Attainment of Sexuality in *Enchytraeus fragmentosus* Bell Under Laboratory Conditions[1]

Enchytraeus fragmentosus Bell (Oligochaeta) was the first of the Enchytraeidae to be reported as lacking sex organs and reproducing solely by fragmentation (BELL[2]). 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 PALUMBO[3]). 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 PHILPOTT[4]).

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 PHILPOTT[4]).

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 (NIELSON[5]; CHRISTENSEN[6]). 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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[1] This investigation was partially supported by the Department of Health, Education and Welfare, Public Health Service, No. AM 08632-04.

[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 Zn^{65} and Mn^{54} 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 metabolism[1]. However, radiation also seems to affect other important metabolic processes[2]. 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).

and 30,000 rads, respectively. 5 min after irradiation 30,000 counts/min of Zn^{65} or 30,000 counts/min of Mn^{54} (as chlorides) were added, and the samples further incubated. At successive times 3 ml were taken from the cultures for measurements of optical density and radioactivity. The cells were harvested by centrifugation, the supernatant decanted and the pellet and tube rinsed 4 times with nutrient broth. Aliquots of the cells and supernatants were taken for radioactivity measurements. The effects of varying the radiation dose are shown in Figure 1.

It seems clear that Mn^{54} is rapidly absorbed in a brief initial phase. Thereafter very little additional metal is taken up by the cells. Irradiation does not seem to affect

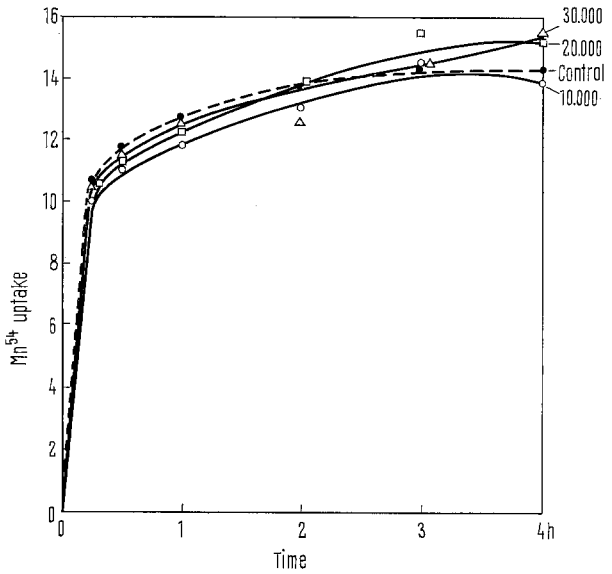

Fig. 1. Effect of various doses of radiation on the time course of Mn^{54} uptake by the cells.

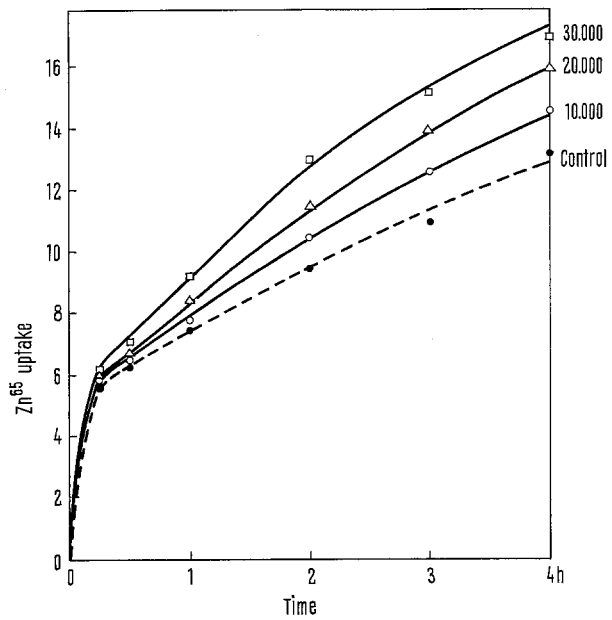

Fig. 2. The uptake of Zn^{65} by the cells observed after irradiation by 10,000, 20,000 or 30,000 rads.

to any significant degree, the uptake of Mn^{54}. In other experiments, where the irradiation dose was increased up to 75,000 rads, we observed the same independence of uptake from irradiation. Zn^{65} uptake after various doses of irradiation is shown in Figure 2. This metal is absorbed by the bacteria less rapidly than manganese but still in considerable quantities and in a rather short time. In contrast to Mn^{54} zinc uptake continues after the initial rapid phase for a long period. γ-irradiation enhances significantly the uptake of Zn^{65}. The effect is small at first, but becomes progressively larger at least up to the third hour and is clearly proportional to the radiation dose. 3 h samples irradiated with 10,000, 20,000, and 30,000 rads contain, respectively, 14%, 27% and 39% more zinc than the controls. In fact the relative uptake by the irradiated cells is even greater, since growth is faster in the controls.

The uptake of bivalent cations by yeasts, plant roots, animals cells, bacteria etc. involves 2 distinct phenomena: (1) binding on the surface of the cells and (2) transport into the cytoplasm[4]. The first process is a chemical phenomenon, involving binding sites with specific affinities for the cations. There is no evidence of an influence exerted by the metabolic state of the cell. Otherwise, active transport into the cytoplasm is a highly specific process which depends on cell metabolism. In our experiments, the process of uptake is different for manganese and zinc. With Mn, there is at first a rapid uptake (in 15 min 75% of the final amount taken up) which is believed to represent surface binding[5]. The rate of manganese uptake decreases thereafter, and the process as a whole is not influenced by irradiation, even at high doses. The uptake of Zn is less rapid at first (43% in 15 min) but continues at an appreciable rate for a considerable time subject to the continuing metabolism of the cells. Thus it seems that in addition to the primary adsorption on the surface, metabolically dependent accumulation of the metal into the cytoplasm is important. This process continues for several hours and is enhanced by irradiation. It is possible that ionizing radiation, in addition to affecting cell division and growth[1], specifically influences the control system of absorption of Zn by the cell. However it is not clear whether the increased radioactivity in the cells represents a higher level of total intracellular Zn or whether it is simply the result of a higher intracellular specific activity caused by enhanced outflow of cellular Zn. In any case, this effect cannot be merely attributed to the well-known general increase of permeability of the membrane induced by irradiation because the enhanced accumulation of radioactive zinc is not observed in the case of manganese[6].

Résumé. L'influence des doses variables d'irradiation γ sur l'absorption (uptake) du manganèse et du zinc par *Bacillus megaterium* a été étudiée. Sans irradiation, l'absorption des deux métaux est rapide au début, puis ralentit fortement par la suite dans le cas du manganèse, tandis que pour le zinc elle continue plus longtemps à un taux relativement élevé.

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[4] A. Rothstein, Int. Conf. Radioisotopes scient. Res. *1* (1957).
[5] A. Rothstein, Fedn Proc. *18*, 1026 (1959).
[6] Z. M. Bacq and P. Alexander, *Fundamentals of Radiobiology*, 2nd edn (Pergamon Press, London 1961).