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Inhibition of Embryo Formation in Certain Nematodes by Roentgen Radiation.*

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Although it is generally recognized that embryonic tissue is more radiosensitive than that of adults,[1] it was thought of interest to determine the effects of roentgen radiation on certain nematodes in which the young develop inside the body of the mother. Such experiments were undertaken in the hope that (a) they might yield information concerning differential susceptibility of cells, and (b) roentgen radiation might prove to be a useful tool in the study of trichinosis. Results of preliminary experiments[2] have been confirmed and extended in this report.

Experiments with Trichinella spiralis. Larvae, removed from infected rat muscle by pepsin digestion, were irradiated with dosages of radiation ranging from 400 r to 6,400 r (H.V.L. ca. 1.5 mm al., 427 r/m). The larvae were then fed to healthy rats and recovered from the intestine 5 days later. Microscopic study of 33 unirradiated female trichinae indicated a mean number of 59.4 worm-shaped embryos (with a standard error of the mean of 4.4) per female. Seventeen females irradiated with only 400 r contained 27.4 ± 5.5 worm-shaped embryos per animal. An irradiation of 1600 r reduced the number of worm-shaped embryos per female (25 examined) to 16.15 ± 3.25. Irradiations of 2000 r (39 examined) and 2,500 r (14 examined) completely inhibited the formation of worm-shaped embryos, but many late cleavage stages were present. Following irradiations of 3,000 r (23 examined), 3,500 r (23 examined) and 3,750 r (20 examined), embryonic development was stopped in early cleavage stages, although the adults themselves were apparently not injured. A dose of 5,000 r (6 examined) inhibited further development of the irradiated larvae.

Inasmuch as the control as well as the irradiated worms at this time contained some undeveloped embryos, the question arose as

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1 Bergonié, J., and Tribondeau, L., *Compt. Rend. Acad. Sci.*, 1906, **143**, 983.

2 Levin, A. J., and Evans, T. C., *J. Parasit.*, 1940, **26**, 6, suppl. abs. No. 52, p. 31.

to their possible development, in time. Other rats were therefore fed with irradiated larvae and examined 2 months later. This allowed time for development of any viable offspring and their migration to the muscles of the host. The muscle tissue was digested and the number of worms determined by dilution counts. (In cases where there were less than approximately 2,000 worms, direct counts were made.) In Fig. 1 the numbers of worms recovered from the muscle at the end of 2 months are compared to the numbers recovered from the intestine at the end of 5 days. It can be seen from these data that the dosages required to prevent the formation of viable larvae which migrate to the muscle are much lower than those necessary to reduce the intestinal infection appreciably. Although the survival of the larvae in the muscle phase following heavy irradiation (3,500 r to 6,400 r) appears in the figure as being zero, actually from 0 to 18, (average of 7) worms were recovered. However, when one considers that the average muscle infection of rats infected with unirradiated nematodes in these experiments (controls) was 541,800, the numbers recovered from rats infected with heavily irradiated larvae are comparatively negligible. The formation of a second generation is prevented by a dosage of radiation almost 2,000 r less than that required to destroy the adults in the intestine. Therefore, it appears possible to produce, experimentally, an intestinal infection of *Trichinella spiralis* in the rat without a consequent muscle invasion.

Experiments with Rhabditis pellio. The experiments with trichinella were limited to irradiation at only one stage in the life cycle and it was impossible to follow the same individual from irradiation to the time when it should produce offspring. For this reason, experiments were performed with *Rhabditis pellio.* One of the authors[3] has cultured a pure line of these nematodes through many generations. It is therefore possible to irradiate the organisms in any stage of its life cycle and to follow the subsequent changes in the same individual.

The developmental stage selected for irradiation was that just prior to maturity. After some few preliminary experiments, it was found that results similar to those obtained with trichina could be obtained by an irradiation dose of 5,000 r. The irradiated organisms grew to maturity and some lived as much as 12 days longer than the controls. Fertilization took place but embryonic development was arrested in early cleavage stages. In later experiments, irradiated females were isolated after copulation and the number of offspring produced was observed from day to day. For example, in one ex-

[3] Sulkin, N. M., *Proc. Ia. Acad. Sci.*, 1940, **47**, 415.

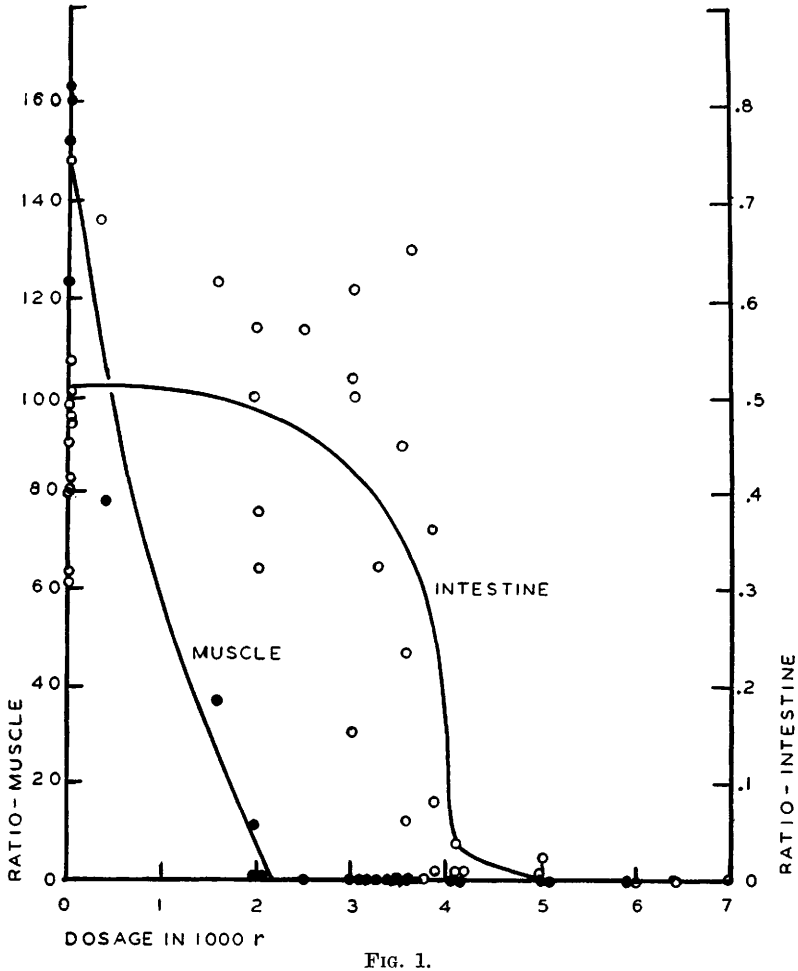

FIG. 1.

Infective larvæ of *Trichinella spiralis* were irradiated, as indicated on the abscissa, and then fed to rats. The ordinates indicate the larvæ-larvæ ratios (number of larvæ recovered divided by the number of larvæ fed). Open circles (○) indicate the larvæ-larvæ ratios of worms recovered from the intestine five days after infection, and the closed circles (●) indicate ratios of worms recovered from the muscles of rats two months after infection. The curves were fitted by inspection.

periment it was found that whereas the controls produced a total of 86.5 ± 7.95 offspring per female, the worms irradiated with a dosage of 4,928 r produced only 2.5 ± 1.5. An irradiation of 6,160 r permitted the mean production of only 0.35 offspring per female, and dosages of 7,076 r and 7,992 r completely inhibited the development of offspring in all cases. Each control and irradiated lot consisted of twenty females, in individual vials.

Discussion. The possibility of using radium radiation in the treatment of trichinosis was tested as early as 1916 by Tyzzer and Honeij.[4] They irradiated infective larvae *in vitro* and in the intestine of the host. It was found that irradiation of the larvae before feeding them to the rat reduced the number containing worm-shaped embryos when examined a few days later. Heavy irradiation of the larvae was fatal to them. Attempts to destroy the trichinae by placing radium needles in the intestine were unsuccessful. Radium radiation applied to the surface of the abdomen of infected rats failed to injure fully developed trichinae in the intestine. The irradiated worms appeared well developed and persisted longer in the intestine than did the controls. Although the experiments failed to demonstrate the therapeutic value of such radiation in the treatment of trichinosis, they indicated that the radiation appreciably modified the development of the parasite.

We can confirm the finding of the above authors, *viz.*, that irradiated adults sometimes persist longer than the controls. This was found to occur in Rhabditis when the dosage was such as to destroy the embryos but permit the adult to survive. It appears probable that the death of the female is hastened by the development of the larvae which apparently destroy the internal organs of the mother. When offspring are destroyed by radiation, the internal organs of the female remain intact and it continues to feed and grow for several days after the controls have become mere shells containing active, growing larvae.

Honess[5] found that a dosage of 800 r given to larvae before feeding them to rats lowered the resulting muscle infection to about 15% of that of the controls. This finding, that even light doses of roentgen radiation given to trichinae before feeding them to rats greatly reduces the number of offspring produced, is in general agreement with our results.

Experimental results have been obtained in these investigations which are in agreement with the recognized factors of radiosensitivity, and with results obtained by other workers in this field. We wish to suggest that it should be possible, by irradiating infective larvae of *Trichinella spiralis* with dosages of 3,000 to 4,000 r before feeding them to rats, to obtain information as to whether an intestinal infection, without the consequent muscle invasion, can produce re-

[4] Tyzzer, E. E., and Honeij, J. A., *J. Parasit.*, 1916, **3**, 43.

[5] Honess, R. F., *J. Colorado-Wyoming Acad. Sci.*, 1940, **2**, 44, *Biol. Abs.*, **14**, No. 14045.

sistance to subsequent infections of Trichinella. Investigations of this type are being carried on at present in this laboratory.

Summary and Conclusions. 1. Immature forms of *Trichinella spiralis* and *Rhabditis pellio* have been irradiated with varying doses of roentgen radiation. 2. It was found that, at proper dosages, the radiation permitted the organisms to grow to maturity, to undergo copulation, and to begin the development of embryos, but the radiation killed the embryos before their development was completed. 3. Based on the foregoing data, it should be possible to ascertain whether an intestinal infection of *Trichinella spiralis,* without the consequent muscle invasion, can produce resistance to subsequent infections of Trichinella.

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Experimental Study of Factors Inhibiting Differentiation of Proctodeum.

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Introduction. It was previously reported (Schechtman[1]) that the ventrolateral blastoporal lip of the early gastrula *(Hyla regilla)* is capable of inducing a proctodeum in the belly epidermis of another embryo. The percentage of positive results was extremely variable: occasionally a group of 6 host embryos might develop an accessory proctodeum in all 6 cases; however, in most groups of 6, positive results occurred in only 1, 2, or 3 cases. This variability persisted in over 200 implantations carried out during the laying seasons of 3 consecutive years. In the entire group slightly more than 50% showed clear accessory proctodea. Accessory proctodea and tails were also produced by centrifuging late blastulae and early gastrulae (Schechtman[2]). An analysis of 983 such embryos by one of us (Cannon, unpublished) showed that only 4% were clearly positive. A striking characteristic of both implanted and centrifuged eggs is that accessory proctodea, with rare exceptions, develop on the belly region.

The aim of the present investigation was to determine the causes behind the great variability shown by the ventral blastoporal lip in

1 Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 48.

2 Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 153.