

The Effect of Praziquantel on Patterns of *Schistosoma mansoni* Eggshell Hatching Breaks

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Abstract: The break of the eggshell formed by water- and praziquantel (PZQ)-induced hatching of *Schistosoma mansoni* eggs was observed by scanning electron microscopy. The break most frequently formed on the long axis of the eggshell opposite the spine, and less frequently along spine side, parallel or oblique to the long axis of the lateral side of the eggshell. An outwardly-curved lip of shell lined the external margins of the hatching orifice. The shell itself was of uniform thickness and fairly smooth. No significant difference was observed in the position breaks between water- and PZQ-hatched eggs. However, PZQ hatching produced smaller hatching orifices and the miracidium frequently failed to escape.

Key words: *Schistosoma mansoni*, Hatching of eggs, Praziquantel

INTRODUCTION

Schistosome eggs hatch easily in water. Although much useful information has accumulated, the exact mechanism of hatching of schistosome eggs remain unknown. Recently one of the authors reported that praziquantel (PZQ) stimulated the hatching of miracidia from *Schistosoma mansoni* eggs which were incubated in phosphate buffered saline (PBS) of 365 mOsM (Katsumata, 1988). The question arises whether there is an essential difference in the mechanism between natural water-induced and PZQ-induced hatching.

Thus far the morphological observations on the hatching of miracidium has provided valuable information on the mechanisms of the break of the eggshell (Kassim and Gilbertson, 1976; Higgins-Opitz and Evers, 1983; Samuelson *et al.*, 1984). These studies encouraged us to compare the break of the eggshell formed by water- and PZQ-induced hatching of *S. mansoni* eggs.

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MATERIALS AND METHODS

The eggs were the Kenya strain of *S. mansoni* which have been maintained in our laboratory for many years. The eggs were collected from the livers of infected hamsters by the digestion technique of Katsumata (1988). Eggs were repeatedly washed with PBS (365 mOsM) in a test tube and allowed to sink under gravity. The sediments contained exclusively the mature unhatched eggs. An aliquot of the egg suspension was either diluted by distilled water or exposed to PBS containing PZQ (a final concentration: 10 ng/ml) to stimulate hatching. One hour later the empty eggshell, miracidium and unhatched eggs were fixed with 2.5% glutaraldehyde in PBS. The specimens were dehydrated in a graded series of ethanol, critical point dried, coated with gold, and observed by JEOL 100 CX scanning electron microscope. Measurements of the length of the eggshell and the length of its hatching break were recorded by using a computer graphic system (Cosmozone, Nikon Ltd., Japan).

RESULTS

The hatching rates of the eggs in water and PZQ solution were about 70% and 20% respectively.

There seem to be a variety of positions for the break. Fig. 1 shows the location of the break in the eggshell formed by water-induced hatching. It formed along the length of the eggshell on the surface opposite the spine (A), on the long axis of the spine side of the eggshell (B), parallel (C) or oblique (D) to the long axis of the right lateral side of the eggshell, and parallel to the long axis of left lateral side of the eggshell (E), when the spine was viewed to go from above to below en face.

Fig. 2 shows the break of the eggshell formed by PZO-induced hatching. The break was noticed in a confined location of the eggshell which did not differ much from the location of the break identified for water-induced hatching. When the hatching was triggered by water, the hatched miracidia swam away from the broken eggshells. However, when the hatching was induced by PZQ, miracidia frequently remain attached to the broken eggshells at any part of their bodies (Figs. 2 B, C, D) or failed to escape from the eggshells (Fig. 2 A).

Table 1 shows the relative frequency of topographical distribution of the break in the eggshell. For both triggers the long axis of the eggshell opposite the spine was the most frequently broken. There was no significant difference in the relative distribution of breaks between water- and PZQ-induced hatching of *S. mansoni* eggs.

Table 2 shows the measurements of long axis of the eggshell and the break formed on the long axis of the eggshell opposite the spine. When the hatching was induced by PZQ, significantly smaller breaks were formed ($p < 0.01$, Students "t" test).

The external margins of the hatching aperture were lined with a lip of outwardly-curved shell (Figs. 1F and 2E). On-end views of the broken shell itself were few (Fig. 1G). The surface of the breaks was relatively smooth and uniformly thick (Figs. 1H and 2F). The present study failed to make it clear whether no piece was lost or broken away.

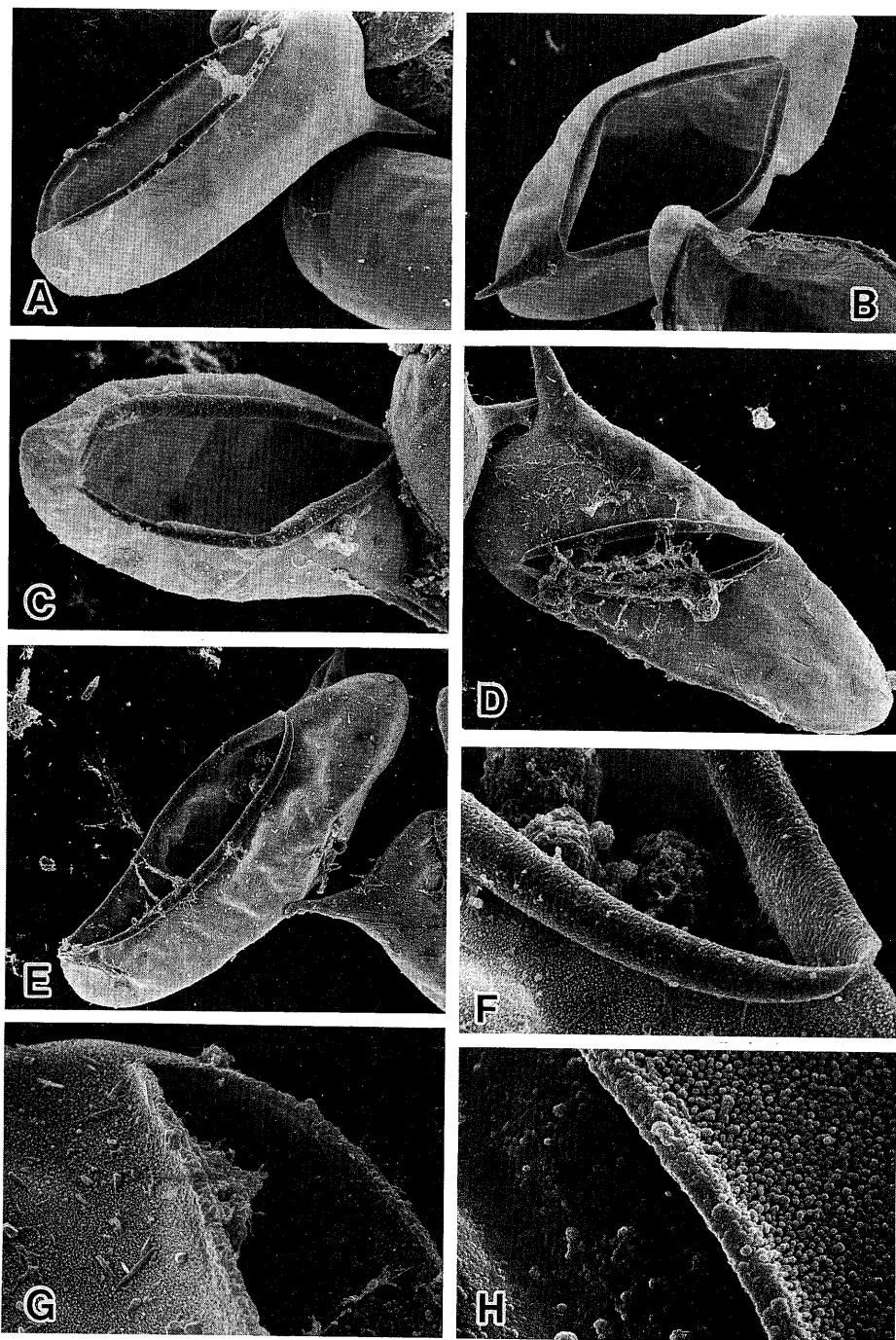


Fig. 1. Scanning electron microscopy of the break on the eggshell formed by miracidial hatching in water. A–E show location of the break. The break formed on the long axis of the eggshell opposite to the spine (A), on the spine side (B), parallel (C) or oblique (D) to the long axis of right lateral side of the shell, parallel to the long axis of the left lateral side of the shell (E), when the spine was viewed to go from above to below en face. The broken area of the eggshell usually curled over (F), but rarely curled less (G). H shows the transverse section of the break. Surface was relatively smooth and uniformly thick.

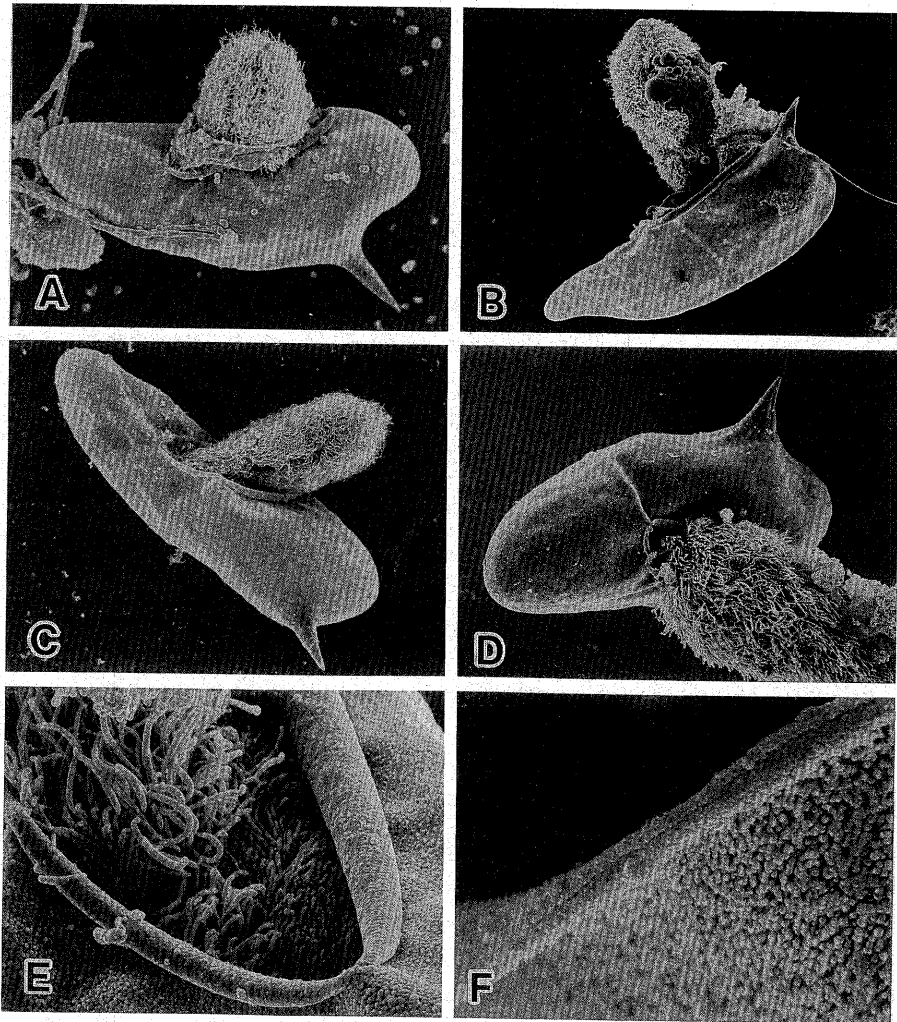


Fig. 2. Scanning electron microscopy of the break on the eggshell formed by miracidial hatching triggered by praziquantel. A–D show the location of the break. The break formed on the long axis of the eggshell opposite to the spine (A), on the spine side (B), oblique to the long axis of the right lateral side of the shell (C), left lateral side of the shell (D), when the spine was viewed to go from above to below en face. note: The miracidium remained within the eggshell (A) or attached the break of the shell at any parts of its body (B, C). The broken area of the eggshell curled over (E). The surface of the transverse section of the break was relatively smooth and uniformly thick (F).

Table 1. Relative frequency of distribution of the break in the eggshell

Trigger of hatching	No. of eggs examined	Location of break in the shell			
		Surface opposite the spine	Spine side	When spine was viewed to go from above to below en face	
				Right lateral side of shell	Left lateral side of shell
water	147	103	11	26	7
praziquantel (10 ng/ml)	129	80	5	37	7

Difference between triggers by $\chi^2=5.987$ (NS)

Table 2. Measurement of long axis of the eggshell and length of the break formed on the eggshell

Trigger of hatching	No. of eggshell examined	Long axis of eggshell (μm)	Length of break (μm)*
water	43	119.5 \pm 11.8	95.3 \pm 13.4**
praziquantel (10 ng/ml)	29	122.5 \pm 8.9	62.1 \pm 19.3**

* Measurement was done on the break formed on the long axis of the shell opposite to the spine alone.

** Difference is statistically significant ($p < 0.01$, students "t" test).

DISCUSSION

The aim of the present study was to compare the water- and PZQ-induced hatching of *S. mansoni* eggs in terms of hatching-break patterns formed on the eggshell. If we could identify any structural difference in the break of the eggshell, we have to accept the idea that the mechanism of PZQ-induced hatching differs from that of natural water-induced hatching. Electron microscopy did not identify any difference except in the length of the break. Both water- and PZQ-induced hatching produced breaks most frequently on the long axis of the eggshell opposite the spine and an outwardly-curved lip of shell appeared at the margins. The failure of miracidia to escape from PZQ-hatched eggs is probably related at least in part to an abnormally small hatching orifice. PZQ paradoxically stimulated the miracidium to swim within the eggshell and allowed them to hatch under high osmotic pressure (Katsumata, 1988), but may have reduced their ability to break free. Therefore, the hatching rate of the eggs exposed to PZQ solution remained low (20%). Although the present study does not prove or disprove the three hypotheses on the mechanism of the hatching of the eggs, viz. osmotic pressure (Bair and Etgens, 1973; Kassim and Gilbertson, 1976), mechanical activity (Samuelson *et al.*, 1984) and proteolytic enzyme (Xu and Dresden, 1986), it encourages us to commence study on the role of the miracidial motility in the hatching of the eggs.

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