

## STUDY OF THE EFFECTS OF GAMMA-RADIATION ON EGGS AND MIRACIDIA OF *SCHISTOSOMA MANSONI*

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### SUMMARY

*Schistosoma mansoni* eggs and miracidia from human feces were irradiated with Cobalt-60, the doses varying from 0.5 to 200 Krad. *B. glabrata* were exposed either to miracidia from irradiated eggs (10 to 200 Krad) or to irradiated miracidia themselves (1 to 50 Krad). In both experiments the miracidia penetrated the snails, but they did not develop. However, when using 0.5-Krad-irradiated miracidia, infection could be detected in 3.2% of the snails.

Groups of *B. glabrata* were exposed to irradiated miracidia (0.5 Krad), and, later on, after periods of respectively, 10, 20 and 30 days, these groups were re-exposed to non-irradiated miracidia. The percentages of infection were, then, seen to be 62.2, 83.3 and 77.7%, respectively. These data show that previous infection with irradiated miracidia could not render the snails immune.

### INTRODUCTION

Many investigators, including LEVIN & EVANS<sup>10</sup>, BACHOFER & PAHL<sup>1</sup>, GOMBERG & GOULD et al.<sup>6</sup>, VILLELA et al.<sup>19</sup>, JARRET et al.<sup>7, 8</sup>, JENNINGS et al.<sup>9</sup>, CHAIA & MURTA<sup>4</sup>, CHAIA<sup>3</sup>, have reported the effects of gamma-radiation on helminth eggs and larvae. The studies with *Schistosoma mansoni* have been carried out with an aim to verify the effects of this radiation on *S. mansoni* cercariae by RADKE & SADUM<sup>13</sup>, SZUMLEWICZ-PERLOWAGORA & OLIVER<sup>17</sup>, SZUMLEWICZ-PERLOWAGORA<sup>15</sup>, OLIVEIRA et al.<sup>11</sup> and on this parasite's intermediate hosts by SZUMLEWICZ-PERLOWAGORA<sup>15</sup>, SZUMLEWICZ-PERLOWAGORA & BERRY<sup>18</sup>.

The present paper studies some effects of gamma-radiation on eggs and miracidia of *S. mansoni*, as well as the influence of such

radiation on the hatching of the eggs and on the penetration power and development of miracidia in the intermediate host, and reports some data from an attempt at immunization of snails with irradiated miracidia.

### MATERIAL AND METHODS

#### 1) *Schistosoma mansoni* strain

The *S. mansoni* eggs were obtained from a patient's feces presenting 3,000 eggs per gram, by using the saline layering technique described by RITCHIE & BERRIOS DURAN<sup>14</sup>. The eggs were counted under microscope and the percentages of viable mature ones recorded from samples of 0.01 ml.

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2) *Biomphalaria glabrata* strain

The *B. glabrata* were laboratory-reared specimens displaying a 15-mm diameter. During the experiment they were kept in plastic aquariums containing 5 litres of dechlorinated water and fed on fresh lettuce.

3) *Irradiation of S. mansoni and miracidia*

Eggs and miracidia of *S. mansoni* were placed into pyrex-glass tubes and then irradiated in Gammacell 220. This apparatus displays the following characteristics: Cobalt-60, half life-5.2 years with dosage of 170 Krad per hour in irradiation position, thus giving an average of 2,890 rad per minute. The present activity of the apparatus is 2,700 curie. Roentgen unit is considered similar to that of rad with a variation of 5%.

4) *Infection of snails*

a) with irradiated eggs

Viable mature eggs were irradiated with gamma-ray different dosages (10, 20, 30, 40, 50, 100, 150 and 200 Krad). After that, the eggs were allowed to hatch according to CHAIA's<sup>2</sup> technique. To a 0.01-sample of miracidia suspension was added a drop of 1% acid fuchsine, the miracidia being counted under microscope. Afterwards, groups of 10 miracidia were placed into glass containers for individual infection of *B. glabrata*, according to PELLEGRINO & KATZ<sup>12</sup>. After 24 hours the snails were put back into the aquariums, the miracidia remaining in the glass container being then counted. Forty-five days after infection, the snails were examined by crushing them between two glass plates. The same techniques previously described were also used for the control group, miracidia from non-irradiated eggs being then employed.

b) with irradiated miracidia

The eggs being hatched, the miracidia were exposed to the action of gamma-rays, the dosage varying from 0.5 to 50 Krad. The same techniques already described were used for infection and examination of the snails.

5) *Attempt to immunize B. glabrata*

Eighty *B. glabrata* were individually infected with 10 miracidia irradiated with 0.5 Krad. Forty-six of them were examined 45 days after infection (by crushing). From the remaining snails, groups of 10 specimens were re-infected with non-irradiated miracidia, 10, 20 and 30 days after the first infection and examined, 45 days later, by crushing.

RESULTS

1) *Irradiation of S. mansoni eggs*

All viable mature eggs irradiated with dosages from 10 to 200 Krad were seen to have hatched. The miracidia obtained could penetrate the snails, but they did not develop. 75% of the snails from the control group (infected with miracidia of non-irradiated eggs), were observed to be eliminating *S. mansoni* cercariae.

2) *Irradiation of S. mansoni miracidia*

The data regarding the effects of 0.5-to-50 Krad gamma radiation on miracidia can be found on Table I. The miracidia showed no to have been altered by radiation (considering gross morphology, movements and

TABLE I

Data showing the effect of different doses of gamma radiation on *S. mansoni* miracidia

Gamma-ray dosage (Krad)	Number of <i>B. glabrata</i>		
	Infected	Examined	Positive for <i>S. mansoni</i> (%)
Control	60	38	30 (78.9)
0.5	50	31	1 (3.2)
1.0	50	29	0
10.0	10	8	0
20.0	10	6	0
30.0	10	8	0
40.0	10	9	0
50.0	10	6	0

penetration power), but it must be pointed out that only the miracidia irradiated with 0.5 Krad could develop (3.2% of snails were eliminating cercariae). There was recorded 78.9% of infection in the control group. The snails infected with irradiated miracidia (0.5 and 1 Krad), as well as the non-irradiated miracidia, were dissected 10 and 20 days later for the search of sporocysts, which, however, could be found only in snails from the control group.

### 3) Attempt at immunization of *B. glabrata*

The data regarding the attempt to immunize *B. glabrata*, by using irradiated miracidia, can be seen on Table II. In the control group, exposed to non-irradiated miracidia, a 96.4 percentage of positivity could be checked 45 days later, whereas, in the group exposed to irradiated miracidia (0.5 Krad), the percentage of positivity was found to be 2.2. As concerns the snails re-infected 10, 20 and 30 days after initial infection, there were found positive percentages of 62.2, 83.3 and 77.7, respectively.

## DISCUSSION

The data obtained show *S. mansoni* eggs to be very resistant to radiation, since even a dosage of 200 Krad could not affect their hatching process nor the penetration power of miracidia. Nevertheless, apparen-

tly normal miracidia (morphology and movement) did not develop after having penetrated the snails. Similar fact was reported by VILLELLA et al.<sup>19</sup> in reference to embryonated eggs of *Ascaris suum*, a dosage of 100,000 to 150,000 rep, being necessary to hinder the development of larvae in guinea pig's lungs.

It is worth mentioning that the behavior of miracidia newly hatched and irradiated (1 to 50.0 Krad) was similar to that of miracidia from viable mature eggs which were irradiated (10 to 200 Krad). Nevertheless, by using miracidia irradiated with just 0.5 Krad, about 3% of the snails could be infected.

LEVIN & EVANS<sup>10</sup>, JARRET et al.<sup>7, 8</sup>, CHAIA & MURTA<sup>4</sup> and CHAIA<sup>3</sup> reported having achieved immunization after previous use of irradiated larvae of *Trichinella*, *Dictyocaulus* and *Strongyloides*, respectively, as "vaccine".

However, our attempt at immunization of snails by using irradiated miracidia (0.5 Krad) did not produce satisfactory results.

## RESUMO

*Estudo dos efeitos da radiação gama sobre ovos e miracídios de Schistosoma mansoni*

Ovos e miracídios de *S. mansoni* obtidos de fezes humanas foram irradiados em bomba de Cobalto-60, com doses que variaram de 0,5 a 200 Krad. *B. glabrata* foram ex-

TABLE II

Attempt at achieving snail immunization through irradiated miracidia

Gamma-ray dosage (rad)	<i>B. glabrata</i>			Challenge								
				10 days			20 days			30 days		
	Infected	Examined	% positive	Infected	Examined	% positive	Infected	Examined	% positive	Infected	Examined	% positive
Control	50	28	96.4	—	—	—	—	—	—	—	—	—
500	80	46	2.2	10	8	62.2	10	6	83.3	10	9	77.7

postas à miracídeos provenientes de ovos irradiados (10 a 200 Krad), bem como a miracídeos irradiados (1 a 50 Krad). Em ambas experiências, os miracídeos penetraram nos caramujos, mas não se desenvolveram. Entretanto, utilizando-se miracídeos irradiados com 0,5 Krad houve infecção em 3,2% dos caramujos.

Grupos de *B. glabrata* foram expostos à infecção com miracídeos irradiados (0,5 Krad). Após 10, 20 e 30 dias foram re-expostos à miracídeos não irradiados. O percentual de infecção foi de 62,2, 83,3 e 77,7% respectivamente. Estes dados mostram que a infecção prévia com miracídeos irradiados não conferiu proteção aos caramujos.

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