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ASPECTS OF THE CROWDING EFFECT IN *BIOMPHALARIA GLABRATA* (SAY, 1818) EVALUATED BY ^{59}Fe UPTAKE

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SUMMARY

The crowding effect in *Biomphalaria glabrata* was investigated using, for quantitative assessment, snail capacity for ^{59}Fe uptake. The results presented showed that: 1) an increase in population of *B. glabrata* corresponds to a decrease of ^{59}Fe uptake; 2) a period of about 3 days after individual isolation is necessary for snail recovery from the crowding effect; 3) no statistically significant difference was found when infected and uninfected snails, kept in crowded or uncrowded conditions, were compared in relation to ^{59}Fe uptake.

INTRODUCTION

The crowding effect has been investigated by different Authors in relation to various organisms. THOMPSON [24] claims that insect excretion products could govern the density of insect populations. SAUNDERS [22] refers that AKEHURST [1] used the word "toxin", BETHE [3] "ectohormone", HUXLEY [9] "parahormone", and LUCAS [16] "ectocrine" to characterize substances existing in water with algae which control the growth of these organisms. KARLSON & BUTENANDT [4] suggest the word "pherhomone" for a substance that, being excreted by animals to external environment, induces a specific reaction to animals of the same species. KIRSCHENBLATT [14] introduced the word "telorgone" that can be split in "homotelorgone" that corresponds to the "pherhomone" and "heterotelorgone" that acts in animals from other species. RICHARDS [19] and ROSE & ROSE [21], working with *Rana pipiens* showed the presence of a homotelorgone in water with tadpoles. KARNAVAR & NAIR [13] described a lipid substance in the feces of *Trogoderma granarium* (*Coleoptera, Dermestidae*) which, when ingested by the larvae, increases their diapause. IKESHOJI & MULLA [10], working with larvae of *Culex pipiens quinquefasciatus* in overcrowding, under axenic and xenic conditions, found heterotelorgones under both conditions. MOORE & WHITACRE [17], working with *Anopheles aegypti* larvae under overcrowding conditions found specific autotoxins (homotelorgones) that decrease the growth and increase the mortality of the larvae.

TURNER [25] was the first to study the crowding phenomenon in populations of snails (*Lymnea*). He observed that overcrowding induces a status of semi-aestivation and a decrease of growth. CHERNIN & MICHELSON [4, 5], RITCHIE et al. [20], and PERLOWAGORA-SZUMLEWICZ [18] observed a decrease of egg laying and growth in *Biomphalaria glabrata* under overcrowding conditions. JOBIN & MICHELSON [11] think that it is possible to

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estimate population growth by means of a mathematical formula. These authors do not believe in crowding substances and paid great importance in food competition, physical attrition among snails and gross pollution. WRIGHT [26] claims that inhibitory substance(s), produced by *Bulinus forskalii*, existis in the water where the snails are reared. In the laboratory, WRIGHT [26] observed that it is impossible to rear 5 to 10 snails in a volume of 200 ml without changing the water. On the other hand, isolated snails, in the same volume of water/snail (20 to 40 ml), thrive very well. A substance soluble in chloroform was isolated by BERRIE & VISSER [2] from the Kajansky lake (Uganda) were a dense population of *Biomphalaria sudanica* was found. It was demonstrated that the substance inhibited the growth of *B. sudanica* and was lethal at higher doses. STURROCK & STURROCK [23] consider that the crowding effect in *Biomphalaria glabrata* may be more important than has been thought previously and the recorded results could not be explained by lack of oxygen and by chemical pollution. GAZZINELLI et al. [8] showed that there is a correlation between the crowding effect when measured by growth inhibition and iron uptake.

In this paper the following aspects of the crowding effect were investigated: a) time necessary to recover from the effects of crowding conditions; b) ^{59}Fe uptake in relation to population density; c) effect of parasitism of *B. glabrata* by *S. mansoni* on ^{59}Fe uptake under normal and crowded conditions. A standardization of the ^{59}Fe uptake method was made by COELHO [6] and COELHO et al. [7].

MATERIALS AND METHODS

Snails

Biomphalaria glabrata, kept in the laboratory for 10 years, were used in the experiments.

^{59}Fe

Ferrous citrate (Ferrutope, Squibb), with a specific activity of 4.98 mc/mg Fe was used within the period when the isotope had at least 30% of its initial activity.

Food

The only source of food was dried and powdered lettuce.

Radio analyzer

A Nuclear Chicago radiation analyzer model 181-B, calibrated to 1.30 Mev (Base level 0.620 x 2 window width 6), was used. The results are expressed in counts per minute (cpm) without any correction except for background.

Water

The water employed in the experiments designed to study ^{59}Fe uptake by the snails was distilled twice before being deionized. This water is free from iron — to avoid isotopic dilution — and chlorine. In other experiments deionized water was used.

RESULTS AND COMMENTS

Experiment 1

Recovery of B. glabrata *from the crowding effect* — One hundred *B. glabrata* (0.6 to 0.8 cm in shell diameter) were introduced into a glass aquarium containing 1 liter of deionized water. Artificial aeration was maintained constant and water temperature kept at 27 ± 0.5°C. The snails remained in the aquarium for one week, without changing the water. After the end of one week, the snails were put individually in 300 ml beakers containing 250 ml of deionized water, changed daily. With the interval of 3 days, starting from day 0, groups of 10 snails were put in a 500 ml beaker containing bidistilled water and 300 ml of deionized water with 2.0 μCi ^{59}Fe. Food (8 mg dried lettuce/snail) was supplied daily. One day later the snails were carefully washed in tap water, dried with filter paper and the radioactivity of each one measured with a radio analyzer. The results obtained were summarized in Table I. There are evidences that a period of about 3 days is enough for the snails to recover completely from the crowding effect. Actually, the mean of counts obtained after 3 days, when compared with day 0 showed a difference statistically significant ($p < 0.001$). On the other hand, no statistical difference between the mean counts of 3, 6 and 9 days was found.

TABLE I

Recovery of *Biomphalaria glabrata* from the crowding effect

Days after individual isolation of snails	Number of snails	Radioactivity (cpm) Mean and standard error	Coefficient of variation (%)
0	10	466 ± 80	17
3	10	736 ± 136	24
6	10	858 ± 402	47
9	10	921 ± 233	25

TABLE II

^{59}Fe uptake in relation to population density

Groups	Number of snails	Radioactivity (cpm) Mean and standard error	Coefficient of variation (%)
A	5	622 ± 180	27
B	5	650 ± 42	6
A	10	445 ± 82	19
B	10	457 ± 92	20
A	20	237 ± 56	24
B	20	234 ± 72	31
A	40	161 ± 42	26
B	40	143 ± 41	27

Experiment 2

^{59}Fe uptake in relation to population density — Groups of *B. glabrata* (4 to 5 mm in shell diameter) were put into poliethylene bags with 200 ml of deionized water. Beakers of 250 ml were used for supporting the bags. The water was not changed within the period of experiment and food (dry lettuce) was supplied as 4 mg/snail/day. Temperature was kept constant (27 ± 0.5°C) and aeration in excess. ^{59}Fe was added in order to have a final amount of 0.6 μCi in each bag.

Two groups (A and B), in the same conditions, were used to check the reproducibility of the results. In each group the bag number 1 contained 5 snails; the number 2, 10 snails; the number 3, 20 snails, and the number 4, 40 snails. After one week the snails were washed carefully, dried and counted individually. The results are shown in Table II.

Estimations were done of the percentage of total iron uptake from each group of snails in relation to the iron offered. The group with 40 snails incorporated 7.5% of all iron offered, the group with 20 snails, 16.9%, the group of 10, 16.2% and the group of 5 snails, 11.4%. These data show that isotopic dilution (food supplied, inactive iron from the shell of snails) had no important role in the results of the experiment.

Statistical analysis (Student test) gave the following information: a) comparison between groups of 5 and 10 snails, $p < 0.01$; b) comparison between groups of 10 and 20 snails, $p < 0.001$; comparison between groups of 20 and 40 snails, $p < 0.001$.

In can be concluded that the increase in population corresponds to a decrease in individual snail incorporation of ^{59}Fe.

Experiment 3

^{59}Fe uptake by infected and uninfected B. glabrata *without interference of the crowding effect* — This experiment was designed to determine the effect of parasitism on the ^{59}Fe uptake by snails. Ten *B. glabrata* (1.5 cm in shell diameter) shedding *S. mansoni* cercariae and 10 uninfected snails (1.5 cm in shell diameter) were isolated individually in beakers containing 300 ml of deionized water. The water was changed daily for a period of 10 days. Infected and uninfected snails were put in two beakers containing 300 ml of bidistilled and deionized water with 2.0 μCi ^{59}Fe per beaker. The temperature was kept at 27 ± 0.5°C and aeration in excess. Dried lettuce was used as food (8 mg/snail). After 24 hr the snails were washed, killed in warm water (70°C) and the soft parts taken off from the shell.

Measurements were done with the soft parts. The results are shown in Table III. As can be seen, ^{59}Fe uptake was greater (753 ± 184) in infected snails than in uninfected controls (624 ± 122), but this difference was not statistically significant. LEE & CHENG [15] observed that infected *B. glabrata* incorporate more ^{59}Fe than uninfected snails.

Experiment 4

^{59}Fe uptake by infected and uninfected B. glabrata *under the crowding effect* — This experiment was designed to verify any difference between infected (*S. mansoni*) and uninfected snails, under the crowding effect, in relation to ^{59}Fe uptake.

Fifty infected snails (1.5 cm in shell diameter) were incubated for 3 days in 1 liter of deionized water. The temperature was kept constant (27 ± 0.5°C), and aeration in excess. Food (8 mg/snail, dried lettuce) was supplied daily. Fifty uninfected snails (1.5 cm in shell diameter) were maintained at the same conditions. Ten snails of each group were put in beakers containing 300 ml of deionized water and ^{59}Fe (2 μCi) was added. After 24 hours the snails were killed in warm water (70°C) and the soft parts taken off from the shells. The results of radioactive measurements of the soft parts are shown in Table IV. Despite the fact that the 2 means were different (infected snails, 509 ± 140); uninfected, 371 ± 151) this difference was not statistically significant.

TABLE III

^{59}Fe uptake by infected (*S. mansoni*) and uninfected *Biomphalaria glabrata* without interference of the crowding effect

Snails	Number of snails	Radioactivity (cpm) Mean and standard error	Coefficient of variation (%)
Infected	10	753 ± 184	24
Uninfected	10	624 ± 122	20

TABLE IV

^{59}Fe uptake by infected (*S. mansoni*) and uninfected *Biomphalaria glabrata* under the crowding effect

Snails	Number of snails	Radioactivity (cpm) Mean and standard error	Coefficient of variation (%)
Infected	10	509 ± 140	28
Uninfected	10	371 ± 151	41

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TABLE V

Activity of overcrowding water from infected and uninfected snails in relation to ^{59}Fe uptake

Overcrowding water	Number of snails	Radioactivity (cpm) Mean and standard error	Coefficient of variation (%)
From infected snails	7	385 ± 27	7
From uninfected snails	7	353 ± 28	8

Experiment 5

Activity of overcrowding water from infected and uninfected snails in relation to ^{59}Fe uptake — Fifty snails (1.5 cm in size diameter), shedding *S. mansoni* cercariae, were introduced in 1 liter of deionized tap water. The temperature was kept at 27 ± 0.5°C and aeration constant. Dried lettuce was given in the amount of 8 mg per snail. The snails were maintained under these conditions for 3 days. Uninfected snails of the same size were kept under the same conditions for 3 days.

The activity of the overcrowding water from infected and uninfected snails was compared. In 2 beakers containing 300 ml of water from each group, 7 snails, which had been previously kept isolated for 5 days, were put in each beaker. ^{59}Fe was added (2 μCi) and, after 24 hr, iron uptake was measured. The results are shown in Table V. As can be seen, no difference between the 2 means (cpm) was observed. Therefore, there is no difference in the production of inhibitory substance(s) from infected and uninfected snails.

RESUMO

Aspectos do efeito "crowding" em Biomphalaria glabrata *(Say, 1818) avaliado pela captação de ferro radioativo (^{59}Fe)*

Os fenômenos decorrentes de pressão populacional foram estudados em *Biomphalaria glabrata* através da captação de ferro radioativo (^{59}Fe) pelos caramujos. Os resultados obtidos permitem concluir que: 1) um aumento da população de caramujos corresponde a uma diminuição individual da capacidade de captar ^{59}Fe; 2) é necessário um período de cerca de 3 dias de isolamento individual para a recuperação do caramujo dos fenômenos decorrentes de uma situação de superpopulação; 3) nenhuma diferença estatisticamente significativa foi encontrada quando caramujos infectados (*Schistosoma mansoni*) e não infectados, em situação de superpopulação e de isolamento, foram pesquisados quanto à capacidade de captação de ^{59}Fe.

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