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The Effect of Ionizing Radiation on *Trichomonas vaginalis*: Increase in Red Pigmented Intracellular Bodies Associated with Irradiated Cells

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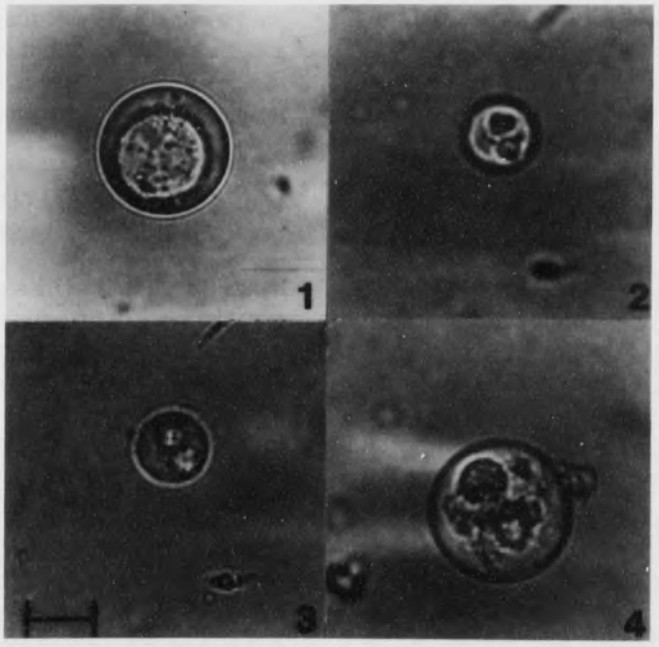
In the course of studies on the effect of ionizing radiation on the viability of the human urogenital parasite, *Trichomonas vaginalis*, red pigmented bodies began to appear in noticeable numbers in the organism's cytoplasm after exposure to high dosages of radiation. These red-pigmented bodies, ranging in appearance from dense granules to vacuolar-like structures, were easily visualized by light microscopy. Further, the number of cells containing these bodies seemed to increase with continued radiation. Review of the literature did not reveal a description of any trichomonad intracellular organelles that were as large as or as densely pigmented as the bodies we found associated with the exposure to ionizing radiation. The increase in the number of such bodies in the cell population in the present study corresponded with the reproductive death associated with an increase in radiation dosage found by Daly et al. (1991) and Hostetler et al. (2004).

The present investigation reports on the general appearance of these pigmented intracellular granules and on a dose-response study of the increase in these bodies by radiation. All methods and materials for culture maintenance, irradiation, radiation source, cell counts, and viability determinations can be found in Daly et al. (1981) and Hostetler et al. (2004). The presence and number of intracellular pigmented bodies were determined by examining trichomonads using wet slide preparations with bright-field light microscopy using oil (970X). For each radiation dose 200-250 cells were examined at a timed interval and compared with the control (unirradiated) cells kept in the dark at ambient temperature.

Initial studies of colony formation determined that a sterilizing dose of gamma radiation for 106 cells of Trichomonas vaginalis occurred at 1600 to 1800 Gy. It was noted that although the trichomonads were no longer viable, as determined by colony counts, they were still intact and highly motile. By contrast, trichomonads kept in the dark became round and were markedly less motile. The viability of this rounded-up control population, however, was close to 100%, and this did not begin to decrease until after 40 hr in the dark at ambient temperature. Trichomonads that were irradiated with more than 1600 Gy possessed a large number of red-pigmented intracellular bodies. These ranged from small (approximately 1 µm: or larger) dark-red objects to large vacuole-like structures that were pink. The larger structures, at times, occupied a major portion of the intracellular space and could be paired or exist as a single body (Fig. 1). Cells with the dense red granules tended to be much smaller than those cells with the larger pink structures. Both of these types of bodies also could be found, although in fewer numbers, in control populations that had not been irradiated. The appearance of these bodies was followed throughout the course of four experiments that extended the radiation dosage to 6100 Gy for the experimental population and 61 hr of testing for the control population in the dark (Fig. 2). The irradiated trichomonads remain actively motile up to 5100 Gy, and the total cell numbers did not begin to decrease until 4600 Gy. After 5100 Gy, no intact cells were found in the irradiated medium. The production of the pigmented bodies in both the irradiated and control cells appeared exponential. The increase in inclusions in the irradiated group coincided with 100% inability to reproduce. The increase in inclusions in the control group also was associated with a diminished reproductive potential as well as lack of motility. The population viability of unirradiated cells after 40 hrs in the dark was reduced to 50-70%. Interestingly, the increase of pigmented intracellular bodies in irradiated cells could be realized only by using cells that were taken from the exponential phase of growth. Cultures that were well into the stationary phase of growth would not produce cell populations that, when irradiated, would contain more inclusions than the unirradiated controls.

The occurrence of the large, pink, vacuole-like, interacellular inclusions in *Trichomonas vaginalis* that had been exposed to high doses of gamma irradiation was an unexpected finding. Retrospectively, these bodies were also found in the unirradiated populations, but with much less frequency. The large inclusions appeared, in terms of sequence and absence, to have formed from smaller, dense, red-pigmented bodies that were easily seen at X470. The vacuole-like pink bodies were larger and more prominent than the largest spherical organelles previously described in trichomonads. We have not found any description in the literature of intracellular bodies produced in cells by chemical or physical agents that correspond to the large pink structures associated with ionizing radiation in this study. In studies involving the effect of certain drugs on *T. vaginalis*, excessive cytoplasmic vacuolation may be found, but these vacuoles do not contain noticeable pigment nor

are they as large as the inclusions described herein (Buchner and Rummel, 1975; Carosi et al., 1977; Ings and Constable, 1975). The smaller, dense, red inclusions may be the same as the presumptive "apoptotic bodies" found within *T. vaginalis* that had been exposed to apoptotic-stimulating drugs (Chose et al., 2002). These bodies were condensed and aggregated nuclear material. Those findings imply that the bodies formed by radiation exposure are also of nuclear origin. The differences that we have found concerning trichomonad bodies of both types are presence of the red pigment and the size of the large bodies. Chose et al. (2002) were reluctant to define their bodies as completely apoptotic in origin because apoptosis originates in the mitochondria, and trichomonads are amitochondrite organisms. Chose and colleagues found no biochemical evidence to substantiate apoptosis as found in other cells. They have suggested that the process reported by them may be a different form of apoptosis not yet elucidated or even paraptosis, a non-apoptotic variant of programmed cell death found in multicellular organisms. Trichomonads are anaerobes and produce hydrogen in organelles called hydrogenosomes (hydrogenosomes are found in a variety of anaerobic free-living and parasitic protozoa–Embley et al., 2003). Iron compounds, such as ferredoxin, are necessary for the hydrogen metabolism of trichomonads (Marczak et al., 1983; Peterson and Alderete, 1984), and it can be posited that the red pigment is a result of a combining of this iron compound with nuclear material. Radiation is also known to affect membrane permeability and cause loss of membrane function, which might allow ferredoxin to enter the nucleus from the cytoplasm. The effect of radiation on the permeability of the outer cell membrane might also explain the larger size of trichomonads with the larger pink bodies. The hydrogenosomes themselves might also be considered as a possible source of the red pigmented bodies because of their requirement for ferredoxin in their metabolic activity. However, given their size and number, it would require coalescence of these bodies and formation of a maximum of 2 such units. Further studies with cytochemical analysis may resolve the genesis of radiation-induced pigmented organelles. We are reluctant to consider at this time that the production of the pigmented bodies is necessarily due to a process of apoptosis, natural or accelerated. Radiation damage may mimic apotic events and result in cell death, but it may not be the same as programmed cell death. As a temporary means of identification until further data is available, we are suggesting the term "thanatic bodies" since they are associated with the inability of *T. vaginalis* to reproduce in culture.



Figs. 1-4. Inclusion bodies found in *Trichomonas vaginalis* irradiated with greater than 1600 Gy of gamma radiation. The trichomonads were actively motile but incapable of reproducing. Large, vacuolar, pink body inside cell (1). Small, dense, reddish bodies (2 & 3). Speculative intermediate phase between small red bodies and large pink ones (4) Other trichomonad organelles are not visible because the plane of focus was on the pigmented bodies. The scale bar represents 10 µm.

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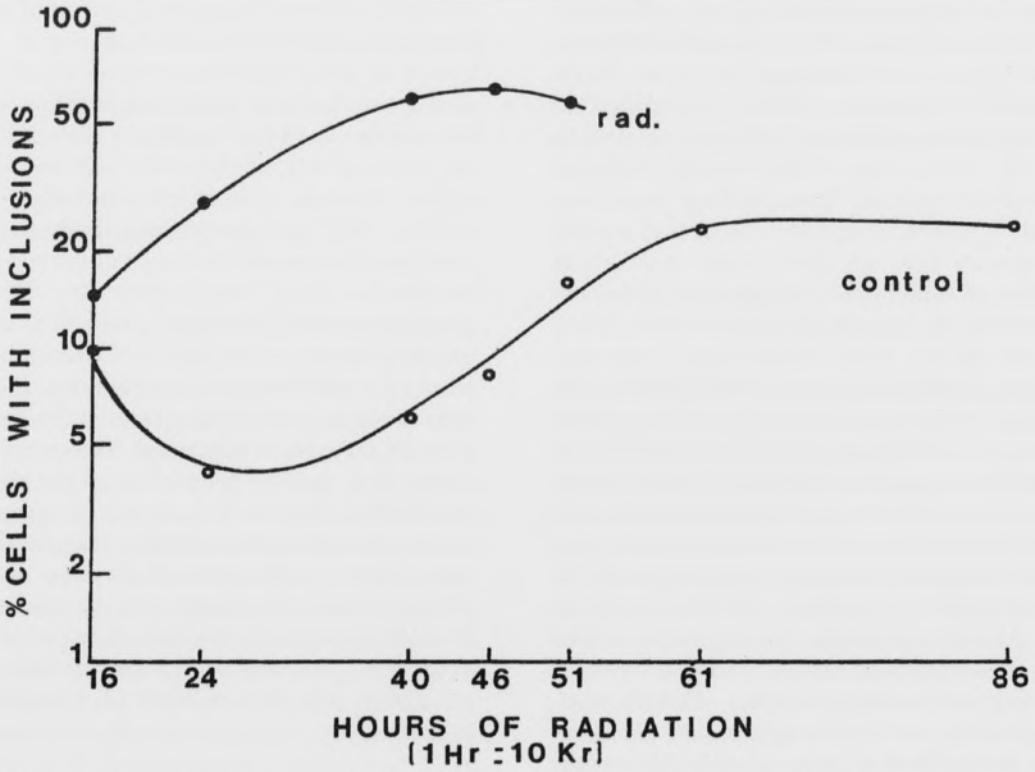

Fig. 5. Semi-logarithmic relationship between the appearance of inclusion bodies (dark red granules and pink vacuole-like structures) in the cytoplasm of Trichmonas vaginalis and gamma radiation greater than 1600 Gy. Unirradiated (control) cells were kept in the dark for a period equivalent to the time needed for a given radiation dose (100 Gy/hr). Points represent the averages for 4 experiments.

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