FISSION-PRODUCT AND CERIUM UPTAKE BY BACTERIA, YEASTS, AND MOLDS[1]

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The metabolism of rare earths and of other significant components of fission products by microorganisms during growth is largely undescribed. A few important specialized studies are available. Both stable lanthanum (Richards and Troutman, 1940) and cerium (Miller, 1959) are known to accumulate in yeast cells, and yeast has been reported to absorb La^{140} preferentially from a mixture of carrier-free Ba^{140}-La^{140} in radioactive equilibrium (Bowen and Rubinson, 1951). Lanthanum is also taken up in significant amounts by resting cells of *Streptococcus faecalis* (Wurm, 1951). Dried spores of five different fungi take up appreciable amounts of cerium in very short periods of time (Miller, McCallan, and Weed 1953*a*). This uptake was reported to be independent of two fungicides (silver and 2-heptadecyl-2-imidazoline) whether presented singly, simultaneously, or consecutively (Miller, McCallan, and Weed, 1953*b*), a conclusion apparently later modified so far as *Neurospora sitophila* is concerned (Miller, 1959). Some evidence is also available that more cerium is taken up in the cytoplasm of the spores than in the cell walls (Owens and Miller, 1957).

Radioactive wastes are inherent to nuclear technology and their management is an increasing concern to industry and to public health. The rare-earth elements are prominent in mixed fission products, ranging from one-fourth to one-half of the total radioactivity during this first year of decay (Hunter and Ballou, 1951). Direct clinical data are sparse or lacking and tentative evaluation of their relative hazard to man (NBS Handbook 69) is largely by extrapolation. The use of many rare earths and fission products is rapidly expanding and additional knowledge of their biological interactions is urgent.

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We therefore compared the uptake of cerium and of mixed fission products by selected bacteria, yeasts, and molds under pure culture conditions. Cerium was used to represent the rare earths that occur among fission products and that have many similar chemical and biological properties. The convenient properties of radioactive cerium-144 also influenced the choice of this element to exemplify the rare earths. This radioisotope is readily available, is long-lived ($T_{1/2}$, 283 days), and is in equilibrium with its short-lived daughter, praseodymium-144 ($T_{1/2}$, 17.5 min). Since the complex decay of this parent-daughter mixture simulates in scope that of the several radioisotopes in fission products, the comparison was done by identical methods. To the extent that the uptakes are similar, the measurements with cerium should reflect the behavior of the rare-earth components; and to the extent that the uptakes of cerium and of fission products are different, they would reflect the behavior of the non-rare-earth components of the mixture.

MATERIALS AND METHODS

Twenty-five organisms including widely differing taxonomic groups were selected for growth in appropriate culture media in the presence of fission products and of cerium. They were taken from the culture collection of one of us (G. T. J.); and convenient availability and familiarity as well as representative taxonomic scope influenced their selection. The bacteria (including Actinomycetes) were grown in nutrient broth; the yeasts were grown in Sabouraud's broth (4% glucose); three fungi that grow well with glucose as a sole carbon source were grown in Czapek-Dox medium (5% glucose); and the other fungi were grown in potato-glucose broth.

Because the rare earths tend to hydrolyze in even mildly acid solutions and thereby precipitate, cerium and fission products were each prepared in nitric acid at pH 1.0. These stock

solutions remained stable indefinitely. These radioactive solutions proved to be stable in all media with or without a complexing agent (citrate) during centrifugation at 34,000 × *g*, a much higher force than the routine procedure later utilized. Nevertheless, 10^{-3} M sodium citrate was routinely added to all media to preclude precipitation of the radionuclides by changes that could occur during growth.

To sterile broths containing 10^{-3} M citrate, cerium or the fission products were added aseptically in sufficient quantities to give an initial activity of 2,000 to 4,000 counts:min:ml of medium. Media were then pipetted to sterile containers in the desired volumes (10 ml for test tube cultures of bacteria and yeast; usually 25 ml in Erlenmeyer flasks for molds). Bacteria and yeast inoculations were made from 24-hr broth cultures in the log phase of growth, two drops per tube or flask. Molds were inoculated with a 1-ml spore suspension in distilled water—a suspension that probably included some mycelial fragments, although the total inoculum was of negligible weight. The following organisms were used: *Aerobacter aerogenes*, *Bacillus cereus*, *Bacillus megaterium*, *Erwinia carotovora*, *Escherichia coli*, *Proteus vulgaris*, *Sarcina lutea*, *Serratia marcescens*, *Streptomyces albus*, *Streptomyces flavovirens*, *Streptomyces viridans*, *Streptomyces viridoflavus*, *Nematospora coryli*, *Saccharomyces cerevisiae*, *Schizosaccharomyces octosporus*, *Schizosaccharomyces pombe*, *Absidia glauca*, *Aspergillus niger*, *Aspergillus novus*, *Byssochlamys fulva*, *Coprinus* sp., *Mucor heterogamus*, *Pythium ultimum*, *Rhizopus nigricans*, and *Spicaria violacea*.

At various periods during growth, the cells were harvested by centrifugation at approximately 18,000 × *g* and washed twice with distilled water. The combined supernatant and washings were made up to a standard volume and 1-ml samples dried in planchets were used for radioassay in a standard counter. Samples were prepared in duplicate and counted for 10 min or until a minimum of 5,000 gross counts was obtained. The use of radioactivity remaining in the supernatant as an index of uptake was verified by satisfactorily accounting for total added activity in both the cell and the supernatant fractions in a large number of cases. Additional verification of the method was gained by comparing the results with those obtained by an entirely different measurement of radioactivity. More than 10% of all recorded results based on beta counting of dried planchets were also measured by gamma counting of liquid samples in a scintillation counter (well-type NaI-Tl crystal). The two methods gave similar results. With the available instrumentation, the beta counting of dried samples gave a slight advantage in counting yield and hence was routinely used.

RESULTS

Absorption data. Aluminum absorption measurements on cerium and fission-product samples taken at two intervals during the course of the uptake measurements are plotted in Fig. 1. Both materials were about 2 months old at the time of delivery, about 4 or 5 months old at the time of the absorber measurements, and the uptake measurements were done when the isotopes were from 3 to 5 months old. The representative slopes are compared more easily in Fig. 1*A*, which is expanded on the abscissa to cover the range of absorbers similar in thickness to counting samples used in the uptake measurements. The slopes are quite similar in this important region indicating that both cerium tracer and mixed fission products emit complex spectra of beta and gamma radiation relatively similar in scope of energy. The decay of cerium-144 and the decay of its short-lived daughter, praseodymium-144, combine to yield at least six levels each of beta and gamma energies ranging from 0.175 to 3.01 and from 0.054 to 2.18 Mev, respectively. The complexity of the radioactive mixture and its changing composition makes a similar definition of the total radiations emitted by fission products extremely difficult. The slopes of the two curves and their similarity over the region that exceeds the thickest samples counted indicate that any error due to absorption is small and similar for the two complex radioactive tracers.

All samples were counted in similar steel planchets and the effect of backscatter would be the same. The effect of self-absorption would differ according to the media. The counting samples (1 ml) from control broths contained the following total solid contents: nutrient broth, 18 mg; potato-glucose broth, 24 mg; Sabouraud's broth, 50 mg; Czapek-Dox broth, 55 mg. In planchets 2.5 cm in diameter these amounts are equal to 3.6, 5.8, 10.0, and 10.1 mg/cm^2. These thicknesses are maximal and apply to the controls; samples of the supernatants from harvested cells would contain less according to the amount of nutrient consumed. Main difficulties

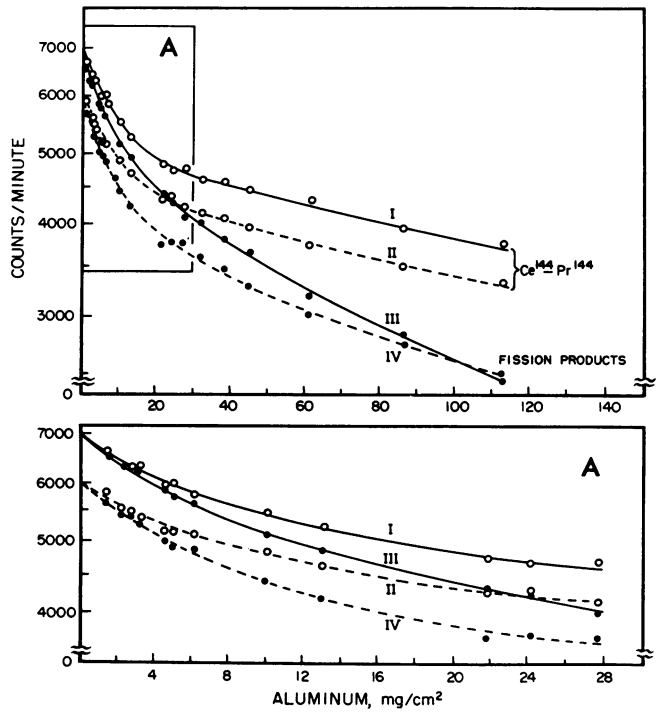

Fig. 1. Counting rates of fission product and cerium samples as functions of aluminum absorber thickness. ○ = Cerium; ● = fission products. *Curves I* and *III* (——), samples counted at 4 months of age. *Curves II* and *IV* (-----), samples counted at 5 months of age. Complex composition of the fission products is reflected in the different slope at the two times, most obvious above 25 mg/cm². The curves quantitate the absorption characteristics of counting samples throughout the period the experimental data were obtained.

Fig. 1A. Region *A* of Fig. 1 expanded on the abscissa to detail the similar slopes within the range of absorber thickness comparable to the counting samples.

in quantitatively evaluating differences of self-absorption by samples from the same media are the wide range in the harvests of different cells and the unknown factors for the conversion of nutrient to cells or to nonvolatile products. This evaluation is practical for the maximal difference between controls and culture tubes. In media of the heaviest concentrations (Sabouraud's and Czapek-Dox broths) this error is negligible. In these media the dry weight of cells ranged from 0.6 to 13.8% of total solids at the time of inoculation. If the conversion from nutrient to cell mass were efficient the medium would be affected only slightly. Check analyses indicated a conversion factor as high as three unlikely for the majority of organisms under our conditions. This factor would account for a maximum of one-fifth of the total solids—that is, a reduction from 10 to 8 mg/cm² with respect to controls versus counting samples. The self-absorption effect would be less than a similar amount of absorber between the source and the detector and for such thin layers would approach linear interpolation. Comparisons by graphic interpolations in this area from Fig. 1*A* show less than 1% difference in counting rate per mg/cm². For bacteria grown in nutrient broth (Table 1), similar calculations involve a reduction from 3.6 to 2.8 mg/cm², also giving counting differences less than 1%.

In some, but not all, cases for Streptomyces (nutrient broth, Table 2) and molds grown on potato-glucose broth (Table 4) the data reflect larger self-absorption counting errors because the total solid content was more significantly reduced during growth. The extremes found on analysis involved a reduction at maximal growth of 3.6 to 1.9 mg/cm² for one Streptomyces and 5.8 to 2.0 mg/cm² for *Coprinus* sp. These data indicate counting differences of approximately 5% for the former and 15% for the latter organ-

TABLE 1

Fission-product and cerium uptake by species of bacteria

Organism Uptake	Uptake (%)			Final pH at 96 Hr	Dry Wt (mg) at 96 Hr
	24 hr	48 hr	96 hr		
Fission products:					
Escherichia coli	62.8	62.3	62.6	6.99	4.7
Aerobacter aerogenes	51.8	53.2	59.2	7.03	4.6
Sarcina lutea	4.2	8.6	10.9	6.24	3.0
Erwinia carotovora	—	11.9	24.5	7.07	4.5
Bacillus megaterium	1.5	8.4	16.8	6.70	2.3
Bacillus cereus	3.3	12.3	18.5	6.63	2.0
Serratia marcescens	0.0	1.7	16.1	6.61	1.8
Proteus vulgaris	2.1	0.2	12.8	6.81	3.2
Cerium:					
E. coli	90.8	94.2	93.9	7.12	4.2
A. aerogenes	78.8	79.3	84.6	7.11	5.2
S. lutea	2.2	19.1	52.8	6.31	2.3
E. carotovora	—	24.7	41.6	7.04	4.9
B. megaterium	12.4	26.5	45.3	6.92	2.8
B. cereus	12.5	22.3	34.9	6.84	2.4
S. marcescens	8.4	11.9	21.0	6.42	1.6
P. vulgaris	6.8	6.4	18.7	6.84	3.1

Medium = nutrient broth + 10^{-3} M sodium citrate; initial pH 6.14; temperature, 29 ± 1 C.

isms at the time involved. However, since uptake was measured by difference in the radioactivity remaining in the supernatant, the effect is to minimize rather than to accentuate the final result. Hence the tabulated results for these organisms may slightly understate the actual amount taken up by harvested cells. Nevertheless, direct comparisons between cerium and fission-product data will still be valid where practically equivalent growth (and hence comparable residual solids in the medium) was obtained. The fact that duplicate measurements of uptake by GM-counting and by scintillation counting gave respectively similar results in those instances in which both techniques were used gives additional validation to the comparison involved. In the scintillation counting the geometry and self-absorption effects were identical.

Bacteria. (1) Eubacteriales:—Uptake data on eight species of bacteria are presented in Table 1. *E. coli* and *A. aerogenes* show remarkably high uptakes, and uptakes reach high levels within 24 hr. For the other six species the percentage of uptake increased during the period from 24 to 96 hr and the 96-hr uptake was considerably above the 24-hr amount. The smaller uptake at 24 hr was correlated with relatively poor growth and as growth increased, in general the uptake increased. For some of these species neither the initial pH (6.14), the medium, nor the temperature used (29 C) was really optimal for growth. The bacteria all grew somewhat, and all altered the initial pH toward alkaline conditions during growth, but some would have undoubtedly grown better had the optimal conditions for each been utilized.

Although uptakes do correlate somewhat with growth, they do not line up precisely so. For instance, *E. carotovora* took up only about one-half the radioactivity removed by *E. coli* and *A. aerogenes* at 96 hr, although the dry weight of the cells was approximately the same. Except for *S. lutea* at 24 hr, which seems statistically insignificant, the uptake of cerium, expressed as percentage of decontamination of controls, was considerably higher than the uptake of fission products.

(2) Actinomycetales:—Three of the four actinomycetes investigated (Table 2) show significant

TABLE 2

Fission-product and cerium uptake by species of Streptomyces

Organism Uptake	Uptake (%)			Final pH at 16 Days	Dry Wt (mg) at 16 Days
	4 days	8 days	16 days		
Fission products:					
Streptomyces albus	25.2	33.5	48.6	8.81	28.6
Streptomyces viridans	23.6	38.0	52.1	8.31	17.0
Streptomyces flavovirens	28.8	29.6	26.0	8.34	16.4
Streptomyces viridoflavus	—	16.8	17.9	8.23	7.1
Cerium:					
S. albus	44.1	65.3	74.3	8.60	21.1
S. viridans	34.8	63.6	71.2	8.36	16.6
S. flavovirens	52.6	60.9	78.6	8.38	18.8
S. viridoflavus	—	12.5	21.0	8.21	7.4

Medium = nutrient broth + 10^{-3} M sodium citrate; initial pH 6.63; temperature, 29 ± 1 C.

TABLE 3

Fission-product and cerium uptake by yeasts

Organism Uptake	Uptake (%)				Final pH at 10 Days	Dry Wt (mg) at 10 Days
	1 day	2 days	4 days	10 days		
Fission products:						
Saccharomyces cerevisiae	20.0	53.3	64.9	68.4	4.21	30.6
Schizosaccharomyces octosporus	0.0	23.2	26.0	46.4	4.57	23.3
Schizosaccharomyces pombe	0.0	4.0	14.6	28.3	4.62	26.7
Nematospora coryli	—	1.2	1.8	25.0	4.47	7.3
Cerium:						
S. cerevisiae	44.5	90.8	97.7	98.2	4.11	33.4
S. octosporus	26.2	34.2	48.4	73.8	4.62	28.3
S. pombe	3.4	13.4	29.3	41.7	4.81	30.7
N. coryli	—	0.0	5.7	36.5	4.49	6.4

Medium = Sabouraud's broth (4% glucose) + 10^{-3} M sodium citrate; initial pH 5.40; temperature, 29 ± 1 C.

cerium uptake, giving approximately 75% decontamination of the medium after 16 days. Two species took up about half of the fission product and two, considerably less. Again, except for *S. viridoflavus* at 8 days, the cerium uptake was much higher than that of the fission products. This exception is also instructive because the lesser uptakes for both radioactive tracers are correlated with lesser growth. Further, the presence of more than 90% of the radioactivity remaining in the medium at pH 8.2 adds validity to our techniques and confirms that citrate or protein complexing prevents precipitation of radionuclides at this relatively high pH.

Yeasts. Two of the four yeasts studied (*S. cerevisiae* and *S. octosporus*, Table 3) showed very high uptakes of both cerium and fission products. The two species that show lesser uptakes eventually take up a significant amount (e.g., after 10 days) but the early lesser uptakes before are definitely correlated with lesser growth. For all yeasts and for all times tested, the uptake of cerium is greater than the uptake of fission products.

Molds. Uptake data on nine other fungi are presented in Table 4. In all species significant uptakes were obtained, particularly at the longer periods of time. Members of the Mucorales (*A. glauca, M. heterogamus, R. nigricans*) and *A. niger* stand out especially, having remarkably high uptakes not only of cerium but also of the fission products.

Under the conditions of our test, fungus growth was vigorous in comparison to that of the bacteria or the yeasts. With dry weights as high as these it can be seen that factors other than growth are also important in the uptake. There is some species selectivity, and the general suggestion is that uptakes are more likely to be high if the fungus produces an acidic product rather than a neutral or basic one. Again, except for *P. ultimum* and *Coprinus* sp. at 4 days time, cerium uptake exceeds that of the fission products.

The specific activity of the shipment of radioactive cerium-144 (7 mc of Ce^{144} per mg of cerium) enables the calculation of the maximal amount of the element that the observed uptake could represent. These amounts were calculated for each observation. For the majority of organisms the absolute uptake was roughly from 1 to 4 × 10^{-3} μg of cerium per mg of dry cells. On the same basis about one-third of the organisms contained less cerium, ranging downward to 1 × 10^{-4} μg per mg, these lesser concentrations of cerium always being correlated with heavier growths. When most of the available cerium (90% or more) is taken up by organisms that differ widely in growth, the lesser concentration of the element in heavier cells appears to reflect dilution throughout the growth rather than cerium binding capacity. Yet many measurements from organisms containing the highest concentrations were made from cultures where appreciable cerium remained available to the cells, suggesting a

TABLE 4

Fission-product and cerium uptake by molds

Organism Uptake	Fission Products			Cerium		
	Uptake (%)	Final pH	Dry wt (mg)	Uptake (%)	Final pH	Dry wt (mg)
At 4 days:						
Pythium ultimum	16.8	4.48	27.1	15.7	5.41	43.9
Absidia glauca	91.9	4.23	51.1	99.0	4.25	50.1
Mucor heterogamus	56.1	4.74	50.8	81.5	4.73	53.1
Rhizopus nigricans	69.0	4.52	30.4	94.7	4.60	30.5
Byssochlamys fulva	46.5	3.96	79.6	71.7	4.01	78.5
Aspergillus niger	47.5	2.50	86.3	64.9	2.37	103.5
Aspergillus novus	19.4	5.14	7.5	58.3	5.68	37.3
Spicaria violacea	16.3	5.12	68.5	18.1	5.21	65.0
Coprinus sp.	20.2	7.08	136.4	15.1	6.97	123.7
At 10 days:						
P. ultimum	23.6	6.75	87.7	30.0	6.30	94.8
A. glauca	91.1	4.41	52.6	97.6	4.46	51.8
M. heterogamus	68.9	4.88	46.3	87.2	4.91	46.2
R. nigricans	73.0	4.58	20.9	96.2	4.87	27.8
B. fulva	62.8	4.31	92.0	85.3	4.30	90.6
A. niger	60.0	1.84	42.4	90.4	1.92	43.1
A. novus	41.8	5.57	31.9	64.5	5.87	58.1
S. violacea	38.6	4.89	93.7	61.5	4.66	84.8
Coprinus sp.	33.8	7.07	152.8	42.4	7.01	161.3
At 20 days:						
P. ultimum	17.7	7.49	96.2	35.7	7.48	102.8
A. glauca	87.1	4.80	61.6	95.9	4.75	62.4
M. heterogamus	72.1	4.92	102.3	84.3	4.96	99.5
R. nigricans	71.6	4.52	24.1	97.8	4.65	25.0
B. fulva	69.3	6.58	92.5	92.5	6.93	104.2
A. niger	83.2	2.33	172.8	96.4	2.41	155.9
A. novus	45.4	5.83	51.9	68.9	6.28	48.4
S. violacea	42.4	5.78	122.0	67.2	6.02	122.7
Coprinus sp.	37.3	6.78	159.8	50.5	7.13	155.5

A. niger, *A. novus*, and *S. violacea* were grown in Czapek-Dox broth (5% glucose) + 10^{-3} M sodium citrate; initial pH 4.92 to 5.18. Other organisms grown in potato-glucose broth + 10^{-3} M sodium citrate; initial pH 5.02 to 5.08; temperature, 29 ± 1 C.

quantity which may have some relationship to binding capacity under the experimental conditions used. It was impractical to attempt analogous quantitation of the absolute elemental uptake of fission products because of the unknown radioisotopic composition. According to the supplier (Isotopes Division, Oak Ridge National Laboratory) the maximal chemical concentration was, however, no more than that of the cerium shipment.

DISCUSSION

Burkes and McCleskey (1947) indicated cerium and lanthanum to be bacteriostatic for a large number of organisms in concentrations varying from 0.0002 to 0.0012 M. Cerium has also shown toxicity to fungus spores in slide-germination tests (McCallan and Wilcoxon, 1934) although the order of toxicity was rather low. Wurm (1951) was unable to test the effect of lanthanum during growth of *Streptococcus faecalis*; under his conditions the rare earth precipitated the phosphate ions required for growth. Still, in the relatively low ionic concentrations of cerium and of fission products used in this study, no toxicity was detected; the dry weight of isotope-treated cultures showed no significant deviation from

controls. Also, the amount of inoculum in all cultures was a negligible part of the final growth. The stability of uninoculated control media indicate that no precipitation by phosphate occurred under these conditions.

An important result in this study is the high degree of cerium and fission-product uptake by so many of these organisms. Most surveys to date, based primarily on aquatic forms (Foster and Davis, 1956; Davis et al., 1958), suggest that algae are the most selective among plants as concentrators of fission products. It now seems that several bacteria, yeasts, and molds must be added to this group. The ecological significance of this finding warrants further investigation. Since all organisms are potential food for some others, the concentration of a radioisotope in any single species points also to the desirability of following in turn possible activity in organisms that feed on them, and following the food chain throughout all trophic levels to ascertain the maximal and minimal extent to which accumulation might occur. The accumulation of large amounts of cerium by *E. coli* and other organisms of common occurrence in soil and sewage may be of possible significance when radioactive wastes are buried or poured down the drain. Also, in rapidly dividing microorganisms adverse mutational effects, due either to the radioactivity of the fission products or to adverse chemical effects of rare-earth cations, are definite possibilities that require further evaluation. The concentrations reported are high enough to deliver a relatively large radiation dose.

The data indicate that cerium is concentrated to a greater extent than the fission products. This is somewhat of a surprise because no known metabolic roles have been established for any rare earths, whereas the elemental scope of the fission products is biochemically broader and includes significant physiological examples. Although no element heavier than molybdenum has been shown to be necessary for metabolic processes, many higher elements do pass through plasma membranes, concentrate in organs and in organisms, and have interesting biological effects that remain largely unexplained. For cerium, the rare-earth fatty liver is one of these (Snyder, Cress, and Kyker, 1959, 1960). The observations are consistent with the speculation that cerium (and possibly other rare earths) may be selectively involved in biological events.

SUMMARY

The uptakes of cerium and of gross fission products by 12 bacteria (including 4 *Streptomyces* sp.), 4 yeasts, and 9 molds were studied under pure culture conditions. Significant uptakes were observed in all cases in which adequate growth was obtained. With few exceptions the uptake of cerium was considerably higher than that of fission products. These findings suggest ecological significance for the lanthanide components of fission products.

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