Effect of Curly Top Disease on Uptake, Transport, and Compartmentation of Calcium

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ABSTRACT

The kinetics of ^{45}Ca uptake by healthy and curly top virus (CTV)-infected tomato roots exhibited an initial exponential phase (first 30 min), but radioactivity increased linearly for the next 1 or 2 hr. Of the activity absorbed in the first 0.5 hr, a significant portion was easily exchanged for nonradioactive Ca^{++}, whereas the remaining part was exchanged very slowly. Diseased roots absorbed more ^{45}Ca during this period, as compared to healthy roots. With intact healthy plants, root radioactivity reached isotopic steady state in ca. 4 hr. Long-term uptake was, again, enhanced in diseased plants, and accumulation continued much longer. However, upward transport in diseased plants lagged behind that in healthy plants, and much less radioactivity eventually reached the upper parts. It appears, therefore, that a substantial amount of calcium taken up by diseased plants, instead of being translocated, becomes diverted into a new (or enlarged) root pool outside the translocation path, which is formed after infection. The over-all tracer efflux was slightly slower in diseased roots, and exhibited four exponential rate components.

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Among the physiological aspects of plant virus infections which have attracted attention, root functions rank behind most other activities, and much of the present knowledge in this area is largely inferential. Because of the opacity of the soil, root symptoms usually remain unobserved and are, therefore, infrequently described. For most types of studies and for the sake of simplicity in experimentation and data interpretation, the natural environment of the root system must be replaced by artificial substitutes; e.g., inert materials, liquid cultures, fog atmospheres, etc. Clearly, these factors impose additional limits to the significance of such research in attempting to extrapolate back to natural conditions.

Recently, observations in this laboratory on several plant species grown in fog-cultures indicated that, in addition to aboveground symptoms, typical and specific root symptoms may indeed occur much more frequently in virus-infected plants than is usually thought. Susceptible cultivars of bean, tomato, and sugar beet infected by the curly top virus develop well-defined root distortions (10). Similarly, tobacco plants infected with the aster yellows pathogen and *Physalis floridana* infected with the virus of potato leaf roll also show distinct root effects (15; A. H. Gold & G. Faccioli, *unpublished data*).

These findings and the general lack of understanding of root pathological physiology prompted us to study the effect of curly top virus infection on ion uptake. The present report deals with the uptake and translocation of ^{45}Ca in the tomato plant.

MATERIALS AND METHODS.—*Plant material.*—Tomato plants (cultivar VF-145) were used throughout. Seedlings were grown in 12.5-cm pots containing U.C. mix (1) in a smog-filtered greenhouse, and were inoculated with curly top virus (CTV) 20-25 days after seeding by means of viruliferous leafhoppers (*Circulifer tenellus* [Baker]). The Giddings strain No. 11 of CTV was used (9). One day after inoculation, the seedlings were removed from the pots, their roots thoroughly cleaned of adhering soil particles, and the plants transferred to plastic fog-buckets using the methods of Gold (*unpublished data*). Plants grew in a modified Hoagland's solution containing 0.2 KNO_3 and 0.1 $MgSO_4$, $Ca(NO_3)_2$, K_2HPO_4, and of minor elements found in the standard Hoagland's solution (11). Plant roots grew suspended in a fog of nutrient solution generated by means of two specially designed glass sprayers.

Intact plants or roots excised 1-3 hr before the experiment began were blotted on filter paper and placed in 250 ml nutrient solution containing 0.05 μc/ml of $^{45}Ca(NO_3)_2$, unless otherwise indicated. Excised root material was cut in ca. 2-cm pieces shortly before it was placed in the radioactive solution. Most experiments with intact plants were carried out in a growth chamber at 25 ± 1 C and 400-500 ft-c (Weston Illumination Meter 756 Quartz Filter, Weston Instruments, Inc., Newark, N. J.), but some preliminary experiments were performed under room conditions at 24 ± 2 C. The pH of the uptake medium was 5.3-5.6, and did not change during the course of the experiments.

Influx experiments.—One healthy and one diseased plant, or 5-6 root pieces, were sampled after various

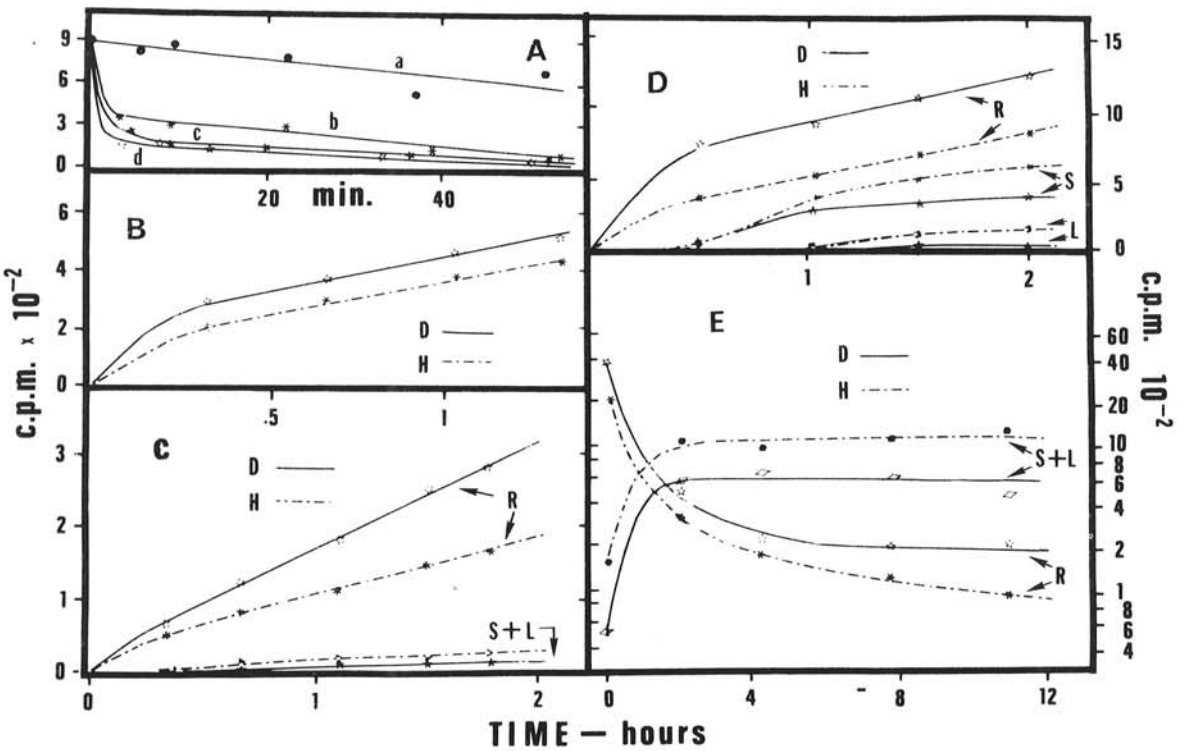
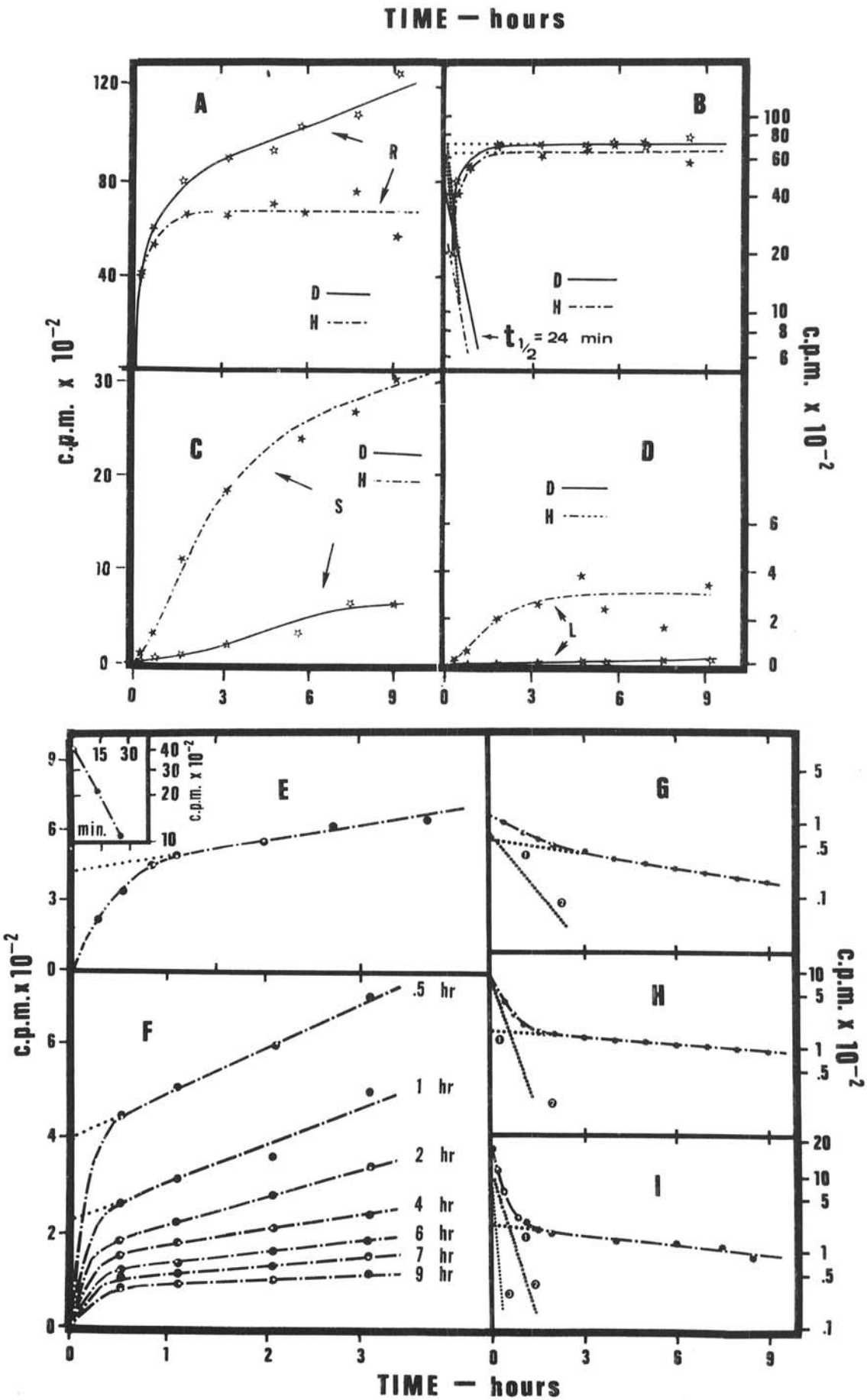


Fig. 1. Radioactivity data are expressed on a mg dry weight basis. **A)** Removal of "free space" ^{45}Ca-activity from roots by washing in (a) H_2O; (b) modified Hoagland's solution (5×10^{-4} M in Ca^{++}); (c) standard Hoagland's solution (5×10^{-3} M in Ca^{++}); (d) as in (c) but with calcium concentration increased tenfold. **B, C, D)** Kinetics of short term uptake of ^{45}Ca by excised roots (B) and by intact plants (C, D). In (C), disease symptoms were more advanced than in (D). R,S, and L refer to root, stem, and leaf radioactivities, respectively. **E)** Translocation of root calcium to the aboveground parts. Plants were allowed a 40-min uptake from a radioactive solution, then transferred to a nonradioactive one.

periods of uptake. Roots were blotted on paper towels and washed in three 200-ml changes of the same nutrient solution. The effectiveness of various solutions with varying concentrations of the ionic species studied in removing surface radioactivity was tested in some preliminary experiments (Fig. 1-A). As a result, an average chase-wash time of 10-15 min in the modified Hoagland's solution was adopted throughout. In the experiment shown in Fig. 2A-D, roots were rinsed for only 3 min and, therefore, external radioactivity may not have been removed completely. Plants were then cut into root stems and leaves plus petioles and dried overnight at 90 C. Excised root samples were washed as outlined above and dried similarly.

Pulse-chase experiments.—After feeding in ^{45}Ca

Fig. 2. (Above) Uptake and distribution of ^{45}Ca in healthy (H) and curly top virus-infected (D) tomatoes. Radioactivity levels are given on a mg dry weight basis. **A, C, D)** Although there is more radioactivity in diseased roots (R), there is less translocation to the stems (S) and leaves (L). Also, whereas in healthy roots ^{45}Ca activity leveled off after the first 3 hr of uptake, diseased roots continued to accumulate radioactivity for a much longer period. In diseased plants, therefore, part of the calcium entering the root must be diverted away from the translocation path. **B)** Analysis of the time course curves of (A) reveals the presence of two separable rate components during the early period of uptake (first 2-3 hr); the slower one, with a half-life of ca. 24 min in both healthy and diseased plants. (Below) Kinetic analysis of early influx and long-term efflux of ^{45}Ca. **E)** Analysis of the early (first 2 hr) uptake of ^{45}Ca by healthy excised roots. The uptake curve consists of a rapid first-order (see inset) component which reaches near-saturation in the first half hr ($t_{1/2}$ ca. 15 min) and a slower component proceeding at a constant rate for at least 2 hr. **F, G, H)** Compartmentation of calcium in the root. The time of uptake was varied from 0 to 3 hr, and the roots were then transferred to nonradioactive nutrient solution for up to 9 hr. Plots of the decrease in "shoulder" and "linear slope" as a function of time spent in the nonradioactive solution are given in G and H. Approximately half of the radioactivity represented by the linear portion (presumably internal pools) of the uptake curve is not easily exchanged for nonradioactive calcium ($t_{1/2}$ 6 hr), whereas the other half is exchanged relatively easily ($t_{1/2}$ ca. 36 min). Likewise, 20% of the radioactivity represented by the "shoulder" (presumably in external compartments) is slowly exchanged ($t_{1/2}$ 9 hr), and 80% is easily exchanged ($t_{1/2}$ ca. 15 min). **I)** Compartmentation and exchange of total root calcium. Roots were allowed a 30-min uptake, followed by transfer to nonradioactive nutrient solution. The decrease in root ^{45}Ca during the first 9 hr of exchange fits a three-exponential curve, with a "free space" (3) ($t_{1/2}$ 4-6 min), a "cytoplasmic" (2) ($t_{1/2}$ 15 min), and a "vacuolar" (1) ($t_{1/2}$ 7 hr) component. The free space component is not present in (H) because no data prior to 0.5 hr are included (see F).


TIME — hours
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TIME — hours

solutions for the desired length of time, individual plants or root samples were removed, blotted on paper towels, and placed in flasks containing ca. 250 ml nonradioactive nutrient (modified Hoagland's) solution. One plant or 5-10 excised root segments were removed at various times, blotted on paper towels as before, and dried at 90 C. Continuous aeration of the solutions was provided throughout, and the old solutions were replaced by new each time a sample was taken.

Radioactivity measurements.—Sample activities were measured in a liquid scintillation spectrometer (Packard Tri-Carb Model 2002) using conventional scintillation fluid (0.3 g 1,4-bis-2[5-phenyloxazolyl]-benzene and 5 g 2,5-diphenyloxazole/liter of toluene). Bray's (5) scintillator was also tested, but it offered no advantage over the one chosen.

In initial experiments, all green plant parts were placed in liquid scintillation vials and counted immediately. Since chlorophyll dissolution noticeably decreased the count rate, data from successive determinations were also plotted semilogarithmically, and initial count rates were obtained by extrapolation. With roots, no such problems were encountered. Later, it was found more satisfactory to grind all plant parts separately in mortars and decolorize with 5.25% Na-hypochlorite solution. Liquid nitrogen improved grinding. After the hypochlorite solution had evaporated completely, 5- to 10-mg samples were weighed into scintillation vials, and 10 ml scintillation fluid were added. The decolorization step could, alternatively, be made on samples inside the vials, but the material adhered onto the walls when dried. With powdered samples up to 40-50 mg/vial count, rates were linear with increasing sample; therefore, no self-absorption corrections were made. Slow sedimentation caused a variable reduction in count rate in successive determinations. Therefore, the vials were prepared and left overnight prior to counting. Later, use of Cab-O-Sil-based thixotropic media eliminated this problem.

Excised root samples of approximately equal weight were placed in scintillation vials and counted directly.

Graphical analysis.—Semilogarithmic plots of ^{45}Ca uptake were analyzed by graphical methods (Fig. 2-B, E, G, H, I). A tangent was drawn to the linear long-term portion of the uptake curve, and the data points at earlier times of absorption were subtracted from this asymptotic line. The differences were plotted on semilog paper and analyzed by the "curve peeling" technique (14, 16) to obtain individual straight line components (Fig. 2-G, H, I). Time-derivatives of the isotope efflux curves (Fig. 3) were constructed by plotting the rate of change in radioactivity for every 10-min interval. These curves, as well as the original efflux profiles plotted on semilogarithmic coordinates, were directly analyzed by the backward projection method.

RESULTS.—*Kinetics of ^{45}Ca influx.*—The time course of ^{45}Ca uptake by roots, stems, and leaves of diseased and healthy tomato plants during a 9-hr period of uptake is shown in Fig. 2-A, B, C, D. It was evident that diseased plants, even when mildly affected, absorbed more radioactivity than healthy plants. Mildly affected plants absorbed less than severely affected ones. The level of ^{45}Ca in the roots of the healthy plants increased during the first 4 hr, and remained nearly constant thereafter. In diseased plants, however, the initial fast isotope absorption was followed by a slower steady increase which continued throughout the 9-hr period.

The movement of radioactivity to stems and leaves was much faster in healthy than in diseased plants (Fig. 1-C, D, E, 2-C, D), even though more radioactivity was present in the roots of the latter. In addition, the lag period of radioactivity translocation to these plant parts was shorter in healthy plants (Fig. 2-C, D). Plants with mild symptoms again behaved intermediately in both respects. It appeared, therefore, that in diseased plants the material taken up by the root, instead of being translocated to the top, was diverted to a new local storage space present in the roots. This pool had a slower turnover compared to the pools normally present in healthy roots.

The amount of isotope taken up into the rapidly equilibrated space(s) was separated by subtracting from the total root radioactivity (Fig. 2-A, B), the part representing the proportional long-term uptake. The resulting curves showed the expected saturation-type kinetics. The relative position of the saturation level as determined from the zero-time intercept of the linear portion of each curve was somewhat higher in diseased plants. This indicated that small differences in uptake into that space also existed. Curve analysis was performed on semilogarithmic plots (Fig. 2-B). At least two processes seemed to be involved during this early uptake phase, as judged by the fact that the zero-time intercept of the graphical component resolved was well below that of the tangent to the linear portion of the original curves. For one of these processes, the rates did not differ in healthy and diseased plants. The data did not allow complete separation of the rate component associated with the other process.

The nature of the rapidly equilibrated pool was further studied in short-term experiments. As can be seen in Fig. 1-B, C, D, 2-E, F, there was an initial (25-30 min) "shoulder" of rapid isotope absorption, followed by a slower, nearly linear increase for 1 or 2 hr. The saturation of the initial phase followed simple first-order kinetics (Fig. 2-E, insert), as would have been expected for a nonmetabolic exchange of ions at the root surface. The "shoulder" was higher in diseased plants (1.5-2.5 times), as compared to healthy plants. With larger plants used in such experiments, the radioactivity was mostly retained in the root system, because of the larger distance the isotope had to travel before reaching the top. This tended to suppress the differences in shoulder size, but revealed differences in the linear uptake rate (Fig. 1-C).

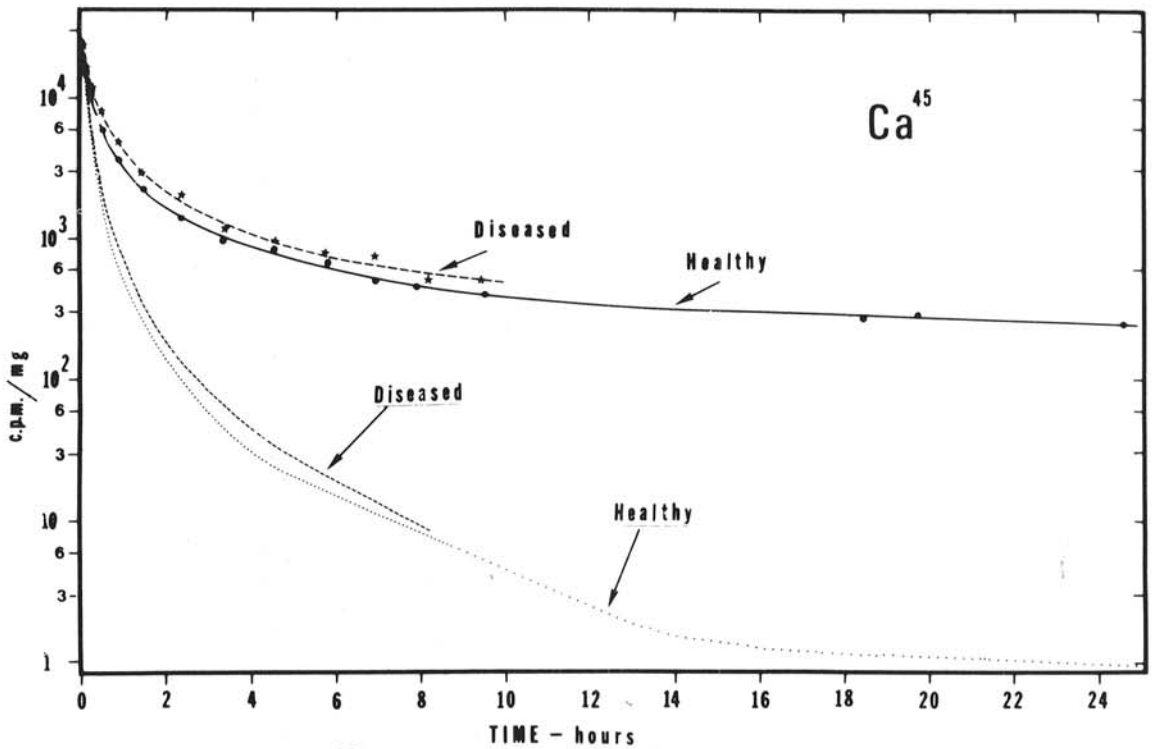

Fig. 3. The kinetics of efflux of ^{45}Ca from excised healthy and diseased roots. Through both uptake (2.5 hr, i.e., more than 5 "cytoplasmic" half-lives) and exchange, roots were maintained in modified Hoagland's solution. Upper curves: Radioactivity retained by the roots at various time intervals after transfer to the nonradioactive medium. Lower curves: First time-derivatives of the upper curves (radioactivity loss/10 min). Graphical analysis (not shown here) resolved four rate components in the kinetic curves. Long-term observations were made only in the case of healthy roots. The longest half-life component becomes predominant only after 10-12 hr and, therefore, could not be resolved from the data available for the diseased roots. Its presence, however, is evidenced by the divergence of the efflux curve and its derivative, a feature that is also evident in the efflux profiles of healthy root material. In the latter, one can see that the data curve and its derivative did become parallel at long times.

Kinetics of ^{45}Ca efflux.—Preliminary experiments with healthy excised roots showed that the exchange of recently absorbed radioactivity was rapid and extensive when the roots were transferred to nonradioactive nutrient solution (Fig. 2-I). The loss of radioactivity up to 9 hr was described by a three-exponential curve. The initial short half-life (9-12 min) component was probably due to washout of free-space radioactivity. The two additional components resolved in this case had half-lives equal to 0.3-0.5 and 6-9 hr. The ratio of the zero-time intercepts of the slowest to the middle half-life component increased with increasing length of feeding in the radioactive solution, which indicates that the space represented by the latter was saturated first. The half-lives given above may, therefore, be taken as indicative of the cytoplasmic and vacuolar exchange rates, respectively (6).

Data from efflux experiments with healthy roots in which the uptake prior to efflux was varied from 0.5 to 3 hr were plotted as a function of absorption time (Fig. 2-F). From this plot, the linear slope, as well as the size of the shoulder, was determined for various periods in the washing solution and plotted semilogarithmically against chase-time (Fig. 2-G, H). It appeared from these plots that about 80% of the shoulder radioactivity was subject to rapid loss (with a half-life of 15 min) upon transfer to the nonradioactive solution. The remaining 20% was exchanged very slowly (with a half-life of 8-9 hr). A residual slow-exchanging component would be expected for a doubly charged cation absorbed onto the cell walls, whereas the easily exchanged portion is probably located in a pool close to the outer surface. The isotope transported internally ("linear slope") seemed also to be partitioned in two pools from which radioactivity was exchanged with a half-life of 50 min and 5 hr, respectively (the latter figure may have been underestimated, as the final slope of the curve in Fig. 2-G was still decreasing).

Elution experiments were then carried out using both healthy and diseased roots. These were fed in radioactive solutions for 2.5 hr; i.e., 5 times the cytoplasmic exchange rate, in order to saturate the cytoplasmic pool (6). The results are shown in Fig. 3. With both healthy and diseased root material, the extent of loss of absorbed isotope was large, but no striking differences were found between the two. This may be explained by the observation that uptake into the new pool present in diseased roots was very slow (note the slow rate of late proportional uptake in Fig. 2-A). As a result, this pool would have become only

slightly labeled during the 2.5-hr uptake. Also, since the isotope seemed to be rapidly lost to the outside upon transfer to the nonradioactive solution (Fig. 2-I, 3), the subsequent transport into this pool would probably be very small. Therefore, the kinetics of efflux from the diseased roots observed here would show only limited influence by the slow compartment, which may in fact explain the slightly slower over-all rate of loss of ^{45}Ca in diseased roots as compared to healthy roots.

In order to obtain kinetic parameters which might be used for calculating the fluxes across the plasma membrane and the tonoplast, the data were analyzed on semilogarithmic plots (analysis not shown). Our earlier experiments (Fig. 2-I) showed that up to 9 hr the kinetics of efflux could be described adequately by equations involving the sum of three exponential terms. With longer observations, however, it appeared that four such terms were needed to fit the data, since a new rate component appeared after 10 hr. This had a half-life on the order of 30-40 hr. Since long-term observations were only taken with healthy roots, the presence of this component in the diseased roots could not be directly revealed by analyzing the kinetic data for the first 10 hr. This could be established indirectly, however, by comparing the efflux curves with their first time-derivatives. As can be seen in Fig. 3, the two curves did not tend toward the same final slopes until after 14 to 16 hr. Such a discrepancy, which was found with both healthy and diseased roots, can be attributed to the influence exerted on the curve tail by a rate component with very long half-life. Table 1 gives the half-life values of these various rate components found in two experiments. These were determined by analyzing the derivative curves, which generally allowed for much better separation of straight lines than the original curves. The last available portion of these curves (between 5 and 10 hr) was assumed to represent one of the components (the second slowest), although to assume it to be straight is not strictly permissible. In two cases in which long-term observations were available, this analysis was found to be in good agreement with a similar analysis made on the actual experimental curves.

With such multiplicity of terms displayed in the efflux curves, a two-compartment model (6, 13), usually applied for calculation of ion fluxes at the membranes, could not be applied here. If the situation is, indeed, as complex as it appears to be in these experiments, a different model and more complex mathematical derivation will be needed. Alternatively, although the calcium concentration in all solutions used was invariant, it is possible that a steady state was not maintained during the long periods of tracer exchange.

DISCUSSION.—Our data support the view that CTV-infected tomato roots, in addition to morphological disturbances, also have physiological abnormalities. With respect to calcium metabolism, disease-induced anomalies include enhanced uptake, reduced translocation, and a concomitant retention in the root. The several kinetic components of uptake revealed here all show abnormal disturbances as a result of infection. Although the distinctions between them were based only on kinetic criteria, it is possible to project some of our results to the physicochemical domain. Thus, the enhancement of early uptake (first 30 min) probably displays some qualitative and/or quantitative difference(s) in the chemical composition of those pools of calcium which are relatively easily accessible from the outside. It has been suggested (2, 3, 7) that calcium is first taken up on the exchange sites of the cell walls. More recent radioautographic data show that ^{45}Ca appears to be particularly associated with cell walls (4). It is also known (8, 12) that the cation exchange capacity of plant roots is due primarily to the free carboxyl groups of pectin. Accordingly, one of the implications of the present results is that the amount and/or degree of esterification of pectic substances in the root cell walls has increased as a result of virus infection. Other possibilities include an increased amount of cytoplasmic calcium or calcium oxalate crystals. Calcium oxalate crystals have been found to acquire considerable quantities of ^{45}Ca beginning after 7.5 min of uptake (4).

The increased slower and continuing uptake must also be related to some chemical pool or physical space. In the healthy plants, there was a very slow net increase of root ^{45}Ca after 3 hr. Since translocation to the top parts continued to occur at later times, this simply reflects the fact that a near-equilibrium isotopic saturation of the accessible root pool(s) had been reached by that time, and, therefore, the amount of isotope taken into the root was equal to that transported to the tops. The increased long-term accumulation of radioactivity in diseased roots, continuing after a certain portion of the root calcium had reached isotopic equilibrium, reflects the

TABLE 1. Half-life of the rate components of ^{45}Ca efflux from labeled tomato roots

Rate component no.	Healthy		Diseased	
	Exp. I	Exp. II	Exp. I	Exp. II
1	>30 hr	>30 hr	>35 hr	>30 hr
2	150 min	80 min	110 min	80 min
3	28 min	25 min	25 min	20 min
4	4-7 min	4-6 min	6-9 min	4-8 min

presence of an additional, slowly accessible root pool. Since the kinetics of linear long-term absorption did not bear any correlation with the translocation of radioactivity to the green parts, it is concluded that this pool is outside the path of translocation. The pool may simply represent an enlargement of normal pool(s) present in healthy roots, or may be a new and distinct one. Its nature is not known, but its location may be in the inner root tissues, which are not rapidly accessible from the outside. Calcium oxalate crystals located in deep layers of the root tissue again represent one possibility. We have not determined the amount of calcium oxalate in healthy and diseased roots, but one report (17) indicates that oxalic acid increased in potato virus X-infected tomatoes.

The failure of the efflux experiments to show significant differences may have been due to an over-all interplay of factors such as pool size(s) and flux rate(s) that may have hidden particular differences in either of the two. Also, the very slow uptake into the new storage root pool would not be expected to have influenced significantly the kinetics of isotope efflux, because of the minimal initial labeling of that pool at the end of the 2-hr period of isotope feeding.

Ion fluxes across the plasmalemma and the tonoplast in plant roots can be estimated from tracer efflux data on the basis of a two-compartment model (6, 13). Our data, however, show that in the case of calcium, this may have to be extended to include one additional pool. The relationships between this and the other cytoplasmic and vacuolar pools would have to be established before any mathematical treatment can be undertaken.

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