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BEHAVIOR OF SCHISTOSOMAL MIRACIDIA AND THE CHANGE IN OSMOTIC PRESSURE OF THE SURROUNDING MEDIUM

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As a preliminary to some studies on vector-parasite relationships to *Oncomelania nosophora*, it is necessary to determine some of the factors influencing the behabior of the miracidia of *Schistosoma japonicum* : hatchability, velocity of swimming, longevity, and infectivity into the host snail.

Such factors might include light, temperature, pH of the surrounding medium and a variation of maturity of miracidia. Each of these factors would requir a large number of experiment.

Sugiura *et al.* (1954), in studying the factors influencing hatching of *S. japonicum* eggs, reported the effects of temperature, light, pH and other several fatcors upon the hatchability of the eggs. The effect of salt concentration on the hatchability of *S. japonicum* eggs was described by Ito (1954). As to locomotion of miracidia, Takahashi *et al.* (1961) reported their responses to thermal, photic, and gravitational stimuli.

The immediate objective of the work reported here was to show the mode of hatching from the eggs and a "reversible" response of miracidium against the change in osmotic pressure of the surrounding medium. Such a study is considered worthwhile because it would contribute knowledge useful in future studies of vector-parasite relationships and would provide an available technique for the studies.

Material and Method

The eggs of *S. japonicum* used in both hatchability and longevity experiments were those obtained in the feces of a dog rinsed sufficiently with excess tap water. The technique described in Takahashi *et al.* (1961) was used for collecting miracidia from the feces.

Salt solution, which had previously been adjusted to a suitable concentration, was used to approximate a desired osmotic tension. Actually, an aliquot of the water-egg suspension was placed in a flask, and a proper amount of salt solution was then added to obtain a desired final concentration. In some cases, sucrose solution was used instead of salt solution by the same technique. A small drop of salt-egg suspension kept in various osmotic tension was picked from flask with a pipette and the number of empty shells from which miracidia had already escaped and that of unhatched ones were counted under microscope. The effect of osmotic pressure on hatchability was expressed by the percentage of the number of unhatched eggs.

A new procedure was designed to be carried out the observation of the *in vitro* survival of miracidia within the eggs shell. This procedure is based on the fact that a very clear-cut "reversible" response of miracidial movement and of their hatching from eggs was observed, namely, that egg submerged in 0.8 per cent salt solution can hatch when the salt concentration of the surrounding medium is diluted with water, though they cannot hatch in 0.8 per cent salt solution. The eggs were kept in a flask which was filled with 0.8 per cent salt solution to prevent them from hatching and were then allowed to remain in the solu-

tion until the observations were made. At 5-day intervals after the immersion a small amount of salt-egg suspension was transferred to a test tube of distilled water, in order to promote their hatching by dilution of surrounding concentration and the observations were performed on the hatchability of these eggs. The *in vitro* survival of miracidia within the shells was estimated on the basis of these observations.

With regard to the effect of the change in osmotic pressure upon free-swimming miracidia, almost the same technique as employed in the egg experiment was utilized for regulating the osmotic pressure of the surrounding medium.

Results

1) The effect of osmotic pressure on hatchability

In order to check the hatchability-suppressing possibilities of salt concentration of the surrounding medium, aliquots of egg-water suspensions were tubed, the salt concentration of each tube was abjusted by adding a proper amount of salt solution, and the appearrance of miracidia was then observed in each tube for 2 hours. A 0.8 per cent salt solution proved to be a suitable concentration for suppressing the hatching of *S. japonicum* eggs.

The effect of salt concentration of surrounding medium on hatching may be assumed as the effect of osmotic pressure, so the observation with sucrose solution was carried out as compared with the salt solution experiment. The result indicated that a 6.0 per cent sucrose solution was the minimal concentration for the suppression of hatching.

The results from these observations indicate that the hatching of *S. japonicum* eggs was influenced at a certain osmotic pressure, at about 4 atm..

A more detailed study should be undertaken in connection with the observation described above. In preparing stock salt-egg suspensions, a series of water-egg suspensions were first made in flasks. The volumes were then determined and a proper amount of salt added to obtain a desired osmotic pressure in the surrounding media. These stock salt-egg suspensions were kept at a room temperature. After 24 hours, the number of emptied shells and that of unhatched ones were counted under microscope. The result is summarized in Table 1. The result indicated that 85.9 per cent of 135 eggs hatched in control, in the eggs submerged in 0.4 per cent salt solution 57.0 per cent hatched. Also in this experiment, 20.8 per cent of eggs hatched in 0.6 per cent salt solution whereas in 0.8 per cent salt solution the percentage which hatched were only 1.1 per cent. It should be noticed that the hatching rate of eggs was 20.8 percent in 0.6 per cent salt solution, in which the osmotic pressure is approximately equivalent to 2.5 atm., but a significant fall of hatching rate was observed in 0.8 per cent salt solution, in which the pressure is about 3.3 atm..

The similar experiment was carried out with sucrose solution for regulating the osmotic pressure. The result of sucrose solution ex-

Table 1. Hatchability of *S. japonicum* eggs after 1 day in NaCl solution (Temperature : 20°C)

Concentration of NaCl solution (%)	No. of unhatched eggs	No. of hatched eggs	Precentage	Osmotic pressure (atm.)
1.0	84	3	3.4	4.1
0.8	87	1	1.1	3.3
0.6	76	20	20.8	2.5
0.4	34	45	57.0	1.7
0.2	30	46	60.5	0.8
0 (control)	19	116	85.9	—

Table 2. Hatchability of *S. japonicum* eggs after 1 day in sucrose solution (Temperature : 20°C)

Concentration of sucrose solution (%)	No. of unhatched eggs	No. of hatched eggs	Percentage	Osmotic pressure (atm.)
10.0	103	0	0	7.0
8.0	98	0	0	5.7
6.0	104	3	2.8	4.2
4.0	58	11	18.6	2.8
2.0	107	28	26.2	1.4
0 (control)	19	116	85.9	—

periment is shown in Table 2. This result shows that 18.6 per cent of eggs hatched in 4.0 per cent sucrose solution, but the percentage which hatched was only 2.8 per cent in 6.0 per cent sucrose solution, in which the osmotic pressure is about equivalent to 4.2 atm.. It confirms the result of salt solution experiment, showing, at about 4 atm. of osmotic pressure, a decrease in hatchability of *S. japonicum* eggs.

2) *The in vitro survival of miracidium within the egg shell*

As a result of the experiment described above, the hatchability of *S. japonicum* eggs was clearly suppressed in 0.8 per cent salt solution, and from this result the outline of the *in vitro* survival experiment was designed. The stock salt-egg suspension was prepared by the same technique as described previously and the salt concentration was adjusted to 0.8 per cent. In order to dilute the salt solution and to promote the hatching rate, a proper amount of suspension was sampled and transferred into a test tube of distilled water 5 days after the stock salt-egg suspension was prepared. Three, 6 and 24 hours after the dilution, the hatchability of eggs included in the diluted suspension was observed. The similar observations were made by the same technique during the period corresponding to 10, 15, 20 and 25 days after the preparation of the stock salt-egg suspension. The result is shown in Fig. 1. About 90 per cent of the eggs, which had been kept in the stock suspension for 5 days, hatched within 6 to 24 hours after dilution. The result is similar to those of control as is shown in Fig. 1. Whereas in the eggs which had been submerged in the salt solution for 10 or 15 days, the rate of hatching was about 70 per cent after 24 hours of dilution and the hatchability falls slightly in these cases. Especially, 15 day-submerged group shown a gradually ascending curve which appears to be associated with their vitality, but, otherwise, the hatchability shows a clear falls in 20 day- and 25 day-submerged groups. In 25 day-submerged group, the rate of hatching was only about 13 per cent even after 24 hours

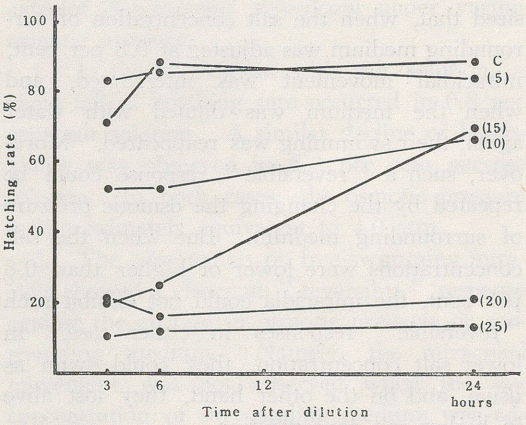

Fig. 1. Hatching rate of eggs immersed in 0.8% NaCl solution

Each curve indicate the result obtained at different times after immersion. C is control and figures in brackets denote the times of immersion in days. At the indicated times, the surrounding medium was diluted to promote the hatchability.

Abscissa : Time after the dilution
Ordinate : Number of hatched eggs ×100/total number of eggs

of the dilution.

These results seem to offer indirect support to that the miracidium is capable of surviving for about one month within its egg shell.

3) *The effect of osmotic pressure on free-swimming miracidia*

The effect of osmotic pressure on free-swimming miracidia : the response of free-swimming miracidia to the change in osmotic pressure was observed.

As an interesting result, it must be emphasized that, when the salt concentration of surrounding medium was adjusted at 0.8 per cent, miracidial movement was interrupted, and when the medium was diluted with water again, their swimming was reappeared. Moreover, such a "reversible" response could be repeated by the changing the osmotic pressure of surrounding medium. But when the salt concentrations were lower or higher than 0.8 per cent, the miracidia could not exhibit such "reversible" responses in either case. In lower salt concentration, they could swim as usual, and on the other hand, they lost alive in higher salt concentration.

The purpose of this experiment was to observe whether the period of time elapsed after miracidial hatching and the time kept them in the 0.8 per cent of salt solution may influence the miracidial response : the recovery rate of miracidial movement. The results of this experiment conducted to observe the recovery rate in different time elapsed after miracidial hatching are given in Table 3. The miracidia observed in this experiment ranged in time from 3 hours to 24 hours after hatching.

In miracidia which were used after 3 hours of hatching (3-hour miracidia after hatching), all of them were capable of recovering their movement, when the period of time immersed in the salt solution was shorter than 60 minutes. In 5-hour miracidia after hatching showed percentages of recoveries ranging from 97 to 79 per cent, and in 24-hour miracidia after hatching gave the lower percentage of recovery. In general, the results show a decrease in recovery rate with an increase in period of immersion,

Table 3. Recovery of miracidia immersed in 0.8% NaCl solution

Minutes of immersion	Test miracidia		
	3 hours after hatching	5 hours after hatching	24 hours after hatching
15	55/55 (100)	73/75 (97)	77/87 (89)
30	124/124 (100)	22/24 (92)	50/83 (60)
60	104/104 (100)	28/32 (88)	46/87 (59)
120	34/46 (74)	27/34 (79)	10/101 (10)

The figures divided by the / signs in this table are No. of miracidia recovered / No. of miracidia used.

Fegures in parentheses represent recovery rates in %.

and in addition, the recovery rate appears to be correlated with the period of time elapsed after their hatching.

Penetration activity of such a recovered miracidium into host snail was histologically normal after salt treatment.

Discussion

So far the role of environmental factors in respect to hatchability of *Schistosoma japonicum* eggs is indicated by the work of several investigators. Sugiura *et al.* (1954) in their investigation observed the effects of several environmental factors : temperature, light, and pH, influencing the rate of hatching of *Schistosoma japonicum* eggs. But they did not observed the effect of osmotic tension.

Kawashima *et al.* (1961), working with the miracidia of *Paragonimus ohirai*, indicated that the locomotive speed of the miracidia in 0.75 per cent salt solution was 0.3 to 0.4 mm per second. While the miracidia could survive for several minutes in the same salt solution.

On the other hand, Rowan (1956) in his studies on the mode of hatching of *Fasciola hepatica* eggs, tested the role of pressure as a factor in the escape of miracidia. His experiment suggested that the pressure changes within the eggs probably would be of interest

only with respect to the phenomonon of hatching.

Although the results of this investigation are necessarily limited in details, they afford some insight into the nature and mode of hatching in *Schistosoma japonicum* eggs. Significant fall of hatchability was observed in a certain osmotic pressure of surrounding medium, and the osmotic tension was also one of the factors involved in the "reversible" response of free-swimming miracidia: namely the experiment on free-swimming miracidia indicated that under a certain osmotic tension at which the hatching was supperesed, their movement was stopped, and when the miracidia had transferred into water, they regained their previous swimming. The evidence for response to pressure changes in hatching of *Schistosoma japonicum* eggs was based on two different experiments: with salt solution and with sucrose solution. We should pay our attention, in these cases, because one is electrolyte and this is different from the other in the chemical character. But only from the viewpoint of osmotic tension, the results obtained from salt solution experiment was similar to those found in the sucrose solution experiment.

The experiment of *in vitro* survival of miracidia within the shell, which was made by the specially designed technique described previousely, indicated that they were able to survive for about one month within their egg shells. This result may suggest that some of miracidia within the shells are able to survive for a month in the field, if the environmental condition is unfavorable to their hatching.

According to Rowan (1956), three basic theories had been reported to explain the hatching phenomenon of the eggs of trematodes: the first is by the muscular activity of the miracidium, the second is that was attributed to the viscous cushion beneath the operculum, and the third is explained by "hatching enzyme". Rowan also stated that cartain theories have been proposed to explain the hatching of eggs of trematodes, but the basic assumptions of all of the theories are the same.

The author, in this observation described here, emphasized that the important essential factor is the change in osmotic tension, because of the fact that both the suppression of hatching and "reversible" response of miracidial swimming occur at a certain osmotic pressure.

Summary

A study was made on the behavior of miracidia of *Schistosoma japonicum* under varing osmotic pressure.

1. In the experiment with salt solution, a rapid fall of hatching rate occurred in 0.8 per cent salt solution. A similar decline of hatchability was observed in 6.0 per cent sucrose solution. In both cases, the osmotic pressure is approximately equivalent to 4.0 atm..

2. The observation on free-swimming miracidia showed a clear-cut "reversible" response against the change in osmotic pressure of surrounding medium. Namely, the miracidial movement was not observed when the salt concentration of surrounding medium was adjusted at 0.8 per cent, but they began to swim again when the medium was diluted.

3. Miracidia could not exhibit such a "reversible" response, when the salt concentrations were lower or higher than, 0.8 per cent.

4. Reversibility of such miracidial response to the change in osmotic pressure decreased with the period of time elapsed after their hatching and with the duration kept in 0.8 per cent salt solution. This fact may be explained in part by their vitality.

5. The results obtained from the observation of the *in vitro* survival of miracidia seem to offer indirect suppert to that they can survive for about one month within the shells.

6. After salt treatment, recovered miracidia could penetrate into host snail.

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日本住血吸虫ミラシジュウムと液の浸透圧

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種々なる浸透圧下における，日本住血吸虫ミラシジュムの行動を観察した．

1．0.8％食塩水，ならびに6.0％蔗糖液に浸漬した日本住血吸虫卵は孵化不能に近かつた．これら溶液の浸透圧は約4気圧に相当する．

2．0.8％食塩水中にミラシジュウムを入れると，その游泳運動は即刻完全に停止するが，水を加えて濃度をうすめると，再び游泳運動を開始する．

3．この游泳停止現象は“可逆的”であるが，0.8％より高濃度の食塩水中では回復不能，低濃度では游泳運動を停止しない．

4．外液の浸透圧の変動に対する，ミラシジュウムの反応にみられた可逆性は，孵化後の経過時間と食塩水中の浸漬時間の延長にともなつて減ずる．このことはミラシジュウムの生活力によつて説明しうるものと考えられる．

5．排卵後宿主体外でのミラシジュウムの生存について調べたところ，卵殻内で約1カ月生存しうることを示唆する結果を得た．

6．食塩水浸漬後，游泳運動を回復したミラシジュウムはミヤイリガイに侵入することができる．