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THE EFFECT OF CHELATING AGENTS ON THE ABSORPTION OF RADIUM BY PLANTS

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SUMMARY

The absorption of radium from solution, by plants, has been compared with that of calcium, active strontium being used as a label for the calcium.

It was found that radium was preferentially retained by the roots and discriminated against in passage to the shoots. However, the uptake and distribution of radium was influenced by ethylenediaminetetra acetic acid (EDTA) and citrate at the concentrations employed in water culture media to keep iron in solution. There was little discrimination against radium after plants had grown for a week in active nutrient solution when EDTA was present, but in the presence of citrate radium moved less rapidly by a factor of about 0.3. In the early stages of treatment, less than a day, the results with citrate were comparable with those of EDTA, whence it is inferred that the decrease in transfer to the shoots is dependent upon the relative rates at which the two complexes decompose.

INTRODUCTION

Numerous studies of the comparative behaviour of calcium and strontium in soils and plants have shown that the two elements are absorbed by plants in a closely similar manner. Thus, although some quantitative differences between the distribution of the two ions in plants are well established, the ratio of strontium to calcium in plant tissues, and hence the concentration of the former element, can be largely inferred from the ratio of the two ions in the nutrient source on which the plants depend [9]. Little comparable information is, however, available for the heavier alkaline earths barium and radium.

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In the past decade interest in the behaviour of radium has been encouraged by the fact that, next to potassium-40, radium-226 is the main naturally occurring radioactive nuclide which enters vegetable foods thereby becoming a source of internal radiation to man and animals. It therefore seemed relevant to consider the extent to which the transfer of radium into plants can be inferred from that of calcium. A preliminary study of this question, by Mistry [7], indicated considerable discrimination against the heavier ion in the transfer to plant shoots but the relationships between the ratio of radium to calcium in the tissue to that in the external solution (*i.e.* the OR) was much less consistent than the corresponding relationships between strontium and calcium. In the present paper, relationships between radium and both strontium and calcium are considered in greater detail; the course of the work was much influenced by the unexpected observation that low concentrations of ferric ethylenediaminetetra-acetic acid (Ferric EDTA), profoundly affect the uptake of radium, when present in the external medium.

EXPERIMENTAL METHODS

Barley, var. Maris Badger, peas, var. Meteor, and maize, var. Canada Cross, grown in water culture were used in different experiments. At the commencement of experiments plants were transferred to fresh nutrient solutions containing radium-226 in activities ranging from 4–33 nC/l (*i.e.* $3.5 - 29.2 \times 10^{-8}$ me/l). In the majority of experiments strontium-85 or strontium-89 were also used, the accompanying carrier strontium being in the range of concentration normally found in AR grade reagents (molar ratio Ca/Sr approximately 1000). On occasions the pH of the solutions was adjusted with *N*/10 sulphuric acid. When treatment periods exceeded 24 hours, transpiration losses from the solution were made up daily with distilled water. Except for Experiment 4, the work was carried out in a greenhouse without supplementary lighting and only limited control of temperature, thus appreciable variations in growth occurred between experiments.

After treatment, entrained solution was removed from the roots by blotting. The leaves and roots were separated, dried at 90°C and wet ashed using nitric and perchloric acids. To avoid the risk of silica precipitation during the subsequent chemistry silica was removed by treatment with hydrofluoric and perchloric acids in platinum dishes. The residues of this treatment were then dissolved in a small quantity of 3M hydrochloric acid and made to known volumes, from which aliquots were taken for analysis.

Calcium was precipitated as oxalate dissolved in dilute perchloric acid and measured by flame emission spectrophotometry.

Radium was estimated by a modification of the method of Goldin [3]. To concentrate radium from the plant extracts, to which alkaline citrate was added, it was coprecipitated with barium and lead sulphates. These were then dissolved in ammoniacal EDTA from which barium and radium sulphates were reprecipitated by acidification with acetic acid; under these conditions, lead and the alpha emitting decay products of radium remained in the supernatant. The precipitate was mounted on 2.5 cm stainless steel discs and its alpha activity was determined before any appreciable formation of radium daughters had occurred (*i.e.* within an hour of separation) the chemical yield of barium, usually about 90%, was determined gravimetrically. Since the quantity of barium used was approximately 3.5 mg, little self-absorption of alpha radiation occurred.

Strontium-89 was measured with a M6 liquid Geiger-Muller counter and strontium-85 with a sodium iodide well scintillation counter. The activity of the strontium isotopes was sufficiently great for radium to contribute less than 1% to the observed counts.

Where measurements of the activities of the isotopes of radium or strontium in plants have been expressed per g dry weight of plant tissue, they have, for convenience, been normalized on a basis of unit activity per ml of solution at the commencement of the experiment.

To compare the behaviour of radium with that of calcium and strontium Observed Ratios (OR's) have been calculated in the manner devised by Comar *et al.*[2] which has been widely used in comparative studies of strontium and calcium. Thus, for example:

$$\text{Strontium/calcium } OR_{(\text{plant/external solution})} = \frac{\text{Strontium/calcium}_{(\text{plant})}}{\text{Strontium/calcium}_{(\text{external solution})}}$$

Since the strontium/calcium $OR_{(\text{plant shoot/external solution})}$ is close to unity the numerical values of the $OR_{(\text{plant shoot/external solution})}$ should be the same when it is based on the comparison of radium with calcium or with strontium; strontium can thus be regarded as a tracer for calcium in a study of the plant shoots. The same is not strictly true with regard to the roots since the strontium/calcium $OR_{(\text{root/solution})}$ is in excess of unity [9] so that the radium/strontium $OR_{(\text{root/solution})}$ exceeds the radium/strontium $OR_{(\text{shoot/solution})}$.

EXPERIMENTAL RESULTS

In Experiments 1 and 2 (Table 1), barley and pea plants, 18 days old, were supplied with radium-226 for 7 days at pH 4 and pH 6. Considerably less than 1 per cent of the absorbed radium reached the shoots in all cases. The OR's show that the radium was retained preferentially to calcium in roots but there was approximately a hundredfold discrimination against radium on translocation to the shoots in both species. The reduction of pH from 6 to 4 increased the

TABLE 1

Comparison of radium uptake for barley and pea plants from nutrient solution at pHs 4 and 6

Species	Initial pH	Duration of experiment days	Dry weights per plant g		Calcium mg/g dry weight		Ra^{226}/g dry weight / Ra^{22} /ml external solution		Observed ratio tissue/nutrient solution	
			Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Experiment 1										
Barley	4		0.22	0.50	73.0	12.3	1060	0.91	2.38	0.012
	6	7	0.22	0.47	84.5	13.0	816	0.77	1.56	0.009
Sign. of diff. (*P*)			NS	NS	NS	NS	0.01	NS	0.05	NS
Experiment 2										
Pea	4		0.22	0.49	36.7	19.8	1083	1.68	5.13	0.014
	6	7	0.24	0.54	30.0	19.0	631	1.16	3.50	0.0098
Sign. of diff. (*P*)			NS	NS	NS	NS	NS	0.05	NS	0.01

Nutrient solution, Na^+, 1.3; K^+, 2; Mg^{++}, 3; Ca^{++}, 8; NO_3^-, 10; SO_4^{--}, 3; PO_4^{---}, 1.3; me/l plus micronutrients. iron 6.2 mg/l as citrate. Ra^{226}, 4.5 nC/l.

TABLE 2

Variation in the uptake of radium and strontium with time for barley plants grown in nutrient solution†. Experiment 3. Age of plants at commencement of treatment, 18 days

Duration of treatment	Plant content nC* Roots Ra^{226}	Roots Sr^{89}	Shoots Ra^{226}	Shoots Sr^{89}	% of root content transferred to shoot in subsequent 24 hours Ra	Sr	% of root content lost from plant in subsequent 24 hours Ra	Sr
6 hours	239	6.1	0.25	0.36	0.44	7	68	87
12 hours	273	6.6	0.78	0.72	0.41	11	60	83
24 hours	440	8.4	2.89	3.80	0.19	20	29	72
7 days	1680	500	6.86	102	0.04	20	20	60

* Activities normalised to an activity of 10 μC/l in the culture solution; actual activities were: Ra^{226} 13.0 μC/l; Sr^{89} 6.76 μC/l.

† Nutrient solution composition shown in Table 1.

retention of radium especially in the roots of barley and peas; calcium was little affected and in consequence the OR's$_{(root/nutrient\ solution)}$ were higher at the lower pH. Because the absorbed radium which reached the shoots of barley plants was not affected significantly, by altering the pH of the nutrient solution, all subsequent experiments were carried out between the pH's 4 and 6. In parallel experiments, broadly similar discrimination was found between radium and calcium in maize and tomato plants (Mistry [7]).

In Experiment 3 (Table 2), the uptake of radium and strontium by barley plants, 18 days old, was compared over periods ranging from 6 hours to 7 days. After each harvest some plants were transferred to tracer-free solutions for a period of 24 hours to show the redistribution and loss from roots of the two ions. As in Experiments 1 and 2 only a very small fraction of absorbed radium reached the shoots but strontium was much more readily transferred. The contrasting behaviour of the two ions was shown when the quantities of ions translocated in the 24 hours after treatment with labelled solutions were expressed as fractions of root content at the end of the treatment period. A much smaller fraction of the radium was translocated, especially after the longer treatment periods. Further evidence that radium became less mobile with time was provided by the much smaller fractional loss from plants in the 24 hours after the longer treatment periods.

The most unexpected results of Experiment 3 were, however, evident from the radium/strontium OR's (Table 3). With treatment periods of 12 hours or less the $OR_{(shoot/external\ solution)}$ was only slightly less than unity, indicating little discrimination between the two ions in their transfer to shoots. However, after 7 days, considerable discrimination against radium was evident as in the earlier experiments; moreover, the preferential retention of radium in roots was considerably greater after the shorter treatment periods.

TABLE 3

Changes with time of the observed ratio ($OR_{tissue/nutrient\ solution}$) with barley grown in different environments. Age of plants at commencement of treatment was 18 days for both experiments

Duration of treatment	Experiment 3		Experiment 4	
	Root	Shoot	Root	Shoot
6 hours	37	0.73	19	0.57
12 hours	44	0.84	11	0.58
24 hours	53	0.69	8	0.14
48 hours	—	—	9	0.15
7 days	3.7	0.07	2.4	0.02

Nutrient solution composition is given in Table 1 (iron as citrate complex) except that the activities used in Experiment 3 were Ra^{226}, 13 nC/l and Sr^{89}, 6.76 μC/l and in Experiment 4 Ra^{226}, 33 nC/l and Sr^{85}, 1.42 μC/l.

The steady decrease with time in the $OR_{(shoot/external\ solution)}$ was also shown in Experiment 4 (Table 3) which differed from Experiment 3 only in that plants were grown in a controlled environment chamber at 20°C. This caused their growth rate during the treatment period to be about twice that in the earlier experiment. Quantitative differences in the results may be attributed to this cause.

In Experiment 5 (Table 4) the transfer of radium and strontium to shoots was examined in plants of greater age than those used in the earlier work. The Observed Ratios were measured over periods of 1, 7 and 21 days in plants 25 days old; observations over the two shorter periods were also made in plants which were 39 days old at the commencement of the experiment. Due to a change in laboratory practice, the concentration of the major nutrient ions differed from that in the earlier studies and whereas iron had formerly been supplied as ferric citrate the EDTA complex was now used. Irrespective of the age of plants and the duration of treatment, the OR's in this experiment were considerably higher than those observed previously. Radium appeared to be transferred preferentially to strontium in the first day and there was only a small degree of discrimination against that ion after 21 days. The results were thus in sharp contrast to those in previous experiments (Table 3), where ferric citrate had been used.

TABLE 4

Observed ratios ($OR_{tissue/nutrient\ solution}$) for barley plants grown in a nutrient solution* containing ferric EDTA (Experiment 5)

Age of plants at commencement of treatment days	Duration of treatment days	Roots	Shoots
25	1	19	1.4
	7	23	1.0
	21	9.1	0.6
39	1	20	1.4
	7	12	0.7

* Nutrient solution, Na^+, 2; K^+, 6; Mg^{++}, 3; Ca^{++}, 3; NO_3^-, 10; SO_4^{--}, 3; PO_4^{---}, 1; me/l plus micro-nutrients. Iron 0.5 mg/l as the EDTA complex. Ra^{226}, 10.5 nC/l; Sr^{85}, 346 nC/l.

This did not appear due to the changed composition of major nutrient ions since Mistry [7] had varied their concentration considerably more widely with only small effects on the extent of discrimination against radium. The possibility was therefore considered that the substitution of ferric EDTA for ferric citrate was responsible for the results shown in Table 4; despite the relatively low concentration of the EDTA complex (9 μmoles/l) it was many orders of magnitude higher than that of radium (5×10^{-5} μmoles/l). Accordingly in Experiment 6, the effect of substituting ferric EDTA for ferric citrate was examined on barley plants 21 days old (Table 5). The dry weight of the plants was unaffected but after treatment for 7 days with radium and strontium the quantity of both ions in roots was significantly lower in the presence of ferric EDTA while that in the shoots was markedly higher, the effects on radium being greatest. Thus EDTA decreased the radium/strontium $OR_{(root/external\ solution)}$ and increased the $Or_{(shoot/externalsolution)}$ to a statistically significant extent. In a number of other experiments in which either ferric citrate or ferric EDTA were used over a period of 7 days the radium/strontium $OR_{(shoot/external\ solution)}$ was consistently higher when EDTA was present.

TABLE 5

Effects of iron EDTA and iron citrate on the uptake and distribution of radium and strontium in barley plants grown in nutrient solution* for 7 days (Experiment 6)

	Dry weights per plant g		Activities in plant nC/g dry weight				Observed ratio tissue/nutrient solution	
	Root	Shoot	Roots		Shoots		Root	Shoot
			Ra	Sr	Ra	Sr		
Ferric EDTA	0.20	0.48	6.7	56	2.1	126	4.6	0.66
Ferric citrate	0.19	0.43	22	79	0.51	94	11.1	0.22
Sign. of diff. (*P*)	NS	NS	0.01	0.05	0.01	0.01	0.05	0.01

* The nutrient solution composition is given with Table 4 except that the activities used were: Ra^{226}, 27 nC/l, and Sr^{85}, 1,060 nC/l. The concentration of iron was 0.5 mg/l.

DISCUSSION

Since radium-EDTA is a less stable complex *in vitro* than calcium-EDTA or strontium-EDTA [1], the marked effect of EDTA on the transfer of radium to plant shoots and the smaller effect of the transfer of strontium appears at first sight surprising. The results of experiments when citrate was present, however, lend further support to the view that complexing agents have a profound effect on the movement of radium in plants. As the citrate ion is subject to relatively rapid biological degradation in culture solutions [4], the fact that the two ions moved similarly to shoots in the presence of citrate, over short treatment periods, and considerable discrimination against radium developed over 7 days, is compatible with radium being translocated considerably more freely in chelated form. A possible explanation of the much greater effect of EDTA on the transport of radium than strontium or calcium is suggested, though not established, by the facts that radium is retained preferentially to the lighter alkaline earths in roots, and that ferric iron can be removed by metabolic processes from the EDTA complex in root tissues [12]. If the sites at which both these processes occur are in close proximity, the extensive complexing of radium would not be surprising, neither would be the transfer of complexed radium to shoots, in view of the fact that translocation of other EDTA complexes is well known [11].

The strikingly different effects of EDTA and citrate on the mobility and transfer of radium in plants but not of calcium and strontium is sufficient evidence that the movement of radium throughout plants, unlike that of strontium, is not primarily determined by the concentration of calcium. The present investigations provide some evidence that variations in pH can significantly alter the relative behaviour of these two ions and it has been clearly demonstrated by Kirchmann *et al.*[6] that the extent of discrimination between them also changed depending upon the external concentration.

This marked contrast between the behaviour of radium and the two lighter alkaline earths is not surprising in view of their ionic radii (Ra: 1.43Å; Sr: 1.13Å; Ca: 0.99Å). On this basis of ionic radii barium can be expected to resemble radium more closely than strontium or calcium. Evidence that this is so has been provided by the work of Smith [10].

The study of nutritional relationships of intact plants grown in solution culture has often aided greatly in the interpretation of the more complex situations which occur in soil. It appears, however, that the procedure may be subject to unusual limitations when radium is compared with the lighter alkaline earths. Numerous organic ligands capable of complexing cations are known to occur in soil and in view of the evidence here presented on the effects of EDTA it is to be suspected that these naturally occurring ligands may profoundly modify the behaviour of radium. A further source of difficulty inevitably besets extrapolation from water culture to the soil; the rate at which calcium diffuses to the soil/plant interface can exert a marked effect on its uptake from the soil and this effect is likely to be still more marked with radium since its greater affinity for exchange sites on the solid phase [5] will lower its rate of diffusion. Thus when plants are growing in soil, especially when the uptake of calcium is limited by the external supply, the ratio of radium to calcium available for uptake at any one time is unlikely to exceed the mean ratio in the solution phase of the soil. On the other hand, as the present results show, when plants are grown in well stirred culture solution the much greater affinity of root surfaces for radium rapidly causes that ion to be absorbed preferentially to calcium from a volume of solution greatly in excess of that of the root system. For this reason the high values for the radium/calcium $OR_{(\text{root/external solution})}$ found in water culture seem unlikely to occur in soil. This limitation, it may be noted, does not beset the use of water culture to predict relationships between strontium and calcium which will occur when plants are grown in soil as the distribution of strontium between solid and solution phase of the soil differs little from that of calcium [8] and the two ions are absorbed in a ratio which is comparable to that in the solution phase adjacent to roots.

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