

Attainment of Sexuality in *Enchytraeus fragmentosus* Bell Under Laboratory Conditions¹

Enchytraeus fragmentosus Bell (Oligochaeta) was the first of the Enchytraeidae to be reported as lacking sex organs and reproducing solely by fragmentation (BELL²). It was described as breaking into 3–11 fragments with each fragment completely regenerated in approximately 10 days.

Testes were subsequently localized in the seventh or eighth segment (VENA and PALUMBO³). Serial cultivation of a cephalic fragment results in the development of a complete male reproductive system and spermathecae at the completion of 2 posterior regenerative cycles. Undifferentiated tissue, in the posterior segment next to the testes, has been observed in a position which would normally contain ovaries if the species were hermaphroditic (VENA and PHILPOTT⁴).

From caudal fragments 78 cephalic lines were successfully established between December 1967 and July 1968. The specimens were isolated in petri dishes lined with moist filter paper and kept at 25°C. Rolled oats were added to the culture as needed. Each worm was observed daily through a binocular microscope at 30× magnification. As soon as the worm fragmented, the cephalic fragment was subcultured in the above manner.

A representative number of worms were fixed in Bouin's solution at the completion of each regenerative cycle for 10 generations. The worms were sectioned at 10 µ and serial longitudinal sections were stained with hematoxylin and eosin.

Of the 78 specimens examined, only one ovum was observed in a worm which had been fixed in July after completing 3 posterior regenerative cycles. From this observation it was apparent that *Enchytraeus fragmentosus* had the morphogenetic potencies to develop male and female gonads in a protandric fashion. The question of functional hermaphroditism had to be resolved by selecting mature worms from stock culture and pooling them into petri dishes.

In September 1968, the cultivation method was changed by lining the petri dishes with a mat of glass wool in place of filter paper. This was done in order to make the daily observations easier by allowing more light to be transmitted through the culture dish. In early October, 1968 the first cocoons containing viable eggs were observed in these dishes while cocoons were conspicuously absent in paper-lined dishes.

The sexual maturity of worms is macroscopically apparent although the worms are only about 1.5 cm

when fully grown. Feulgen whole mounts of sexually mature worms demonstrate sperm in the spermathecae. Serial longitudinal sections show a well defined clitellum at the genital segments. The ovaries are fully developed and functional in the position which was suggested earlier (VENA and PHILPOTT⁴).

It is difficult at this time to say whether the detection of viable eggs is due to the different cultivation method or seasonal fluctuations in the sexual maturity of an enchytraeid (NIELSEN⁵; CHRISTENSEN⁶). 2 observations seem to indicate that the cultivation method supports sexuality. First, mature worms transferred to glass wool deposit cocoons within a short period of time (in some cases, within 24 h). Secondly, about 10% of the asexual products from a mature individual achieve sexual maturity and deposit cocoons at the end of the second generation (20 days).

Experiments are in progress to determine if the modified cultivation method enhances the reproductive physiology of *E. fragmentosus*.

Zusammenfassung. Es wurde früher beschrieben, dass *Enchytraeus fragmentosus* Bell (Oligochaeta) keine Sexualorgane besitzt und sich nur durch Fragmentation fortpflanzt. Jetzt wurden männliche und weibliche Sexualorgane entdeckt, die sich wahrscheinlich als Folge veränderter Zuchtbedingungen entwickeln.

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² A. W. BELL, *Science* 129, 1278 (1959).

³ J. VENA and M. PALUMBO, *Science* 156, 1762 (1967).

⁴ J. VENA and M. PHILPOTT, *N. J. Acad. Sci. Bull.* 13, 19 (1968).

⁵ C. O. NIELSEN, *Oikos* 6, 153 (1955).

⁶ B. CHRISTENSEN, *Nature* 184, 1159 (1959).

Uptake of Zn⁶⁵ and Mn⁵⁴ by Irradiated *Bacillus megaterium*

The most prominent effect of ionizing radiation on bacteria is the apparent killing of a number of cells. This so-called lethal action was recognized long ago as specific inhibition of cell division rather than as a general inhibition of metabolism¹. However, radiation also seems to affect other important metabolic processes². In this paper we report on the influence of γ -irradiation on the uptake of zinc and manganese, essential elements for the bacterial cell but toxic at high intracellular concentrations. It was previously reported that combined zinc chloride and γ -irradiation, exert a synergistic lethal effect on *B. megaterium*³. This action is eventually due to an increased uptake of the toxic metal, induced by radiation.

Cultures of *B. megaterium* (strain Elstre) were grown for 18 h at 35°C in nutrient broth (Difco) while being aerated by shaking. At the beginning of the experiment, the optical density, as measured in a Bausch and Lomb Spectronic spectrophotometer, was 0.2 corresponding to the mid-logphase growth. Samples of the culture were either used as controls or irradiated at room temperature with γ -rays from a cobalt-60 source. Samples were exposed for 2, 4 or 6 min, corresponding to 10,000, 20,000

¹ M. R. ZELLE and A. HOLLAENDER, *Radiat. Biol.* 2, 365 (1955).

² E. POLLARD and C. VOGLER, *Radiat. Res.* 15, 109 (1961).

³ M. KIORTSIS, *Nature* 217, 746 (1968).