

## IMMUNIZATION OF RATS WITH IRRADIATED *STRONGYLOIDES* LARVAE

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### SUMMARY

No apparent modification has been observed in the structure of *Strongyloides* worms (*S. ratti* + *S. venezuelensis*) recovered from rats infested with larvae irradiated with 10 to 40 kiloroentgens there has been recorded just a reduction in the number of worms recovered 30 days after the infection with the irradiated larvae. The worms from larvae irradiated with 50 kiloroentgens presented no genital apparatus and were smaller than the normal ones. The percentage of worms from irradiated larvae decreased as the dosage of irradiation increased. The larvae could stand irradiation with 140 kiloroentgens quite well.

Rats vaccinated three times with 10,000 irradiated larvae (140 kr.) and a 15 day interval between one vaccination and another got immune to *Strongyloides* infection.

### INTRODUCTION

Investigators have obtained a certain degree of immunity in hosts after infection with irradiated helminth larvae. As regards strongyloidosis however no studies in this line seem to be so far available. JENNINGS et al.<sup>6</sup> have studied the action of X rays on larvae of *Nippostrongylus brasiliensis*. JARRET et al.<sup>4</sup>, achieved immunization experimenting with irradiated larvae of *Dictyocaulus viviparus*. MILLER<sup>10</sup> has successfully vaccinated dogs with irradiated larvae of *Ancylostoma caninum*. RADKE & SADUN<sup>12</sup> and SZUMLEWICZ-PERLOWAGORA & OLIVER<sup>14</sup> have studied the action of irradiation on *Schistosoma mansoni*.

The present paper studies the action of irradiation on *Strongyloides* larvae and the vaccination of rats with these irradiated larvae.

### MATERIAL AND METHODS

In this experiment there have been used 114 albino rats weighing from 150 to 180 g and divided into several groups. Sixty-eight rats (A, B Table I), subdivided into 6 groups, were subcutaneously infected with about 3,300 irradiated larvae of *Strongyloides* (*Strongyloides venezuelensis* + *Strongyloides ratti*). The rats from group 1 were infected with larvae irradiated with 10 kiloroentgens; those from groups 2, 3, 4, 5 and 6 were infected with larvae irradiated with 20, 30, 40, 50 and 60 kiloroentgens, respectively. Simultaneously, 11 rats taken as controls were infected with equal number of normal larvae from the same sample as those employed above. The rats from groups A and B, as well as their control groups, were sacrificed, respectively, 8 and 30 days after the inoculation of larvae, the worms reco-

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TABLE I

Results obtained in rats infected with larvae irradiated (*Strongyloides venezuelensis* + *Strongyloides ratti*) with 10 to 60 kiloroentgens

| Group | Rats used in the experiment |               |   |                 |        |
|-------|-----------------------------|---------------|---|-----------------|--------|
|       | no. of rats                 | Kiloroentgens | no. of irradiated larvae injected per rat | Worms recovered |        |
|       |                             |               |   | Average number  | %      |
| 1     | 5 A                         | 10            | 3,300                                     | 1,858 A         | 56.2 A |
|       | 8 B                         |               |   | 189 B           | 5.7 B  |
| 2     | 5 A                         | 20            | 3,300                                     | 1,320 A         | 40.0 A |
|       | 6 B                         |               |   | 59 B            | 1.7 B  |
| 3     | 5 A                         | 30            | 3,300                                     | 1,356 A         | 41.0 A |
|       | 6 B                         |               |   | 21 B            | 0.6 B  |
| 4     | 5 A                         | 40            | 3,300                                     | 1,356 A         | 41.0 A |
|       | 6 B                         |               |   | 2 B             | 0.06 B |
| 5     | 5 A                         | 50            | 3,300                                     | 365 A           | 11.0 A |
|       | 6 B                         |               |   | 3 B             | 0.09 B |
| 6     | 5 A                         | 60            | 3,300                                     | 210 A           | 6.3 A  |
|       | 6 B                         |               |   | 1 B             | 0.03 B |

A — Rats sacrificed 8 days after infection. The average number of worms recovered from 6 rats of the control group was 1,943 — or better — 58.8%

B — Rats sacrificed 30 days after infection. The average number of worms recovered from 5 rats of the control group was 688 — or better — 20.8%

vered being then counted by the method of MCGEE et al.<sup>9</sup>. Other rats (Table II) were infected three times with about 10,000 *Strongyloides* larvae irradiated with 140 kr., a 15-day interval being left between one infection and another. Eight days after each infection, some of the rats were sacrificed and the worms recovered were counted by the method referred to above. Fifteen days after the last infection with irradiated larvae, the rats and their control groups were infected with about 3,000 normal larvae. The animals were then sacrificed 8 days after the last infection with irradiated and counted.

*Strongyloides* sample — The larvae used in this research work were obtained from a sample of *Rattus rattus norvegicus* isolated by BRENER & CHAIA<sup>2</sup> and kept in laboratory by successive passages into albino

rats. This sample was later on found to comprise not only larvae of *S. ratti* but also of *Strongyloides venezuelensis*. The rats' feces were mixed with granulated charcoal bone and 48 hours later collected by BAERMANN'S<sup>1</sup> method. The larvae in several 0.1 ml samples were then counted under dissecting microscope, the required number of larvae being then subcutaneously injected in the rats.

*Worm examination* — The worms were collected by the method of MCGEE et al.<sup>9</sup> and then fixed in Bles's fixative, placed in 70% alcohol with 5% glycerin added and, finally, mounted in Jelly glycerin, a technique employed by LITTLE<sup>8</sup>. Seventy worms (40 of them from normal larvae and 30 from larvae irradiated with 50 kr.) were drawn in camera lucida and then measured with the help of a curvometer (Table III).

TABLE II

Results obtained in rats infected with irradiated larvae (140 kr.) and re-infected, later on, with normal larvae (*Strongyloides venezuelensis* + *Strongyloides ratti*)

| Group | no. of larvae injected per rat | no. of rats | Day* of infection | Rats sacrificed |                      |       |
|-------|--------------------------------|-------------|-------------------|-----------------|----------------------|-------|
|       |                                |             |                   | no.             | Worms recovered      |       |
|       |                                |             |                   |                 | Mean number          | %     |
| 1     | 10,000 <sup>+</sup>            | 30          | 1                 | 4               | 5 <sup>va</sup>      | 0.05  |
| 2     | 10,000 <sup>+</sup>            | 26          | 15                | 3               | 0.3 <sup>va</sup>    | 0.003 |
| 3     | 10,000 <sup>+</sup>            | 23          | 30                | 3               | 0.3 <sup>vn</sup>    | 0.003 |
| 4     | 3,000 <sup>ln</sup>            | 20          | 45                | 20              | 6.9 <sup>va+vn</sup> | 0.23  |

The mean number of worms obtained in 5 control rats of group 4, infected with 3,000 normal larvae, was 962, which corresponds to 32.0% recovery.

- \* day of infection
- + larvae irradiated with 140 kr.
- ln normal larvae
- va abnormal worms
- vn normal worms

*Irradiation of larvae* — The larvae were placed into plastic tubes in volumes, at most, 1 cm high and then irradiated into Gama-cell 220. This apparatus displays the following characteristics: Cobalt-60, half life — 5.2 years with a dosage of 287 kilorads per hour in irradiating position, thus giving an average of 4,717 roentgens per minute. The present activity of the apparatus is 3,457 curies. For gamma emissions, the roentgen is considered similar to the rad, with approximate variation of 5%.

RESULTS

The percentage of worms from rats infected with larvae irradiated with 10 to

60 kr. (A, Table I) and sacrificed 8 days after infection ranged from 56.2 to 6.3%. In another group of rats infected with larvae irradiated with 10 to 60 kr. (B, Table I), but sacrificed 30 days after the inoculation of the larvae, the percentage of worms recovered ranged from 5.7 to 0.03% while in the control group is was 20.8%. For further details, see Table I.

The worms from larvae infected with 10 to 40 kr. were structurally normal, whereas those from larvae irradiated with 50 kr. (Fig. 1) presented no genital apparatus and were smaller than the normal ones, the variation in length being statistically significant (Table III). The average normal worm was 2.244 mm while the average worm from irradiated larvae was 2.120 mm.

TABLE III

Measure of normal worms and of worms from larvae irradiated with 50 kiloroentgens

| Worms             | no. of measures | Worm length (mm)             |
|-------------------|-----------------|------------------------------|
| Normal .....      | 40              | 2.575 — 1.818 (2.254 ± 0.15) |
| Irradiated* ..... | 30              | 2.727 — 1.666 (2.120 ± 0.32) |

\* Worms from larvae irradiated with 50 kr.

The numbers in brackets are the mean numbers and their respective standard deviations

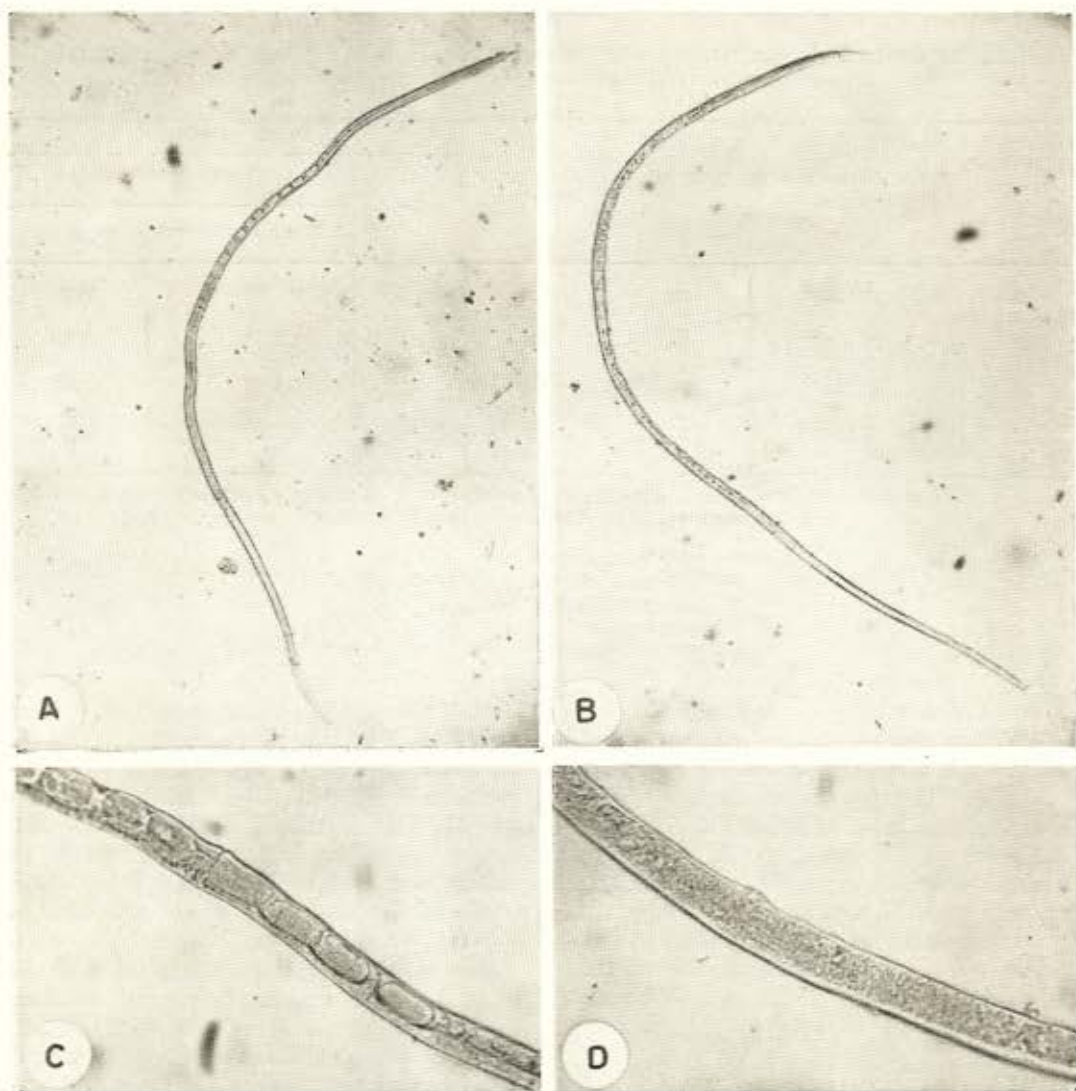


Fig. 1 — Parthenogenetic females of *Strongyloides*. A) Normal worms 36  $\times$ . C) Aspect of the uterus of a normal female worm 150  $\times$ . B) Worm from larvae irradiated with 50 kiloroentgens 36  $\times$ . D) Aspect of the uterus of worm B 150  $\times$ .

The percentage of worms recovered from rats in groups 1, 2 and 3 (Table II) were, respectively, 0.05, 0.003 and 0.003%. In group 4, the percentage of worms recovered was 0.23%, while in its control group such percentage was 32.0%. The genital apparatus was lacking in worms from groups 1 and 2, whereas the only worm recovered from group 3 was structurally normal. As concerns group 4, there have been found worms presenting both normal and abnormal structures (Table II).

#### DISCUSSION

The worms from larvae irradiated with 10 to 40 kiloroentgens presented apparently normal structure. The worms from larvae irradiated with 50 kr. on the contrary, presented no genital apparatus. These abnormalities, however, cannot be said to be transitory or permanent, since the worms in this case were very early eliminated from the host (Fig. 2). The development of their genital apparatus may have been tempora-

rily hindered by the irradiation. In fact, a worm presenting normal structure has been recovered from a rat infected with 30,000 infected larvae (Group 3, Table II).

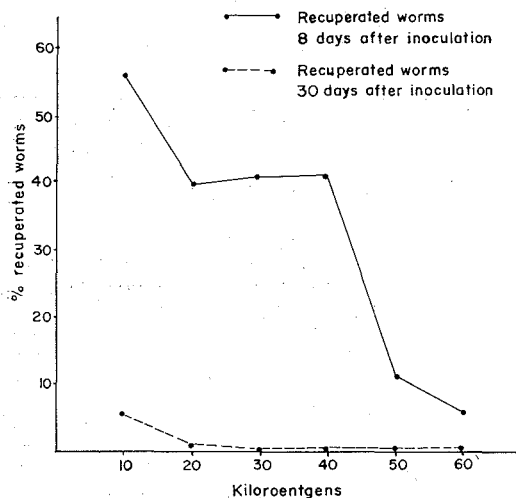


Fig. 2 — Percentage of recovery of *Strongyloides* worms from irradiated larvae. The worms were collected 8 and 30 days after infection.

Sterilization of worms through the irradiation of larvae has already been referred to by LEVIN & EVANS<sup>7</sup> who experimented with *Trichinella spiralis* and by MILLER<sup>11</sup> with regard to *Ancylostoma caninum*. SZUMLEWICZ-PERLOWAGORA & OLIVER<sup>14</sup>, observed a temporary paralyzation in the development of *Schistosoma mansoni* worms. As mentioned above, *Strongyloides* worms from larvae irradiated with 50 kr. were smaller than the ordinary worms, the difference between them being statistically significant (Table III). SZUMLEWICZ-PERLOWAGORA<sup>15</sup>, also recorded morphological changes in *S. mansoni* worms from irradiated cercariae.

SHELDON<sup>13</sup> succeeded in immunizing rats with *Strongyloides ratti* dead larvae kept in saline suspension. JARRET et al.<sup>5</sup> found it of little practical value to immunize calves with adult worm antigen of *Dyctiocaulus viviparus*. Comparing the results obtained with normal and with irradiated larvae of *A. caninum* in the immunization of dogs, MILLER<sup>10</sup> concluded the latter to be superior to the former. CHAIA & CUNHA<sup>3</sup> obtained partial immunization in rats by infecting them with normal larvae of *Strongyloides*.

JARRET et al.<sup>4</sup> achieved immunization in calves after infection with *D. viviparus* irradiated larvae. In our research work we got to immunize rats after 3 vaccinations with irradiated filarioid larvae (140 kr.). Data from another experiment of ours now in progress show that just one vaccination with irradiated *Strongyloides* larvae was sufficient to immunize the rats.

JARRET et al.<sup>4</sup> suggest two vaccinations with irradiated larvae of *D. viviparus* for the immunization of calves. These investigators got better results with lower dosages of irradiation (20 kr.) than with higher ones (60 kr.).

As with a 60 kr. irradiation dosage the percentage of worms recovered was still large, we suggest higher dosage of irradiation (140 kr.) on the larvae to be used in the vaccination of rats (Table I).

The present study does not seem to indicate the worms from irradiated larvae to be responsible for the immunization since with a dosage of 140 kr. the percentage of worms recovered was very low (0.005%), even in the rats sacrificed eight days after infection. Rats would not resist an infection with a number of normal larvae similar to that of irradiated ones used in the immunization of hosts (30,000 larvae). When infected with a smaller number of larvae, the immunization is not complete (CHAIA & CUNHA<sup>3</sup>). When immunity is acquired by the rats, the irradiated larvae seem to be destroyed in the very site of infection.

Studies are being performed to show the destination taken by irradiated larvae when injected in rats as well as their role in the immunization of such animals.

#### RESUMO

#### Imunização de ratos com larvas irradiadas de *Strongyloides*

Não houve aparentemente modificações nas estruturas dos vermes de *Strongyloides* (*S. ratti* + *S. venezuelensis*) provenientes de uma infecção por larvas irradiadas com 10 a 40 kiloroentgens. Apenas, houve uma redução do número de vermes recuperados 30 dias após a infecção das larvas irradiadas. Os vermes provenientes de infecção com larvas irradiadas com 50 kr. não pos-

suiam o aparelho genital e o seu percentual de recuperação foi reduzido. Êstes vermes, foram menores do que os vermes normais. O percentual de recuperação de vermes provenientes de larvas irradiadas, foi tanto mais reduzido quanto maior foi a dosagem de irradiação das larvas. As larvas suportaram muito bem uma irradiação de 140 kr.

Ratos vacinados três vêzes com 10.000 larvas irradiadas (140 kr.), com intervalos de 15 dias entre uma e outra vacinação tornaram-se imunes à infecção pelo *Strongyloides*.

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