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ISOTOPIC EQUILIBRATION OF CALCIUM-45 WITH LABILE SOIL CALCIUM

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INTRODUCTION

Except in soils which contain free calcium carbonate, extraction with normal ammonium acetate at pH 7 usually provides a measure of the availability of calcium to plants which is adequate for practical purposes. The generally unequivocal results obtained by this procedure in soils of widely contrasting type have encouraged the conclusion that the calcium which is displaced by ammonium acetate is all of equal availability to plants and furthermore that calcium which is not displaced by this procedure is of negligible availability. Studies of the isotopic exchange of calcium have likewise suggested that the calcium in soils which is available to plants can be regarded as a single physico-chemical entity; when soils are suspended in labelled calcium solutions there is evidence of a single rapid exchange reaction (Blume and Smith [2]). In this respect calcium contrasts with phosphate for which fast and slow exchange reactions have been identified (McAuliffe [7], Russell and Marais [13]).

None the less there is evidence that the behaviour of labile calcium in the soil may be considerably more complex. Allaway [1], and also Mehlich and Colwell [8], found that all the calcium held on clay surfaces may not be equally available to plants despite the fact that it can be displaced with N NH_4Ac; the extent of this effect varies between different clay minerals. No published evidence however suggests that these effects are large. Thus it appeared reasonable to assume that if calcium-45 were incorporated in soil,

isotopic equilibrium would rapidly be attained with all the labile soil calcium which is accessible to plants. However, when this procedure was adopted in experiments designed to provide information on the effect of calcium on the absorption of strontium-90 by plants anomalous results were obtained; they suggested that the isotopic equilibration of calcium in soil might be considerably more complex. The present investigation was therefore undertaken.

Isotopically exchangeable calcium in a series of soils was measured and compared both with the quantities displaced by normal ammonium acetate and with estimates of the labile soil calcium, obtained by growing plants in the soils at various intervals of time after the addition of carrier-free calcium-45.

EXPERIMENTAL METHODS

Soils

Five soils which differed widely in their content of calcium were used (Table 1). For convenience the soils were numbered in order of increasing content of exchangeable calcium. Before use the soils were passed through a 2-mm sieve, air dried, and thoroughly mixed in a bin shaker revolving twenty times per minute. For some experiments calcium oxide was added to the soils in a finely divided form and mixed in the shaker for one and a half hours.

TABLE 1

The origin and calcium content of the soils investigated
(All calcium values in me per 100 g soil)

Soil no.	1	2	3	4	5
Place of origin	Harwell Berks.	Larne N. Ireland	Banbury Oxon.	Sandford Oxon.	Dorchester Oxon.
Geological description	Greensand	Basalt	Middle Lias	Kimmeridge	Gault
pH (in N/10 KCl)	5.8	4.7	7.2	7.4	7.3
Isotopically exchangeable calcium*	10.4	15.3	26.0	26.9	39.8
Calcium extracted by N NH_4Ac**	11.0	14.7	22.1	24.0	37.1
Calcium extracted by 5N HCl **	11.7	16.2	38.4	330.0	56.3
Total calcium by alkali fusion	29.6	69.9	57.1	335.0	62.7
Calcium present as $CaCO_3$ ***	0	0	12	240	8

* The mean values from four or more experiments carried out over a period of eighteen months are shown. The maximum deviation from the mean in any single experiment never exceeded 5%.

** By the method of Peech *et al.* [9].

*** By estimation of the CO_2 released when the soil was treated with 11 N HCl.

Determination of isotopically exchangeable calcium

Five-gram aliquots of soil suspended in 60 ml of labelled 0.004 *N* calcium chloride solution were shaken continuously for varying periods on an end-over-end shaker. The resultant suspensions were centrifuged. Calcium in the solution was estimated by titration with versene (Cheng and Bray [4], Mason [6]) and calcium-45 by precipitation of the oxalate which was slurried onto aluminium dishes for counting beneath an end-window Geiger-Muller counter.

The content of isotopically exchangeable calcium in the soil (E-value) was calculated from the equation

$$E = y\frac{x_t}{y_t} - x$$

where x and y are the amounts of Ca and Ca^{45} in the initial solution and x_t and y_t the amounts after shaking with the soil for time t. In addition the extent of sorption or desorption (S) of calcium by the soil could be calculated from the equation $S = x - x_t$.

This method had previously been employed in studies of isotopically exchangeable phosphate (Russell, Russell, and Marais [13]). For the reasons explained by these authors, values for exchangeable calcium obtained in this way are valid only if the calcium sorbed by the soil during the equilibration period remains in isotopic equilibrium with that in the solution Evidence on this question was obtained by varying the concentration of calcium in the solution by a factor of three; sorption was greatly increased but the E-value was not significantly altered. It could, therefore, be assumed that the sorbed calcium remained exchangeable. Furthermore, although the addition of potassium and magnesium to the equilibrating solution

TABLE 2

The effects on isotopically exchangeable calcium and sorption produced by the addition of calcium and potassium and magnesium to the equilibration solution (All values in me Ca per 100 g soil)

Ions present in solution (me/l)			Soil				
Ca	K	Mg	1	2	3	4	5
Isotopically exchangeable calcium							
4.15	—	—	10.01	15.89	27.49	27.47	39.99
4.15	4.15	4.15	11.79	15.35	27.53	27.13	41.48
12.45	—	—	10.68	15.59	27.57	26.80	41.11
	S.D. ($P = 0.05$)		0.81	NS	NS	NS	NS
Sorption of calcium							
4.15	—	—	0.52	0.95	−3.09	−3.54	−2.98
4.15	4.15	4.15	−3.19	−2.63	−6.83	−7.55	−8.46
12.45	—	—	0.45	2.09	−1.69	−2.91	−2.49
	S.D. ($P = 0.05$)		0.26	0.41	0.41	0.41	0.26

greatly increased the desorption of calcium the E-Values for Soils 2 to 5 were unaffected. In Soil 1 however this treatment increased the E-value to a small but significant extent; some of the calcium displaced by potassium and magnesium had therefore not been exchangeable.

Plant-culture methods

In some experiments barley and cabbage plants were grown in pot culture. Acid washed silica sand was mixed with the soil in the proportions of 2 : 1. Carrier-free calcium-45, together with nutrients, in some experiments, were incorporated in the sand—soil mixture. The mixture for each pot (630 grams) was prepared separately in a planetary mixing machine. Seven barley or five cabbage plants were grown in each pot, which was placed in a shallow plastic dish to which water, and if necessary, nutrients could be added. The pots were set out at random in a greenhouse and rerandomised twice per week to avoid position effects. Shoots and roots were harvested separately; the latter were carefully separated from soil by sieving and washing in demineralised water.

Analysis of plant samples and determination of Larsen values

The plant material was digested in nitric and perchloric acids. Inactive calcium was determined by the potassium permanganate/oxalate method (Vogel [14] and Peech *et al.*[9]) and calcium-45 was determined by the method already described. When experiments lasted for several months all samples were stored in the deep freeze so that they could be analysed in random order at the conclusion of the experiment.

The extent to which the added calcium-45 had been diluted by soil calcium on absorption by the plant can be estimated by the "Larsen" procedure using the equation: –

$$L = \frac{Y_f(X_p - D)}{Y_p} - X_f$$

where X_f and Y_f are the quantities of calcium and calcium-45 added to the soil, X_p and Y_p the total plant content and D is the calcium content of the seed (Larsen [5], Russell, Russell, and Marais [13]). These measurements are here referred to as L-values.

EXPERIMENTAL RESULTS

Isotopically exchangeable calcium in soil

The extent of isotopic exchange in Soils 1 to 5 over periods of 1, 7, and 14 days was compared (Fig. 1). In all soils an initial fast exchange reaction occurred within one day; thereafter there were only small changes. However, there was a marked secondary exchange reaction in Soil 4 which contained a high level of calcium

carbonate; a small but statistically significant secondary exchange reaction was also observed in Soil 3.

In a further experiment the extent of isotopic exchange was measured over a period of 42 days in Soils 1 and 4. In Soil 1 no increase in isotopic exchange was observed but in Soil 4 exchange

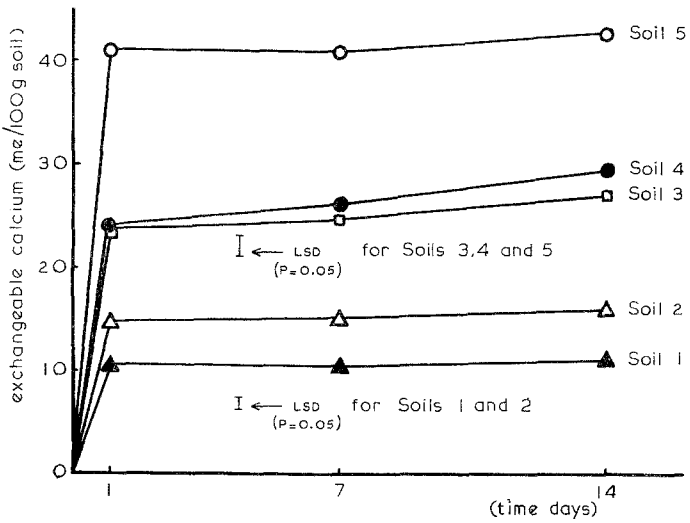

Fig. 1. The effect of the duration of equilibration on isotopically exchangeable calcium in Soils 1–5.

continued at a slow rate, the increment between the 14th and 42nd day being 11 per cent of the total; this effect was statistically significant. Since the secondary exchange reactions were confined to soils containing calcium carbonate it may be inferred that they were due to the slow penetration of labelled calcium into the crystal lattice of calcium carbonate.

Despite the small changes in the E-value of Soils 3 and 4 after the initial period it was convenient for the purpose of characterising each soil in a standard way to use the E-values after 7 days shaking; the initial fast reaction was then complete and the values were changing but slowly. In subsequent discussion E-values determined after 7 days equilibration are alone given.

The effect on the E-value of the addition of lime to air-dry soil. Lime (CaO) was added to the soil at a rate of 5.36 me Ca per 100 g soil ,equivalent to 1½ tons incorporated to the depth of 6 inches per acre of land (*i.e.* 3750 kg to the depth of 15 cm per

hectare). The limed soil was kept air-dry for 20 days and the E-value was then determined. Expressed as percentages of the quantity of calcium added the increments in E-value caused by the addition of lime, were 63, 89, 36, 4, and 0 per cent respectively for Soils 1 to 5 (Table 3). Thus only a fraction of the added calcium remained

TABLE 3

The effect of lime on isotopically exchangeable calcium
(Values in me Ca per 100 g soil)

Soil	1	2	3	4	5
pH (in *N*/10 KCl)	5.8	4.7	7.2	7.4	7.3
E-value	10.68	15.23	25.25	26.61	40.13
Increase in E-value induced by lime *	3.40	4.78	1.95	0.22	0.00

* Lime added to soil at rate of 5.36 me Ca per 100 g soil.

isotopically exchangeable. This may have been due to the precipitation of calcium as carbonate especially in Soils 3, 4, and 5 which were of highest pH and contained the largest amount of isotopically exchangeable calcium.

Comparison of the E-value with chemical measurements of soil calcium. The quantities of calcium extracted from soil by *N* ammonium acetate are compared with the isotopically exchangeable calcium (Table 1). The results for Soils 1, 2, and 5 by the two methods are within 8 per cent. For Soils 3 and 4 the E-values were 18 and 12 per cent higher than the quantity of calcium extracted with ammonium acetate. Blume and Smith [2] also found higher E-values in soils containing calcium carbonate.

The calcium content of soil, as determined by extraction with 5*N* hydrochloric acid, the total calcium measured by fusion analysis, and the quantity of calcium present in the form of carbonate are also shown in Table 1. The results indicate that even in those soils which contain no calcium carbonate and relatively little isotopically exchangeable calcium there is still a considerable amount of calcium in other forms.

Absorption of soil calcium and calcium-45 by plants

In a preliminary experiment barley and cabbage were grown in Soils 1 to 5 which had been labelled 49 days previously with carrier-free calcium-45 (3.64 μc per pot). The soil was kept moist

from the time the tracer was added until the conclusion of the experiment. A standard nutrient application was given to all soils. It consisted of an initial application of 63 mg phosphate as KH_2PO_4 added with the tracers, and 10 ml 1% KNO_3 applied weekly. Replication was five-fold. Barley was harvested after 35 days; because of the slower initial growth of cabbage it was harvested after 42 days.

The L-values were measured for each crop in each soil. The specific activity of calcium (calcium-45/inactive calcium) was determined in the roots and shoots separately and also in ammonium acetate extracts of the soils at the conclusion of the experiment. The results are shown in Table 4. The E-values, which had been previously determined for the soils, are shown for comparison. In each soil the two crops gave similar L-values; these exceeded the E-values by from 15 (Soil 3) to 35 per cent (Soil 1). Thus an appreciable quantity of the soil calcium, which did not undergo isotopic exchange with calcium-45 in solution, was accessible to the plants.

TABLE 4

Comparison of isotopically exchangeable soil calcium in Soils 1 to 5 with Larsen values found when barley and cabbage plants were grown for 35 and 42 days respectively

The specific activities of calcium in shoots and roots and in ammonium acetate extracts of the soil at the end of the experiment are also shown

Crop	Soil					S.D.
	1	2	3	4	5	(P=0.05)
Isotopically exchangeable calcium	10.4	15.3	26.0	26.9	39.8	
Larsen value (me Ca/100 g soil)						
Barley	14.10	19.27	29.80	32.09	48.73	
Cabbage	14.11	18.45	30.26	32.01	48.56	2.39
Specific activity of calcium (mμc Ca^{45}/mg Ca)						
Barley, shoot	6.37	4.89	2.94	3.02	1.94	
Root	5.78	3.21	2.61	1.65	1.48	0.56
Ratio: shoot/root	1.05	1.52	1.12	1.83	1.31	
Cabbage, shoot	6.34	5.11	2.96	2.84	1.78	
root	3.28	4.06	2.29	2.21	1.94	0.56
Ratio: shoot/root	1.93	1.25	1.29	1.28	0.91	
NH_4Ac extract of soil at end of experiment	6.07	4.49	2.50	1.72	1.66	

The most interesting result of this experiment was that the specific activity of the calcium which was present in the roots was

always significantly lower than that in shoots. Because all the soils contained calcium which was not isotopically exchangeable (Table 1), consideration was given to the possibility that this unexpected result was due to soil particles, which contained unlabelled calcium, remaining attached to the roots. This question was carefully examined. The quantities of soil attached to the roots were found by weighing; they never exceeded 10 mg soil per g dry root tissue and were frequently less. There was no correlation between the amount of soil remaining on the roots and the difference in specific activity of calcium between the roots and shoots. Furthermore, if all the calcium in the soil adhering to the roots had not been labelled with calcium-45, the specific activity of calcium in the roots would have been decreased by at most 4 per cent, which was considerable smaller than the difference found in these experiments. It was therefore concluded that the lower specific activity in the roots found in this (Table 4) and subsequent experiments (Tables 5 and 6, Figures 2 and 3) could not be attributed to contamination with soil calcium.

An alternative explanation was suggested by the fact that the calcium in plant roots has, on average, been absorbed more recently than that in the shoots; this is because calcium moves only unidirectionally from the root to the shoot (Biddulph [3] Rediske and Selders [10], Russell and Squire [12]). The specific activity in the shoots would, therefore, be higher than that in the roots if the equilibration of calcium-45 with soil calcium had continued throughout the growth of the plants, thus progressively lowering the specific activity of the soil calcium available to the plants. Experiments were therefore undertaken to study the manner in which the specific activity of calcium changed with time; plants were harvested after different periods of growth and the effect of adding calcium-45 to the soil at different intervals before planting was also examined.

Changes with time in the ratio in which calcium-45 and stable calcium enter plants. Table 5 shows results for an experiment in which barley and cabbage were grown for varying periods in Soils 1 and 4. Nutrients were added at the rate of 10 ml 1% KNO_3 and 10 ml 0.09% KH_2PO_4 per pot. The effect of the addition of lime at a rate of 5.36 me Ca per 100 g soil was also studied. There were five replicates. Plants were sampled 29, 48, and

71 days after sowing except for cabbage in Soil 4 which made slow initial growth and were sampled after 48, 71, and 92 days.

TABLE 5

Effect of lime and of the duration of the growth of barley and cabbage plants on the Larsen values for Soils 1 and 4

The ratio of the specific activity of calcium in shoots to that in roots is also shown

Soil	Crop	Lime † added	Sampling occasion ‡			S.D. ($P=0.05$)
			1	2	3	
Larsen value (me Ca per 100 g soil)						
1	Barley	–	11.59	13.30	13.79	
		+	14.51	16.81	17.54	
						1.40
	Cabbage	–	11.67	12.65	12.22	
		+	14.81	17.34	16.39	
4	Barley	–	28.41	34.40	34.62	
		+	33.73	38.07	36.09	
						3.26
	Cabbage	–	28.58	27.39	28.69	
		+	30.58	30.99	32.24	
Ratio of specific activity, shoot/root						
1	Barley	–	1.24 ***	1.20 **	1.17 **	
		+	1.15 *	1.01	1.07	
	Cabbage	–	1.36 ***	1.26 ***	1.65 ***	
		+	1.06	1.30 **	1.20 *	
4	Barley	–	1.61 ***	1.36 ***	1.47 ***	
		+	1.52 ***	1.36 ***	1.32 ***	
	Cabbage	–	1.69 ***	1.66 ***	1.28 ***	
		+	1.41 *	1.40 ***	1.28 **	

† Lime as CaO was added at a rate of 5.36 me Ca per 100 g soil equivalent to 1½ tons per acre.

‡ Except in cabbage grown in Soil 4 the plants were sampled after 29, 48, and 71 days; due to slow initial growth cabbage grown in Soil 4, was sampled after 48, 71, and 92 days.

* Specific activity of shoots significantly greater than that of roots ($P < 0.05$).

** Specific activity of shoots significantly greater than that of roots ($P < 0.01$).

*** Specific activity of shoots significantly greater than that of roots ($P < 0.001$).

Barley grown in both soils gave a significant steady increase in L-value with the increasing age of the plants. In cabbage by contrast the L-values were more variable and in only one comparison was the final L-value significantly greater than the initial; there was thus some indication of an interspecific difference. Lime increased the L-value in both soils; the increment expressed as a

percentage of the lime added to the soil was on average 69 for Soil 1 and 60 for Soil 4. This suggests that not all the added calcium remained available to the plants. When lime was not added the specific activity of calcium in the roots was always lower than that in the shoots to a statistically significant extent. The presence of lime sometimes, but not invariably, reduced this effect.

The effect of the period for which calcium-45 has been present in the soil. Table 6 shows the results for an experiment in which barley was planted either 2 or 32 days after calcium-45 had been added to the soil. A nutrient solution containing magnesium sulphate, potassium nitrate, sodium nitrate, and potassium dihydrogen phosphate was added at two levels: a high level, the quantity of cation added equal to the amount of exchangeable calcium in the soil and a low level which was one quarter of this amount. Replication was five-fold. Plants were harvested 32 days after planting.

In both soils, at the low level of nutrient the L-values were greater when the calcium-45 had been present in the soil for 32 days prior to sowing the seed. This tendency also appeared at the

TABLE 6

Effect of the addition of nutrients and the period for which calcium-45 had been mixed with Soils 1 and 3 on the L-value of calcium in shoots and roots of barley plants

Soil	Level of nutrient	Interval between addition of calcium 45 to soil and planting of barley (days)		S.D. ($P = 0.05$)
		2	32	
Larsen Value † (me Ca per 100 g soil)				
1	Low	10.94 *1.0392*	11.73 *1.0688*	
	High	12.27 *1.0882*	12.70 *1.1032*	
				0.0265
3	Low	24.67 *1.3918*	26.49 *1.4225*	
	High	25.70 *1.4099*	27.00 *1.4311*	
Ratio of specific activity, shoot/root				
1	Low	1.43 ***	1.20 ***	
	High	1.13 **	1.23 ***	
3	Low	1.27 ***	1.17 ***	
	High	1.10 *	1.14 **	

† The statistical analysis of L-values was carried out on a log basis: the transformed values are italized.

* Specific activity of shoots significantly greater than that of roots ($P < 0.05$).

** Specific activity of shoots significantly greater than that of roots ($P < 0.01$).

*** Specific activity of shoots significantly greater than that of root s ($P < 0.001$)

high level of nutrient though it was less marked. The high level of nutrient caused the L-value to be greater but this effect was significant only in Soil 1.

The specific activity of calcium in the roots was again significantly lower than in the shoots. When calcium-45 had been added only two days before planting the addition of nutrients markedly reduced the difference in the ratio of specific activity between roots and shoots, but when it had been already present for 32 days there was little difference. Thus, in the latter case, it appears that equilibrium of the added calcium-45 with soil calcium had been attained prior to growing the plants.

The results shown in Table 6 therefore suggest that added nutrients can enhance the equilibration of added calcium-45 with soil calcium when plants are not growing in the soil. Further evidence of this effect was obtained when nutrient solutions were added to labelled soils in which plants were not grown. After 13 weeks the specific activity of calcium was lowered by about 25 per cent if the following nutrients were added once weekly per 10 g soil: – 55 mg $MgSO_4$, 106 mg KNO_3, 38 mg $NaNO_3$, 20 mg KH_2PO_4. No significant change in specific activity of calcium occurred when 1/8th of these quantities was added.

The effects of depleting the soil calcium to varying extents. A more prolonged experiment was carried out with ryegrass grown in pots of different sizes so that the soil became depleted of calcium to varying extents. The use of ryegrass had the further advantage that the shoots could be sampled at successive intervals so that the changing ratio of calcium-45 to calcium absorbed by the roots of the same plant could be followed. Soils 1 and 5 were used in pots containing either 20 or 210 g of soil mixed with sand. In view of the results of the previous experiment nutrients were added in solution at the rate of 10 ml per week irrespective of pot size. The total quantity of nutrient cations applied over the 17 weeks of the experiment was identical with that added at the high level in the previous experiment.

The first sample of the shoots was taken after 32 days. Successive samples were taken after 60, 80, and 122 days; the samples taken on these occasions contained little tissue which had developed before the previous sampling. The soil was sampled on all harvesting occasions, except that at 60 days. The experiment was carried out in four-fold replication.

The quantity of calcium absorbed by plants at the end of the experiment represented the following percentage of the exchangeable calcium originally present in the soil: –

Soil	1	5
Small pots . . .	31	16
Large pots . . .	6	2

The depletion of calcium was thus considerably greater in the small than in the large pots. Moreover, the figures for the small pots underestimate the depletion of the soil in which the majority of the roots were growing because the application of water and nutrients at the base of the pots led to the greatest root development in the lower 2 cm of soil. At the end of the experiment young secondary roots were proliferating in the upper few cm of the small pots, though not of the large ones. This was attributed to exhaustion of the soil in which the primary roots had developed.

The specific activity of calcium in plants and the soil is shown in Figures 2 and 3 for Soils 1 and 5 respectively. In the small pots the specific activity of calcium in the shoots reached a minimum value at the 80 day sampling and then rose at the final harvest; this increase may have been due to active root development in the relatively undepleted upper layers of soil. The values both for the roots and the soil in which the plants had grown decreased throughout the experiment and were considerably lower than those for the shoots at the final harvest. In the large pots the specific activity of calcium in the plant roots was again lower than that in the shoots but there was little difference between the values for the the shoots and for the soil.

It is concluded that the much lower specific activity of calcium in roots than in the soil on all but one occasion in both sizes of pot was due to the fact that there was local depletion of the soil in the neighbourhood of the active roots.

The horizontal dashed lines on Figures 2 and 3 show the ratio of the calcium-45 added to the total soil calcium estimated by alkali fusion. This is the lowest possible value for the specific activity of calcium either in the soil solution or in plants unless calcium of a considerably higher specific activity had been previously absorbed. In the small pots the specific activity of the calcium in roots sank

apparently below this value, as did also the specific activity in soil extracts for the small pots of Soil 1.

These results show clearly that the action of plant roots caused

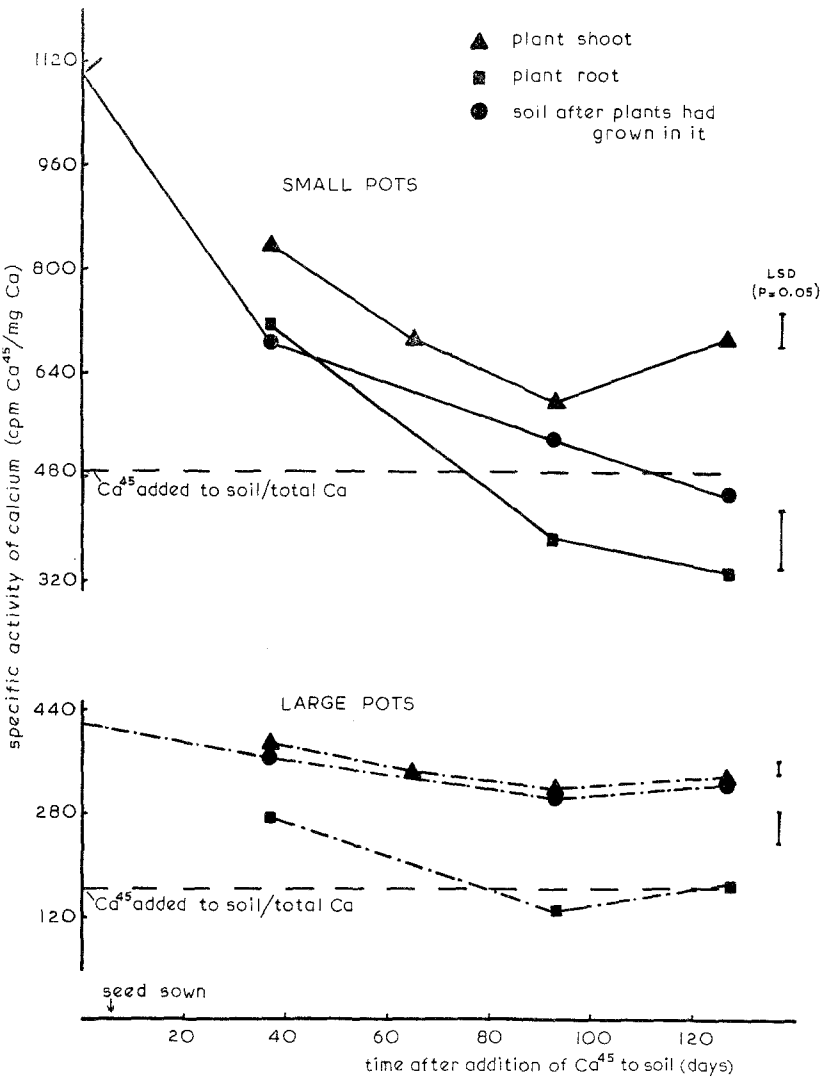

Fig. 2. The specific activity of calcium in the shoots and roots of ryegrass and in N NH_4Ac extracts of Soil 1 in which the plants had been grown. Plants were grown for varying periods in two sizes of pots.

Significant differences are shown ($P = 0.05$).

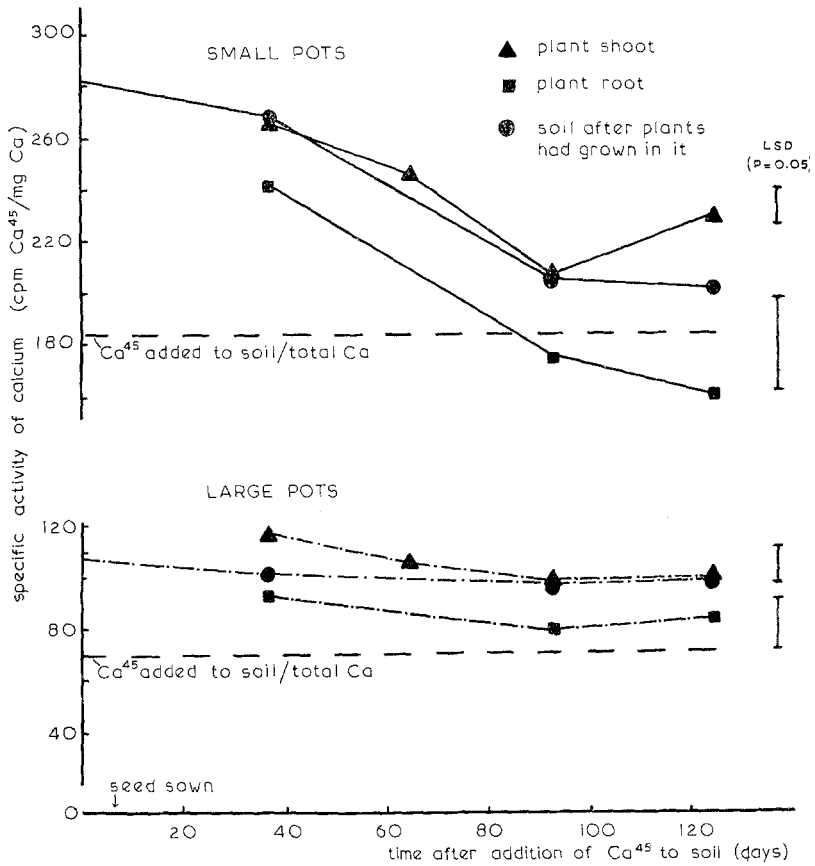

Fig. 3. The specific activity of calcium in the shoots and roots of ryegrass and in the *N* NH_4Ac extracts of soil 5 in which the plants had been grown. Plants were grown for varying periods in two sizes of pots. Significant differences are shown ($P = 0.05$).

the specific activity of labile calcium in soil to fall progressively and that the depletion of the soil enhanced this effect.

DISCUSSION

Measurements of isotopically exchangeable calcium made by suspending soils in labelled solutions (E-values) suggest that added calcium-45 equilibrates in the soil in a relatively simple manner. Equilibrium was rapidly attained except in soils where the slow secondary exchange could be attributed to the presence of calcium

carbonate (Fig. 1). The total amount of isotopically exchangeable calcium was very similar to the amount extracted by *N* NH_4Ac (Table 1), and it could not be increased by changes in the concentration of calcium, potassium and magnesium ions in the solution with which the soil was shaken (Table 2). These results accord readily with the view that the calcium immediately accessible to plants in soil can be regarded as a simple homogenous source or labile pool.

When plants were grown in the soil, however, this interpretation of the behaviour of labile calcium proved inadequate. The specific activity of calcium which plants absorbed was lower than that which could be expected from measurements of isotopically exchangeable calcium or ammonium acetate extraction. Moreover, the specific activity of the calcium entering plants decreased with time (Fig. 2 and 3), the extent of this effect being enhanced both by the addition of nutrients (Table 6) and by the exhaustion of the soil (Fig. 2 and 3). In the extreme case it caused the specific activity of calcium both in soil extracts and in plant roots to fall below the ratio of the calcium-45 added to the total calcium as measured by alkali fusion.

At first sight the results of measurements of the E-value appear irreconcilable with observations made when plants were grown in the soil. The E-values changed little after 7 days and were scarcely affected by the addition of electrolytes. By contrast the specific activity of calcium absorbed by plants decreased with time and this was accelerated by the addition of nutrients. It appears probable that this difference was due to the contrasting concentrations of calcium in the solution phase in the two types of experiments. For the determination of E-values, the concentration of calcium in the solution was raised considerably above that in the soil solution to permit accurate measurement. When plants were grown for L-value determinations, no calcium was added to the soil, and moreover the continued equilibration of calcium was accelerated by the depletion of the soil calcium through absorption by the plants. Since this continued equilibration appears to be dependent on a low calcium concentration in the solution phase, the measurement of exchangeable calcium by the method here described appears to provide incomplete information on the behaviour of calcium under normal conditions.

It is of interest to consider how much of the soil calcium which is not initially in an exchangeable form may eventually be absorbed by plants. Estimates of this can be made in the following way. If no "extra" calcium became exchangeable while plants were growing the mean specific activity of the calcium absorbed by plants would be the ratio of the added calcium-45 to the exchangeable soil calcium as measured by the E-value technique. The "extra" calcium which became exchangeable can, therefore, be calculated from the total quantities of calcium-45 and stable calcium absorbed by the plants, the quantities of calcium-45 added to the soil in which they were grown and its content of isotopically exchangeable calcium. Calculations of the "extra" calcium which became exchangeable have been made on this basis for the experiments illustrated in Tables 4, 5, and 6, and Figures 2 and 3. When the quantity of "extra" calcium is expressed as a percentage of the total calcium in the soil, measured by alkali fusion, it is found that except under the extreme exhaustion condition when plants were grown in small pots for prolonged periods the "extra" soil calcium in no case exceeded 0.8 per cent of the total soil calcium; the average being 0.2 per cent. Under conditions of extreme exhaustion the values rose to 3.1 and 1.7 per cent for Soils 1 and 5 respectively. It is thus apparent that only a very small fraction of the non-exchangeable calcium in soils was absorbed by plants. In no case did the "extra" calcium, expressed as a percentage of the exchangeable calcium initially present, reach 10 per cent and it was usually considerably lower than this figure.

It is known that the availability of calcium to plants is dependent on its state of combination in the soil (Russell,[11]) and although the present experiments provide no direct evidence on the origin of the "extra" calcium, it would be reasonable to assume that it is derived from sparingly soluble compounds. The relatively slow rate of equilibration of calcium present in these forms will depend on the solution and diffusion rates of the calcium ions, which in turn will vary with soil moisture and other factors.

The present results indicate the importance of assessing the velocity at which calcium can enter plants from the soil relative to that at which labile calcium adjacent to the root can be replenished by diffusion. If the latter process is slower depletion will rapidly occur. The fact that under exhaustion conditions the specific ac-

tivity in plant roots was found to be considerably below that of samples of soil suggests that diffusion of calcium was the limiting factor; it points to the fact that equilibria observed in even quite a small volume of soil in which plants are grown may not reflect at all closely the conditions at the soil/plant interface. In this connection it is of interest to note that Wiersum [15], has concluded that 5 per cent or less of the total volume of soil is usually in direct contact with plant roots and functions as a source of plant nutrients. The "extra" calcium which was rendered exchangeable as a result of the activity of plant roots in the present experiments was always a considerably smaller fraction of the total calcium in the soil.

If the rate of migration of calcium to the plant/soil interface exerts a considerable effect on the equilibrium of labile calcium with sparingly soluble forms, the ratio in which calcium-45 and stable calcium are absorbed by plants could vary depending on the quantity of calcium which enters per unit area of root surface. Interspecific variations could therefore arise. Significant differences in the specific activity of absorbed calcium were on occasions observed between barley and cabbage in the present investigation; there is, however, insufficient information on the pattern of absorption in their roots to show whether this explanation is applicable.

Although the simple concept that plants absorb calcium from a single homogenous source in the soil is inadequate to explain the results obtained in the present experiments, there is no reason to believe that the acceptance of this concept will lead to erroneous interpretations of practical situations. Under field conditions it appears that either the amount of calcium extracted by normal ammonium acetate or the E-value will be equally good measures of the size of the labile pool of calcium in soils. The secondary equilibration of calcium-45 with sources of calcium over and above that described as exchangeable is too small to invalidate such assessments. It is mainly of importance in assessing the results of tracer studies, especially if the object is to compare changes in the availability of calcium with that of other ions which have been added to the soil.

SUMMARY

When soils are suspended in solutions of labelled calcium chloride isotopic exchange with the labile soil calcium occurs rapidly. This may be followed by a slow secondary exchange reaction, but its magnitude is not great and equilibrium is nearly, if not completely, attained within 7 days.

When, however, plants are grown in soil throughout which carrier-free calcium-45 has been thoroughly mixed, it is found that the calcium-45 absorbed by the plants has equilibrated with a quantity of soil calcium larger than that which undergoes isotopic exchange when soils are suspended in solutions of labelled calcium chloride. The analysis of plants grown for varying periods shows that equilibration can continue for several weeks, and that the quantity of soil calcium with which the calcium-45 is associated can be increased both by the addition of electrolytes to the soil and by growing plants under "exhaustion" conditions. In 5 soils the "extra" calcium which equilibrated with calcium-45 in this way never exceeded 3.5 per cent of the total soil calcium, and was usually considerably lower.

The continued equilibration of calcium-45 with soil calcium causes the specific activity (Ca^{45}/stable Ca) of the calcium entering plants to decrease. Because the calcium in plant roots has, on average, been absorbed more recently than that in shoots, the latter show higher specific activities.

The causes of these effects are discussed and consideration is given to their significance in the interpretation of results of experiments involving the use of calcium-45 as a tracer in soils.

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