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Bull. Wld Hlth Org. } 1970, 42, 569-574

Cercarial Production from *Biomphalaria alexandrina* Infected with *Schistosoma mansoni*

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The present paper deals with longitudinal and cross-sectional methods of counting cercariae shed from Biomphalaria alexandrina *in Egypt, both experimentally and naturally infected with schistosomes. By the longitudinal method, the daily output of cercariae was counted from the first shedding from experimentally infected snails and from the day of collection from naturally infected ones. The results show that the size of the snails at the time of shedding exerts a very large effect on the output of cercariae and that the numbers obtained in the laboratory are not representative of cercarial output in the field. By the cross-sectional method, the cercarial output in the first 24 hours from infected snails collected in different months from the field was counted. The results show that output is size-specific. When the size-specific output is adjusted to the size-composition of infected snails taken from the field, it is estimated that the daily output from infected snails in the field may be 957.7 cercariae. However, this number may vary with the season.*

Cercarial production is one of the most important factors constituting the transmission dynamics of schistosomiasis. In spite of a review by McClelland (1965), much still remains to be learned. The present paper deals with results obtained by the longitudinal counting of *Schistosoma mansoni* cercariae from experimentally and naturally infected *Biomphalaria alexandrina* and by cross-sectional counting of the output of cercariae during the first 24 hours from *Biom. alexandrina* collected in the field, with particular reference to the effects of snail size on cercarial production.

MATERIALS AND METHODS

Laboratory-bred *Biom. alexandrina* were divided into two groups. Snails in group 1 were maintained under crowded conditions and those in group 2 received better care and were less crowded. When they were 5 weeks old, the snails were individually exposed to 2–4 *S. mansoni* miracidia. At the end of the cercarial incubation period, 29 cercarial-shedding snails in group 1 and 15 in group 2 were obtained. They were 5 mm–6 mm and 9 mm–10 mm in diameter, respectively, at the time of first shedding.

A total of 25 naturally infected *Biom. alexandrina* was collected in the field at the end of May 1967 when snails in groups 1 and 2 started to shed cercariae. Among these snails, 8 (group 3) were 5 mm–9 mm in diameter, 10 (group 4) were 9 mm–12 mm and 7 (group 5) were 12 mm–15 mm. These 5 groups of snails were maintained individually in 200-ml bottles. Cercarial counts were made for each snail daily during the remaining life of the snail.

From May 1967 to May 1968, *Biom. alexandrina* were collected monthly in the field under uniform conditions; they were then measured and placed individually in 20-ml test-tubes for 24 hours. The cercarial output during the first 24 hours was determined for some of the collections over a period of 6 months. Snails that shed no schistosome cercariae were crushed individually and examined under the microscope; the crushed snail was considered positive for infection if 1 cercaria or more was seen.

A cercarial count was carried out by means of the filtration–ninhydrin technique (McClelland, 1961), but before filtration, the number of cercariae was roughly estimated under a magnifier. If the number was below 1000, the total was counted, but if the number was larger the same dilution method as used by Chu, Sabbaghian & Massoud (1966) was followed.

A small piece of fresh lettuce was provided as food for the snails and was renewed at each change of water or each counting. The range in water temperature in the laboratory was 23°C–25°C in late May and October, 25°C–27°C in June, 26°C–28°C in July–September and 21°C–23°C in November.

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TABLE 1

DURATION OF INFECTION, TOTAL CERCARIAL OUTPUT AND DAILY CERCARIAL OUTPUT OF *SCHISTOSOMA MANSONI* FROM *BIOM. ALEXANDRINA*

Source of infected snails	Group	Size of snails (mm)	No. of snails	Duration of infection in days (mean ± SD) [a]	Total cercariae per snail (mean ± SD) [a]	No. of cercariae per snail per day (mean ± SD) [a]
Laboratory	1	5–6	29	34.9 ± 17.86	5 391.8 ± 4 368	133.4 ± 76.45
	2	9–10	15	38.8 ± 13.07	21 049.4 ± 9 494	535.4 ± 149.4
Field	3	5–9	8	76.8 ± 39.12	9 806.5 ± 9 050	127.8 ± 76.38
	4	9–12	10	68.5 ± 28.64	10 846.0 ± 6 895	167.9 ± 90.73
	5	12–15	7	53.4 ± 23.65	42 523.7 ± 34 100	1 006.8 ± 921.9

[a] SD = Standard deviation.

RESULTS

Longitudinal counting of cercariae

The mean duration of infection, the mean total cercarial output and the mean daily cercarial output per snail for the five groups of snails are presented in Table 1.

Duration of infection. The mean duration of infection was 34.9 days ± 17.86 days for 29 snails in group 1, and 38.8 days ± 13.07 days for 15 snails in group 2. The difference in the duration of infection between these two groups of snails is not statistically significant. For the naturally infected snails, the mean duration of infection after collection was 76.8 days ± 39.12 days for 8 snails in group 3, 68.5 days ± 28.64 days for 10 snails in group 4, and 53.4 days ± 23.65 days for 7 snails in group 5. As the duration of infection before collection was not known, comparisons among these three groups would not be possible. Nevertheless, it might show that smaller infected snails from the field survived longer than larger snails. It was evident, however, that naturally infected snails survived longer than experimentally infected snails under laboratory conditions.

The maximum duration of infection was 130 days in a naturally infected snail and the minimum duration of infection was only 1 day by an experimentally infected snail.

Total number of cercariae shed per snail. For the experimentally infected snails, the mean total number of cercariae per snail was 5391.8±4368 for 29 snails in group 1 and 21 049.4±9494 for 15 snails in group 2. The result showed that snails 9 mm–10 mm in diameter shed 4 times as many cercariae as those 5 mm–6 mm in diameter. For the naturally infected snails, the mean total number of cercariae per snail was 9806.5±9050 for 8 snails in group 3, 10 846.0±6895 for 10 snails in group 4 and 42 523.7±34 100 for 7 snails in group 5. These results show that cercarial output is related to snail size.

The maximum output was 112 354 from a naturally infected snail and the minimum was 72 from an experimentally infected snail.

Daily output of cercariae. For the experimentally infected snails, the mean number of cercariae per snail per day was 133.4±76.45 for 29 snails in group 1 and 535.4±149.4 for 15 snails in group 2. The result shows that the daily output from snails 9 mm–10 mm in diameter was 4 times higher than that from snails 5 mm–6 mm. For the naturally infected snails, the output was 127.8±76.38 for 8 snails in group 3, 167.8±90.73 for 10 snails in group 4 and 1006.8±921.9 for 7 snails in group 5, indicating that the daily output of cercariae is related to the size of the snail.

The maximum output during any one day was 8900 cercariae from a naturally infected snail and the minimum number was zero.

Patterns of cercarial shedding. Beginning with the day on which cercariae were first counted, the average number of cercariae shed per snail per day in a period of 10 days was calculated throughout the life of snails in the five groups. Table 2 shows that in groups 2–5, the peak of shedding was reached within the first 10 days after first counting but in group 1 it was reached after 20–30 days of patency. The rate of shedding from experimentally infected snails was steady for the first 40 days and then the rate declined. For the naturally infected snails, the intensity of shedding declined slowly after 20 days

TABLE 2
CERCARIAL SHEDDING FROM *BIOM. ALEXANDRINA* DURING 10-DAY PERIODS IN THE LABORATORY

No. of days after first shedding or counting of cercariae	Experimentally infected snails				Naturally infected snails					
	Group 1 (5–6 mm in diameter)		Group 2 (9–10 mm in diameter)		Group 3 (5–9 mm in diameter)		Group 4 (9–12 mm in diameter)		Group 5 (12–15 mm in diameter)	
	No. of snails (mean)	No. of cercariae per snail per day (mean)	No. of snails (mean)	No. of cercariae per snail per day (mean)	No. of snails (mean)	No. of cercariae per snail per day (mean)	No. of snails (mean)	No. of cercariae per snail per day (mean)	No. of snails (mean)	No. of cercariae per snail per day (mean)
0–10	28.0	116.2	15.0	655.5	7.6	263.4	10.0	636.8	7.0	2 675.5
11–20	23.2	207.4	14.5	563.4	7.6	226.7	9.9	334.8	7.0	1 044.0
21–30	19.7	213.8	12.2	538.9	7.6	164.5	9.0	164.3	6.1	427.3
31–40	16.9	121.8	9.3	463.5	7.6	148.3	9.0	93.2	5.6	174.3
41–50	9.3	80.2	6.4	205.1	6.6	93.0	8.0	31.4	3.9	27.2
51–60	4.0	135.7	1.6	18.0	5.9	73.6	6.0	13.8	2.0	12.8
61–70	0.4	30.5	0	0	5.0	56.1	6.0	17.2	2.0	0.9
71–80	0	0			5.0	70.6	4.0	21.7	1.0	6.6
81–90					4.9	71.6	3.6	4.8	1.0	8.2
91–100					2.0	66.2	2.0	3.1	0	0
101–110					2.0	37.1	1.0	0		
111–120					2.0	15.7	0	0		
121–130					1.0	0.5				
131–140					0	0				

for the snails in group 3 and abruptly after 10 days for snails in groups 4 and 5.

Out of 44 experimentally infected snails, 6 died during the active shedding stage and in 38 others the cercarial output declined gradually until shedding stopped for 1 or more days, then some cercariae appeared again for a few days. This pattern was repeated until the snail died.

Out of 25 naturally infected snails, 2 died during the active shedding stage, shedding declined uninterruptedly in 3 snails until they died, 6 died after period of shedding similar to that of the majority of experimentally infected snails. Nine snails after shedding cercariae for some time ceased shedding for 10–20 days, then a few cercariae reappeared for 1 or more days. This pattern was repeated until the snails died. Three snails after ceasing to shed cercariae resumed daily shedding until they died. The 2 remaining snails shed very few cercariae at the start and occasionally shed few cercariae during the remainder of their life. A complete cessation of shedding from infected snails was not observed among 69 snails under study.

Daily output of cercariae for the first 10 days. Beginning from the day of collection, the average number of cercariae shed per snail per day for the first 10 days from 25 naturally infected snails (groups 3–5) is given in Table 3. Results obtained showed that the maximum cercarial output occurred on day 4 with an average of 1529.7 cercariae per snail and that the average number during the first 10 days was 1150.2 cercariae. The number shed during the first 24 hours was 1205.0 which is quite close to the average for the first 10 days. Thus, the first 24-hour cercarial output from the field-collected snails could be used as a means of counting cercariae cross-sectionally.

Cross-sectional method of counting cercariae

The cercarial output during the first 24 hours from snails collected in May, June, August, September, October and November 1967 was correlated with the size of the snails, a size-specific cercarial output being obtained (Table 4). The results demonstrated that cercarial output increased with the snail size up to a diameter of 15 mm and then declined in larger snails.

TABLE 3

DAILY CERCARIAL OUTPUT DURING THE FIRST 10 DAYS FROM 25 INFECTED *BIOM. ALEXANDRINA* COLLECTED IN THE FIELD

Days after collection	Mean no. of cercariae per snail	Range in numbers of cercariae
1	1 205.0	8–8 000
2	987.3	1–8 600
3	1 183.5	83–6 300
4	1 529.7	148–8 025
5	1 349.1	96–6 475
6	1 289.6	59–8 900
7	994.8	1–3 175
8	1 268.5	36–8 900
9	958.8	34–7 250
10	735.4	11–4 000

TABLE 4

CERCARIAL OUTPUT FOR THE FIRST 24 HOURS OF SIZED *BIOM. ALEXANDRINA* AFTER THEIR COLLECTION IN THE FIELD IN MAY, JUNE, AUGUST, SEPTEMBER, OCTOBER AND NOVEMBER 1967

Size (mm)	No. of snails	No. of cercariae shed per snail	
		Mean	Range
5.1–9.0	25	227.0	1–1 368
9.1–11.0	15	570.5	45–2 500
11.1–12.0	11	854.0	10–2 050
12.1–13.0	17	1 190.1	2–3 500
13.1–14.0	26	1 516.8	50–8 000
14.1–15.0	10	1 603.3	111–3 500
15.1–17.0	10	995.2	2–2 166

From June 1967 to May 1968, a total of 587 infected *Biom. alexandrina* was collected from the field. Their sizes and numbers infected are shown in the following tabulation:

Size (mm)	*No. of infected snails*
5.1–9.0	83
9.1–11.0	128
11.1–12.0	91
12.1–13.0	90
13.1–14.0	110
14.1–15.0	56
15.1–17.0	29

From the size composition of groups of naturally infected snails and the size-specific cercarial shedding potential as shown in Table 4, the over-all cercarial shedding potential of naturally infected snails was estimated as 957.7 cercariae per snail per day.

DISCUSSION

Development of sporocysts and cercariae in trematodes is related to the sizes of the intermediate hosts (Mathies & Cort, 1956) and *S. mansoni* is no exception. Barret & Barbosa (1959) demonstrated that the mean daily cercarial output from *Biom. glabrata* 13 mm–16 mm in diameter was more than double the number from snails 7 mm–10 mm in diameter. The studies described in this paper showed that experimentally infected snails 9 mm–10 mm in diameter shed 4 times more cercariae than those only 5 mm–6 mm in diameter. From naturally infected snails, the cercarial output is size-specific and increases with size up to a diameter of 15 mm and then declines in larger snails.

From available information, it appears that there is a wide range in maximum total cercarial output from *Biom. glabrata*; the highest maximum being 563 375 (Barbosa, Coelho & Dobbin, 1954) and lowest maximum 37 568 (Schreiber & Schubert, 1949). For *Biom. alexandrina* in the present study, the highest maximum output was 112 354 cercariae from a naturally infected snail; its pre-collection output was, of course, unknown.

Available information on the daily cercarial output of *S. mansoni* from various intermediate hosts is summarized in Table 5. Data show that even from *Biom. glabrata*, the numbers range from 380 to 4598, i.e., a 12-fold difference. The average daily output from *Biom. sudanica*, namely, 499, estimated by McClelland (1967) was quite close to the average (535) obtained from our experimentally infected *Biom. alexandrina* 9–10 mm in diameter. Even among the African species of *Biomphalaria*, the range is from 133 to 1006 with a 7.5-fold difference; both the upper and lower values of the range were obtained in the present study. Such a great variation may be due to a difference in size of snails at the time of shedding. Since these numbers were mainly obtained by longitudinal counting of cercariae from snails of a certain size-range under special conditions, the size-composition of infected snails in the field was unavoidably disregarded. Thus, these results are unlikely to be representative of the cercarial shedding potential of snails in the field.

By the longitudinal method of counting, snails 9 mm–10 mm in diameter are estimated to shed 535

TABLE 5
SUMMARY OF DATA ON DAILY CERCARIAL OUTPUT OF *SCHISTOSOMA MANSONI* FROM VARIOUS SPECIES OF SNAILS

Snail host	Daily cercarial output		Authors
	Maximum	Average	
Biom. glabrata (formerly *Australorbis glabratus*)	7 500	—	Faust & Hoffman (1934)
	5 721	1 300	Giovannola (1936)
	4 158	698	Schreiber & Schubert (1949)
	—	380	Standen (1949)
	17 600	4 598	Barbosa, Coelho & Dobbin (1954)
Biom. pfeifferi	1 000	—	Gordon, Davey & Peaston (1934)
Biom. angulosa	4 168	280	Sturrock (1965)
Biom. sudanica	1 500	499	McClelland (1967)
Biom. alexandrina	—	290	Standen (1949)
Biom. alexandrina			
5 mm–6 mm in diameter	1 379	133	Present study
9 mm–10 mm in diameter	2 196	535	Present study
Naturally infected snails, 12 mm–15 mm in diameter	8 900	1 006	Present study

cercariae per snail per day, and by the cross-sectional method, snails 9 mm–11 mm in diameter are estimated to shed 570.5 cercariae per snail per day. If the size-composition of experimentally infected snails is adjusted to that of naturally infected snails, it is possible that the daily cercarial outputs as estimated by these two methods would be in closer agreement. Thus, by the cross-sectional method, the daily cercarial output from infected *Biom. alexandrina* becomes 957.8, which seems to be closer to the shedding potential of snails in the field. However, there are still limitations to this method since cercarial output is known to vary with the season (Shattock, Fraser & Garnett, 1965). This point is considered in another paper (Chu & Dawood, 1970).

The duration of *S. mansoni* infections in snails has been reviewed by McClelland (1965). The results of our study show that naturally infected snails survive longer under laboratory conditions than do experimentally infected snails. The mean length of life of infected snails in the field is not known, although Hairston (1965) estimated from successive field samplings in the Nile Delta area that it is 18.27 days, assuming that no new infection appears during the interval between collections.

Gordon, Davey & Peaston (1934) reported that in *Biom. pfeifferi* the number of cercariae discharged by an infected snail on the first day of shedding was always small but that the output increased rapidly and after a few days large numbers were shed. Faust & Hoffman (1934) observed 4 infected *Biom. glabrata* and found that the peak of shedding occurred later than 45 days after the first cercariae were shed. Sturrock (1965) found that in *Biom. angulosa* cercarial production reached a peak between day 15 and day 30 of patency and then declined to a lower, but steady, level. In our experimentally infected snails, the peak of shedding was reached between day 20 and day 30 of patency for snails 5 mm–6 mm in diameter, but within the first 10 days of patency for snails 9 mm–10 mm in diameter. Possibly, snail size may affect the timing of maximum output. A peak which is reached immediately after the start of cercarial shedding will have epidemiological importance; since the cercarial incubation period is short and man–water contact is long and frequent in warm seasons, cercarial production will be increased by an early development of the shedding peak. Other factors which may affect the peak of cercarial shedding are temperature and season. Our other studies have shown that when the parasite matures in a cold season, the peak of shedding may not be reached for 2 or 3 months after the first shedding is observed.

ACKNOWLEDGEMENTS

The authors express their appreciation to Dr Farag Rizk, Under-Secretary, Ministry of Health, and Dr Ayusha Abd el Aziz, Director General of Endemic Diseases Department, Ministry of Health, UAR, and director of the Egypt-49 Project, for their interest and encouragement during the study. The assistance received from Dr M. H. Soussa and other members of the project is also greatly appreciated.

RÉSUMÉ

PRODUCTION DE CERCAIRES PAR *BIOMPHALARIA ALEXANDRINA* INFECTÉ PAR *SCHISTOSOMA MANSONI*

On a procédé au laboratoire à une étude longitudinale et transversale de la production de cercaires par *Biomphalaria alexandrina* porteur d'une infection expérimentale ou naturelle à *Schistosoma mansoni.* Cette recherche visait surtout à préciser les relations entre la taille des mollusques et l'intensité des émissions de cercaires.

Dans un premier temps, on a mesuré quotidiennement, dès la première émission, la quantité de cercaires libérées par des mollusques infectés expérimentalement dont 29 (groupe 1) atteignaient 5-6 mm et 15 (groupe 2) 9-10 mm de diamètre. Des numérations identiques ont été effectuées, à dater du jour de la récolte sur le terrain, à partir de 25 mollusques porteurs d'une infection naturelle: 8 spéciments (groupe 3) de 5-9 mm de diamètre, 10 (groupe 4) mesurant 9-12 mm, et 7 (groupe 5) dont la taille atteignait 12-15 mm.

Les mollusques du groupe 2 ont émis 4 fois plus de cercaires que les mollusques du groupe 1, bien que la durée de l'infection ait été quasi identique dans les deux cas. Chez *Biomphalaria* infecté naturellement, on a noté également une corrélation entre la taille et la production de cercaires; dans les conditions du laboratoire, la survie de ces mollusques a été plus longue que celle de leurs congénères infectés expérimentalement. Les quantités moyennes de cercaires libérées ont été de 133, 535, 128, 168 et 1007 par mollusque et par jour respectivement dans les groupes 1 à 5. Le nombre maximal de parasites produits au cours d'une journée (par un mollusque infecté naturellement) a été de 8900. Le clocher d'émission a été atteint dans les 10 jours suivant le début des numérations dans tous les groupes, sauf dans le groupe 1 où il n'a été observé qu'après 20-30 jours.

On a d'autre part compté les cercaires émises pendant les 24 heures suivant la récolte sur le terrain par des *Biomphalaria* — dont la taille a été mesurée — infectés par *S. mansoni.* Les numérations ont mis en évidence une relation spécifique entre la taille du mollusque et l'intensité de la production de cercaires, cette dernière augmentant proportionnellement aux dimensions de l'hôte jusqu'à 15 mm, puis diminuant chez les mollusques de plus forte taille.

En se basant sur la répartition selon les dimensions de 587 *Biom. alexandrina* infectés recueillis sur le terrain et sur l'aptitude à la production de cercaires caractéristique de chaque groupe de taille, on évalue à 957,7 cercaires par mollusque et par jour le potentiel d'émission d'une population de mollusques porteurs d'une infection naturelle.

REFERENCES

Barbosa, F. S., Coelho, M. de V. & Dobbin, J. E. (1954) *Publ. avuls. Inst. Aggeu Magalhães*, **3**, 79-92

Barret, A. C. & Barbosa, F. S. (1959) *An. Soc. Biol. Pernambuco*, **16**, 13-18

Chu, K. Y. & I. K. Dawood (1970) *Bull. Wld Hlth Org.*, **42**, 575-580

Chu, K. Y., Sabbaghian, H. & Massoud, J. (1966) *Bull. Wld Hlth Org.*, **34**, 121-130

Faust, E. C. & Hoffman, W. A. (1934) *Puerto Rico J. publ. Hlth*, **10**, 1-47

Giovannola, A. (1936) *Proc. helminth. Soc. Wash.*, **3**, 60-61

Gordon, R. M., Davey, T. H. & Peaston, H. (1934) *Ann. trop. Med. Parasit.*, **28**, 323-418

Hairston, N. G. (1965) *Bull. Wld Hlth Org.*, **33**, 45-62

Mathies, A. W. & Cort, W. W. (1956) *J. Parasit.*, **42**, 429-431

McClelland, W. F. J. (1961) In: East African Institute for Medical Research, *Annual report, 1960-61*, Nairobi, Government Printer, p. 12

McClelland, W. F. J. (1965) *Bull. Wld Hlth Org.*, **33**, 270-276

McClelland, W. F. J. (1967) *Exp. Parasit.*, **20**, 205-218

Schreiber, F. G. & Schubert, M. (1949) *J. Parasit.*, **35**, 91-100

Shattock, M. S., Fraser, R. J. & Garnett, P. A. (1965) *Bull. Wld Hlth Org.*, **33**, 276-278

Standen, O. D. (1949) *Ann. trop. Med. Parasit.*, **43**, 268-283

Sturrock, R. F. (1965) *Ann. trop. Med. Parasit.*, **59**, 1-9