PRELIMINARY INVESTIGATIONS OF A MULTIPLE RADIOISOTOPE TECHNIQUE FOR THE EVALUATION OF ROOT ACTIVITY

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I INTRODUCTION

Since the beginning of the era of modern agriculture, research workers have continuously been aware of the important role that the root systems of plants play in plant nutrition. The inherent physiological characteristics of the rooting portion of the plant play a very important part in the utilization of soil water and nutrients. Since these underground organs supply the major portion of all water and nutrients, every investigation which involves some phase of plant nutrition should be based on a thorough understanding of the characteristic rooting pattern of the particular plant. Not only should the extent of the root system be known, but the zones of relative root activity must also be ascertained in order to make accurate recommendations of plant population, fertilizer placement and depth of water application.

Until recent years, the methods available for making such needed root evaluations have been extremely laborious and time consuming which restricted the number of observations which might be made. However, the introduction of radioisotopes into agricultural research has offered a new method for making these needed measurements on the many crop plants.

It was the objective of this experiment to investigate the possibility of using a radioactive tracer technique to characterize the relative root activity in the various zones of the rooting volume.

II REVIEW OF LITERATURE

Early Techniques in Root Evaluation

Considerable time and effort has been devoted to the development of techniques whereby the characteristic rooting patterns of the many different plants may be observed and studied.

The early techniques used for root evaluation have been basically the same, that is, direct observation of the roots of the specimen. The first and most widely used methods excavated the roots of the specimen under study with spades, icepicks, hand trowels, picks and water jets. These in situ excavation methods, which employed both the hand tools (1, 41, 42, 4, 6)[1] and the spray of water (13, 16, 24, 25, 26, 35, 37, 38) have enabled the separation of a good proportion of the main roots for many plants. However, due to the manual labor involved the number of observations that can be made is extremely limited.

In an attempt to reduce some of the manual labor involved in making one observation, these in situ excavation methods, as well as the numerous methods reviewed extensively by Miller (23) have continuously been modified. The more recent trends appear to be more toward representative sampling by taking soil cores (19, 7, 20, 9), prisms (34), blocks (17, 33, 36, 28, 18, 45, 46, 21, 11) and monoliths (5, 43, 8, 10) rather than

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[1]Numbers in parenthesis refer to "Literature Cited," page 46.

removing the entire root system.

These early techniques have made possible some excellent studies. Because of the continuous modification of these techniques it is definitely apparent that a less tedious and time consuming method is needed for root evaluation.

Recent Developments in Root Evaluation

In an attempt to abate some of the tedious and time consuming operations involved in the physical extraction of the roots a new approach has been introduced. This approach is based on the ability of a plant to absorb certain chemical elements variously located throughout the rooting zone. Sayre and Morris (32) were among the first to investigate this possibility by placing lithium in patterns around the plants under consideration. The subsequent presence of lithium in the aerial portions of the plant served as an index of lateral root extension.

A similar approach using phosphorus-32 placed in patterns around grape vines was used by Lott and his associates (22). Since all the placements were shallow, a positive test obtained by tissue testing was considered an indication of lateral root extension. At first, this appears to be a logical interpretation; however, Sayre and Morris (32) recognized the possibility that a negative test in the uptake of lithium might also be interpreted to mean that the extent of the elemental absorptive capacity was represented rather than maximum extension of the roots. It might also be well to keep this in mind in the use of phosphorus or other elements in the evaluation of root extension.

The findings of Lott, et al. (22) were substantiated by the work of Boggie and co-workers (2) in their study of root development using radioactive tracers. Their placements consisted of 150 microcuries of phosphorus-32 placed at depths of 10, 30, 60 and 90 centimeters. They concluded that the phosphorus-32 in the various portions of the rooting zone could be used in determining maximum rooting depth and possible lateral extension.

Using a similar technique, during the study of root penetration and distribution of eight grasses, Burton, et al. (3) placed 0.2 millicuries of phosphorus-32 at depths of 2, 4, 6, and 8 feet. The roots of all the grasses were reported to have reached depths between 2 to 8 feet within three months as indicated by the phosphorus-32 activity in the above ground parts of the plants. This experiment was extended and root cores were taken from some of the treated plots. They concluded that the phosphorus-32 uptake decreased rapidly with increased depth and root yields could not be correlated with phosphorus-32 uptake. This poor correlation is not surprising since the plants were approximately 23 months old when the root samples were taken. It seems probably that at this age the older and larger roots, which would make the largest contribution to the total root system in both volume and weight, would be cutinized or suberized and cease to absorb while continuing their functions as conducting organs. Although there was no correlation between root yields and the phosphorus-32 uptake, the data received might have been applicable in determining the portion of the active absorbing roots within a given portion of the rooting zone.

Emphasizing this possibility, Hall and co-workers (15) in what appears to be the first extensive effort to correlate root activity with isotope uptake, interpreted all of their data on the basis of root activity and what appeared to be the actively absorbing roots. They predicted and sketched the root activity in the various zones of placement throughout the growing season for four different crop plants. The isotope, phosphorus-32, was placed at four depths (4, 8, 12 and 16 inches) in circles of 5 different radii. This study involved twenty plants per replication. By sampling the sites, the root activity, based on the phosphorus in the plants, was traced throughout the growing season for cotton, corn, peanuts and tobacco. Throughout the experiment their interpretations appeared to be sound. However, their predictions of root activity have not been tested.

III MATERIALS AND METHODS

The data presented in this thesis were obtained from a laboratory study conducted at Oklahoma State University during 1958 and 1959. The objective of this experiment was to investigate the possibility of using a radioactive tracer technique to characterize the root activity in various root zones of an individual plant. Analyses of aerial and underground portions of the plant were used as the criteria to determine if a relationship existed between the uptake of an isotope and the quantity of roots which occurred in the different zones of placement.

Selection of the Isotopes

As previously suggested by several investigators (3, 15) the uptake of an isotope placed in the rooting zone of a growing plant could possibly lead to an index of the activity of the roots within these zones of placement. With this point of view in mind, the rooting zone of each plant under study was divided into three layers and a radioisotopic tracer was incorporated within each zone.

It was definitely apparent that the isotopes used in this work would have to be relatively long half-lived isotopes which would be taken up into the plant in appreciable amounts, and they would have to possess physical characteristics which could be used to identify each of the isotopes in the presence of one another. A survey of the nutrient elements which were commercially available in long half-lived radioisotopes showed that there were several isotopes which could be used, e.g., Ca^{45}, P^{32}, Fe^{59}, Fe^{55}, C^{14} and others. However, if a combination of these isotopes were used in the zones of placement the problem of availability of the individual element would have to be taken in account when comparing zones. To avoid the use of factors to compensate for selective ion absorption it appeared desirable to use three isotopes of a single element. Unfortunately, the number of radioisotopes commercially available for any one essential nutrient element is very limited.

However, it has been established through the study of the uptake of radioactive waste (14, 29, 30) and fission materials originating from atomic detonations (27, 31) that there are some non-essential elements which have the ability to substitute in the plant for one of the essential nutrient elements. The most widely investigated by-product of fission, strontium, has this ability and can be partially substituted in the plant for calcium. Strontium was selected as the element to be used in this experiment since appreciable amounts of this element may be taken up by the plant and, in addition, it has three long half-life radioisotopes which are commercially available.

The three isotopes of strontium used in this experiment were Sr^{89}, Sr^{85} and Sr^{90} + Y^{90}. Strontium-90 has a half-life of 19.9 years and emits a .61 mev beta particle. Present with Sr^{90} is its daughter product, Y^{90}, which has a half-life of 61 hours and emits a 2.18 mev beta particle. Strontium-89 is also a beta emitter, 1.46 mev, which has a half-life of 53 days. Strontium-85 has a half-life of 65 days and emits a .51 mev gamma ray (40).

Radioisotope Counting System

All the counting was performed with equipment procured from Nuclear-Chicago Corp. The counting system consisted of a model 186 scaler and a model DS5-1P (scintillation) detector probe equipped for the use of either a XTB anthracene crystal 3/16 inch thick by 1 1/2 inches in diameter or a XT150 sodium iodide crystal 1 1/2 by 1 1/2 inches. The detector was housed in a model 3053 lead shield.

Gamma Detection

In all but one of the forage samples analyzed there were present two or more of the introduced isotopes which resulted in the presence of both beta and gamma radiation. It was possible to discriminate against either type of radiation through the choice of crystals with the counting system previously described since the system was equipped with both an anthracene crystal, sensitive to beta particles, and a sodium iodide crystal, sensitive to gamma rays.

The gamma rays emitted by the Sr^{85} were detected by using the sodium iodide crystal. An aluminum absorber with a density of 1882 mg./cm^2 was placed between the crystal and the sample. The aluminum absorber appeared necessary to prevent any possible detection of the beta particles present. The overall counting efficiency of this geometrical arrangement used for the detection of gamma radiation was 5.7 per cent.

Beta Detection and Separation by Absorption

The ability to differentiate between the beta and gamma radiation, which ultimately resulted in the separation of the Sr^{85} from the Sr^{90} +

Y^{90} and the Sr^{89}, was simplified through the use of the two crystal counting system. However, the separation of the two beta emanations was not as easily accomplished and necessitated the development of an absorption separation procedure which could be used to identify combinations of these two isotopes as they occurred in the forage samples.

This procedure, which is outlined below, is based on the addition of different proportions of $Sr^{90} + Y^{90}$ and Sr^{89} curves to form new curves containing different percentages of these two isotopes.

Beta Absorption

Quite fortuitously, the combined effects of a continuous spectrum and scattering leads to an approximate exponential absorption law for beta particles of a given maximum energy (12). For this reason, the absorption curves, activity versus thickness ($mg./cm.^2$) of absorber traversed, are generally plotted on semilog graph paper. Figures 1 and 2 represent typical $Sr^{90} + Y^{90}$ and Sr^{89} absorption curves respectively. The shape of these curves depends on the shape of the beta spectrum and the geometrical arrangement of the sample, sample holder, absorber and detector.

The closer the sample and absorber are to the detector, the more nearly a straight line plot the semilog absorption becomes for a beta particle emitter. However, in most counting systems the distances between the sample and absorber and detector will be some finite distance and this will result in some curvature toward the vertical axis even for isotopes which emit only one beta energy spectrum. Such curvature can readily be seen in Figure 2.

In order to discuss the effects of geometry on these beta absorption

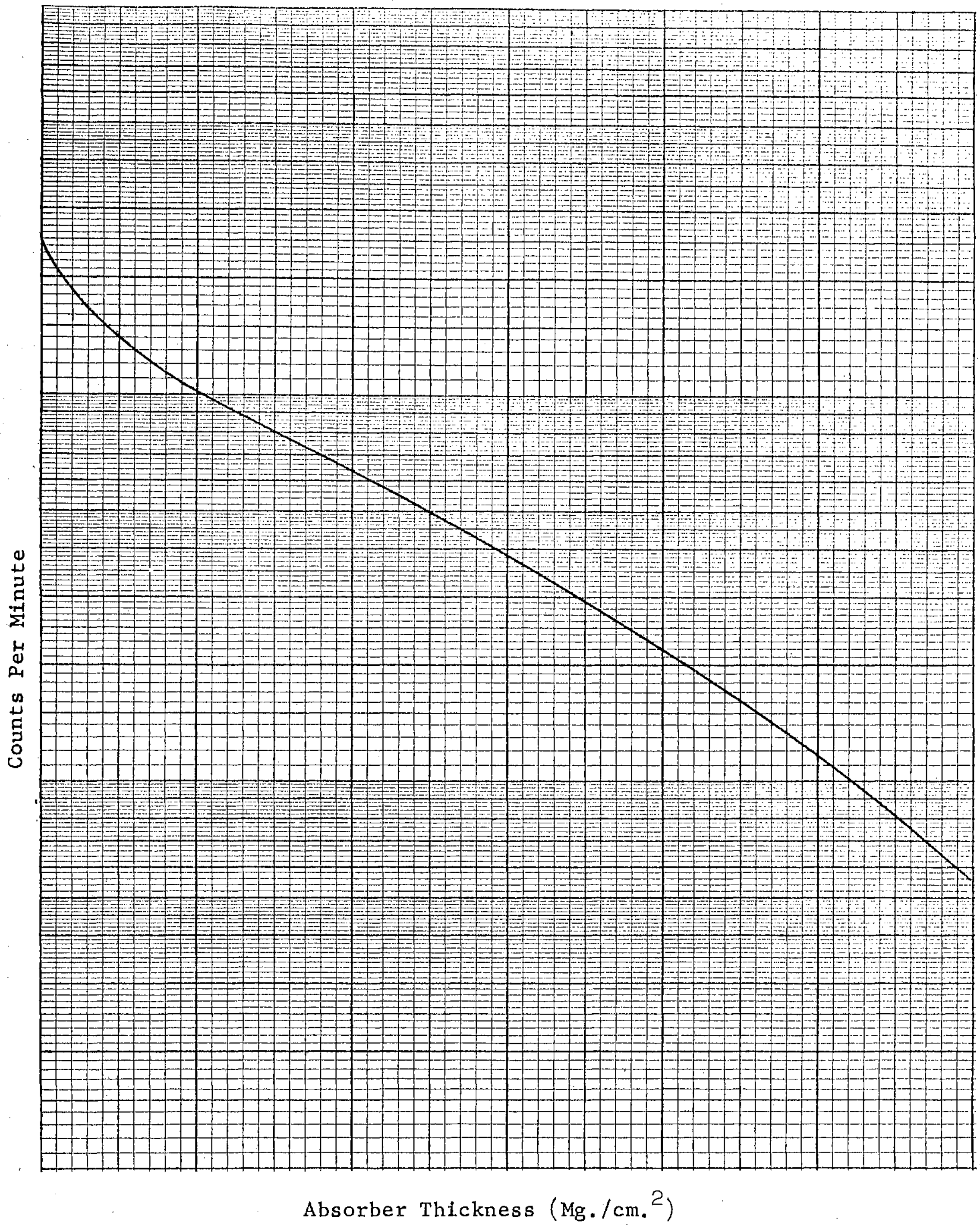

Figure 1. Pure Sr^{90} + Y^{90} Absorption Curve

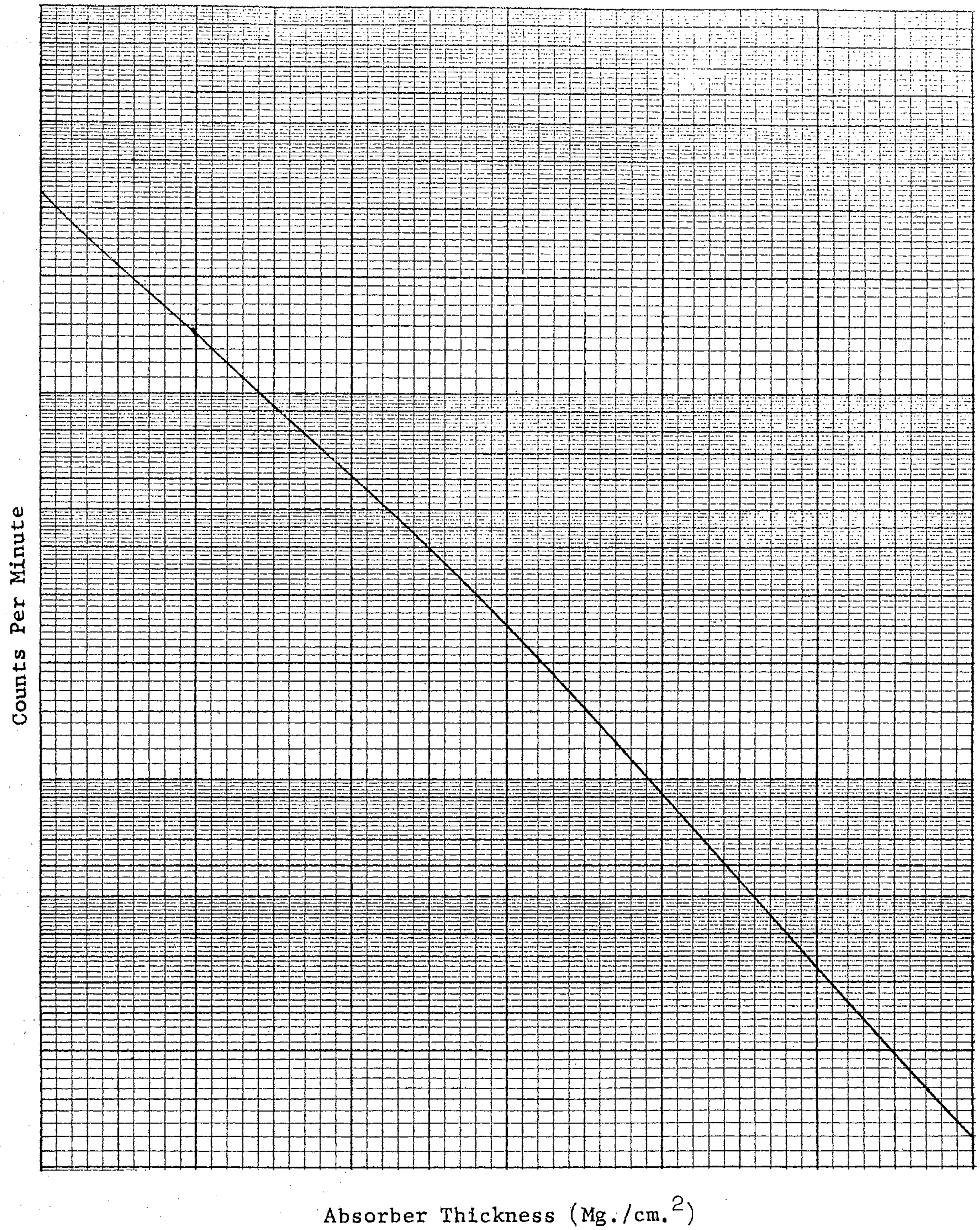

Figure 2. Pure Sr^{89} Absorption Curve

curves, it will be advantageous to consider three possible cases: Case 1 where the distance between the source, absorber and detector is an infinitesimal distance, Case 2 where the source has been displaced downward from the absorber and detector a sufficient distance to cause geometrical effects below the absorber and Case 3 where both the source and detector have been shifted away from the absorber enough to cause geometrical effects both above and below the absorber. For convenience in later discussions, the distance from the source to absorber will be defined as p and the distance from the absorber to the detector will be defined as q.

Case 1, (p small, q small): In the case where the distance between the source, absorber and detector is infinitesimal the exponential absorption law can be represented by the equation $A_d = A_o e^{-ud}$, where A_o represents the "apparent source strength" through an absorber of zero thickness (disregarding the aforementioned effects of geometry) and A_d represents the detectable activity through an absorber thickness d with an absorption coefficient u. Note that where $d = 0$, $A_d = A_o$ which means that A_o is not the activity of the source material since counter efficiency, solid angle between source and detector, etc., must be taken into account. For a given geometry, all such factors are constant so A_o is some constant proportion of the source activity.

In making such a plot of A_o versus d, the transformation $\log A_d = \log A_o + (-ud)$ or, $y = c_1 - ud$ can be made, where $y = \log A_d$, and c_1 is a constant equal to $\log A_o$. If values are assigned to A_o and ud it is thus seen that for this idealized curve a linear function (straight line) y versus d results (Figure 3).

If the source strength is increased or decreased, that is, if activity

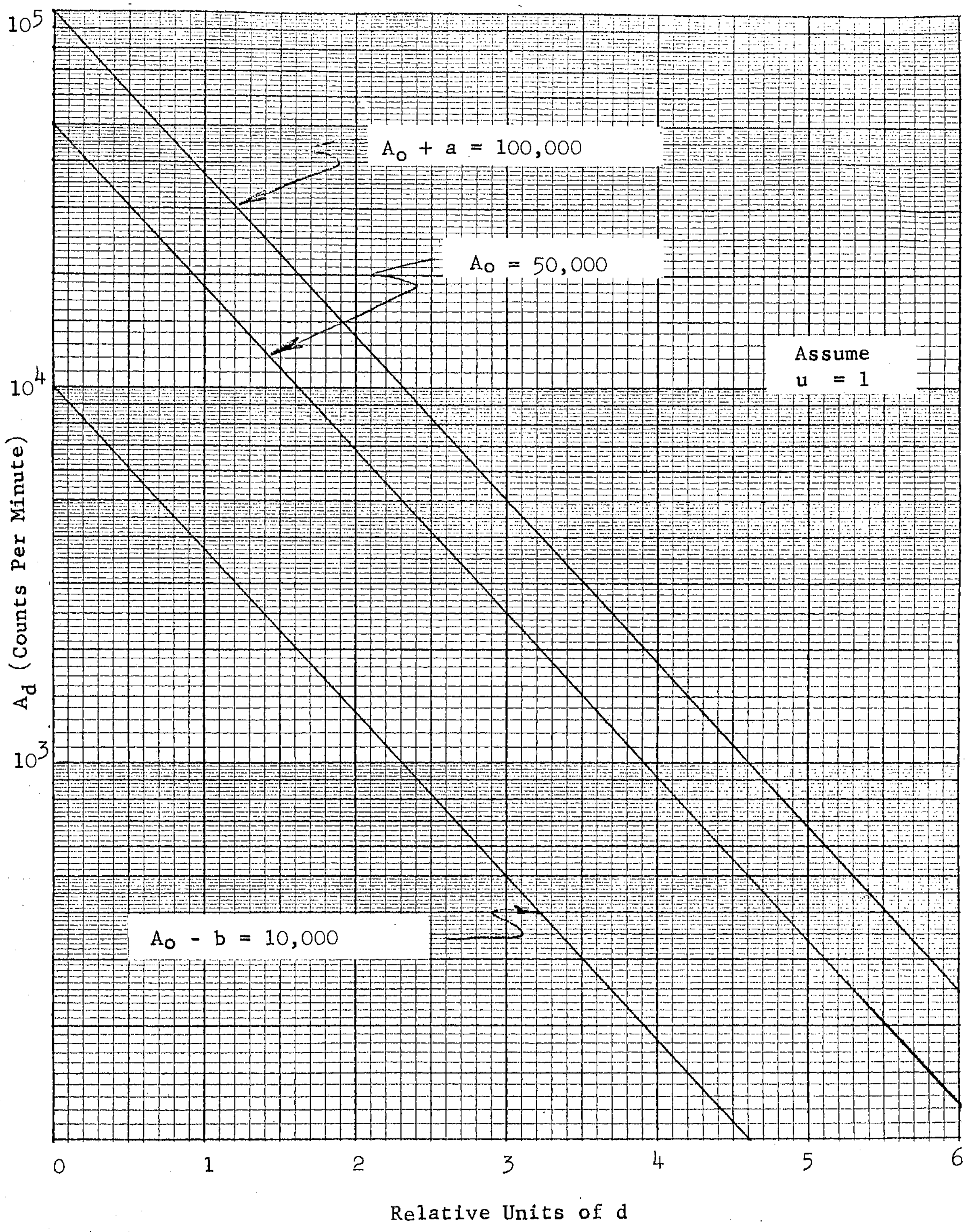

Figure 3. Ideal Absorption Curve

is added to or subtracted from A_o new curves are obtained:

$$y = \log (A_o + a) - ud = c_2 - ud$$

$$y = \log (A_o - b) - ud = c_3 - ud$$

These curves will have the same slope as the original curve, but have different axis intercepts. An examination of the foregoing equations shows that for all d's the vertical distance between any two curves is constant. Hence, the curves are parallel as is seen in Figure 3.

Case 2, (p large, q small): Experimental curves, in which distances between components are not infinitesimal, for reasons mentioned earlier do not generally appear as straight lines. Due to preferential scattering and absorption of specific energy components of the beta spectrum by the sample holder, shield, air and other geometrical features, certain portions of the spectrum appear to be intensified or diminished in the counting procedure. This accounts for the curvature as is shown in Figure 1.

In comparing this Case, where the source is some distance from the detector and absorber to Case 1, A_o as viewed at the plane of the absorber will appear to be changed by some "increment" for a given value of d by the scattering and absorption processes. They may serve to increase or decrease A_o since the experimental curves depart above or below the theoretical straight line for different values of d.

Since by the very nature of beta scattering, the number of particles undergoing scattering or absorption in an element of volume is proportional to the number of particles entering the volume, values can be assigned to compensate for these processes. If x_1 is designated as the proportion by which A_o changes resulting from the scattering and absorption between absorber and source for a given absorber d_1, the apparent source strength now becomes $x_1 A_o$ as viewed at the bottom of the absorber. Note that with

$d = 0$, A_d is not the same in Case 1 as Case 2 since for Case 1, $A_{d_o} = A_o$; for Case 2, $A_{d_o} = x_o A_o$. If curves are examined where the source strength is $A_o + a$ and $A_o - b$ where $d = d_1$, the apparent source strengths will be $x_1(A_o + a)$ and $x_1(A_o - b)$ respectively.

The functions for any i value of d can be represented by

$$y = \log x_i A_o - ud_i = \log x_i + \log A_o - ud_i = \log x_i + c_1 - ud_i$$

$$y = \log x_i(A_o + a) - ud_i = \log x_i + \log(A_o + a) - ud_i = \log x_i + c_2 - ud_i$$

$$y = \log x_i(A_o - b) - ud_i = \log x_i + \log(A_o - b) - ud_i = \log x_i + c_3 - ud_i$$

An examination of these equations of discrete functions shows that the distance between ordinates along two given curves is constant for all d_i. This means all curves where source activity is the parameter are geometrically parallel.

Case 3, (p large, q large): In the case where the geometry has been altered to produce geometrical effects both above and below the absorber, curves will result which are comparable to experimental curves.

As pointed out in the discussion of Case 2, the apparent source strength at the plane of the absorber will be changed due to the processes of scattering and absorption below the absorber by a factor of x. These same types of processes will also appear to diminish or intensify in the absorber to detector space the component $xA_o e^{-ud}$ traversing the absorber. By the same argument put forth in Case 2, this increase or decrease can be compensated for by a factor which in this discussion will be termed z.

If x_1 and z_1 are designated as the proportionate increases and decreases in A_o resulting from the scattering and absorption respectively below and above the absorber d_1, the components activating the detector will not become $z_1(x_1 A_o e^{-ud_1})$. If consideration is again given to sample strengths $A_o + a$ and $A_o - b$ with $d = d_1$, the components activating the

the detector will now be $z_1[x_1(A_o + a)e^{-ud_1}]$ and $z_1[x_1(A_o - b)e^{-ud_1}]$ respectively.

The functions to be plotted can now be represented by

$$y = \log z_i x_i A_o - ud_i = \log z_i x_i + \log A_o - ud_i = \log z_i x_i + c_1 - ud_i$$

$$y = \log z_i x_i(A_o + a) - ud_i = \log z_i x_i + \log(A_o + a) - ud_i = \log z_i x_i + c_2 - ud_i$$

$$y = \log z_i x_i(A_o - b) - ud_i = \log z_i x_i + \log(A_o - b) - ud_i = \log z_i x_i + c_3 - ud_i$$

The numerical values of x_i and z_i for a given d_i in this development may be extremely complicated functions but for a given geometry, absorber and isotope they will remain contant. Hence no time needs to be spent on trying to evaluate x_i and z_i.

The curves containing the x and z terms are viewed here as discrete functions. Again note that the distance between the ordinates along two given curves is constant for all d_i. Hence all curves where source activity is the parameter are geometrically parallel. This property enables one to construct a curve for any activity of a source provided one curve is known for the geometry and the particular isotope. Figure 4 shows curves of $Sr^{90} + Y^{90}$ for several assumed source strengths by using the curve of Figure 1 for the general shape.

Isotope Separation by Absorption

Theory of Curve Addition: As previously pointed out, each isotope has a characteristic absorption curve regardless of its apparent source strength A_o. In view of this fact, it should be reasonable to expect that when two beta emitting isotopes, B and C, are mixed together the resulting absorption curve should exhibit slope changes common to both components.

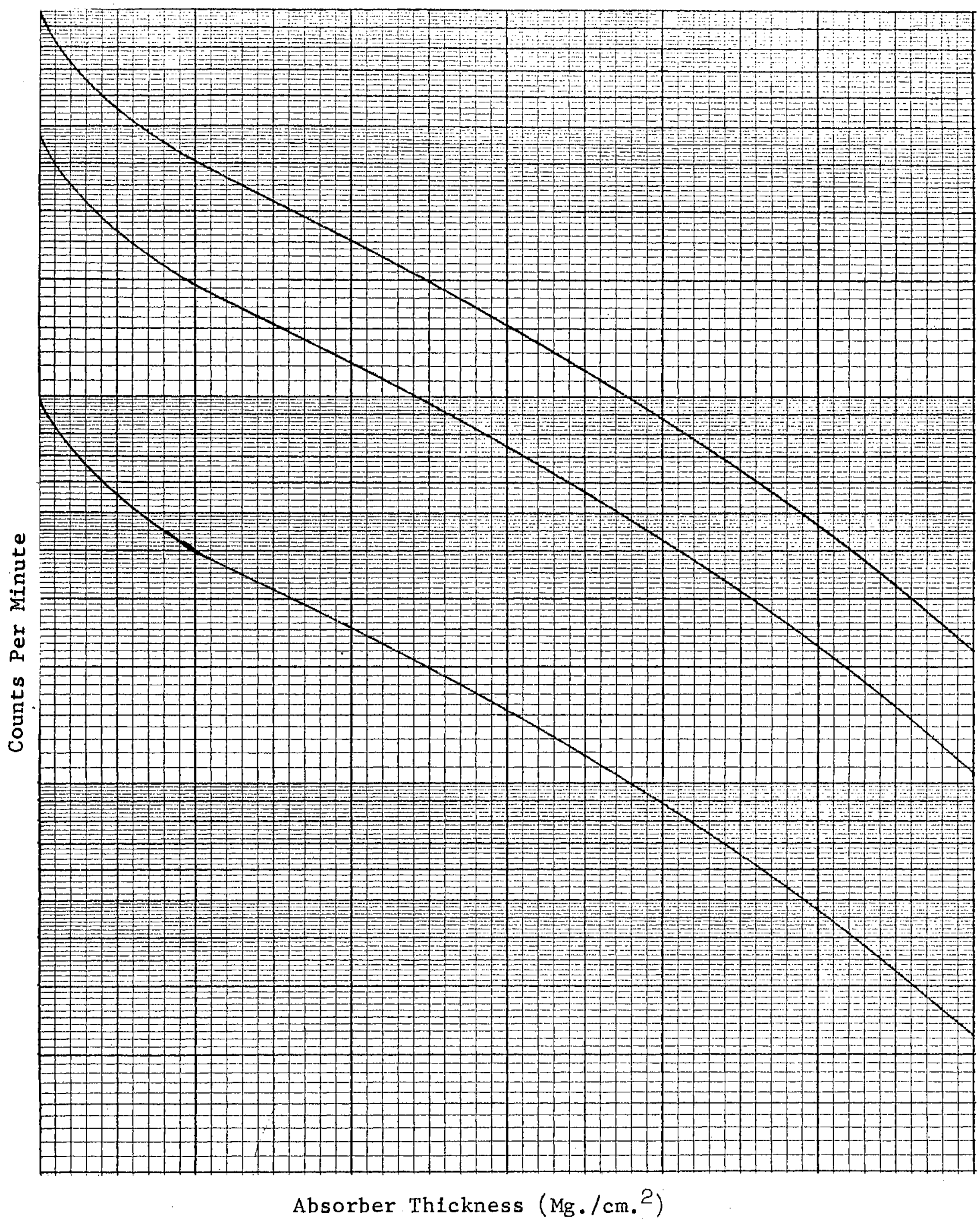

Figure 4. Comparison of Absorption Curves of Different Source Strengths

If an absorption curve is prepared on a mixture of these two isotopes the resulting curve should lie somewhere between the B and C curves when the ordinates for the zero absorber thickness are aligned for all three curves. The shape of this curve will approach the shape of either the B or C curve depending on which makes up the largest percent of the mixture. The curve resulting from different mixtures of these two isotopes can be expressed by the equation

$$y_i = z_{i_B} x_{i_B} h B_o e^{-ud_i} + z_{i_C} x_{i_C} j C_o e^{-ud_i}$$

where h and j represent the percentages of the isotopes B and C which were present in the mixture. The other symbols take the same values as before. The z and x factors take a further subscript denoting the radioisotope to which they pertain.

Since the values of x and z may be very complicated functions and may not be determined easily, experimental methods may be used to obtain values of $z_{i_B} x_{i_B} h B_o e^{-ud_i}$ and $z_{i_C} x_{i_C} j C_o e^{-ud_i}$ in order to add the curves together in different proportions. The preceding section shows that these values may be obtained directly through the use of experimental curves for isotopes B and C. Any desired curve may be obtained when the ordinate for the zero absorber thickness is aligned at values on the vertical axis corresponding to the desired h and j for the isotopes B and C respectively. The method used for preparing curves of different proportions of B and C (Sr^{90} + Y^{90} and Sr^{89} for this experiment) is outlined below.

Establishment of the Pure Isotope Curves: In constructing the experimental curves small samples of Sr^{90} + Y^{90} and Sr^{89} were evaporated to dryness in stainless steel sample pans, 1 inch in diameter by one-fourth inch deep. To insure uniform deposition in the pan, the samples were evaporated under an infrared lamp on a 16 revolutions per minute sample spinner. The evaporated samples used to construct the pure curves produced a count of approximately 2500 counts per minute at the zero absorber thickness in the constant geometry used for all subsequent counting.

Absorption curves were taken on both the Sr^{90} + Y^{90} and the Sr^{89} samples using the counting system previously described plus a model C-101 Nuclear-Chicago aluminum absorber set ranging in densities from 1.4 to 1882 mg./cm.^{2}. One hundred thousand counts were collected at each absorber thickness to provide a reliable counting error of less than 0.6 percent (39).

The counts per minute versus absorber density was then plotted on semilog graph paper to give Sr^{90} + Y^{90} and Sr^{89} absorption curves. (Hereafter these initial curves will be termed <u>pure curves</u>. See Figures 1 and 2).

Establishment of the Reference Curves: Reference curves which contained different percentages of the two isotopes were then prepared for isotope separation. The combinations are listed in Table I. In all cases the Sr^{90} was in equilibrium with its daughter, Y^{90}.

TABLE I

Reference Curves

100% Sr^{90}	
90% Sr^{90} + 10% Sr^{89}	40% Sr^{90} + 60% Sr^{89}
80% Sr^{90} + 20% Sr^{89}	30% Sr^{90} + 70% Sr^{89}
70% Sr^{90} + 30% Sr^{89}	20% Sr^{90} + 80% Sr^{89}
60% Sr^{90} + 40% Sr^{89}	10% Sr^{90} + 90% Sr^{89}
50% Sr^{90} + 50% Sr^{89}	100% Sr^{89}

In preparing these curves for isotopic separation the pure curves previously constructed were divided into 13 density intervals ranging from 0-600 mg./cm.^{2} inclusively at 50 mg./cm.^{2} increments. The points where the curves intersected the 13 density intervals were marked with a small dash parallel to the horizontal axis as illustrated in Figure 5. These density increments, represented by the small dashes, provided convenient references at identical d_i values with which to obtain values from the pure curves to construct curves representing the different base percentages (Table I). The pure curves were then traced onto plastic sheeting and a vertical reference line was constructed representing the zero absorber thickness. (The curves on the plastic sheeting will subsequently be called <u>templates</u>).

Curve Addition: In order to obtain values to add the curves together in different percentages, the pure Sr^{90} + Y^{90} and Sr^{89} templates were laid over separate pieces of semilog graph paper and their reference axis aligned with the zero absorber thickness ordinate on the graph paper. Then the zero absorber thickness of the two curves were aligned with values corresponding to desired percentages of Sr^{90} and Sr^{89}. For example, in preparing the 10% Sr^{90} + 90% Sr^{89} curve, the zero absorber thickness of the Sr^{89} was aligned with the point on the graph paper equivalent to 90,000 counts per minute while the Sr^{90} curve was aligned with the point equivalent to 10,000 counts per minute. See Figure 6. The small dashes previously marked on the curves were then read as counts per minute on the vertical axis (Figure 6). The counts per minute corresponding to these points of intersection were recorded at all 13 density increments for both the Sr^{90} and the Sr^{89} curves. The counts at a given d value for the Sr^{89} were then added to the corresponding d value counts

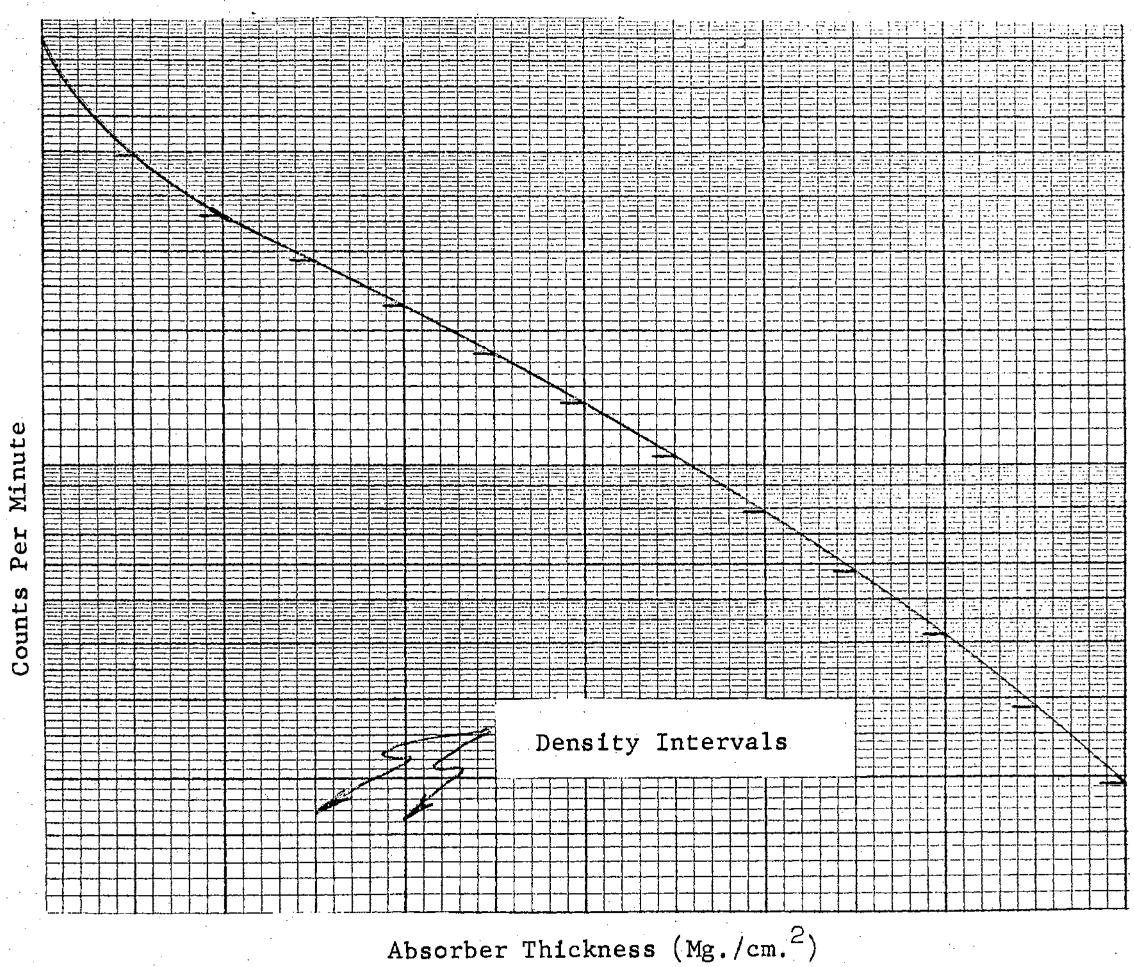

Figure 5. Construction of the Pure Curve Template.

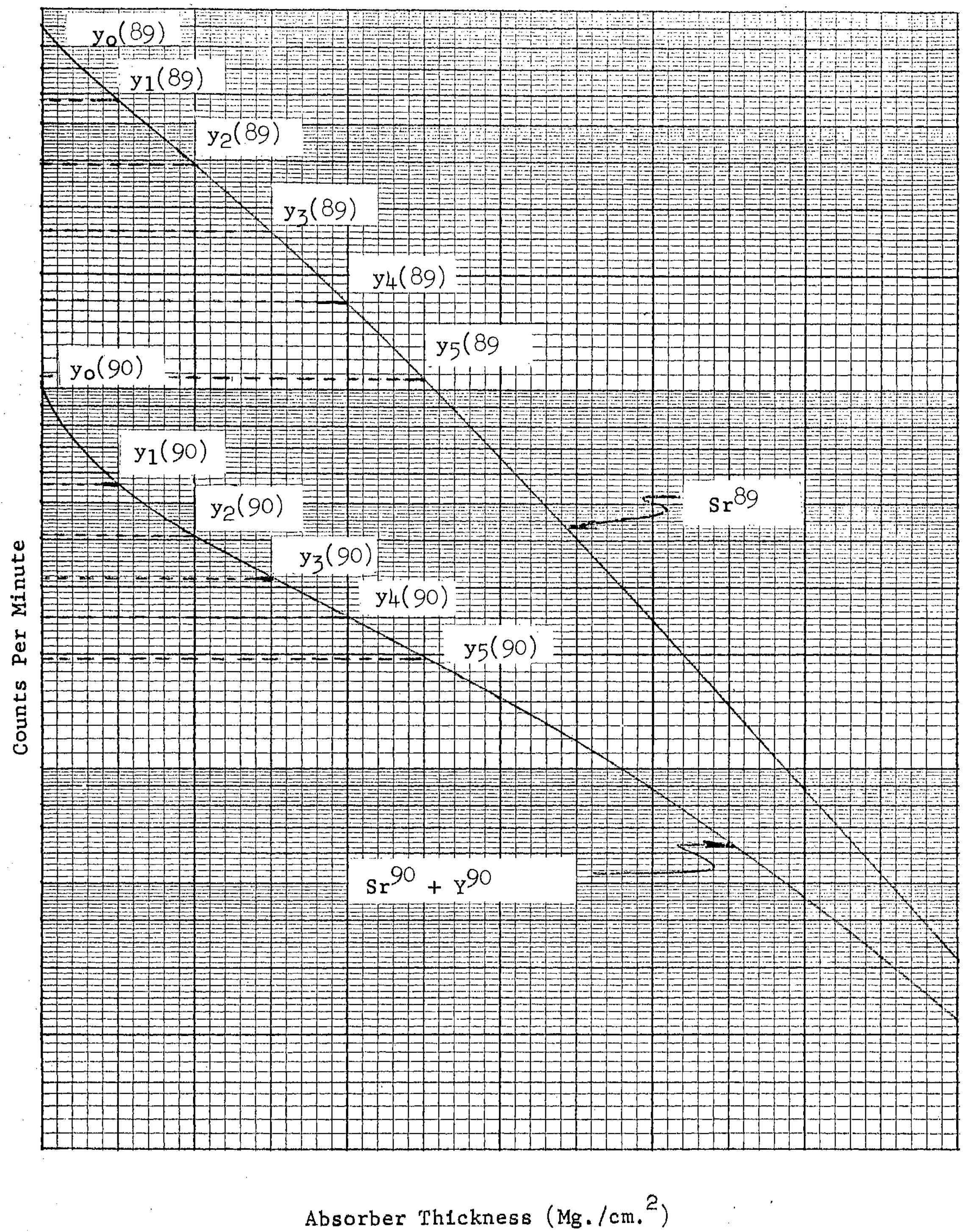

Figure 6. Curve Addition

for the Sr^{90}, e.g., $y_o(89) + y_o(90)$, $y_1(89) + y_1(90)$ . . . . . $y'_{12}(89) + y_{12}(90)$. These sums were then replotted (activity versus absorber thickness) to form a curve representing 10% Sr^{90} + 90% Sr^{89}. This process was repeated at 80,000 cpm, 70,000 cpm, ... 10,000 cpm for the Sr^{89} and at 20,000 cpm, 30,000 cpm, ... 90,000 cpm for the Sr^{90} until all 9 curves were prepared.

These prepared curves represented a set of reference curves which could be used to identify unknown proportions of these isotopes as they occurred in experimental samples.

These reference curves were then traced onto plastic sheeting to provide a set of transparent templates. These templates when placed over curves obtained from the forage samples, were used to determine the relative proportion of each isotope in the sample.

Identification of the Isotopes in the Forage Samples

In preparing the forage curves for identification of isotope make-up, it was not necessary to collect points at all the available absorber densities. Therefore, five points of 100,000 counts were collected using absorber densities of 0, 20, 104, 431 and 1882 mg./cm.^{2}.

Each point was converted to counts per minute and plotted on semilog graph paper after the background had been subtracted. The counts per minute traversing the 1882 mg./cm.^{2} absorber was considered a measurement of background. The zero absorber density was used as a reference to align the reference template while the other three points were used in selecting the template which gave the best fit.

Preparation of the Separation Layers

In order to prevent the movement of isotopes from one zone to another through the root growth medium, a separation layer of some sort was necessary for this experiment. The layers would need to be impermeable to water and ions but permeable to the roots. After considerable experimentation, a paraffin-rosin layer was developed which possessed all the characteristics needed for this experiment.

The layers used were composed of a 1:1 mixture (by weight) of paraffin and rosin which was prepared by melting and mixing the two components. After the mixture had reached the boiling point, 75-80 milliliters of the liquid was poured on the surface of water (heated to 60° C) one-half inch deep in a pan which was 12 inches in diameter and 1 1/2 inches deep. The mixture was allowed to cool and was then cut into an eight inch square to be fitted in the special pots to be described later. In order to make a water tight seal with the sides of the pot, the layers were sealed to the sides of the pots with a heated soldering iron.

The layers used were approximately one millimeter thick. Previous observations had shown that when the layers were penetrated by a root they formed a seal which restricted the movement of water and nutrients from the upper zones into the underlying zones.

Design and Construction of the Pots

The pots used in this experiment were constructed of fifteen pound tar construction paper. The pots had a six inch square base and were nine inches high.

The pots were constructed from 24 inch square cut-outs which were cut and folded as illustrated in Figure 7.

Upon final shaping the containers were stapled together and waterproofed with a paraffin coating.

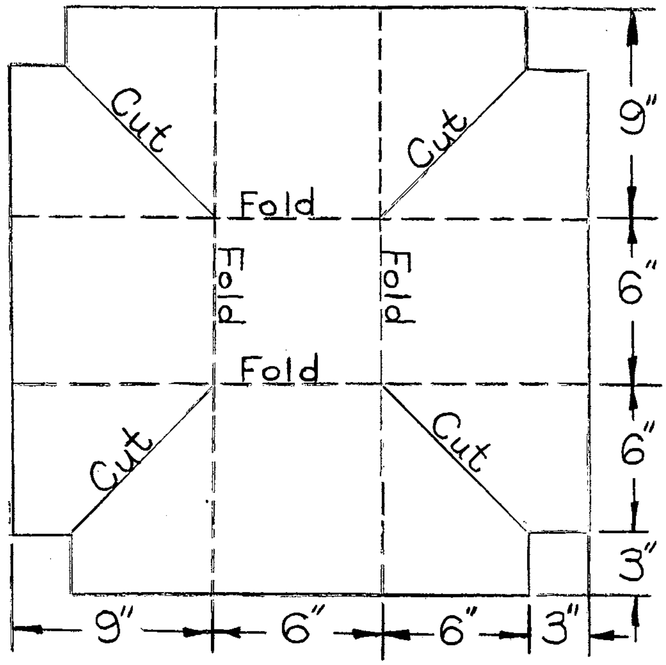

Figure 7. Design of the Pots

Pot Preparation for Plant Culture

A three day period was devoted to the preparation of all the pots with one day being devoted to completing one zone in all the pots before proceeding to the next. This procedure appeared to be necessary to avoid loss of water from the underlying zones. Sterile silica sand (1960 grams), equivalent to 72 cubic inches, was placed in each zone. One hundred and sixty milliliters of a modified number 2 Hoagland nutrient solution was added to each zone. This was equivalent to the amount of water this quantity of sand will hold against the pull of gravity. The composition of the nutrient solution is given in Table II.

TABLE II

Composition of the Nutrient Solution

Salt	gm./liter
NH_4NO_3	.95
KNO_3	.61
$MgSO_4 \cdot 7H_2O$	.49
$NH_4H_2PO_4$	.12
Ferric Citrate	.005

The minor elements were added by supplying 1 cc per liter of solution containing the following:

Salt	gm./liter
H_3BO_3	.60
$MnCl_2 \cdot 4H_2O$	.40
$ZnSO_4$	.05
$CuSO_4 \cdot 5H_2O$	.05
$H_2MoO_4 \cdot 4H_2O$	.02

Radioisotope treatments were as follows: 150 disintegrations per second of Sr^{85} per gram of sand to the bottom zone, 150 disintegrations per second of Sr^{89} per gram of sand to the middle zone and 100 disintegrations per second of $Sr^{90} + Y^{90}$ per gram of sand in the top zone. The activity of the radioisotope solution added to each of the zones was determined in the constant counting geometry which was maintained throughout the experiment. Each radioactive solution contained as carrier 0.00078 grams of stable $SrCl_2$ per microcurie of radioisotope.

Between the zones was placed one of the paraffin-rosin layers, previously described. Also incorporated in each zone was a tube to provide a means of adding water and nutrients as the need might arise (Figure 8). The tubes were hard rubber construction with the bottom sealed and the bottom two inches perforated. The tubes were sealed to the layers at the time they were sealed in the pots with a heated soldering iron. It was felt that these vents, open to the atmosphere, also provided a means for exchange of gases.

Experimental Procedure

On February 13, 1959, an additional 2000 grams of sand and 160 milliliters of nutrient solution was added to the top portion of the pots to provide a medium for the planting of $1\frac{1}{2}$ x $1\frac{1}{2}$ x $1\frac{1}{2}$ inch plugs of Ladino clover, Trifolium repens L.

The experiemnt was conducted in the laboratory under florescent lights adjusted with an automatic time switch to a daily light period of 13 hours.

The experiment consisted of 30 pots, 24 treated pots and 6 untreated pots which served as an index of moisture conditions and root penetration in the early stages of the experiment.

The surface layer in each pot was watered with distilled water throughout the experiment. Sufficient water was added at intervals to the untreated top zone to insure a favorable moisture condition for plant growth. It was deemed unnecessary, by visual observation, to provide any additional water to the three underlying zones since the moisture conditions did not appear to change appreciable from the the planting date to the date the experiment was terminated. This is an interesting observation in itself.

The first harvest date was 14 days after the planting date. Harvest dates are listed in Table III. The entire aerial portion of the plant was

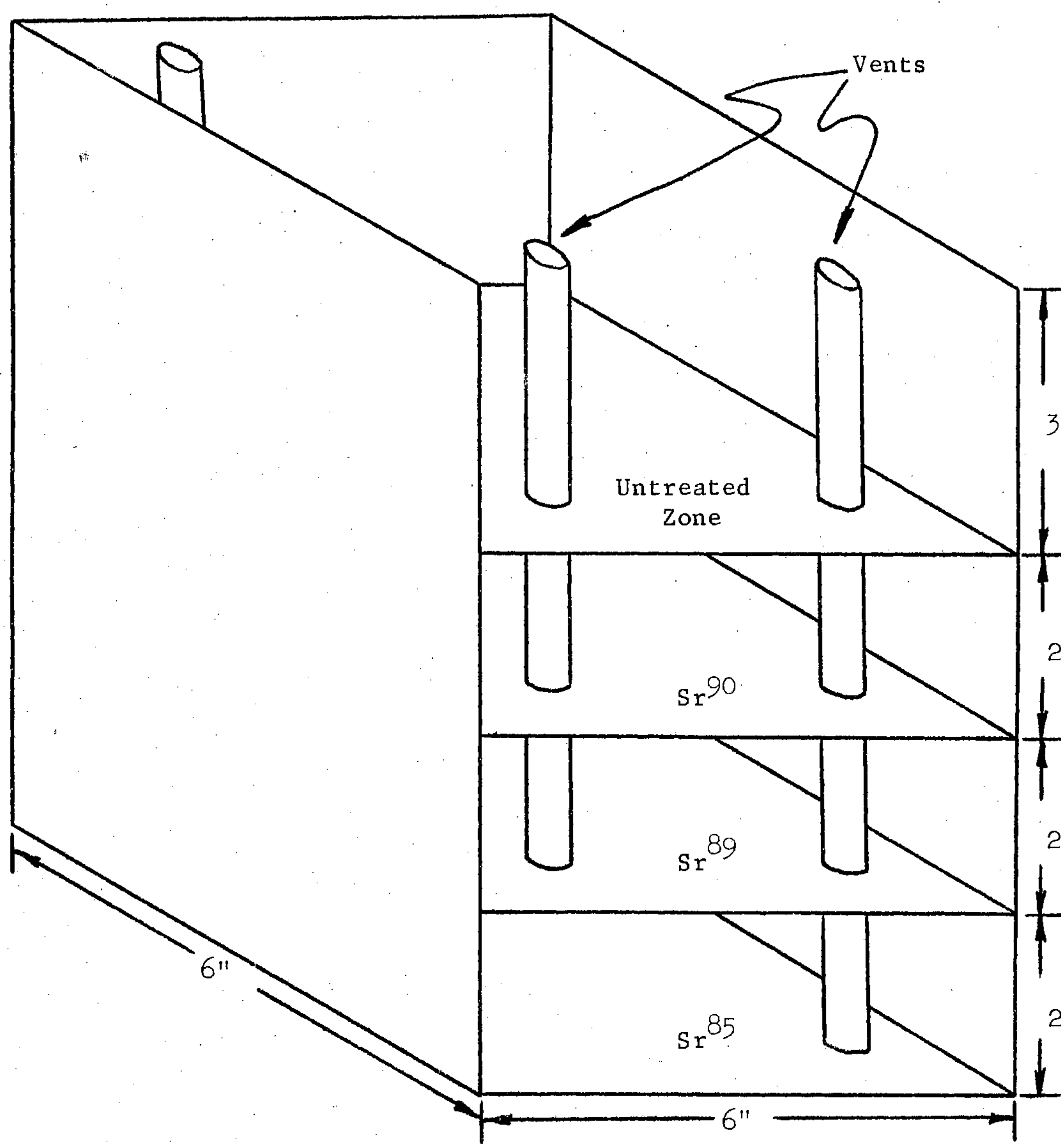

Figure 8. Diagram of Assembled Pot.

TABLE III

HARVEST DATES

Harvest Date	Pot Number*	No. Days Between Planting and Harvest
2/27/59	21	14
3/17/59	26	32
3/28/59	25	43
3/28/59	24	43
3/28/59	28	43
4/5/59	19	51
4/5/59	30	51
4/11/59	23	57
4/11/59	27	57
4/11/59	18	57
4/20/59	11	66
4/26/59	10	72
4/26/59	14	72
5/3/59	17	79
5/3/59	20	79
5/10/59	9	86
5/10/59	22	86
5/10/59	29	86
5/17/59	7	93
5/17/59	8	93
5/17/59	12	93

*The plant materials from pots 13, 15 and 16 were lost during digestion of the plant samples.

clipped and added to the lower leaves which had withered and had been collected prior to the harvest date. This plant material was dried at 85 degrees centigrade in a forced-air, low-draft drying oven for 12 hours. After drying, the plants were weighed and the yields were recorded. The dried plant materials were then stored for radioisotope determinations.

The pots were then disassembled and the roots were collected from each zone. The roots were then placed in 200-ml. beakers half filled with water and stored for physical determinations.

Analysis of Forage Materials

The entire aerial portion of the plant was digested with 20 ml. of a nitric-perchloric acid mixture (3 parts of concentrated HNO_3 and 1 part of 72% $HClO_4$). Upon complete digestion the samples were evaporated to dryness in order to free the samples from acids corrosive to the stainless steel sample pans. The residue was then dissolved in distilled water and the volume of the solution was reduced to two or three ml. The solution was filtered and brought up to 10 ml. volume in a volumetric flask. A sample, generally 2 ml., which provided a fairly active sample was withdrawn and evaporated in a stainless steel sample pan under an infrared lamp on a 16 rpm sample spinner.

The samples were then stored for at least two weeks to allow the Sr^{90} and Y^{90} to reach equilibrium before they were counted. At the end of this period the samples were counted with the counting system previously described.

Physical Measurements on Roots

The roots were freed of sand particles and were blotted with absorbent tissue to remove any excess water and were immediately weighed to obtain the "wet" weights.

The volumes of the recovered root systems were obtained by using a water displacement technique.

The roots were then dried at 75 degrees centigrade for 12 hours and the "dry" weights were then determined. For each of these measurements the percent of the roots in each zone for a given pot was calculated.

Data Analysis

The counts per minute contributed by the two beta emitters were determined by multiplying the counts per minute traversing the zero absorber thickness by the percents contributed by each of these two isotopes as determined by the procedure previously discussed. These values were then summed with the counts per minute contributed by the Sr^{85}. The percent of each isotope present in a given forage sample was then calculated.

IV RESULTS AND DISCUSSION

Evaluation of the Methods for Root Measurement

Evaluation of the three methods of root measurement showed the "dry" weight method to be the most accurate followed by the "wet" weight and the volume determinations. In making the "wet" weight and volume determinations there was the possibility of leaving excess water on the roots which could account for some error. Error in the volume determination could have resulted from the small volume of roots found in the zones.

There was very close agreement between the three percentages in all the pots, only in one pot, pot 9, did the three percentages vary over a range greater than 12 percent. (Tables IV, V and VI).

Root Measurements Versus Strontium Uptake

Agreement between the physical root measurements and the predicted root activity for 10 of the first 12 pots harvested is shown in Table IV. When a 15 percent range between the four percentages was considered tolerable, 9 of these pots agreed for all three measurements and 10 pots were in agreement for the "dry" weight determinations. These pots were harvested in the first 72 days of the experiment and a major portion of all the roots in the pots were light in color, fine and fibrous (Figures 9, 10, 11 and 12). In this initial stage of the experiment the zone of greatest activity was in the upper zone as indicated by the strontium

TABLE IV

PERCENT OF RECOVERED ROOTS AND STRONTIUM UPTAKE FOR ROOTS WHICH ALL APPEARED TO BE ACTIVE ABSORBERS*

Pot	Layer	Root Concentration			% Sr. Uptake	No. Days Between Planting & Harvest
		% Volume	% Dry Wt.	% Wet Wt.		
24	1	100	100	100	100	14
21	1	67	67	73	67	14
21	2	33	33	27	33	14
26	1	61	67	59	81	32
26	2	34	28	36	16	32
26	3	5	4	5	2	32
28	1	50	48	49	50	43
28	2	31	34	32	45	43
28	3	19	18	19	6	43
30	1	90	96	99	88	51
30	2	10	4	1	12	51
19	1	46	47	48	40	51
19	2	43	43	42	56	51
19	3	11	10	10	5	51
23	1	50	56	58	55	57
23	2	50	44	42	45	57
27	1	75	69	76	79	57
27	2	17	17	11	16	57
27	3	8	14	13	5	57
11	1	46	56	53	53	66
11	2	44	32	38	32	66
11	3	10	12	9	14	66
10	1	59	54	62	56	72
10	2	29	24	27	35	72
10	3	12	22	11	9	72

* Note the agreement between the percent strontium uptake and the percent roots recovered for the pots listed in Table IV. These 10 pots were among the first 12 pots harvested in the initial 72 days of the experiment.

Figure 9. Roots recovered from pot 30. Note the fine fibrous roots exhibited in both dishes.

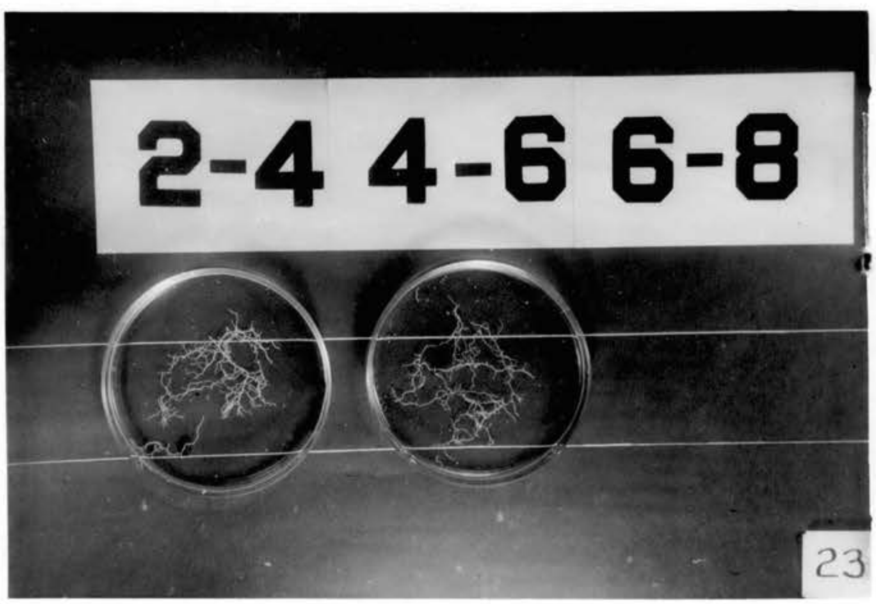

Figure 10. Roots recovered from pot 23. Note the fine fibrous roots exhibited in both dishes.

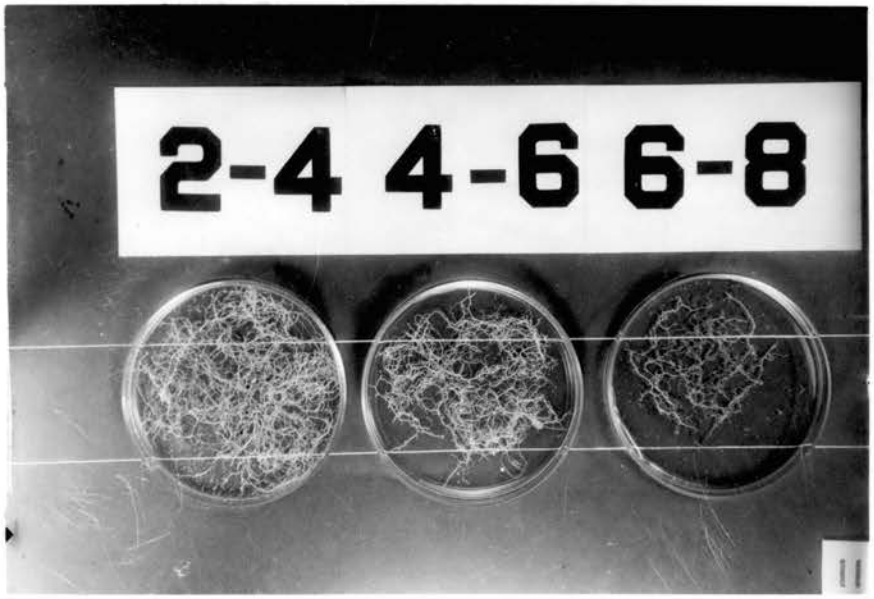

Figure 11. Roots recovered from pot 11. Note the fine fibrous roots exhibited in all the dishes.

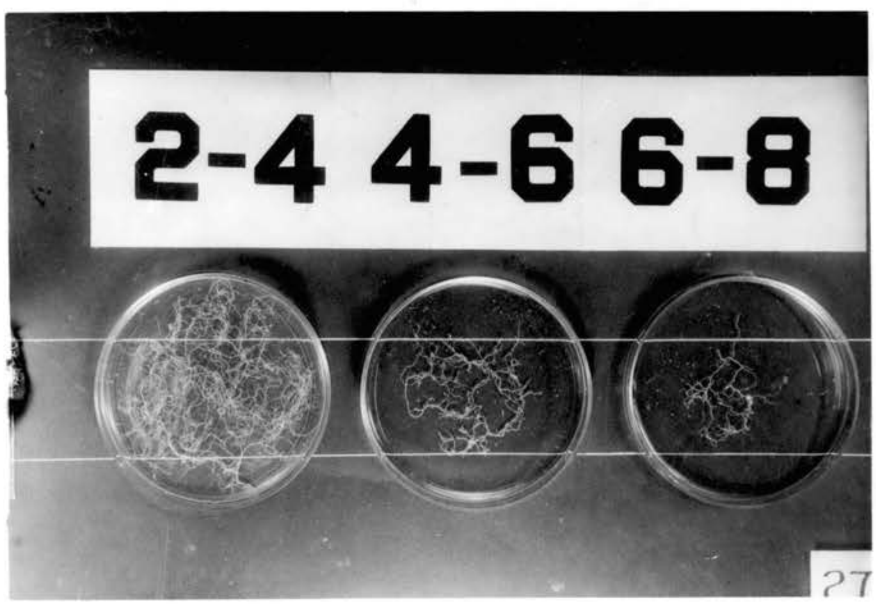

Figure 12. Roots recovered from pot 27. Note the fine fibrous roots exhibited in all the dishes.

uptake and the quantity of roots in these zones.

Four other pots, pots 7, 9, 12 and 20, harvested at later dates also agreed for the 15 percent range. The percent of strontium uptake and the percent of the roots recovered from these pots are listed in Table V. The agreement between the percentages for these four pots were not as close as the pots reported in Table IV. Observations of the roots showed that for the most part they were light in color, fine and fibrous in nature, but a small portion had become coarse and woody.

Many of the roots in the top zone of the remaining pots had become dark, coarse and woody and no longer appeared to be active absorbers (Figures 13 and 14), whereas, the roots in the middle zone were still light and fine in texture. When a pronounced amount of these dark coarse roots became visible the percent uptake varied widely from the percent of the roots recovered from a given pot as shown in Table VI. Visual observations of these pots revealed that the zone of greatest activity had shifted from the top zone to the middle zone. The radiostrontium determinations also showed the same indications.

From the results presented in Tables IV, V, and VI, two distinct sets of data are evident, that is, either agreement or disagreement between the percent uptake and the roots which occurred between the wax layers. In order to interpret these data perhaps it would be worth while first to review the events which would be expected to occur after a root penetrated one of the paraffin-rosin layers.

Consider a root emerging into a zone of isotope placement. Upon emerging, the root would be expected to be an active absorber and the more active the root the greater the absorption. As the root ages and branches out it will continue to function as an absorber until the cell

TABLE V

PERCENT OF RECOVERED ROOTS AND STRONTIUM UPTAKE FOR ROOTS OF WHICH A MAJOR PORTION APPEARED TO BE ACTIVE ABSORBERS*

Pot	Layer	Root Concentration % Volume	% Dry Wt.	% Wet Wt.	% Sr. Uptake	No. Days Between Planting & Harvest
20	1	33	40	34	45	79
20	2	50	49	61	49	79
20	3	17	11	5	6	79
9	1	71	62	82	56	86
9	2	24	36	18	40	86
9	3	6	2	1	4	86
7	1	29	33	37	41	93
7	2	50	45	50	51	93
7	3	21	22	17	7	93
12	1	85	82	90	68	93
12	2	14	17	9	28	93
12	3	1	1	1	4	93

* Note that some agreement exists between the percent strontium uptake and the percent of roots recovered for the pots listed in Table V. It should also be noted that the percentages listed in this Table do not agree as well as the percentages listed in Table IV.

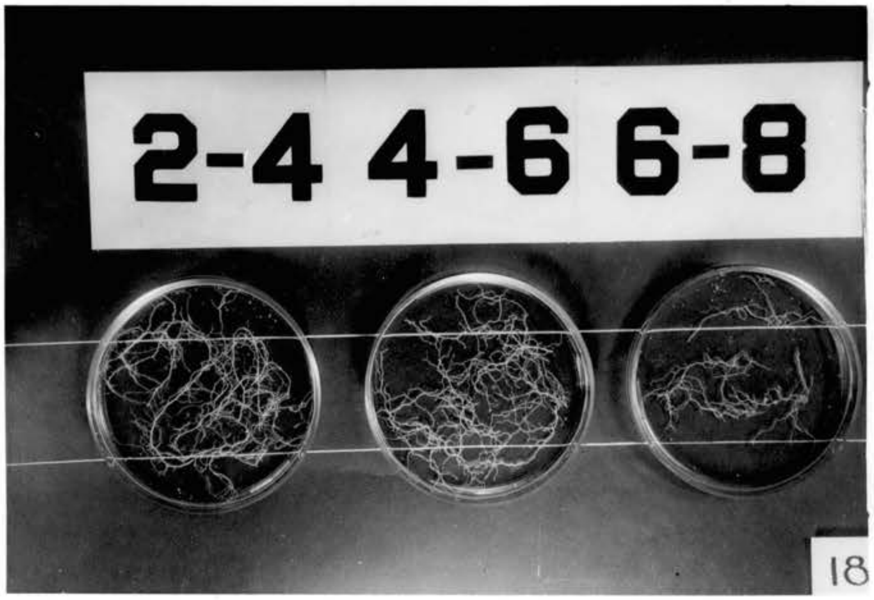

Figure 13. Roots recovered from pot 18. Note the large coarse roots which appear in the 2-4 inch dish as compared with the fine fibrous roots appearing in the remaining dishes.

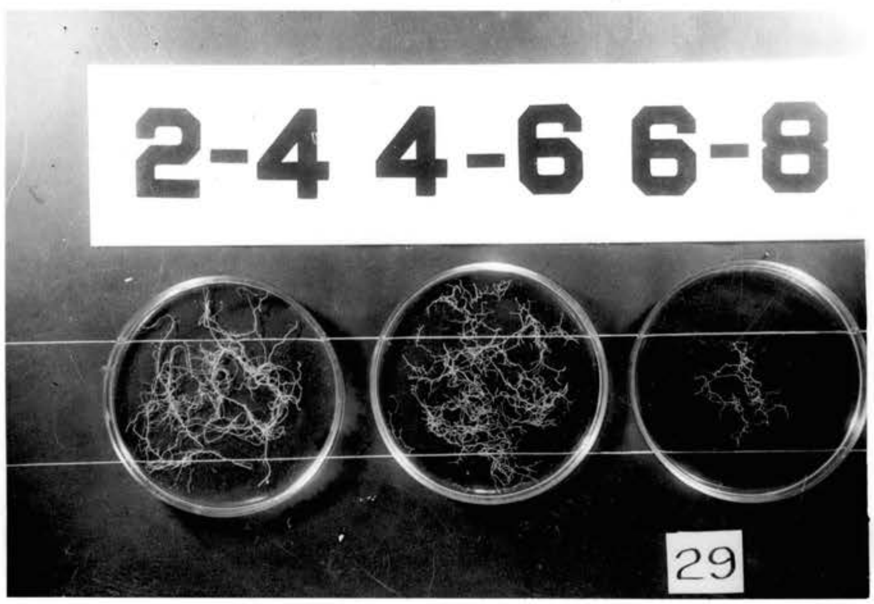

Figure 14. Roots recovered from pot 29. Note the large coarse roots which appear in the 2-4 inch dish as compared with the fine fibrous roots appearing in the remaining dishes.

TABLE VI

PERCENT OF RECOVERED ROOTS AND STRONTIUM UPTAKE FOR ROOTS OF WHICH A LARGE PORTION APPEARED TO BE INACTIVE*

Pot	Layer	Root Concentration % Volume	Root Concentration % Dry Wt.	Root Concentration % Wet Wt.	% Sr. Uptake	No Days Between Planting & Harvest
25	1	50	52	58	44	43
25	2	28	24	18	52	43
25	3	22	24	24	4	43
18	1	46	45	35	40	57
18	2	31	37	42	54	57
18	3	23	18	23	5	57
14	1	45	48	48	45	72
14	2	22	18	19	50	72
14	3	32	34	33	5	72
17	1	45	53	45	27	79
17	2	32	29	29	69	79
17	3	23	19	25	3	79
22	1	45	41	52	35	86
22	2	36	35	28	62	86
22	3	18	24	20	3	86
29	1	56	58	63	35	86
29	2	38	35	33	59	86
29	3	6	7	4	5	86
8	1	62	62	73	54	93
8	2	23	21	15	40	93
8	3	15	16	12	6	93

* Note the wide variation between the percent of strontium uptake and the percent roots recovered listed in this Table as compared with the percentages listed in Tables IV and V.

walls are infiltrated with fatty, waterproof substances (41). The root will gradually decrease in absorption until the root becomes completely suberized, at which time it will cease to function as an absorbing organ even though it will continue functioning as a conductor and continue to expand in size.

Weaver and co-workers (44) in a discussion of these events stated that "even among fibrous-rooted species, including cereals, it must be kept distinctly in mind that the number of roots in any given area of soil is no criterion of their activity. It seems probably that with increasing age the older roots are cutinized and suberized and unless new branches are put out, the seat of maximum absorbing activity is transferred to soil layers of ever-increasing depth, inhabited by the younger parts of the root system."

In view of this discussion, it seems that a close relationship can not be expected between the uptake of strontium and the quantity of roots which occurred in the various zones of placement. However, a thorough study of both the physical determinations and the visual observations obtained in this experiment showed that due to the suberization of the older roots and the downward movement of the seat of maximum absorption that two distinct comparisons can be made: (1) a comparison when all the roots in a given pot appeared to be active absorbers and (2) a comparison when only a portion of the roots in a given pot appeared to be active absorbers.

In making such comparisons it appears logical to assume in the first case that as long as a major portion of the roots were still active that some relationship should exist between ion uptake and the quantity of roots which occurred in the various zones of placement. The results reported in Table IV shows this assumption to be valid, that is, as long as the roots within a given pot were light in color, fine and fibrous, which appears to be the characteristics of active absorbing roots (Figures 9, 10, 11 and 12), the physical measurements were in agreement with the predicted values.

In the second comparison, when a large portion of the roots in a given zone were suberized (Figures 13 and 14), it does not seem feasible that an absorption technique can be used to measure the root concentrations. The inability to make such a comparison has been previously pointed out in the work of Burton and co-workers (3). The data presented in Table VI verifies this assumption.

Obviously, these old suberized roots made a major contribution to the total root system and even though they were actively absorbing from the time of emergence to the date they became suberized the growth attained after suberization more than offset the added absorption which occurred due to the longer life in the zones of placement.

Due to these old suberized roots it does not seem feasible that an absorption technique can be used as an indication of root concentrations. However, the ability to measure the zones of activity which occurred from the date of isotope introduction to the date of harvest by the uptake of the radioisotopes appears to be a logical interpretation of the data presented in this discussion. It will generally be the root activity which is of interest in experimental work so the multiple radioisotope technique appears to have promise.

To recapitulate, the results obtained in this experiment showed that as long as all the roots in any given pot were light in color, fine and fibrous in nature the predicted root activity and the physical measurements were in agreement. As the roots in the upper zone became dark, coarse and woody the zone of activity shifted into the middle zone and the predicted values varied widely from the physical determinations of the gross root concentration. This indicated that the uptake of strontium and the root concentrations could be compared as long as the largest portion of the roots were actively absorbing. As the older roots became suberized this comparison could no longer be made. Since good agreement existed between the strontium uptake and the root concentrations in the pots where the roots all appeared to be active absorbers, this appears to be an indication that the uptake of strontium can be used to measure root activity. Furthermore, it is believed that since this comparison can be made that the uptake of strontium can be used as a tool for measuring root activity even though some of the roots are suberized and are no longer absorbers.

Since the results of this experiment showed that a multiple tracer technique is possible for measuring root activity of growing plants it will now be worthwhile to consider some of the refinements that will be needed before such a technique will be applicable to extensive research work in the field.

The largest refinement needed at the present appears to be in the use of long half-life isotopes, especially Sr^{90}, in the field. However, this problem for the use of three isotopes may be solved in the near future since there is a fourth radioisotope of strontium, strontium-82, which might be substituted for strontium-90. At the present to the author's knowledge this isotope is not commercially available, but if and when it does become available it would solve the problem of using the extremely long half-life isotope, Sr^{90}.

Other studies which will need due consideration before this technique will be applicable to field work includes methods of placement and mobility of strontium in various soil classes. An extensive study needs to be made on methods of isotope placement in order to utilize fully the time saving features which this technique offers. Without rapid and simple methods of isotope placement this technique might well have to forfeit some of its labor saving features. Studies also need to be conducted on the mobility of strontium in various soil classes before this technique can be used with assured accuracy.

V SUMMARY AND CONCLUSIONS

This thesis reports the results of a laboratory experiment conducted at Oklahoma State University in 1958 and 1959. The objective of this experiment was to investigate the possibility of using a radioactive tracer technique to characterize the root activity of individual plants.

The rooting zone of the plants under study were divided into three zones separated by a paraffin-rosin layer. Within each zone was incorporated one of three radioisotopes of strontium, $Sr^{90} + Y^{90}$, Sr^{89} and Sr^{85}.

Ladino clover, Trifolium repens L., plantings were made in the prepared pots on February 13, 1959. The entire aerial portion of the plants were harvested and the percent of the three radioisotopes present in the forage was determined for each pot. The roots from each zone were collected and "dry" weight, "wet" weight and volume determinations were made.

A comparison was made between the percent strontium uptake and the percent of the roots which occurred in the various zones of placement. The results showed a very close agreement between the uptake of strontium and the roots which occurred in each zone when all the roots in a given pot appeared to be actively absorbing. However, when a portion of the roots became dark and woody and no longer appeared to be active the uptake and the root concentrations varied widely.

Since good agreement existed between the strontium uptake and the root concentrations in the pots where the roots all appeared to be active, it is believed that the uptake of strontium can be used as a measure of root activity. If this comparison is valid, it seems logical to assume that even though a portion of the roots are suberized and are no longer absorbing that the uptake of strontium can be used as a measure of root activity.

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