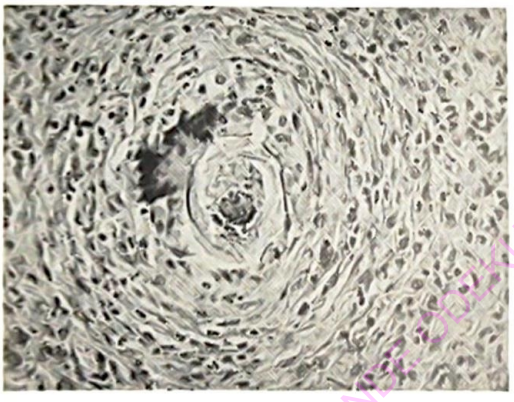

FIG. 3. A fading eosinophilic bifringence around a degenerating egg of *S. japonicum* on Day 32. Note the scanty cellular remnants and minimal deposition of connective tissues (× 57·4).

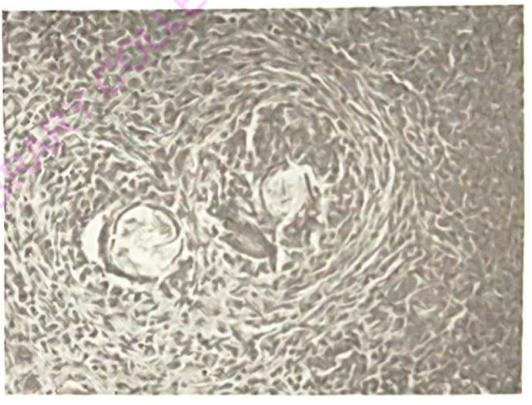

FIG. 4. *Schistosoma mansoni* eggs (Day 32) with concentric rings of collagenous materials and a mixture of eosinophil and mononuclear 'cuffing' (× 44·8).

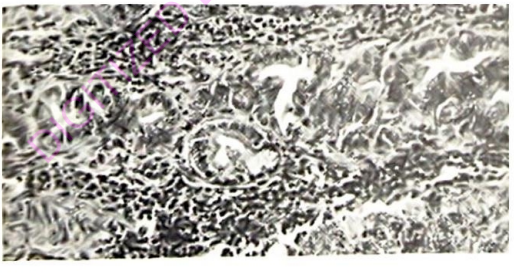

FIG. 5. *Schistosoma japonicum* egg-induced bile duct hyperplasia with an extensive inflammation of the periductal walls and adjacent liver parenchyma on Day 32 (× 53·3).

21·5 μm) than those of *S. japonicum* (205·8 ± 18·6 μm) ($P < 0·001$). Also, the involution of granulomata around eggs of *S. japonicum* occurred faster than those around eggs of *S. mansoni*. Thus, by Day 100, *S. japonicum* lesions have regressed to a mean size of (102·4 ± 9·1 μm) whereas those of *S. mansoni* remained at least two times as large (200·6 ± 11·8 μm).

The cellular composition around the eggs of both parasite species varied with time. On Day 32 when the peak mean size of granuloma was reached, massive infiltration of neutrophils, lymphocytes and plasma cells featured predominantly around *S. japonicum* egg. However, around *S. mansoni* eggs, it was a preponderance of eosinophils (Fig. 2) and epithelioid cells, moderate number of lymphocytes and few giant cells that were observed. In addition, fibroblasts were found concentrically around eggs of *S. mansoni*. By Day 32, the deposition of collagenous fibres (Fig. 4) was obvious and these became increasingly abundant, thereafter, until they replaced the bulk of the initial cellular component. In contrast, fibroblasts were relatively sparse around *S. japonicum* eggs and the amount of connective tissue and scarring produced were correspondingly scanty (Fig. 3). Sometimes, but very characteristically, *S. japonicum* eggs were lodged at the periphery of the granuloma (Fig. 1) rather than being circumscribed by its cellular constituents.

Hoeppli phenomena (Fig. 3) were more frequent around exogenous eggs of *S. japonicum* than around eggs of *S. mansoni* in the liver of the recipient mice. About 3·1% of intact eggs of *S. japonicum* with matured miracidia were observed with this eosinophilic bifringence by Days 16 and 32.

In general, the pathologic changes induced by *S. japonicum* eggs in the liver of recipient mice were earlier in onset and more severe at every granulomal stage than those of *S. mansoni*. Among these changes are the formation of abscesses with necrotic centres (Fig. 1); bile duct hyperplasia (Fig. 5); portal endophlebites, structural distortion of the liver parenchyma, and extensive periportal and acute inflammation both near to, and at sites spatially remote from the immediate physical location of the intact egg.

Discussion

The recognition of the important role of schistosome eggs and of the differences in the pathogenicity induced by eggs of the three major species of human schistosomes have led to various attempts to develop a model which will enable a satisfactory comparison of the nature and sequence of histopathologic events caused by each of these species in the mammalian host. The use of the lung model has proved unsuitable for investigating the aetiology of *S. japonicum* lesions as it fails to elicit pulmonary granulomata around eggs of this species in the unsensitized mice (Warren *et al.*, 1975, 1978). Also Edungbola and Schiller (1979) enumerated other limitations of the lung model.

In the current study, the induction of typical hepatic granulomata in mice with exogenous eggs of *S. japonicum*, supports the suggestions by Edungbola and Schiller (1979) that the target organ is one of the important parameters that should be considered while interpreting the biological progression of schistosome egg lesions in the mammalian tissue.

That the mean size of hepatic granulomata induced by eggs of *S. japonicum* was significantly smaller than those of *S. mansoni* conforms with previous reports (Warren & Domingo, 1970; Warren *et al.*, 1975, 1978; Meleney *et al.*, 1952) that granulomata having the largest and smallest mean sizes occurred around eggs of *S. mansoni* and *S. japonicum* respectively, while that of *S. haematobium* intermediates between these two.

Although the granulomata induced by *S. mansoni* eggs were larger than those of *S. japonicum*, the size of granuloma *per se* showed no direct correlation with either the period of onset or the severity of pathologic changes manifested. Thus, *S. japonicum* eggs induced changes which were more severe and which occurred faster. Lichtenberg, Erikson and Sadun (1973) also noted that the size of the egg lesion was not related to the degree of pathogenicity and for this reason, they questioned the relevance of granulomal measurements as criteria for assessing the degree of severity caused by different schistosome species.

The ability of *S. japonicum* eggs to evoke

more severe and fatal damage than other human schistosomes, have received considerable attention (Meleney *et al.*, 1973; Lichtenberg *et al.*, 1973; Erickson *et al.*, 1971). The egg laying capacity (Moore & Sandground, 1956), the granulomal size (Moore & Warren, 1976), and the differences in the cytotoxic and antigenic properties (Lichtenberg *et al.*, 1973), are among factors thought to account for the most virulent effects of *S. japonicum* eggs in the mammalian tissues.

Since all the variables, except the parasite species, are controlled, the results of this study lend stronger support to the contention that the greater virulence of *S. japonicum* eggs is probably due to their cytotoxic and/or antigenic uniqueness.

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