the increase of a single alkaline phosphomonoesterase, or of a complex of phosphomonoesterases, or of one enzyme in a complex having many characteristics in common. This is among other aspects of the problem now being more fully investigated (*6*).

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Synergistic Action of Ethylenediaminetetraacetate and Radiation on Yeast

When radioisotopes are used as radiation sources in studies with growing cultures of microorganisms, it is essential that the isotope be kept evenly distributed throughout the suspension. This is particularly true with isotopes emitting alpha particles whose ranges are only a few microns. The use of plutonium and polonium as sources of alpha radiation in growing cultures is complicated by their tendency to be adsorbed on or taken up by the cells. The chelating agent ethylenediaminetetraacetate (EDTA) is known to depress the deposition and promote the excretion of plutonium in animals (*1, 2*). The present investigation was initiated to determine whether EDTA would also be effective in decreasing or preventing the uptake of plutonium in growing cells and permit the use of plutonium as an alpha radiation source in cultures of microorganisms.

Growth studies were conducted with a diploid strain of *Saccharomyces cerevisiae* cultured at 30°C in an autoturbidimeter which automatically recorded changes in the optical density of the suspension (*3*). Plutonium in 0.2*N* nitric acid was added directly to a sterile, chemically defined medium. Sodium EDTA was used at a concentration of 3×10^{-4} *M* in all tests except those designed to evaluate the effects of varying concentrations of this compound.

The growth of yeast in the presence of plutonium and EDTA is shown in Fig. 1(*A*). The increase in optical density of the cultures is indicated by a plot of autoturbidimeter readings against time. Growth was not altered by EDTA, but it was delayed approximately 3 hours by 0.5 μc of plutonium per milliliter. With EDTA and plutonium present, a synergistic effect was observed and the inhibition of growth was much more pronounced.

Because other work in our laboratories had shown that EDTA was metabolized by yeast, it appeared that this additional inhibition possibly resulted from EDTA carrying chelated plutonium into and concentrating it in the cells. To test this supposition the concentration of plutonium associated with the cells was tested in both growing and nongrowing cultures. Under both conditions less plutonium was associated with the cells when EDTA was present than when it was absent from the medium. From this it appeared that the effects from EDTA were not due to an increased radiation dose in cells exposed to EDTA and plutonium.

Since the EDTA appeared to augment the radiation effects from plutonium, it was necessary to determine whether this was specific for plutonium or was a general synergistic effect with any radiation. Beta radiation from tritium was used because the tritium would not be chelated by the EDTA and would be uniformly distributed through the culture. Tritium oxide was added to the sterile medium, and growth curves were determined as before. Figure 1(*B*) shows growth curves for yeast grown in the presence of tritium and EDTA. As before, EDTA had no effect on growth in the control tubes. Tritium, at 90 mc/ml of growth medium, produced a marked inhibition of growth. This inhibition was doubled by the presence of EDTA with this and lower concentrations of tritium. The effect of EDTA thus appeared to be that of a general synergistic action with radiation.

By employing higher concentrations of sodium EDTA it was possible to obtain greater synergistic effects of EDTA with radiation. However, higher concentrations of EDTA also inhibited growth in unirradiated control cultures.

These results suggested either that radiation increased the sensitivity of yeast to EDTA or that EDTA increased the sensitivity of yeast to radiation. If radiation increased the sensitivity of yeast to EDTA, then exposure of yeast to x-radiation with subsequent growth in EDTA should result in a decreased rate of growth. However, EDTA in the growth medium did not affect the inhibition of growth produced by a single exposure of the inoculum to 300,000 r delivered either at the rate of 2000 or 13,000 r/min. Also, incubation of yeast in EDTA for 2 hours prior to x-radiation did not

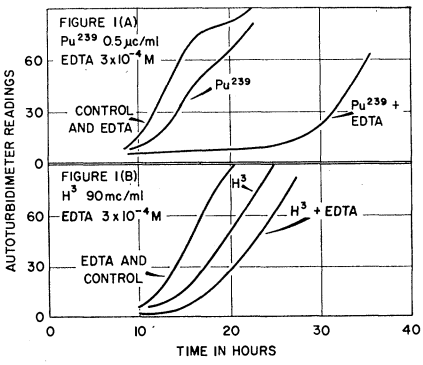

Fig. 1. Growth of yeast in the presence of (*A*) plutonium and EDTA and (*B*) tritium and EDTA.

alter its ability to grow either in liquid culture or on nutrient agar.

It is not possible at the moment to specify the mode of action by which EDTA amplifies radiation sensitivity. It appears probable, however, that the effect is produced by a general change in the electrolyte balance of the cell rather than by a specific deficiency of calcium since the addition of calcium to some of the cultures did not alter the effect of the EDTA. An effect of electrolyte concentration on the radiosensitivity of the respiratory system of yeast has been observed by Bair and Stannard (*4*). The ability of EDTA to produce chromosome aberrations and to increase the rate at which aberrations are produced by radiation administered at low dose rates has been reported by Wolff and Luippold (*5*), as have also more generalized effects on ionic balance which in turn affects chromosome behavior (*6, 7*).

Because EDTA increases the apparent radiosensitivity of yeast, its use in radiation studies may be limited to those in which this property is of interest (*8, 9*).

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