Preliminary Report of X-Ray Effects on the Nematode *Rhabditis strongyloides*

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Numerous articles have appeared in the literature dealing with the effects of irradiation of nematodes where the emphasis has been on lethal effects. In our investigation we are concerned with the nonlethal effects of the characters of adult individuals of a bisexual nematode, *Rhabditis strongyloides*. This worm appears to be different, in respect to observable changes, from animals, plants, and bacteria used in conventional genetic studies.

The nematodes used in this study were obtained from Norman D. Levine, of the College of Veterinary Medicine, University of Illinois. These roundworms came from skin lesions resembling scabies found infesting a dairy cow on a farm near Lincoln, Illinois.

Several worms were obtained from under scabs and introduced to sterile nutrient agar plates poured about 1 mm thick. About one week later these Petri dish cultures were sprinkled lightly with granulated blood fibrin. The following day they were teeming with worms in all stages of development. By the use of blood fibrin it was possible to control the production of worms at will and to maintain a pH of about 8 in the culture. A new generation of worms can be produced every 3 days. Some cultures were allowed to dry slowly at room temperature. After several weeks or months in the dry state they revived within a few hours whenever a film of water was added. Dry cultures were also scraped from the Petri dishes as a dry flakelike dust and sprinkled lightly on fresh nutrient agar. Within 24 hr the worms revived. From the above stocks, cultures were taken for the purpose of studying irradiation effects.

Irradiation of wet cultures

On February 14, 1948, a wet culture was separated into four portions and exposed as follows: 100 r, 400 r, 1,600 r, and 6,400 r, respectively, with 200 kv, no filter, dosage rate of 112 r/min. The portions were then cultured on fresh agar and observed each week until May 14, 1948, when they were again recultured. Since no changes in the worms were observed, they were transferred to fresh culture media every 3 months until December 18, 1948. Control cultures were given the same culture treatment. By supplying a sprinkling of blood fibrin to the cultures, it was possible after successive generations to increase the size of female worms from about 1.5 mm in length to 2.5 mm. Old females change from the oviparous to the ovoviviparous condition. No structural changes were noted either in the controls or in the irradiated worms.

Another set of wet cultures was exposed on December 18, 1948, to 10,000 r, 20,000 r, and 40,000 r units, respectively, at 100 kv, no filter, dosage rate of 1,600 r/min. Observations were made as follows: December 20, 1948, no changes were noted in culture No. 1, given 10,000 r units. Additional blood fibrin was sprinkled on the worms December 28, 1948, and they were unchanged the following day. When examined again January 7, 1949, the irradiated worms and controls were normal.

Culture No. 2, which had been given 20,000 r units, was treated like Culture No. 1, and no changes were observed until January 8, 1949, when many larvae appeared with marked changes in the refractive index of the rhabditin granules of the intestine. Normally the intestine of *Rhabditis strongyloides* appears dark throughout its length in transmitted light but is white in reflected light. The chemical composition of rhabditin is unknown, but it is the substance associated with this color change. Some larvae had a normal black band in the middle of the intestine, with the ends clear; others were light in the middle and dark on the ends, or dark at one end and light throughout the rest of the length, or with scattered patches of dark throughout the entire length, or completely light, like "dauer" larvae. Such larvae belonging to other species have been reported to be carried under the elytra or attached to the integument of certain insects, such as dung and carrion beetles.

Culture No. 3, which was given 40,000 r units, received the same treatment as Culture No. 1, but by December 20, 1948, no adult worms were to be found, and only a few active larvae. On December 28, 1948, young males and females appeared that seemed to be completely sterilized, as the reproductive systems were quite vacuolate. The following day numerous worms of both sexes were seen with looped or spiral-like intestines. Eggs appeared in assorted sizes, some with very thin shells, others with heavy albuminous coats similar to ascaris eggs. On January 5, 1949, numbers of worms similar to "dauer" larvae were noted on the cover of the Petri dish. Changes in rhabditin granules appeared similar to those in Culture No. 2. The dauerlike larvae had an unshed molt retained like a sheath, the intestines were light, and the bulbus of the esophagus was greatly reduced.

Irradiation of dry cultures

Dry cultures, which had been saved from controls during the year 1948, were separated into 4 portions and exposed to 10,000 r, 20,000 r, and 40,000 r units, respectively, at 100 kv, no filter, dosage rate of 2,750 r/min. Each culture was then sprinkled on a fresh sterile nutrient agar plate on February 21, 1949. Six days later all 3 cultures were teeming with adults, larvae, and eggs on the surface of the agar. The same effects were obtained with 40,000 r units in the dry state as were previously obtained with 20,000 r units on wet cultures.

Cultures derived from irradiated individuals of *Rhabditis strongyloides* contained many (up to about 50%) individuals with visible changes. These changes are the same as occur occasionally in normal cultures. The radiation-induced changes occurred in cultures many generations removed from the irradiated animals. We are therefore dealing with genetic effects. The animals were kept in crowded mass cultures; thus, the radiation-induced genetic changes observed have been perpetuated for generations in a highly competitive milieu. The genetic response to irradiation of nematodes is strikingly different from that observed in insects, mammals, and various

plants and bacteria, in all of which manifest changes in fully viable individuals are rather exceptional.

Radiation effects in nematodes have been studied before, but the emphasis has not been on the characters of the adult individuals, as in our investigation. Alicata and Burr (*1*) tried to kill larvae of *Trichinella spiralis* by irradiation with radiocobalt. They found that a dose of 2,000 r per day was not lethal. The eggs of *Parascaris equorum* have been the object of numerous investigations with irradiation, as in the work of Zuppinger (*2*). However, the effects studied were mainly killing and delay of mitosis. As far as we know, changes in the adults like the ones we observed have not been previously described.

References

1. ALICATA, J. E., and BURR, G. O. *Science,* 1949, **109**, 595.
2. ZUPPINGER, A. *Strahlentherapie,* 1928, **28**, 639.

The Order of Utilization of Phosphorus Compounds in the Egg by the Chick Embryo[1]

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In the course of some experiments in these laboratories on autoradiography with P^{32}, we observed that early chick embryos invariably showed a higher deposition of P^{32} than later ones. The result could indicate that the embryo first utilizes the inorganic phosphorus available in the egg for building the phosphorus compounds in its tissues, for we injected P^{32} as NaH_2PO_4. To check on this hypothesis we gathered data on the specific activity of various phosphorus fractions in the embryo.

TABLE 1

SPECIFIC ACTIVITY*

	Average acid soluble	Residual phosphorus	Phosphatide
2-day embryo	10.71	20.3	1.67
4-day "	5.45	8.94	0.50
6-day "	2.23	0.59	0.59

* Specific Activity $= \frac{\text{Counts/}\mu\text{g of phosphorus}}{\text{Counts/ml of original } NaH_2PO_4 \text{ sol}} \times 10^4$

New Hampshire brown eggs from the University of Maryland farm were set aside in three groups to be incubated in a modified Buckeye incubator for 2-, 4-, and 6-day periods. Each of these eggs had received 0.1 ml of an isotonic solution of NaH_2PO_4 with an activity of 0.2 μc. The injections were made from a 1-cc tuberculin syringe with a ½-in. No. 27 needle through a small hole, drilled in the blunt end of the egg into the air chamber while the egg was supported in a cotton-filled cup. We used a No. 54 drill in a small table drill press controlled by a foot pedal. The eggs were sealed with sterile paraffin. The isotonic NaH_2PO_4 was prepared from radioactive KH_2PO_4, procured from Oak Ridge, following standard procedure. The radioactivity of the solution was determined by comparison with Bureau of Standards Ra D + E standard, No. 26. All radioactivity measurements were made with a mica end-window Geiger-Müller tube, window thickness 3.0 mg/cm², feeding into a Tracerlab Autoscaler. The experimental technique was essentially that reported by Branson *et al.* (*1*).

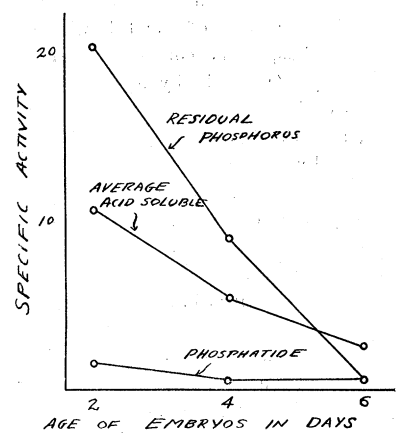

FIG. 1. Behavior of the specific activities for 3 phosphorus fractions.

At the end of each incubation period the embryos were removed, frozen immediately in liquid air, and ground to a powder. The powder was extracted several times with cold 10% trichloracetic acid, followed by extraction with cold 5% acid. Separation of the acid solubles into their component parts was not carried out. However, the residue from the acid solubles was extracted with alcohol and ether to remove nucleoproteins and phosphatides. The scheme of separation was based on that of Hevesy *et al.* (*2*).

The phosphorus was determined spectrochemically according to the procedure of Kitson and Mellon (*4*). The results are given in Table 1 and Fig. 1. The values in Table 1 are the averages of 15 2-day old embryos, 11 4-day old embryos, and 11 6-day old embryos, respectively. The embryos were pooled prior to chemical extraction to insure sufficient material for proper handling.

The eggs to be used for autoradiography were prepared in the same manner. The viable embryos were dropped immediately into liquid air. They were allowed to thaw, and the material was prepared following method C of Holt *et al.* (*3*). Ten micron sections were used on Agfa Triple-S Pan film. The sections were given a thin coat of 1% collodion to avoid sticking and the production of artifacts.

Fig. 1 shows that the specific activities of the 3 phosphorus fractions decrease rapidly as the embryo ages. This behavior can be understood only on one tenable hypothesis: the embryo first uses the small amount of

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