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THE RELATIONSHIP BETWEEN Ca^{45}, TOTAL CALCIUM AND FISSION PRODUCT RADIOACTIVITY IN PLANTS OF PORTULACA OLERACEA GROWING IN THE VICINITY OF THE ATOM BOMB TEST SITES ON ENIWETOK ATOLL

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During the course of the A.E.C.-U. of W. re-survey of the Eniwetok area in July and August of 1949 an extensive collection was made of plants of the species Portulaca oleracea. Special attention was given to this species because it occurred on all three "test" islands, and particularly because it was a dominant pioneer species re-inhabiting the denuded areas adjacent to the bomb craters. Re-population by August of 1949, some 15 months after the tests, was only partly successful. Plants nearest the craters showed signs of distress which resembled those of calcium deficiency, i.e., young leaves die back at the tips and margins, the stalks then dying from the terminal bud downward. The severity of the factor or factors responsible for the injury was sufficient to keep plants from becoming established in the immediate vicinity of the craters. At a distance of 30 to 100 yards, only scattered patches of dead or "unhealthy" plants occurred and from this distance outward injury became progressively less severe until normal plants were encountered at approximately 300 yards.

Preliminary analyses carried out for the original report showed that plant No. 574 collected at a distance of 20 yards from the tower base on Runit Island contained only 9.3 mg Ca/gm of dry matter while a similar specimen, plant No. 578 collected at 200 yards contained 47.5 mg Ca/gm of dry matter. Plants usually die from calcium deficiency when the concentration is as low as 10 mg Ca/gm of dry matter. A concentration of 47.5 mg of Ca/gm of dry matter is well within the normal range for many plants. This report is the result of an attempt to evaluate some of the factors responsible for the abnormal calcium relations of Portulaca oleracea growing in the vicinity of the test sites.

Plants of Portulaca oleracea in random stages of maturity and senescence were collected along a line directed from the bomb craters outward on each of the three islands utilized in the tests of April and May of 1948, i.e., Engebi, Aomen, and Runit. The transect line was in a general south-easterly direction in all three instances as was dictated by the geographic features of the islands with respect to the crater site. Collections were made during July and August of 1949 and analyses were made during January to June of 1951.

The plants were pressed flat and air dried in the field. They were not washed, but merely subjected to a light brushing to remove adhering macroscopic soil particles at the time of collection. Subsequent autoradiographs showed that fission products from the soil had become dispersed upon the plants and had persisted there. As the plants had grown from seed since the time of the "tests", the radioactive particles had been splashed or otherwise mechanically deposited upon them by rain drops or other climatic agencies. In a general way, the frequency of the radioactive particles upon the plants should reflect their frequency in the surrounding soil, and the distribution at any specified distance should be random.

The presence of calcium 45 in the coral sands adjacent to the bomb craters was shown in the original report[1]. The calcium 45 would, of course, be limited to the area in which the neutron flux was dense enough for a significant conversion of the isotope calcium 44 into calcium 45. The calcium 45 in the coral sands could then be absorbed by the roots, if present in a soluble form, as it is indistinguishable to the plant from the other isotopes of calcium.

In a general sense there were then three sources of radiation to which the plants were subjected; namely, the general background of radiation due to fission products in the soil, the fission products lodged upon the plants, and the calcium 45 absorbed by the plants from the coral sands. The latter provided a source of "internal" radiation.

Jacobson and Overstreet[2] have studied the absorption of fission products by plants and have showed that when Y, Ce, Zr + Cb, Te, and Pu were fixed on clays there was considerable fixation of the isotopes on the roots of the plants (barley and peas) grown therein,

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[1] Sec. VI Report No. UWFL 23. "Radiobiological Survey of Bikini, Eniwetok and Likiep Atolls. July, Aug. 1949." Applied Fisheries Lab. Univ. of Washington.

[2] L. Jacobson and R. Overstreet. Soil Sci. 65: 129-34 (1948).

but translocation was very limited as is generally the case with most heavy elements. Strontium, perhaps because of its relationship to calcium, proved somewhat of an exception since of the initial dose of that element administered to the soil 1.6% was translocated. The adsorption of fission products by roots was pronounced, i.e., to the extent of depleting the clay colloids of a large part of their "fixed" isotopes.

Methods and Results

The dry plant material was placed in 125 ml Erlenmeyer flasks with 24/40 ST necks and weighed. The material was then wet ashed in nitric acid using ST joints as reflux chimneys. Ashing was carried to dryness, the ash weighed and then dilute hydrochloric acid added to 10 ml. Three separate aliquots of one ml each were placed on stainless steel disks and three successive "counts" recorded for each. A composite average was then determined and the results expressed as <u>total radioactivity</u> in disintegrations per minute per gram of dry matter. The material on the three plates was then returned to the remainder of the sample. <u>Total calcium</u> was then precipitated as oxalate and the oxalate titrated with $MKnO_4$[3]. The results were expressed as mg Ca/gm dry matter. The solution was saved for the next analysis.

<u>Radioactivity due to calcium 45</u> was then determined by removal of fission products by a scavenging procedure aimed at the separation of calcium from all other elements[4]. Calcium 45 was then "counted" as calcium oxalate which had been plated onto stainless steel disks at a thickness of less than 1 mg/cm^2. No correction for self-absorption was considered necessary. Geometry factors for P^{32} were used in the absence of better factors.* The results were expressed

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[3]Kolthoff & Sandell. Textbook of Quantitative Inorganic Analysis, The MacMillan Company, New York, 1941.

[4]W. W. Meinke. UCRL 432, Aug. 30, 1949. Chemical procedure Used in Bombardment Work at Berkeley.

*Calc. from Nat'l. Bureau of Standards reference solutions of P^{32}.

as d/min/gm dry matter. The calcium 45 activity was corrected for decay so as to correspond to the time of "counting" of the total activity. Calcium 45 activity was then subtracted from total activity to give radioactivity due to fission products. Both calcium 45 activity and fission product activity were then corrected for decay back to August 1 of 1949, the time of collection of the plants. In correcting for the decay of fission products a half life value of 275 days was used. This value was taken from UWFL report # 23 and represents a value obtained from sample XE-38, (the gut of a damsel fish taken off Runit island), whose decay curve was similar to a sample of coral sand collected on Engebi island.

By use of the various measurements and calculations, Figures 1, 2, and 3, for Engebi, Aomen and Runit islands, respectively, were constructed. They represent in graphic fashion the relationship between (1) the activity due to fission products on the plants, (2) the activity due to calcium 45 in the plants, (3) the total calcium in the plants, and (4) the distance from the tower bases at which the plants were collected. It is recognized that uncertainties associated with the decay correction factor for fission products may render uncertain the absolute values for fission product activity. However, the relative values should be reliably reported.

The results as portrayed in Figures 1, 2, and 3 show a striking dependence between the radioactivity due to fission products lodged upon the plants and the total calcium present in the plant's tissues. When radioactivity due to fission products is high the calcium content of the tissues is invariably low, and when fission product activity is lower calcium content is correspondingly higher. There is no similar dependence between total calcium content and the combined radioactivity due to fission products plus calcium 45.

Fission product activity decreased to some extent with distance from the tower bases within the 300 yard zone studied, but randomness of distribution caused local departures from a straight line relationship. The rather tortuous curves depicting fission product activity with increasing distance have almost exact mirror images in the curves representing total calcium in the tissues. This inverse relationship between fission product activity and total calcium content is so striking that it is necessary to presume a causal relationship between them. As the frequency of occurrence of specks of fission material upon the plants is proportional to their frequency in the soil, the fission products in the soil must be considered to be contributing radiation to the plants in amount proportional to some factor of the activity which resides upon the plants. As the plant in question is a shallow rooted one, and in habit lies prostrate upon the ground, it is in a position to effectively absorb radiation emitted from the surface layer of the soil.

The radiation dosage which the plants were receiving from fission products lodged upon them at the time of collection, can be approximated from a simple calculation. The data show that plant #592 collected at the 100 yard station on Aomen island would represent a typical example. Figure 4 is an autoradiograph of the plant collected at this station. Calcium 45 activity is manifest by the overall background of radiation within the stem and leaf tissues while the fission products appear as discrete spots lodged at random over them. The root area is indicated by the intense blackening of the film in the basal portion. Assuming from the data of Fig. 2 that the tops possess a total activity of 23,000 c/min/gm dry matter or 600 c/min/gm (or cc) of fresh material, and assuming that 50% of the radiation is absorbed and that E_{av} is 1×10^6 ev,[5] then:

$$\frac{600 \text{ d/min/cc} \times .5 \text{ absorbed} \times 1 \times 10^6 \text{ ev/d} \times 1 \times 10^4 \text{ min/week}}{52.5 \times 10^{12}} = \text{approx. } 0.06 \text{ rep/week.}$$

This does not take into consideration radiation from the fission products lodged

[5] This is 1/3 of E_{max} for Pr^{144} the radioactive daughter of Ce^{144} which is present as a fission product, p. 29 - UWFL-23.

upon the roots which contribute radiation to these organs in great excess of that received by the tops.

Jacobson and Overstreet[6] state that 0.1 uc of fission products per gram of soil caused marked injury to the roots of plants when grown therein for a period of three months. It is obvious from Fig. 4 that the roots of Portulaca oleracea have accumulated fission products in great excess to the tops. It is unfortunate that more direct comparisons between the radiation received by the roots of these plants and those of Jacobson and Overstreet cannot be made. However, our observations on the fixing power of plant roots for ruthenium is in agreement with those of Jacobson and Overstreet and our autoradiograph (Fig. 4) shows a marked accumulation of fission products by Portulaca oleracea roots. Our only soil analysis, i.e., Runit coral sand (surface layer) at a distance of 200 yards from the crater, showed an activity in excess of 0.01 uc/gm of sand at the time of collection of plants. Because of the ability of the roots to adsorb fission products when they are present in the soil (Fig. 4), it is then highly probable that the radiation received is sufficient to cause some injury to the roots.

A consideration of the mechanical effects of the bombs upon the physical environment of the plants is desirable before any general conclusions are formed as alteration of the environment might be responsible for some of the observed responses. The Portulaca plants growing in the immediate vicinity of the craters showed signs of distress which very closely resembled those of calcium deficiency as it appears in laboratory grown plants. This, at first thought, may seem anomalous as the basic medium of the coral atolls is calcium carbonate. However, an explosion of that nature and magnitude seems capable of the oxidation of organic matter and the expulsion from the immediate vicinity of all finely divided particles rendering the site raw and impoverished of the accumulated debris formulating

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[6] Jacobson, L. and Overstreet, R. The uptake by plants of Plutonium and some products of nuclear fission. Soil Sci. 65:129-134 (1948).

the "top soil" upon which the roots feed. All which remained was the coarse coral sand which had to be invaded and repopulated as a pioneer site.

Because of the abundant rain in that area and the porosity of the coral sands in the denuded site, soluble minerals available for shallow rooted plants, such as Portulaca, were certainly at a premium. The solubility of $CaCO_3$ in distilled water is sufficient to supply Ca at only 0.00014 Molar. Laboratory plants cannot be successfully grown at Ca concentrations much below 0.0005 M. However, the presence of carbonic acid markedly increases the solubility of calcium. Pertinent sources of carbonic acid, other than sea water, are from the CO_2 metabolically produced by the roots and from decaying organic matter. Since the decaying organic matter was largely removed or destroyed soluble calcium originating from this means would be slight. If insufficient the plants would die at a susceptible age. But even so, the next plant invasion may profit from them providing it can establish itself and acquire the minerals released at the death and decomposition of the former inhabitants. The establishment of the natural vegetation for that area must then await the slow re-development of the barren site. Portulaca, as a dominant pioneer species, was just becoming established. Even though the first invaders were only partially successful, their presence would aid in a re-concentration of the meager supply of available plant nutrients, insuring an ever increasing tempo of establishment and succession leading toward the re-development of a suitable environment and a stable community.

In the absence of previously accepted experimental evidence indicating that the absorption of calcium may be reduced by radiation from an external source, an explanation based on the destruction of the "habitat" and the subsequent poverty of certain mineral nutrients therein may seem appealing. That the plants in question were suffering from extreme calcium deficiency, the severity of which was sufficient to be the immediate cause of their death, seems evident. And it appears to the authors that the major cause of the calcium deficiency can be attributed to radiation damage. That roots are especially sensitive to ionizing radiation has been pointed out by many investigators[7], but no selective mechanism for the supression of calcium absorption by radiation has been suggested. Nevertheless it is undesirable to dismiss the curves of Figs. 1, 2, and 3 without serious consideration.

When a regression curve is attempted wherein calcium content of the tissues is plotted against fission product activity on the tops of the plants, the spread of points is too wide to indicate a striking dependence between the two. However, the amount of activity in the surface of the soil, and the ability of the roots to acquire within or upon them a significant portion of the fission products in the soil suggests that injury to the root system by radiation was possible and that at least one specific part of that injury was to the calcium absorption. The results indicate the desirability mechanism of controlled experiments to determine the effects of chronic beta radiation in the root environment on the absorption of various mineral nutrient by plants.

Summary

Analyses were made for total radioactivity, radioactivity due to calcium 45, and for total calcium on plants of the species Portulaca oleracea collected along a transect from the craters outward on Engebi, Aomen and Runit Islands. Collections of plants were made approximately 15 months after the 1948 atom bomb tests at Eniwetok Atoll. At that time the front of the re-invading pioneer plant species had reached to within approximately 100 yards from the crater centers. Vegetation was spotty to 300 yards, but signs of successful re-establishment were evident beyond. Clearing of the islands prior to the tests had been responsible for the destruction of many plant habitats. The effect of the bombs was to further denude the areas and to remove from them the accumulated debris which constituted the so-called "top soil". The leaching effects of the frequent rains on the barren

[7] Dugger, B. M., Biological Effects of Radiation. McGraw Hill, 1936.

exposed coarse coral sands undoubtedly rendered it very difficult for shallow rooted pioneer species to re-establish themselves. Extreme calcium deficiency was manifest in the invading plants and was one of the major factors contributing to the exclusion of plants from the area.

The radiation dosage received by the plants was undoubtedly at the level where injury is expected to become apparent. Most of the radiation received by the plants had its origin in the fission products scattered about in the surface of the coral sands. Some fission products had lodged upon the plants presumably being transported by mechanical means such as splashing rain drops, or wind action, etc., but by far the greatest concentration of fission products occurred within or upon the roots. It is estimated that the radiation received by the roots from this source was comparable to the amount reported by Jacobson and Overstreet to cause injury to the roots, i.e. ranging up to 0.1 mc/gram of soil.

The "internal" radiation received by the plants was due largely to calcium 45 acquired by direct absorption from the coral sands. Such plants receiving internal radiation, were limited to that area receiving a high enough neutron flux for a significant conversion of the calcium 44 in the soil to calcium 45. The activity within the plants which was due to calcium 45 was relatively low and was not correlated with injury symptoms.

There may be two contributing factors for the low calcium content of the plants growing near the bomb craters. They are: (1) the destruction of habitat resulting in a paucity of soluble calcium in the soil, and (2) an effect of radiation from fission products lodged upon the roots upon the absorption of calcium. There exists in the literature some evidence that radiation may reduce root extension, but prior to the present report there has been no concrete evidence that radiation may reduce calcium absorption. The very striking correlation between fission product radioactivity and reduced calcium content of tissues as herein demonstrated, suggests the probably fruitfulness of an investigation of the effects of radiation from fission products upon the absorption of various mineral nutrients by plants.

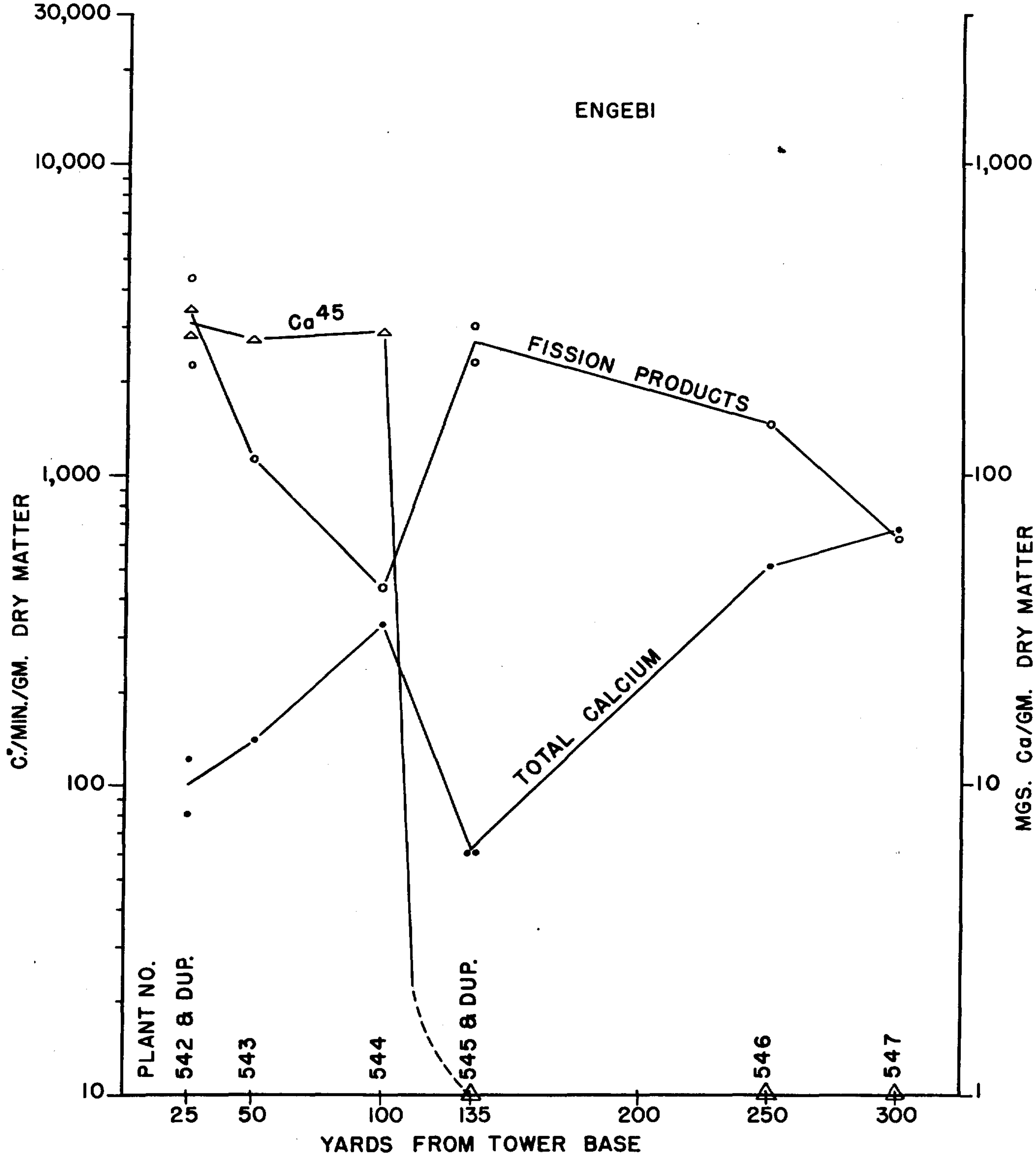

Fig. 1—Diagrammatic representation of the calcium 45 radioactivity, the fission product radioactivity, and the total calcium concentration of the plants of the species Portulaca oleracea collected at various distances from the tower base on Engebi Island. An apparent inverse relationship between fission product radioactivity and total calcium concentration is evident.

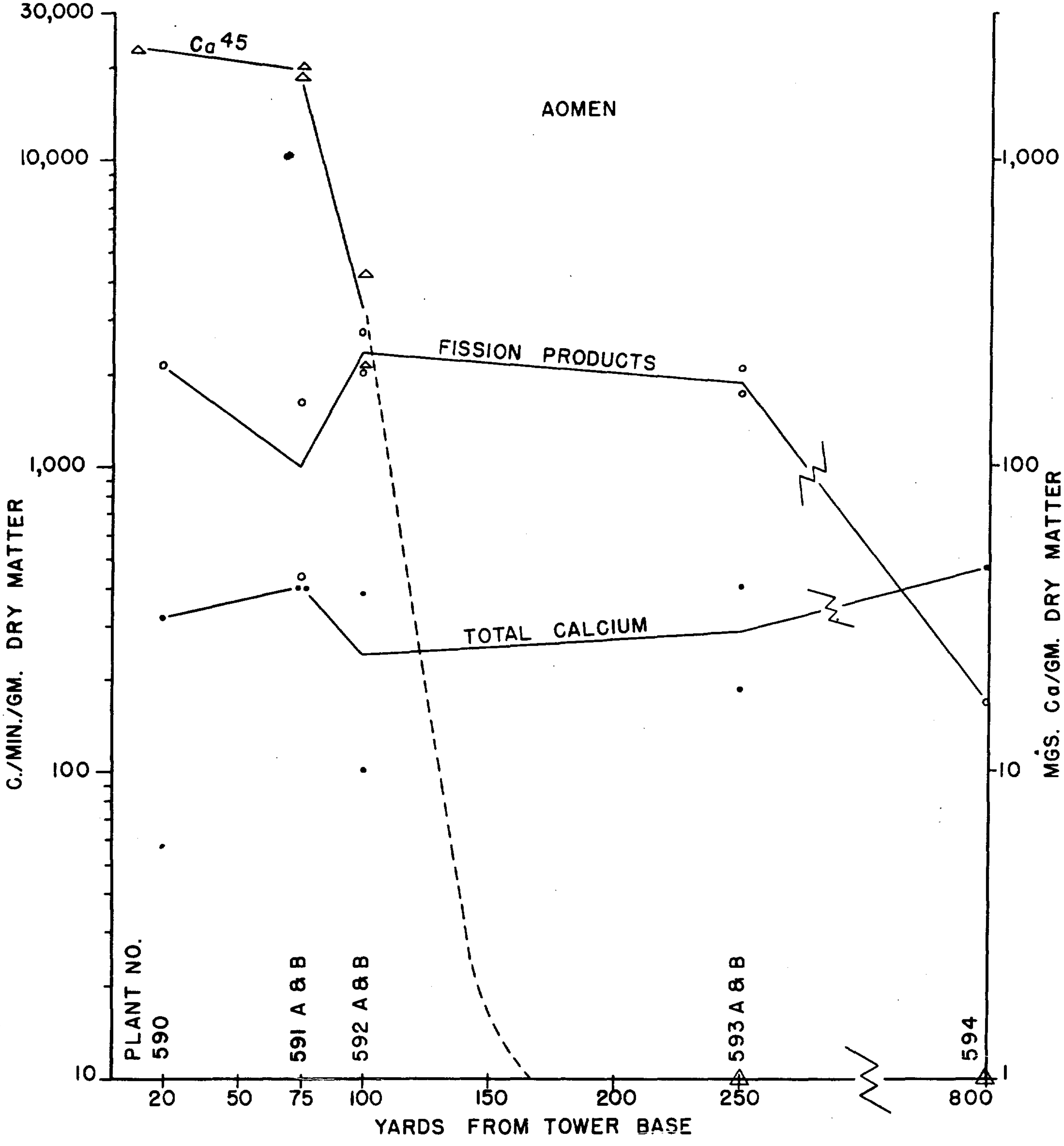

Fig. 2—Diagrammatic representation of the calcium 45 radioactivity, the fission product radioactivity, and the total calcium concentration of the plants of the species Portulaca oleracea collected at various distances from the tower base on Aomen Island. An apparent inverse relationship between fission product radioactivity and total calcium concentration is evident.

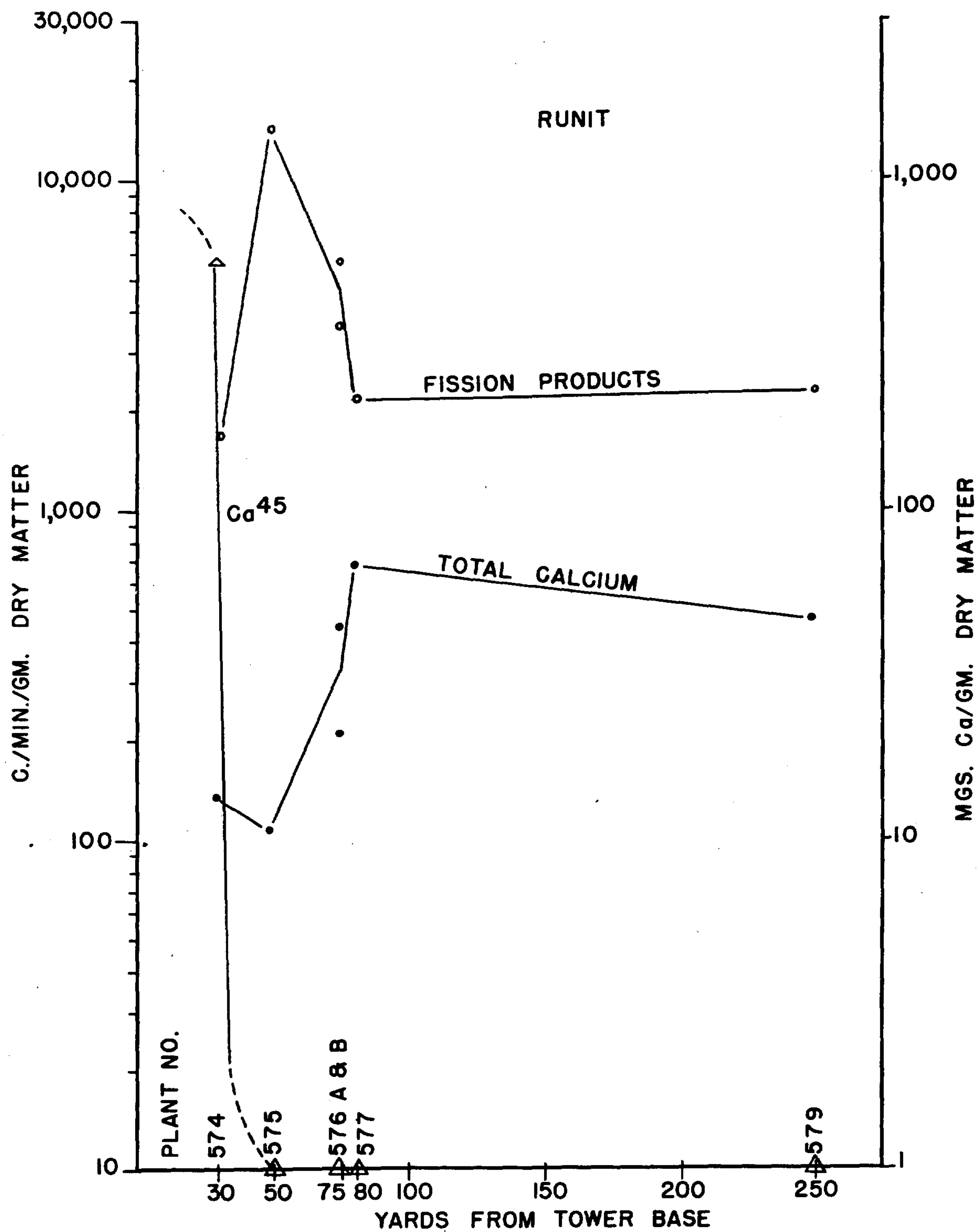

Fig. 3—Diagrammatic representation of the calcium 45 radioactivity, the fission product radioactivity, and the total calcium concentration of the plants of the species Portulaca oleracea collected at various distances from the tower base on Runit Island. An apparent inverse relationship between fission product radioactivity and total calcium concentration is evident.

Fig. 4—Autoradiograph showing the presence of calcium 45 and "specks" of fission products on Portulaca oleracea collected 100 yards from the tower base on Aomen Island. The general image of the plant is due largely to the radioactivity of calcium 45 while the discrete spots scattered at random over the plants are due to fission product radioactivity. The intense activity at the base is due to fission products adsorbed in the root crown.

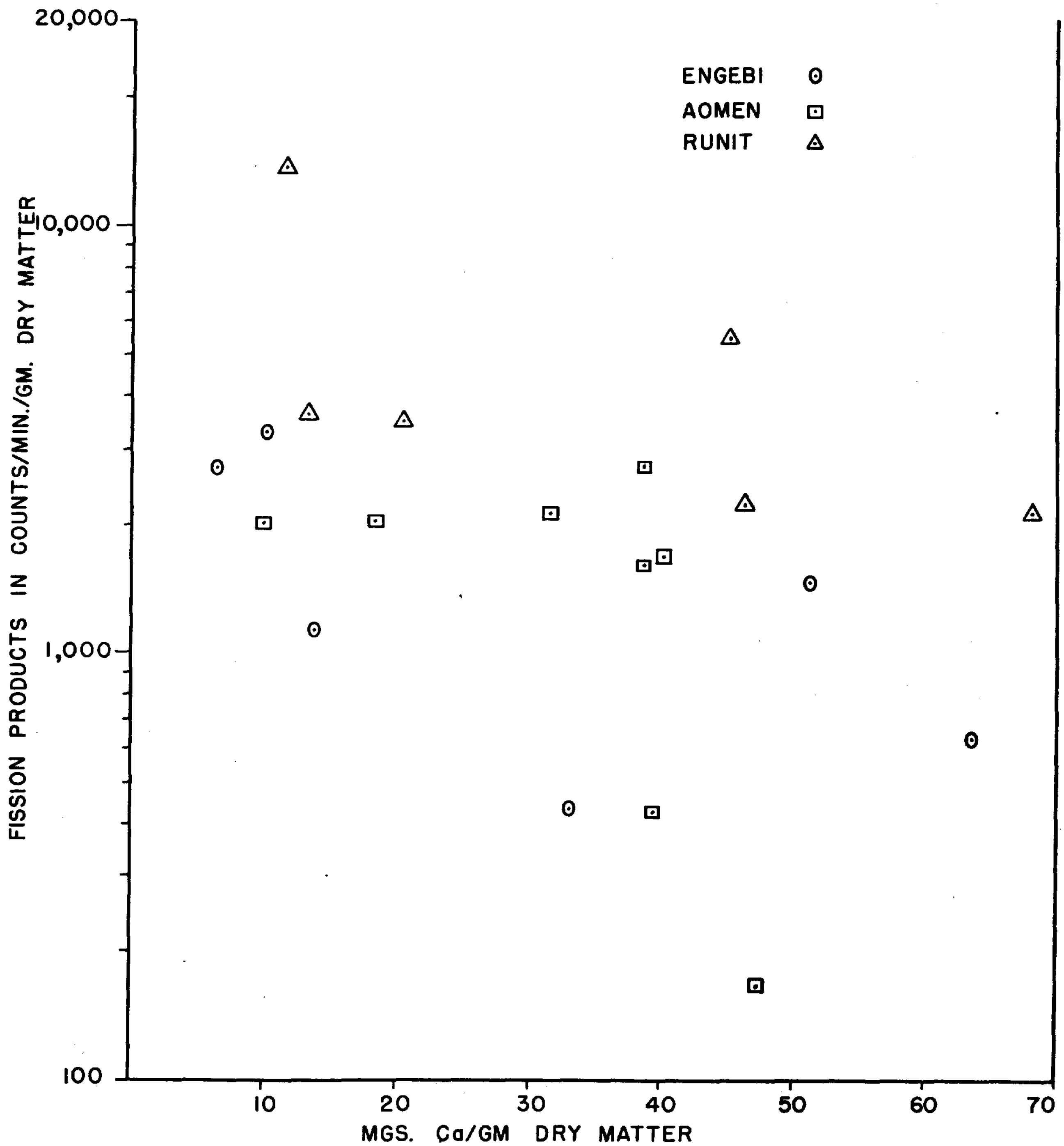

Fig. 5—Regression graph showing the degree of dependence between the total calcium concentration of Portulaca oleracea and the fission product radioactivity of the "specks" lodged upon the plants. (The spread of the points is so great that a regression line was not attempted.) It is assumed that the fission product radioactivity upon the plants does not act directly in reducing the calcium concentration of the plants, but that a high value indicates a correspondingly high value for fission products in the soil, and the magnitude of this radioactivity may then control the calcium absorption by the plant. See text for discussion.

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